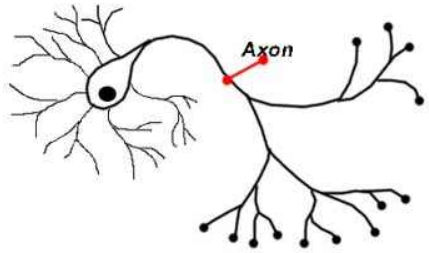
3. Bioelectric Phenomena
3-2 Hodgkin-Huxley Model
Ion Channels
Recent Tools

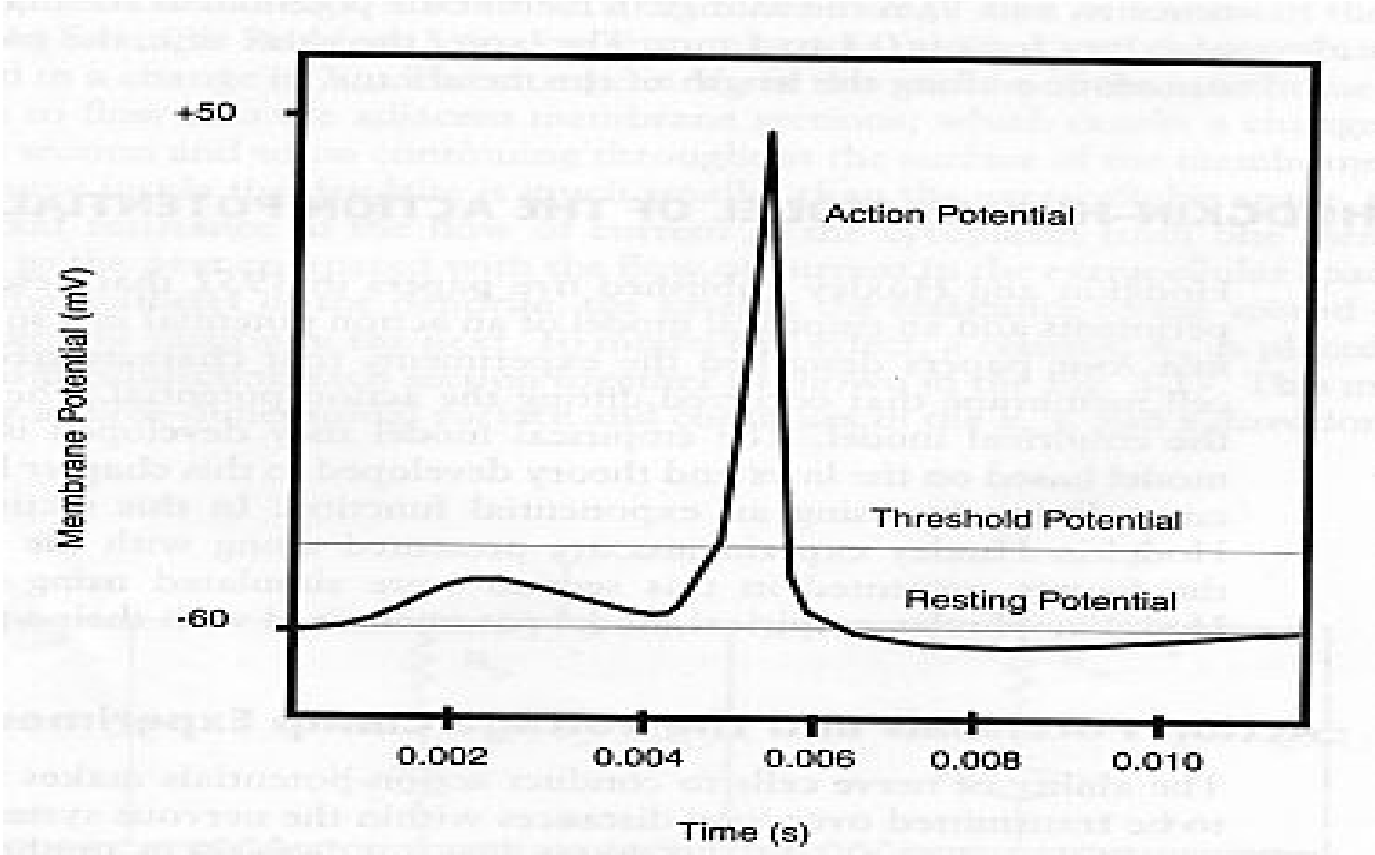
Hodgkin-Huxley model of the Action Potential

-An action potential does not decrease in amplitude as it is conducted away from its site of initiation.(all or none)

- An action potential occurs when V_m reaches the threshold potential at the axon hillock.



- Once V_m reaches threshold, time- and voltage-dependent conductance changes occur in the active Na^+ and K^+ gates that drive V_m toward E_{Na}, then back to E_K, and finally to the resting potential.



Stylized diagram of an action potential once threshold potential is reached at approximately 5 ms. The action potential is due to voltage- and time-dependent changes in conductance. The action potential rise is due to Na+ and the fall is due to K+ conductance change.

- The action potential moves through the axon at high speeds and appears to jump from one Node of Ranvier to the next in myelinated neurons. (Saltatory Conduction)

- This occurs because the membrane capacitance of the myelin sheath is very small, making the membrane appear only resistive with almost instantaneous changes in Vm possible.

- To investigate the action potential, Hodgkin and Huxley used an unmyelinated squid giant axon in their studies because of its large diameter and long survival time of several hours in seawater at 6.3^{o}C

Voltage Clamp

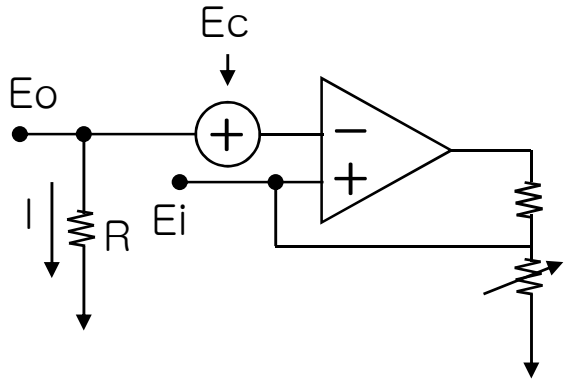
- The problem: two dynamic mechanisms in the channel during action potential: time and voltage
- Hodgkin and Huxley's contribution: set voltage constant and obtain the time dependent changes of ionic currents during action potential
- Method: Remove space variable(space clamp) and voltage variable(voltage clamp) by inserting long inner electrode and setting transmembrane-voltage constant by feedback.
- Two active channels are studied: K+ and Na+ channels
- Separatation of K+and Na+ channels was done by selection of external solution.
-

Voltage clamping

- Let displacement current 0

  I = C(dV/dt) = 0

  By forcing a constant voltage,



Give feedback using a Potentiostat to make

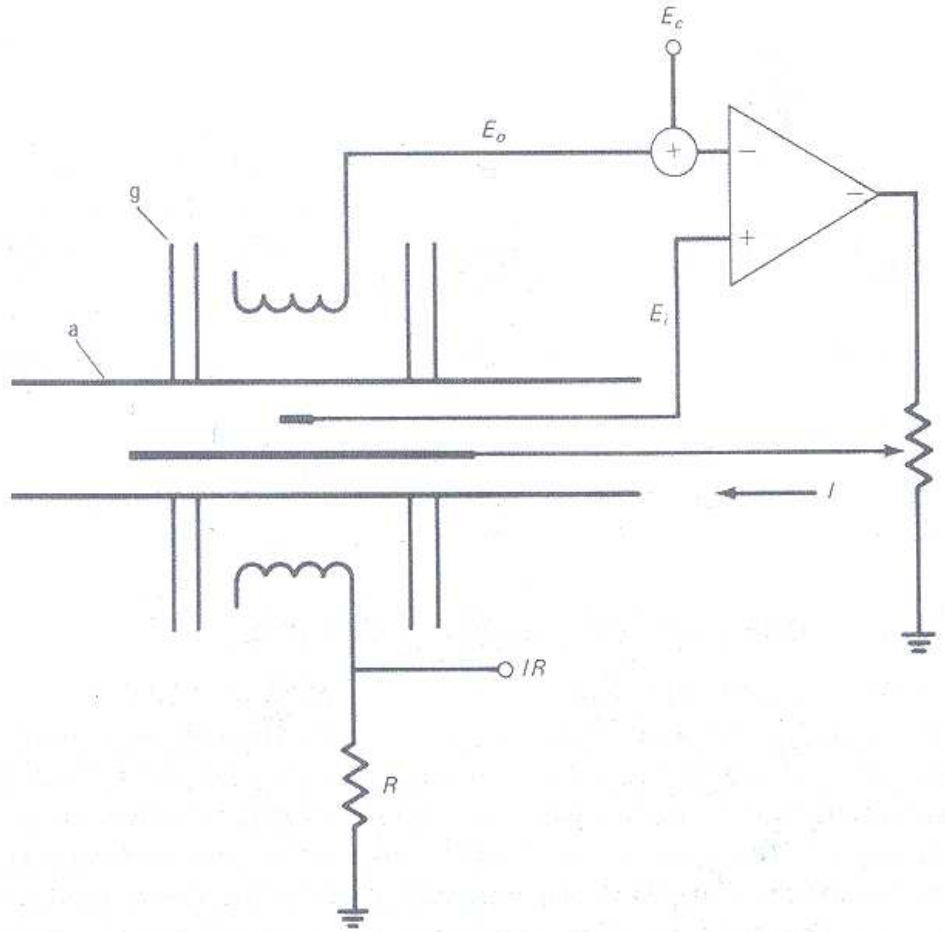
Ei−(Eo+Ec)=0, then

Ei – Eo = Ec: voltage clamping

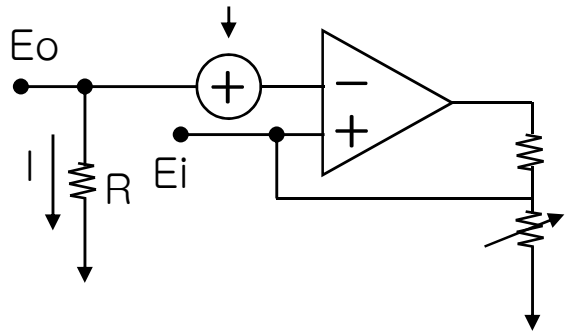
Ei,Eo:membrane potential

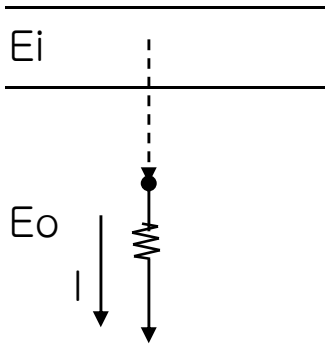
Ec : control voltage

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.



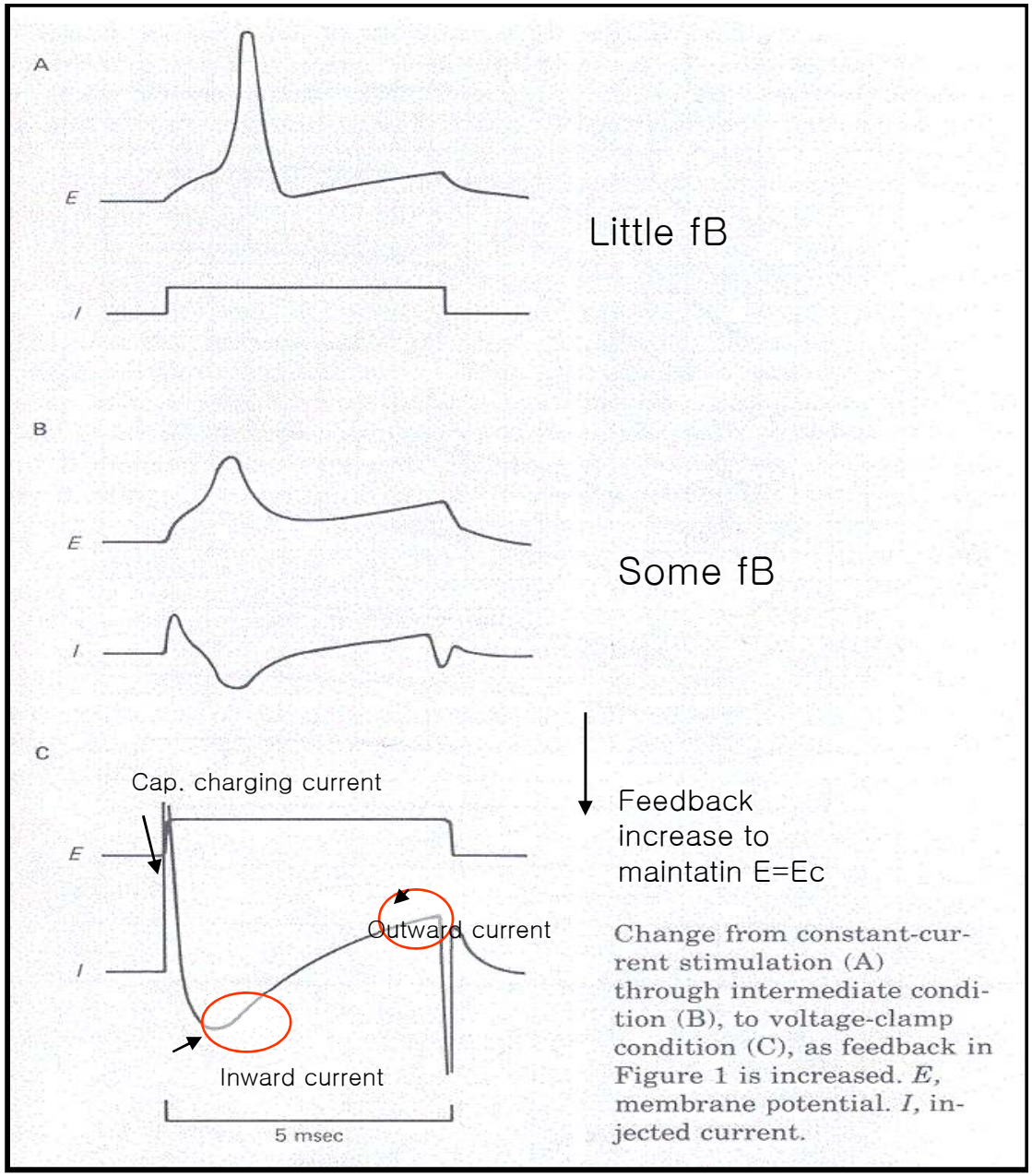


Inner electrode

outside electrode

Gnd

I : transmembrane current flowing to ground



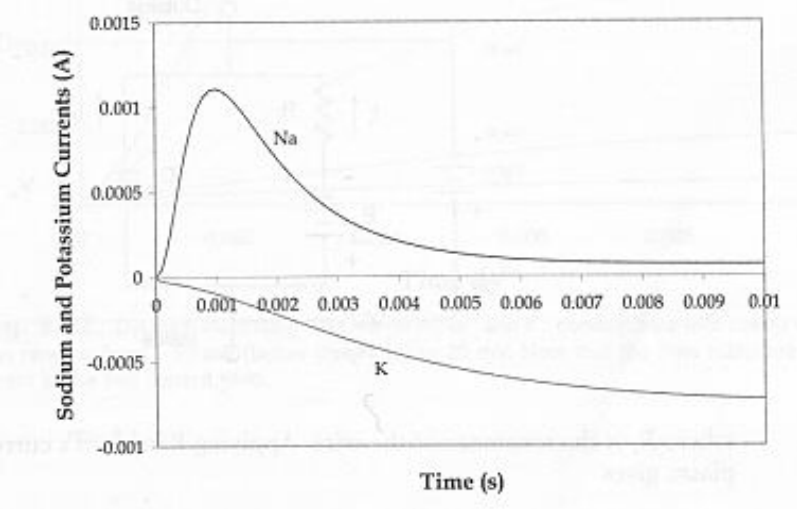
–The initial pulse is the displacement current through capacitor due to the step increase in voltage

– the leakage current through passive gates: $I_i = \frac{V_c - E_l}{R_l}$

This can be obtained by measuring the transmembrance current at below threshold(before activation of Na and K active gates)

–Subtracting both the capacitive and leakage current from Im leaves only the Na+ and K+ currents.

To separate the Na+ and K+ currents, they chose a Na+ free solution (they substituted a large impermeable cation for Na+).



(The results after subtractions) Diagram illustrating sodium and potassium currents due to a –20mV voltage clamp

(inward current is positive)

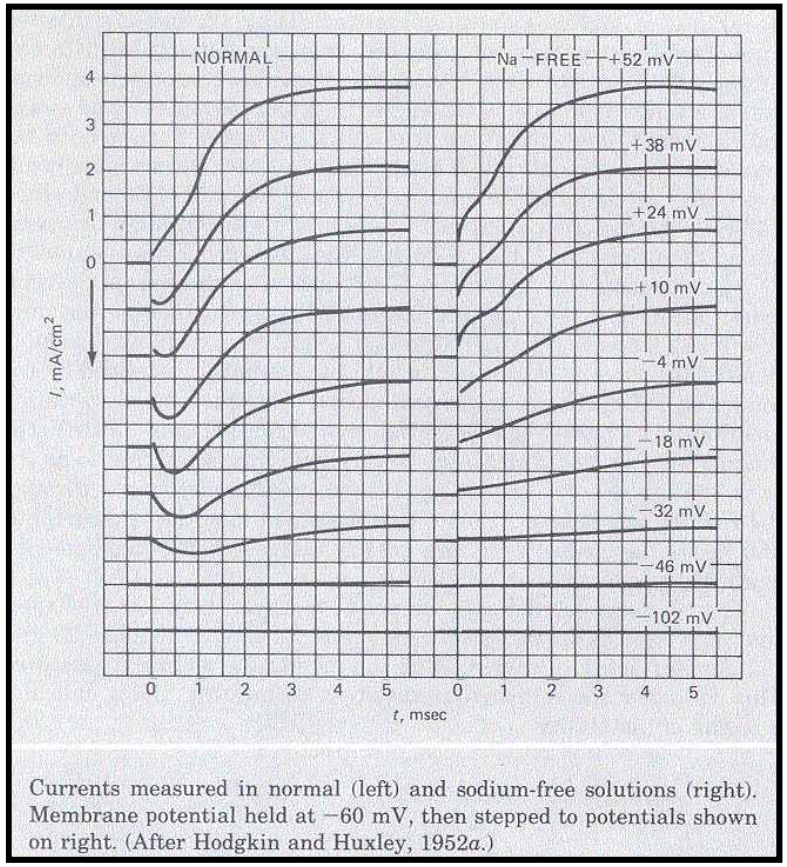
then to get G

$$I_K = \frac{V_m - E_K}{R_K} = G_K (V_m - E_K)$$

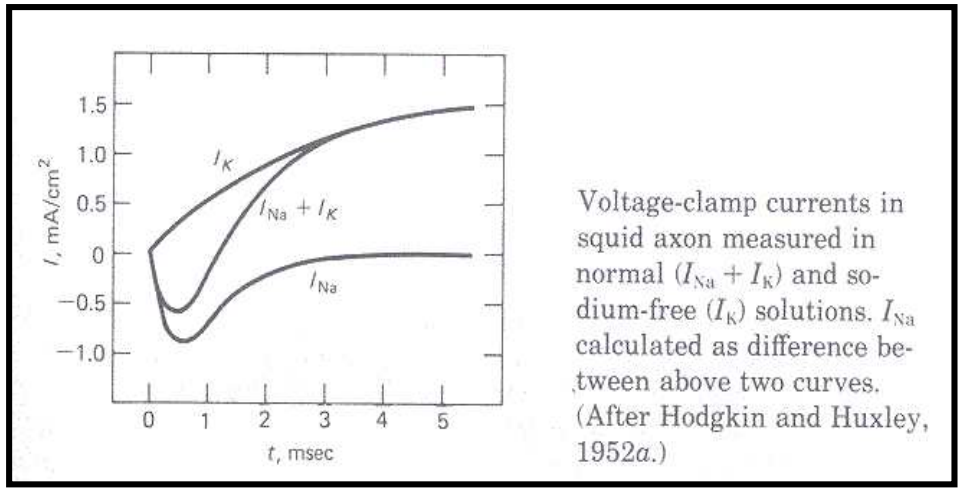
$$I_{Na} = \frac{V_m - E_{Na}}{R_{Na}} = G_{Na} (V_m - E_{Na})$$

- 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 while the voltage clamp is maintained.
- Once the Na^+channels close, they cannot be opened until the membrane has been hyperpolarized to its resting potential. The time spent in the closed state is called the refractory period.

Normal vs. Na free solution



Currents measured in normal (left) and sodium-free solutions (right). Membrane potential held at −60 mV, then stepped to potentials shown on right. (After Hodgkin and Huxley, 1952*a*.)



Voltage-clamp currents in squid axon measured in normal ($I_{Na} + I_K$) and sodium-free (I_K) solutions. I_{Na} calculated as difference between above two curves. (After Hodgkin and Huxley, 1952*a*.)

They did it for many voltages

(inward current is negative)

The independent Na+ and K+ channels

- From the Voltage clamp:

"The ionic current is divided into the initial Na+ influx and the later K+ outflux and these are independent."

- Later, these have been verified through experiments using toxins: tetrodotoxin(TTX)(Na block) and tetraethylammonium(TEA)(K block)

Equations Describing G_{Na} and G_K

The Differential Rate Equations

$$(1-m) \underset{\beta_m}{\overset{\alpha_m}{\rightleftharpoons}} (m) \qquad \frac{dm}{dt} = \alpha_m \cdot (1-m) - \beta_m \cdot m$$

$$(1-h) \underset{\beta_h}{\overset{\alpha_h}{\rightleftharpoons}} (h) \qquad \frac{dh}{dt} = \alpha_h \cdot (1-h) - \beta_h \cdot h$$

$$(1-n) \underset{\beta_n}{\overset{\alpha_n}{\rightleftharpoons}} (n) \qquad \frac{dn}{dt} = \alpha_n \cdot (1-n) - \beta_n \cdot n$$

$$h(t) = h_\infty - (h_\infty - h_0) \cdot e^{-t/\tau_h}$$

- α, β : gate open or close rate

m, h, n are the fraction of open gates and (1-m), (1-h), (1-n) are fraction of closed gates.

- The rate constants α and β are solely functions of voltage and change instantaneously with any change in voltage.
- The gating parameters m,h, and n will change along exponential time courses. For example, the inactivation gates, h, will respond according to:

$$h(t) = h_s - (h_s - h_0) \exp(-t/\tau_h)$$

Here h_s is the steady-state value of h at the new voltage and h_0 is the steady-state value of h prior to the voltage-step. τ_h is the exponential time constant.

To explain the sigmoidal (S-shaped) kinetics of the rising phase of the sodium and potassium currents, Hodgkin and Huxley proposed that the Na channels were controlled by three activation (m) gates and the K channels were controlled by four activation (n) gates. By fitting the time courses of the Na and K conductances in response to voltage-clamp step changes in membrane voltage, Hodgkin and Huxley were able to obtain the values for the rate constants at different voltages from the following relations:

- Potassium

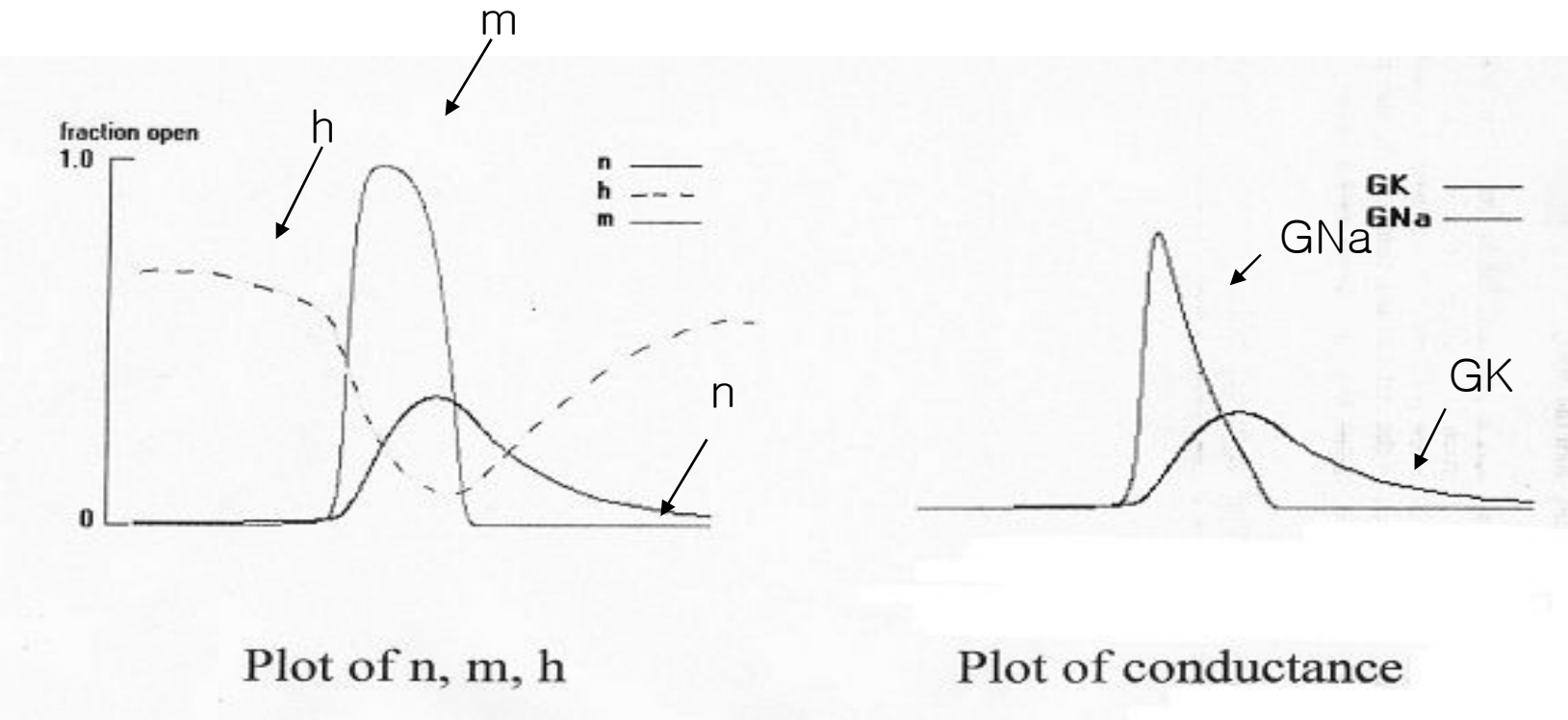
$$G_K = \overline{G}_K n^4$$

Sodium

$$G_{Na} = \overline{G}_{Na} m^3 h$$

i.e., maximum channel conductance multiplied by rate constants.

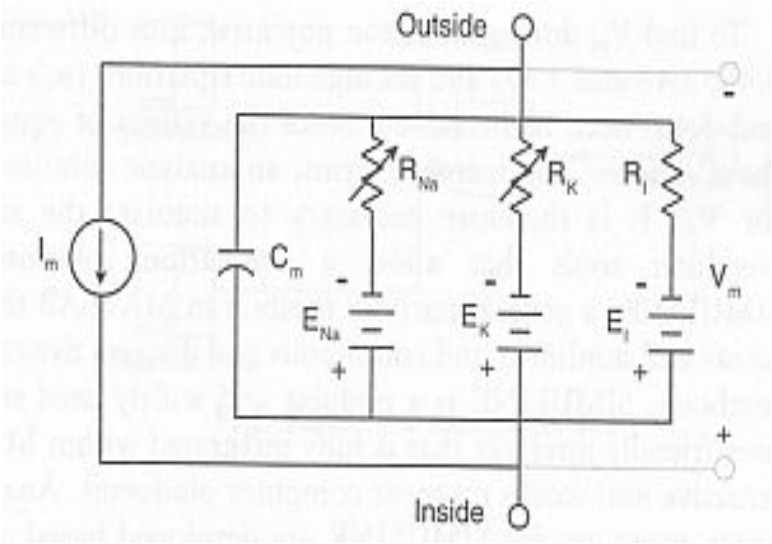
Hodgkin Huxley model



Circuit model and Equation
for the Time Dependence of the Membrane Potential

$$I_m = G_{\mathrm{K}}(V_m - E_{\mathrm{K}}) + G_{\mathrm{Na}}(V_m - E_{\mathrm{Na}}) + \frac{(V_m - E_l)}{R_l} + C_m\frac{dV_m}{dt}$$

(G_K and G_{Na}: the voltage-time-dependent conductance)



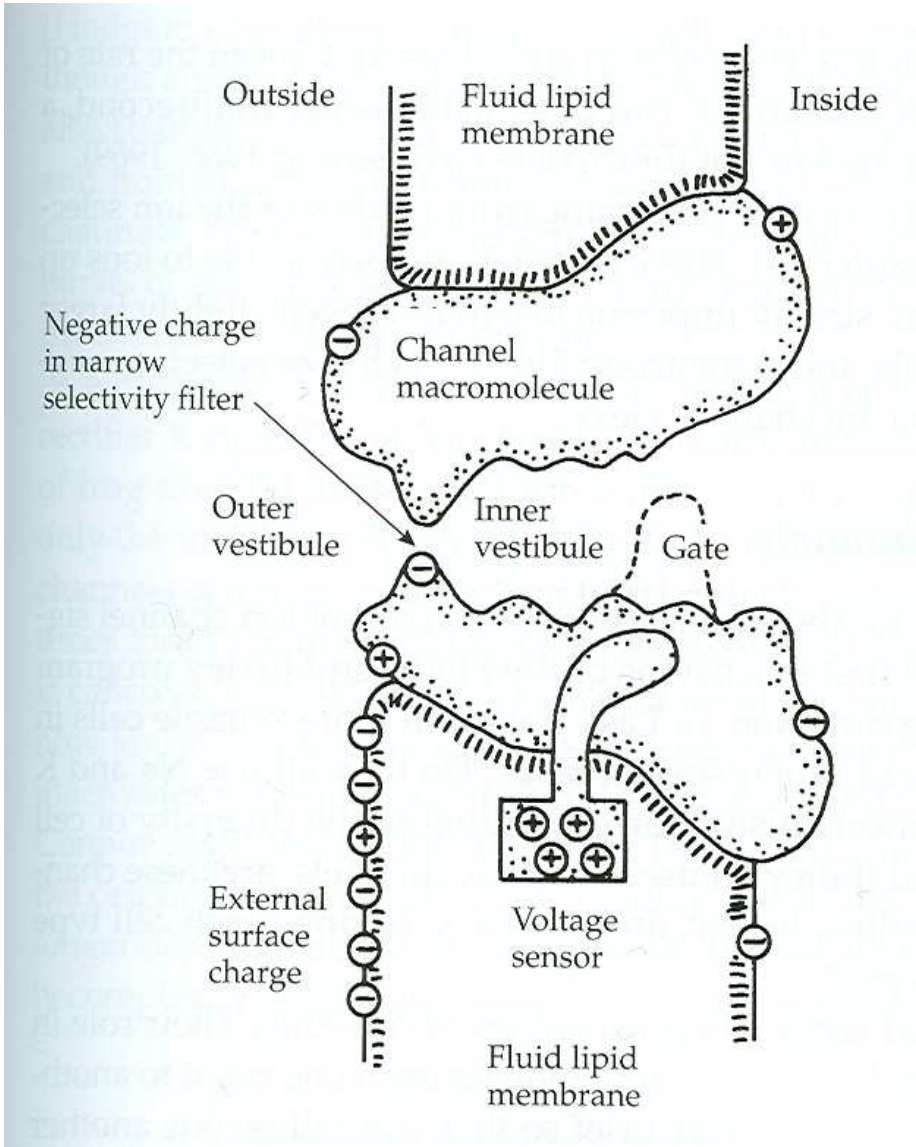
Circuit model of an unmyelinated section of squid giant axon..

- **We need to solve all differential equations regarding n, m and h including algebraic expression for alphas and betas.**
- **Analytic solution is not possible due to the nonlinear nature of n and m.**
- **Simulation is necessary**
- **APSIM is a VB (virtual basic) based software that simulates action potentials. It uses** a simple finite difference approximation to solve the series of differential equations.

Ion Channels

◆ Recent efforts to study Ion channels

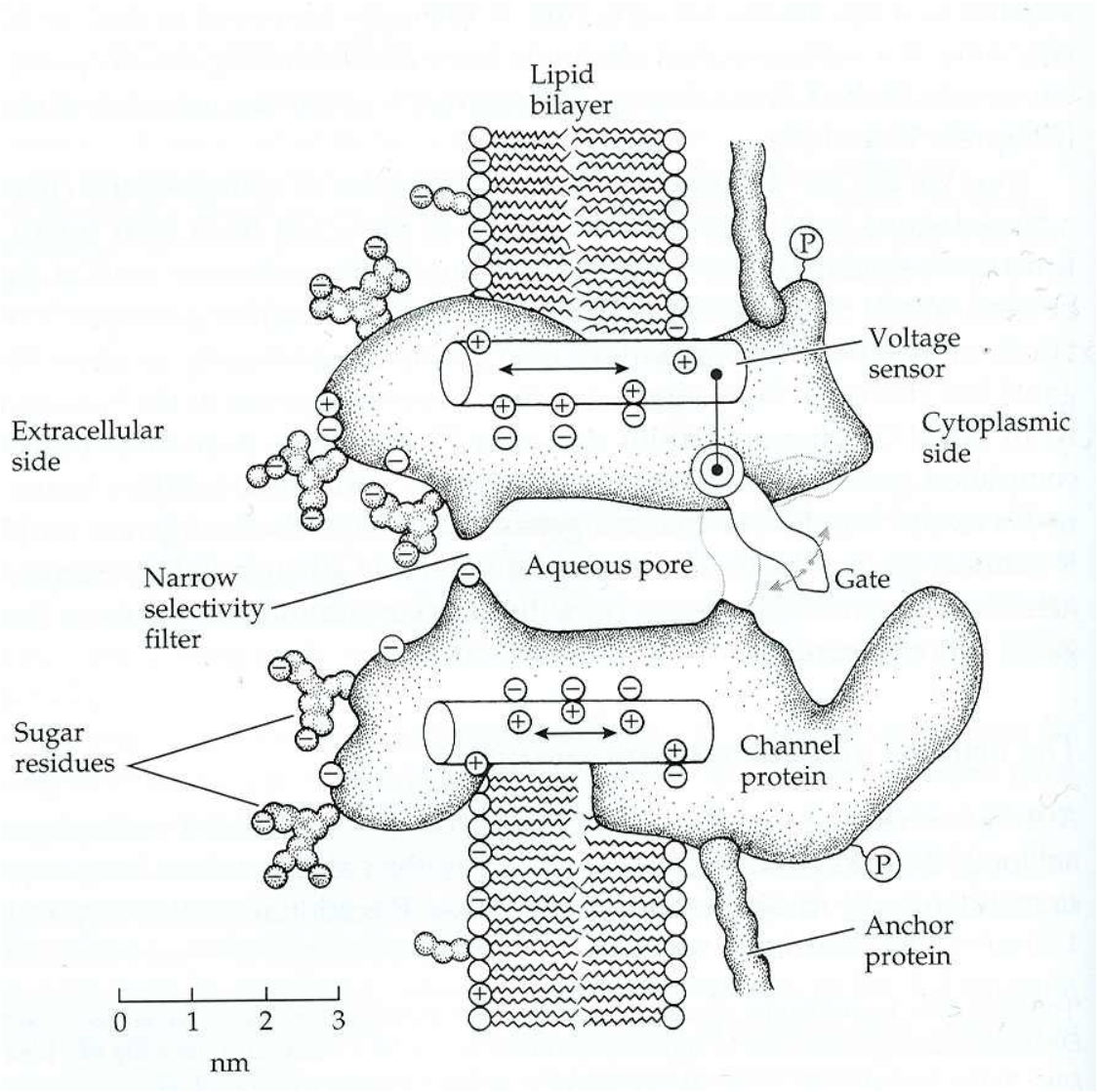
1. Hodgkin-Huxley Model(1952)
2. Discovery of Channel Blockers(1965~1975)
3. Patch-clamping (1981) revealed functional characteristics of various ion channels
4. Some of the molecular structures were made known by molecular biology and genetic technologies(late 1990's)
5. Channel staining in Immune cell chemistry helped locate the ion channels.(2000's)



From Ion Channels of Excitable Membranes, third edition, by Bertil Hille, Sinauer, 2001

1975: after pharmacology and biophysics experiments:

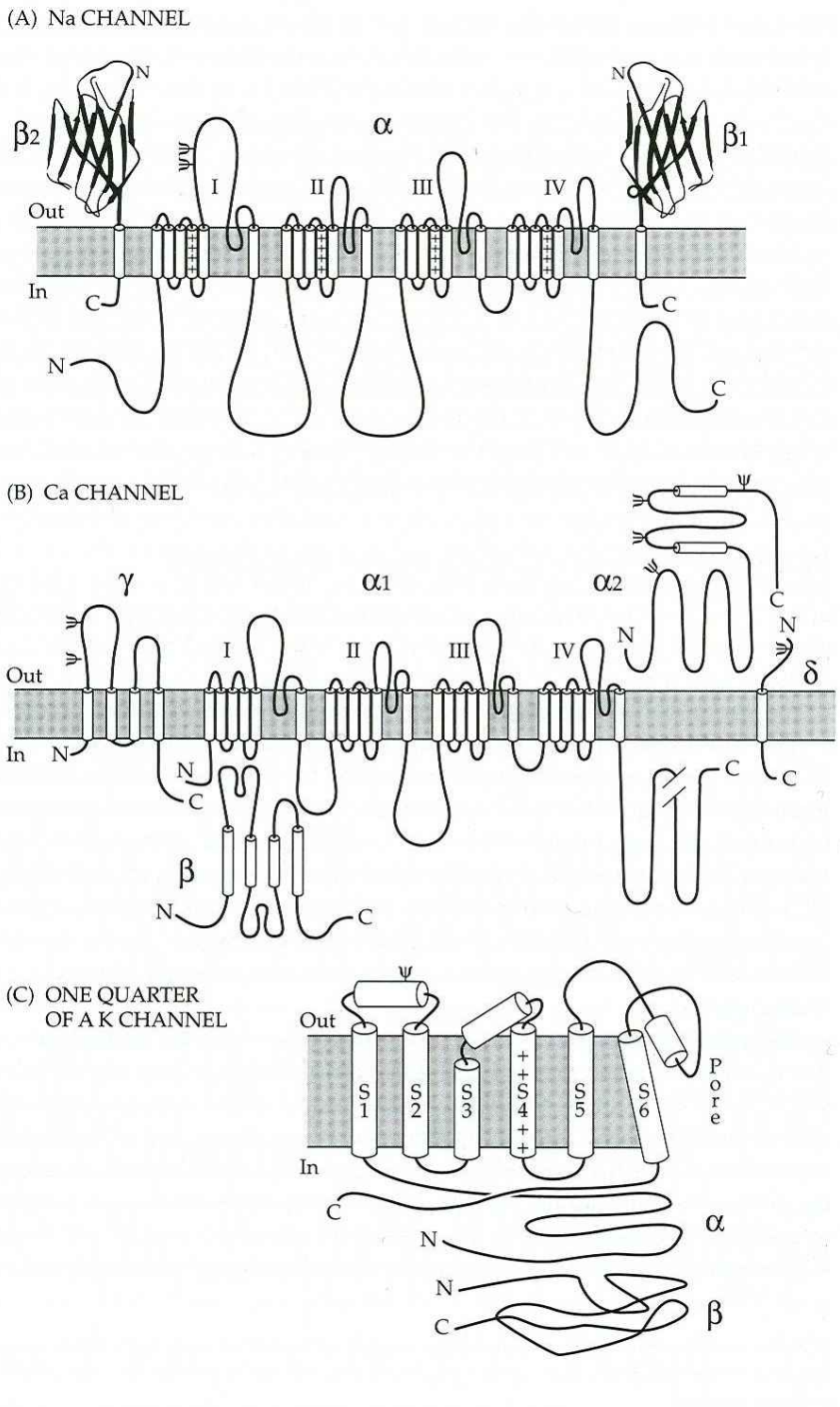
Fig. 3.6 Early Cartoon of a Voltage-Gated Channel
The channel is drawn as a transmembrane protein with a hole through the center and is labeled with terms in use in 1975. The functional regions – gate, voltage sensor, vestibules, and selectivity filter –were deduced from voltage-clamp experiments. This diagram was drawn with results from Na channels in mind. [From Hille 1977c.]



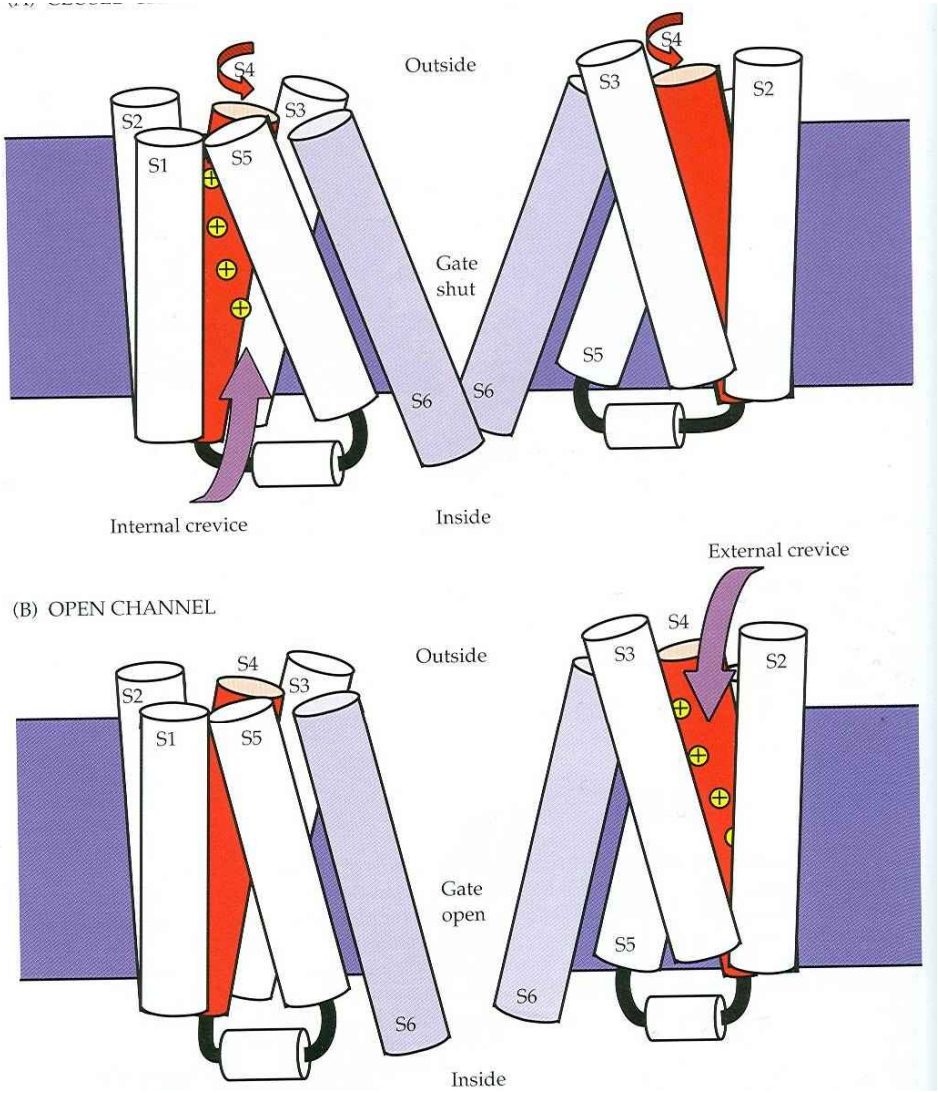
After gene array test and protein analysis, they learned that a long protein constitutes a channel. The chain penetrates membrane at least 24 times for Na or Ca channels. Four small loops act as selectivity filter for a specific ion.

Fig. 3.14 A 1991 Working Hypothesis for a Channel

Fifteen years after Figure 3.6 was drawn, we had learned that there is significant channel mass in the intracellular and extracellular spaces; that there are four alpha-helical segments with positively charged residues acting as voltage sensors; and that channels are heavily glycosylated and tied to other intracellular proteins. [From Hille 1992.]



This diagram shows the four loop filters. More important, it shows alpha and beta peptide chains. The Alpha-Peptide chain penetrates the membrane 24 times. They are grouped into four subunits numbered I/II/III/IV. Each group consists of 6 segments S1/S2/S3/S4/S5/S6. Among these S4 is the voltage sensor.

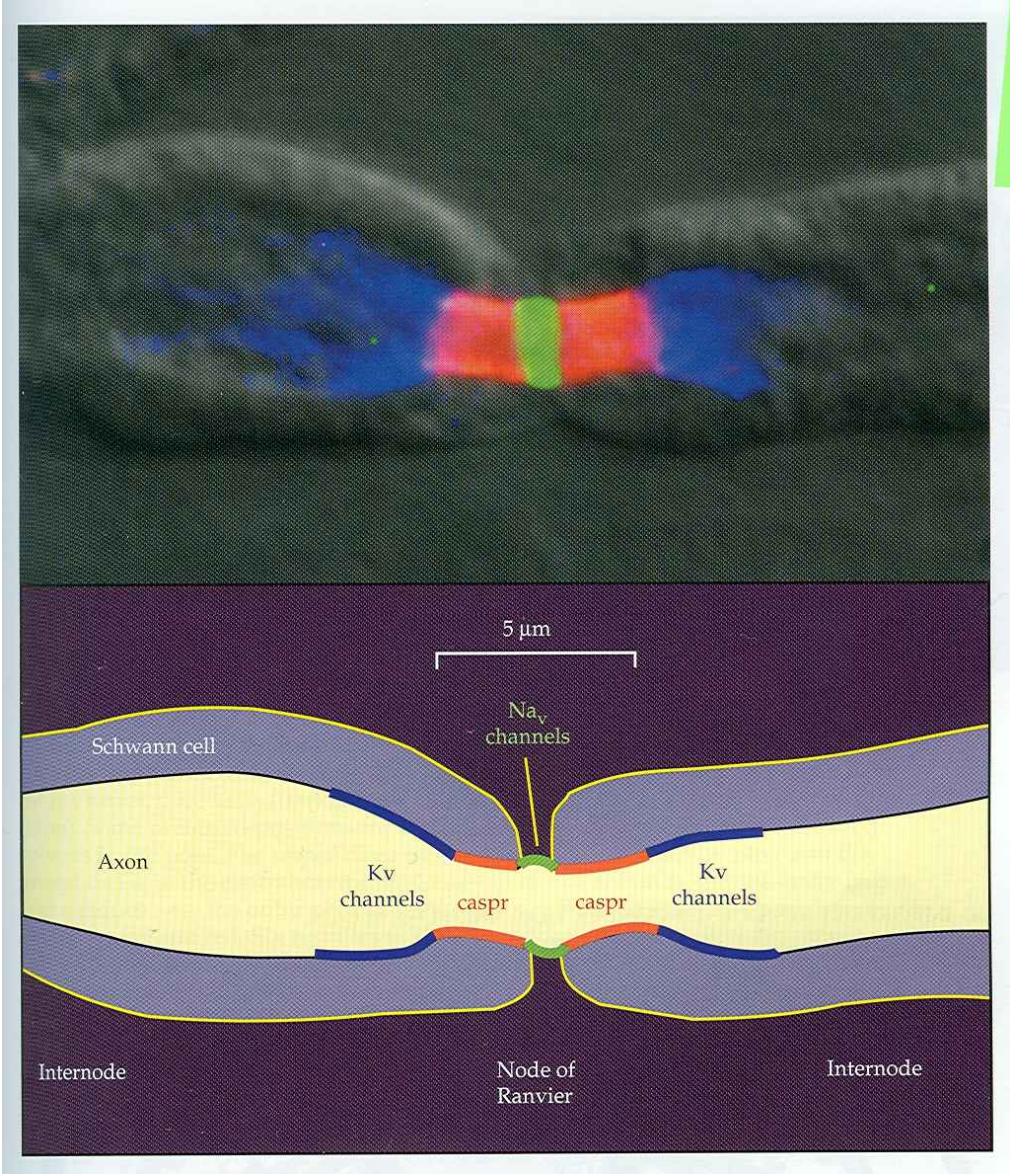


Voltage-gated channel의 S4 segment는 Voltage sensor의 많은 Gating charge를 운반한다. 이들은 Membrane potential의 변화에 반응해서 물리적으로 형태가 변화한다. Positive한 이들 Gating charge들은 Negative potential에서 세포 내부의 용액과 접촉하고 Positive potential에서는 외부의 용액과 접촉한다.

S1, S2, S3, S4로 구성되는 전체를 Voltage-sensing module이라고 하며, Channel이라 함은 이들 4개의 Transmembrane segment와 S5, S6 2개의 Transmembrane segment, 그리고 Core pore-forming complex인 P-loop를 말한다. S4가 주요 Voltage sensor이고 나머지 Module들은 선택적으로 세포 내, 외부로의 노출을 돕는 역할을 하는 정도라고 볼 수 있다.

Plate 7. Models for opening of a voltage-gated channel.

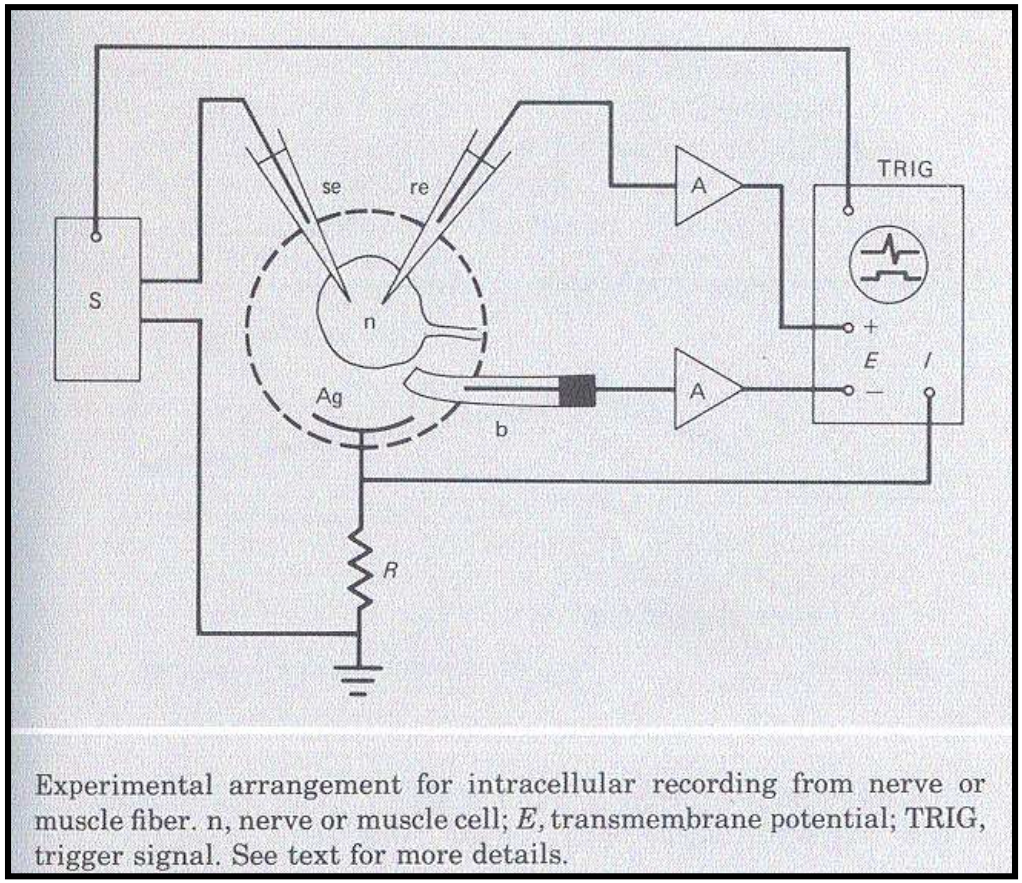
The model shows two of the four subunits of a K channel, each with its six transmembrane helices (cylinders S1 to S6). The S4 segments are red and their gating charges yellow. The narrow selectivity filter formed by P-loops is not shown. (A) The channel is closed by the S6 segments crossed at the inner end. In a closed channel, the gating charge is mostly exposed to the inner solution via the internal crevice (arrow). (B) During a depolarization, the S4 segments rotate and the gating charges shift into the external crevice (arrow). This motion also favors a movement of the S5 and S6 segments that opens the pore. Because of the crevices in this model, a rotation of S4 suffices for voltage sensing without any outward movement. [After Cha, Snyder et al. 1999.]



Myelination은 Na, K Channel을 새롭게 분포시키는데(Rasband and Shrager 2000), Na Channel은 Node of Ranvier에 집중적으로 분포한다. Myelin이 없는 경우보다 I_{Na} peak값이 10~15배에 이르고 Resting 상태에서의 Conduction도 10~100배에 이른다. K Channel도 Myelin으로 부분적으로 덮여있는 Node 주위에 많이 분포한다. Internode에는 대부분이 다양한 종류의 K Channel이고 Na Channel은 군데 군데 있는 정도이다. 이렇게 Node에 Channel들이 모여있는 것은 Protein scaffold, cytoskeletal elements, 복잡한 membrane protein들 때문이다. Plate 1은 Immunocytochemical 연구를 통해 얻은 것이다.

more on the Neural Recordings

Intracellular recording

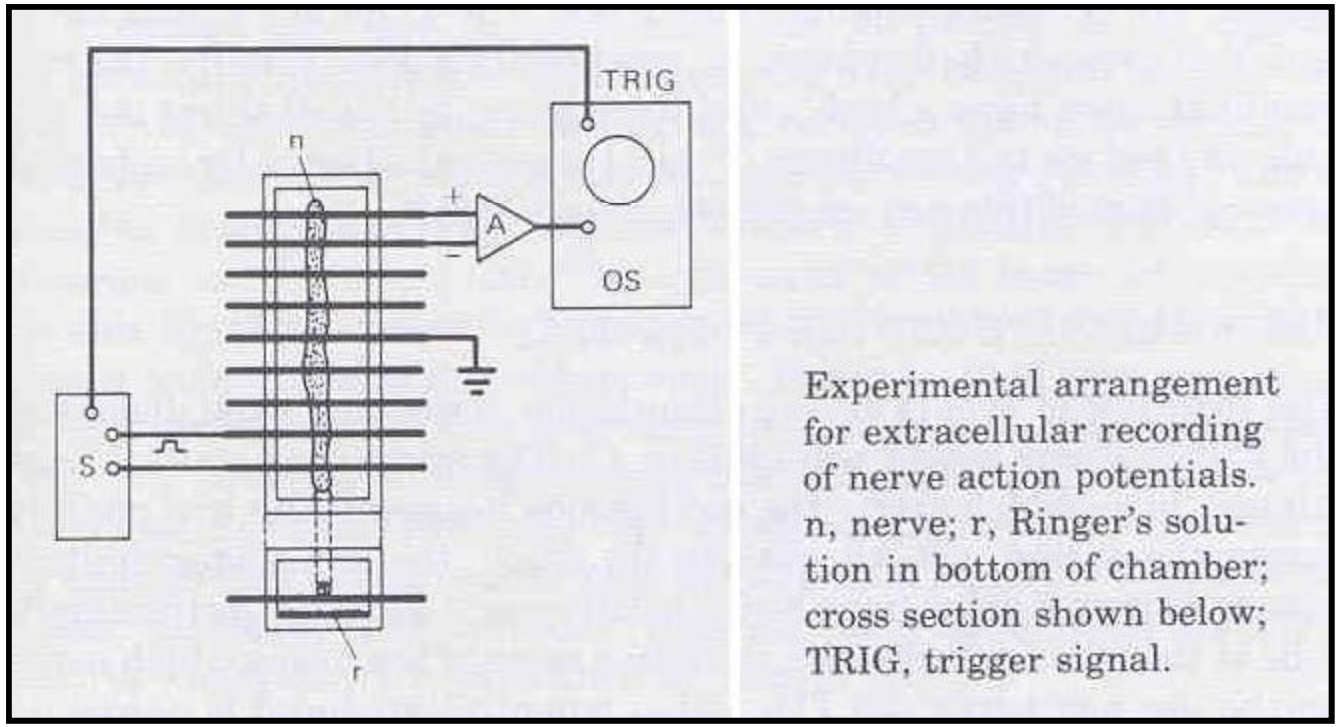


Experimental arrangement for intracellular recording from nerve or muscle fiber. n, nerve or muscle cell; *E*, transmembrane potential; TRIG, trigger signal. See text for more details.

- High Zin($10^{10}\Omega$), low gain DC Amp is needed.
  - Cf. high gain AC Amp is for extracellular work.

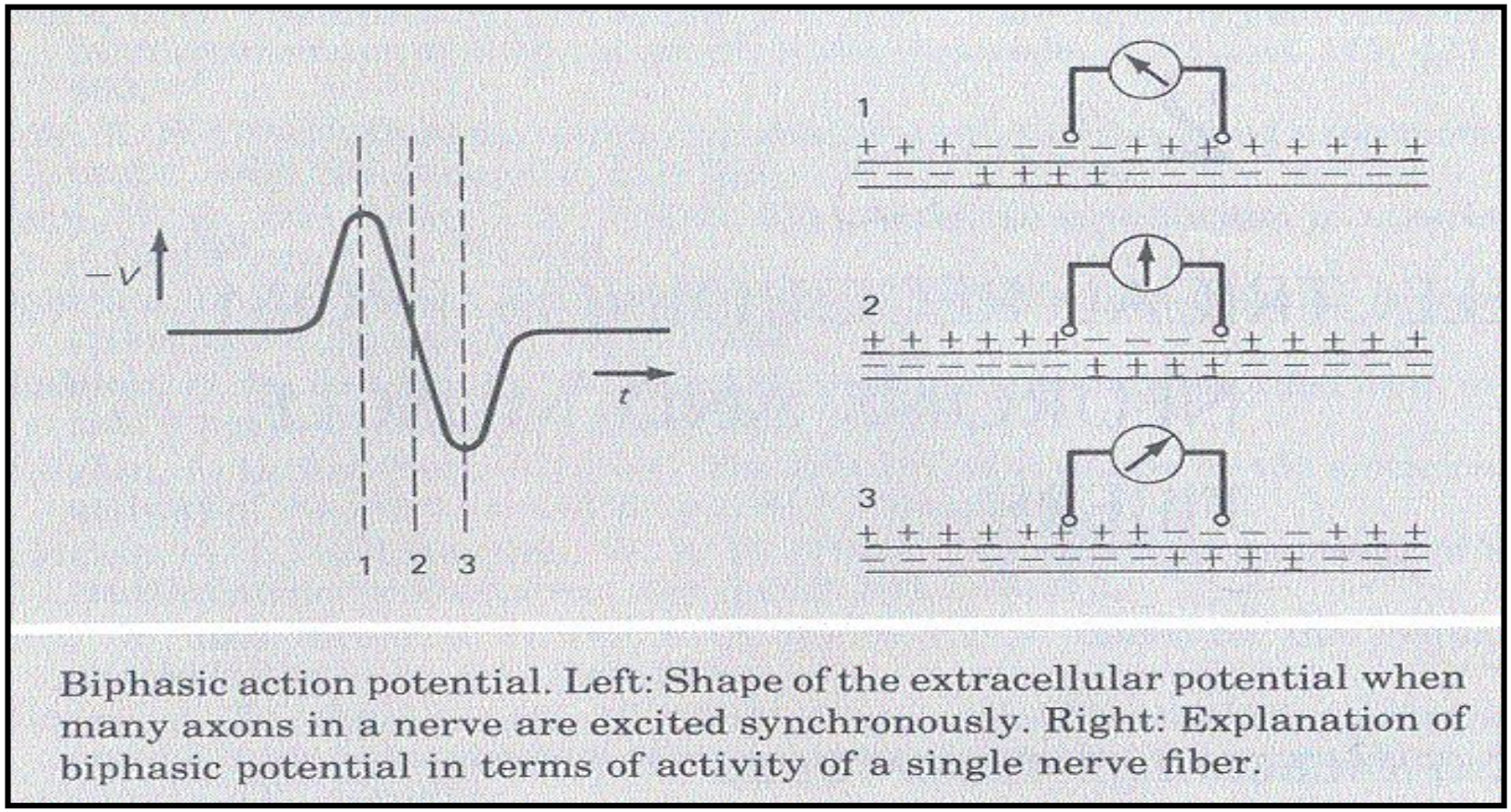
Figure 12

A typical extracellular recording setup. Stimulus and recording. Conduction delay can also be considered.



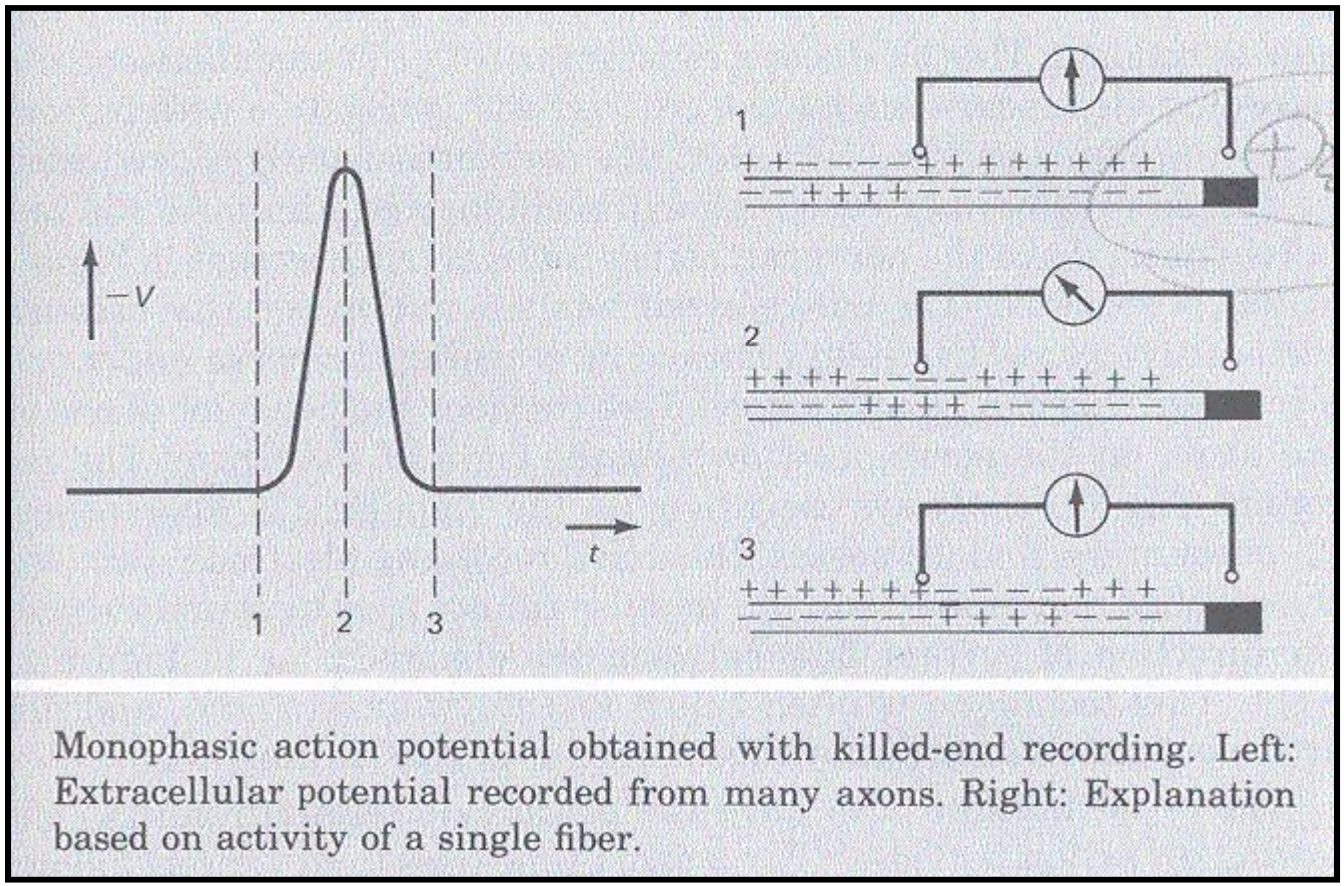
Experimental arrangement for extracellular recording of nerve action potentials. n, nerve; r, Ringer's solution in bottom of chamber; cross section shown below; TRIG, trigger signal.

Extracellular recording: Biphasic AP using bipolar electrodes.



Biphasic action potential. Left: Shape of the extracellular potential when many axons in a nerve are excited synchronously. Right: Explanation of biphasic potential in terms of activity of a single nerve fiber.

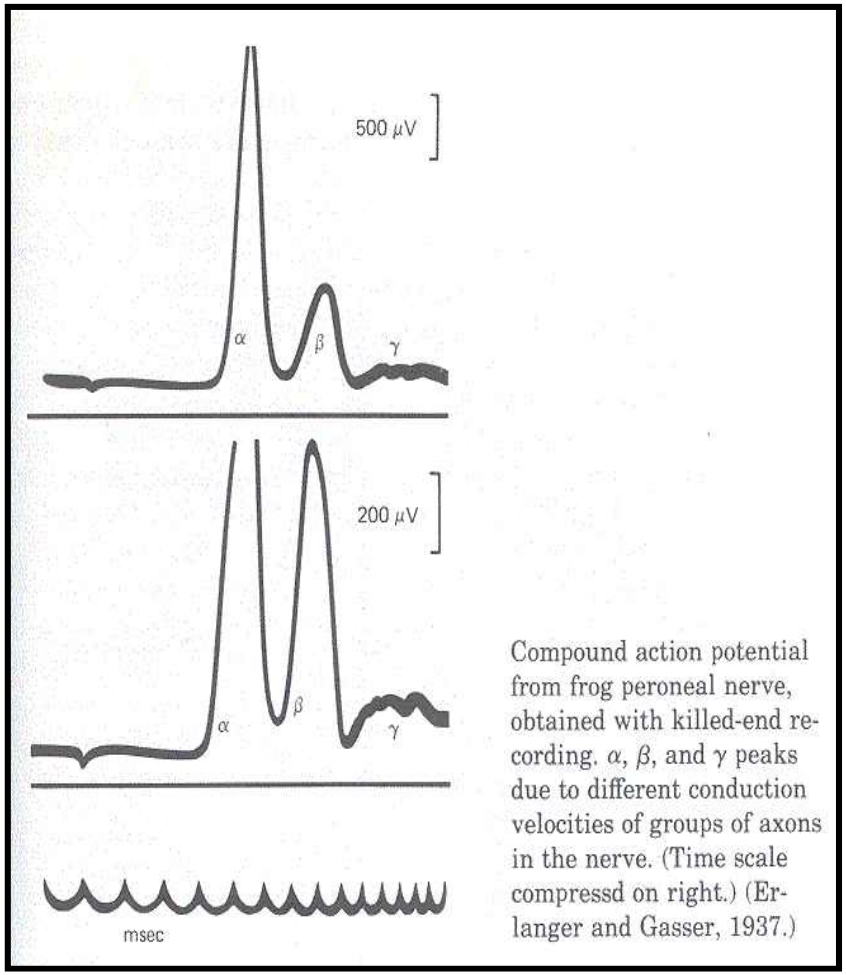
Monophasic AP recorded using killed-end recording.



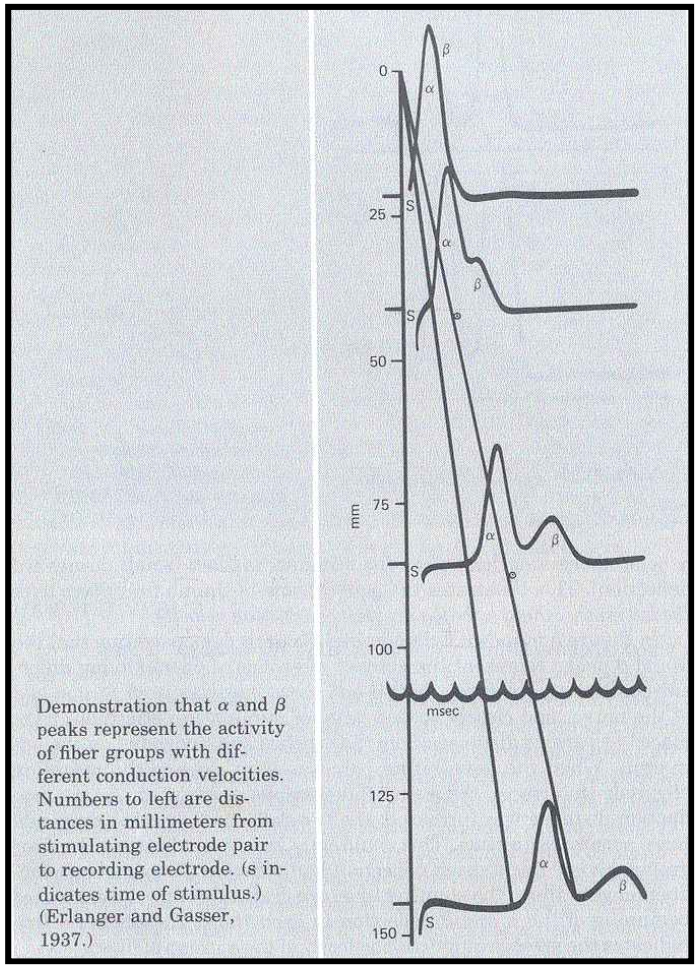
Monophasic action potential obtained with killed-end recording. Left: Extracellular potential recorded from many axons. Right: Explanation based on activity of a single fiber.

Killed-end: always charged to + and unchanged.

Compound AP and individual AP's separated in time.



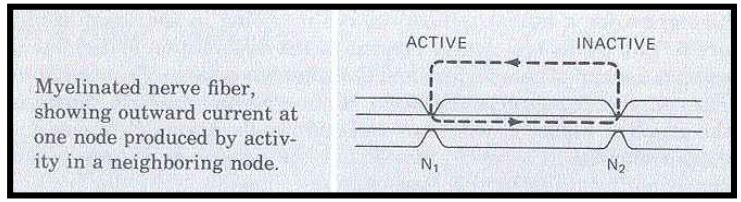
Compound action potential from frog peroneal nerve, obtained with killed-end recording. α, β, and γ peaks due to different conduction velocities of groups of axons in the nerve. (Time scale compressd on right.) (Erlanger and Gasser, 1937.)



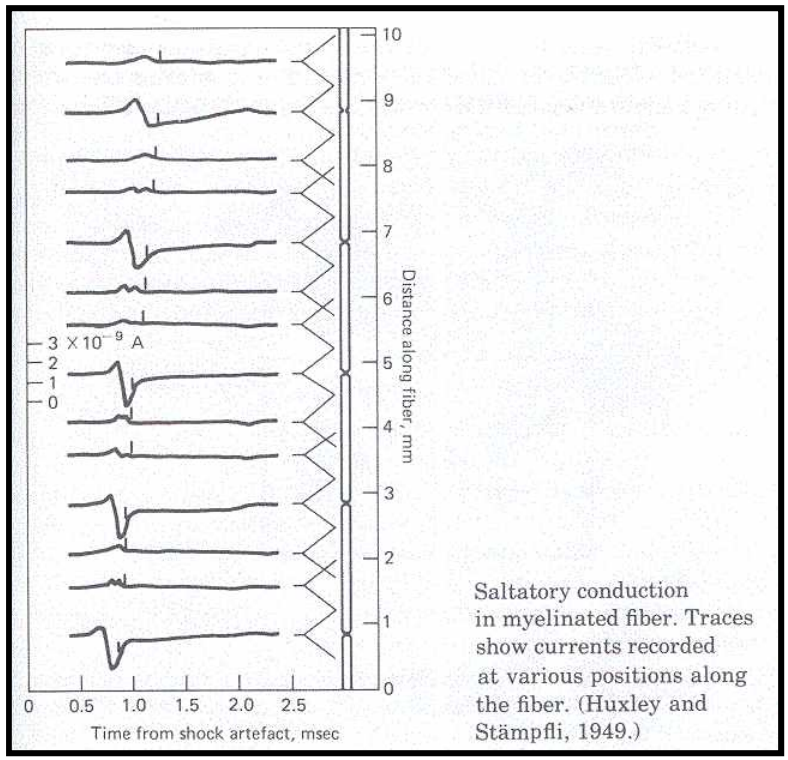
Demonstration that α and β peaks represent the activity of fiber groups with different conduction velocities. Numbers to left are distances in millimeters from stimulating electrode pair to recording electrode. (s indicates time of stimulus.) (Erlanger and Gasser, 1937.)

Myelinated nerve,the node of Ranvier, Saltatory conduction.

- Very little current for the segments with myeline sheath
- Almost no depolarization during the myelinated segment (potential is decremental)
- The non-decremental A.P.jumps over to next Ranvier node.(Saltatory conduction)
- Found in humans and higher animals.

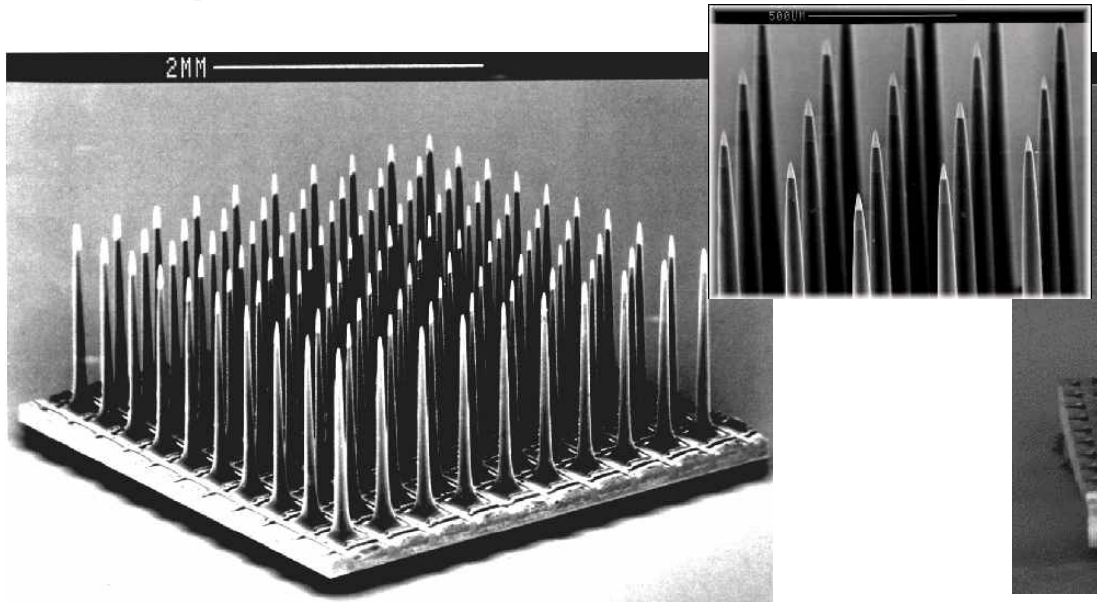


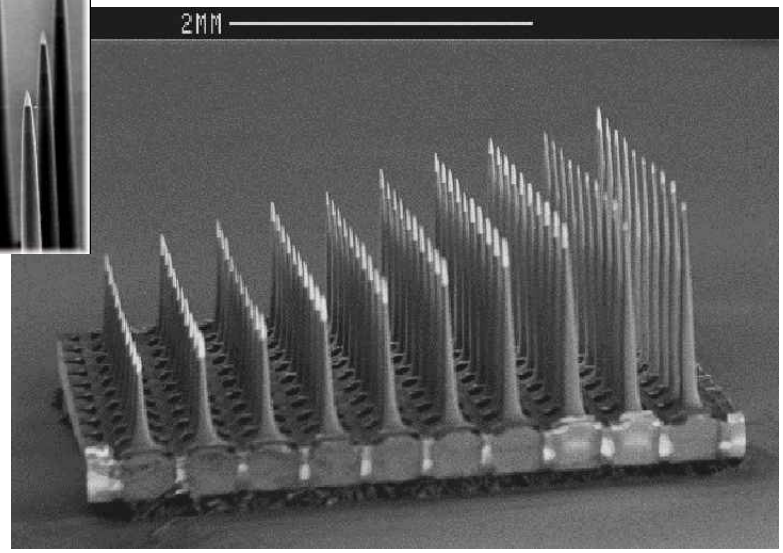
Myelinated nerve fiber, showing outward current at one node produced by activity in a neighboring node.



Saltatory conduction in myelinated fiber. Traces show currents recorded at various positions along the fiber. (Huxley and Stämpfli, 1949.)

Examples of Neural Recording Devices: MEA(MicroElectrode Array)





"Slant" Array

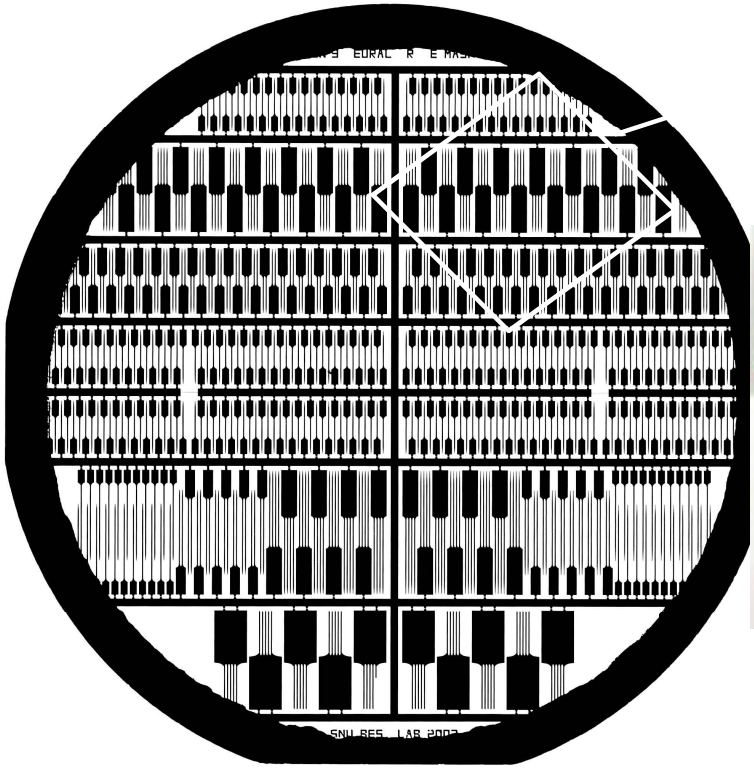
•This is a silicon based array of 100 penetrating microelectrodes, designed to be implanted in the cerebral cortex. It provides a means to stimulate and/or record from large number of neurons simultaneously.

Chronic Connector

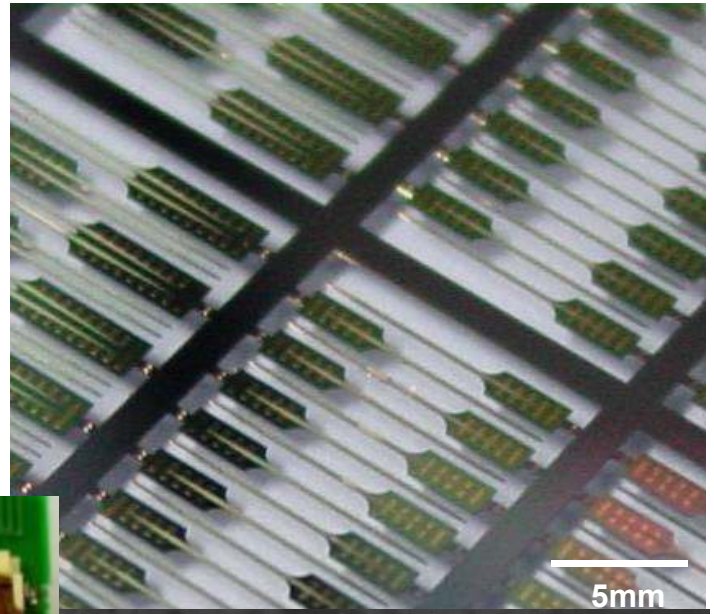
•This is a variant of the UEA and contains 100 penetrating microelectrodes of various lengths (typically from 0.5 mm to 1.5 mm in length). The USA is designed to be implanted in the peripheral nervous system and to provide a means for simultaneous stimulation and recording from large number of nerve fibers.

SNU Depth Probe

(A)



(B)



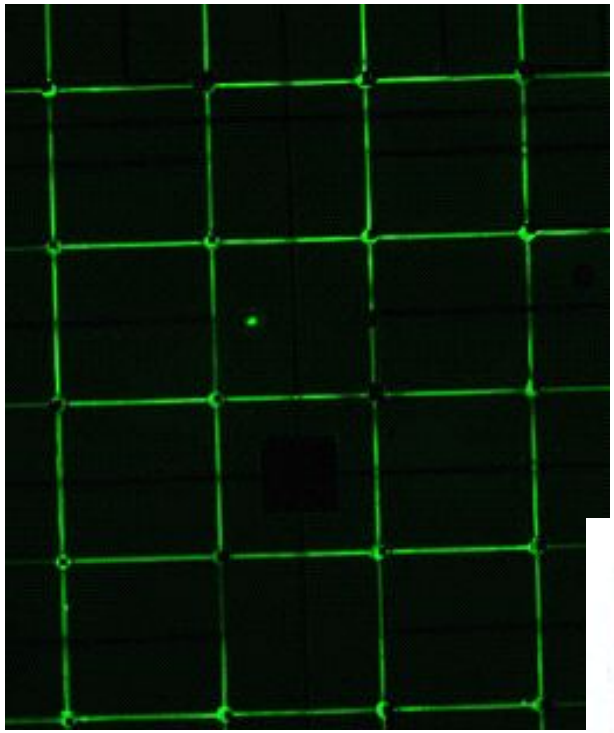
(C)

A) Released whole wafer image,

B) Fabricated 1 shank neural probe

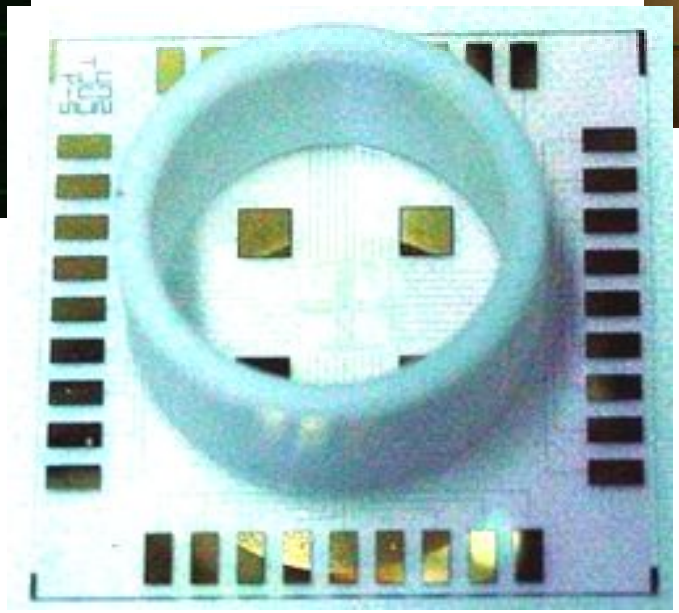
C) SEM Image - electrode sites and probe tip

Neural Network Research with Planar Microelectrode Array

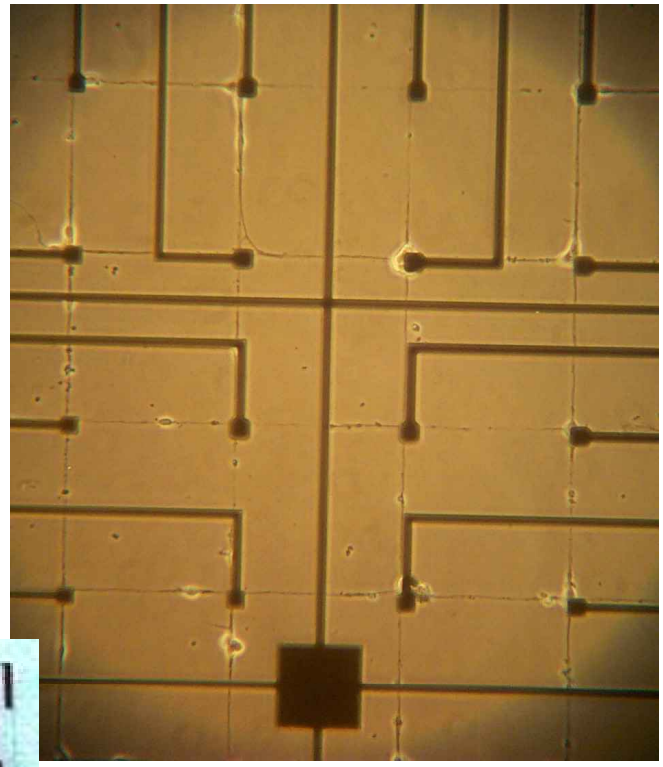


poly-l-lysine
(containing FITC)
Microstamped MEA

SNU Cult:
Culture Plate
type MEA



Culture Plate



Cultured hippocampal
neurons In low density