

# 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

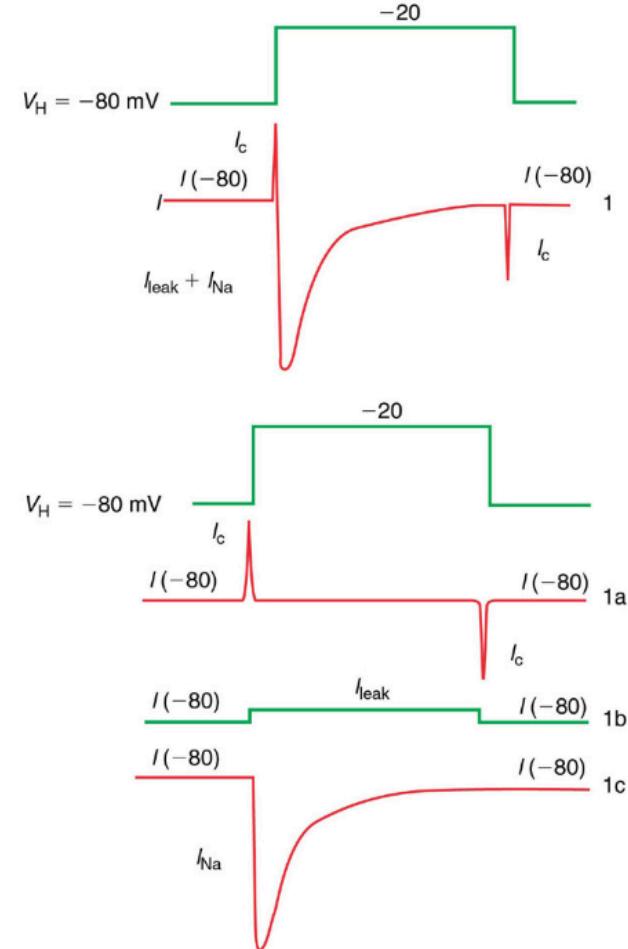
- Microelectrodes are sharp and will impale the cell
  - Only allow voltage difference between inside and outside of membrane to be measured
- Patch clamps are used to measure electrical signals on a patch of membrane or the whole cell
- Patch clamps are positioned onto the membrane and a small suction 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

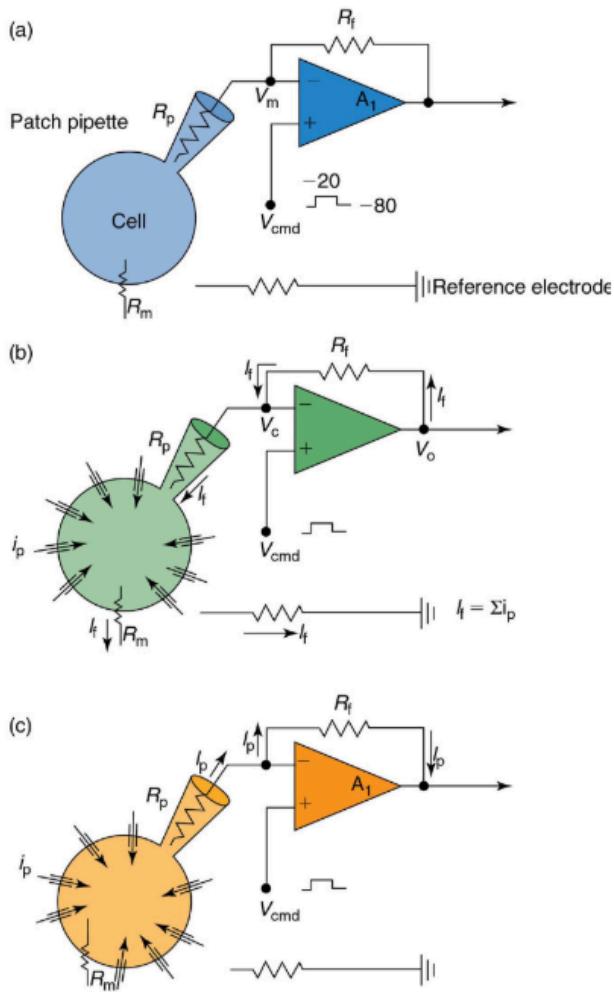
- $I_c = V_B - V_A$
- $I_c = \text{injected current}$



- - Here  $K^+$  and  $\text{Ca}^{2+}$  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,  $-80\text{mV}$  to  $0\text{mV}$  = a  $+80\text{mV}$  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

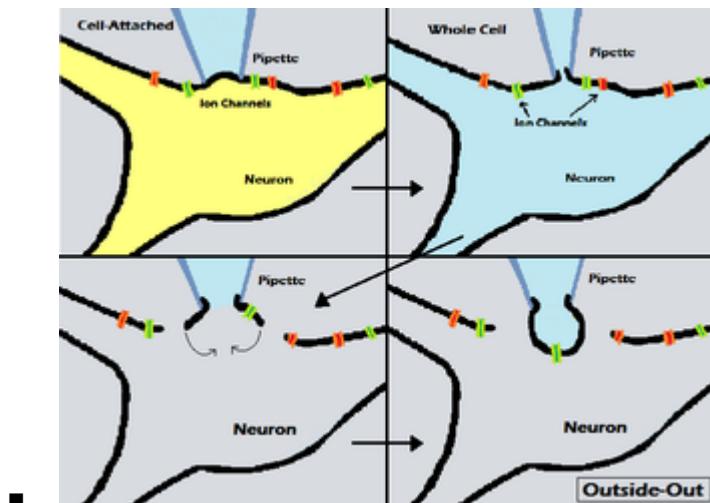


- Voltage clamping in patch clamp
  - $V_m$  and  $V_{cmd}$  (common voltage) are both sent to the amplifier  $A_1$  which compares the two
  - $A_1$  sends  $V_o$  (voltage that can evoke a current that has the size to counterbalance cell current)
    - $V_o = R_f I_f$
    - $R_f$  = resistance for 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



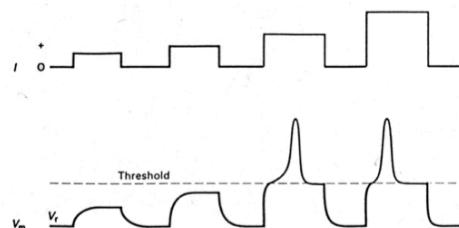
- Intracellular enviro = pipette
  - Extracellular enviro = bath
- It can be used to look at effects, especially for ligand-gated ion channels and external ligands (e.g. transmitters or antagonists)
  - Often used for concentration jumps
    - Concentration jumps are when application of agonist is very 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

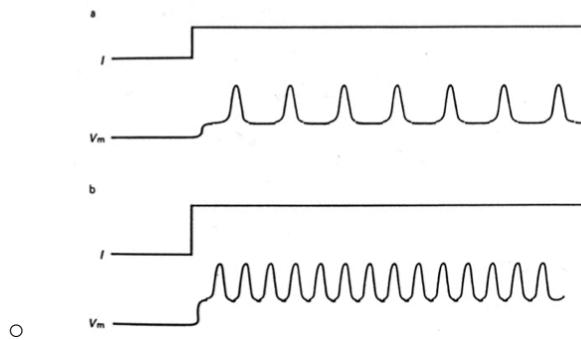
- Ions in solution are surrounded by a cloud of water molecules attracted by the net charge of ion → energetically unfavorable, thus improbable
- Ion channels are large integral membrane proteins that form aqueous pores through plasma membrane to allow ions to cross
  - Plasma membrane act as selective 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

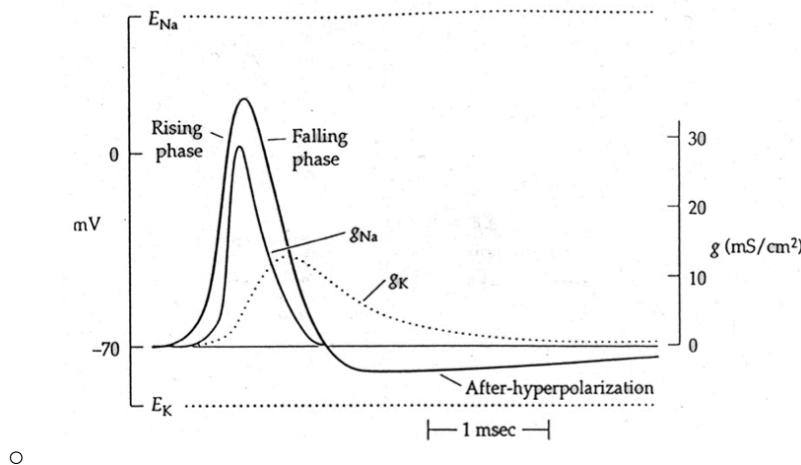


- - Strength-latency relationship and refractory period allow encoding of info in form of frequency code

## Frequency coding



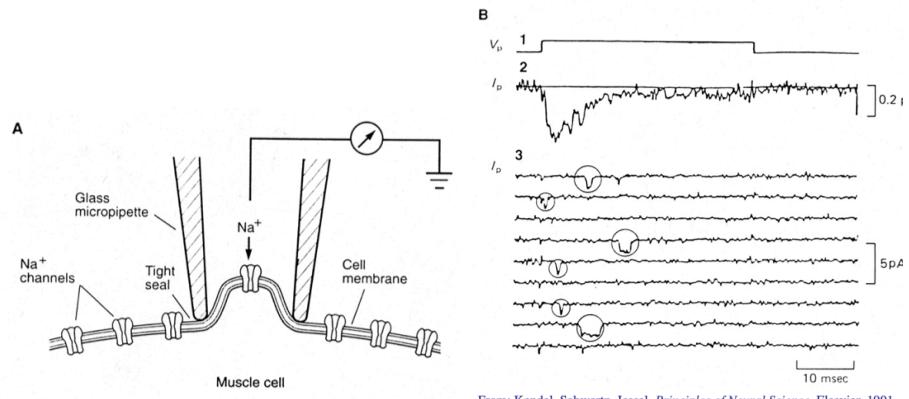
- Conductances can never be negative, membrane potential changes with potassium and sodium at different times for AP duration



- Depolarisation that initiates an AP causes transient change in membrane  $\rightarrow$   $K^+$  permeability to  $Na^+$  permeability
- Armstrong and Hille's Working Hypothesis:
  - Ions are passing through aqueous pores called channels
  - Ion channels are proteins
  - Channels for  $Na^+$  and  $K^+$  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

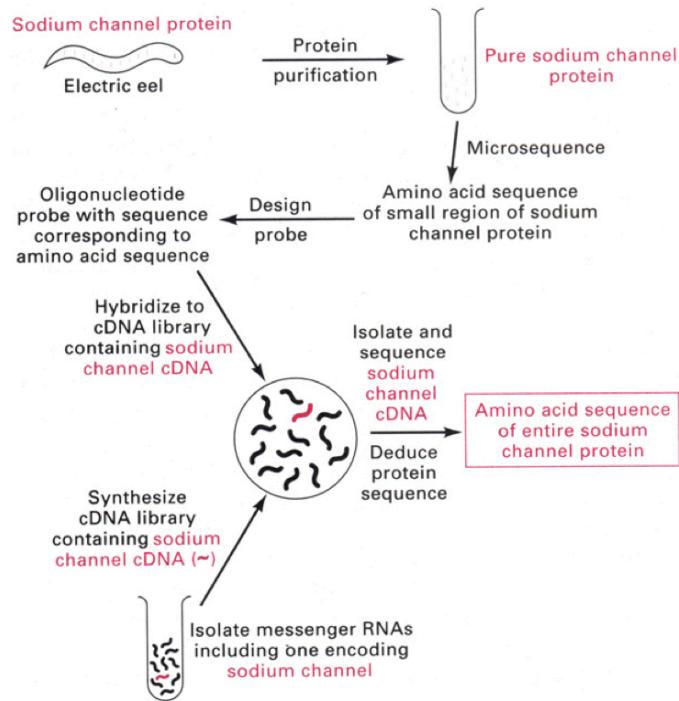
- Ion channels theory can be experimentally tested:
  - Patch-clamp technique  $\rightarrow$  single channel measurements
    - Voltage clamp



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

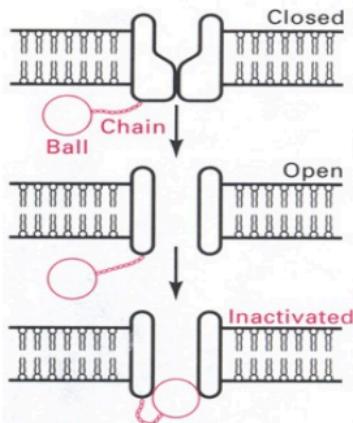
- Hydropathy plot was obtained by assigning to each amino acid in the protein a hydrophobicity and hydrophilicity value
  - Every amino acid is hydrophobic or hydrophilic
  - Hydrophilic lie more on outside of protein, hydrophobic lie on the inside
  - Apply this knowledge to membrane protein
- To cross 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

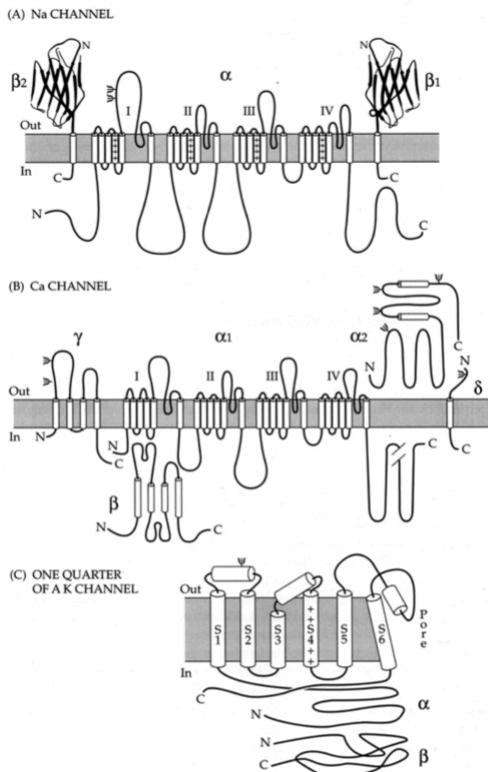
### *The inactivation domain*



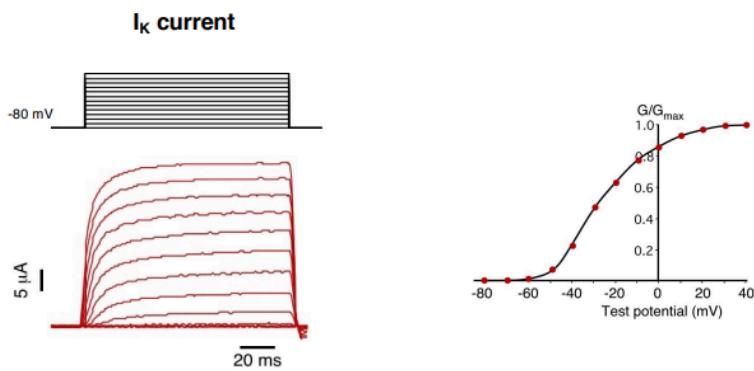
- Inactivation is linked with N-terminus

- 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

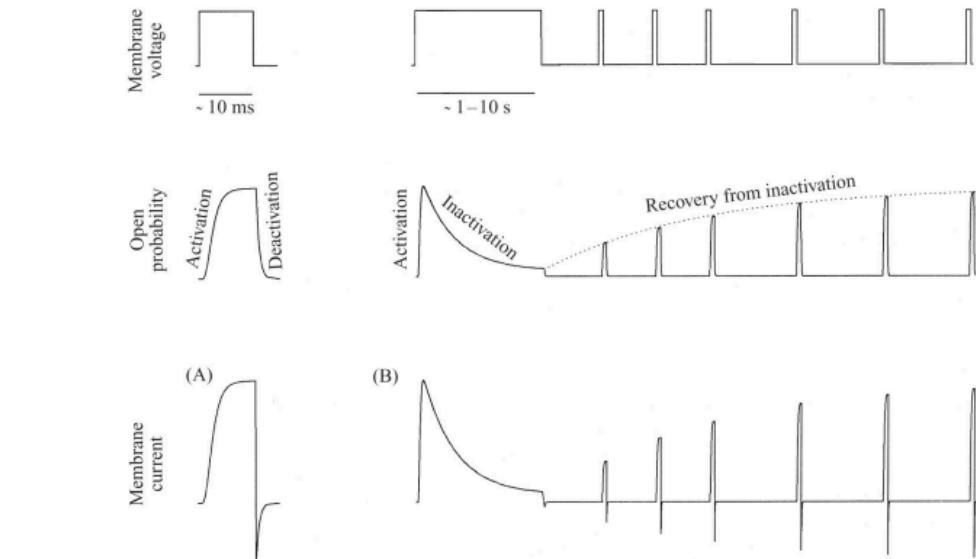
## Potassium Channel



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

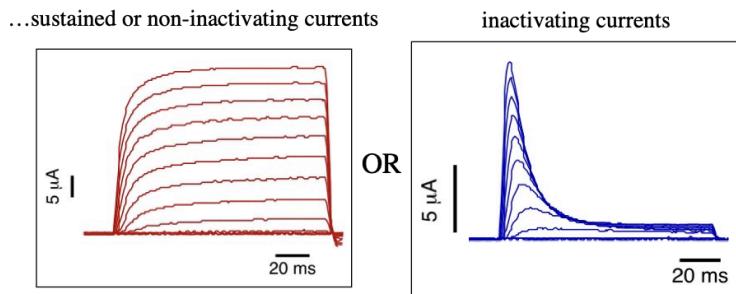


- ○ Terminologies

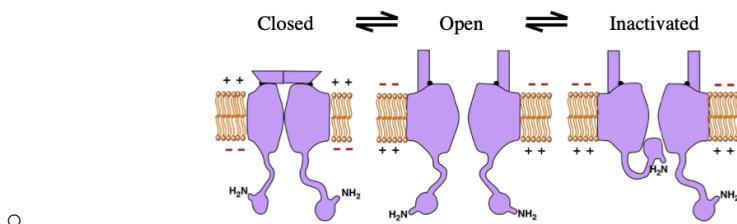


- ○ Voltage steps
- ○ Open probability
  - Deactivation for voltage-gated ion channels (Desensitisation is for another thing)
  - Activation upwards curve and inactivation downwards current
  - Longer pulse may have both activation and inactivation
  - Recovery from inactivation → open probability are 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...

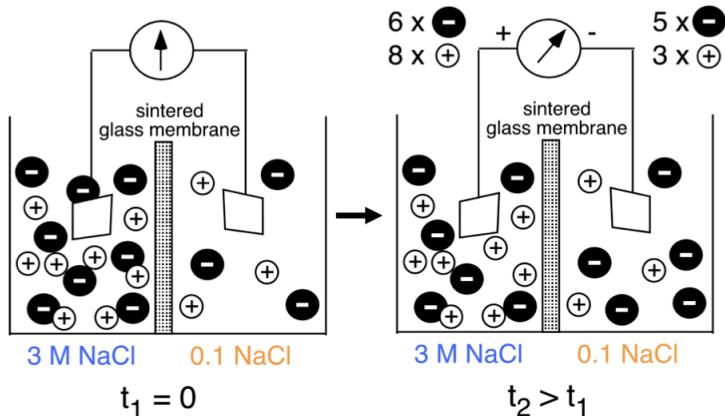


Model for potassium channel inactivation



- 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

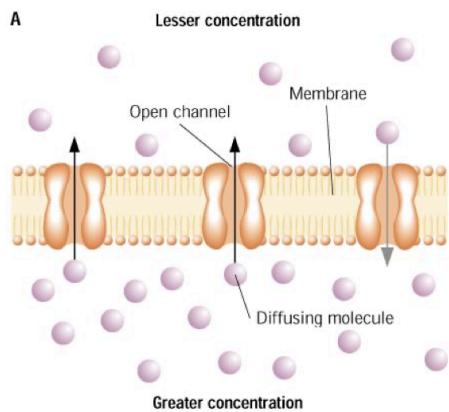
Excitable cells (e.g. neurons and muscles) behave like batteries

Membrane potential → difference in electrical potential across the membrane

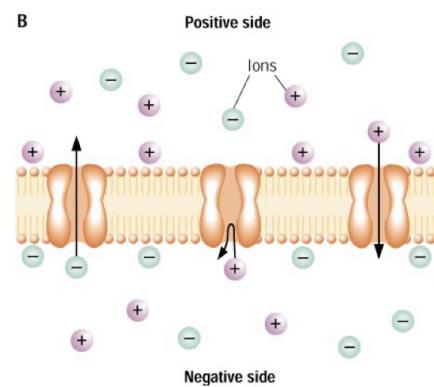
- Results from the separation of negative and positive charges at the interface of the membrane with extracellular space and with intracellular space
- Due to the movement of ions across the membrane

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

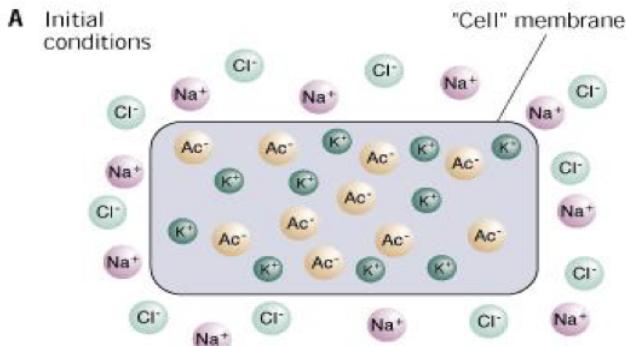
High conc of NaCl in seawater, squid live in the sea, need to maintain isotonicity (osmotic pressure), thus squid need higher conc of Na and Cl to balance the seawater osmotic pressure

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 → 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 → 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:

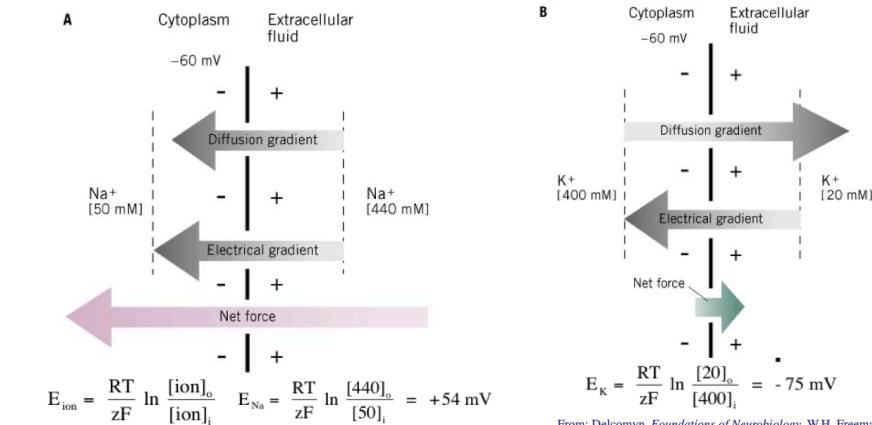
**Squid Giant Axon**

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

- - 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

- - $\text{Na}^+$ :  $(-60\text{mV}) - (+54\text{mV}) = -114\text{mV}$ 
    - 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

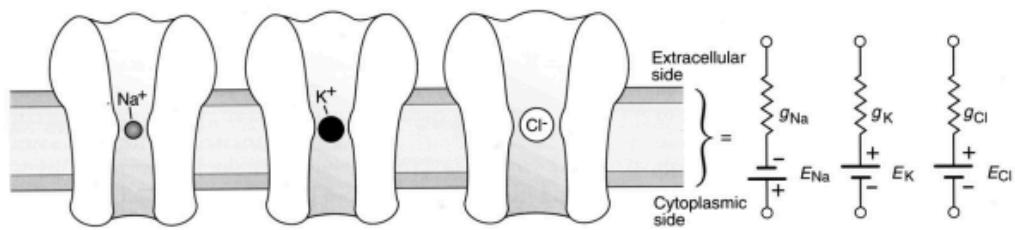
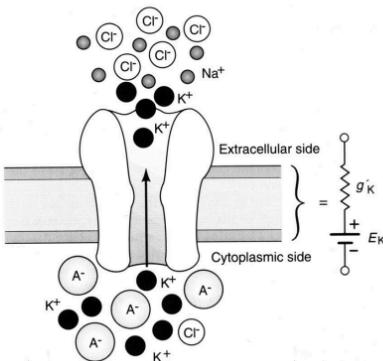
### **Experimental Evidence**

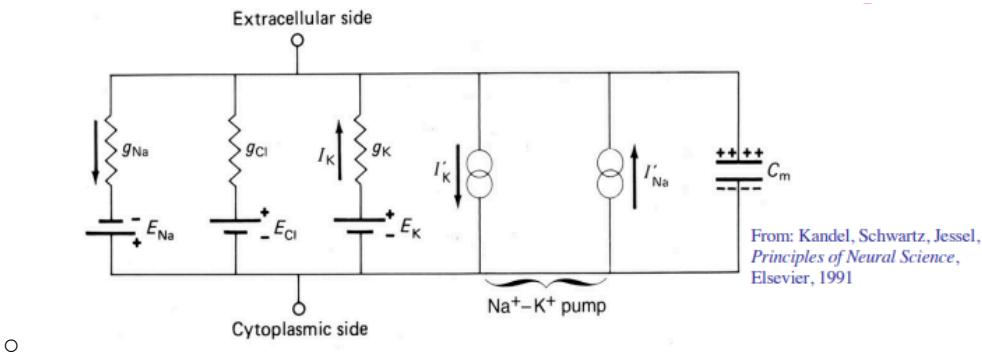
- Supports theoretical picture that resting potential mostly dependent  $\text{K}^+$  distribution
  - Membrane potential almost entirely independent of external concentration of sodium because the ratio between permeabilities of  $\text{Na}^+$  and  $\text{K}^+$  is so small that small changes in extracellular 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

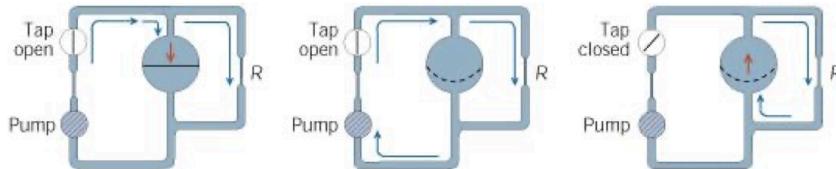
- 3 main features of neurons are used for electrical signalling
  - Ion channels → resistors
  - Concentration gradient of relevant ions across the membrane → batteries
  - Ability of the membrane to store charge → capacitors
- Neurons can be modelled by an electrical circuit consisting of resistors (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



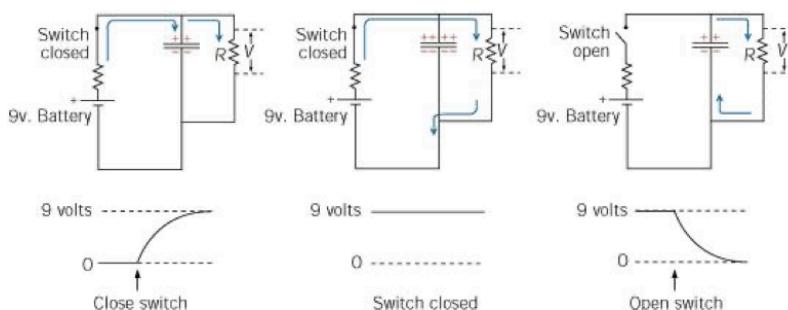
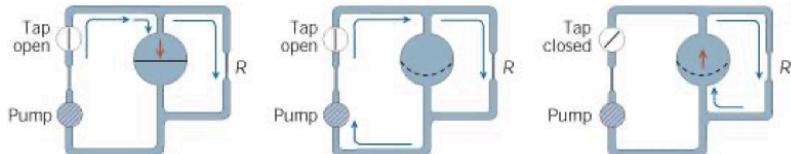
From: Delcomyn, *Foundations of Neurobiology*, W.H. Freeman & Co., 1997

- - 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

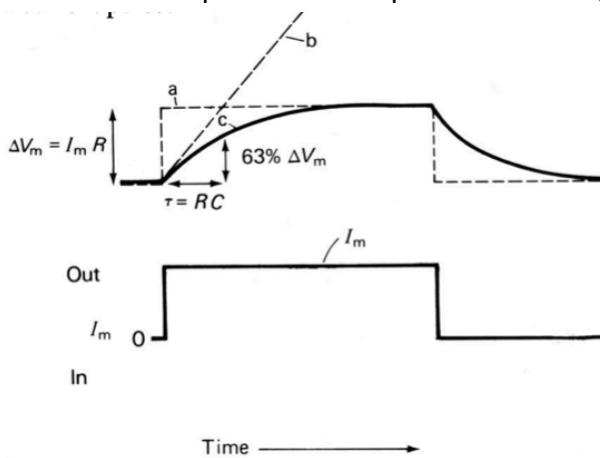
- Pure lipid membrane (no proteins) has a capacitance of  $0.8 \mu\text{F}/\text{cm}^2$  = specific capacitance
  - Can use specific membrane capacitance and total capacitance of all the membrane to find surface area



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

- 

- 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



- 

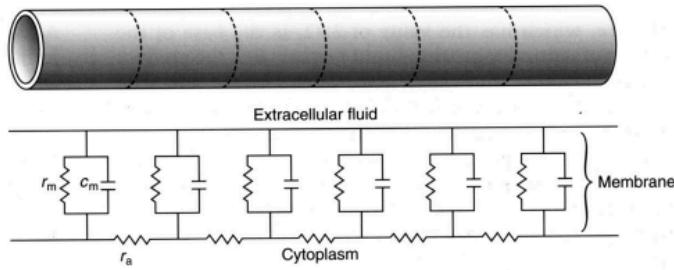
- Capacitance of the membrane reduces rate at which membrane potential changes in response to current pulse

- 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

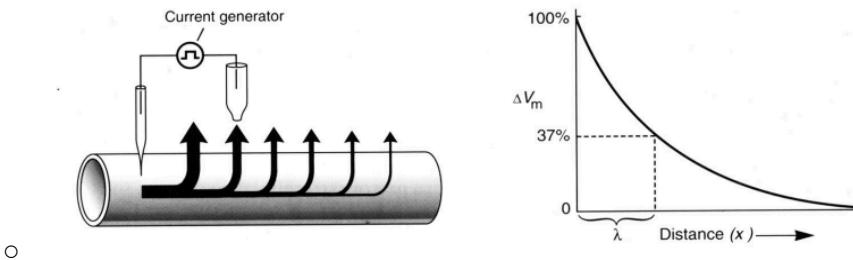
$$\tau = R \cdot C$$

- ○ 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



- - Axial resistance are the resistors connected in series on cytoplasm side
  - Resistance that applies to 1cm segment of an individual neuronal process with certain diameter (d)
    - $r_m \Rightarrow$  membrane resistance
    - $r_a \Rightarrow$  axial resistance

- Length and resistance
  - Longer cylindrical process length = more internal resistance (axial resistance)
  - Longer cylindrical process length = less membrane resistance because more channels are available for current to leak through the membrane



- - Inject current into axon
  - Current flows out across membrane
  - More current flows near site of injection than distant sites as axial resistance ( $r_a$ ) increases with distance from site of injection
  - $V_m = I_m r_m \Rightarrow$  change in membrane potential produced by current becomes smaller moving away from injection site as current flowing out decreases
  - Decay with distance = exponential shape
- Membrane length constant

$$\lambda = \sqrt{r_m / r_a}$$

- 
- Distance that a current will travel away from the site where it is injected/begins
  - Measure of efficiency of the electrotonic conduction in a given neuron
- Qualitatively corresponds to distance along neuronal process at which a constant applied voltage will decay to about 37% ( $1/e$ ) of its original value
- Efficiency influences spatial summation and propagation
  - Spatial summation is the summation of 2 or more inputs from different locations occurring at the same time
- Cells with high  $R_m = \lambda$  is large

- Postsynaptic potential can spread farther because relatively few ions are lost across the membrane
- Cells with low  $R_m = \lambda$  is small
  - Huge ionic leak, fewer ions travel and carry current farther down
- Cells with high  $R_a = \lambda$  is small
  - Axons with small internal diameter, more resistance to internal flow
  - Current will not travel far
- Cells with small  $R_a = \lambda$  is large
  - Axons with large diameter, does not impede flow of current
- Larger  $\lambda \rightarrow$  potential can travel further down axon and still be effective in eliciting a postsynaptic response even if very far from synapse
  - No significant loss of amplitude
  - Hence if 1 postsynaptic potential has a larger  $\lambda$ , then when it summates with a distant postsynaptic potential, the amplitude will still be great than if the  $\lambda$  was small
- Specific axial resistance ( $R_a$ )  $\rightarrow$  internal longitudinal resistance of 1cm length of a cylindrical process  $1\text{cm}^2$  in cross-sectional area
  - Calculated from  $r_a$  (axial resistance)
  - Independent of geometry
  - Consider **resistance decrease as cross-sectional area increase**
- Specific membrane resistance ( $R_m$ )  $\rightarrow$  resistance of  $1\text{cm}^2$  of membrane
  - Calculated from  $r_m$
  - Independent of geometry
  - Consider **membrane resistance goes down as lateral surface area (the surface area around the axon) goes up**
    - $R_m$  depends primarily on resting permeability of the membrane to  $K^+$  and  $Cl^-$

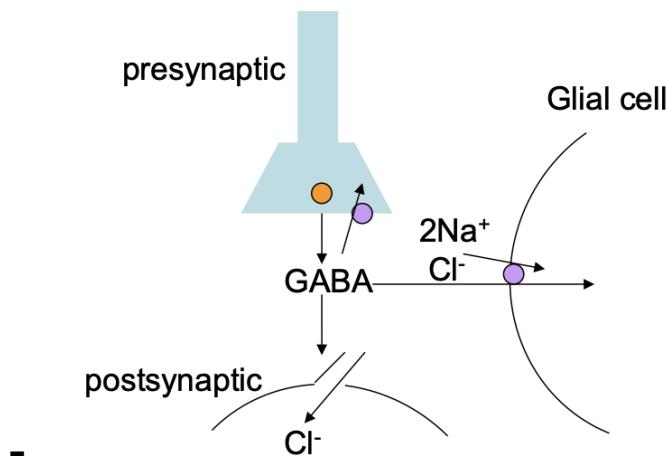
Note: Neuronal action potentials do not summate!! EPSP and IPSP do

$\Rightarrow$  Postsynaptic potentials are graded and can sum, amplitude is proportional to the strength of the stimulus but it is usually small, and they have no refractory periods

## Active Transport

- Active transport is the transport of substances across membranes, against their electrochemical gradient
- Energy equation
  - $RT \ln(\frac{C_2}{C_1})$
- Active transport requires energy
  - $\sim 5.9\text{ kJ/mole}$  to change concentration by  $\sim 10$ -fold
  - $\sim 5.8\text{ kJ/mole}$  for moving against electrical gradient
- Power sources:
  - ATP hydrolysis
    - ATP splitting:  $ATP \rightarrow ADP + Pi$

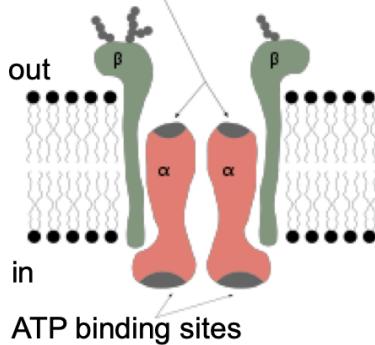
- 50 kJ/mole
- Ion gradients → transport an ion down its electrochemical gradient generates the energy for the transport of the one we want against its electrochemical gradient (e.g.  $\text{Na}^+$  - glucose transporter)
  - Transport of 1  $\text{Na}^+$  gives 5.9 kJ from the concentration gradient
  - Transport of 1  $\text{Na}^+$  gives 5.8kJ from electrical gradient (into cell as it is attracted by negative charge inside)
  - Total = 11.7 kJ/mole
- Function
  - Establish transmembrane ion gradients and voltages
  - pH regulation
    - Many intracellular reactions are very pH-dependent, so pH regulation crucial
  - Solute accumulation
  - Termination of synaptic transmission
    - For glutamate and GABA synaptic action is terminated by transport into cells against their conc gradient



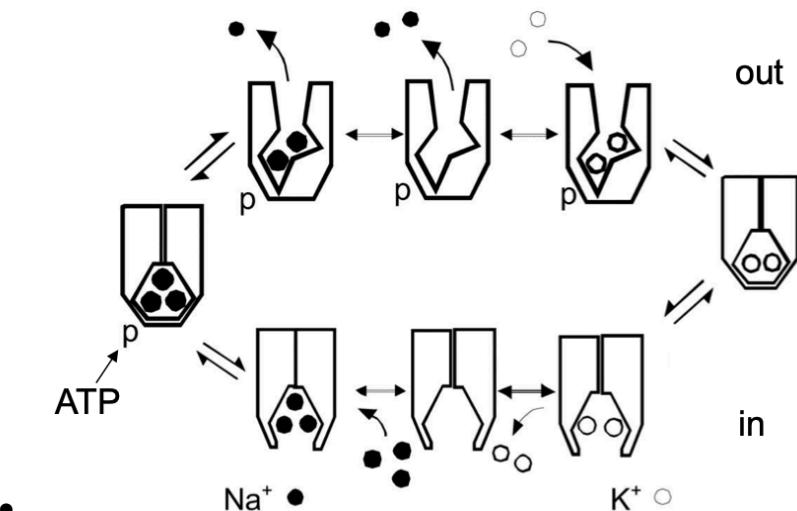
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- Energy:
  - Energy obtained form 1 positive charge entering cell = 5.8 kJ/mole
    - $2\text{Na}^+$  and  $1\text{Cl}^-$  in = net 1 positive in
  - $2\text{Na}^+$  going down conc gradient = 11.8kJ
  - $1\text{Cl}^-$  going down conc gradient = 5.9 kJ
    - Total energy gained = 23.5kJ/mole
  - Energy needed to move GABA = 5.9 kJ for 10-fold gradient
    - GABA is neutral, so no need to consider electrical gradient
  - Roughly  $10^4$  gradient
    - Similar for glutamate
  - Second messenger regulation
- Transporters:
  - Sodium-potassium ATPase
    - Structure:
      - P-type ATPase
      - 2 alpha subunits, 2 beta subunits
        - Alpha subunits bind to ATP insid
        - Ouabain (inhibitor) binds outside

- Beta subunits are not needed for the pumping of ions

### Ouabain binding sites

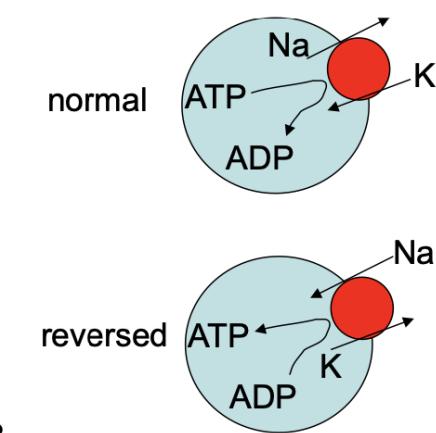


- Observations leading to conclusion that ATPase is the pump:
  - ATP not hydrolysed unless ions are transported
  - ATPase found wherever  $\text{Na}^+$  and  $\text{K}^+$  are pumped
  - ATPase and pump are both in membrane
    - Both inhibited by ouabain and stimulated by  $\text{Na}^+$  and  $\text{K}^+$
- Mechanism:
  - 3  $\text{Na}^+$  binds inside
  - Sodium allows phosphorylation of the pump on an aspartate residue
  - Phosphate has 2 negative charges → transporter change conformation after binding
  - Faces the outside
  - Transporter binding site favors potassium
  - 2  $\text{K}^+$  binds outside
  - Potassium allows dephosphorylation
  - Conformation change →  $\text{K}^+$  transported inside



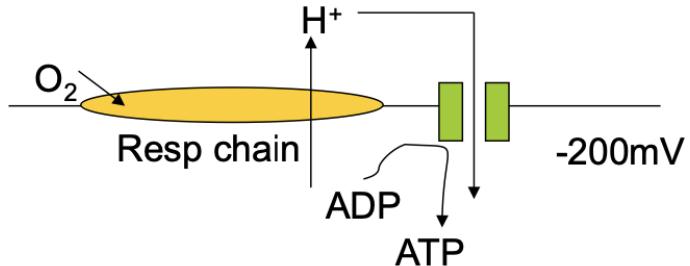
- Energy needed:
  - To move 2  $\text{K}^+$  = ~0
    - Roughly at equilibrium potential at resting potential so moving in and moving out forces are balanced → no energy needed for any movement

- To move 3 Na<sup>+</sup> = 3 x 11.7kJ = 35kJ
- Energy from ATP = 50kJ/mole
- Ca<sup>2+</sup>-ATPase
  - Types:
    - Plasma membrane calcium ATPase (PMCA)
    - Sarcoendoplasmic reticulum calcium ATPase (SERCA)
      - Number of ions transported by the two types may different
  - Mechanism
    - Aspartate phosphorylated during the carrier cycle
      - ATP only hydrolyzed when Ca<sup>2+</sup> is pumped
    - Extrude 1 Ca<sup>2+</sup> from cytoplasm to outside of the cell or into sarcoendoplasmic reticulum, and 2 H<sup>+</sup> in / out
      - Conformational change moves the ion
      - ATP builds up calcium conc gradient across cell
  - High affinity for calcium but works slowly
    - Sodium-calcium exchanger has lower affinity but works faster
    - Km = 150nM
  - Sequence homologous to Na pump
    - P-type ATPase
  - Energy needed:
    - No energy needed for charge transfer → Ca<sup>2+</sup> cancels 2H<sup>+</sup>
    - Energy to move 2H<sup>+</sup> (2-fold conc change) in = 3.6 kJ
    - Energy to move Ca<sup>2+</sup> (10-fold conc change) into SR or out of cell = 5.9 kJ
    - ATP = 50 kJ / mole
      - After moving 2 H<sup>+</sup> = 46.4
      - 46.4 / 5.9 = 7.9
      - Ca<sup>2+</sup> can change by 10<sup>7.9</sup> fold
        - If 10mM Ca<sup>2+</sup> in SR, an lower cytoplasm [Ca<sup>2+</sup>] to 10<sup>-2</sup>/10<sup>7.9</sup>
- Reversal of ATPases
  - ATP hydrolysis is tightly coupled to ion movements
  - With the right ion gradients, Na<sup>+</sup>-K<sup>+</sup> and Ca<sup>2+</sup>-ATPases can run backward getting energy from the ion gradients and using it to make ATP

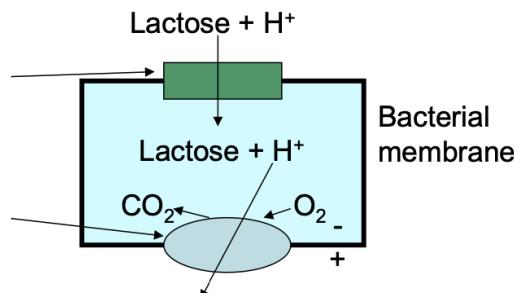


- Example:

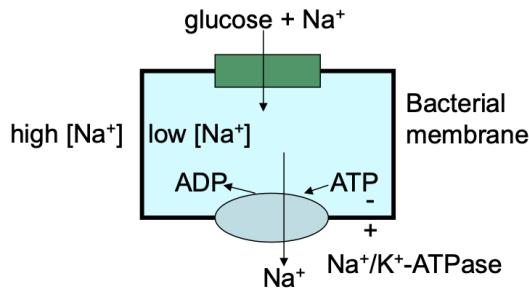
- $H^+$  - ATPase
  - Usually runs backwards, making ATP at the expense of proton gradient (generated by proton pumping fuelled by metabolism)



- $H^+$  co-transport came first
  - Energy from proton gradient and voltage gradient set up by respiratory chain
  - Mitochondria  $\rightarrow$  endosymbiosis
- F-type
  - Made of F0 membrane-spanning part and F1 catalytic subunits which bind ATP
- V-type ATPase
  - Found in vesicles, Golgi apparatus, bacteria, fungi
  - Pumps protons into vesicles and has many subunits
- Ion-coupled active transport
  - Powered by co-transport of ion, usually sodium or proton transport down gradients
  - Lac permease
    - Accumulate lactose, energy comes from  $H^+$  gradient and voltage gradient set up by respiratory chain



- Found in bacteria
- Glucose
  - Accumulate glucose in cell



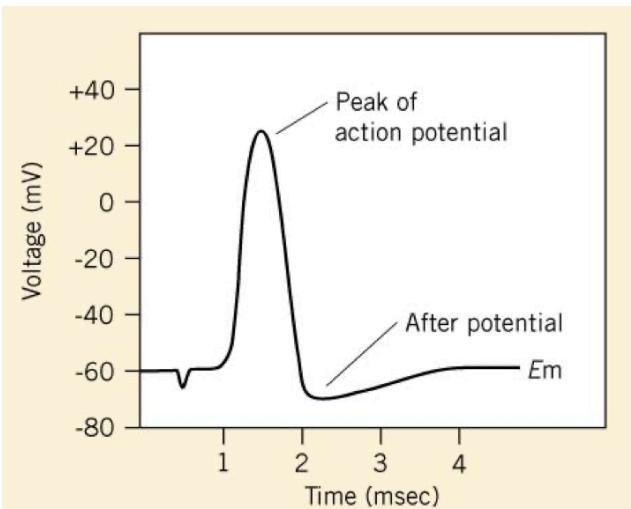
- Powered by Na<sup>+</sup> gradient
- Na<sup>+</sup>/Ca<sup>2+</sup> exchange
  - 3 sodium enter for 1 calcium leaving
  - Energy:
    - Energy gained from Na<sup>+</sup> gradient = 17.7 kJ/mole
    - Energy gained from 1 net positive charge entering because inside cell is negative = 5.8 kJ/mole
      - Total energy gain = 23.5 kJ/mole
    - Energy needed to pump Ca<sup>2+</sup> up conc gradient is 5.9 kJ mole per 10-fold gradient
      - 23.5/5.9 = 4
    - So 10<sup>4</sup> gradient can be powered
      - Intracellular calcium can be lower to 2x10<sup>7</sup>M

## Action Potential

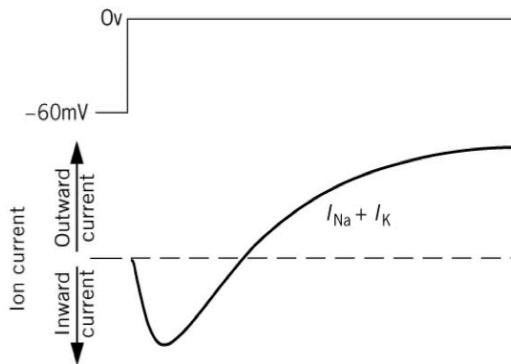
$$V = IR$$

$$R = 1/G$$

- Generation and propagation are different
- Action potential is a brief, transient reversal of the membrane potential that sweeps along the membrane of a neuron
  - Electrical signal

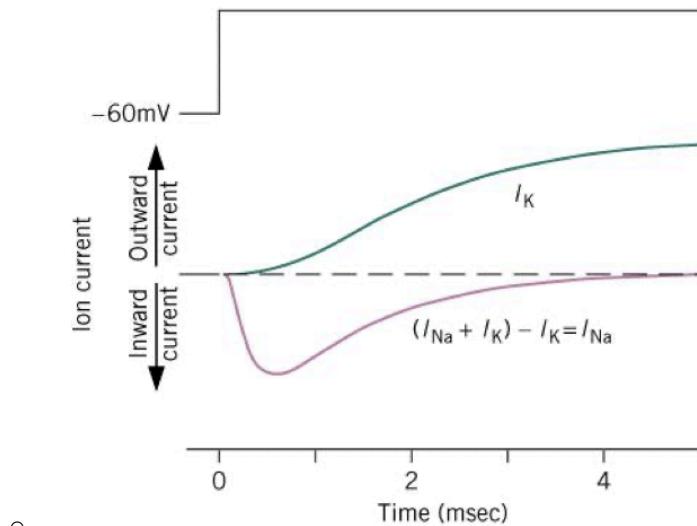


- Phases:
  - Depolarisation
  - Peak or overshoot
    - Positive signal
    - Between +20mV and +40mV
    - Below equilibrium potential for sodium (~50mV)
  - Repolarisation
    - Return of membrane potential towards negative / resting state
  - Afterhyperpolarisation
    - Undershoot
    - Short period, membrane potential more negative than at rest
- Characteristics:
  - Temporary
  - All or none
    - After reaching the threshold, AP will be fired or not occur at all
    - No change in amplitude no matter how much you exceed the threshold by
  - Threshold
  - Refractory period
    - Impossible/ more difficult to initiate another AP
- Ions responsible for AP:
  - Initiation of AP briefly switches its predominant permeability from  $K^+$  to  $Na^+$  → Different voltage-gated ion channels open during the different phases of the action potential
  - Conclusion from Hodgkin, Huxley, and Katz's experiment:
    - Method: Voltage-clamp technique
      - Decide at which voltage to fix membrane potential
      - Researcher in control of voltage → command voltage
      - Clamp (hold) the membrane at a particular potential
    - Used squid axon
    - Penetrate axon with electrodes
      - 1 to inject current
      - 1 to read current
    - If there are ion movements across the membrane that would change the potential, the voltage clamp amplifier detected any movement of potential away from command potential and set a current that will bring membrane potential back to clamped value (feedback)
      - By measuring current produced by device to keep membrane potential constant, we can measure the ion currents produced by the neurone under the different conditions
      - Sign inverted for injected current
    - We can separate different current components by:
      - Modifying concentration gradient of 1 of the critical ions
      - Substituting 1 of the critical ions with impermeant one
      - Selective pharmacological blockade of the different voltage-sensitive channels (TTX for sodium, TEA for potassium)
    - Demonstrated the existence of 2 types of voltage-gated currents



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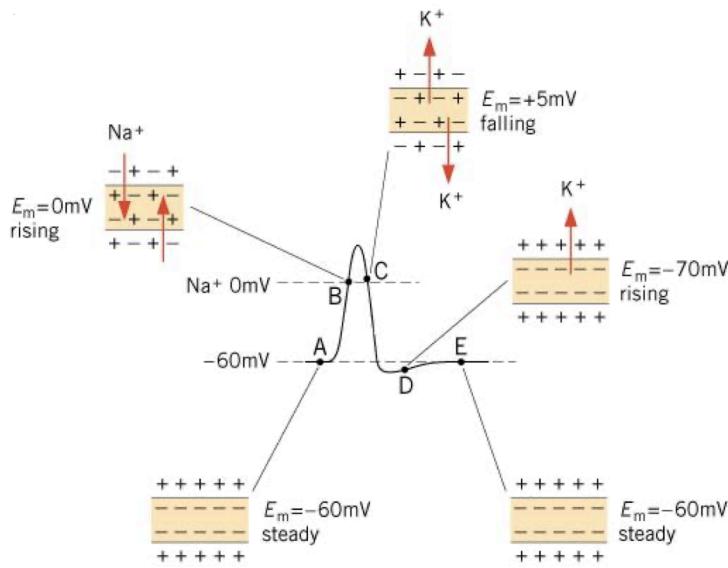
- Inward current followed by slower and sustained outward flow of current



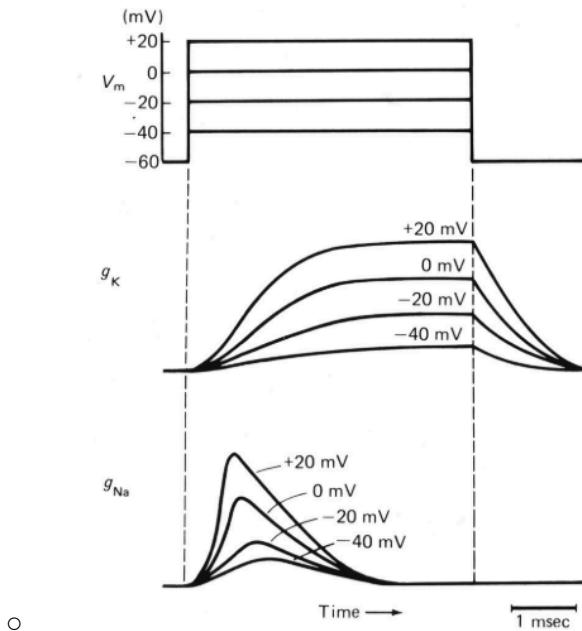
○

- $\text{Na}^+$  current is gone
- Only current observed is potassium
- Subtracting  $K^+$  current from the current for both  $\text{Na}^+$  and  $K^+$  → got the specific current for both ions

- Changes in permeability occur due to different voltage-gated ion channels opening during different phases of AP

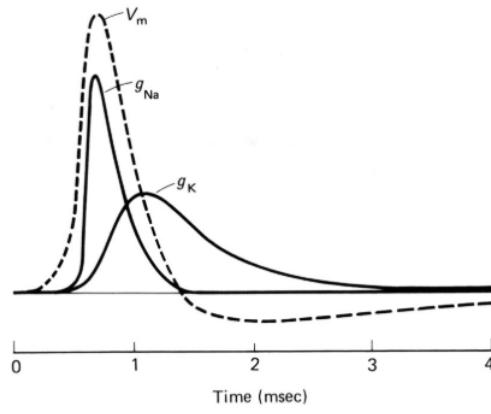


- - Inactivated sodium ion channels cannot conduct any more
    - Inactivation is not closing
- Conductance to ions
  - $$g_K = \frac{I_K}{(V_m - E_K)}$$
  - $$g_{Na} = \frac{I_{Na}}{(V_m - E_{Na})}$$
  - I = Current
  - $V_m$  = command potential
  - E = equilibrium potential from Nernst equation
- Voltage step and conductance

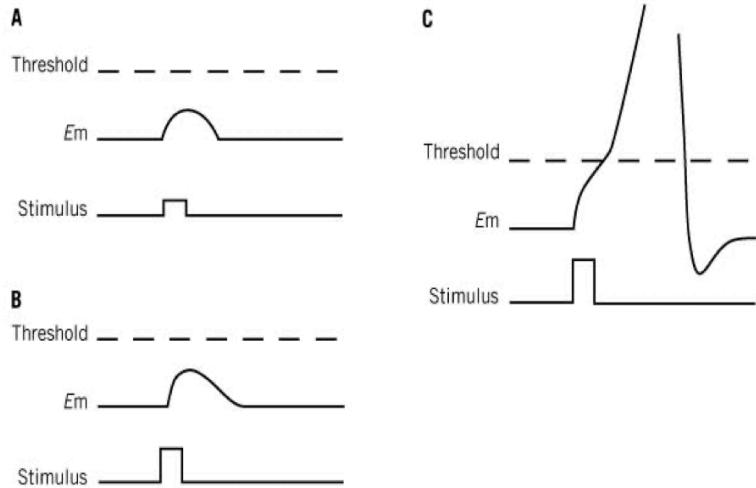


- Both  $\text{K}^+$  and  $\text{Na}^+$  open in response to depolarising voltage steps
- Greater depolarising step = greater extent of conductance and faster opening
- Different rates of onset and offset at all levels of depolarisation for  $\text{K}^+$  and  $\text{Na}^+$  channels

- $\text{Na}^+$  channels close more rapidly than  $\text{K}^+$  channels
- $\text{Na}^+$  inactivate, so decrease in conductance during a maintained depolarising voltage step
  - Channel  $\rightleftharpoons$  Open  $\rightleftharpoons$  Inactive
  - Inactive state going back to channel state will need time and a negative  $V_m$
- Currents for  $\text{K}^+$  and  $\text{Na}^+$  will reverse their directions when potential approaches one of the ion's equilibrium potential
- Shape of AP matches the conductance curves for  $\text{K}^+$  and  $\text{Na}^+$  at different phases which means that **different phases of the AP results form the opening and closing of voltage-gated  $\text{K}^+$  and  $\text{Na}^+$  channels**



- Features:
  - All or none
    - AP occurs and reaches full amplitude, or doesn't occur at all
      - Amplitude independent of the magnitude of stimulus that stimulated it
    - Small depolarisation = small inward current of  $\text{Na}^+$ , but also increases outward currents of  $\text{K}^+$  voltage channels and leak channels due to changing electrochemical driving forces
      - **Outward potassium current opposes depolarising sodium current when depolarisation not great enough**
    - Large enough depolarisation = great voltage sensitivity and rapid  $\text{Na}^+$  voltage-gated channel activation process ensure that the membrane potential reaches threshold, where the  $\text{Na}^+$  current exceeds the increase in outward  $\text{K}^+$  channels



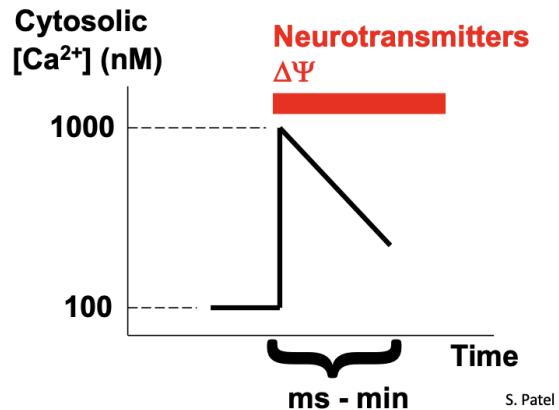
- Threshold is the specific value of  $V_m$  at which the net ionic current ( $I_{Na} + I_K + I_{leak}$ ) just changes from outward to inwards, positive charge on the inside of membrane
- Refractory period
  - More difficult to excite a neurone to generate another AP
  - Caused by inactivation of  $Na^+$  channels and elevated  $K^+$  conductance immediately after an AP
  - Absolute refractory
    - Membrane is **repolarising and immediately afterward AP**
    - No new AP can be generated because threshold = infinite
  - Relative refractory
    - Threshold higher than normal (on its way back to normal level)
    - New AP can be generated if stimulus strong enough
    - More potassium outward current counterbalancing any sodium current coming in
  - Refractory period important for the direction that AP travels towards
  - Refractory period prevent AP from summatting
- Action potential propagation
  - AP can move along the length of an axon  $\Rightarrow$  long distance communication
  - Propagation = depolarisation  $\rightarrow$  excitation of regions of membrane adjacent to location of AP
    - Local spread followed by the active regenerative process
  - New action potential will not be generated backwards but will be generated forward if current is strong enough to reach threshold
  - **Membrane capacitance and axon diameter affect the velocity of AP propagation**
  - If axial resistance is large, it will oppose to passage of AP
    - Axons with small diameters will have high axial resistance  $\rightarrow$  hinder speed of propagation of action potential
    - $R_a$  large = small axon, current flowing through is smaller, takes longer to change the charge on the membrane = slow
  - If membrane capacitance is large, more charge must be deposited on membrane to change the potential across the membrane
    - $C_m$  large = current must flow for longer to produce a given depolarisation = slow

- Rate of passive spread varies inversely with  $R_a C_m$
- Strategies for rapid AP propagation
  - Large axons = increase diameter of axon core
    - $R_a$  decreases in proportion to square of axon radius
      - So  $R_a$  decrease a lot, decreases by the squared of its value
    - $C_m$  increase in proportion to radius
    - Decreases  $R_a C_m$
  - Myelin is a way to overcome the fact that humans and other mammals with complex nervous system cannot afford to have large axons (too much energy)
    - Decrease  $C_m$  as capacitance is inversely proportional to thickness of insulating material
    - Decreases  $R_a C_m$
    - Saltatory conduction → insulation provided by myelin forces the depolarising current farther down the axon, thereby allowing AP to skip parts of the membrane
      - Nodes of Ranvier have greater density of sodium channels

## Calcium Signalling

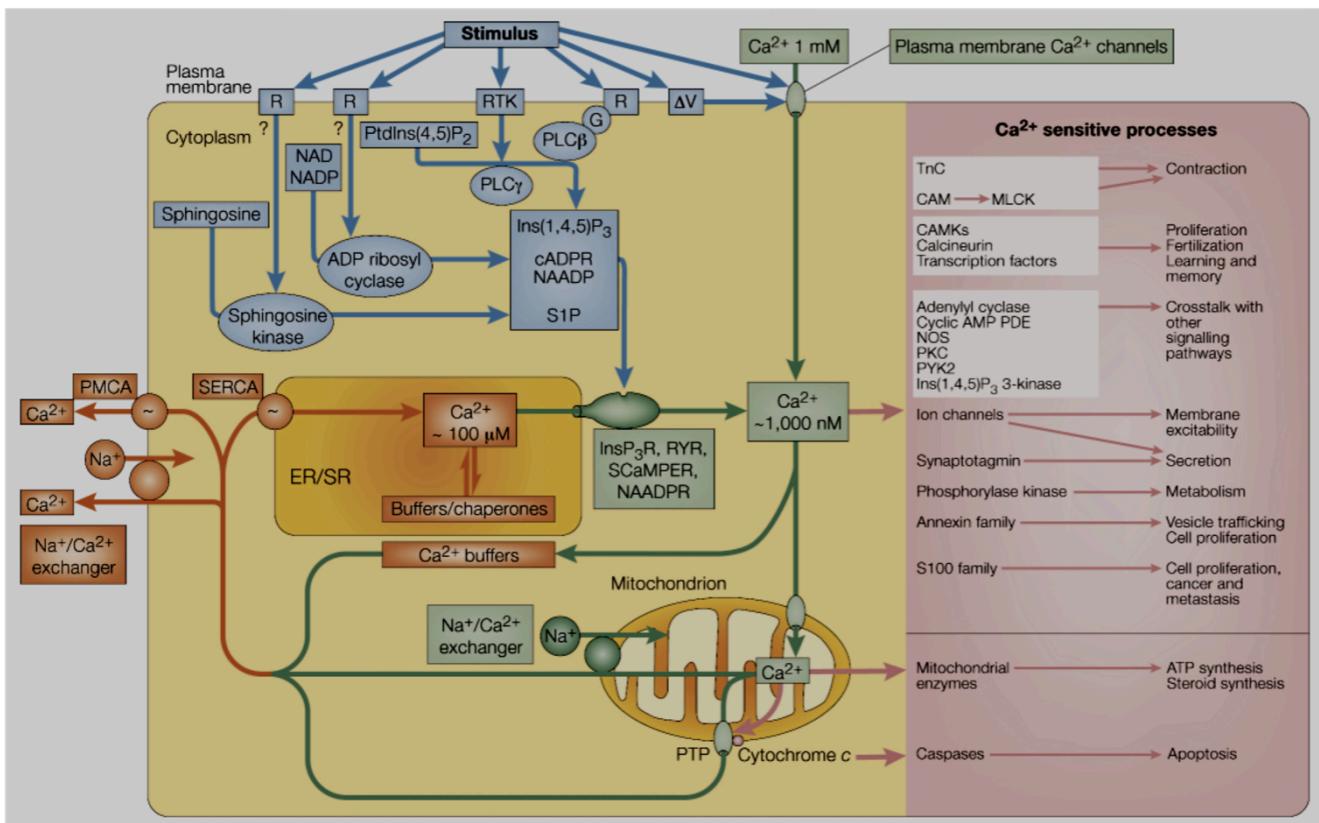
### Calcium basics

- Calcium is a unique signalling molecules
  - Not synthesized from precursors
  - Cannot be broken down
- Calcium is versatile
  - Calcium controls a broad range of neuronal fuctions
    - NT release
    - Membrane excitability
    - Synaptic plasticity
    - Changes in gene expression
    - Growth and differentiation of neurons in development
    - Programmed neuronal death
- Important facts and number
  - Extracellular calcium concentration → 1-2mM
  - Intracellular calcium concentration → 50-100 nM
  - Diffusion constant for  $\text{Ca}^{2+}$  ( $D_{\text{Ca}}$ ) depends on ion size and medium
    - $D_{\text{Ca}}$  (water) =  $\sim 600 \mu\text{m}^2/\text{s}$
    - $D_{\text{Ca}}$  (cytoplasm) =  $\sim 200 \mu\text{m}^2/\text{s}$
  - Calcium is estimated to migrate no further than 0.1-0.5 $\mu\text{m}$  and lasts only  $\sim 50 \mu\text{s}$  before encountering a binding protein

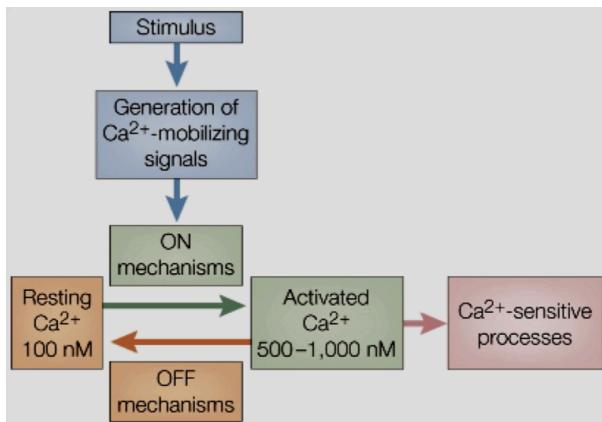


- Rapid increase in calcium which then quickly declines

- Ca<sup>2+</sup> signals are highly regulated at 2 levels:
  - Spatially
  - Temporally
- Large concentration gradients are useful for signalling purposes
  - Calcium has tendency to move into cell cytoplasm from extracellular environment and from intracellular stores such as the ER (with high Ca<sup>2+</sup> conc)
- We want the calcium to not last long in cells as it is toxic (e.g. can activate caspases which cause cell death)
  - A variety of homeostatic mechanisms are involved in maintaining calcium at low levels



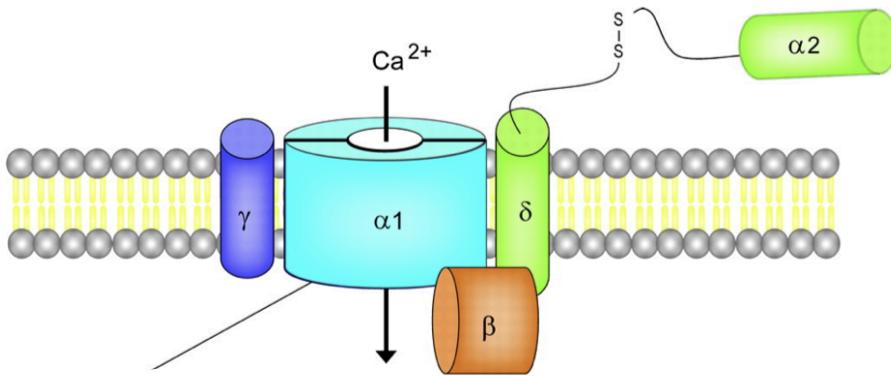
Due to these cascades (calcium sensitive processes), calcium is responsible for many processes



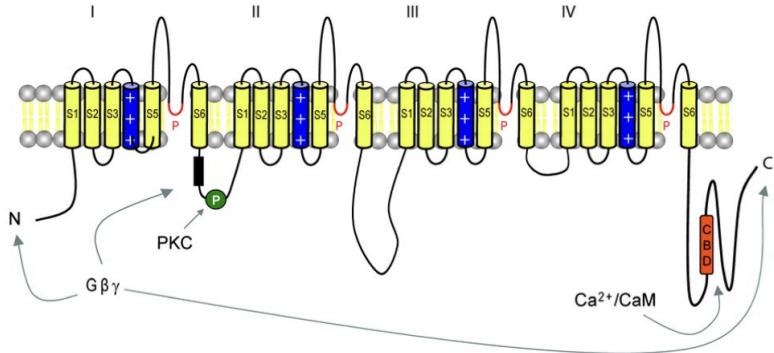
## On Mechanism Extracellular

- **Voltage-gated Calcium Channels**

- In the plasma membrane
- Contains pore-forming alpha-1 subunit that determines their main biophysical and pharmacological properties

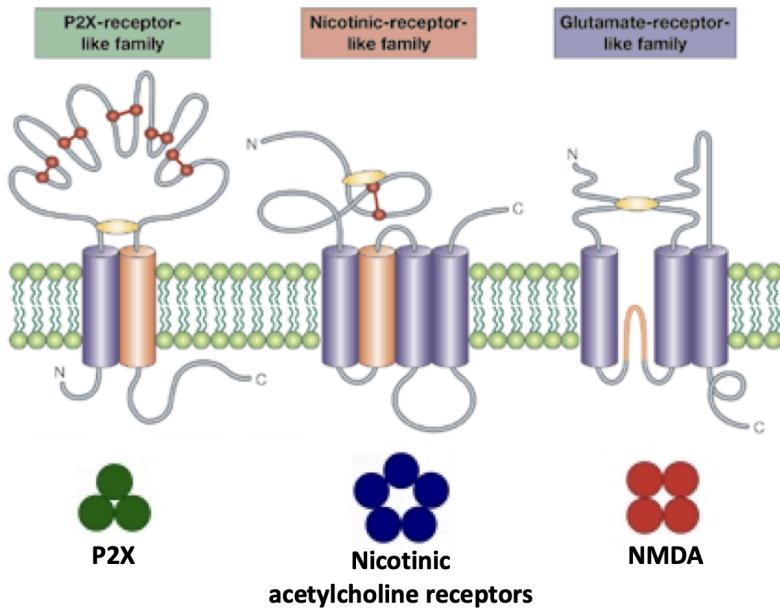


- 
- The alpha-1 subunit consists of:
  - Intracellular amino and carboxyl termini
  - 4 domains all linked with a single very long polypeptide
  - Each domain contains 6 transmembrane spanning regions and a hairpin loop of amino acid that dips into membrane (but does not fully cross it)
    - S4 has highly positively charged amino acids → voltage sensing, highly selective only for  $\text{Ca}^{2+}$
    - Hairpin loops line pore



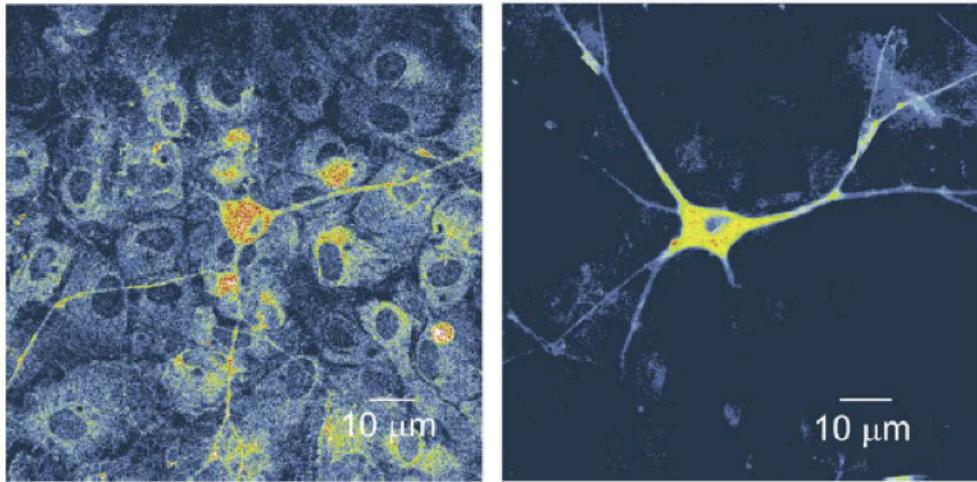
Benarroch EE, Neurology 2010;74:1310-1315

- - Additional subunits acting as chaperones and modulate alpha subunit function
  - Regulatory regions (e.g. N and C terminus) responsible for certain types of calcium channels with beta, gamma subunits → important for neuromodulation
- **Ligand-gated, calcium-permeable receptors**
  - Respond to different NT (e.g. ATP, ACh and glutamate)
  - Have different topologies and stoichiometries



- - Large extracellular loop for P2X → gated by ATP
  - Extracellular N and C terminus for nicotinic receptor
  - NMDA receptor = tetrameric, glutamate + glycine gated

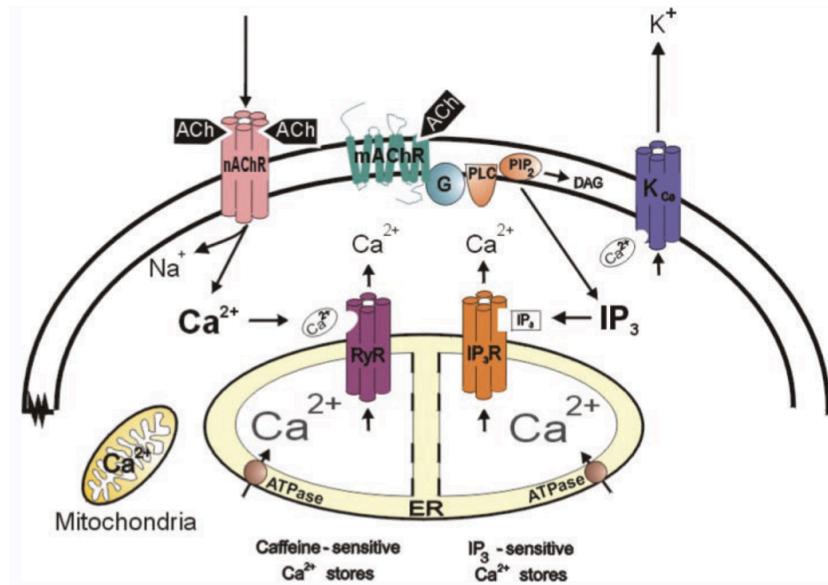
## On Mechanism Intracellular



(Hippocampal neuronal cultures stained with fluorescent thapsigargin which binds to SERCA pumps and fluorescence tryanodine which binds to ryanodine receptors)

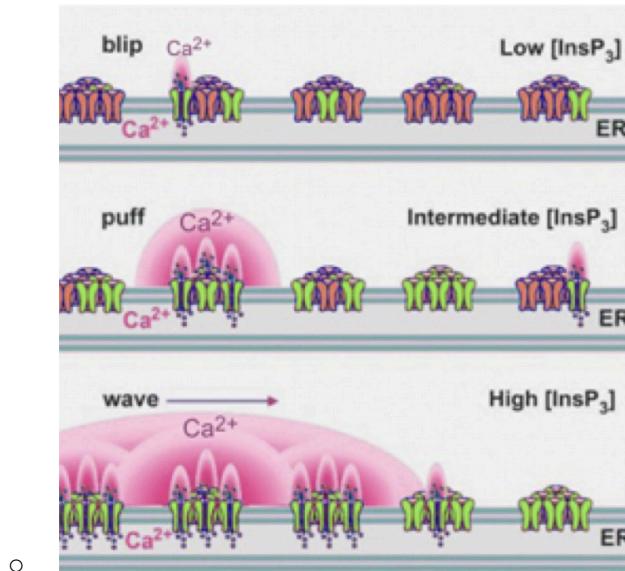
ER are calcium stores distributed throughout neuron (in cell body and dendrites) → Calcium signalling can happen independently in dendrites

The same neurotransmitter can activate multiple  $\text{Ca}^{2+}$  sources in neurons via distinct signalling pathways. This can lead to different final effects (depending on the location of the receptors and their effectors) can signal amplification, and synergistic effects



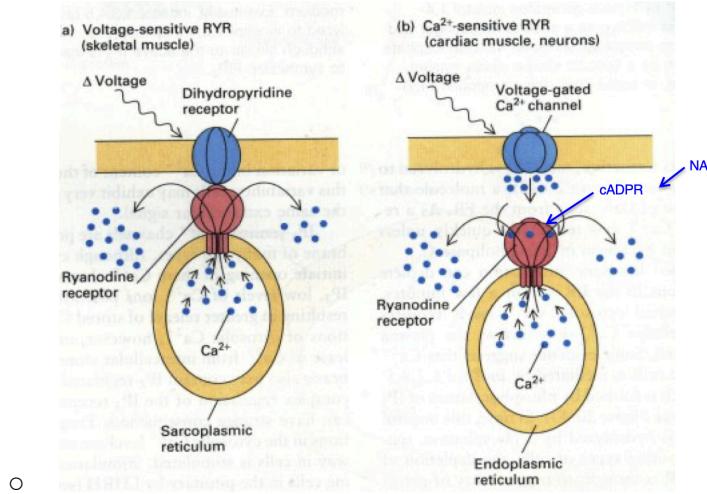
- $\text{IP}_3$  Receptors
  - $\text{IP}_3$  is synthesized from  $\text{PIP}_2$
  - $\text{IP}_3$  receptors sit on surface of ER
  - Formed by 4 subunits

- For each subunit there are 3 paralogs, that can form homo- or heteromers → molecular variability
- Large N-terminal IP<sub>3</sub>-binding domain on cytoplasmic side
- C terminus intraluminal (in the ER)
- Further ATP and calcium binding sites on their cytoplasmic side
- Inhibited by heparine
- Modulated by calcium in biphasic manner
  - Response to IP<sub>3</sub> is optimal when free calcium concentration in cytoplasm is ~300nM, but substantially inhibited at lower or higher calcium concentration
  - Reversible biphasic regulation by cytosolic Ca<sup>2+</sup> is a characteristic shared by all 3 mammalian IP<sub>3</sub> receptors



- - IP<sub>3</sub> binding to receptor on smooth ER leads to release of Ca<sup>2+</sup> into cytoplasm
  - Higher concentration of IP<sub>3</sub> will lead more sustained activation of receptors → to greater calcium elevation in cytoplasm

- Ryanodine Receptors
  - Leads to **calcium-induced calcium release (CICR)**



- - In muscles:

- As voltage-gated ion channel open, opening mechanism is mechanically coupled to activation of ryanodine receptors sitting in skeletal muscle sarcoplasmic reticulum
- Triggers opening of ryanodine receptors on SR → increase in calcium levels
- In neurons
  - Calcium flow through voltage-gated calcium channel
    - Primary cytoplasmic calcium concentration elevation
  - Calcium act as ligand and bind to ryanodine receptor, activating it
    - Secondary, more sustained calcium release
  - Large transmembrane proteins sitting in ER membrane
  - Formed by 4 subunits
    - 3 genes that can form homomers: RyR1, RyR2 and RyR3
  - Are activated by  $\text{Ca}^{2+}$ , cADPR, or by direct coupling to L-type VGCC in muscles
  - Have a large N-terminal domain on the cytoplasmic side
  - Are stimulated by caffeine
  - Ryanodine is a plant alkaloid and blocker
  - **CICR generates oscillations** in concentration of cytosolic free  $\text{Ca}^{2+}$  that underlie the waves that propagate via  $\text{Ca}^{2+}$  diffusion in many cell types
    - Can occur spontaneously or result of stimulation by external signal
    - Represent most widespread oscillatory phenomenon at cellular level
    - Seen in astrocytes
    - Waves can be localised to soma or dendrite, or move from cell to cell through gap junctions
    - Can underlie some pathological symptoms (e.g. migraines)

## Off mechanism

Calcium buffer and NCX are the first mechanisms to decrease  $\text{Ca}^{2+}$  conc, the remaining is taken care of by PMCS

- **Re-uptake in ER by SERCA pump** → ER and Mitochondria are sinks and sources for  $\text{Ca}^{2+}$ 
  - SERCA pump
    - Transports 2  $\text{Ca}^{2+}$  per ATP
      - Energy consuming
    - 3 different genes
    - High density in ER membrane
    - Activated by high cytosolic  $\text{Ca}^{2+}$  concentration and inhibited by high ER  $\text{Ca}^{2+}$  concentration
    - Inhibited by phospholamban and inhibition is relieved by phosphorylation
- Re-uptake in mitochondria by uniporter or  $\text{Na}^+/\text{Ca}^{2+}$  exchanger (NCX)
- **Expelling of calcium out of cells by plasma membrane PMCA pump and NCX**
  - PMCA
    - Transports 1  $\text{Ca}^{2+}$  per ATP consumed with a rate of  $\sim 30\text{Ca}^{2+}$  per second

- 4 different genes and multiple splice variants
- 10 transmembrane spanning regions
- Released from inhibition in response to elevation of intracellular calcium
  - Autoinhibitory domain with calmodulin binding site
  - Bound to calmodulin → inactive
  - $\text{Ca}^{2+}$  bind to calmodulin
  - Activated by  $\text{Ca}^{2+}$ -bound calmodulin
- Regulated by phosphorylation of PKC and PKA
- NCX
  - **Powered by electrochemical gradient of  $\text{Na}^+$** 
    - Transports 3 $\text{Na}^+$  in against 1  $\text{Ca}^{2+}$
    - Operates without ATP
  - Antiporter membrane protein
  - 9 transmembrane spanning regions and a large cytosolic loop
  - Rate of ~2000-5000  $\text{Ca}^{2+}$  per second
  - Low affinity for  $\text{Ca}^{2+}$
  - Activated by high concentrations of cytosolic  $\text{Ca}^{2+}$
- **Calcium buffers:**
  - Most calcium that enters cytoplasm is rapidly bound to various **cytosolic buffers**
  - **Parvalbumin** → has 4 binding sites for calcium, common amino acid motif favours calcium binding
    - High affinity for calcium, but slow off-rate (unbind very slowly)
    - Relatively high affinity for  $\text{Mg}^{2+}$ , intracellular high conc of  $\text{Mg}^{2+}$ , at rest most parvalbumin bound to  $\text{Mg}^{2+}$ 
      - Will take time to unbind from magnesium and bind to calcium
      - This gives time for calcium to act
    - After  $\text{Ca}^{2+}$  unbinds it will be thrown out or back into cytoplasm
  - Calbindin-D28k
  - Cytosolic buffers are first loaded and then unloaded thus influencing the amplitude and duration of cytosolic  $\text{Ca}^{2+}$  signal
  - Buffer capacity varies considerably between cells

## Experiment

Demonstrating involvement of  $\text{Ca}^{2+}$  in cellular response (does it act on a channel?):

1. Ionophores → mimic response of an ion
  - a.  $\text{Ca}^{2+}$  ionophores mimic  $\text{Ca}^{2+}$
2.  $\text{Ca}^{2+}$  channel blockers/inhibitors should inhibit response
3.  $\text{Ca}^{2+}$  chelators (e.g. EGTA) should prevent or suppress the response
  - a. Bind to calcium

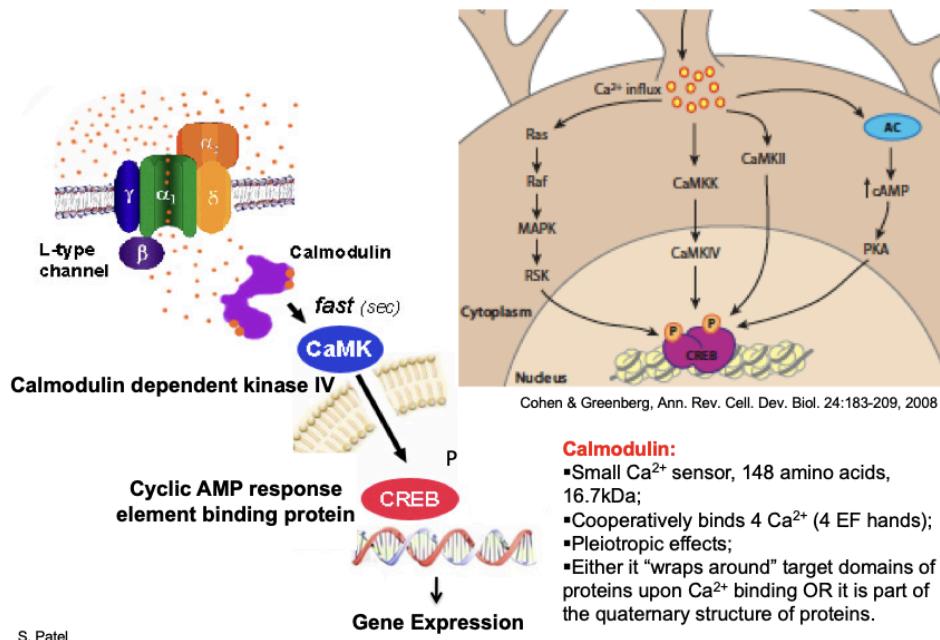
Response should correlate with a rise in intracellular free  $\text{Ca}^{2+}$  as revealed by  $\text{Ca}^{2+}$  sensitive dyes (e.g. FURA-2, Fluo-4)

## Calcium effect on postsynaptic cell:

- Calcium mediates signal transduction from synapse to nucleus
  - Extracellular calcium enters postsynaptic cell via:
    - Synaptic and extrasynaptic ligand-gated channels
    - Voltage-gated channels
    - **NMDA receptor and L-type voltage-gated channel are major routes for calcium entry**
  - CICR can also amplify calcium signals via ryanodine receptors
- Calcium in various locations (channel mouth, cytoplasm, nucleus) can signal to transcription factors
  - Gene products influenced by calcium activity contribute to neuronal development and plasticity
  - Allows nucleus to communicate adaptive changes to synapse

## Calmodulin

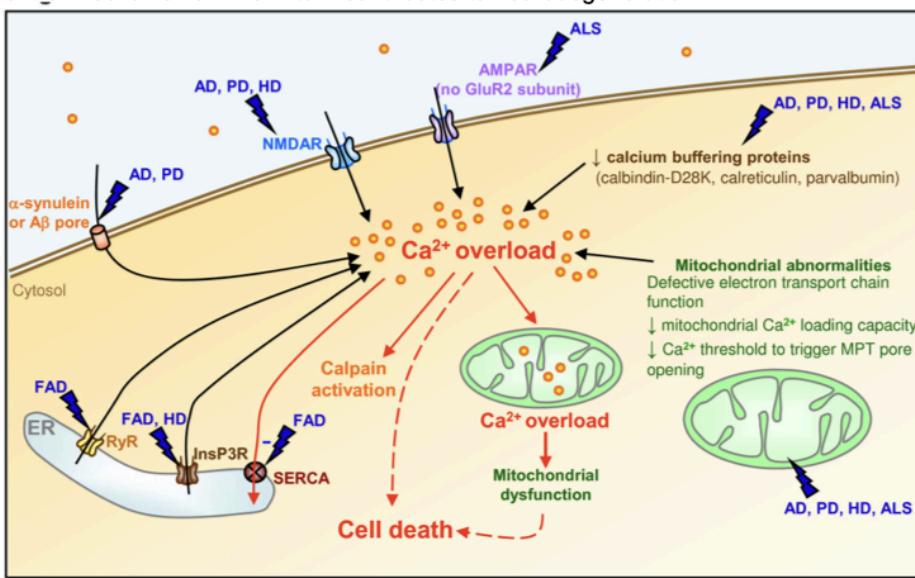
### Calmodulin is the calcium sensor for gene expression



S. Patel

## Dysfunctional aspects of $\text{Ca}^{2+}$ signalling: $\text{Ca}^{2+}$ overload in neurodegenerative diseases

AD/FAD, PD, HD, and ALS affect cytosolic calcium levels by deregulating different homeostatic control mechanisms. This in turn contributes to neurodegeneration.



AD: Alzheimer's disease; FAD: familial Alzheimer's disease;

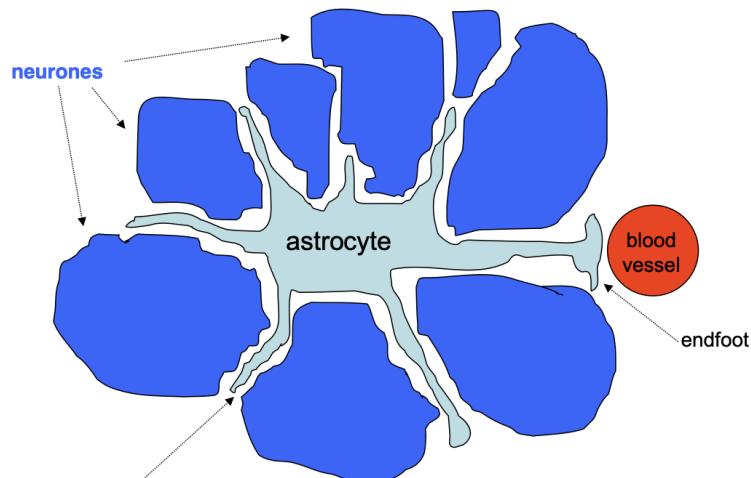
PD: Parkinson's disease; ALS: amyotrophic lateral sclerosis;

HD: Huntington's disease;

Marambaud et al. Molecular Neurodegeneration 2009. 4:20

## Homeostasis and Glia

- Astrocyte function:
  - Buffering  $[\text{K}^+]_o$  via Na/K pump and ion channels
    - Also spatial buffering
  - Taking up and recycling glutamate and other transmitter
  - Preventing extracellular glutamate reaching neurotoxic levels

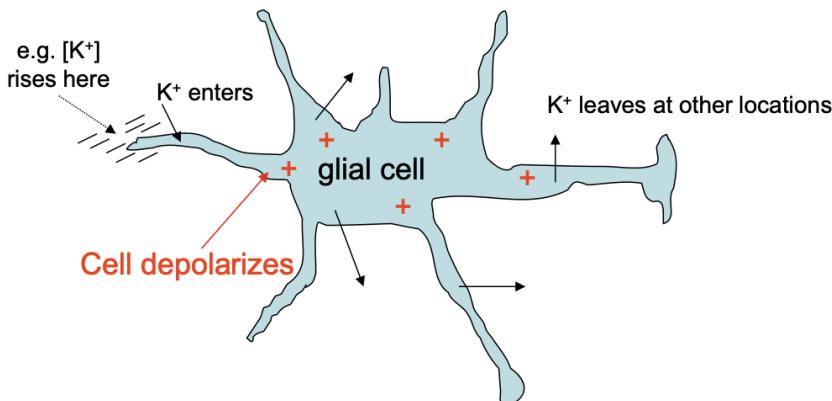


- Extracellular volume is small.
  - Need for a system to control the environment around neurons

- Control conc of ions and neurotransmitters
- Endfoot → contact with blood vessels

## Removal of $K^{\pm}$

- Changes of  $[K^+]$  in extracellular space
  - Cause: AP in neuron 1 → hyperpolarisation =  $K^+$  efflux
  - Effect: Rise in extracellular potassium in extracellular space → Neuron 2 takes up the  $K^+$  (diffusion into neuron 2 down a conc gradient)
  - Result: Depolarisation of neuron 2, possibly causing AP and hence leading to mixing of info being coded by 2 neurons → can get epileptic discharge
- Glial cells minimize extracellular potassium changes by:
  - Taking up  $K^+$  through ion channels
  - Taking up  $K^+$  through Na/K pump
- Nernst potential = no net flux

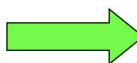


- - One end with the higher local extracellular potassium will depolarise due to influx of  $K^+$
  - The other end does not have high extracellular potassium concentration
  - Potassium will diffuse out of the glial cell at the other end of the glial cell → **Spatial buffering**

The result is:

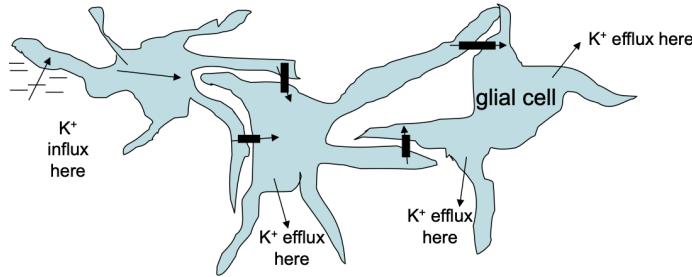
an inward  $K^+$  current where  $[K^+]_o$  is high

an outward  $K^+$  current where  $[K^+]_o$  is low

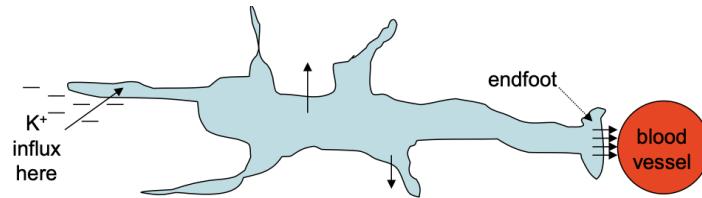


i.e. SPATIAL BUFFERING

- Spatial buffering is facilitated by:
  - Gap junctions between glial cells → allows  $K^+$  to be buffered to a larger area



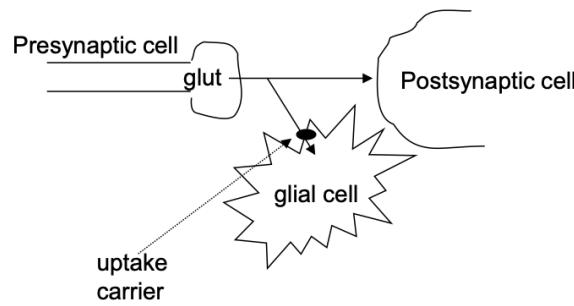
- Astrocyte endfoot →  $K^+$  leaves via endfoot to blood vessel, not deposited around other neurons



- Evidence:
  - Resulting depolarisation from injecting potassium is much larger when applied to the endfoot
  - Specialisation of endfoot membrane with potassium being deposited there
- Over 90% of cell's  $K^+$  conductance is in the endfoot → accumulated  $K^+$  is buffered to blood vessel
- High  $K^+$  conductance results from large number of  $K^+$  channels present

## Removal of Neurotransmitter

- Neuromuscular junction → ACh
  - Inactivated by AChE
- Glutamatergic synapse → glutamate
  - No extracellular enzyme to inactivate it
  - **Glutamate is removed by uptake into glia**
  - Inside glia: glutamate is converted to glutamine (by **glutamine synthetase**) or enter the Krebs cycle



- Glutamate uptake is important:

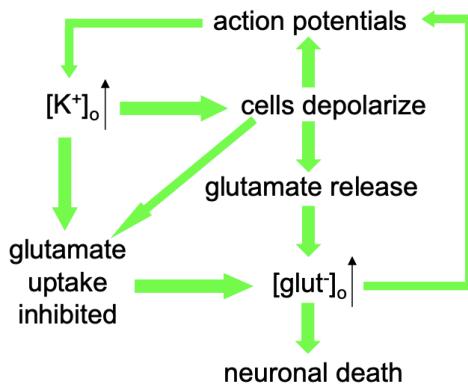
- Terminates synaptic transmission
  - Essential for sending brief signals through neurons
- Recycles synaptically released glutamate
- Prevents glutamate-induced neurotoxicity
  - Cause:
    - Glutamate activates NMDA receptors →  $\text{Ca}^{2+}$  influx ⇒ increase in  $\text{Ca}^{2+}$  levels
    - $\text{Ca}^{2+}$  dependent enzymes will “eat” cell and trigger release of apoptosis-inducing cytochrome C from mitochondria
    - Ion fluxes resulting from glutamate acting on neurons and glia lead to water movements → cells swell and die (e.g. dendrites have very thin membranes, swell easily)
- Removal mechanism:
  - No ATP needed to accumulate glutamate against a conc gradient
    - Gets energy by **co-transporting  $\text{Na}^+$  ions** down their electrochemical gradient into cell
  - 3  $\text{Na}^+$  transported in for every glut in
  - More  $\text{Na}^+$  than glut are transported
    - Uptake generate inward current
    - Uptake inhibited by depolarisation because the the  $\text{Na}^+$  gradient across the membrane is diminished, hence the glutamate transporter cannot rely on this gradient to transport glutamate in when there is depolarisation.

## Failure of $[\text{K}^+]_o$ and $[\text{glut}]_o$ homeostasis

Occurs in anoxia and ischaemia



Positive feedback:



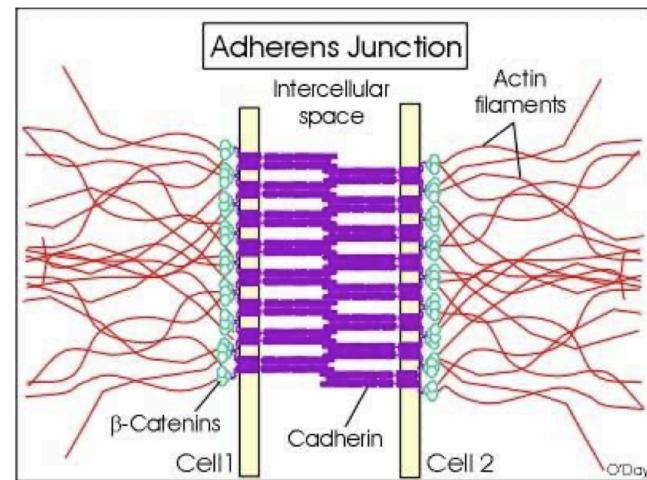
- - Cycle of potassium and glutamate imbalance

## Other Astrocyte Functions

- Astrocytes can communicate with neurons via  $\text{Ca}^{2+}$
- $[\text{Ca}^{2+}]$  rises in astrocytes are suggested to release gliotransmitters from vesicles or through ion channels
  - Glutamate, D-serine, ATP
- May alter neuronal excitability or modulate synaptic transmission
- $[\text{Ca}^{2+}]$  rises in astrocytes also generate vasoactive messengers, derivatives of arachidonic acid
  - Regulates blood vessel diameter
  - Capillary pericytes regulate blood flow
- Control energy supply to brain
- Regulate pH
- Secrete substances that control synapse formation
- Secrete D-serine → co-agonist with glutamate for NMDA receptors
- Release glutamate and ATP to modulate neuronal function
- Control blood flow

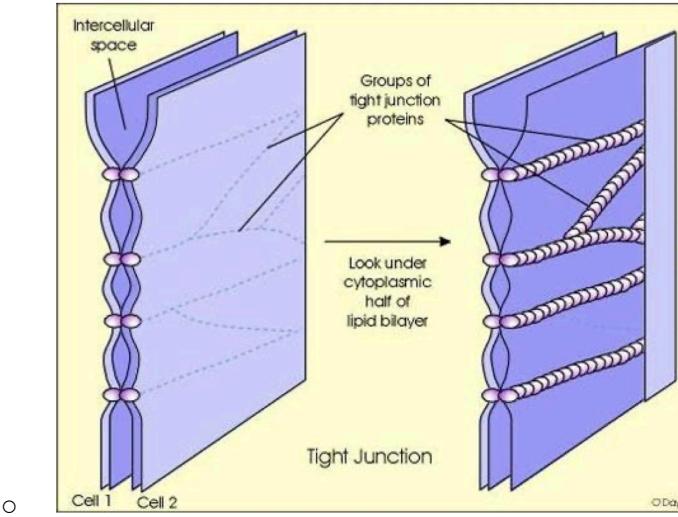
## Cell Junctions

- Adhering junctions
  - Join cells mechanically
  - Found in tissues subject to mechanical stress (e.g. heart, skin)
    - Heart needs to beat constantly, always under mechanical pressure experience strong forces, need to stay together



- Cadherin glue 2 cells together
- Open meshwork of cadherin, things can diffuse through extracellular space (e.g. glucose diffuses between 2 cells and get to places which may not be well supplied by arteries)
- Junctions designed to hold cells together

- Impermeable junctions (tight junctions)
  - In epithelia (e.g. gut)
  - Selectively reabsorb stuff
    - Allow transporters to absorb glucose from gut
    - Passive transporters export it to extracellular space/blood
  - Inhibit diffusion of transporters from 1 side of the cell to the other
    - Fluid mosaic model
  - Provide sealing of extracellular space in gut epithelium
    - Cannot leak back
    - Things cannot leak in



- Hemijunctions
  - Pannexin hemichannels may be opened by lower extracellular calcium or ischaemia
    - Releases intracellular constituents like ATP and glutamate

## Gap junctions

- Made of connexons
  - Hexagonal symmetry → 6 subunits for each connexon
  - Different variations depending on the arrangement of subunits
- Allow electrical impulse to travel through whole of junction
  - Discovered looking at neuromuscular junction (NMJ)
  - Hyperpolarisation of muscle → hyperpolarisation in nerve
    - Communication goes both ways
  - Remove calcium in extracellular space → no effect on flow of current through junction
    - Not chemical synapse
- Heart
  - Gap junctions between all cells of the heart
  - Mechanism
    - Impulse initiated in the SA node
    - Impulse spreads through atrium → contraction of atrium

- Current carried by potassium from 1 cell to another via gap junction
- Bundle of his → large diameter, lower resistance
  - Spread from atrium to ventricle
- AV node → not many gap junctions, causes delay between impulse spreading from atrium immediate to the ventricle, delay the contraction of ventricle, ventricle has time to fill completely
  - Clinicians look at delay
  - Some people don't have enough junctions in AV node → delay is too much or indicate that sodium is not enough
  - Bundle branches start spontaneously beating if no junctions in AV node, safety system to ensure heart beat
- Gut
  - Gap junctions couple cells of gut → peristalsis
- Cortex
  - Pyramidal cells have gap junctions → 1 pyramidal cell fires, and adjacent cell receives some depolarisation, synchronisation of neuronal firing
- Formation of gap junctions
  - Gap junctions between all cells of body except for a few
  - Gap junctional connexons float around in the membrane till they meet another connexon in an opposed membrane when they bind and open
    - Promiscuous: bind to connexons across cell and species barriers
    - Small % of connexons may be open when not bound to partner
    - Open state → diffusion can occur
      - Constantly open is not good because you lose solutes and the concentration gradient is disrupted
    - Closed → twisted conformation, nothing can pass through
- Experiment of forming of gap junctions
  - Baths with extracellular fluid
  - 2 cells put into bath
  - 1 cell has recording electrode + current passing electrode, the other only has recording
  - Pass constant current to make voltage of 2 cells different
  - 2 cells are pushed together
  - Voltage for cell only with recording electrode shows depolarisation in quanta (little steps)
    - Change in voltage towards positive direction = formation of gap junction
  - Inject calcium into cell
  - Quantum lowers as the gap junctions close and cells uncouple (no more depolarisations)
- Allows small sized substances to pass through
  - Metabolic cooperation
  - Experiment:
    - Thymidine kinase adds phosphate to thymidine

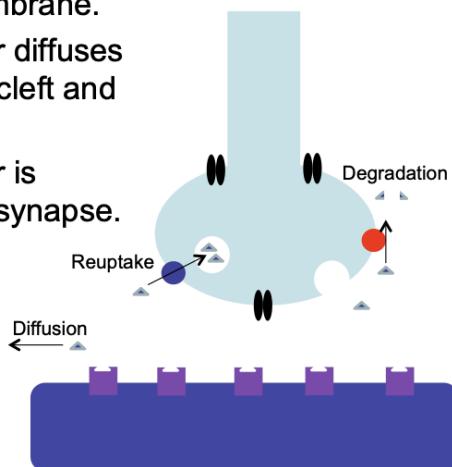
- Phosphate thymidine can enter nucleus
- Mutant cell lacks thymidine kinase (which adds phosphate to thymidine)
  - No silver grains in nucleus to show accumulation of radioactive labelled thymidine
- Normal cell
  - Silver grains showing accumulation of radioactive thymidine in nucleus
- Mutant cell coupled to normal cell
  - Mutant cell gains shows accumulation of thymidine in nucleus
  - Gap junction allows phosphorylated thymidine to pass from normal cell to mutant cell
- Calcium wave
  - IP<sub>3</sub> diffuse between cells → spreading wave of calcium release due to wave of IP<sub>3</sub> which diffuses between cells
  - Rise in calcium activate ATP release which acts on P2X/P2Y receptors to raise intracellular calcium further
  - Rise of calcium may fall with distance, giving a mechanism for differential gene expression according to location → could be relevant to specifying positional information in development
- Modulation of gap junction
  - Change pH
  - Plot magnitude of conductance against pH
    - **Very basic pH** → lots of conductance
    - Acidic pH closes gap junctions → low conductance
      - Acidic pH may indicate cell death, thus cells want to uncouple otherwise may cause electrical imbalance in other cells
  - High intracellular calcium closes gap junctions
    - Too much calcium indicates issue with cell, don't want this to be coupled to other normal cells
  - Regulated by voltage, hormones, protein kinase

## Synaptic Transmission

### Presynaptic Transmission

## Events leading to Synaptic Transmission

1. Action potential invades synaptic terminal.
2.  $\text{Ca}^{2+}$  channels open.
3. Vesicles fuse with presynaptic membrane.
4. Neurotransmitter diffuses across synaptic cleft and binds receptors.
5. Neurotransmitter is eliminated from synapse.



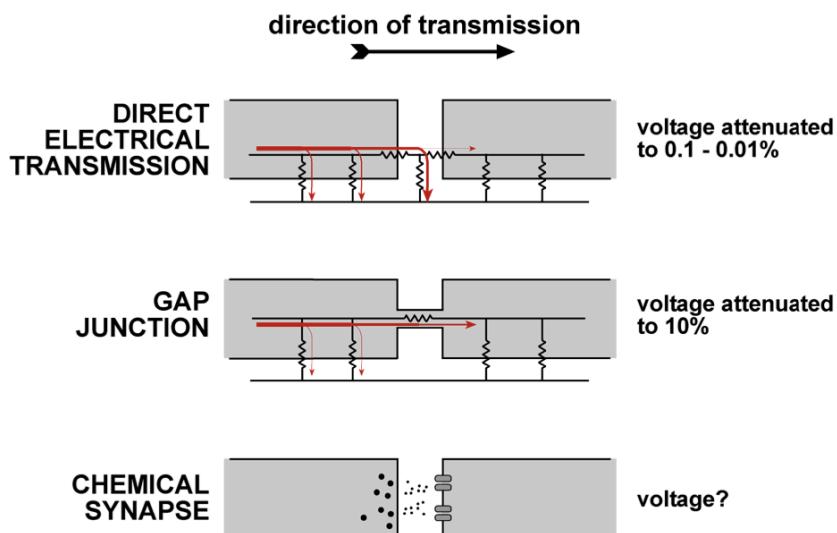
Golgi: Reticular theory (wrong):

- The NS was a large interconnected network comprised of the fused cytoplasm

Cajal: Neurone doctrine:

- Application of cell theory to nervous system (neurons were separate entities)

## Chemical vs. electrical transmission



Gap junctions:

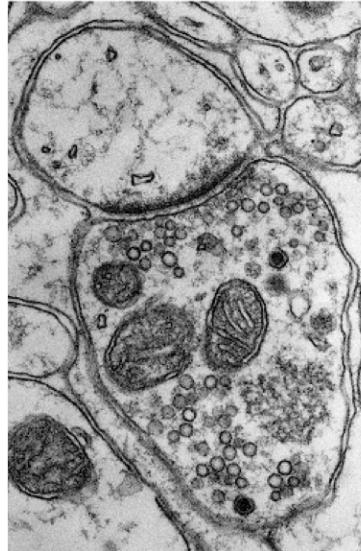
- Same sign signal
- Reciprocal
- Fast
- 2nd messengers diffuse
- Time course determined by presynaptic cell

Chemical synapse:

- Same sign or inverted
- Unidirectional
- Fast or slow
- The same signal (chemical) can be decoded differently by different targets
- Repertoire of interactions allows complex information processing

## CHEMICAL SYNAPSE - THE EVIDENCE

- LANGLEY – Curare blocked transmission in ciliary ganglion
- ELIOT – extract from adrenal glands mimicked the action of sympathetic nerves
- LOEWI – ‘vagusstoff’ (literally “vagus stuff”) liberated from one heart slowed the next one. Later identified as ACh
- DALE (& FELDBERG) – ACh was released at the neuromuscular junction and sympathetic ganglion
- ECCLES – last proponent of electrical transmission ‘converted’ to chemical synapses
- ROBERTSON – Electron microscopy first used to image ultrastructure of the synapse.



## $[Ca^{2+}]_o$ is required for synaptic transmission

4	Be
9.012	
12	Mg
24.31	
20	Ca
40.08	
38	Sr
87.62	
56	Ba
137.3	
88	Ra
(226)	

Inhibits synaptic transmission

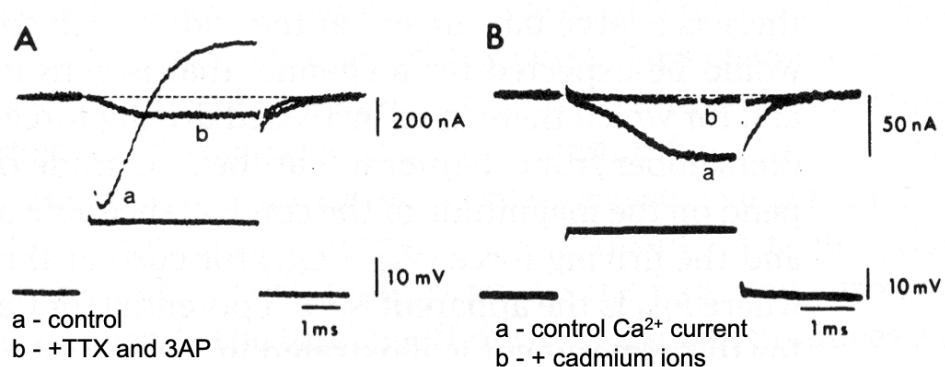
Locke and Ringer showed that extracellular calcium was required for nervous regulation of the heart.

Will partially support synaptic transmission

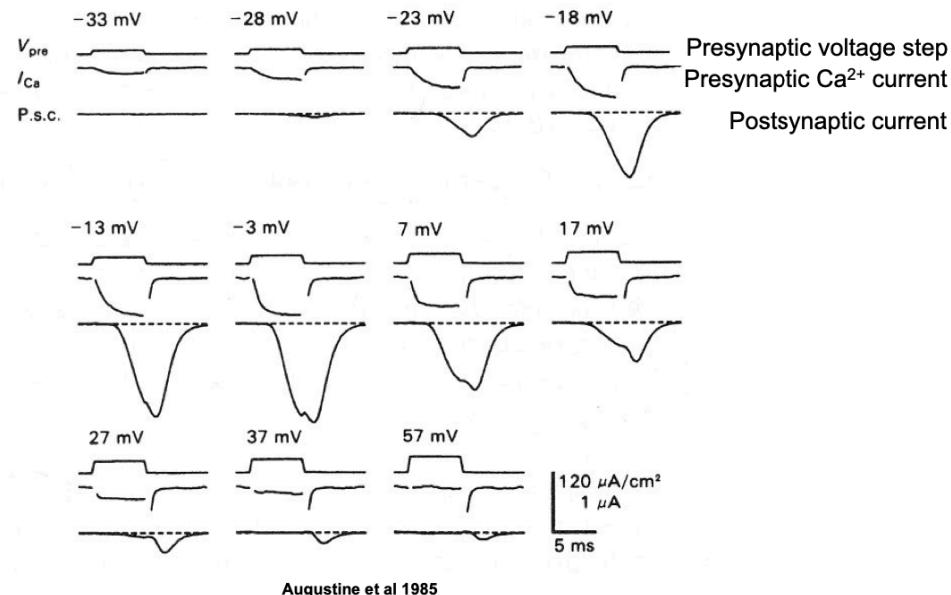
but, release is asynchronous

## SQUID GIANT SYNAPSE

Presynaptic calcium currents



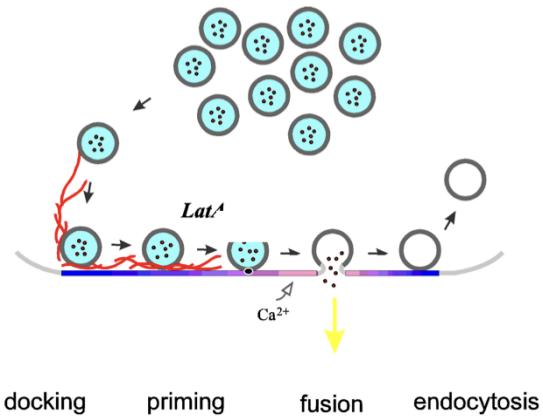
**RELATIONSHIP BETWEEN PRESYNAPTIC  
CALCIUM CURRENT AND TRANSMITTER RELEASE IN THE SQUID**



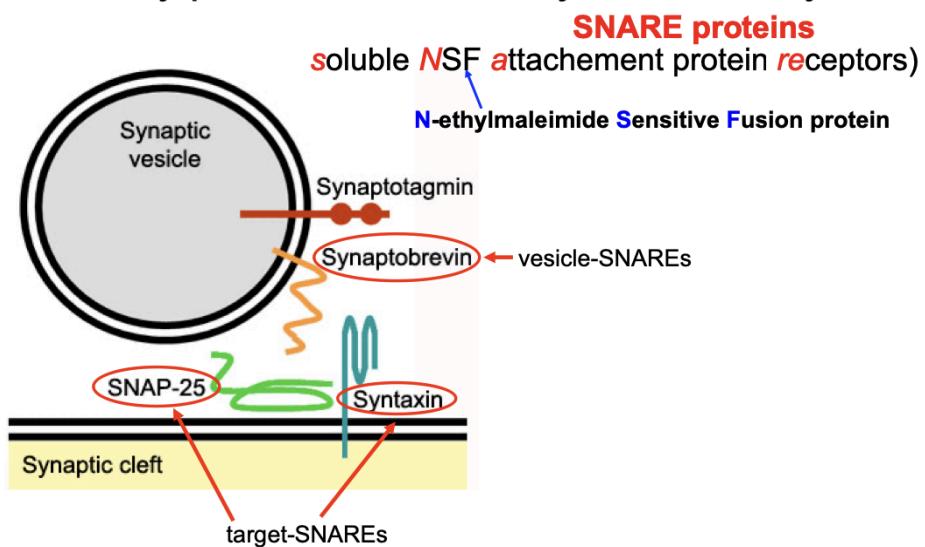
Augustine et al 1985

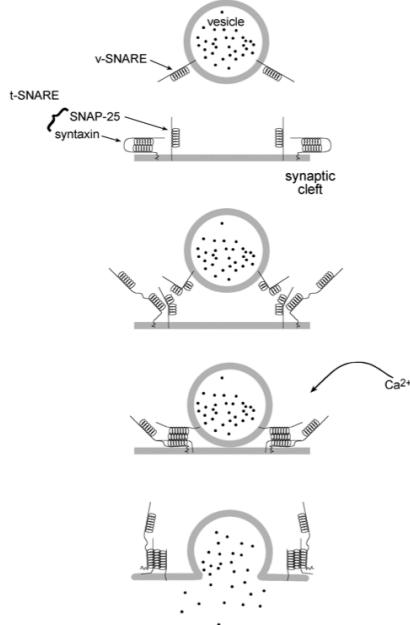
Bigger presynaptic Ca<sup>2+</sup> current = greater postsynaptic current → Ca<sup>2+</sup> important in signalling / eliciting a response in the postsynaptic current

## Neurotransmitter release mechanisms



### Key proteins of the exocytic machinery





NSF acts post-fusion to dissociate SNARE complexes for recycling of synaptic vesicles.

SNAREs form a tight complex consisting of a bundle of four  $\alpha$ -helices

⇒ one from v-SNARE

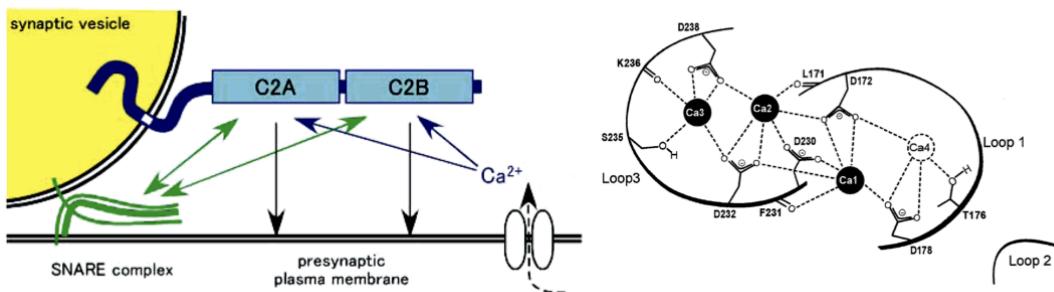
⇒ three from t-SNARE

→ energy gained from zippering to form the stable “*trans*” SNARE complex likely drives membrane fusion.

## Neurotransmitter release probability

- $\text{Ca}^{2+}$  central to release probability of NT
  - Rate of spontaneous vesicle fusion low in basal conditions
  - $\text{Ca}^{2+}$  influx increase rate of exocytosis (vesicle fusion)
  - Neurotransmitter are released within 100 microseconds of AP arrival
  - NT release displays non-linear dependence on extracellular calcium
- $\text{Ca}^{2+}$  channels provide  $\text{Ca}^{2+}$  microdomains for NT release
- $\text{Ca}^{2+}$  sensor must transduce the rise in intracellular calcium to trigger exocytosis machinery

## Synaptotagmin I is a major $\text{Ca}^{2+}$ sensor for exocytosis



Calcium regulates multiple steps in the vesicle fusion pathway but SYAPTOTAGMIN has attracted special interest. It could be the CALCIUM SENSOR for transmitter release.

- binds calcium cooperatively
- low affinity for calcium  $\mu\text{M}$
- it changes conformation on binding calcium
- genetic disruption leads to lack of synchronous vesicle release
- MAY NOT BE ONLY SENSOR

Synaptotagmin has high affinity for calcium → **Synaptotagmin may be the calcium sensor for NT release**

Synaptotagmin important because it binds to calcium and allows interaction with vesicle snare and target snares

## Neurotransmitter

Synaptic vesicles can fuse spontaneously without nerve stimulation → small postsynaptic response

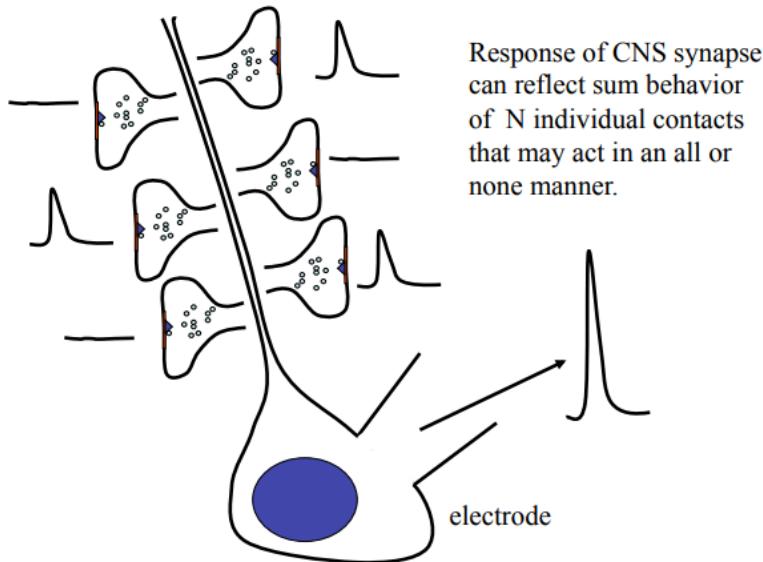
AP increases probability of vesicle fusion by promoting  $\text{Ca}^{2+}$  influx

Not all nerve stimulation will result in successful release of neurotransmitters

- Multiple release sites at nerve endings ensure depolarisation of target cell
- Giant synapses in the auditory system (e.g. calyx of Held)
- NMJ →  $10^3$  release sites

**Response of CNS synapse can show sum behaviour of # individual contacts that may act in an all or none manner**

Each release site can act independently from each other



- At synapses with only a single release site, changing the % of release does not affect the amplitude of the response
- At synapses with multiple release sites, changing % can change the response amplitude as summation could be different depending on how many synapses are activated

Depending on synapse, some release sites release the same NT (focus on this more simplicity sake) but others release different.

### Probability of # Synapse Release

At synapses with only a single release site, changing the probability of release site.

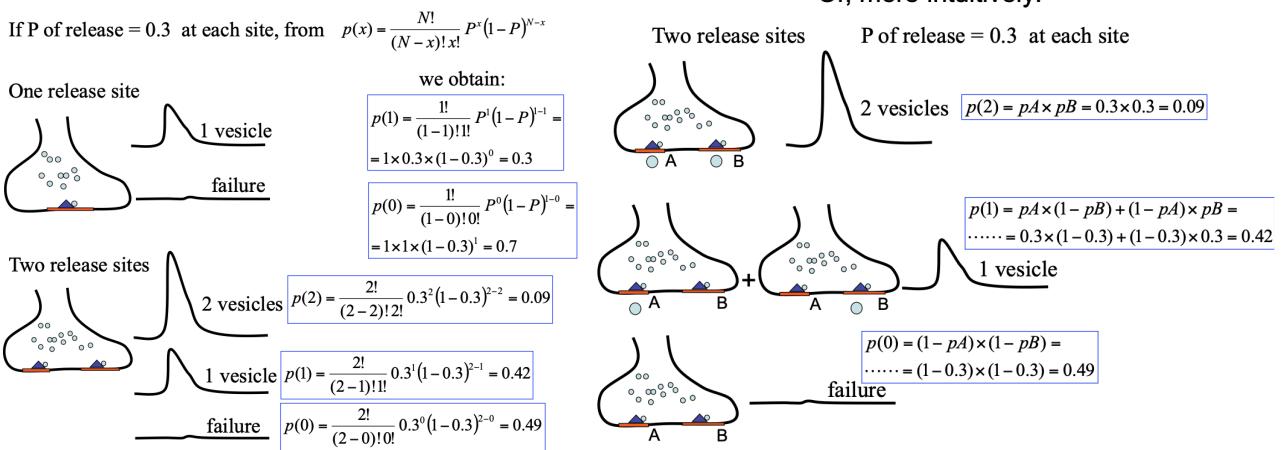
$$p(x) = \frac{N!}{(N-x)!x!} P^x (1-P)^{N-x}$$

N= number of release sites

P= probability of release at each site

p(x)= probability of observing x released vesicle out of N

$0! = 1$



## Quantal release = binomial model

Variance very high for intermediate probability of release

## Postsynaptic Current Size

Assuming a binomial distribution the mean amplitude of the synaptic response is

$$\bar{I} = NPQ \quad \text{Where } N \text{ is the number of release sites, } P \text{ is the probability of release and } Q \text{ is the quantal size and } \bar{I} \text{ is the mean current amplitude}$$

The variance for a binomial distribution is

$$\sigma_I^2 = NQ^2P(1-P) \quad \text{or} \quad \sigma_I^2 = Q(NPQ) - (NPQ)QP \quad \text{or} \quad \sigma_I^2 = Q(NPQ) - (NPQ)\frac{(NPQ)}{N}$$

$$\text{since} \quad \bar{I} = NPQ$$

the relationship between variance and mean current is

$$\sigma_I^2 = Q\bar{I} - \frac{\bar{I}^2}{N}$$

Measuring the mean current and its variance under different release probability conditions (i.e. changing the extracellular  $\text{Ca}^{2+}$  concentration) provides estimates of Q and N, while P can be derived from  $\bar{I} = NPQ$

Amplitude of current initiated: **I = NPQ**

To apply equation:

- Variability of quantal content (e.g. conc of NT in each vesicle) not taken into account

- Synapses with multiple release sites assumed to have % release and quantal size uniform across all sites
- Individual quantal events summate linearly

### **Variance of mean current will be affected by intrasite variation and intersite variation:**

- Intrasite
  - Variations in NT content in each vesicle
  - Stochastic properties of postsynaptic receptors
  - Multivesicular release (more than 1 vesicle release from same site)
- Intersite
  - Intrasite variation
  - Variations in average quantal size across sites
  - Number of sites from which a vesicle is successfully released (variance is low at very high or very low % of release)
  - Differences in probability of release at different sites

### **Probability of Release**

Docked synaptic vesicle → number of readily releasable vesicle available

Limited docked synaptic vesicles = depletion at high stimulus frequency

When multiple release sites are involved, facilitation = increase in release %

Short term plasticity = history dependent change in responsiveness:

- Residual  $\text{Ca}^{2+}$  facilitate transmission when not all quanta are released on the first stimulus
- If transmission is robust on the first stimulus most readily releasable vesicles will be gone and depression results

Transmitter is released and very rapidly reaches postsynaptic membrane

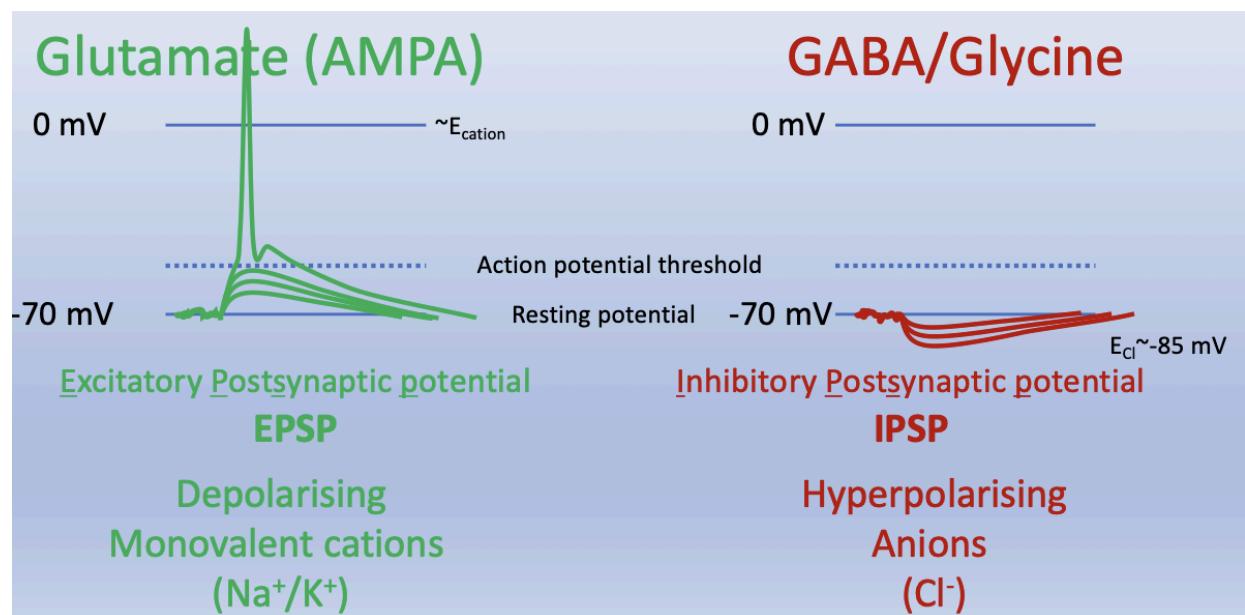
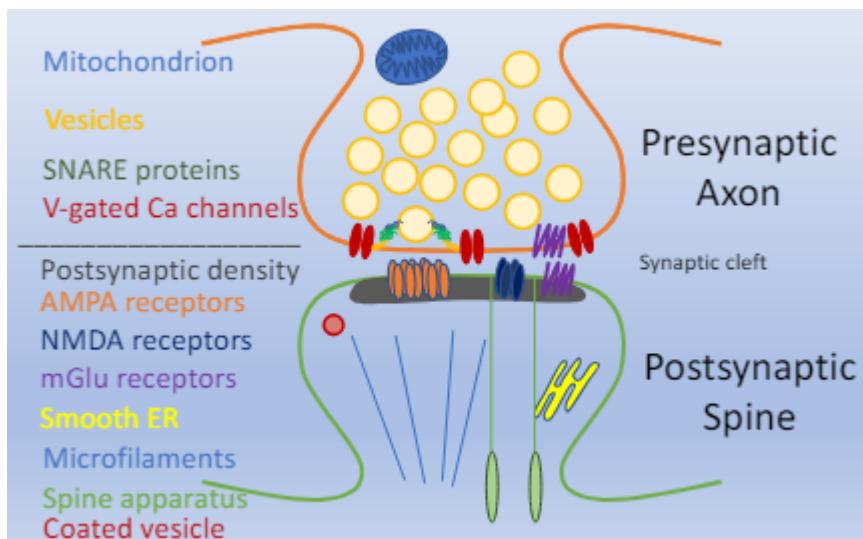
Concentration falls very rapidly because of diffusion away from release site

Diffusion applies to all neurotransmitters with similar diffusion coefficients, but it is not the only factor determining lifetime of transmitter in cleft

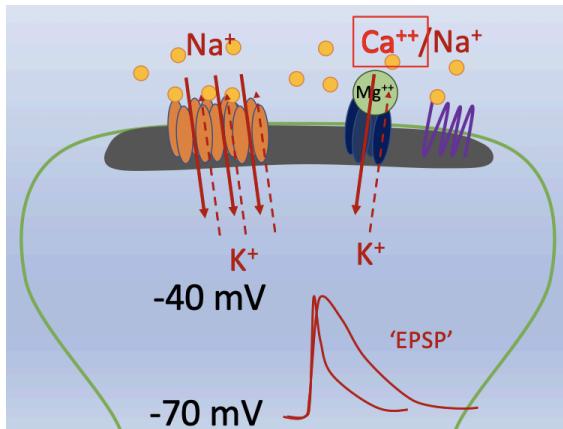
- ACh
  - Hydrolysed by extracellular enzymes
  - Block of AChE prolongs decay of synaptic potentials
- Glutamate
  - Taken up by family of sodium gradient powered transporters, either into neurones or glial cells
  - Glial cell route is thought to be quantitatively most important
  - Glutamate converted to glutamine in glial cells and sent back to glutamatergic neurones for reconversion to glutamate
- GABA

- Taken up by 3 transporters: GAT-1/2/3
  - GAT-1 = located mostly in axon terminals
  - GAT-3 = glial transporter
  - GAT-2 = less abundant and localised in neuronal and glial cells in some parts of the brain → typically distant from synaptic cleft
- Glycine
  - Taken up by GlyT1/2
    - GlyT1 = located in astrocyte
    - GlyT2 = located in presynaptic neuronal membrane

## Postsynaptic Transmission

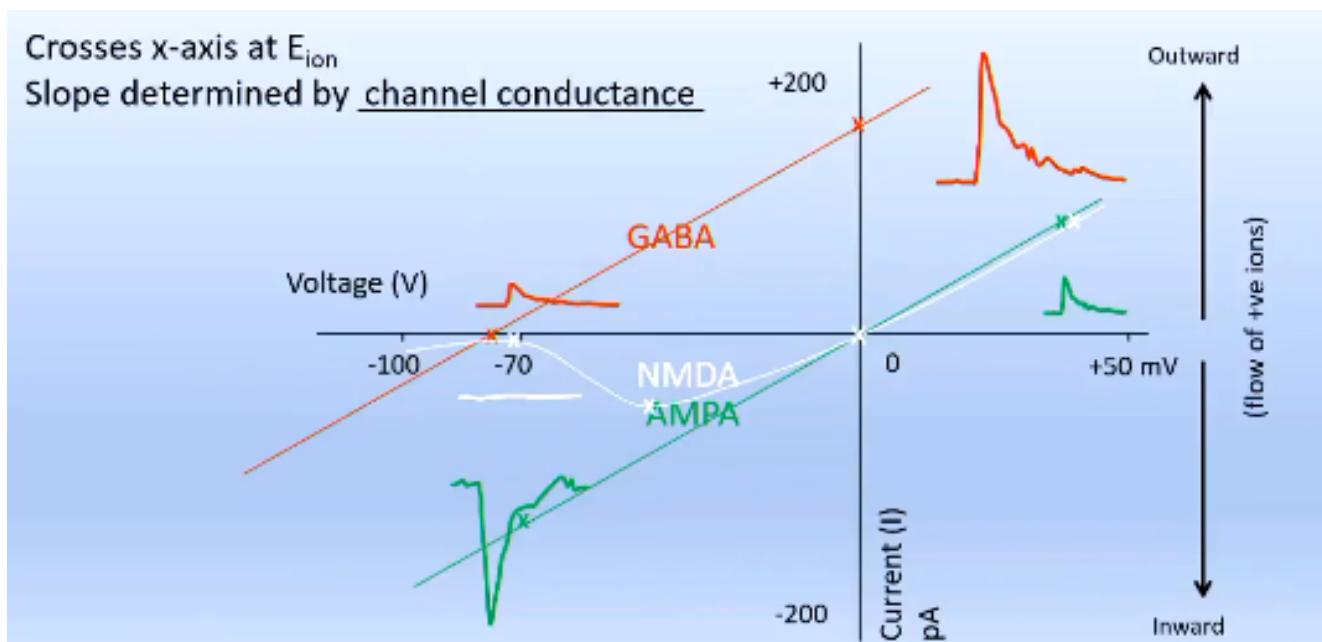


- AMPA receptors open to monovalent cations: sodium, which moves in, and potassium, which moves out (rarely calcium)
  - Depolarisation
  - Graded synaptic potentials (excitatory - EPSP)
  - Grading of EPSP → threshold reached = AP generation
- GABA open to anions: Cl<sup>-</sup> and sometimes bicarbonate
  - Hyperpolarisation
  - Graded synaptic potentials (inhibitory - IPSP)



1. Glutamate binds to AMPA and mGlu receptors
2. AMPA opens to allow inward flow of Na<sup>+</sup> and outward flow of K<sup>+</sup>
  - a. Net flow of inward current → EPSC (downward = inward current)
  - b. EPSC cause EPSP
3. NMDA also open but immediately blocked by magnesium
4. Membrane potential reach ~ -40mV
5. Magnesium propelled out of NMDA channels → Na<sup>+</sup> + Ca<sup>2+</sup> move in
  - a. EPSP for NMDA receptors is longer in duration
    - i. Rise time is slow and decay time is slower
    - ii. Coincidence detector: detect presynaptic activity (release of glutamate, and hence binding of glutamate to NMDA) and postsynaptic activity (depolarisation of postsynaptic membrane)

## I-V Curve



### AMPA

- 0mV = reversal potential for AMPA
  - No net flow of current (no net movement of sodium and potassium)
- Outward current = positive membrane voltage
  - $K^+$  close to its rev potential
- Inward current = negative membrane voltage
  - $Na^+$  closer to its rev potential

### GABA

- -85mV = reversal potential for GABA
  - No net flow of current
- Outward current = positive membrane voltage
  - Inward flow of negative = outward current
- Inward current = negative membrane voltage
  - Outward flow of negative = inward current
  - Hyperpolarised voltages make GABA excitatory → outward flow of negative charges (inside membrane becomes more positive / depolarised)
  - **GABA isn't simply an inhibitory neurotransmitter, it depends on the voltage of the membrane to determine whether GABA binding causes inward or outward flow of chloride**

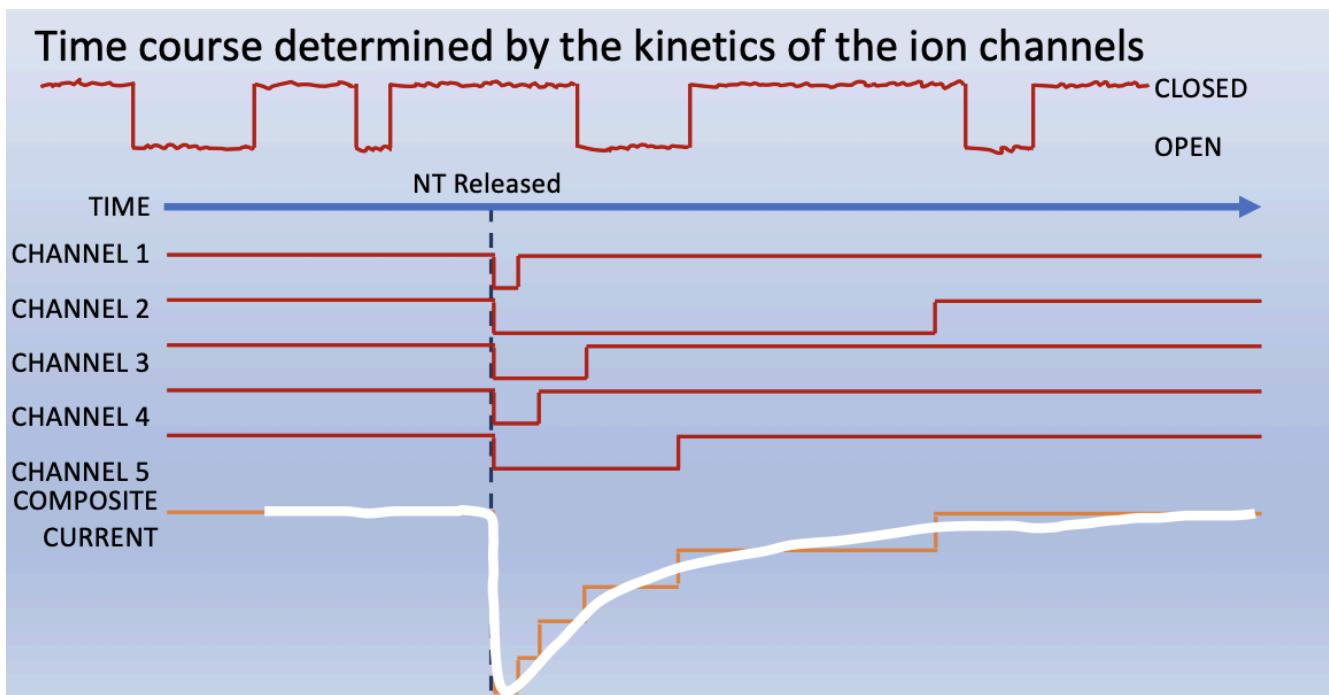
### NMDA

- 0mV = reversal potential for NMDA
  - Same as for AMPA as both allow for influx of sodium and potassium
- Unblocked similar to AMPA current
- No influx of ions at resting membrane potential (-70mV) which is because of  $Mg^{2+}$  block not allowing any flow of ions through the channel
- After -40mV the  $Mg^{2+}$  block is relieved
  - This allows the subsequent  $Na^+$  movement inwards
  - Starts to have inward current

- NMDA currents last longer than AMPA

Curves always cross x-axis at equilibrium potential (reversal potential)

Slope is determined by channel conductance (if individual channel allow for ions to pass through, the slope will be steeper)



### Fast initiation of current and slow decay

Individual AMPA channels can only have inward currents or none (usually open or close)

Multiple channels work together to produce synaptic current

- Closing of channel closes at different times but opens at the same time → hence the initial steep inward current (fast), and slow exponential decay of the current

### Synaptic Response

1 vesicle (quantum) of glutamate saturates the postsynaptic receptor

<b>Amplitude at single synapse (quantal amplitude):</b> <ul style="list-style-type: none"> <li>• Number of post synaptic channels per synapse</li> <li>• Conductance of postsynaptic channels</li> </ul>	<b>Overall amplitude:</b> <ul style="list-style-type: none"> <li>• Number of synapses (n)</li> <li>• % of release at each synapse (p)           <ul style="list-style-type: none"> <li>◦ AP does not always lead to release of NT</li> </ul> </li> <li>• Quantal amplitude (q)</li> </ul>
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<p>Amplitude at a single synapse = q</p> <p>(Different from NMJ, NMJ amplitude determined by pres-synaptic due to very large numbers of post-synaptic receptors)</p>	<p><math>q \times n \times p</math></p> <p>p and n are presynaptic factors q is postsynaptic factor</p> <p>Variation in response determined by number of synapses (n)</p> <p>1 synapse: No variation in successful responses 2 synapses: Variation</p> <ul style="list-style-type: none"> <li>• 2 successful response → summate</li> <li>• 1 successful, 1 unsuccessful</li> </ul> <p>N synapses: Huge variations</p> <p><b>Coefficient of variation (CV) =</b> Standard deviation of response / mean response, noise at synapse</p> <ul style="list-style-type: none"> <li>• <b>Number of synapses</b></li> </ul> <p>Central synapse excitatory neurotransmission has a low % of release → could have all failures (quite often)</p>
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## Synaptic Plasticity

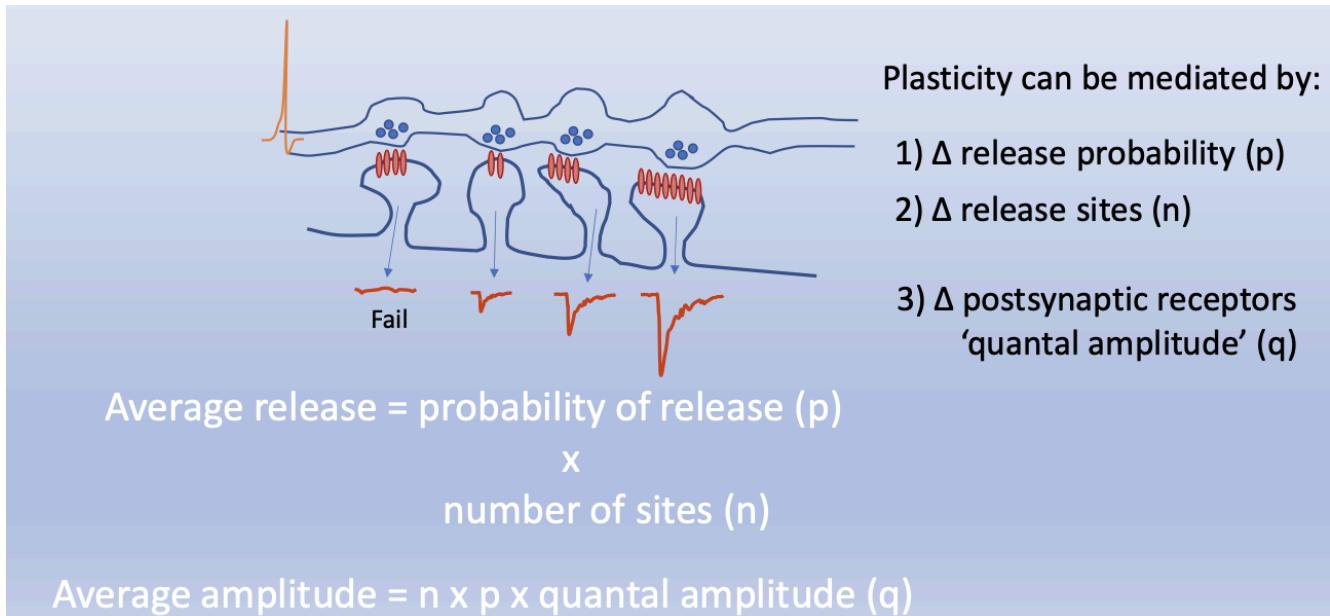
### Short Term Plasticity (STP)

Activity-dependent changes in synaptic efficacy (strength)  
Can increase and decrease

For a neuron:

Average release at the neuron = probability of release (p) x number sites (n)  
 Average amplitude of synaptic potential =  $n \times p \times q$  (quantal amplitude)

- Plasticity can be mediated by release of probability, release sites, number of sites

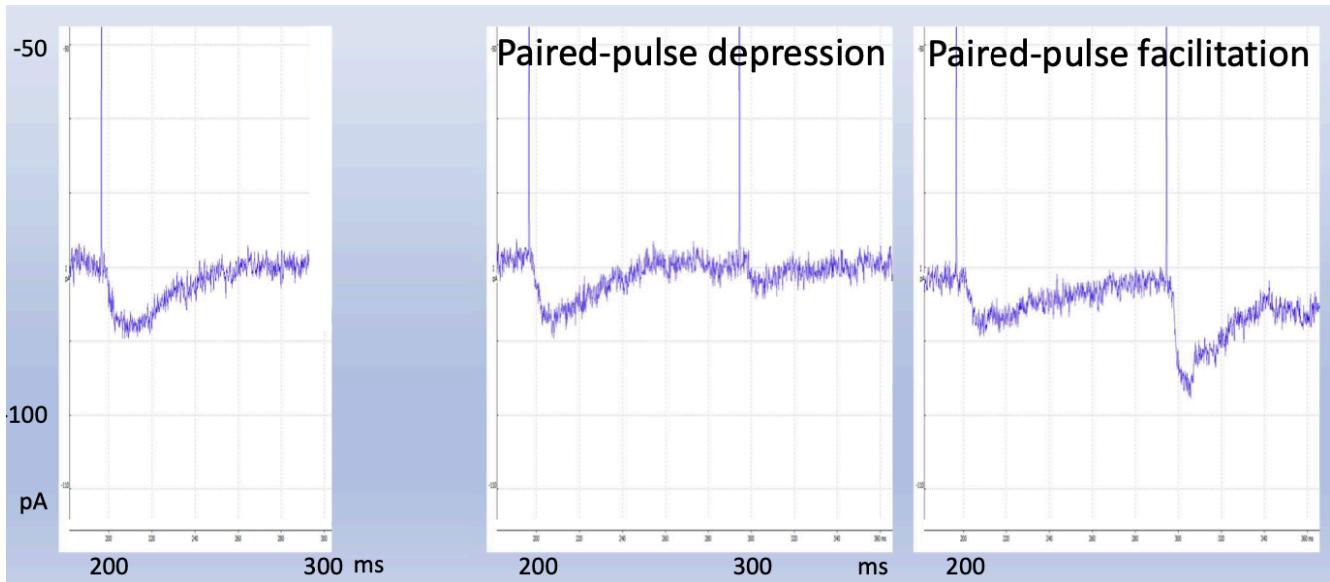


Very short-term plasticity: Paired pulse facilitation or depression (ms - sec)

Short-term plasticity: Post-tetanic potentiation or frequency dependent depression (minutes)

Long-term plasticity: Long term potentiation or depression (LTP/LTD) (hours - years)

## Paired-pulse Plasticity



Stimulate 2 times in quick succession (same synapse fired) → second response smaller than first (depression) or larger than first (facilitation)

Response different from summation, current returns fully to baseline before second response

Average amplitude of synaptic potential =  $n \times p \times q$   
⇒  $p$  changed

Facilitation caused by residual calcium from the presynaptic terminal → increase % release on second response

- First AP % release is low
  - Second AP % release will increase
1. Calcium influx and build up in presynaptic terminal
  2. Probability of release quite low for basal activity
  3. Not a lot NT release
    - a. Still has many readily release NT vesicles
  4. Small # synapses activated
  5. Second AP comes along
  6. Residual calcium + calcium influx
  7. % of NT release increase
  8. More NT release
  9. More synapses activated
  10. Larger current

Depression caused by depletion of readily release pool of NT vesicles → decrease % release on second response

- First AP % of release is high
- Second AP % of release will decrease

Typically only 1 vesicle is ready for release, the rest are in reserve pool

1. High probability of release
2. Calcium influx
3. High prob of release
4. Many release of NT
5. Many synapse activated
6. Second AP
7. Not many readily release vesicles left
  - a. Depletion of readily release pool
8. Less synapses activated than before
9. Smaller current

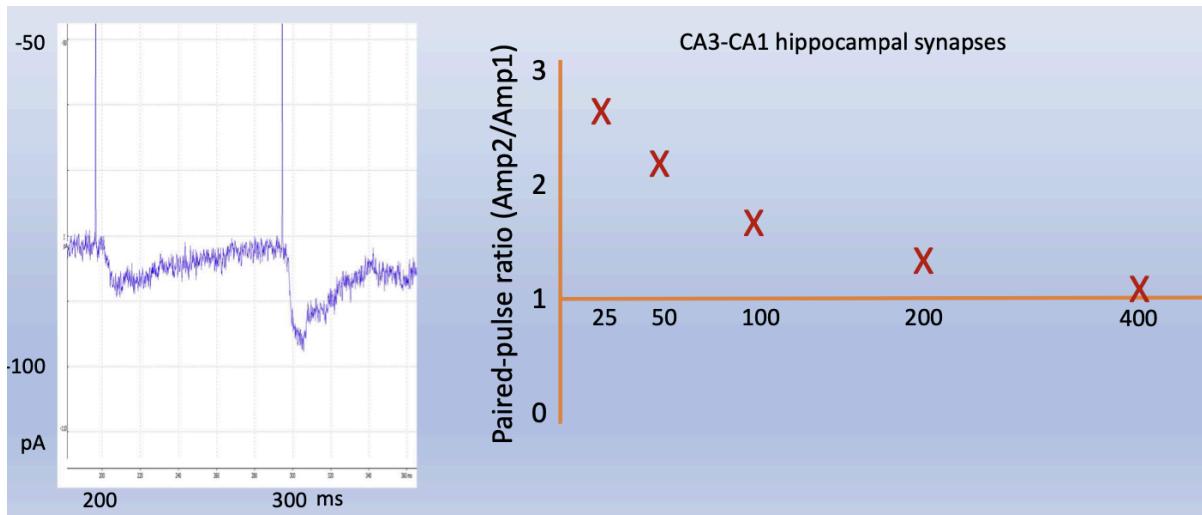
Alternative explanations for paired-pulse depression

1. Postsynaptic receptor desensitisation
2. Metabotropic receptors on presynaptic side
  - a. Homosynaptic
    - i. Glutamate released downregulate subsequent release
  - b. Heterosynaptic
    - i. For example: GABA released from interneurons downregulate release

## CA3-CA1 hippocampal synapse

### Time-dependent reaction

- As time drags on, decreased paired-pulse ratio due to residual  $\text{Ca}^{2+}$  cleared
  - Less likely to have facilitation
- Overall paired-pulse ratio is determined by balance between residual  $\text{Ca}^{2+}$  and depletion of readily-releasable pool of NT

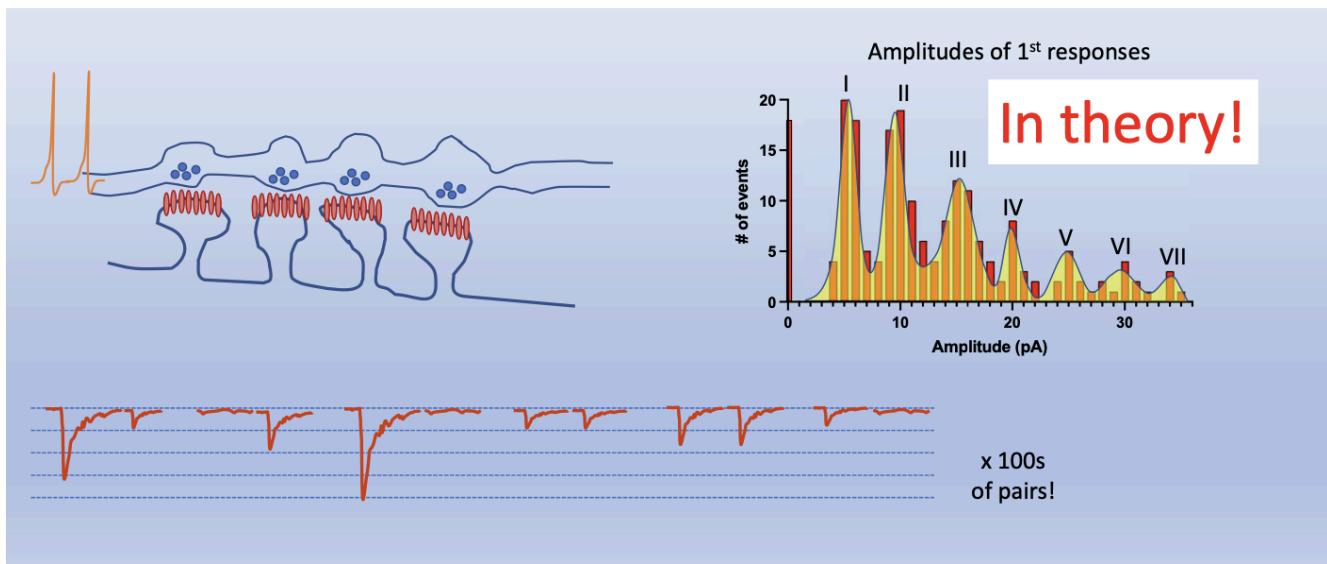


### Experimental evidence for paired-pulse facilitation:

1. Fluorescent calcium dyes in large terminals
  - a. Include calcium dye in preparation, intensity indicates concentrations of calcium
2. Calcium buffers
  - a. BAPTA → fast calcium buffer, inhibits both responses (fail to release NT)
    - i. Release of NT dependent on  $\text{Ca}^{2+}$
  - b. EGTA → slow calcium buffer, inhibits only second response (residual calcium)

## Quantal analysis

Measure sizes of single responses



Synapses have same number of channels

1. Paired-pulse stimulation applied to axon terminal
2. Measure responses of synapse for the 2 stimulations
3. Do it many times
4. Get amplitude frequency distribution
5. Plot how many times you see an event/response at each amplitude
  - a. And number of times u see failures

Position of peaks determined by: q

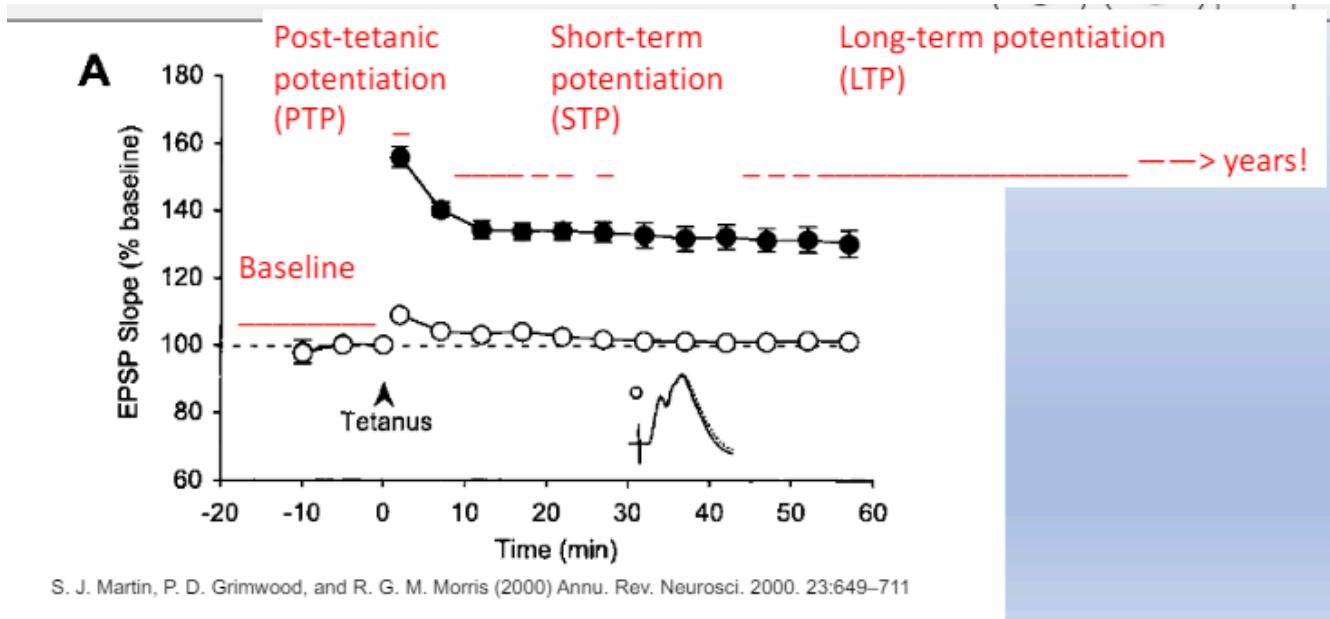
Relative height (number of peaks and failures) determined by: p and n

- n and p (presynaptic)
  - Decrease in p (% of release) heighten peaks at smaller amplitudes while shorten peaks at higher amplitudes because lower % release = less likely to generate current = lower amplitude of EPSP generated
    - Also increase peak at 0 amplitude because more failures of release, so less likely to have EPSP
  - Decrease in n (number of synapses) heightens peaks at smaller amplitudes because less AMPA receptors = less currents generated = lower amplitude of EPSP
    - Increase peak at 0 because less likely to have EPSP
- q (postsynaptic)
  - Decreased q (quantal amplitude) shifts every peak to the left because all responses will have smaller amplitude due to smaller amplitude for all synapses of the neuron

## Long Term Plasticity

- **Activity-dependent, long-lasting increase in synaptic efficacy (hours to years)**
- Attractive model for learning and memory

- Requires synchronous activity
- First experiment done on hippocampus → Hippocampus only have dendrites on 1 side and axon terminals on other side



- Only active pathway show potentiation
- Apply tetanus (high freq, 100Hz, burst of activity)
  - PTP
  - STP ~20 minutes
  - LTP
    - Activity remains higher than baseline

## LTP

Properties:

- Activity dependent
- Stable and long-lasting
- Input specificity: only active synapses undergo synaptic plasticity
  - Stimulating electrode in extracellular space
  - Axons surrounding electrode fire, LTP induced only in active pathways
- Cooperativity/associativity: overcoming induction threshold
  - Cooperativity
    - Weak synapses cooperate to overcome threshold for releasing  $Mg^{2+}$  block
  - Associativity
    - Weak synapses associate with strong synapse to overcome threshold for releasing  $Mg^{2+}$  block

**Input specificity, cooperativity/associativity all encoded by NMDA**

Basal transmission:

1. Bind to AMPA and NMDA
2. Sodium through AMPA receptor, none through NMDA
  - a. AMPA underlie basal activity

Induction of LTP/LTD on postsynaptic side:

1. During tetanus → repeated activation of AMPA receptors
2. Depolarisation
3.  $Mg^{2+}$  is released from NMDA channel
4.  $Na^+ + Ca^{2+}$  influx through NMDA channel
5.  $Ca^{2+}$  activate calcium calmodulin
6. Calmodulin activate CaMKII
7. CaMKII phosphorylates other CaMKII
  - a. Autophosphorylation → long lasting effects, carry on after stimulation
8. CaMKII phosphorylate AMPA receptors (LTP)
  - a. Improve conductance
  - b. More AMPA inserted into postsynaptic density from periphery
9. CaMKII phosphorylate GluR1 (LTP)
  - a. Increases its conductance
  - b. Alters GluR1 mobilisation, more inserted into postsynaptic density

CaMKII important for LTP, elimination of CaMKII = elimination of LTP

- CaMKII mutant mice have impaired LTP
- Affects long-term memory (water maze, learn visible platform, don't know where platform is anymore when you hide it)

## LTD

- Prolonged low-frequency stimulation induces depression (~5Hz)
- Can be induced by correctly timing the activation of presynaptic axons and postsynaptic neurons (aka STDP)
- Mechanism
  - LTD requires modest increase in  $Ca^{2+}$  (Malenka RC 1994)
    - Modest increase optimal for LTD
    - Large increase optimal for LTP (large depolarisation ⇒ more NMDAR activated  
⇒ good for LTP)
  - Activation of  $Ca^{2+}$  dependent protein phosphatase cascade which involves a protein named protein phosphatase 1 (PP1)
    - Loading CA1 pyramidal cells with PP1 enhances LTD (Morishita et al., 2001)
  - Dephosphorylation of postsynaptic PKA substrates
  - AMPA receptors are removed from membrane (endocytosis back into neuron)

## Unsilencing

Silent synapses = no release

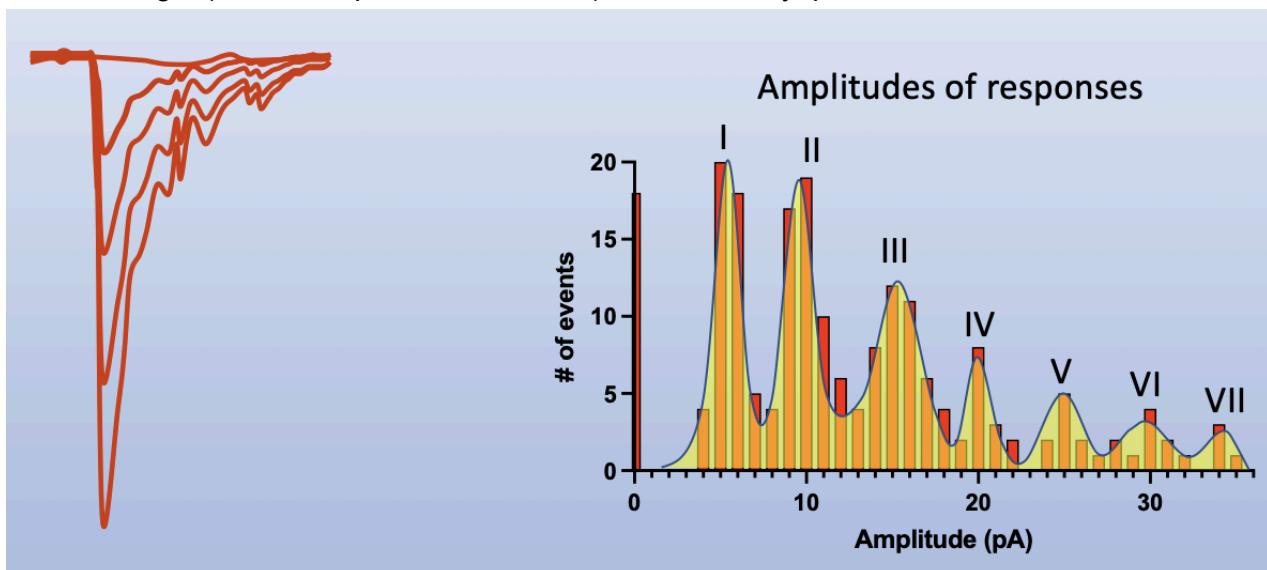
Postsynaptic silent synapse: No AMPAR only NMDAR, thus at basal level of activity, only slow NMDA (not enough depolarisation to cause fast NMDA response)

Unsilencing → Inserted AMPA receptors → each response increase (the graph will shift to the right similar to increased q)

## Quantal Analysis and Unsilencing

Position of peaks determined by: q

Relative height (number of peaks and failures) determined by: p and n



I - amplitude when 1 synapse activated

II - amplitude when 2 synapses activated

III - 3 synapses activated

etc...

Increase q : Shift peaks to the right (peaks have larger amplitudes due to larger single synapse amplitudes)

Increase p: Increase peaks at right end, more likely to get release from multiple sites than 1 site, hence increase in peaks for the greater amplitudes (which indicate that more than 1 synapse are activated)

Increase n: Increase peaks at right end

Retrograde messengers can act as signals from post-synaptic to presynaptic to influence % of release

## Late Phase LTP

Late LTP → protein synthesis

Synaptic tagging important = highlights that the synapse has undergone LTP and highlights for late LTP

# Neuromodulation

Neurotransmission vs Neuromodulation

- Neurotransmission
  - Precise; Point to point
  - Direct gating of ion channels
  - Direct postsynaptic effects lasting tens of ms
  - No secondary effects
  - Postsynaptic electrical effects are fast and strong
- Neuromodulation
  - Diffuse transmission
  - Effects mediated by second messengers
  - Postsynaptic effects lasting several hundreds of ms to hours
    - **Typically involve a G-protein**
  - Postsynaptic electrical effects are slow and weak

Transmitters responsible for neuromodulation:

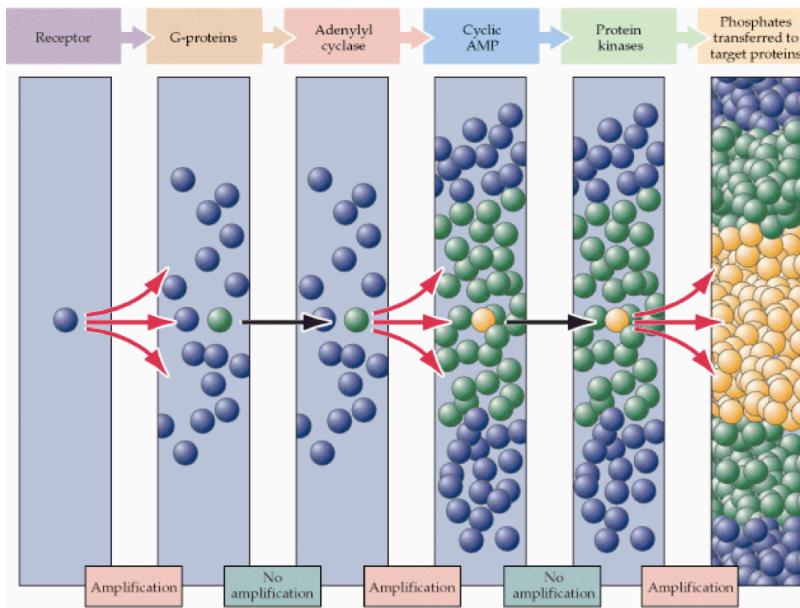
- Monoamine: Effect over large area
  - Noradrenaline → maybe responsible for global functional state change
  - Dopamine → wide range of function (e.g. executive functions, reward)
  - Serotonin → maybe responsible for global functional state change (arousal e.g. switch from sleep to arousal)
  - Histamine
- Acetylcholine
- Glutamate
- GABA
- ATP
- Neuropeptides
- Opioids
- Cannabinoids

Neuromodulation speed depends on receptor mediating action of neurotransmitter

- Ionotropic = fast
- Metabotropic = slow
  - Receptor and G-protein are two different things for a GPCR
  - G protein alpha subunit types

- Gs
  - Activate adenylyl cyclase
  - Increase cAMP levels
  - Activate protein kinase A (PKA)
  - Increase in protein phosphorylation
- Gq
  - Activate phospholipase C
  - Increase in diacylglycerol (DAG) and IP<sub>3</sub> concentration
  - DAG level increase activate protein kinase C
  - IP<sub>3</sub> level increase lead to Ca<sup>2+</sup> release
  - Increase in protein phosphorylation and activate calcium binding proteins
- Gi
  - Inhibit adenylyl cyclase
  - Decrease in cAMP levels
  - Inhibit protein kinase A
  - Decrease protein phosphorylation
- Different types of G-protein types formed by different alpha, beta and gamma subunits
  - Beta-gamma subunit can also interact with final protein
- Most neurons express more than 1 G-alpha subunit type

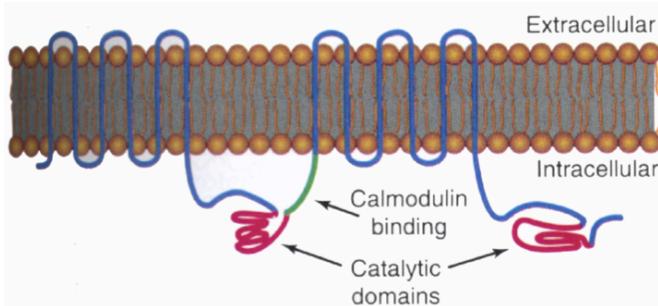
### GPCR cascade = good for signal amplification



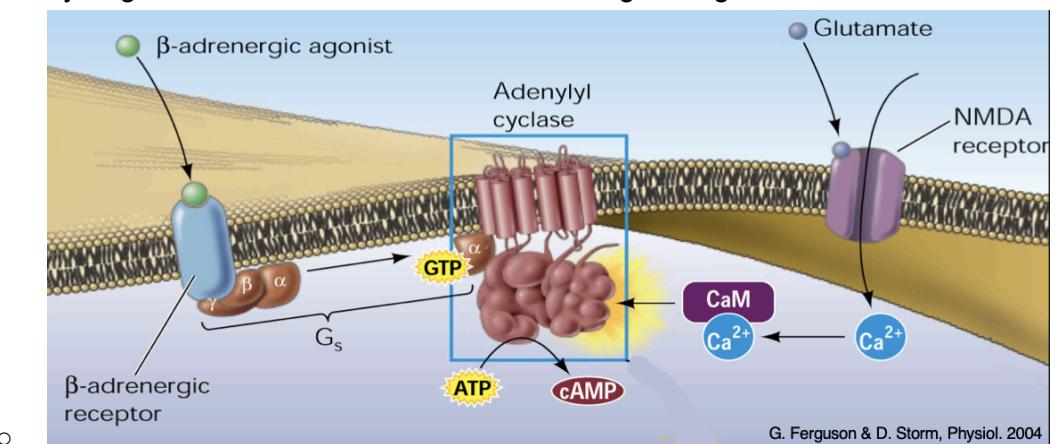
Convergence or divergence of signals mediated by G-protein

- 1 receptor may activate several G-proteins / 1 receptor activate 1 G protein which activates multiple effectors → divergence
- Many receptors may activate 1 G-protein / Many receptors activate different G-proteins but all activate 1 effector → convergence

## Adenylyl cyclase structure



- Adenylyl cyclase can be co-stimulated or inhibited by other signals
  - AC 1 can be stimulated by calcium level increase as it **could be stimulated by  $\text{Ca}^{2+}$ -calmodulin**
- Therefore, these complex signal transduction cascades are useful in signal integration
  - Coincidence detectors
    1. Effectors in neurons receive signals from 2 stimuli from 2 activated metabotropic receptors within reasonable time window
    2. Enzyme will sum up and produce effect that is a result of combined action of both pathways inside cells.
      - Synergistic effect, increased effect, more long-lasting.



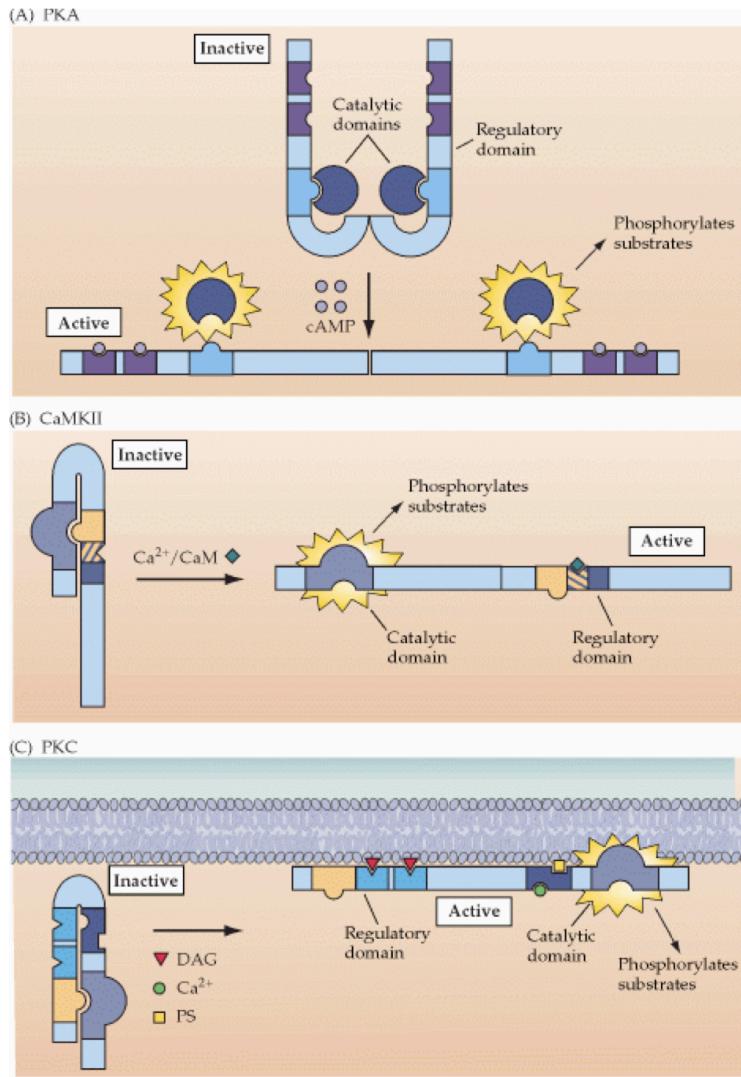
## Second messengers

- Small diffusible molecules that are produced in response to receptor activation
- Bind and activate effector molecules such as protein kinases, ion channels and a variety of other proteins, thus continuing the signalling cascade
- Some are stored in special organelles and released when needed (e.g.  $\text{Ca}^{2+}$ )
- Production and release can be localised to limit space and time of signal activity
- Hydrophobic molecules: Diffuse from plasma membrane into intermembrane space to reach and regulate membrane associated effectors

- DAG
  - PIP2
- Hydrophilic molecules: located in cytosol
  - cAMP
  - cGMP
  - IP3
  - $\text{Ca}^{2+}$
- Gases: Can diffuse both through cytosol and across cellular membranes
  - NO
  - CO

## Protein kinases

- Specialised domains with specific functions
- Each of the kinases has homologous catalytic domains responsible for transferring phosphate groups to substrate proteins
  - Catalytic domains inactive due to presence of autoinhibitory domains that occupy catalytic site
  - Binding of II messengers to appropriate regulatory domain of kinase removes autoinhibitory domain → catalytic domain activated
- Some kinases (e.g. PKC and CaMKII) the autoinhibition and catalytic domains are part of the same molecule, others (e.g. PKA) the autoinhibitory and catalytic domains are separate subunits



### Loewi's experiment:

Connected 2 chambers with hearts inside, fluid between 2 chambers can be transferred

- 1 heart had vagus nerve attached to it
- Another heart had none

Heart 1 contraction force and rate decrease when the vagus nerve was stimulated

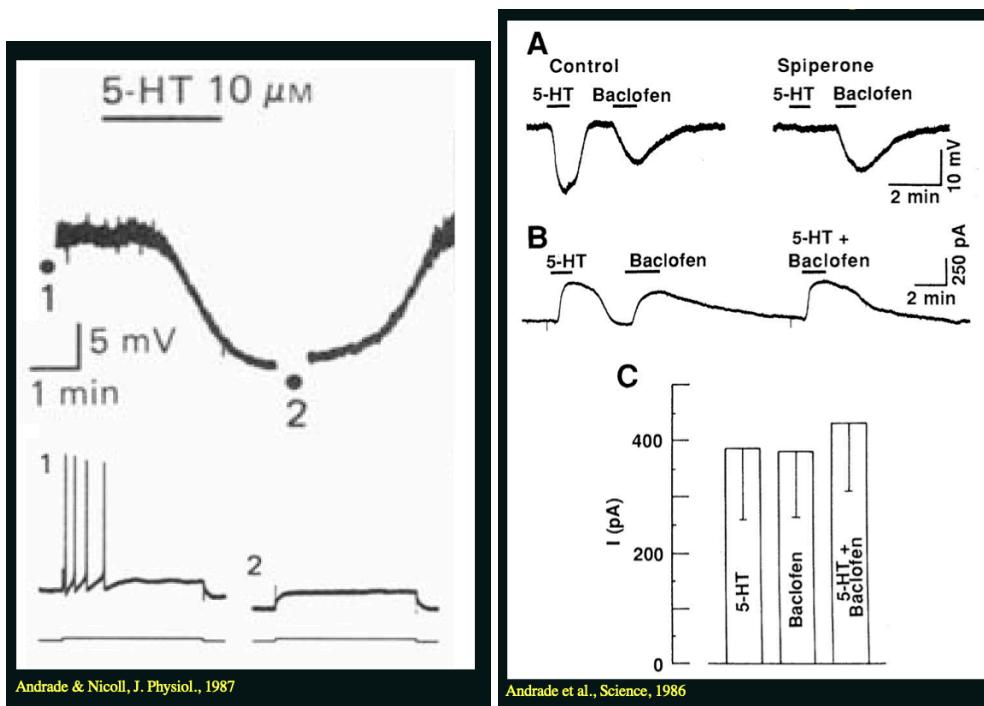
Heart 2 contraction force and rate also decreased → inhibitory effects of vagus transferred, must be some substance that could be transferred

1. Stimulation of the vagal nerve
2. Release of ACh
3. ACh binds to muscarinic AChR
4. Activation of G-proteins
5. Beta-gamma subunits binds to  $\text{K}^+$  channels (GIRK)

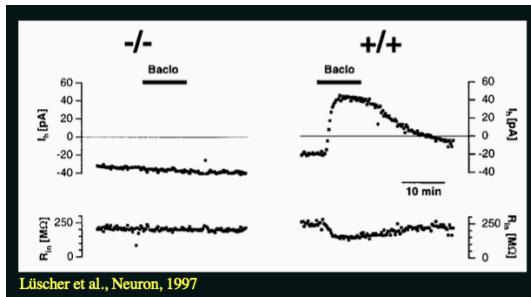
- a. GIRK: G-protein inward rectifying potassium channels
- 6. Membrane potential goes negative as it approaches equilibrium potential for potassium ( $K^+$  flows out)
  - a. **Membrane hyperpolarisation**
- 7. Move away from threshold potential for AP generation
- 8. Heart beat rate slows down
  - a. Action is relatively fast (30-100msec) and localised

## Postsynaptic Modulation

- Serotonin
  - o Membrane resting potential hyperpolarises when 5-HT binds to 5-HT<sub>1A</sub> receptors
  - o Beta-gamma subunit bind to GIRK and leads to increase membrane permeability to potassium
  - o Reduce cell excitability
- GABA receptors exerts same effect as serotonin and shares same target
  - o Baclofen = GABA-B (metabotropic) agonist
- GABA + 5-HT = convergence of effect



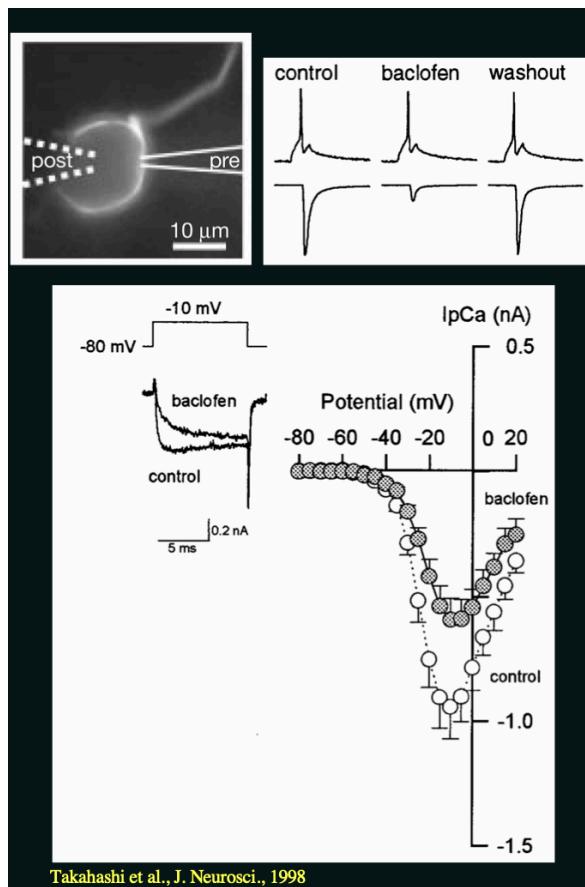
Mice lacking GIRK2 gene do not display  $K^+$  current and a change in membrane resistance in response to GABA-B agonists



## Presynaptic modulation

### GABA

- Presynaptic terminal with GABA-B receptors and GABAergic neurons form axo-axonal synapse
- Release of GABA inhibits presynaptic calcium channels directly which is mediated by G-protein beta-gamma subunits

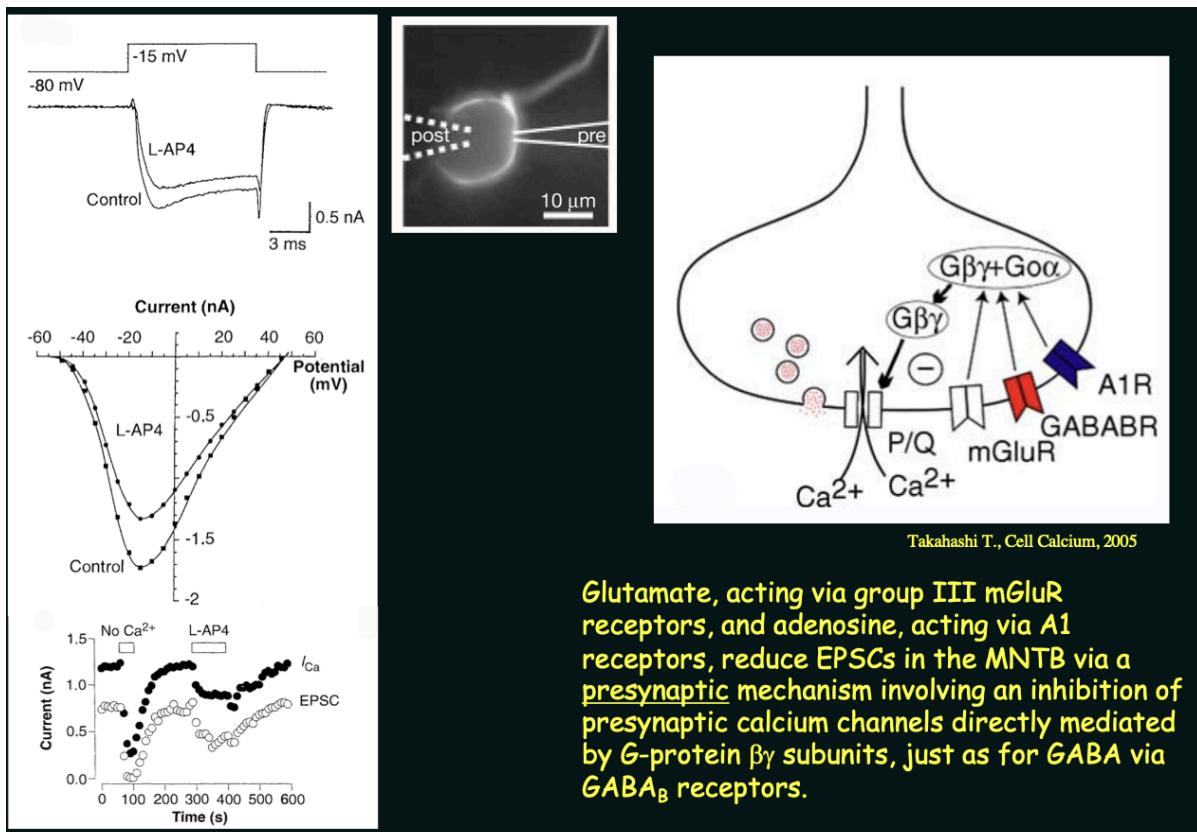


- Top: Presynaptic current is detected, but no postsynaptic current is detected in calyx of held (squid axons) injected with baclofen
- Bottom: Calcium current is smaller when cell is injected with baclofen (GABA-B agonist)

- Less release of NT
- EPSCs reduced in postsynaptic membrane

## Glutamate

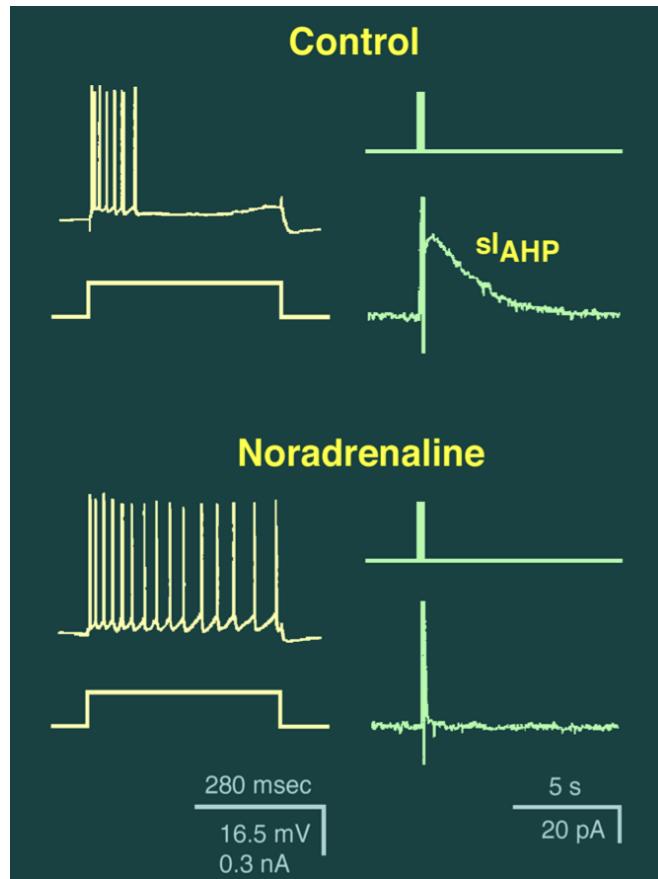
- Autoinhibition: glutamate released from the presynaptic terminal
- Glutamate act via group III mGluR receptors and adenosine act via A1 receptors
- Inhibit presynaptic voltage-gated  $\text{Ca}^{2+}$  channels directly which is mediated by G-protein beta-gamma subunit binding to channel
- Less release of NT
- EPSCs reduced in postsynaptic membrane



## Modulation of signal encoding

- Serotonin and noradrenaline make neurons more excitable
- Modulation of spike frequency adaptation and of a  $\text{Ca}^{2+}$ -activated  $\text{K}^+$  current by NA in hippocampal pyramidal neurons
  - Normal neuron → has adaptation
    - Extended excitation in postsynaptic terminal activated  $\text{Ca}^{2+}$  channels
    - Increase in  $\text{Ca}^{2+}$  concentration
    - Activate  $\text{Ca}^{2+}$ -dependent  $\text{K}^+$  channels

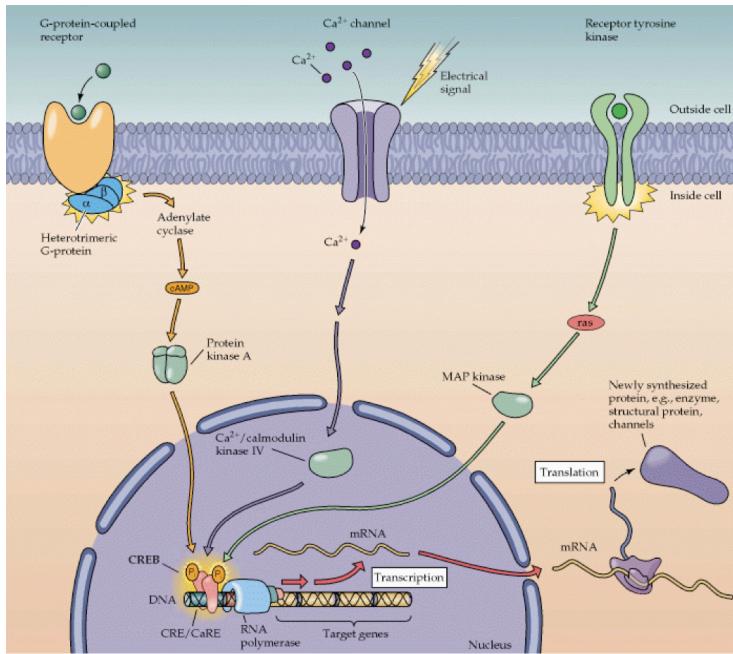
- Increase in  $K^+$  levels → hyperpolarisation → voltage of membrane shifted away from threshold needed to AP firing and adaptation
- Inject noradrenaline → adaptation inhibited
  - NA abolish  $K^+$  currents
    - NA bind to receptor
    - NA activate Gs alpha subunit
    - Gs increase cAMP → PKA activated
    - PKA phosphorylate for calcium-activated potassium channel
    - Lower open probability
    - Less  $K^+$  current
  - Neuron stays depolarised for the whole fo the voltage step



- NA may underlie attention → enhanced state of excitability and reduced adaption

Neuromodulation may have long-term effects due gene expression changes → remodelling of neurons

- Long term LTP



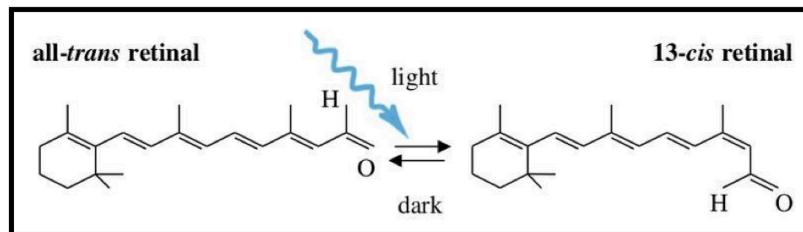
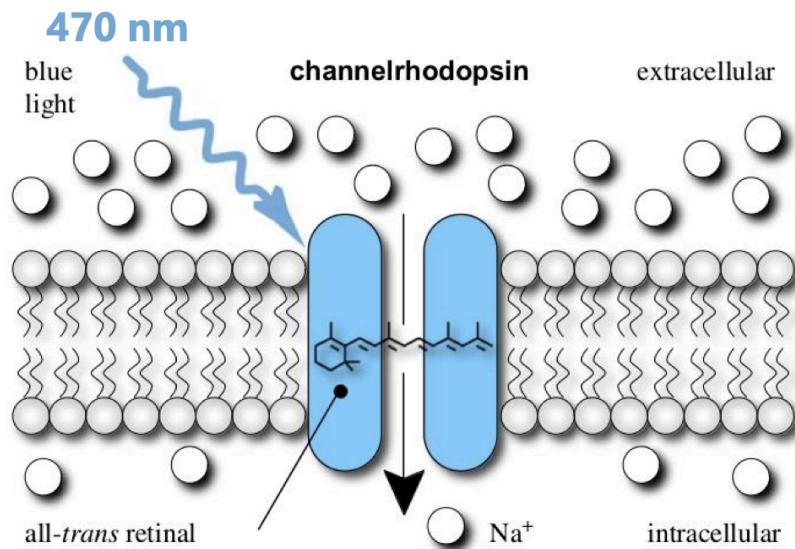
# Optogenetics

Optogenetics is the delivery of light to neurons expressing light-sensitive ion channels/pumps. It targets specific neurons with genes coding for light-sensitive ion channels

Alters cell function

## Development of optogenetics

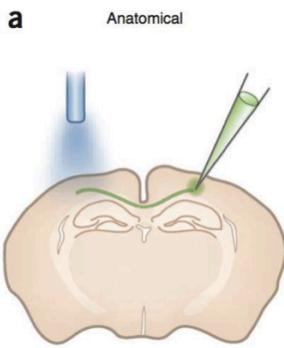
- Channel rhodopsins function as “visual” proteins direction the algae towards or away from light
  - These algae rely on light to move around → phototaxis
- Channel rhodopsins are a subfamily of **retinylidene** proteins that function as light-gated ion channels
- Light absorption triggers a subsequent conformational change of the protein and gating of the channel



- Open of channel allows influx of sodium and some calcium (cation influx) → more positive membrane potential
- Optogenetic control in vitro
  - Neurons grown in culture were transduced with ChR2
  - YFP (yellow fluorescent protein) was expressed under same promoter as ChR2
    - Effectively tagged neurons expressing ChR2 → to monitor expression
  - Activation of ChR2 by blue light induced a depolarising current in these cells
    - Short pulses of blue light were sufficient to induce AP
- Mice experiment, optogenetic control ex vivo (functional organ removed from animal)
  - A viral construct containing ChR2 was infused into hippocampi of adult mice
  - ChR2 was under control of EF-1 alpha promoter
  - ChR2 was expressed in adult mouse hippocampus
  - Blue light could drive spiking in hippocampal neurons in slice
    - Can time spiking
- Mice experiment, optogenetic control in vivo
  - Transgenic mouse line was generated to express ChR2 in cortical neurons
  - Hole in skull and fibre optic cable inserted
  - Reliably caused spiking in living animal
- Different optogenetic actuators
  - ChR2
    - Excite cells in response to light
  - NpHR
    - Inhibit target cells in response to light by pumping  $\text{Cl}^-$

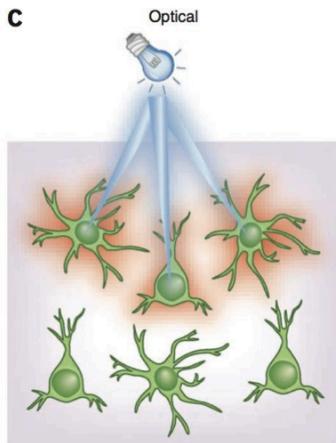
- Arch
  - Inhibit target cells in response to light
  - Arch lets  $H^+$  out, hyperpolarization
- OptoXR → range of function depending on G-protein coupled to it

## Precision



Physical delivery of virus  
to a given anatomical  
location; uncovers  
**circuit connectivity**  
**patterns** by making use  
of axonal projections.

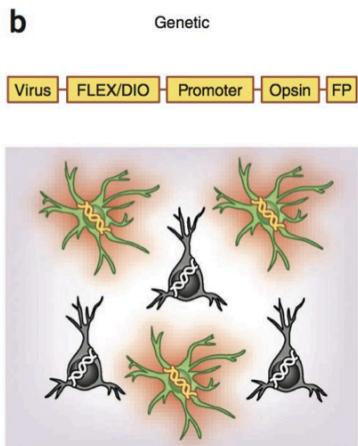
Delivering light to target location which the cells project to  
Uncover which cells in the given anatomical location projects to the other area that you shine light on



Directing the illumination source to a given set of cells or even individual neurones; useful when the targets of interest are **separated in space**.

High spatial precision with optic genetics through laser targeting

- Broad light activation
- Focal activation with 2-P

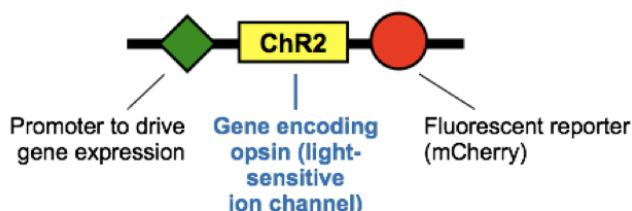


Cell types can be addressed if the cell type of interest has a **known genetic identity**.

- Express ChR2 only in cells with a certain gene (e.g. cre/lox method)

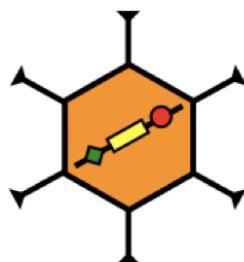
## Delivering Genetic Construct

### 1. Piece together genetic construct (Transgene)



**On its own, AAV is biologically inert (no chance of causing infection or disease) and non-replicating**

### 2. Package construct into virus



**Adeno-associated virus (AAV)** is a small virus which can be used as a **vector** to deliver target genes into mammals

**It takes at least two weeks for ChR2 to be expressed fully in transduced neurons**

### 3. Inject virus into brain structure

**AAV** will be infused bilaterally into the **entorhinal cortex**

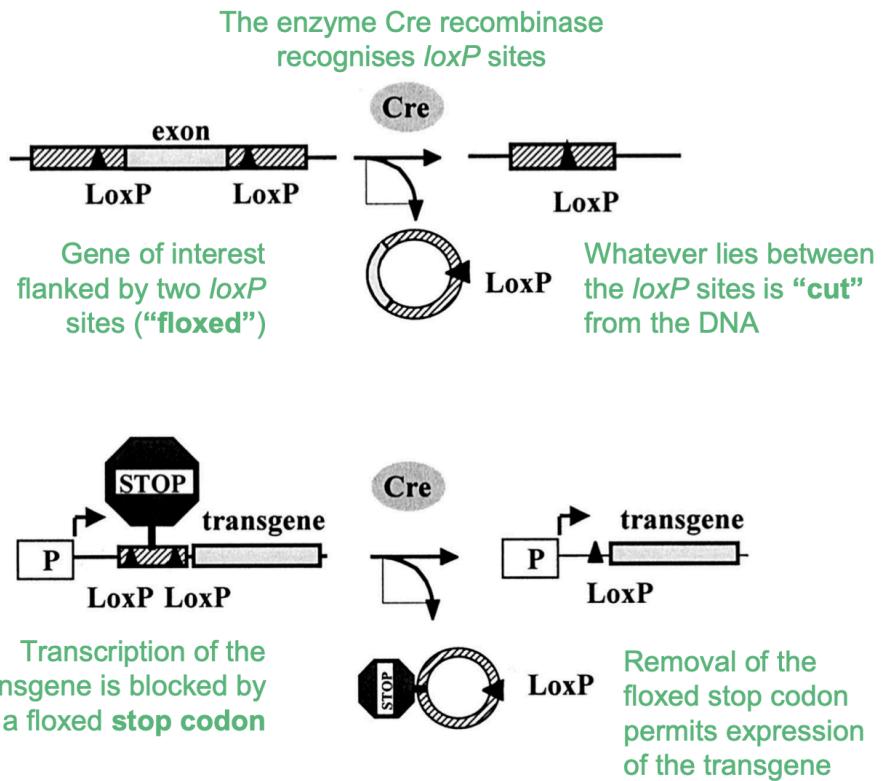


Cell type specific promoter → target a particular cell

Transduction is the process by which foreign DNA is introduced into another cell via viral transducer

## Cre/Lox Recombination Method

- Cre gene encodes enzyme Cre recombinase
  - Cre protein recombines DNA when it locates specific sites in DNA molecules, the loxP sites (cleaves loxP sequences)
  - Knock-out NMDAR in hippocampus
- When cells that have loxP sites in their genome also express Cre, the enzyme catalyses a recombination event between the loxP sites
- Promoter followed by STOP codon flanked by 2 loxP sites, which is then followed by ChR2 gene (floxed-stop ChR2)
- When virus containing the transgene encoding ChR2 transduces a cell expressing cre, the loxP sites + the STOP codon they flank are cut out, gene expressed
  - Only get ChR2 in cells expressing cre enzyme
  - Virus is basically non-specific
- Cell specificity achieved



- Deliver ChR2 to target site, deliver cre gene to projection / source site, this way only the pathway you want to target will express ChR2

Targeting specific compartments of cell:

- Short peptides encoding intracellular retention signal, targeting or anchor signals can be used to target optogenetic actuators to specific subcellular compartments (localise expression to a specific subcellular compartment)
  - Myosin-binding domain of melanophilin or the C-terminus of neuroligin-1 → ChR2 in soma and dendrites
  - C terminus of Kv2.1 subunit restricts expression to soma and proximal dendrites
  - Ankyrin G-binding domain of intracellular loop II-III of voltage-gated sodium channels → localise ChR2 to axon initial segment
- Want to be specific in targeting single cell
  - For example: localise ChR2 to cell body → Selectively activate single cell

## Application

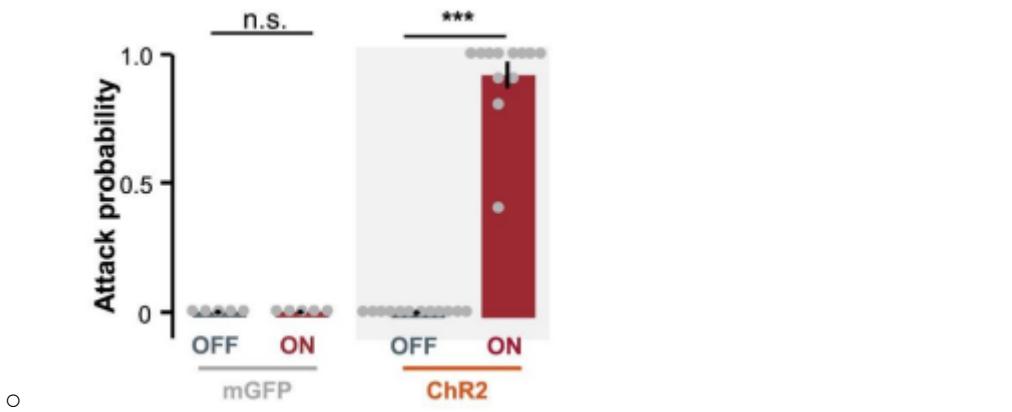
Optogenetics permits the activation or inhibition of specific sub-populations of neurons using light, and does so without affecting surrounding neurons or fibers

Used to study causal relationships between activity of targeted brain pathways and behaviour they may regulate

## Hypothalamus

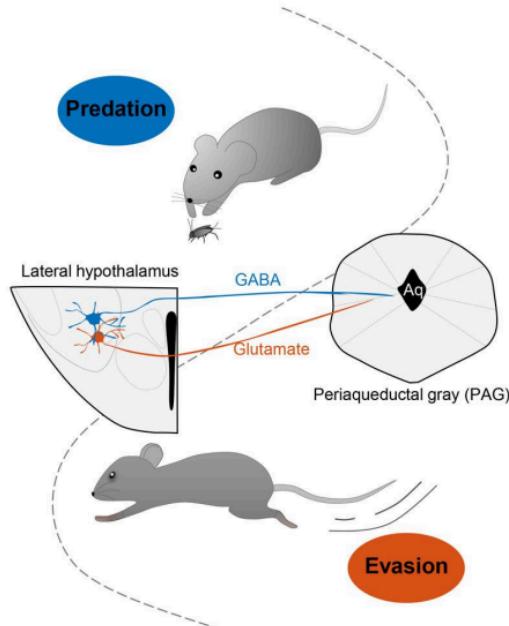
Hypothalamic circuit is important for predation and evasion

- PAG can elicit biting and attack responses in mice (electrical stimulation of LH and PAG shown to elicit biting attack responses)
- ChR2 inserted into lateral hypothalamus-PAG pathway and effects observed on predation assay
  - Inject RetroAAV which affects synaptic terminals
  - Gene product is transported back up the axon into cell body
  - Label all cells projecting to PAG with cre
  - Insert ChR2 gene floxed by loxP sites into cell
  - ChR2 only expressed in cells with cre (aka those projecting to PAG)
- Experiment: Put mice with crickets
  - Projection activated → mice attacks crickets
  - Aggressive attack behaviour triggered when light switched on



- - Shining light on mGFP → control
  - Demonstrate that light itself has no effect on animal behaviour
- Slice PAG, record from neurons in PAG, but stimulate lateral hypothalamus
  - Mixed GABA and glutamate projections from LH to PAG
- Experiment: GABAergic pathway sufficient to trigger predation
  - V-gat (particular GABA transporter) cre mouse → all GABAergic neurons will express cre
  - Couple with viral injection of ChR2
  - Just GABAergic express ChR2
  - Light stimulation of GABAergic neurons → trigger attack behaviour
- Experiment to show circuit is necessary for predation
  - V-gat cre animal
  - Virus with GtACR1 delivered into lateral hypothalamus
    - GtACR1 = Light gated chloride channel (inhibitory)
  - Light stimulates channel → inhibition of GABAergic neuron → loss of hunting behaviour

- Experiment demonstrating glutamatergic neurons important for evasion
  - V-glut2 cre mouse
  - Virus with GtACR1 delivered into lateral hypothalamus
  - Blue light activates channel → inhibition of glutamatergic neurons → no preemption of evasion behaviour (only run away when robot hits the mouse)
- Experiment with glutamatergic neurons #2
  - V-glut2 cre mouse
  - Deliver ChR2 into glutamatergic LH neurons
  - Activate neuron → trigger escape behaviour
  - Activate neuron → mice in process of hunting switches to evasion behaviour

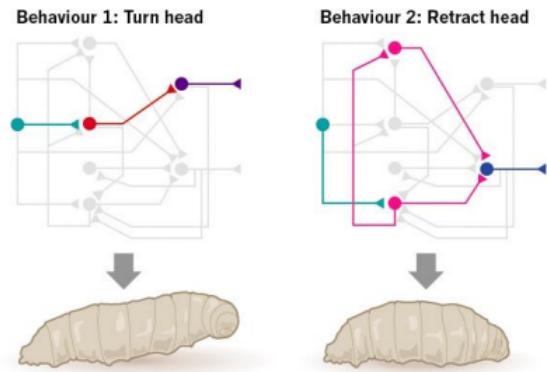


- Limitation
  - Studies are not specific in the population of neurons they activate
  - Other pathways from LH may be important not the LH-PAG specifically
    - Only first experiment targeted LH-PAG specifically

## Functional Connectome

Optogenetics used to activate single neurons and in combination with other techniques (e.g serial electrophysiology) can be used to create functional map of brain

- 1 neurons may have several pathways



Smith 2017

- Activating single neuron may not lead to single response
- Serial EM to assess connectivity of neurons → reconstruct connectome (complete map of neuron connections)
- Chettih and Harvey experiment
  - Express ChR2 in cell bodies of single pyramidal neurons in V1 (expression is sparse)
  - Express calcium indicator (fluoresce in presence of calcium)
    - Useful because when neuron activates, calcium influx
  - Mice watch video
  - Periodically stimulating 1 neuron of interest, look at how stimulated neuron impact function of other neurons
    - Neuron surround stimulated neuron = excited
    - Further away = inhibition (may be due to inhibitory interneuron)
  - Effect also dependent on functional properties of neuron
    - Neurons with same response properties to stimulated neuron = excited
      - Clarify signal
    - Neurons which are quite similar = inhibited more strongly
      - Sensory discrimination → eliminate nearby possibilities to reduce confusion, precision

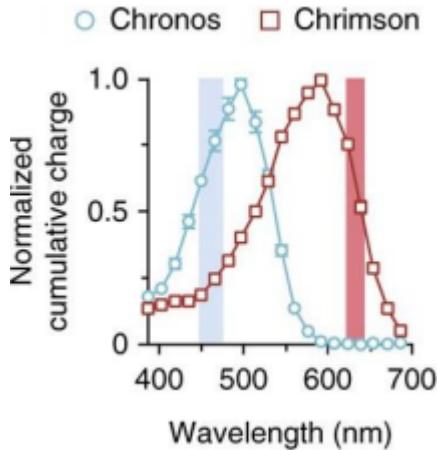
## Limitations and Modifications

### Limitations

- Insertion of fibre optical cables damage brain (limit therapeutic potential)
- Light cause heating → affect brain function
- Channels open stochastically, new conductance may affect functional properties of cell
- Depolarisation caused by alien channels may affect integration and plasticity
  - Pattern of release not naturalistic

### Modifications

- Optogenetic actuators are being developed with modified kinetics and/or activation wavelength



- ○ Can activate both at same time as chronos and chrimson (2 types of optogenetic actuators) have different sensitivities to different wavelengths
- ○ Target 2 population of neurons at the same time → high degree of control
- Far-red shifted opsins may negate need for fibre optic cables
  - Light in far-red is less defracted by tissues → penetrate more easily
    - ChRmine opens in response to far-red wavelengths
  - ChRmine expressing parvalbumin neurons
  - Light travels through skull and brain
  - Epileptic mouse
  - AMplifier detects seizure activity
  - 50% of red light activated during epilepsy
  - Trials with red light activated → seizure duration shorter
    - Terminate / shorten seizures
- Vision restoration
  - Retinitis pigmentosa → loss of photoreceptors = complete blindness
  - Virus expressing ChrimsonR injected into remaining retinal circuitries, express ChrimsonR in ganglion cells
  - Goggles scan environment and recode light into appropriate wavelength and intensity and yield partial restoration (e.g. detect object, grasp object)
- New actuators
  - Opto-XR
    - Modify intracellular domain of rhodopsin and replace it with other components to trigger different pathways (e.g. G-protein)
    - Opto-XR → Rhodopsin molecules
      - Activate opto-beta2-adrenal receptor → Gi signalling
      - Activate opto-alpha1-adrenal receptor → Gq signalling
  - Opto-RTK
    - Dimerise → autophosphorylation
    - Remove lox domain
    - Attach lox (light sensing) domain in intracellular part
    - RTK pathway now under control of light

# **Revision & Exam Techniques**

Section A is a single question, all subsections must be answered

Section B - pick 1 of the 3 questions

Use separate answer books for Sections A and B