3-2. Bioelectric Phenomena

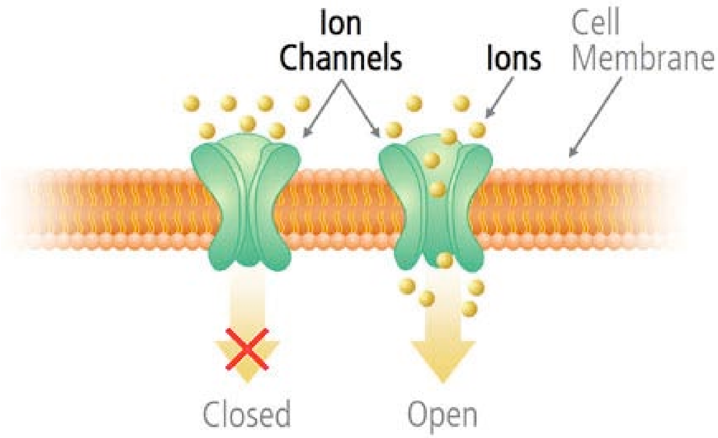
Resting potential & Action potential
H-H modeling

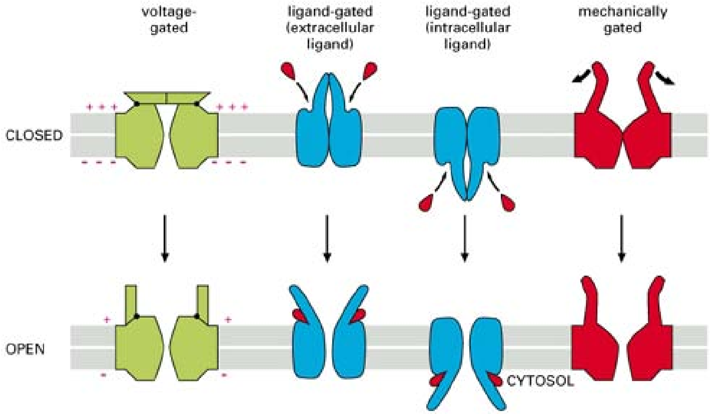
Contents

1. Ion channels
2. Resting membrane potential
   - Nernst Equation
   - The Goldman Hodgkin Katz Equation
3. Action potential
4. Voltage clamp
5. Hodgkin-Huxley model

Ion channels

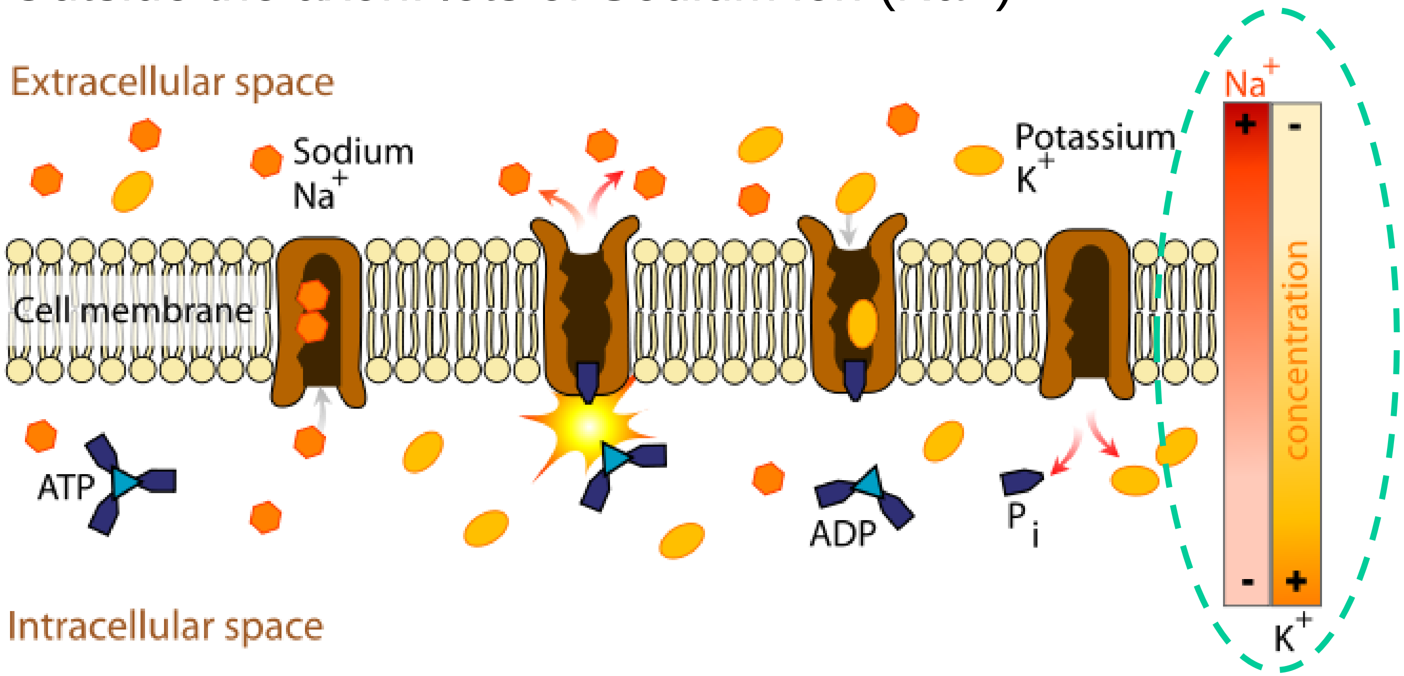
- Flow gates of ions
- Control of voltage gradient across the cell membrane
- Two types of ion channels
  - Resting ion channel
    - Normally open, maintain of the resting membrane potentials
  - Gated ion channel
    - Normally closed, opening by membrane potential changes, ligand binding, or membrane stretch





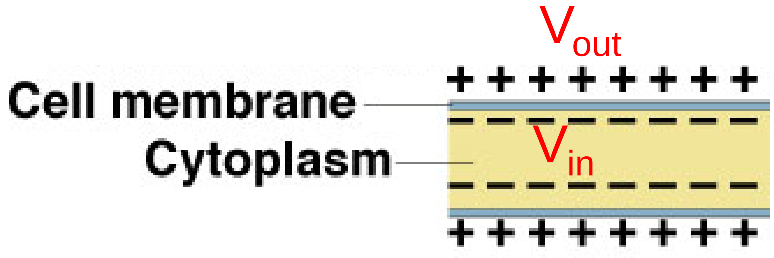
Ion distribution

- Ion distribution across the cell membrane
  - Inside the axon: lots of potassium ion (K+)
  - Outside the axon: lots of Sodium ion (Na+)



Membrane potential

- Difference of electrical potential across the membrane (V_m)
- $V_m = V_{in} - V_{out}$



- Distribution of charges across cell membrane
  - Outside membrane: *positively(+)* charged
  - Inside membrane: *negatively(-)* charged
  - Maintained by membrane which blocks ion diffusion

Resting membrane potential

- Membrane potential of neurons at rest (V_r)
  - Electrical potential across the membrane in the absence of signaling
- Range: - 40 ~ - 90 mV
- Caused by ion distribution
- Maintained by resting ion channels
  - In glial cells: selective for single (potassium) ion only
  - In nerve cells: selective for several ion species

Resting potential video clip

Resting membrane potential

- The resting potential is the result of an unequal distribution of ions across the membrane.
- Then…which factors mainly determine the distribution and movement of ions??

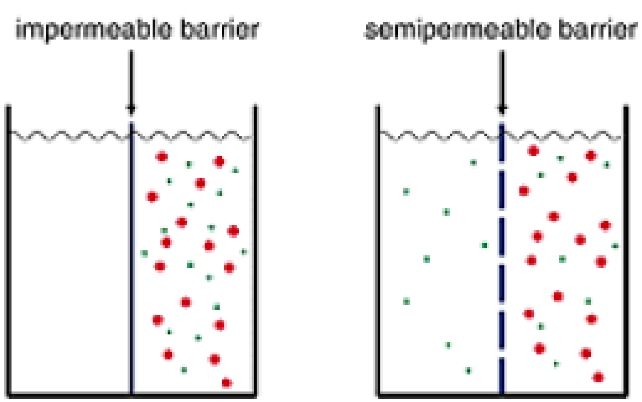
➡ **Ionic permeability**

Equilibrium (Nernst) potentials

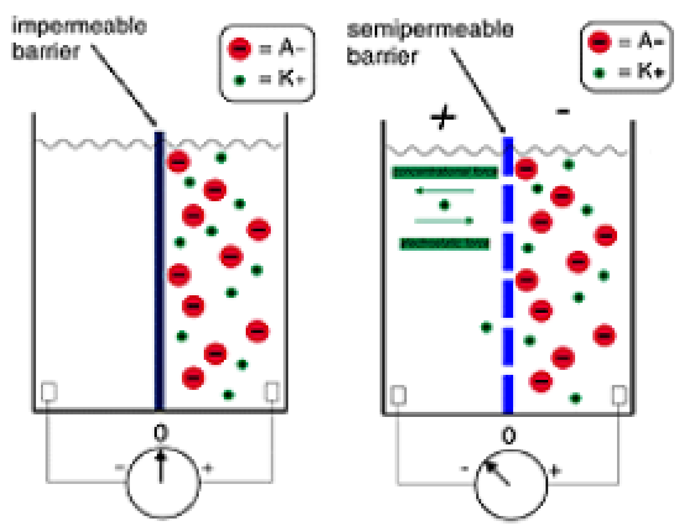
- $E_x = \frac{RT}{zF} \ln \frac{[X]_o}{[X]_i}$, *Nernst Equation*
  - R: gas constant / T: temperature in degrees Kelvin / F: Faraday's constant / [X]: ion concentrations

$$E_{Na} = v_i - v_o = 26 \ln \frac{[Na^+]_o}{[Na^+]_i} \text{mV}$$

$$E_{Cl} = v_i - v_o = -26 \ln \frac{[Cl^-]_o}{[Cl^-]_i} = 26 \ln \frac{[Cl^-]_i}{[Cl^-]_o} \text{mV}$$



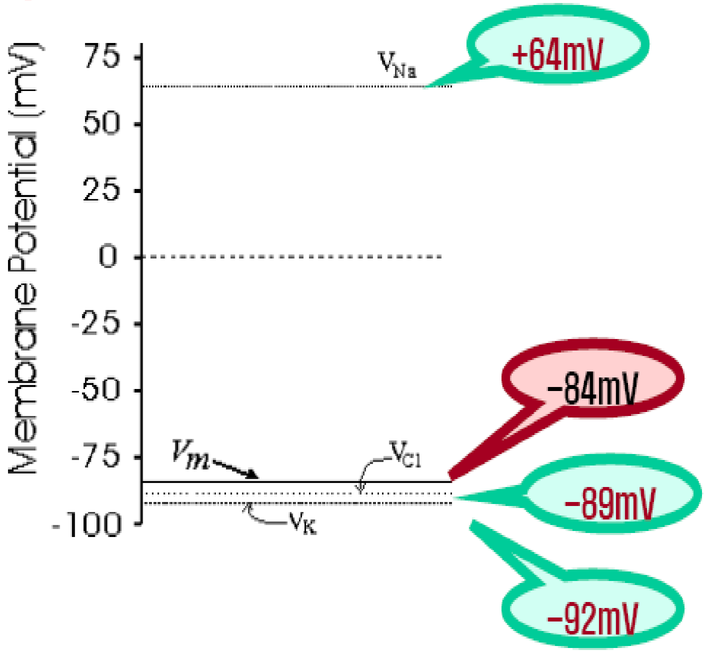
Diffusion



The flow of a single ion

- If the plasma membrane were permeable only to any single ion of K^+, Na^+, and Cl^-, the potential difference across the membrane could be calculated by the Nernst equation.

ion	cytoplasm	extracellular	V_{eq}
Na^+	12	140	+64mV
K^+	135	4	−92mV
Cl^-	5	150	−89mV



The flow of multiple ions

- Why was the resting potential -84 mV, when E_K = -92 mV?
  - This cell, as in many other cells in the nervous system, is permeable to more than one ionic species at rest
- How can we quantify the contribution of multiple ionic species?

➡ **The Goldman Hodgkin Katz Equation (GHK eq.)**

Goldman Hodgkin Katz Equation

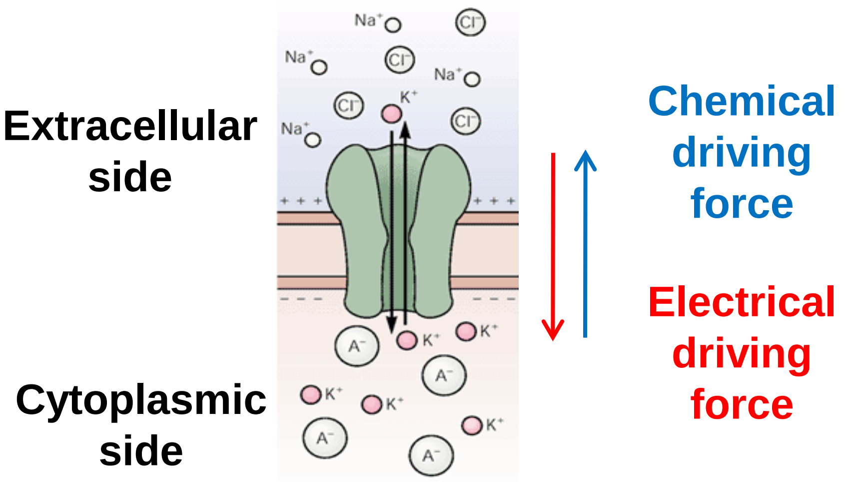
$$V_m = \frac{RT}{F} \ln \frac{P_K[K^+]_o + P_{Na}[Na^+]_o + P_{Cl}[Cl^-]_i}{P_K[K^+]_i + P_{Na}[Na^+]_i + P_{Cl}[Cl^-]_o}$$

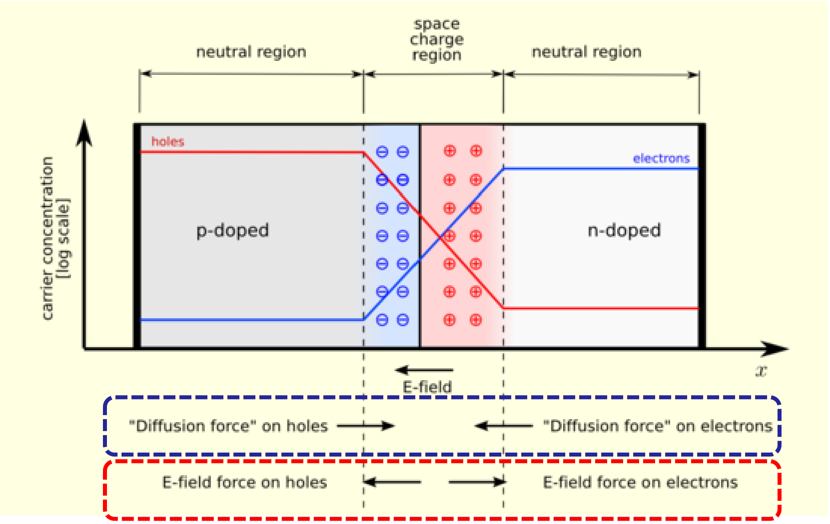
- Because the membrane has a finite permeability, the actual electrical potential at any point in time is a compromise between their combined influence.
- GHK equation provides a reasonable prediction of the electrical potential difference across the plasma membrane of a cell at rest.

Ion	permeability (cm/sec)
Na+	1×10^{-9}
K+	1×10^{-7}
Cl–	1×10^{-8}

Balancing the ion diffusion by electric field

- Two forces of ion driving at resting channels
  - Chemical driving force
    - Diffusion by concentration gradient
  - Electrical driving force
    - Electrophoresis by electrical potential difference
- These driving forces are similar to 'diffusion and E-field forces' of p-n junction.

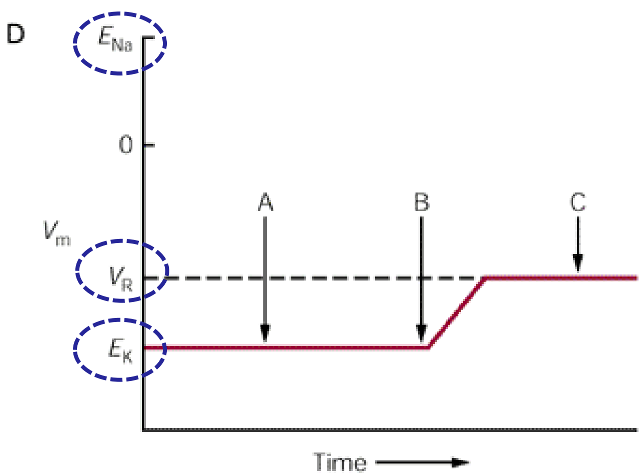


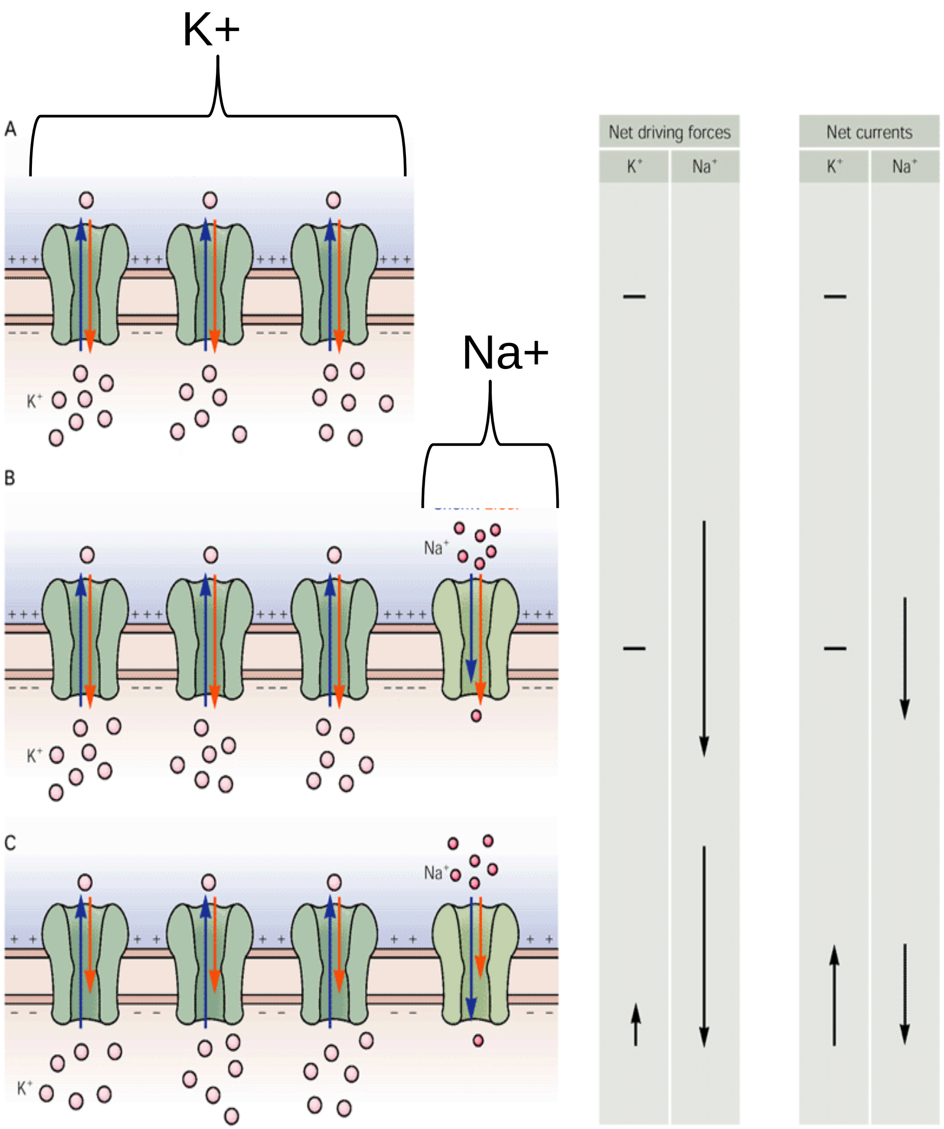


Resting membrane potential

The resting potential of a cell is determined by the relative proportion of different types of ion channels that are open, together with the value of their equilibrium potentials

Chemical driving force
Electrical driving force





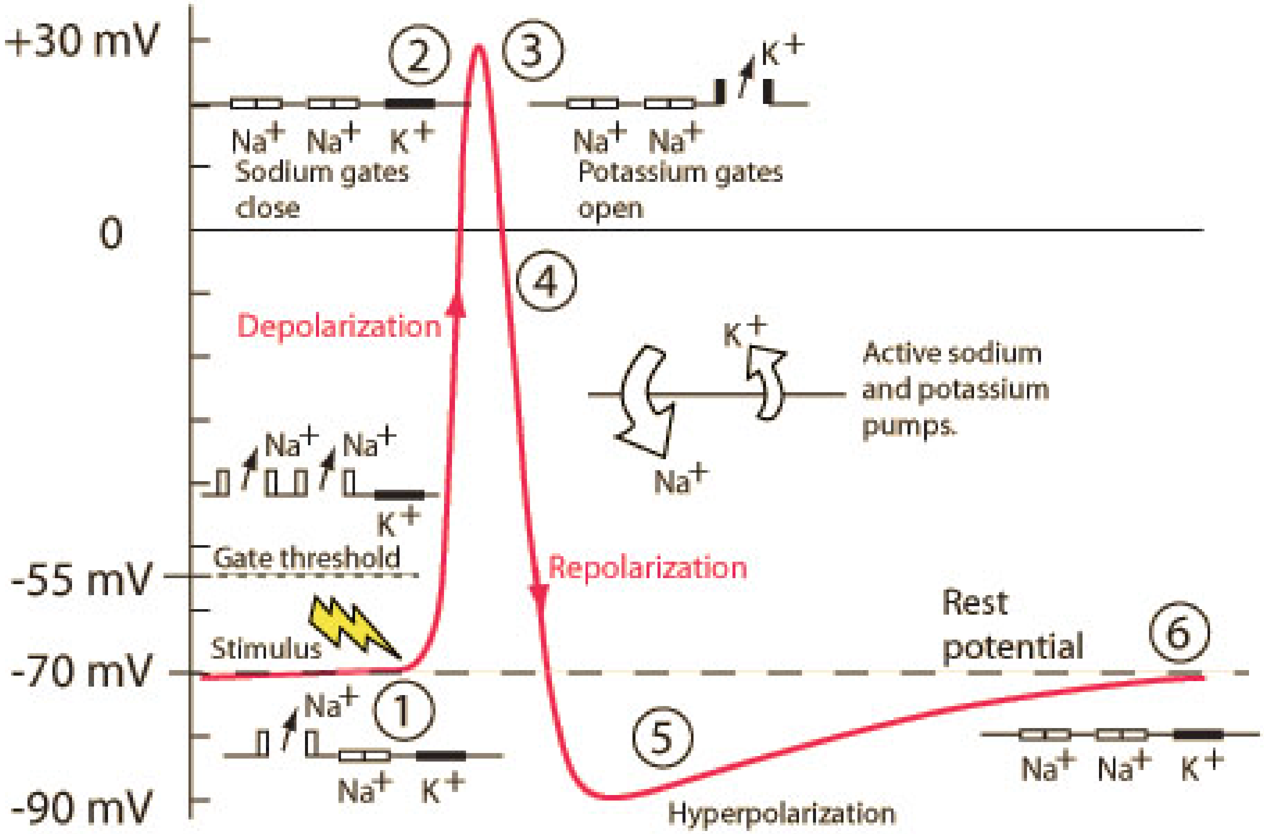
Membrane stimulation

- Threshold
  - Critical level when cell responds actively with the opening of voltage-gated ion channels.
  - Opens both the Na+ and K+ voltage-gated ion channels.
- Sub-threshold stimulus

  → **Equilibrium potential**
- Supra-threshold stimulus
  - Volatage-gated sodium channels become more permeable, and sudden Na^+ influx causes the cell to depolarize.
  - K^+ flows out of cell and the inside of the cell again hyperpolarizes.

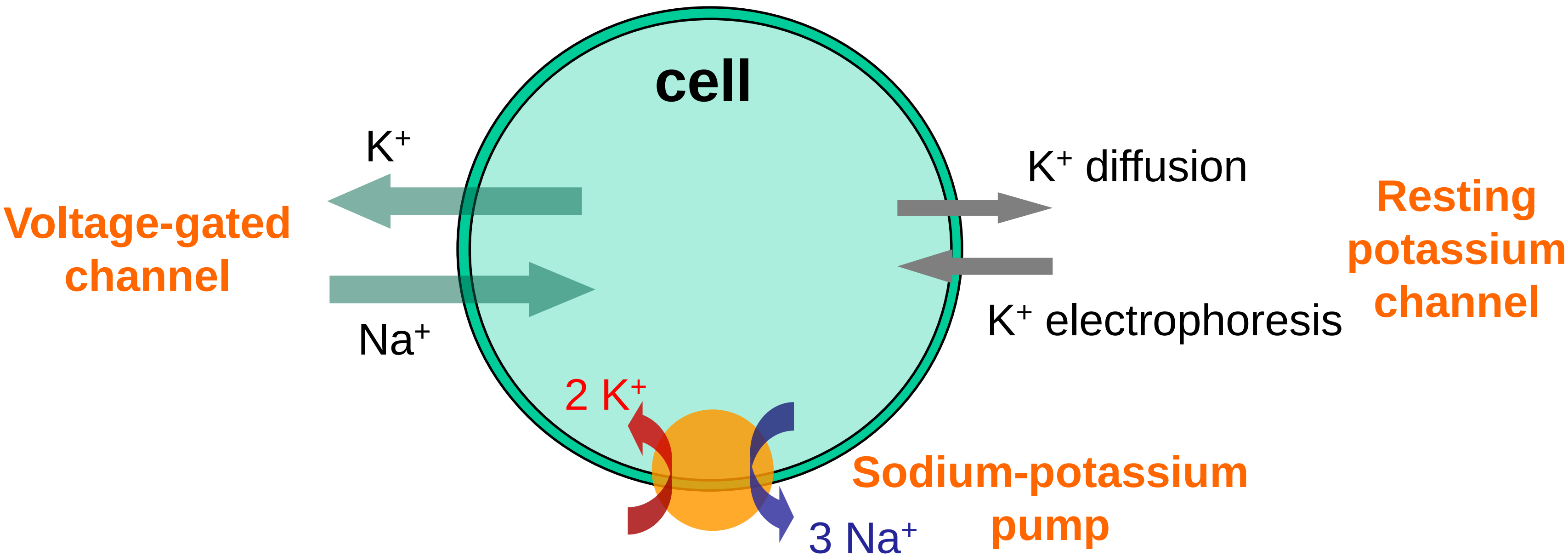
  → **Action potential**

Action potentials (APs)

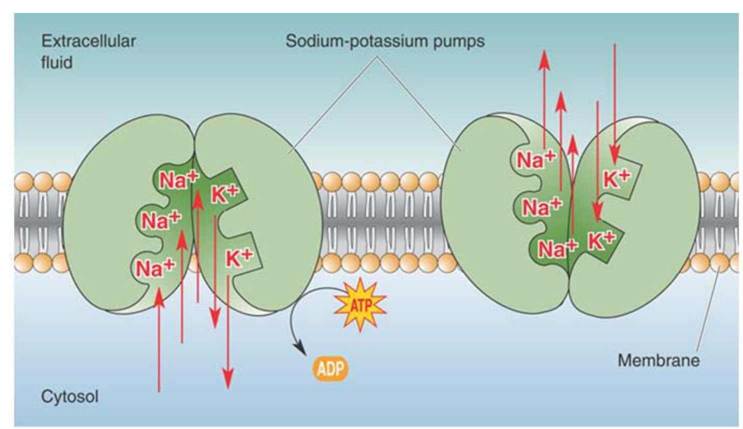


Action potential video clip

Multiple fluxes in a neuron

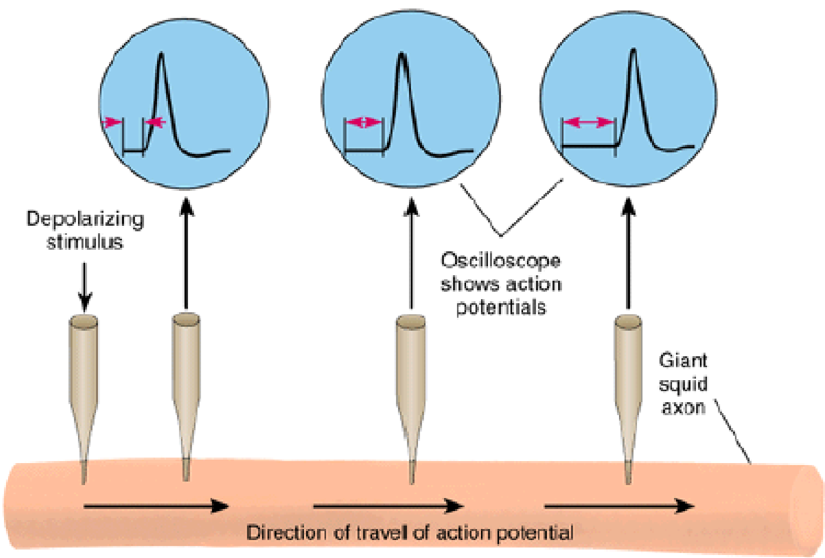


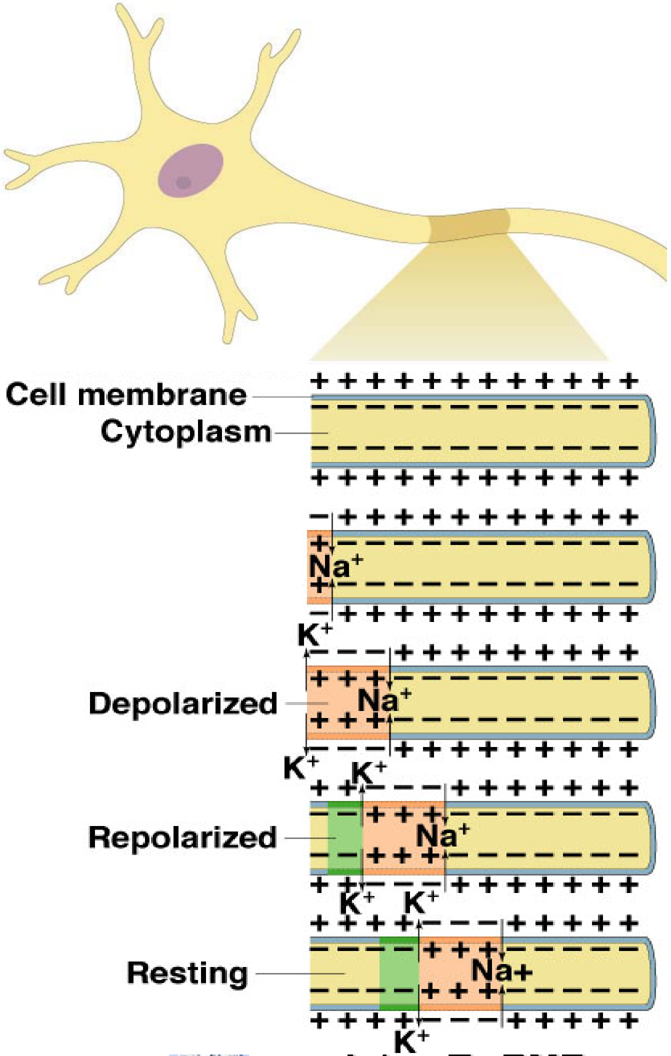
- Sodium-potassium pump:
  - Take out three Na+ / Insert two K+
  - To keeps the voltage steady



Propagation of APs

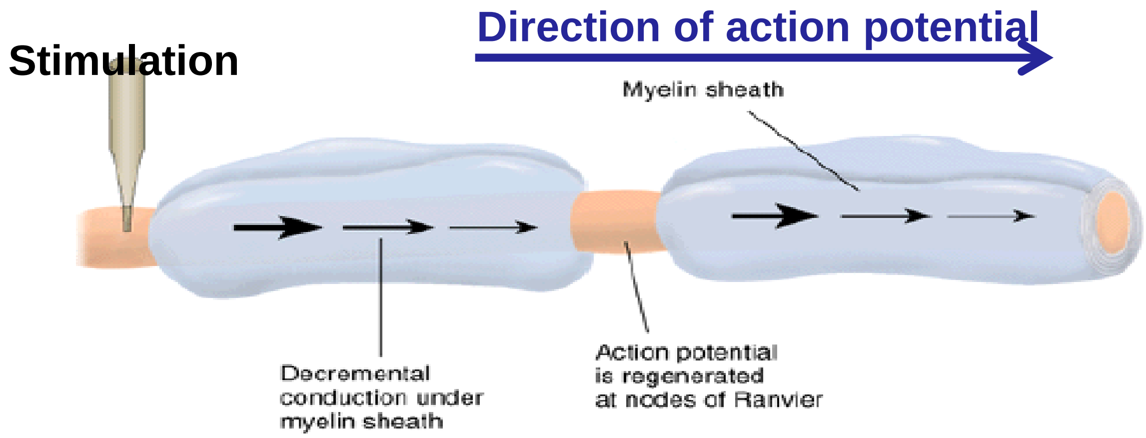
- Propagation
  - Reproduced at different points along the axon membrane.
  - Positive charges can depolarize the next region of the membrane.
  - Axon diameter affects the velocity.





Conduction of APs

- Saltatory conduction
  - The jumping of an impulse between the “Nodes of Ranvier” thus dramatically increasing it’s speed.
  - This occurs because the membrane capacitance of the myelin sheath is very small.
  - Only occurs in axons having Myelin.
    - 1~100 m/s, depending on the fiber species and its environment.



Other characteristics of APs

- Refractory Period
  - Brief period of time between the triggering of an impulse and when it is available for another.
  - NO NEW action potentials can be created during this time!
- All or None Response
  - If an axon is stimulated above its threshold it will trigger an impulse down its length.
  - The strength of the response is not dependent upon the stimulus.
  - An axon cannot send a mild or strong response. It either responds or does not!!!

Voltage Clamp experiment

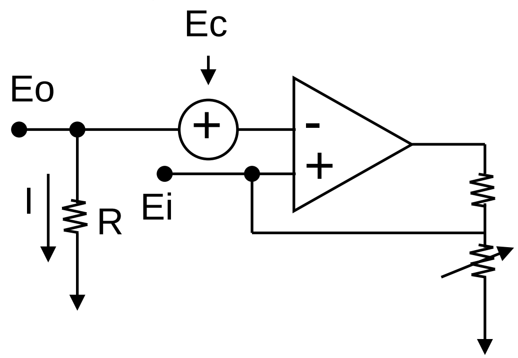
- Goal:
  - Set voltage constant and obtain the time dependent changes of ionic currents during Aps
- The problem:
  - Two dynamic mechanisms in the channel during AP: time and voltage
- Method:
  - Remove space variable(space clamp) and voltage variable(voltage clamp) by inserting long inner electrode and setting transmembrane-voltage constant by feedback.
  - Two active channels: K+ (rising phase), Na+ (falling phase)
  - Separation of K+ and Na+ channels was done by selection of external solution.

Voltage Clamp

Let displacement current 0

$I = C(dV/dt) = 0$

By forcing a constant voltage,



give feedback using a Potentiostat to make

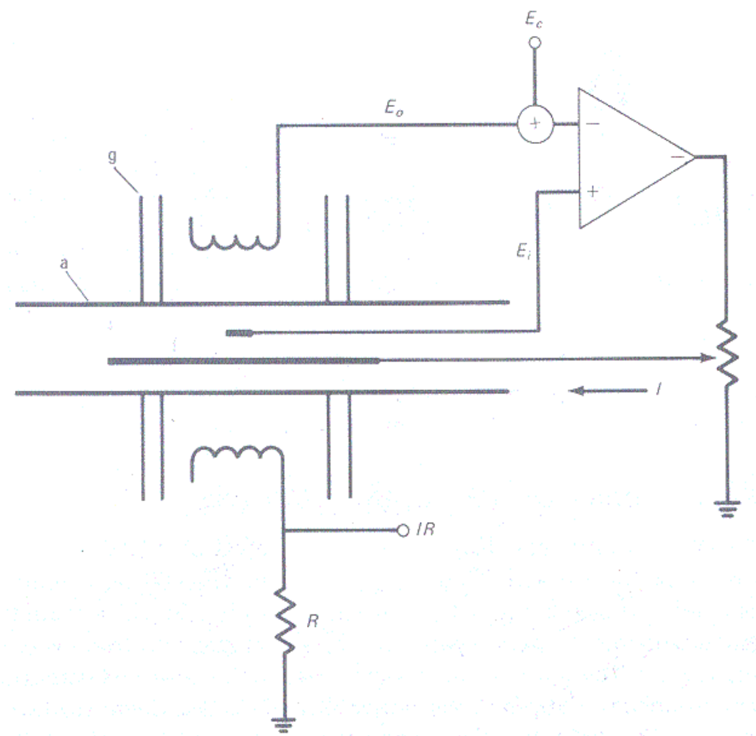
Ei-(Eo+Ec)=0, then

Ei – Eo = Ec, voltage clamping

Ei,Eo:membrane potential

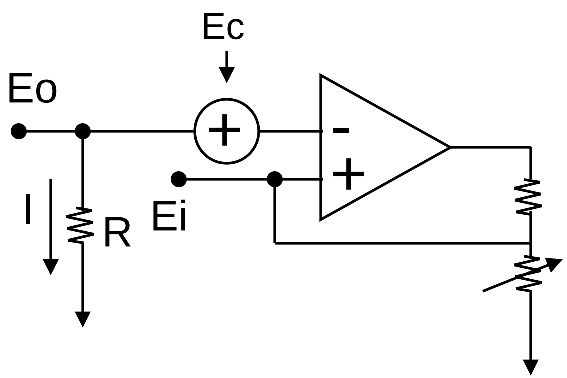
Ec : control voltage

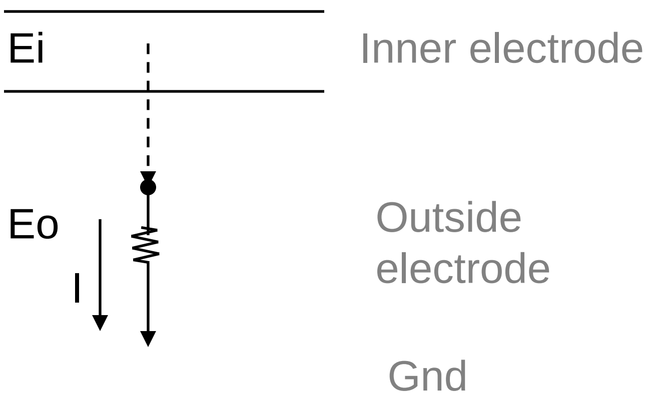
Then the current Eo/R is the current injected to maintain constant Vm(=Ei-Eo).



Voltage-clamp circuit for use with squid axon. a, axon; g, plastic guards. Uninsulated portion of internal voltage electrode indicated by short heavy line; uninsulated portion of current-injecting electrode indicated by long heavy line. E_i, internal potential; E_o, external potential; E_c, command potential. Feedback tends to make $E_i - E_o = E_c$. Injected current, I, measured as voltage drop across series resistor R.

4. Voltage clamp



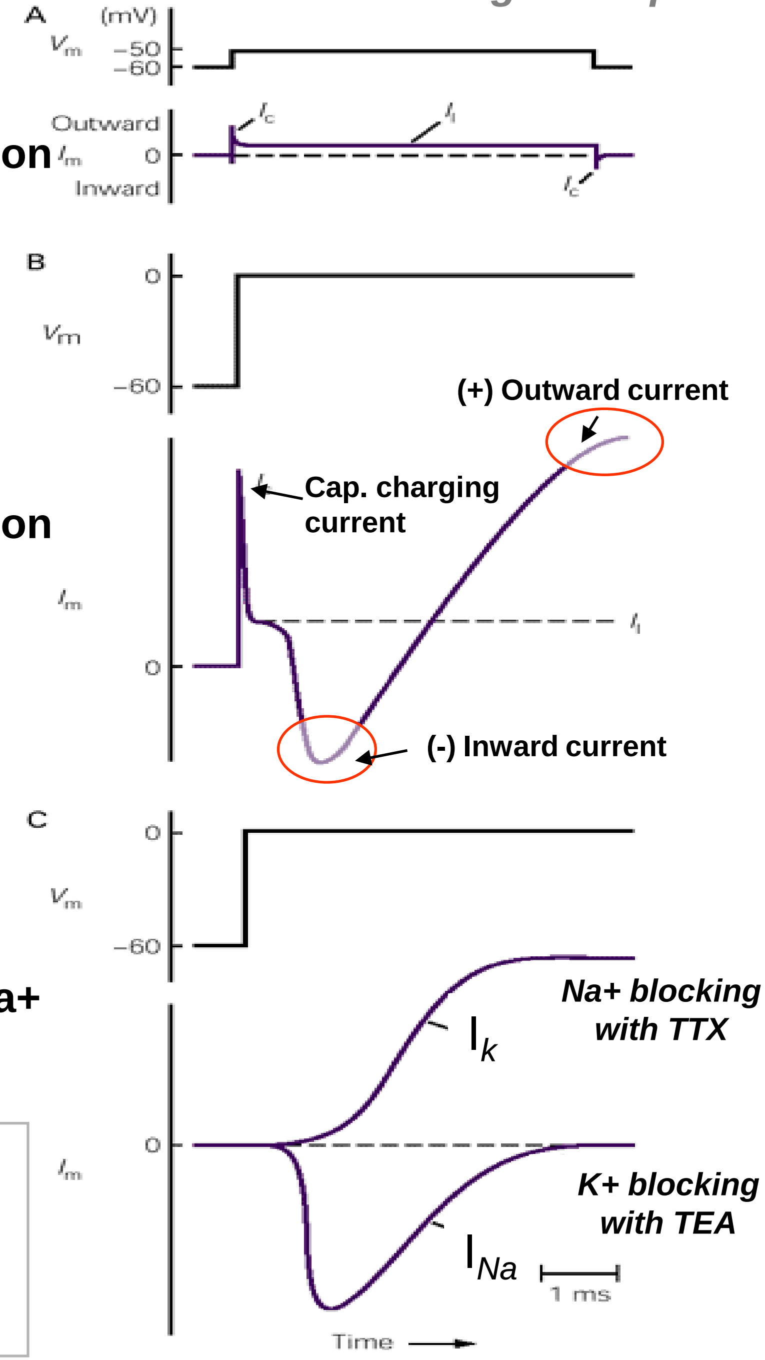


I : transmembrane current flowing to ground

Small depolarization

Large depolarization

Pure K+, Na+ current



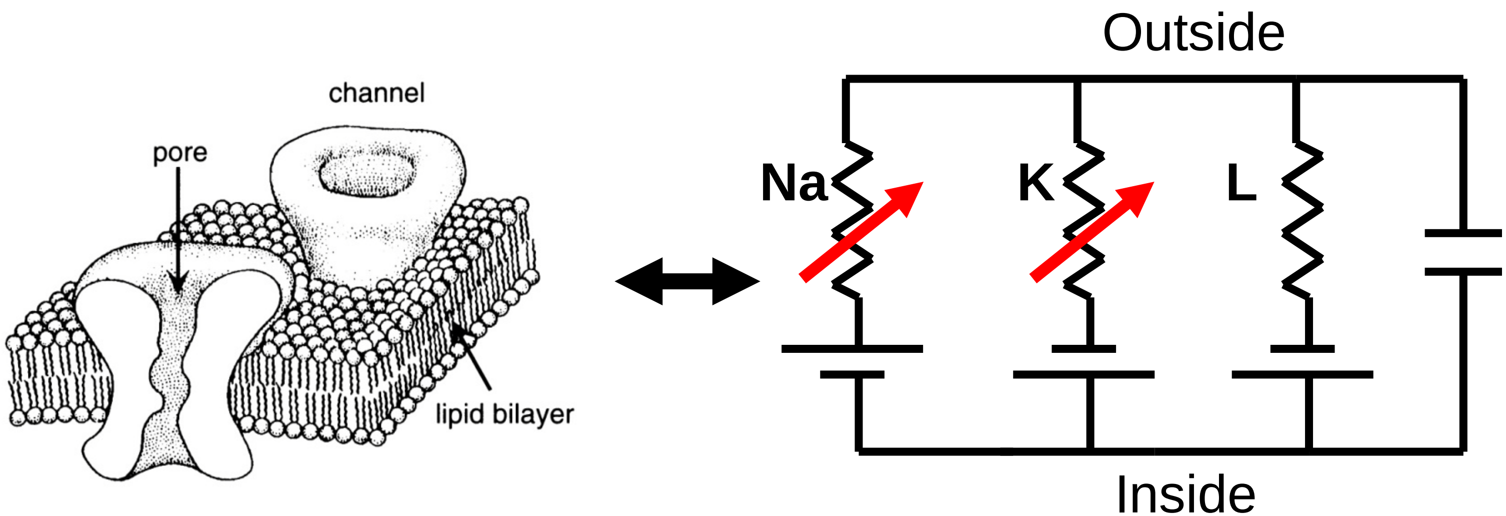
- I_c: Cap. charging current
- I_l: leakage current
- I_k : K+ current
- I_{Na}: Na+ current

Voltage Clamp

- The initial pulse is the displacement current through capacitor due to the step increase in voltage.
- The leakage current through passive gates.
- For all clamp voltage above threshold, the rate of onset for opening Na^+ channel is more rapid than for K^+ channels, and the Na^+ channels close after a period of time while K^+ channels remain open.

Neuron membrane model

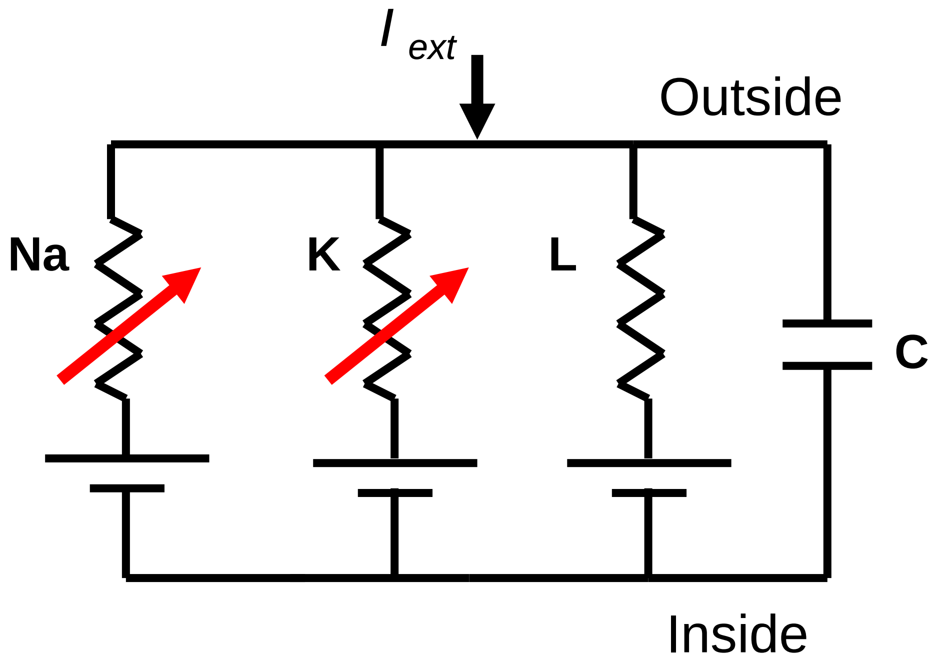
- Hodgkin Huxley model
  - In an active membrane, some conductances vary with respect to time and the membrane potential.



Currents in an Active Membrane

$$I_{ext} = I_{Na} + I_k + I_L + I_C$$

$$\left.\begin{aligned} &\text{Ohm's Law}: \mathrm{V} - \mathrm{V}_{\mathrm{Na}} = I_{Na} R_{Na} \\ &R_{Na} = \frac{1}{g_{Na}} \end{aligned}\right\} \Rightarrow$$



$$I_{Na} = g_{Na}(V - V_{Na})$$

$$I_k = g_k(V - V_k)$$

$$I_L = g_L(V - V_L)$$

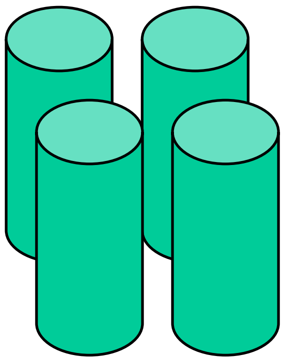
$$I_C = C\frac{dV}{dt}$$

$$I_{ext} = g_{Na}(V - V_{Na}) + g_K(V - V_K) + g_L(V - V_L) + C\frac{dV}{dt}$$

$$C\frac{dV}{dt} = g_{Na}(V_{Na} - V) + g_K(V_K - V) + g_L(V_L - V) + I_{ext}$$

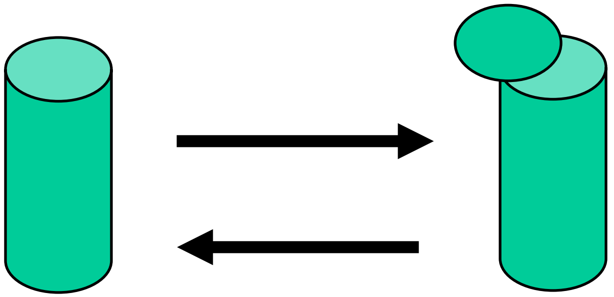
The Potassium Channel

- The potassium has 4 similar sub units



  - Each subunit can be either “open” or “closed”

Protein 3D
Configurations



  - The channel is open if and only if all 4 subunits are open.

The Potassium Channel

- The probability of a subunit being open: n
- The probability of the channel being open: n^4
- The conductance of a patch of membrane to K^+ when all channels are open: $\bar{g}_K$ (Constant obtained by experiments)
- The conductance of a patch of membrane to K^+ when the probability of a subunit being open is n: $g_k = \bar{g}_K n^4$

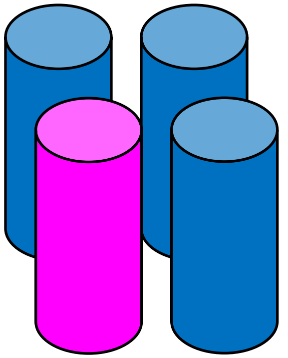
$$C\frac{dV}{dt} = g_{Na}(V_{Na} - V) + g_K(V_K - V) + g_L(V_L - V) + I_{ext}$$

↓

$$C\frac{dV}{dt} = g_{Na}(V_{Na} - V) + \bar{g}_K n^4 (V_K - V) + g_L(V_L - V) + I_{ext}$$

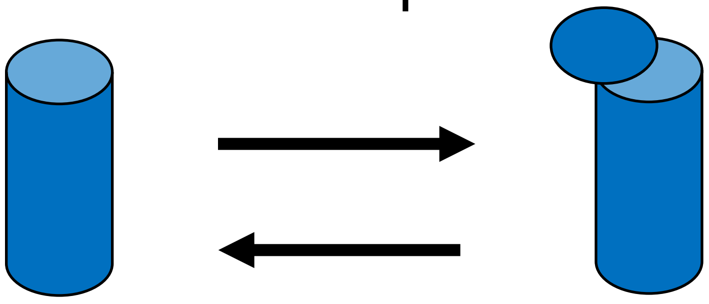
The Sodium Channel

- The potassium has 3 similar fast subunits and a single slow subunit



  – Each subunit can be either "open" or "closed"

Protein 3D Configurations



  – The channel is open if and only if all 4 subunits are open

The Sodium Channel

- The probability of a fast subunit being open: *m*
- The probability of a slow subunit being open: *h*
- The probability of the channel being open: $m^3 h$
- The conductance of a patch of membrane to Na^+ when all channels are open: $\bar{g}_{Na}$ (Constant obtained by experiments)
- The conductance of a patch of membrane to Na^+ :

$$g_{Na} = \bar{g}_{Na}\, m^3 h$$

$$C\frac{dV}{dt} = g_{Na}(V_{Na} - V) + g_K(V_K - V) + g_L(V_L - V) + I_{ext}$$

↓

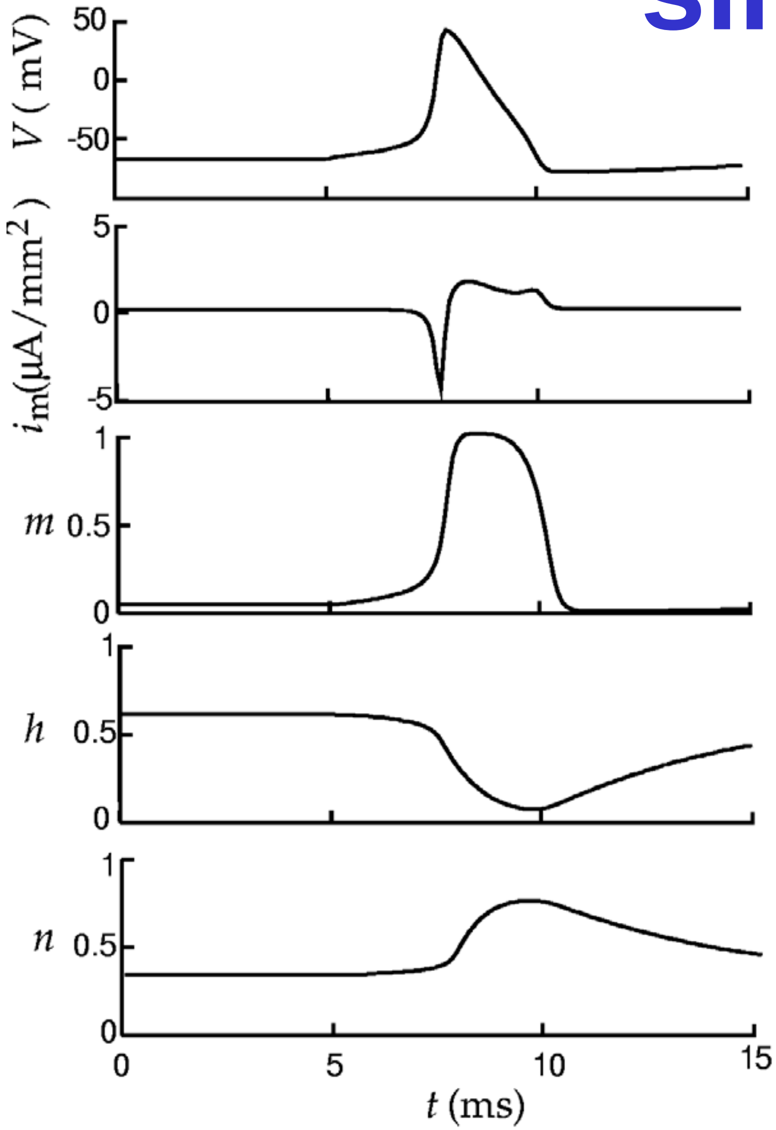
$$C\frac{dV}{dt} = \bar{g}_{Na}\, m^3 h\,(V_{Na} - V) + \bar{g}_K\, n^4\,(V_K - V) + g_L(V_L - V) + I_{ext}$$

Hodgkin Huxley model

$$C\frac{dV}{dt} = \bar{g}_{Na} m^3 h (V_{Na} - V) + \bar{g}_K n^4 (V_K - V) + g_L (V_L - V) + I_{ext}$$

- HH model in single compartment form adds a persistent K and transient Na channel to the simple leakage model
- Simulation is necessary due to the nonlinear nature of n and m.
- APSIM is a VB (virtual basic) based software that simulates action potentials.

Hodgkin Huxley model simulation



- Initial rise in V is due to current injected at t=5ms which drives current up to about –50mV
- At this point *m* rises sharply to almost 1 while *h* is also, transiently, non-zero.
- This causes an influx of Na^+ ions and a large rapid depolarisation to about 50mV due to +ve feedback because *m* increases with V
- However, increasing V causes *h* to decrease shutting off Na current
- Also, *n* increases activating K^+ channels and ion flow outward
- Finally, values return to initial values

Any other changes in the neuron during APs? Cellular Volume Change

Action potential

Na^+ influx & K^+ efflux

↓

Change in $[Na^+ + K^+]_i$

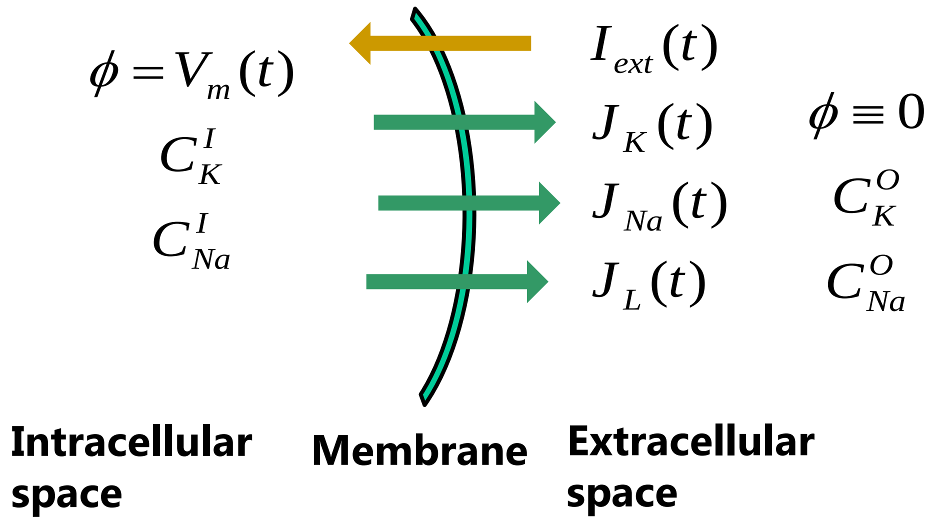
↓

H_2O flux by diffusion

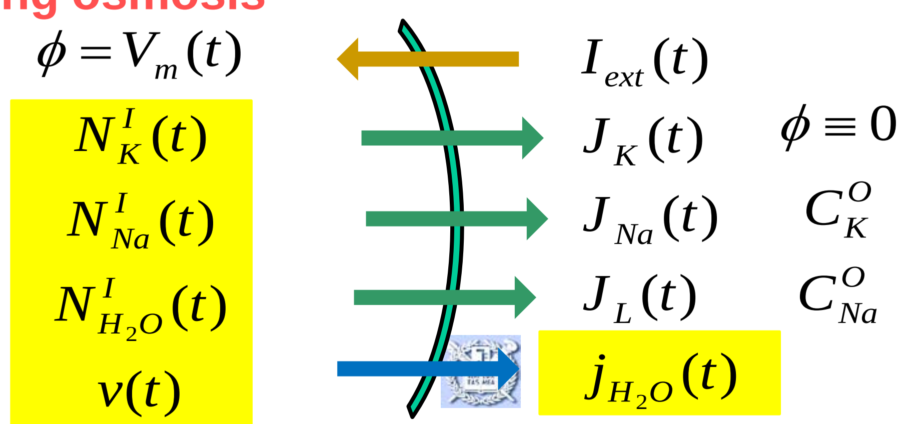
↓

Cellular volume change

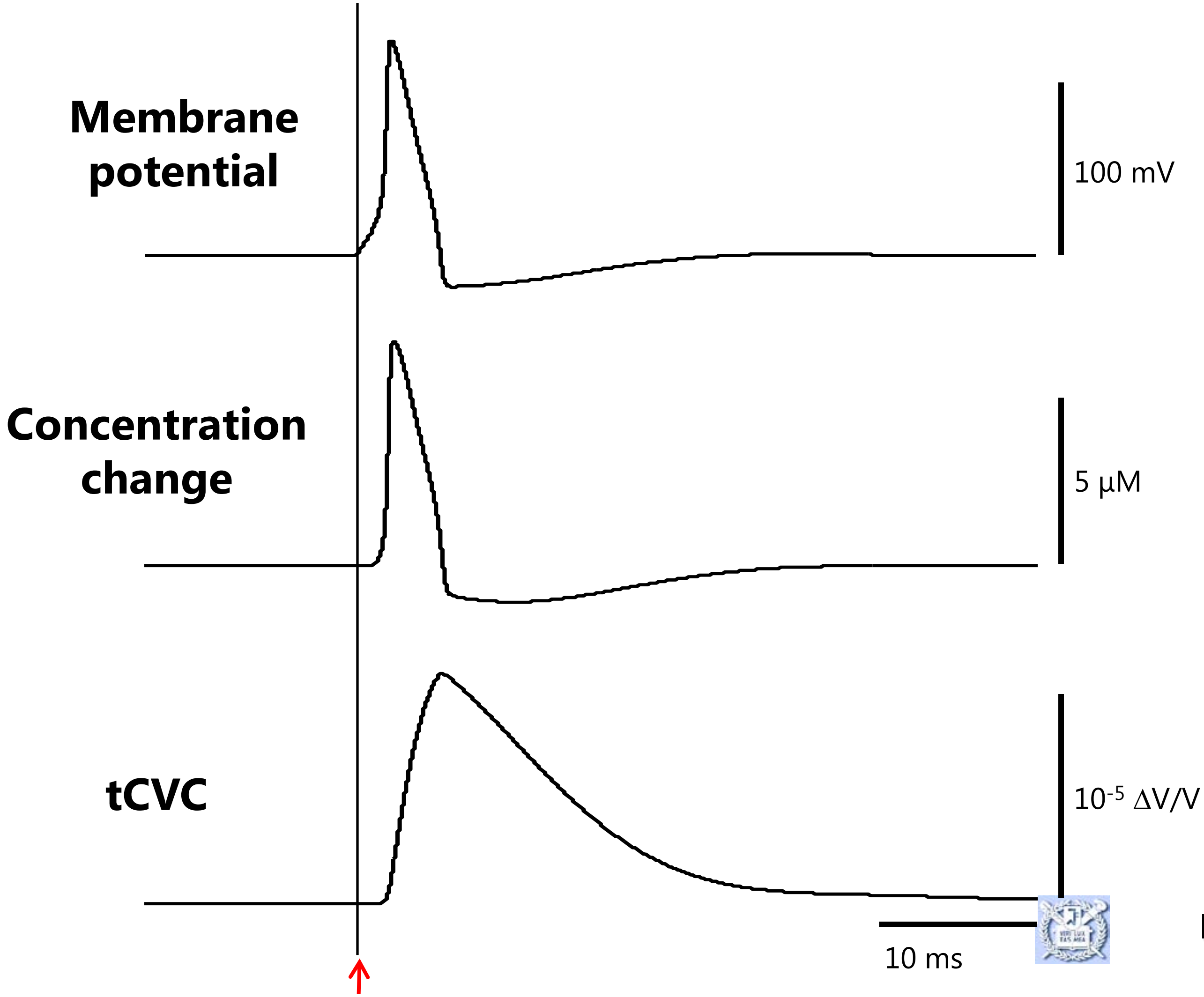
Conventional H-H model



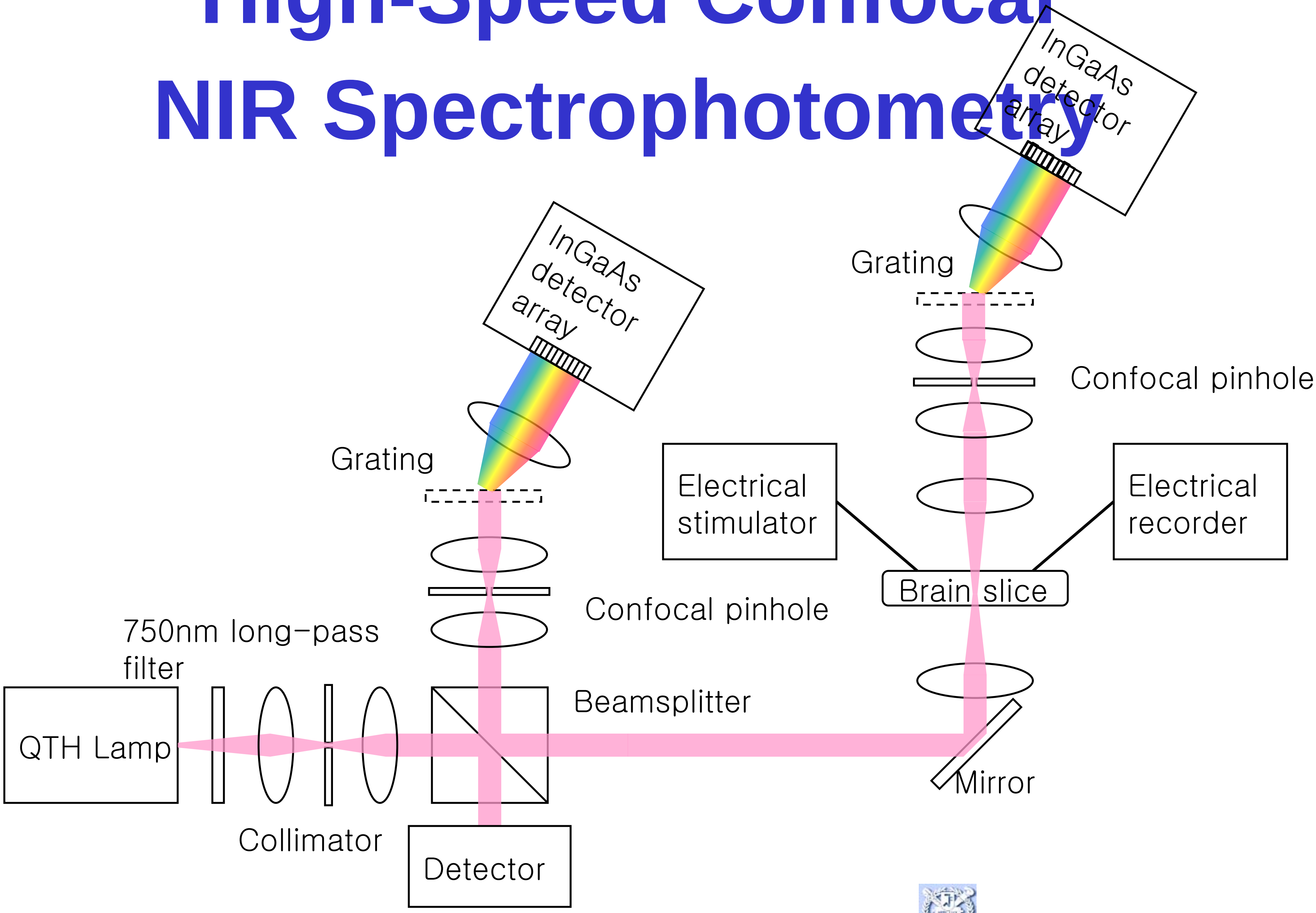
Including osmosis

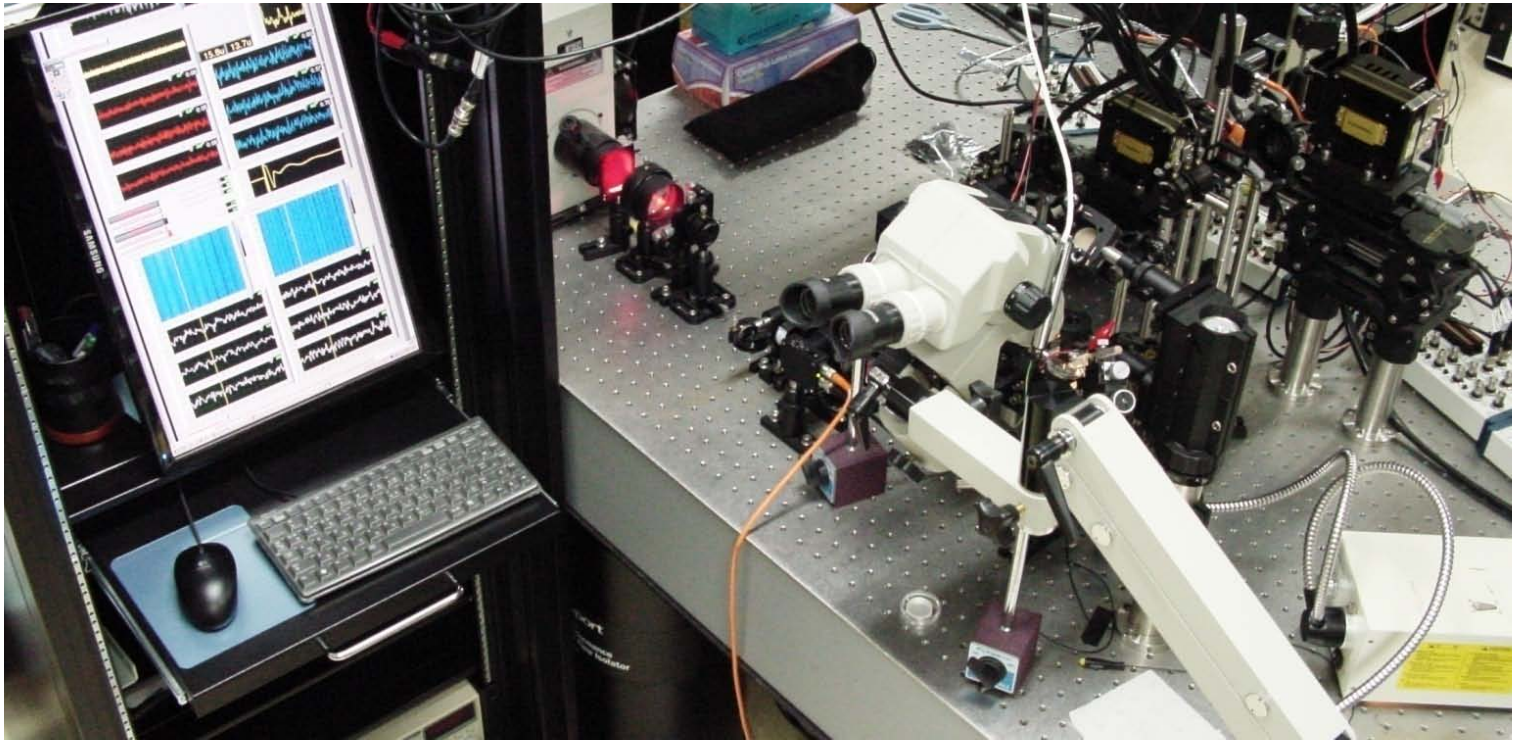


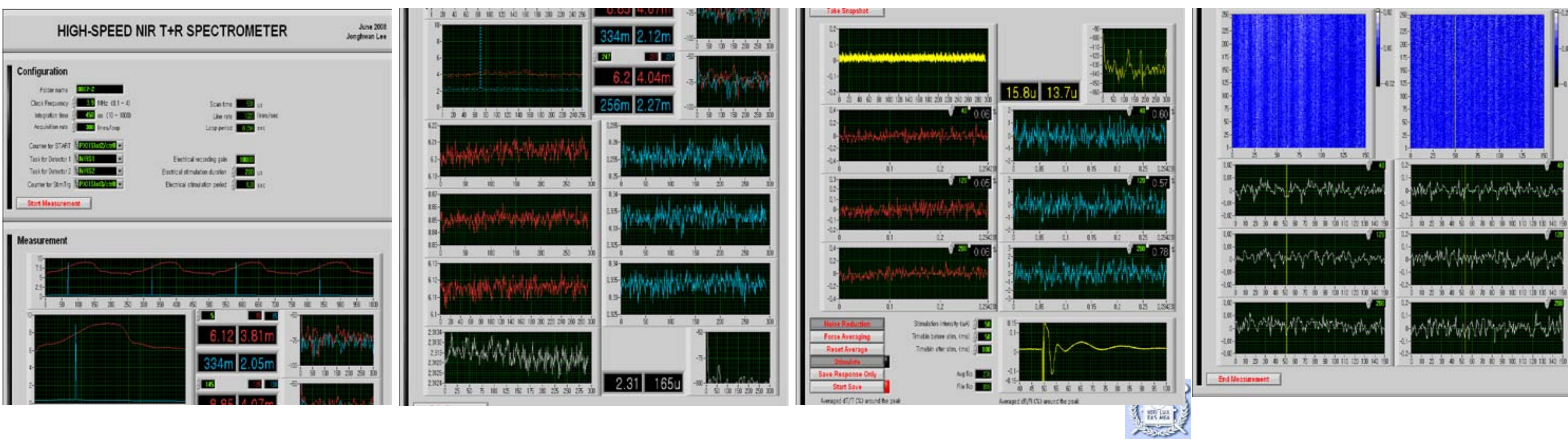
Transient Cellular Volume Change (tCVC) results



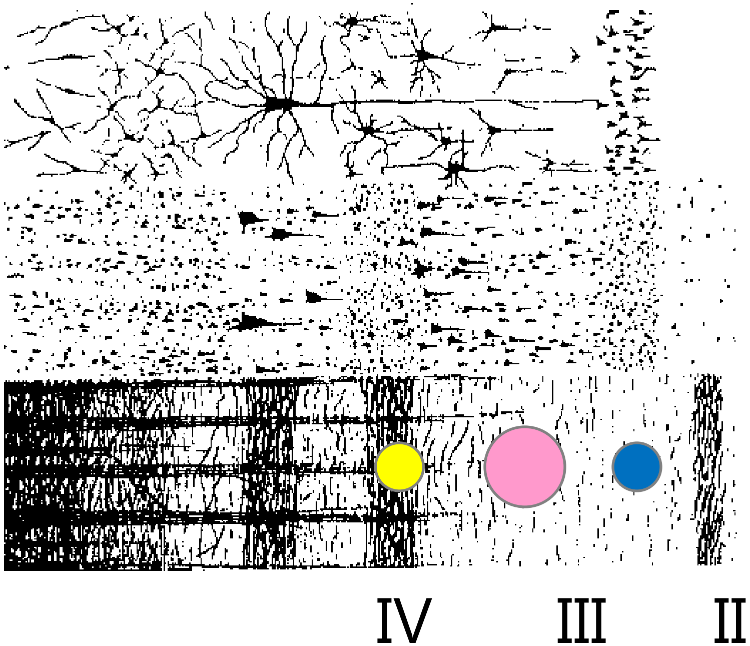
High-Speed Confocal NIR Spectrophotometry

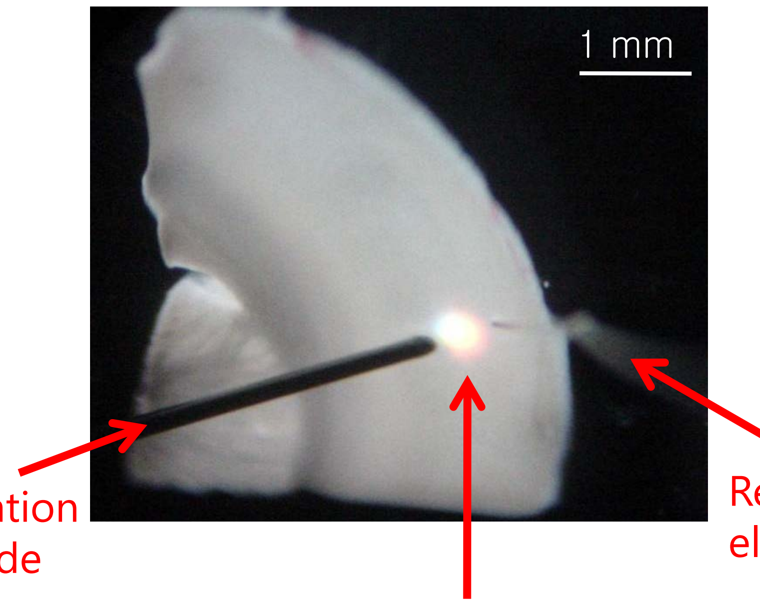





HIGH-SPEED NIR T+R SPECTROMETER
June 2008
Jonghwan Lee
Configuration
Start Measurement
Measurement
Take Snapshot
Noise Reduction
Force Averaging
Reset Average
Stimulate
Save Response Only
Start Save
End Measurement

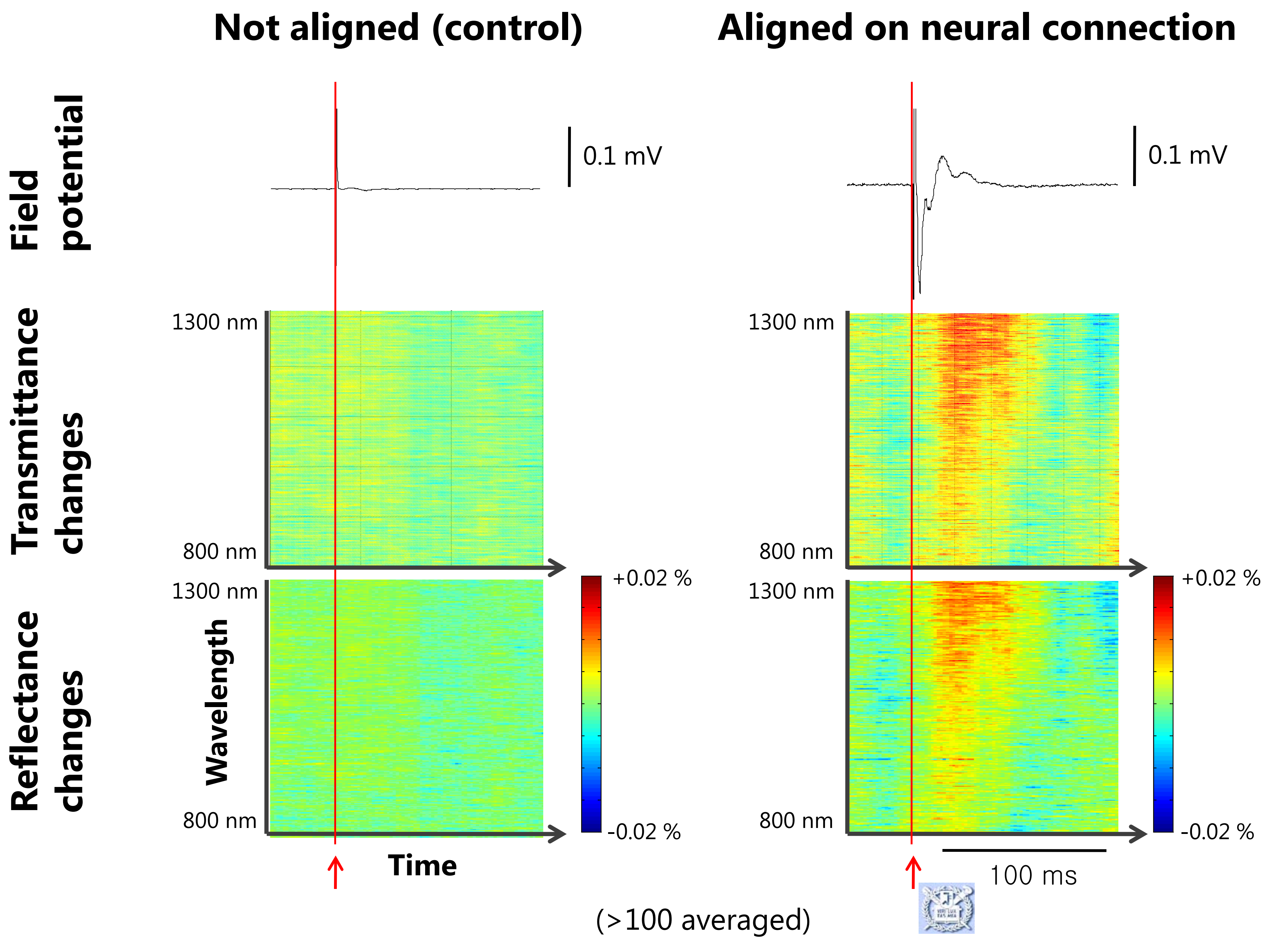
Rat Cortical Slices





- Electrical stimulation
- Optical recording
- Electrical recording

Heimer



Not aligned (control)
Aligned on neural connection
Field potential
Transmittance changes
Reflectance changes
0.1 mV
0.1 mV
1300 nm
800 nm
1300 nm
800 nm
1300 nm
800 nm
1300 nm
800 nm
Wavelength
+0.02 %
-0.02 %
+0.02 %
-0.02 %
Time
100 ms
(>100 averaged)