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

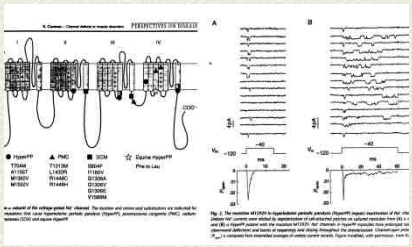
Channels, resting and action potentials

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

1

Cont...Slide 2 of myotonias

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



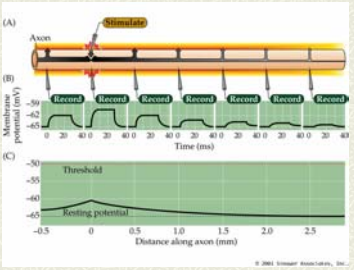
Reasons for studying channels 1:

- **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)

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



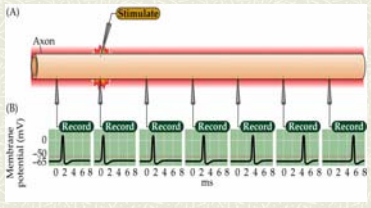
Purves *et al.*

Reasons for studying channels (handout) 2:

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

AP propagation along an axon

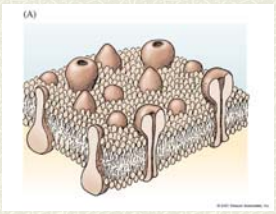
- Constant amplitude
- Active propagation
- Time delay with increasing distance
- Bi-directional



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



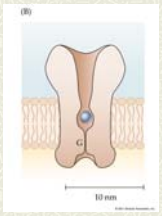
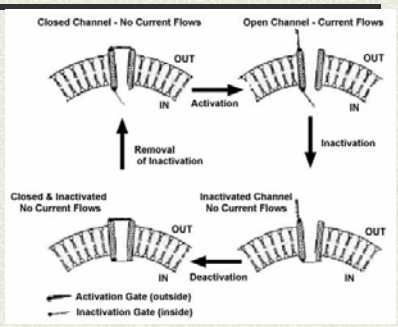


Fig. 2.1

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

SEQUENCE

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



After Levitan and Kaczmarek, 1997

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

Voltage-activated

Stretch-activated

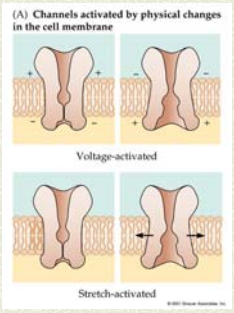
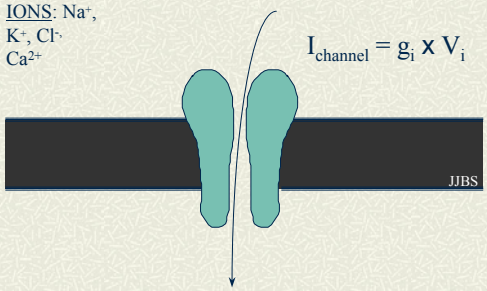


Fig. 2.2

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

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

$I_{channel} = g_i \times V_i$



cont. Modes of channel activation

Chemical activation – extracellular ligand

Chemical activation – intracellular, usually by 2nd messenger

(Cytoskeletal – role of integrins, cell adhesion molecules)

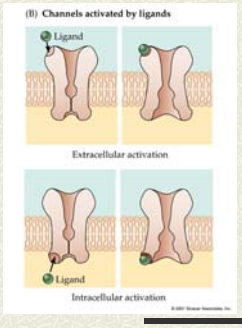
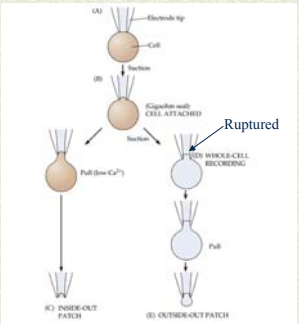


Fig. 2.2

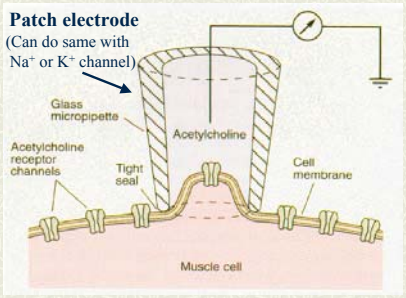
Patch clamp recording configurations



- 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")



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)

Examples of patch clamp recordings

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

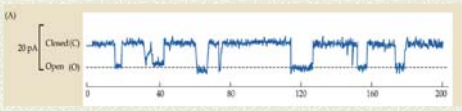
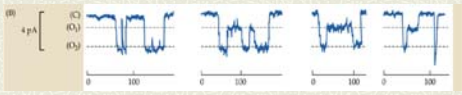


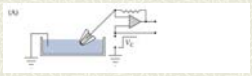
Fig. 2.4

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



Convention: downward = inward current

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

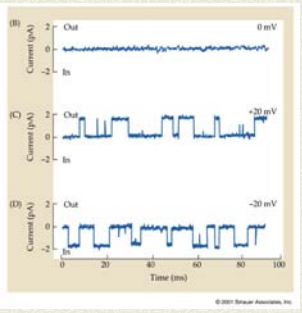
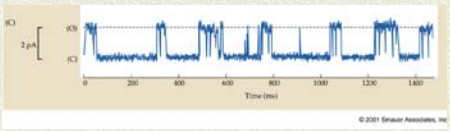


Fig. 2.6

cont…Examples of patch clamp recordings

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



cont. Fig. 2.4

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)

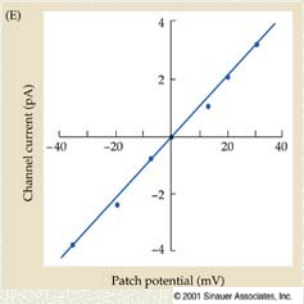


Fig. 2.6

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

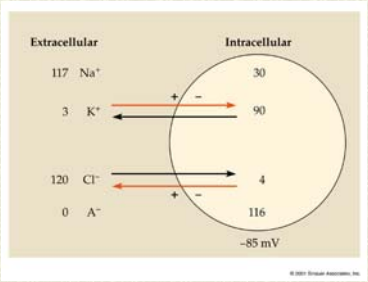


Fig. 5.1

If, 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

Baker, Hodgkin, and Shaw (1962)

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

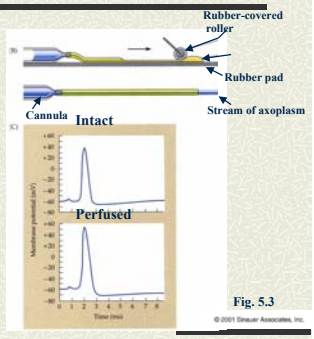
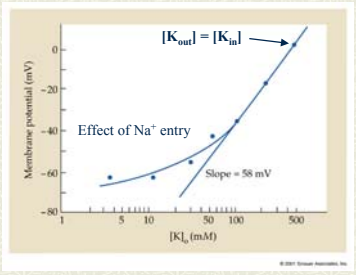


Fig. 5.3

Dependence of V_m on $[K^+]$



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

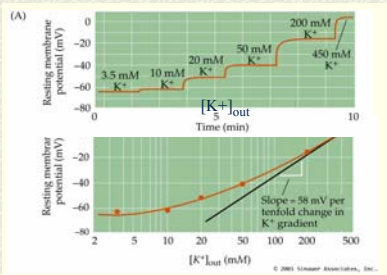
Ion	Concentration (mM) Axoplasm	Blood	Seawater
Potassium	400	20	10
Sodium	50	440	460
Chloride	60	560	540
Calcium	0.1 μM[a]	10	10

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

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

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)

The Nernst Equation

Which simplifies to:

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

Simplifies (at 20°C) to:

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

ln > log conversion x 2.31
R = gas constant
T = temperature in °Kelvin
F = Faraday constant
z = valancy

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}
- **$V_{df} = V_m - E_{ion}$**
- The equilibrium potential for a particular ion is given by the Nernst equation (electrical and chemical considerations)

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+}

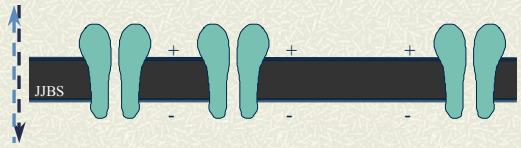
About the Nernst equation

- Refers to a single ion at 20° 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

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 ($V_m - E_{K+} = 0$)



K+

- 80 mV

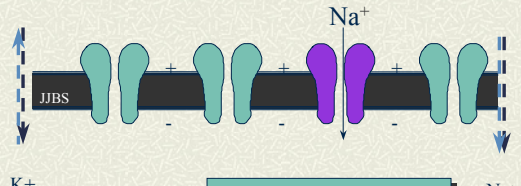
V_m is at steady state

- 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*)

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

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



K+

- 80 mV

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

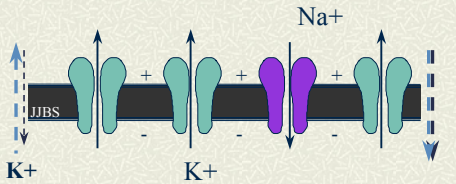
Na^+

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)

So Vm goes more +ve.

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



K+ **K+** Na+

Inside - 70 mV

*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.

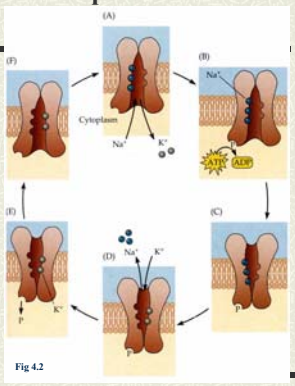


Fig 4.2

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

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 - 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

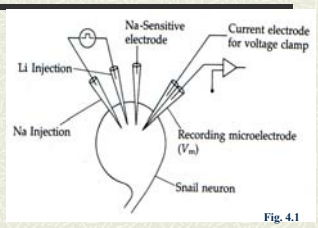


Fig. 4.1

….*Cont.* Active transport

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 - Na^+/K^+ pump

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

(C) Ouabain blocks pump

(D) K^+-free medium

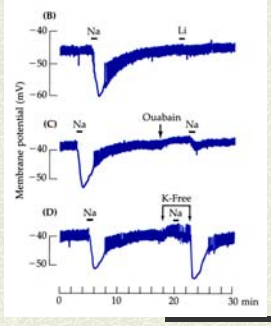


Fig.4.1

….*Cont.* Active transport

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)

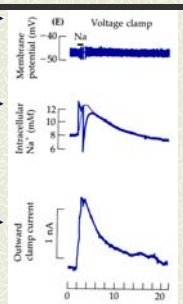


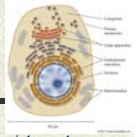
Fig. 4.1

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

Reminder about the Action Potential…(Ch.6)

Calcium Pumps

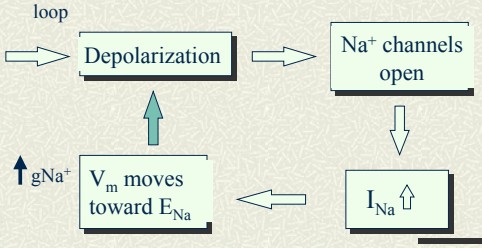


- High buffering capacity for intracellular calcium (Ca^{2+}) essential to role in multitude of specific processes
- Examples: vesicle fusion and release of NT; 2nd messenger; muscle contraction; activation of ion channels; regulation of cytoplasmic enzymes; *etc.*
- Ca^{2+} entry through plasma membrane (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

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. 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

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