

# Introduction to Electricity

- Charge (Q) is measured in coulombs (C)
  - Charge of 1 electron is  $1.6 \times 10^{-19}$  C
  - 1 coulomb is  $6.25 \times 10^{18}$  electrons
  - Charge of 1 mole of univalent ions = Faraday constant
    - F = 96,500 coulombs per mole
  - $Q = It$ 
    - Q = total charge
    - I = current
    - t = time for current flow
- Current is the flow of electrons or ions from 1 place to another
  - Rate of change of charge with time
  - Measured in amperes (A)
  - $1A = 1C/1s$
- Potential difference is measured in volts (V)
  - Difference between the amount of charge in 2 places
  - High voltage difference = more flow (increase in A)
- Resistance
  - Reduce amount of ions that can flow through
  - For any given potential difference the current that flows through an element of a circuit is determined by its resistance
  - Smaller conductance = high resistance = smaller flow of ions

## Ohms' Law

$$V = IR$$

→ Resistance is ohm ( $\Omega$ )

$$G (\text{conductance}) = \frac{1}{R}$$

→ Conductance is measured in siemen (S)

Therefore:  $I = GV$

## Circuits

Elements in an electrical circuit can be arranged in series, parallel or in combination of series and parallel

Resistors in series:

$$V = IR_1 + IR_2 = I(R_1 + R_2)$$

$$\frac{V}{I} = (R_1 + R_2)$$

$$R = R_1 + R_2$$

- Current is the same throughout the circuit
- Voltage drops proportionally to the resistance as it passes through each resistor
- Resistances in series add

Resistors in parallel:

$$I = I_1 + I_2 = \frac{V}{R_1} + \frac{V}{R_2} = V\left(\frac{1}{R_1} + \frac{1}{R_2}\right)$$

$$\frac{1}{R} = \frac{1}{R_1} + \frac{1}{R_2}$$

- Resistances in parallel add as reciprocals
- Voltage difference across 2 resistors are the same

## Capacitance

- Capacitor = insulator (does not conduct) placed between 2 conductors
  - This allows for the storage of charge
- When a capacitor is connected to a battery (a voltage difference is applied) electrons build on 1 plate due to the current flow, repelling electrons from the other plate.
  - 1 plate = positively charged
  - 1 plate = negatively charged
- Once capacitor is fully charged up, electron flow stops and charge is stored on the plates
  - An electrical potential difference is stored across the capacitor
- Charge stored in a capacitor is proportional to applied voltage
  - $Q = CV$
  - C = capacitance, indicating how much charge can be stored for a given charging voltage
  - Capacitance measured in farad (F)
    - 1F = can store 1C of charge given a 1V potential difference
- Factors affecting capacitance
  - Plate area
    - Large plate = more charge stored
  - Plate spacing
    - Closer plates = more charge stored
  - Dielectric material
    - Dielectrical material = more charge stored
- Capacitors can be connected in series or in parallel
  - Series:  $C = C_1 + C_2 + \dots$
  - Parallel:  $\frac{1}{C} = \frac{1}{C_1} + \frac{1}{C_2} + \dots$
- Current flow in a circuit with a capacitor is dependent on time
  - When a capacitor is initially uncharged and a voltage is applied across it, such as by connecting it to a voltage source through a resistor, current begins to flow into the capacitor.

- During this charging process, the current gradually decreases over time as the capacitor becomes increasingly charged.
- This is because as the capacitor charges up, the potential difference (voltage) across it increases, reducing the potential difference between the plates and thus reducing the driving force for current flow.

## Methods for Studying Ion Channels

Ion channels in membrane are either closed or open

Channels are inserted in parallel in the membrane

- Conductances sum
  - $G_m = G_1 + G_2 + G_3$
  - $\frac{1}{R} = \frac{1}{R_1} + \frac{1}{R_2} + \dots$

### Patch Clamping

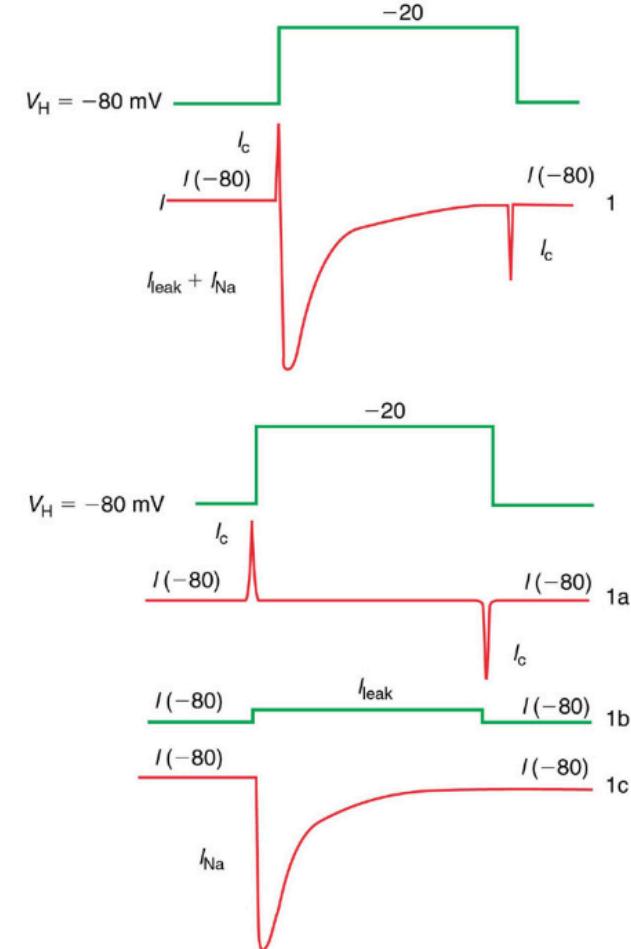
- Microelectrodes are sharp and will impale the cell
  - Only allow voltage difference between inside and outside of membrane to be measured
- Patch clamps are used to measure electrical signals on a patch of membrane or the whole cell
- Patch clamps are positioned onto the membrane and a small suction is applied
  - Isolate and measure the current flow for the channels suctioned only
  - Near infinite resistance between inside of pipette and outside  $\Rightarrow$  current cannot flow outside the pipette
  - Electrode is inserted through the pipette to pass current through cell
- Patch clamps are made of glass
  - Glass sticks well to the membrane as the membrane is made of lipids
- When a voltage is applied, the ions will start to flow through the ion channel suctioned by the patch clamp, and current generated by the ion flow can be measured

### Voltage Clamp

- Voltage clamp is when circuit is stably fixed to a predetermined voltage
  - Voltage difference between cell and extracellular space is fixed
- Electrode inserted into cell, current injected into cell
- Voltage clamp = feedback system
  - Feedback amplifier compares voltage across membrane with imposed command voltage

- Amplifier injects current with equal size and duration to the cell, but different sign to the synaptic current to oppose the synaptic current, counterbalancing the change in voltage that is caused by the synaptic current

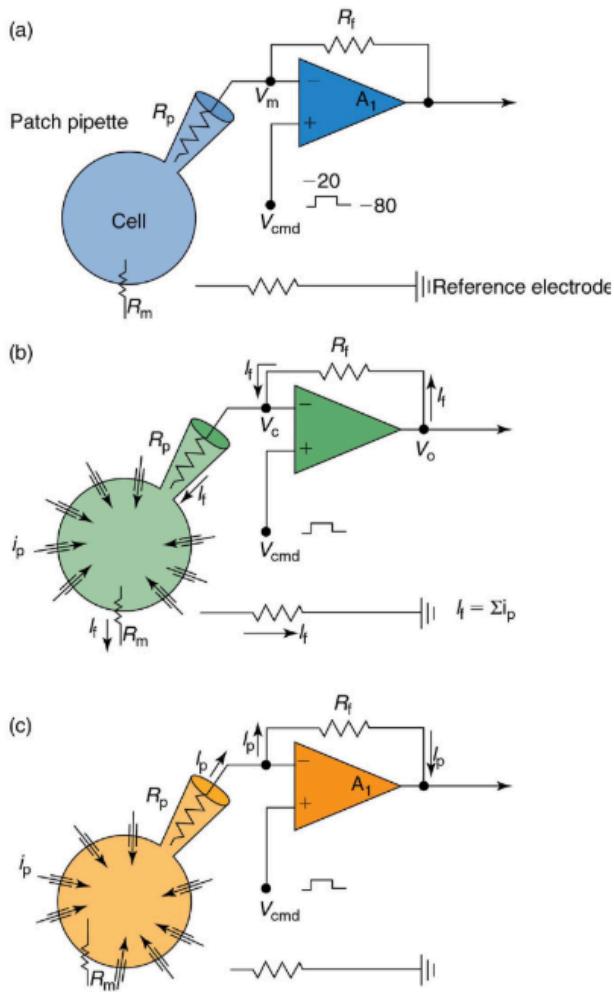
- $I_c = V_B - V_A$
- $I_c = \text{injected current}$



- - Here  $K^+$  and  $Ca^{2+}$  channels are blocked
  - $I_{\text{Na}}$  (sodium current) is downwards as opposed to upwards (which represents depolarisation) because here the  $I_{\text{Na}}$  is actually the current injected to oppose the sodium current, thus while the size and shape will be the same, it will be in the opposite direction
  - $I_c = \text{capacitative current}$
  - $I_L = \text{leak current}$ , it is linearly proportional to the  $\Delta V_m$  (membrane potential change, -80mV to 0mV = a +80mV change)

- Current monitor (ampere meter) measures the current it takes to hold the voltage (aka the current needed to counterbalance synaptic current)
- Definitions
  - $i$  = current that flows through a single channel when it opens
  - $g$  = single channel conductance
    - $g = i/V$
    - $V = \text{voltage / driving force amount}$
  - $P$  = fraction of time the channel spends in open state

- Can be brief
- $N$  = number of channels
  - Patch clamping,  $N$  is number of channels within pipette tip area

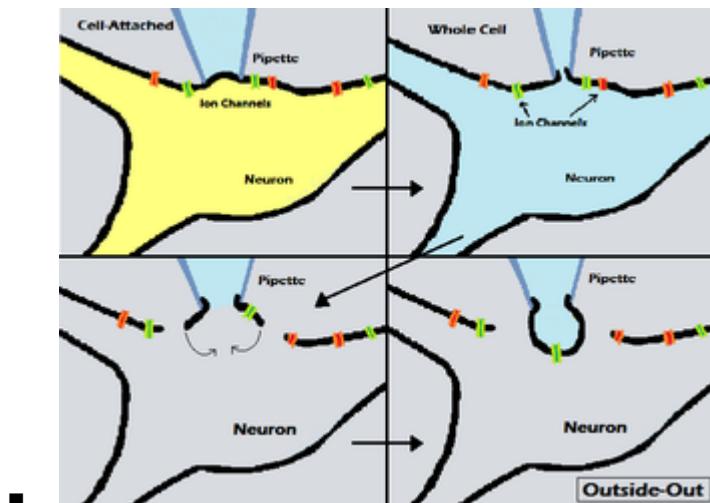


- Voltage clamping in patch clamp
  - $V_m$  and  $V_{cmd}$  (common voltage) are both sent to the amplifier  $A_1$  which compares the two
  - $A_1$  sends  $V_o$  (voltage that can evoke a current that has the size to counterbalance cell current)
    - $V_o = R_f I_f$
    - $R_f$  = resistance for feedback resistor
    - $I_f$  = feedback current, current required to oppose cell current
  - $I_f$  sent to cell
  - $I_p$  (whole cell current) flows through circuit and is measured as a voltage change of whole cell to  $V_{cmd}$

## Configurations

- Cell attached

- Record activity of all the channels contained in the small patch of membrane
  - Intracellular enviro = cell itself
  - Extracellular enviro = pipette
- The resistance between the inside of the pipette and the external solution is virtually infinite, no current can flow between the membrane and glass of the pipette, and current cannot flow from inside of pipette to the outside of cell
  - Electrical isolation of membrane patch under tip of pipette → leaked current cannot be measured
  - Augments signal to noise ratio
- **Can be used to measure single channel current ( $i$ )**
  - However,  $V_m$  is not known since  $V_m = V_i - V_e = V_i - V_p$  ( $V_p$  is pipette voltage) and we don't know cell internal voltage ( $V_i$ )
- Inside-out
  - Pull from cell-attached configuration → the membrane breaks off and the inside of the membrane now faces the outside of the pipette
    - Extracellular enviro = Pipette
    - Intracellular enviro = Bath
  - Inside of membrane is accessible, can be used for looking at effects of internal metabolites on channels (e.g. second messengers)
    - Can test rapid changes in composition of intracellular enviro on channels
- Whole cell
  - Break the "bleb" suctioned by pipette → Electrode now connected to the whole cell
  - Measures contribution of all channels to total membrane current
  - Cytoplasm of cell diffuses out and is replaced by pipette solution → control ion concentrations at expense of loss of metabolites important for channel function or modulation (lose some features of cell)
  - **Total current flowing through population of identical channels can be measured**
    - $I = Np_o i$ 
      - $p_o$  = fraction of channels are in open state
- Outside-out
  - Pull from whole-cell configuration → membrane is elastic and elongate as you pull, eventually when it breaks the ends to will fuse, the outside of the membrane faces the outside of the pipette



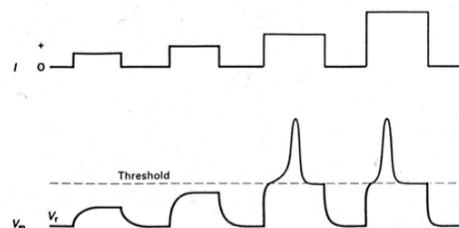
- Intracellular enviro = pipette
  - Extracellular enviro = bath
- It can be used to look at effects, especially for ligand-gated ion channels and external ligands (e.g. transmitters or antagonists)
  - Often used for concentration jumps
    - Concentration jumps are when application of agonist is very rapid and the duration of application is short (e.g. using theta tube with top compartment filled with normal solution while bottom is agonist, move the tube up and down very fast)
      - Rapid changes of the extracellular solution
    - Concentration jumps mimic what occurs at a synapse

All configurations except cell-attached, allow for control of intracellular environment  
 Allows for single cell recordings and recordings from cells too small to be impaled

## Structure of Ion Channels

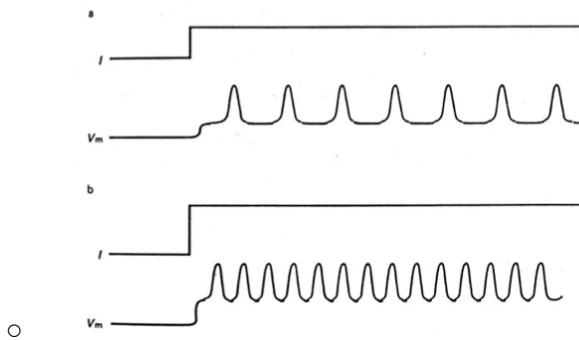
- Ions in solution are surrounded by a cloud of water molecules attracted by the net charge of ion → energetically unfavorable, thus improbable
- Ion channels are large integral membrane proteins that form aqueous pores through plasma membrane to allow ions to cross
  - Plasma membrane act as selective barrier
- Basic signalling unit is the action potential (all or nothing)
  - Threshold for generation of action potentials guarantees that small random variations in membrane potentials are not interpreted as meaningful information
  - All or nothing → once threshold is crossed, AP will be formed; below the threshold, nothing will happen, also AP = full size to guarantee that nothing will be lost along the way

The all-or-none law

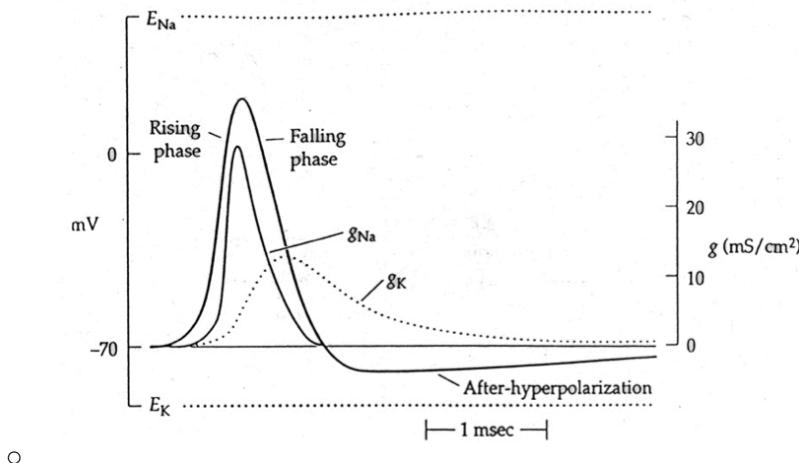


- - Strength-latency relationship and refractory period allow encoding of info in form of frequency code

## Frequency coding



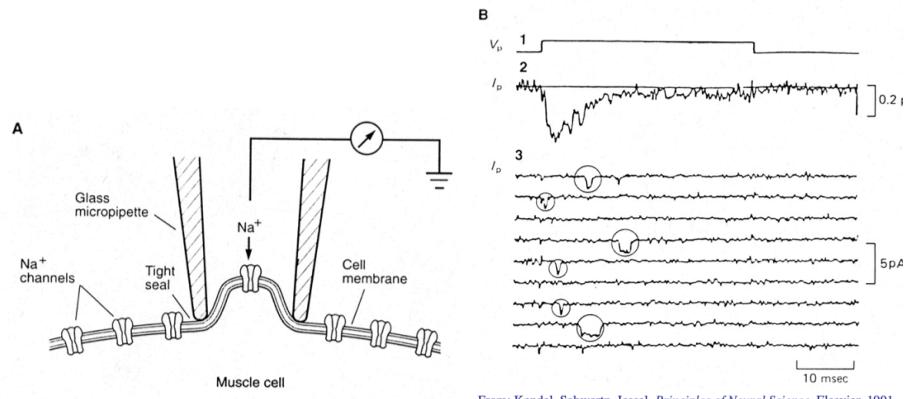
- Conductances can never be negative, membrane potential changes with potassium and sodium at different times for AP duration



- Depolarisation that initiates an AP causes transient change in membrane  $\rightarrow \text{K}^+$  permeability to  $\text{Na}^+$  permeability
- Armstrong and Hille's Working Hypothesis:
  - Ions are passing through aqueous pores called channels
  - Ion channels are proteins
  - Channels for  $\text{Na}^+$  and  $\text{K}^+$  are different
  - Swinging gates open and close in response to voltage
  - Use electric currents to measure gating, permeation and block
  - Channel blockers are molecules that enter the pores and physically plug them

## Ion Channel Experiments

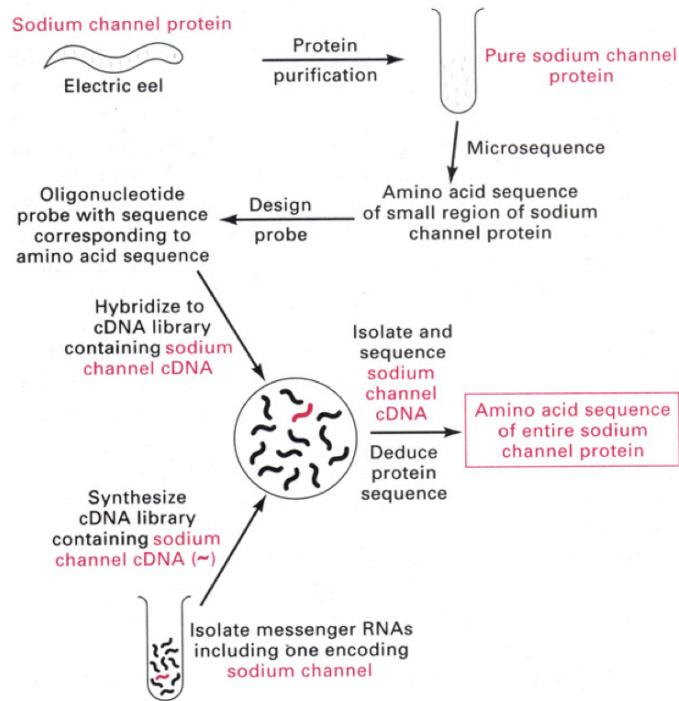
- Ion channels theory can be experimentally tested:
  - Patch-clamp technique  $\rightarrow$  single channel measurements
    - Voltage clamp



From: Kandel, Schwartz, Jessel, *Principles of Neural Science*, Elsevier, 1991

- Molecular cloning → voltage-gated ion channels and structure-function studies
  - Using electrical eel

### Cloning of a $\text{Na}^+$ channel via protein purification



- 1. Purify protein so only have pure sodium channel proteins
  2. Proteolytic digest of channels to obtain stretches of amino acids (20-25 aa) which represent the sodium channel
  3. Obtain a mixture of oligonucleotides with sequence corresponding to the amino acid sequence
  4. Obtain mRNA from rat brain and synthesize cDNA (complementary DNA),
  5. Label oligonucleotides radioactively
  6. Hybridise the rat cDNA with the eel oligonucleotides
  7. The hybridised DNA will appear positively labelled and represents the DNA for the sodium channel
  8. Isolate and sequence this sodium channel cDNA to get amino acid sequence

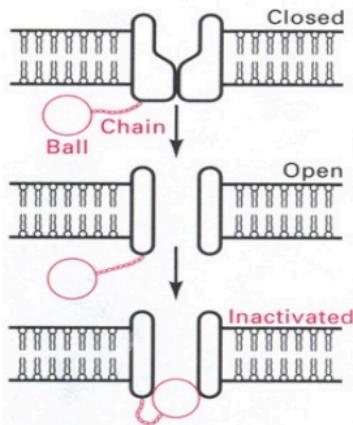
- Hydropathy plot was obtained by assigning to each amino acid in the protein a hydrophobicity and hydrophilicity value
  - Every amino acid is hydrophobic or hydrophilic
  - Hydrophilic lie more on outside of protein, hydrophobic lie on the inside
  - Apply this knowledge to membrane protein
- To cross the bilayer, membrane protein needs alpha helices (~20 amino acids), length of the channel protein is not found in globular proteins
- Running average over 19aa are calculated (e.g. amino acids 1-19) for each amino acid in the sequence (e.g. after doing it for 1-19, the next one is 2-20) and this average is plotted against its position (e.g. if average is 1.5 for amino acids 1-19, 1.5 is plotted on the amino acid 1 position)
- When hydrophobicity is bigger than 1.5 and the sequence is longer than 20 = alpha helix
  - Identified 4 domains each with 6 alpha helices
- **Structure-Function studies** (how do we know it's an ion channel)
  - Xenopus oocytes
  - Convert sodium channel alpha subunit cDNA into cRNA
  - Inject mRNA into oocyte and see if we can record current using patch-clamp
    - Some oocyte are injected with water → should see no response
    - Some injected with pure RNA of alpha subunit only → small current
    - Some injected with rat brain mRNA
  - Open probability / current is greater in oocytes with pure sodium channel mRNA
  - Open probability is shorter in oocytes with total rat brain mRNA
    - Not completely resembling the oocytes with pure sodium channels
    - Due to the presence of other associated subunits
- Transfection of clonal cell lines and site-directed mutagenesis
  - cDNA encoding channel protein is constructed and then modified (mutated)
  - Mutated cDNA used to transfet mammalian cells or to produce messenger RNA which is injected into xenopus oocytes
  - Mutations of alpha subunit of sodium channel changes channel kinetics
    - Determine what role specific amino acids play
- Crystallisation → high resolution 3D structure of ion channels

## Voltage-gated Ion Channels

- Water filled pores
- Sensitive to voltage changes, opens and closes in response to changes in membrane potential
- Flow of ions = passive
  - Equilibrium set up across membrane is determined by electrochemical driving force across membrane
- Selectivity filter (e.g. discriminate on basis of ionic charge, diameter and weak electrostatic interaction of the ions with the amino acid residues that line the wall of the channel)
- Gating
  - Transition between close and open states in response to voltage changes

- Involved conformational changes in channel structure
- Gating current
  - 100 times smaller than ionic current
  - Gating charge is the charge on the voltage sensor, gating current is the flow of current within the channel protein
  - Very brief
  - Immediately after change in voltage, gating current flows, but only while channel protein is undergoing the movement to new conformation (ionic current flow only after new conformation)
- Ion channels proteins have defined functional domains → voltage sensor
  - S4 segment = voltage sensor
    - Every 4th amino acid was a positively charged one
  - In helix form, the positively charged residues would be arranged in spiral around the helix
  - Change in electric field across the alpha-helix leads to uncoupling of positive residues from their partners → displacement or rotation of helix
    - Move in direction that is perpendicular to plasma membrane
  - Movement of charge in direction of imposed electric field
    - S4 contains amino acids, H, R and K which are positively charged at physiological pH
- Channel has 3 states
  - Open, close, inactivated
    - Delayed rectifier potassium channels do not typically inactivate like other voltage gated ion channels → undergo slower process of activation and deactivation rather than fast inactivation
  - Ball and chain model
    - It is the process by which a channel enters a non-conducting state following a depolarisation
    - They are unable to open for a period of time
    - Inactivation results from the block of the open channel by part of the channel protein located in the amino-terminal region of each subunit

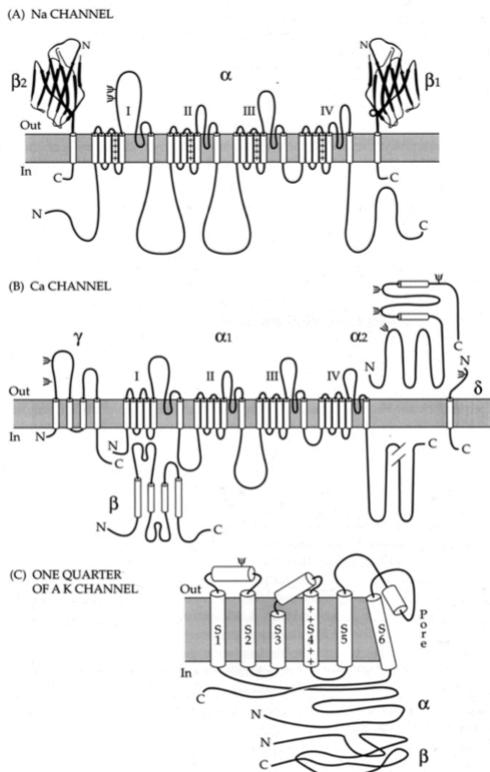
### *The inactivation domain*



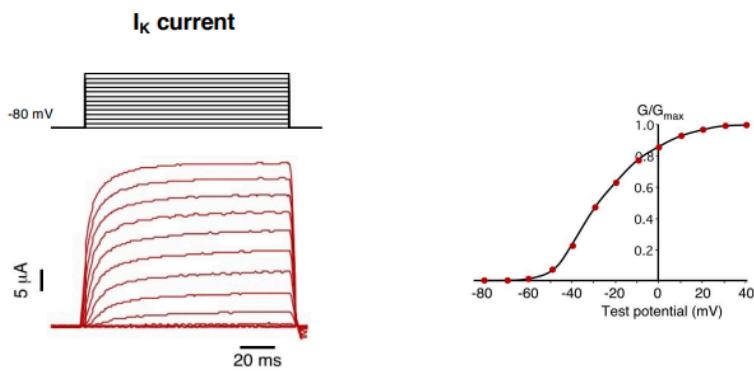
- Inactivation is linked with N-terminus

- Removal of amino-terminal around 20 amino acids of the Shaker potassium channel by mutagenesis eliminates rapid inactivation
- Synthesize the part you removed to create ShB and add it into the bath
- The inactivation is back after adding the ShB peptide into the bath

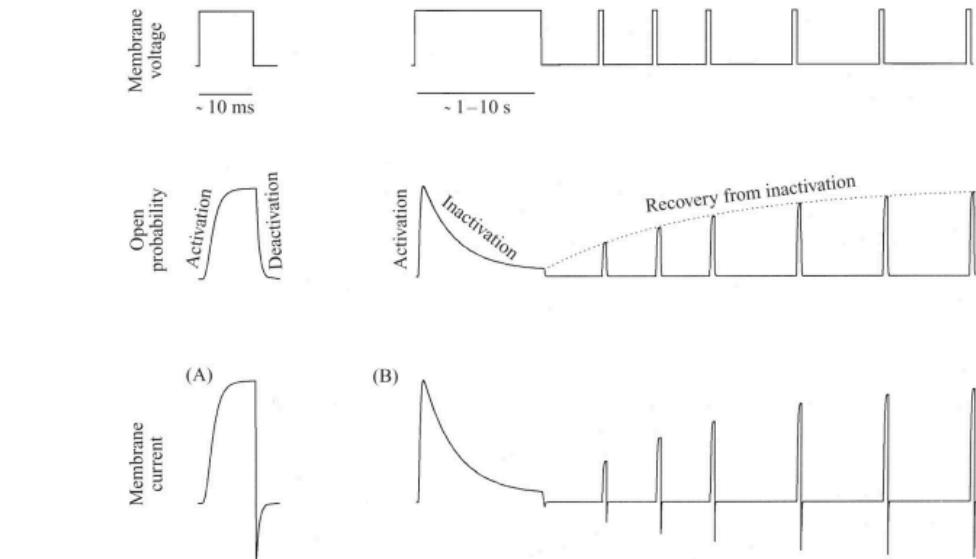
## Potassium Channel



- ○ Potassium channels are tetramers
  - Homomer - 4 identical subunits come together
  - Heteromer - 4 different subunits come together
- Relative conductance increases as current becomes more positive

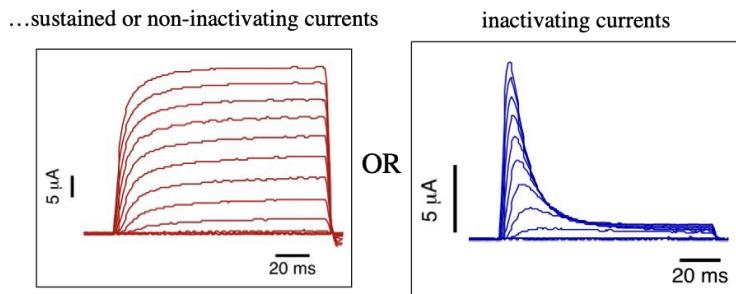


- ○ Terminologies

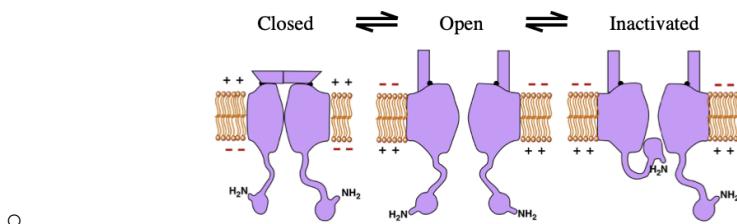


- ○ Voltage steps
- ○ Open probability
  - Deactivation for voltage-gated ion channels (Desensitisation is for another thing)
  - Activation upwards curve and inactivation downwards current
  - Longer pulse may have both activation and inactivation
  - Recovery from inactivation → open probability are not as great
- Membrane current → similar to open probabilities
- Tail current → measure channel properties
  - Channel open at 20mV, inward flow
  - Invert flow when -100mV, channel close
    - Outward current turns into inward current when current is more negative than -80mV for potassium through leak channels because the reversal potential for potassium is around -80
  - -40mV, channels do not completely close after going to -80mV, a few channels open still
- Defined functional domains
  - Pore domain
  - Use blocker CTX (block outside) and TEA (block from inside)
  - Use 2 different ion channels with different conductance
  - When pore domain is exchanged, single channel conductance is exchanged as well
    - Pore domain is important for conduction properties
- Inactivating and non-inactivating potassium currents

Once activated, potassium channels can mediate...

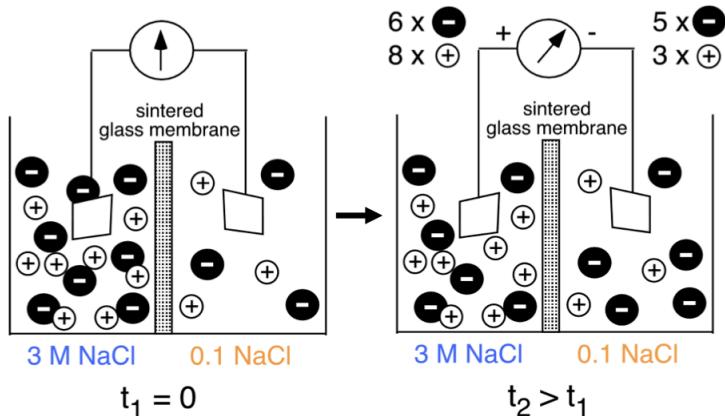


Model for potassium channel inactivation



- Water is stripped off by ion channel

## Bioelectricity 1



Adapted from Hille, Ion channels and excitable membranes, 3rd edition, page 320

The central membrane is equally permeable to both ions ( $\text{Na}^+$  and  $\text{Cl}^-$ )

- First Tank:
  - ◆ Electroneutrality on both sides (equal numbers of positive and negative charges on both sides)
  - ◆ No movement
- Second Tank:
  - ◆ Chloride ions are 57% faster than sodium ions
  - ◆ Chloride ions move from the compartment of higher conc to lower conc (left to right) ⇒ both compartments become not electrically neutral
    - Diffusion potential created

- ◆ Resulting electric field accelerates  $\text{Na}^+$  motion and slows  $\text{Cl}^-$  movement as the right side becomes more negatively charged  
⇒ Both diffuse at the same rate
- ◆ Concentration gradient diminish, and diffusion potential declines
- ◆ Steady state reached when ion movements result in no net change of numbers on each side
- Magnitude of diffusion potential mainly depends on:
  - Concentration gradient
  - Electrical potential difference
    - Ions will flow in the direction and to side of the membrane that has a potential opposite to the charge it carries
    - Strength of the electrical field (size of potential difference across the membrane) is proportional to the force propelling ions to move
    - Ions on the side of the membrane with a net charge opposite to their own will be impeded from moving through the channel
  - Difference in the mobilities of ions
    - Mobility of ions in solution depends on
      - Size:
 
$$D_s = \frac{k_B T}{6 \pi r_s \eta}$$
        - $r > \Rightarrow D_s \downarrow = \text{mobility small}$
        - $r < \Rightarrow D_s \uparrow = \text{mobility high}$
      - Interaction with solvent
        - Ions have hydration shells (water molecules surround ions) → contribute to size and thus mobility
      - Molecular weight has a **smaller effect** because mobility is inversely proportional to molecular weight
    - Absolute temperature
      - Higher temperatures increase the energy and therefore the movement of the molecules, increasing the rate of diffusion

## Plasma membrane

- Lipid bilayer, hydrophobic
- 6-8 nm thick
- Proteins embedded or associated with the lipid bilayer to allow ion movement across: Ion channels
  - Water-filled pores across the membrane, specialized in letting ions go through
    - **NOT ACTIVE TRANSPORT, no energy used**
    - Movement is **passive** and according to the concentration gradient
  - Main features:
    - Conduct ions
      - Ions in solution are strongly hydrophilic

- Low chance of passing through pure lipid membrane (thermodynamically unfavourable)
- Selective to different ions (selectivity)
  - Selectivity filter (part of channel that is very narrow) decides which ions go through
  - Amino acids residues in the channel replace hydration water that stabilised ions in solution, so ions are stripped of their hydration shell and are stabilised by the amino acids
    - Different amino acid residues stabilise different ions, thereby creating selectivity
      - For example: some amino acids residues in the channel stabilise sodium the best, hence channel is more selective to sodium
  - Open and close in response to specific signals (gating)
- Two main groups:
  - Gated
    - Ligand (e.g. AMPA)
    - Voltage (e.g.  $\text{Na}^+$ )
  - Non-gated
    - Still preserve selectivity, let through predominantly one type of ions
    - Non-gated channels in an axon are permeable to potassium

## Membrane Potential

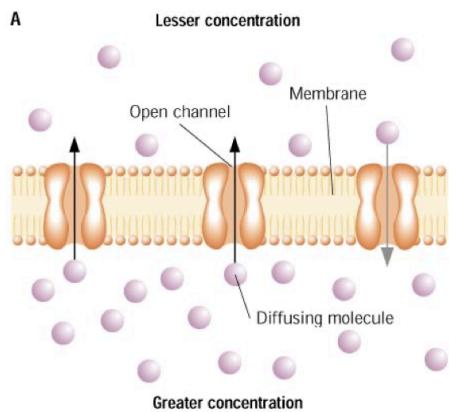
Excitable cells (e.g. neurons and muscles) behave like batteries

Membrane potential → difference in electrical potential across the membrane

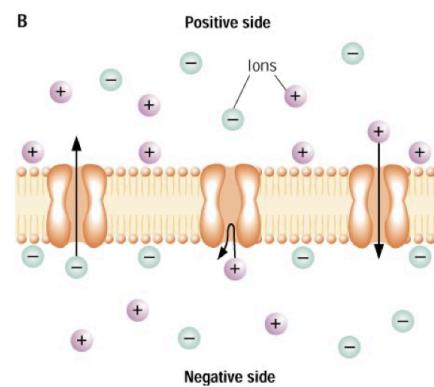
- Results from the separation of negative and positive charges at the interface of the membrane with extracellular space and with intracellular space
- Due to the movement of ions across the membrane

Resting membrane potential exclusively refers the membrane potential to when neurons are electrically inactive

- Potential difference doesn't change / changes negligibly concentration of ions
- Around -60mV
- 3 factors inducing an ion to cross a membrane:
  - Difference in concentration of ion



- - Electrical potential difference across the membrane



- - Action of an ion pump
    - Active transport
    - Against electrochemical gradient

- Julius Bernstein was the first to suggest resting potential exists across the membrane of every neuron
- 3 possible source of membrane potential:
  - Physical disruption of the membrane with a sharp needle
    - Membrane potential quickly declines to 0mV
    - **An intact membrane is essential for the resting potential to be maintained**
  - Change in the concentration of an ion across the membrane
    - Resting membrane potential changes
    - **Relative concentrations of ions inside and outside of the neuron are important**
  - Chemical inhibition of metabolic activity of the neuron
    - Resting membrane potential slowly declines to 0mV
    - **Energy-requiring processes are necessary to maintain a membrane potential over a long time**
- 3 factors are responsible for the generation and maintenance of resting potential:
  - Selective permeability of neuronal membrane
    - Arises from the selectivity of ion channels
  - Unequal distribution of ions across membrane

- Action of energy-requiring ion exchange pumps located in the cell membrane
  - Essential to maintain NOT to generate resting membrane potential

**Squid Giant Axon**

<b>Ion</b>	<b>[Inside]</b>	<b>[Outside]</b>
K <sup>+</sup>	400 mM	20 mM
Na <sup>+</sup>	50 mM	440 mM
Cl <sup>-</sup>	51 mM	560 mM

**Cat Motor Neuron**

<b>Ion</b>	<b>[Inside]</b>	<b>[Outside]</b>
K <sup>+</sup>	150 mM	5.5 mM
Na <sup>+</sup>	15 mM	150 mM
Cl <sup>-</sup>	9 mM	125 mM

Most ion channels sitting in excitable cell membranes are gated potassium selective channels → membrane permeable to K<sup>+</sup>

K<sup>+</sup> low concentration outside cell, high inside cell

Na<sup>+</sup> high concentration outside cell, low inside cell

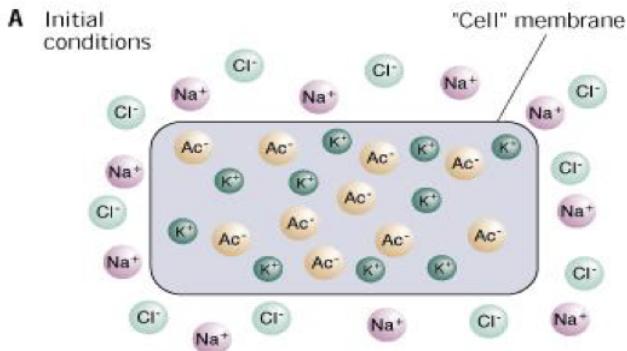
High conc of NaCl in seawater, squid live in the sea, need to maintain isotonicity (osmotic pressure), thus squid need higher conc of Na and Cl to balance the seawater osmotic pressure

Cl<sup>-</sup> relatively low inside neuron, so negatively charged proteins inside neuron also important for counterbalancing the high K<sup>+</sup> conc inside (aka great positive charge)

## Generation of Resting Potential

Mostly dependent K<sup>+</sup> distribution

-60mV



1. Outside of cell is electrically balanced due to equal numbers of  $\text{Cl}^-$  and  $\text{Na}^+$  ions
  - o Inside of cell is electrically balanced due to equal numbers of positive  $\text{K}^+$  ions and negatively charged proteins
  - o Acetate mimics protein inside the artificial cell above as it is negatively charged and big
2. Presence of non-gated, selective channels permeable to  $\text{K}^+$  ions
3.  $\text{K}^+$  ions move through potassium-selective channels down the  $\text{K}^+$  concentration gradient
  - o Efflux > influx initially as flowing due to large  $\text{K}^+$  conc gradient
4. The electrical charge outside of cell becomes more positive as  $\text{K}^+$  is +1 charged
5. Electrical potential counteracts the effects of concentration gradient forcing  $\text{K}^+$  ions to move
6. Equilibrium point with rate of  $\text{K}^+$  moving out and moving in reached
  - o Net flow is zero, efflux = influx

Equilibrium potential  $\rightarrow$  electrical potential difference present across the membrane that balances the concentration gradient

- Net flow of ions is zero
- Diffusion gradient is equal to the electrical gradient

Nernst Equation  $\rightarrow$  Calculate equilibrium potential

By the **Nernst Equation:**

$$E_{\text{ion}} = \frac{RT}{Fz} \ln \frac{[\text{ion}]_o}{[\text{ion}]_i}$$

$E_{\text{ion}}$	= Equilibrium potential for "ion"
R	= Universal gas constant [8.314 J/(mol *K)]
T	= Temperature, in Kelvin (273 +°C)
F	= Faraday constant (charge per mole: 96500 coulombs/mol)
z	= Valence (electrical charge) of the ion
ln	= Natural log (log to the base e)
$[\text{ion}]_o$	= Outside concentration of the ion under consideration
$[\text{ion}]_i$	= Inside concentration of the ion under consideration

- Defines the electrical potential across a membrane required to balance the chemical gradient so that there is no net movement of ions
- Equilibrium potential = constant x natural log of ratio of external to internal surroundings
  - o Constant takes into account thermal energy of the cell and surroundings, and the current carried the ion (charge)
- Features:
  - o Applies to only 1 type of ion at a time
  - o Equilibrium potential it provides for an ion is independent of the membrane potential
- Common ions and eq potential:

**Squid Giant Axon**

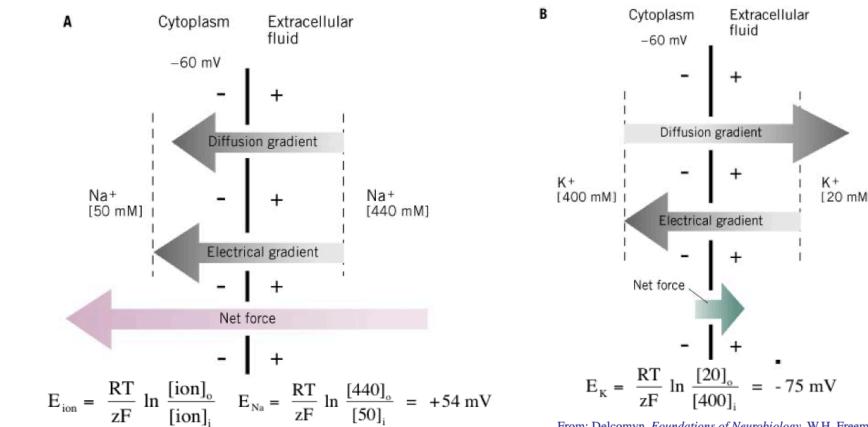
Ion	[Inside]	[Outside]	
K <sup>+</sup>	400 mM	20 mM	E <sub>K</sub> = -75 mV
Na <sup>+</sup>	50 mM	440 mM	E <sub>Na</sub> = +54 mV
Cl <sup>-</sup>	51 mM	560 mM	E <sub>Cl</sub> = -60 mV

**Cat Motor Neuron**

Ion	[Inside]	[Outside]	
K <sup>+</sup>	150 mM	5.5 mM	E <sub>K</sub> = -83 mV
Na <sup>+</sup>	15 mM	150 mM	E <sub>Na</sub> = +58 mV
Cl <sup>-</sup>	9 mM	125 mM	E <sub>Cl</sub> = -66 mV

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- The equilibrium potential for chloride is quite similar to the resting membrane potential
- Nernst equation is important because the value of equilibrium potential relative to the membrane potential determines the direction in which the ion will flow
  - $V_{DF} = V_m - E_{ion}$ 
    - $V_{DF}$ : Driving force for ion to move
    - $V_m$ : membrane potential
    - $E_{ion}$ : equilibrium potential of ion
    - Signs and direction of flow
      - $V_{DF} = 0 \Rightarrow$  no driving force to move in or out
      - For cations:
        - $V_{DF} > 0 \Rightarrow$  outward flow
        - $V_{DF} < 0 \Rightarrow$  inward flow
      - For anions:
        - $V_{DF} > 0 \Rightarrow$  inward flow
        - $V_{DF} < 0 \Rightarrow$  outward flow
  - Calculation example:



From: Delcomyn, *Foundations of Neurobiology*, W.H. Freeman & Co., 1997

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- $\text{Na}^+$ :  $(-60\text{mV}) - (+54\text{mV}) = -114\text{mV}$ 
  - Drive for entering cell is strong
- $\text{K}^+$ :  $(-60\text{mV}) - (-75\text{mV}) = +15\text{mV}$ 
  - Small drive to exit cell at resting potential
- When membrane potential of a neuron is different from equilibrium potential → ion movement not at equilibrium

- At resting potential of a neuron, ion movements are at equilibrium even though the resting membrane potential is not = to their equilibrium potentials
- Therefore, there are other factors present that counteract ion movement brought by unbalanced passive forces so that efflux can = influx

■ **Permeability of membrane**

- Both the driving force and membrane permeability need to be taken into consideration for the movement of ions
  - Low permeability to sodium ions at resting potential
    - Hence, though sodium driving force into cell is strong at the resting potential, sodium doesn't rush into the axon
    - The small amount that does enter is balanced by action of sodium-potassium pump which transports  $\text{Na}^+$  out of the cell, thus maintaining the conc gradient
  - Given many open  $\text{K}^+$  channels at rest, there is a small but constant  $\text{K}^+$  efflux due to its driving force to leave (+15mV)
    - This is counteracted by sodium-potassium pumps
      - Moves 3  $\text{Na}^+$  out and 2  $\text{K}^+$  in → maintain resting concentration of ions

### **Goldman-Hodgkin-Katz equation → Calculate resting potential**

$$E_m = \frac{RT}{F} \ln \frac{P_K[\text{K}^+]_o + P_{\text{Na}}[\text{Na}^+]_o + P_{\text{Cl}}[\text{Cl}^-]_i}{P_K[\text{K}^+]_i + P_{\text{Na}}[\text{Na}^+]_i + P_{\text{Cl}}[\text{Cl}^-]_o}$$

where  $P_K$ ,  $P_{\text{Na}}$ , and  $P_{\text{Cl}}$  are the permeabilities of  $\text{K}^+$ ,  $\text{Na}^+$ , and  $\text{Cl}^-$ , respectively, and  $E_m$  is the membrane potential.  $R$ ,  $T$  and  $F$  are the same constants used in the Nernst equation.

- Also known as “constant field equation” as one of the assumptions is that electrical field of membrane potential is constant across the span of the cell membrane
- To determine the resting potential of a neuron, the concentration difference and membrane permeability of each ion that can cross the membrane must be taken into account
  - Nernst equation only deals with one ion only and doesn't consider permeability of membrane to that ion, hence not suitable
  - Goldman-Hogkin-Katz good
- Membrane highly dependent on distribution of ion that can most readily pass through membrane
  - If permeability for  $\text{Cl}^-$  and  $\text{K}^+$  is 0, then membrane potential =  $\text{Na}^+$  membrane potential as only Na can pass through

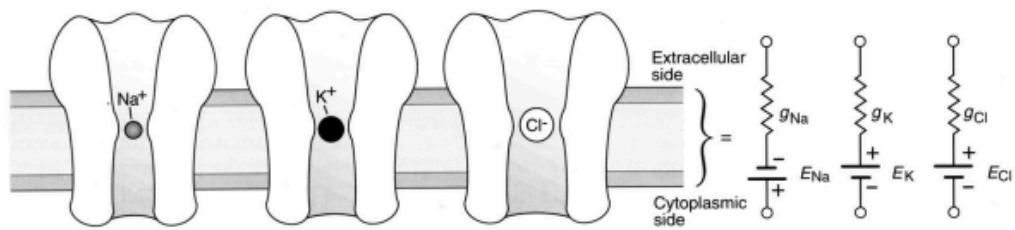
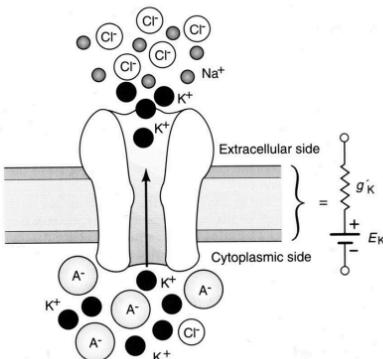
### **Experimental Evidence**

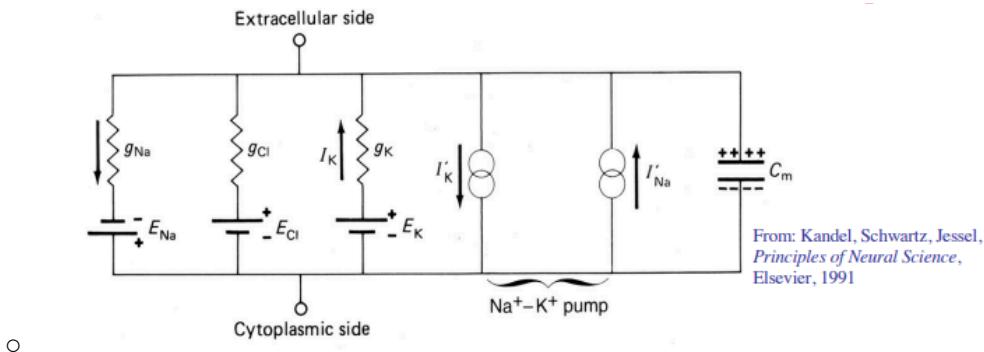
- Supports theoretical picture that resting potential mostly dependent  $\text{K}^+$  distribution
  - Membrane potential almost entirely independent of external concentration of sodium because the ratio between permeabilities of  $\text{Na}^+$  and  $\text{K}^+$  is so small that small changes in extracellular conc of sodium will be negligible
  - Changes in external concentration of potassium have significant effects on membrane potential

- Intracellular recording
  - Electrode is placed inside the neuron
  - Electrodes pick up the electric signal and measure the resting potential of a neuron

## Bioelectricity 2

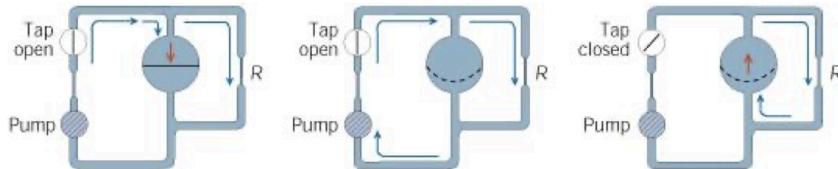
- 3 main features of neurons are used for electrical signalling
  - Ion channels → resistors
  - Concentration gradient of relevant ions across the membrane → batteries
  - Ability of the membrane to store charge → capacitors
- Neurons can be modelled by an electrical circuit consisting of resistors (conductors), batteries and capacitors
  - $R = 1/G$ 
    - $G$  = Conductance → any object through which electrical charges can flow
      - $G$  (Siemens, S)
  - $V = IR$ 
    - $V$  (volts)
    - $I$  (amperes, A)
- Advantage of using an electrical circuit → provide a quantitative understanding of how a current flow through a circuit generates signals in nerve cells
- Each ion channels act as a conductor / resistor and a battery (potential difference across it)
  - $g_K$  is the electrical symbol for a single potassium leak channel
  - $E_K$  = equilibrium potential of  $K^+$





- Most abundant types of ion channels are in parallel
- Under steady-state conditions,  $\text{Na}^+$  and  $\text{K}^+$  fluxes are driven by  $\text{Na}^+-\text{K}^+$  pumps
  - 3  $\text{Na}^+$  out and 2  $\text{K}^+$  in
- The leak channels adjust to meet the pump ratio
  - 3  $\text{Na}^+$  in and 2  $\text{K}^+$  out
- Lipid membrane = capacitor

## Capacitance



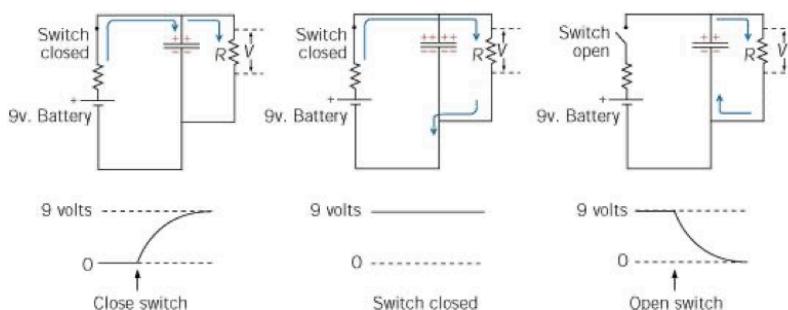
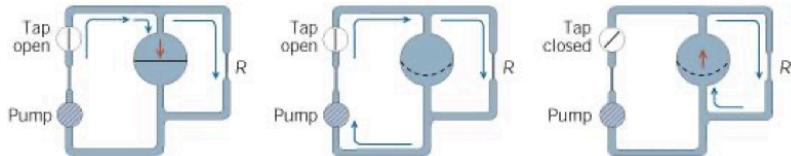
From: Delcomyn, *Foundations of Neurobiology*, W.H. Freeman & Co., 1997

- - When tap is opened, water begins to flow
  - Initially, some water will displace the diaphragm in the centre and some will flow through constriction on the right
    - Not a lot will flow through the right though because the resistance is higher
  - Only when the diaphragm in the reservoir is stretched to its full extent by the water pressure applied to it, the water flowing through the tap will flow through constriction R
  - When tap is closed, water will continue to flow for a short time in the right-hand loop because the rubber diaphragm will now give up the water that had been pushed up against it
    - Reservoir with diaphragm is a capacitor
- **Capacitance is the ability of a capacitor to store electrical charge**
  - $C = Q/V$
  - Defined as the amount of charge held by a capacitor per unit of voltage
- Membrane can act as a capacitor because positive and negative ions can accumulate on the two sides of the membrane, separated by the non-conducting lipid bilayer
  - Membranes have about the same thickness, the main factor making neuron capacitance abilities different = surface area
    - Larger surface area = more charge stored

$$C = \epsilon \epsilon_0 A/d$$

$\epsilon$  = dielectric constant  
 $\epsilon_0$  = polarizability of free space  
 $A$  = Area of plate (or conductive surface)  
 $d$  = thickness of insulator

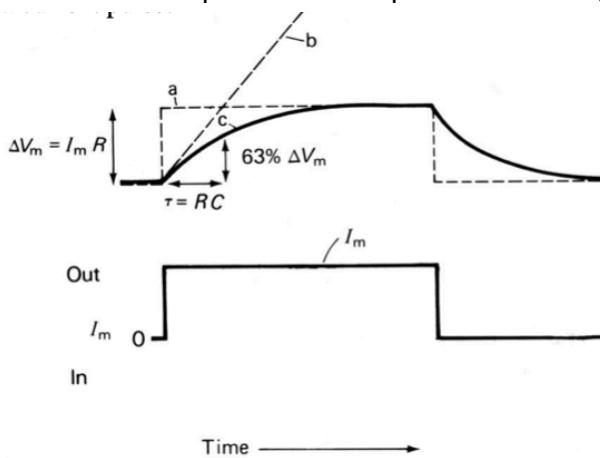
- Pure lipid membrane (no proteins) has a capacitance of  $0.8 \mu\text{F}/\text{cm}^2$  = specific capacitance
  - Can use specific membrane capacitance and total capacitance of all the membrane to find surface area



- Pump = Electromotive force (i.e. battery);
- Tap = Switch;
- Rubber diaphragm = Capacitor;
- Narrowed regions of pipe = Resistance.

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- When switch is closed, current from battery flows through a circuit consisting of a resistor ( $R$ ) and a capacitor in parallel
- Initially, some current flow through  $R$  and some flow to capacitor
- The current to capacitor will cause a build-up and separation of charges, charging capacitor
- Only when capacity is fully charged will current flow through resistor  $R$
- When switch is opened again, current will continue to flow for a short time in the right hand loops because capacitor will now give charge



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- Capacitance of the membrane reduces rate at which membrane potential changes in response to current pulse

- Line a: Instantaneous change in membrane potential if the membrane had **only resistive** properties
- Line b: Slow ramp-like change in membrane potential if membrane had **only capacitive** properties
- Line c: actual change in the membrane potential in response to a rectangular current pulse with **both capacitive and resistive** properties

## EPSP Spread

$$\tau = R \cdot C$$

- - Describes the rising phase of the potential change
  - It is the time it takes for a potential to build up and also decay
  - $\tau$  is **Membrane resistance x Membrane capacitance**
    - **Membrane time constant**
    - Can be measured experimentally
    - Qualitatively, it is the time it takes for  $V_m$  to build up to about 63% ( $1 - 1/e$ ) of its final value to a rectangular step of current
    - Membrane resistance is determined by magnitude and number of leak channels
  - $\tau$  can change the time course of synaptic signals, thereby influencing the integrative properties of the neuron (aka temporal summation)
    - Synaptic sequence of events
      - **Presynaptic:** AP → Increase in  $Ca^{2+}$  levels → Release of NT
      - **Postsynaptic:** NT binding → Opening of ligand-gated channels → flowing of synaptic current → change in post-synaptic potential
    - This generation of potential includes 2 phases: rising and falling
      - Rising = determined by active and passive properties of the membrane
      - Falling = determined by passive only
        - **Falling phase has a time course determined by  $\tau$**  (membrane only)
        - Larger  $\tau$  = longer duration of synaptic potential → more likely to summate temporally
- Voltage decrease in amplitude with distance from its site of initiation within a neuron
  - Resistance across a dendrite
    - Cytoplasm of dendrite offers significant resistance to flow of current
      - **Axial resistance / Internal resistance**
      - Axial does not equal axonal
        - Axial = any cable-like processes
    - Membrane of dendrite has resistance to flow of current due to lipid membrane not being conductive, but it offers some flow due to leak channels
      - **Membrane resistance**
    - Both axial and membrane resistance apply to 1cm segment of an individual neuronal process with a certain diameter