ZOO332H1S
Lecture 3,4
Jan. - 2003
(AJE)

Channels, resting and action potentials

Erwin Neher (left) and Bert Sakmann in their laboratory (1985).

1

Reasons for studying channels 1:

- **Fascinating stuff in Zoology – adaptation to environmental stress**
- "…there is a constant struggle…between the instinct of the one to escape its enemy and the other to secure its prey." – Charles Darwin
- Skin of newt (*Taricha)* contains TTX, these newts are generally avoided by snakes since they are toxic
- *Thamnophilis sirtalis* can eat these newts – why?
- Why do nociceptive fibres in DRG of rat express TTX-resistant sodium channel $Na_v1.9$ (NaN)?

Geffeney *et al*.(2002); Fang *et al*.(2002)

Reasons for studying channels (handout) 2:

Cannon (1996) on myotonias (side one); channel mutations and recordings (myotonias) (side 2).

after S.C. Cannon (1996) TINS **19** (1), 3-12.

The myotonias (an impairment of muscle relaxation; muscle stiffness, difficulty relaxing grip after handshake or opening eyes after squinting in bright sunlight - membrane excitability is enhanced, repetitive discharges that persist several seconds; not blocked by curare) and periodic paralyses (reduced membrane excitability; attacks of weakness, EMG silent, membrane depolarized at -60 to -50mV from normal value of -90mV, APs can't be generated) have dramatic effects on muscle function and also provide a model system in which to study mechanisms of electrical excitability in muscle. APs are initiated at the neuromuscular junction and propagate both longitudinally along the muscle fibre and radially into the transverse tubule system (T-tubule) where voltage-sensitive L-type Ca^{2+} channels undergo a conformational change that triggers release of Ca^{2+} from the sarcoplasmic reticulum. This coupling between arrival of input at the neuromuscular junction and release of Ca^{2+} from the SR is essential for the rapidity and fidelity of motor responses. Derangements in this electrical coupling occur in the myotonias and periodic paralyses, and the molecular basis for these defects has been shown recently to arise from mutations of ion channels that are expressed selectively in skeletal muscle. The functional consequences of the mutations are being characterized physiologically and, for some disorders, have been shown to be sufficient to cause the stiffness or weakness that occurs in these muscle diseases.

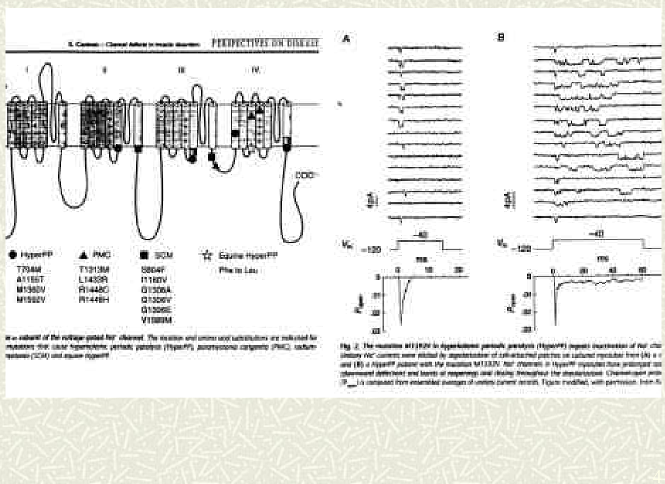
ABLE 1. Classification of hereditary myotonias and periodic paralyses

enotypic assification	Cl^- channel			Na^+ channel		Ca^{2+} char
cus	7q35	7q35	17q23–25	17q23–25	17q23–25	1q31–32
eritance	AR	AD	AD	AD	AD	AD
nctional defect	Reduced Cl^- conductance	Reduced Cl^- conductance	Slowed inactivation	Slowed and incomplete inactivation	Incomplete inactivation	Enhanced inactivati
netrance	100%	100%	100%	100%	100%	Male 100% Female 50
yotonia ntensity pecial features	Severe Constant	Moderate Constant	Variable Fluctuates	Moderate Paradoxical Cold-aggravated	Mild	None
eakness Degree pecial features	None	None	None	Mild to moderate Cold-aggravated	Moderate to severe ↑[K^+] worsens	Severe ↓[K^+] wor
sorder	Generalized myotonia (Becker's)	Myotonia congenita (Thomsen's)	Sodium-channel myotonia	Paramyotonia congenita	Hyperkalemic periodic paralysis	Hypokalen periodic paralysis
enotypic lassification	←enhanced excitability→ (pure myotonia)			←mixed→	←reduced excitability→ (pure paralysis)	

dium-channel myotonia includes: myotonia fluctuans, K^+-aggravated myotonia and myotonia permanens.
breviations: AD, autosomal dominant; AR autosomal recessive.

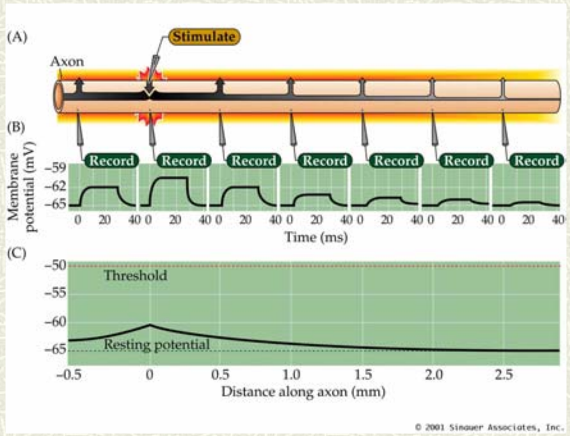
Cont...Slide 2 of myotonias

- Single amino acid substitutions
- Dramatic effects possible
- Effects on channel properties



Passive current flow in an axon

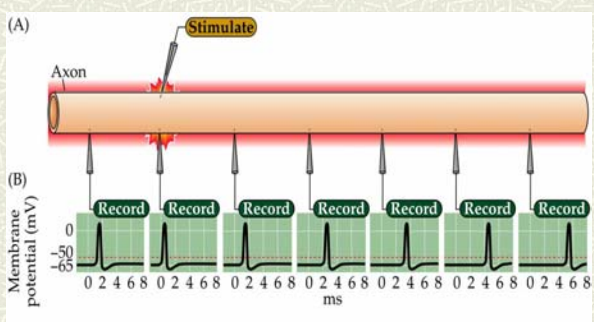
- **Sub-**threshold stimulus
- Passive current flow
- Decreasing amplitude of potential change with distance
- Very short time delay along axon



Purves *et al.*

AP propagation along an axon

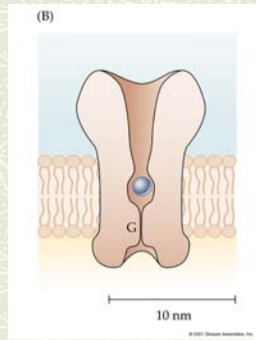
- Constant amplitude
- Active propagation
- Time delay with increasing distance
- Bi-directional



Purves *et al.*

Ions get through membranes via channels

- Lipid bilayer (fluid mosaic model)
- Membrane-spanning proteins form channels
- Membrane channel in cross-section
- “G” channel gate – selectivity; probability of opening; gating



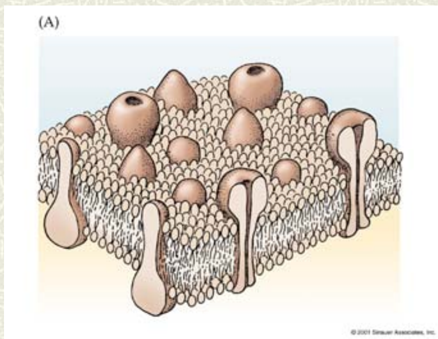


Fig. 2.1

Conductance changes are caused by changes in channel open probability . . .

Voltage-activated

Stretch-activated

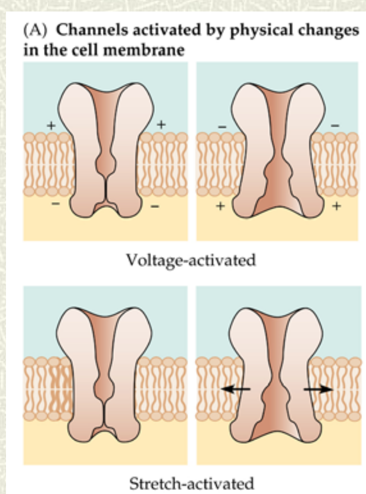


Fig. 2.2

cont. Modes of channel activation

Chemical activation – extracellular ligand

Chemical activation – intracellular, usually by 2nd messenger

(Cytoskeletal – role of integrins, cell adhesion molecules)

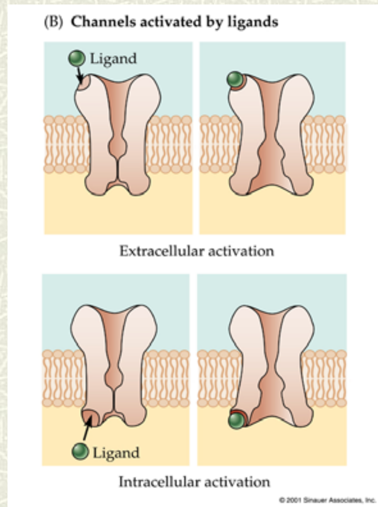
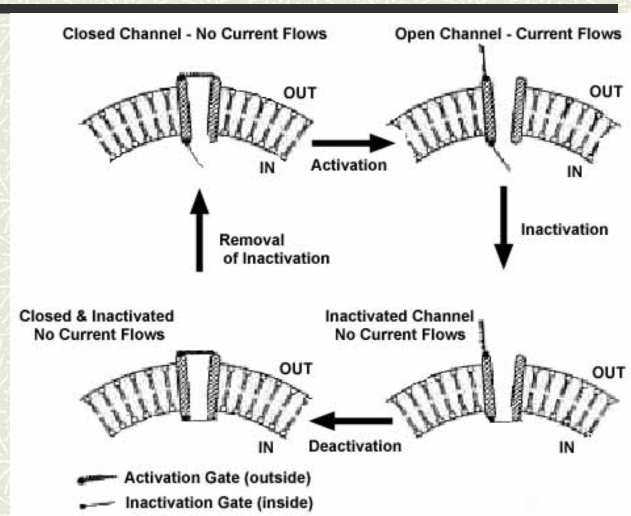


Fig. 2.2

V-sensitive Na^+ channel opening and closing (a "classic")

<u>SEQUENCE</u>

- **at rest**
- **depolarizing**
- **inactivated (depolarized)**
- **deactivated (closed)**
- **repolarize and remove inactivation**

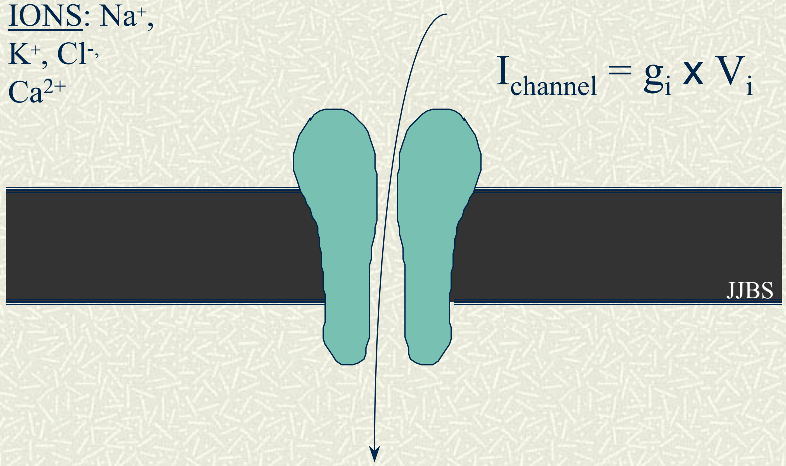


After Levitan and Kaczmarek, 1997

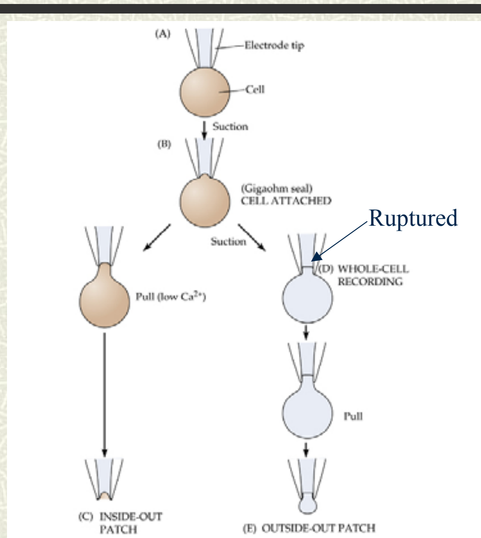
Current through individual channels follows Ohm's Law (supports data from whole membrane)

IONS: Na^+, K^+, Cl^-, Ca^{2+}

$$I_{channel} = g_i \times V_i$$



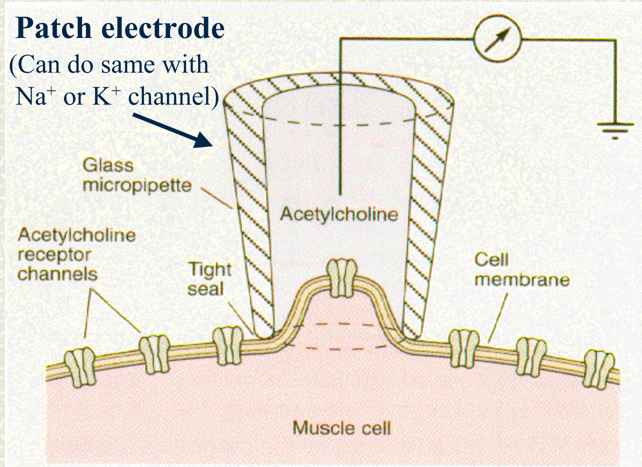
Patch clamp recording configurations



- Tip of electrode makes gigaohm (10^9 ohms) seal
- Cell-attached patch
- Inside-out patch
- Whole-cell recording
- Outside-out patch (tricky)

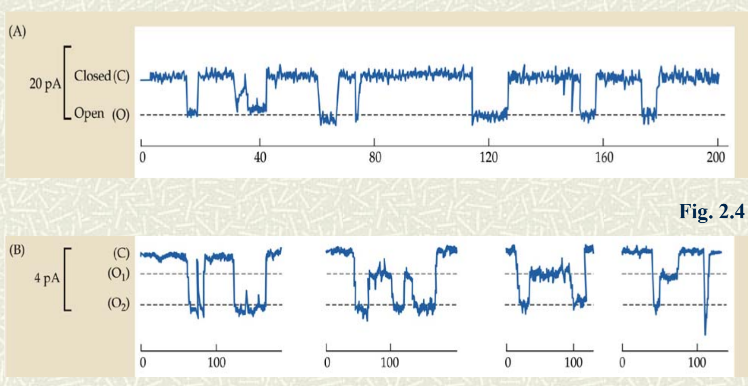
Fig. 2.3

E.g., NMJ AChR channel (another "classic")



Examples of patch clamp recordings

Glu-activated channel, locust leg muscle – cell attached patch

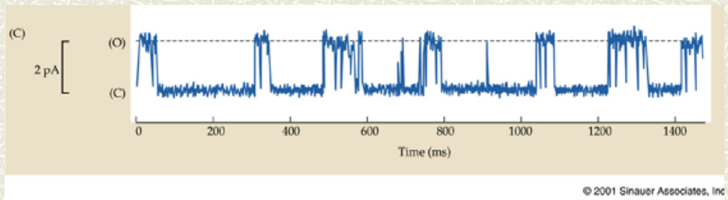


AChR, outside-out, rat embryonic heart; max at 3pA and substate of 1.5pA

Convention: downward = inward current

cont…Examples of patch clamp recordings

Glycine-activated chloride channel; outside-out patch; chick cultured spinal cord cells



cont. Fig. 2.4

Summary: Ions moving across neuronal membrane...

• Channels - v-gated, stretch, ligand, 2nd messenger, and "resting channels" (responsible for resting permeability)

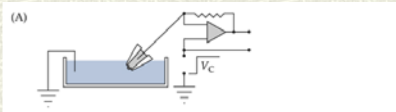
• Why do ions flow across the membrane through channels? (permeability & conductance)

• What are some of the factors that determine the conductance level of a particular channel?

• How do species of channels differ?

• An all-or-none event? (popcorn)

Effect of potential on currents



N.B. Points:

• recording set-up (outside-out)

• spont'ly active 'non-specific' K^+ channel

• equal $[K^+]$ in and out

• make inside electrode +20 mV

• make inside electrode –20 mV

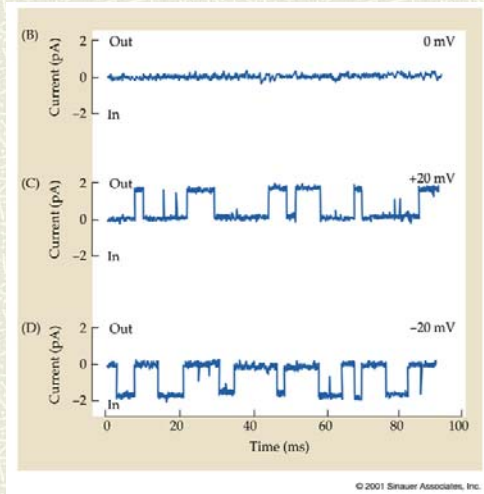


Fig. 2.6

Effect of potential on currents

• Channel currents as a function of applied voltage:
Linear relationship between current and voltage
• *i.e.,* amount current proportional to voltage (potential) across the membrane

Ohm's Law: **V = IR or I = γV**

(where γ is channel conductance)

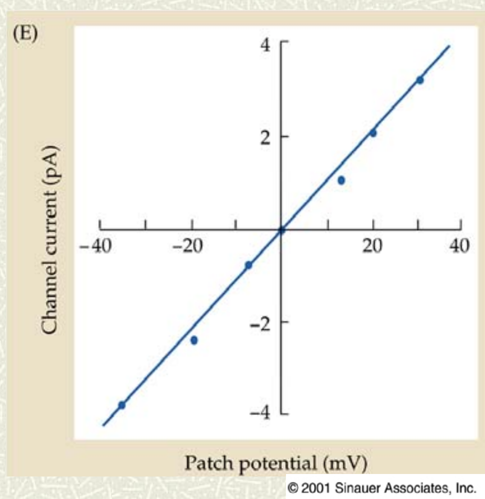


Fig. 2.6

BUT, $[K]_i$ is not = $[K]_o$ and resting potential not = 0

- Model Cell
  - Semi-permeable to K^+, Cl^-
  - Impermeable to Na^+, A^-,
  - Electrical neutrality
  - Charge separation at membrane
  - Membrane as a capacitor

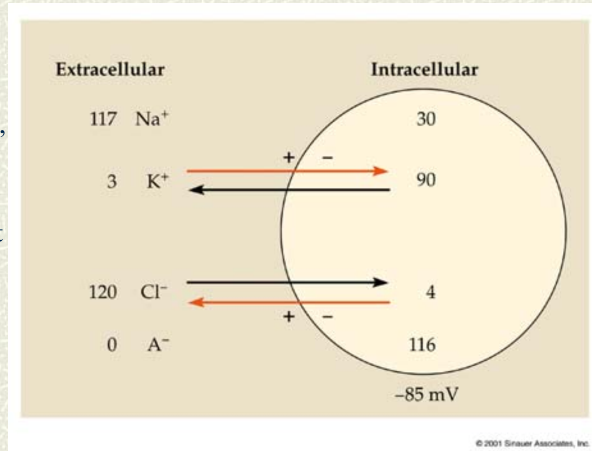


Fig. 5.1

Baker, Hodgkin, and Shaw (1962)

- What's essential for an AP?
  - Squid giant axon
  - Cannulate and extrude axoplasm
  - Refill with appropriate salt solution
  - Stimulate

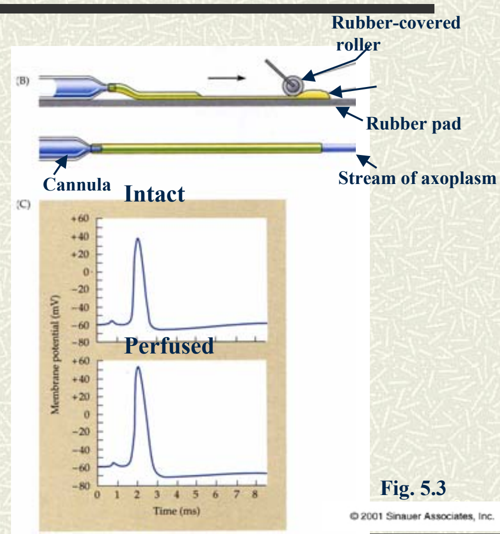


Fig. 5.3

Ion concentrations – squid giant axon

- Note blood salt ($[Na^+]$) approximates that of sea water
- Quite different from terrestrial mammal
- Use Nernst to calculate resting potential

Table 5.1
Concentrations of ions inside and outside freshly isolated axons of squid

	Concentration (m*M*)		
Ion	**Axoplasm**	**Blood**	**Seawater**
Potassium	400	20	10
Sodium	50	440	460
Chloride	60	560	540
Calcium	0.1 μ*M*[a]	10	10

Source: After Hodgkin, 1964.
[a] Ionized intracellular calcium from Baker, Hodgkin, and Ridgeway, 1971.

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Mammalian neuron		
Potassium (K^+)	140	5
Sodium (Na^+)	5–15	145
Chloride (Cl^-)	4–30	110
Calcium (Ca^{2+})	0.0001	1–2

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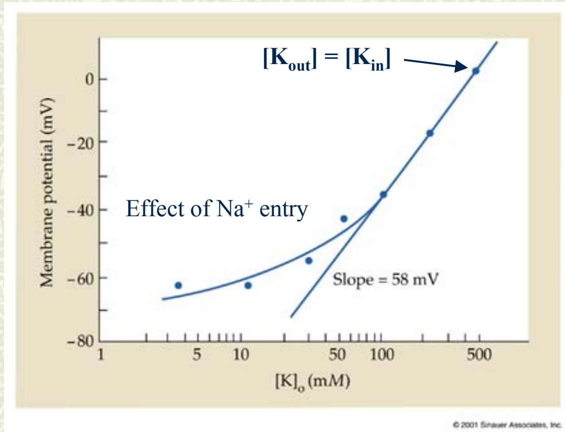
<u>If,</u> V_m is due to K^+ diffusion across a semi-permeable membrane….

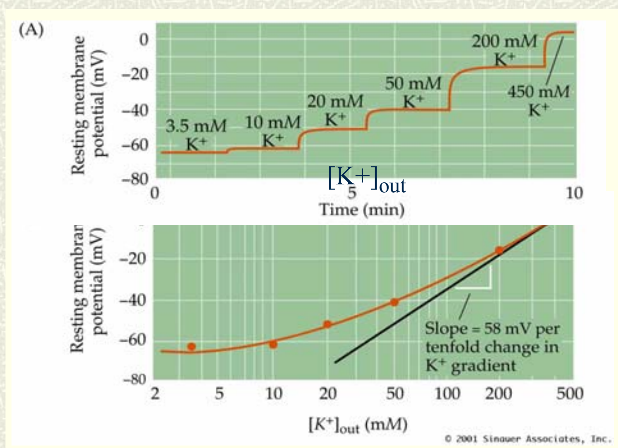
- Changing $[K^+]$ should change V_m according to the Nernst Equation
- Internal $[K^+]$ for squid axon is about 400 mM, so if external $[K^+]$ is same. V_m should be zero

Dependence of V_m on $[K^+]$



Cont… Dependence of V_m on $[K^+]$ - Nernst Equation

- Same story, extra figure to show change of $[K^+]_o$



Driving force on ions in solution

From previous example – why is K^+ going across the membrane?

1. Intermittent permeability through channel
   - Open channel >> permeability
   - Permeability + ions >> conductance
2. Equal concentrations of K^+ both sides (no chemical gradient)
3. Provided electrical gradient (+20 mV, -20mV)

What is $V_{driving\ force}$ for an ion?

- It's *not* V_m!
- It depends on how far away V_m is from the *equilibrium potential* for the ion, E_{ion}
- $\mathbf{V_{df} = V_m - E_{ion}}$
- The equilibrium potential for a particular ion is given by the Nernst equation (electrical and chemical considerations)

About the Nernst equation

- Refers to a single ion at 20^o C (but…)
- Is voltage when that ion is in thermo-dynamic equilibrium (electrical and chemical forces balance)
- Each ion may have a different E_i
- Membrane voltage may not equal any value of E_i

The Nernst Equation

$$E_{ion} = \frac{RT}{zF} \ln \frac{[ion]_{out}}{[ion]_{in}}$$

Simplifies (at 20^{o}C) to:

$$E_{ion} = 58 \log \frac{[ion]_{out}}{[ion]_{in}}$$

Which simplifies to:

ln > log conversion x 2.31
R = gas constant
T = temperature in ^{o}Kelvin
F = Faraday constant
z = valancy

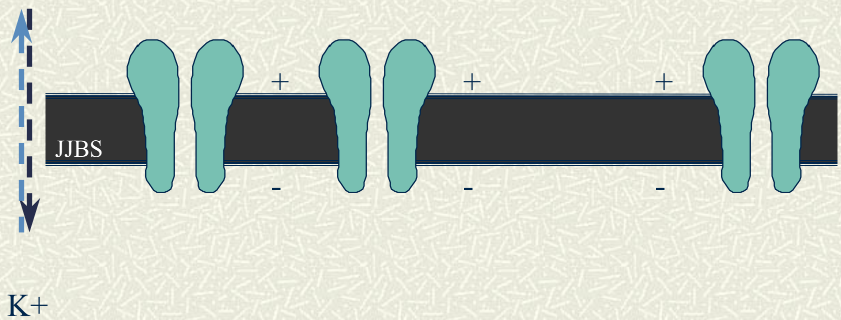
Current for an ion is zero at the equilibrium potential (also known as the reversal potential)

- *E.g.*, E_{K+} is typically -80 mV, so I_{K+} at this value for V_m is zero, *whatever the membrane conductance*
- E_{Na+} is about +50 mV, so Na^+ is **not** at equilibrium at -80 mV . . .
- . . . and I_{Na+} will depend on (V_m - E_{Na+})
- *and on* g_{Na+}

What about actual V_m?

- Would equal E_{K+} if membrane only permeable to K^+, *e.g.* glial cells (and muscle cells (excitable)) come close
- If membrane permeable to other ions which are *not* at their equilibrium potential, then they will cross membrane and change V_m
- So V_m will be compromise between values of E_i
- At E_i, diffusive flux and currents are equal and opposite because concentration gradient balances electrical gradient

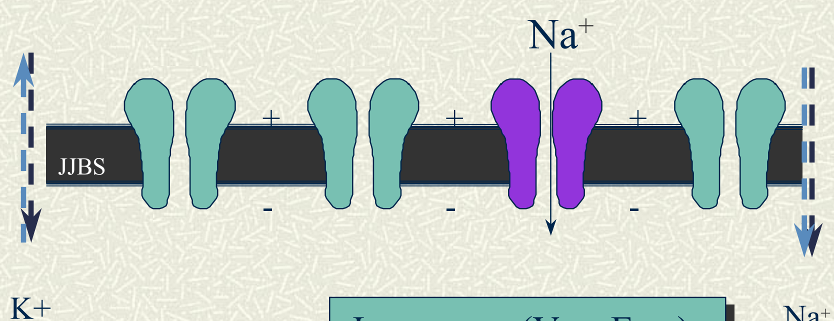
At E_{K+}, I_{K+} is zero ($V_m - E_{K+} = 0$)



- 80 mV

But there are some leaky resting Na^+ channels

The voltage and concentration gradients are inward for Na^+,
(and quite powerful) so there would be a *slight* Na^+ current

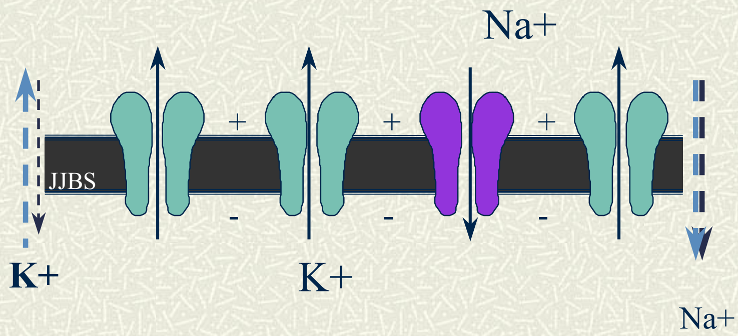


- 80 mV

$$I_{Na+} = g_{Na+} (V_m - E_{Na+})$$

So Vm goes more +ve.

Which means K^+ is no longer at equilibrium, (its voltage gradient is smaller)



Inside - 70 mV

V_m is at steady state

- When $I_{K+} = - I_{Na+}$
- or when *NET* current across membrane = 0 (all ions)
- This value of V_m is defined by the Goldman-Hodgkin-Katz equation (also known as the *Constant Field Equation*)

$$V_m = 58 \log \frac{P_K [K^+]_{out} + P_{Na} [Na^+]_{out} + P_{Cl}[Cl^-]_{in}}{P_K [K^+]_{in} + P_{Na} [Na^+]_{in} + P_{Cl}[Cl^-]_{out}} \text{ mV}$$

How is the membrane potential maintained?

• Ions enter and leave during an action potential (relatively few must cross for an AP, but deal with 1000's of APs/minute)

• Concentrations in the cytoplasm must be kept constant

• Active transport of ions - the source of resting neuronal membrane potential (and indirectly, the AP)

• Primary active transport uses energy provided by hydrolysis of ATP (Na/K exchange pump); average $3Na^+$ out for $2\ K^+$ in

• active transport and experiments (Ch. 4 – Pg. 61- 68)

Cont...Process of active transport

(A) Inward-facing binding sites have a high affinity for Na^+ and a low affinity for K^+. Previously bound K^+ ions are released and 3 Na^+ ions are bound.

(B) Na^+ binding is followed by ATP binding and phosphorylation of the enzyme.

(C) The phosphorylated enzyme undergoes a conformational change such that its binding sites face the extracellular solution.

(D) outward-facing sites have a low affinity for Na^+ and high affinity for K^+, and they bind two K^+ ions.

(E) K^+ binding leads to dephosphorylation.

(F) Dephosphorylation is followed by a return to the starting conformation.

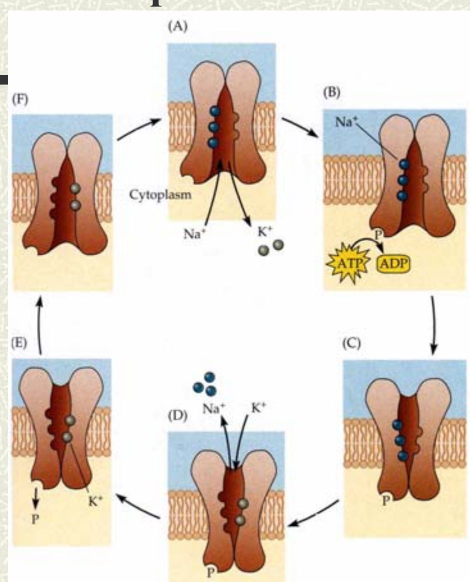


Fig 4.2

Role of Active Transport of Na^+ and K^+

- Perpetual task of extruding Na^+ and intake of K^+
- Essential to maintain viability of nerve cells
- Hydrolysis of ATP - pump action coupled: 3 Na^+ out for 2 K^+ in
- Specificity: **requires** Na^+ inside; not as specific for K^+ outside (other X^+ can substitute)

…*Cont.* Active transport - Na^+/K^+ pump

Notes *re*. Exp'al Setup:

- electrodes
- inject Na^+ into cell by passing current through pipette
- current flow is **between two electrodes** (Na^+ and Li^+ filled electrodes) and **NOT** through the cell membrane

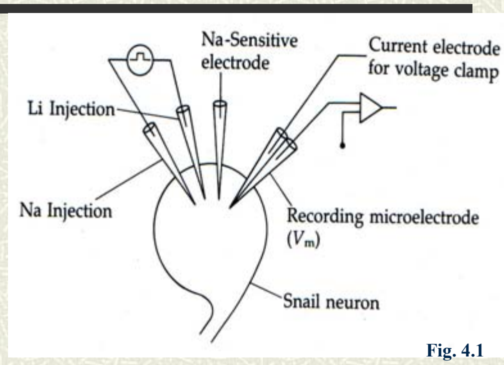


Fig. 4.1

…*Cont*. Active transport - Na^+/K^+ pump

(B) note membrane potential change with Na^+, but none with Li^+

(C) Ouabain blocks pump

(D) K^+-free medium

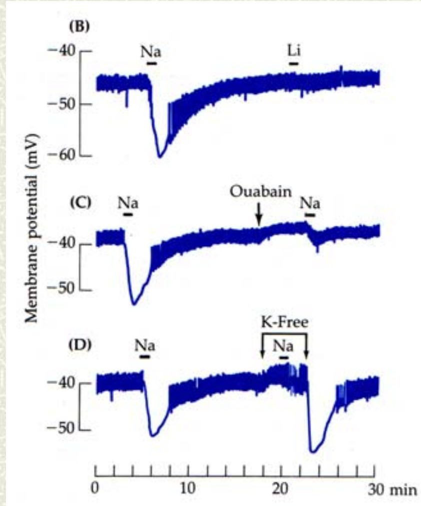


Fig.4.1

….*Cont*. Active transport

Evidence that membrane potential change due to action of the Na^+/K^+ pump:

1. Input resistance did not decrease (expected if hyperpol'n was due to an increase in gK^+ or gCl^-)

2. Effect of ouabain reduced or eliminated hyperpol'n

3. Replacement of external K^+ eliminated effect of Na^+ injection (until K was replaced in bath)

….*Cont.* Active transport

What about **pump rate and exchange ratio** ? (*I.e.*, how much Na^+ and K^+ are moving?)

Use the **voltage clamp technique** !! (has also been done using radioactive isotopes)

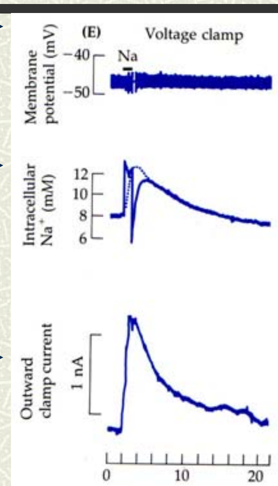
….*Cont.* Active transport

1. Membrane potential clamped

2. Intracellular Na^+ measured

3. Measure current: (i) outward, (ii) amplitude and duration (*see 2.*), & (iii) total charge leaving only about 1/3 of charge injected in form of Na^+ ions.

4. All this fits >>> 3:2 pump ratio (Na^+ out/ K^+ in)



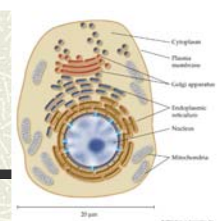
1.
2.
3.

Fig. 4.1

Recapping Active Transport - Na^+/K^+ pump

- constant transport of Na^+ & K^+ essential for viability
- hydrolysis of ATP used to drive Na^+/K^+ pump (*i.e.*, pump acts as an ATPase)
- pump specific for Na^+_{out}; but not same requirement on K^+_{in} (in absence of K outside activity is about 10% of normal)
- ouabain - commonly used glycoside which blocks pump

Calcium Pumps



- High buffering capacity for intracellular calcium (Ca^{2+}) essential to role in multitude of specific processes
- Examples: vesicle fusion and release of NT; 2nd messenger; muscle contraction; activation of ion channels; regulation of cytoplasmic enzymes; *etc.*
- Ca^{2+} entry through plasma membrane (specific channels) but also released from intracellular stores (ER, SR, mitochondria)
- FURA2, Arsenazo III, aequorin – dye indicators of free Ca^{2+}
- Ca^{2+} ATPase responsible for expulsion across plasma membrane and also into intracellular compartments
- $[Ca^{2+}]_i$ *ca.* 10-100 nM; $[Ca^{2+}]_o$ *ca.* 2-5 mM

cont. Calcium pumps

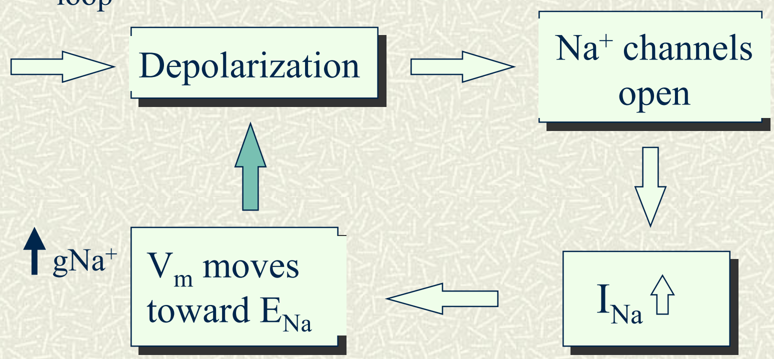
- SR ATPase: high density in membranes, rapid recovery from muscle contraction
  - Analogous to that described for Na/K ATPase; high affinity binding of 2 Ca^{2+}; enzyme then phosphorylated, conformational change and release of Ca^{2+} on other side
- Plasma membrane Ca^{2+} ATPase has single high affinity site for Ca^{2+} and only one Ca^{2+} expelled
- Na^{+}-Ca^{2+} Exchange – transporter molecule coupled to inward movement of Na^{+} down [ ] gradient = energy to drive Ca^{2+} uphill
- NCX transport system – one Ca^{2+} out for 3 Na^{+} in
- Although NCX exchanger has lower affinity for Ca^{2+} it has higher density in membrane and *ca*. 50 greater capacity

Reminder about the Action Potential…(Ch.6)

Positive feedback cascade

Note: AP - depends on passive current, but ions moving cause majority of ΔV_m

- Voltage-gated Na^+ channels open in a positive feedback loop



Cont... the AP, but….

- How is the inflow of Na^+ stopped? ***Na^+ - inactivation***
- What about K^+ ? ***Voltage-gated K^+ channels &***
- Introduced the "microscopic" level of ionic current flow (channels)
- "Macroscopic" currents - voltage clamp ("whole" cell)
  - Classical analysis by Hodgkin and Huxley, 1952

More on the AP next lecture