Department of
Life Sciences
and Systems Biology

UNIVERSITÀ
DI TORINO

Cellular and Molecular Biophysics

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CFU 5 LM Biotecnologie Industriali- 6 LM Fisica - A.A. 2024/25

Corso di laurea in LM Biotecnologie Industriali- LM Fisica

Electrical properties of cell membranes_02

Action potential.
Hodgkin and Huxley model
Signal propagation.
Patch clamp technique

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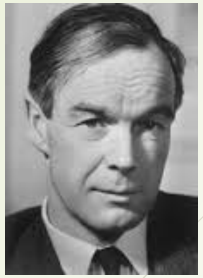
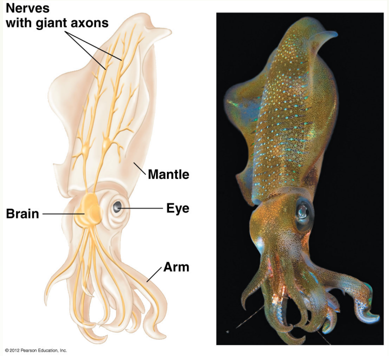


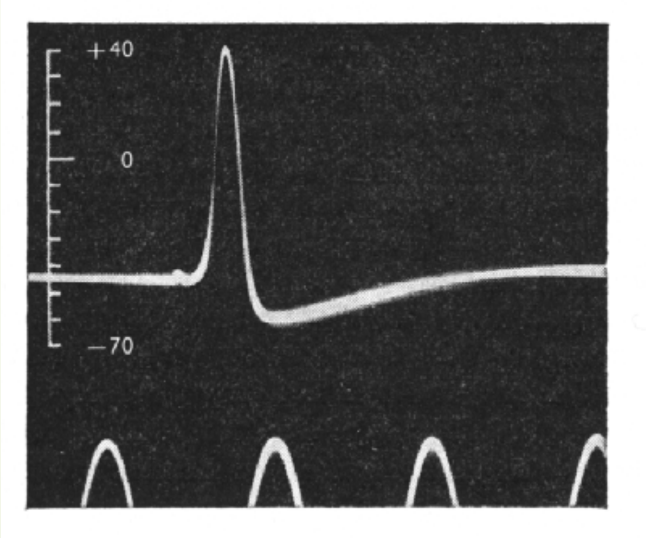
Hodgkin

Huxley

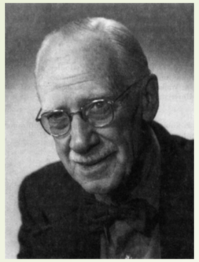


1952: they proposed the existence of voltage-dependent channels !!

(the structure of biological membranes was still unknown…)



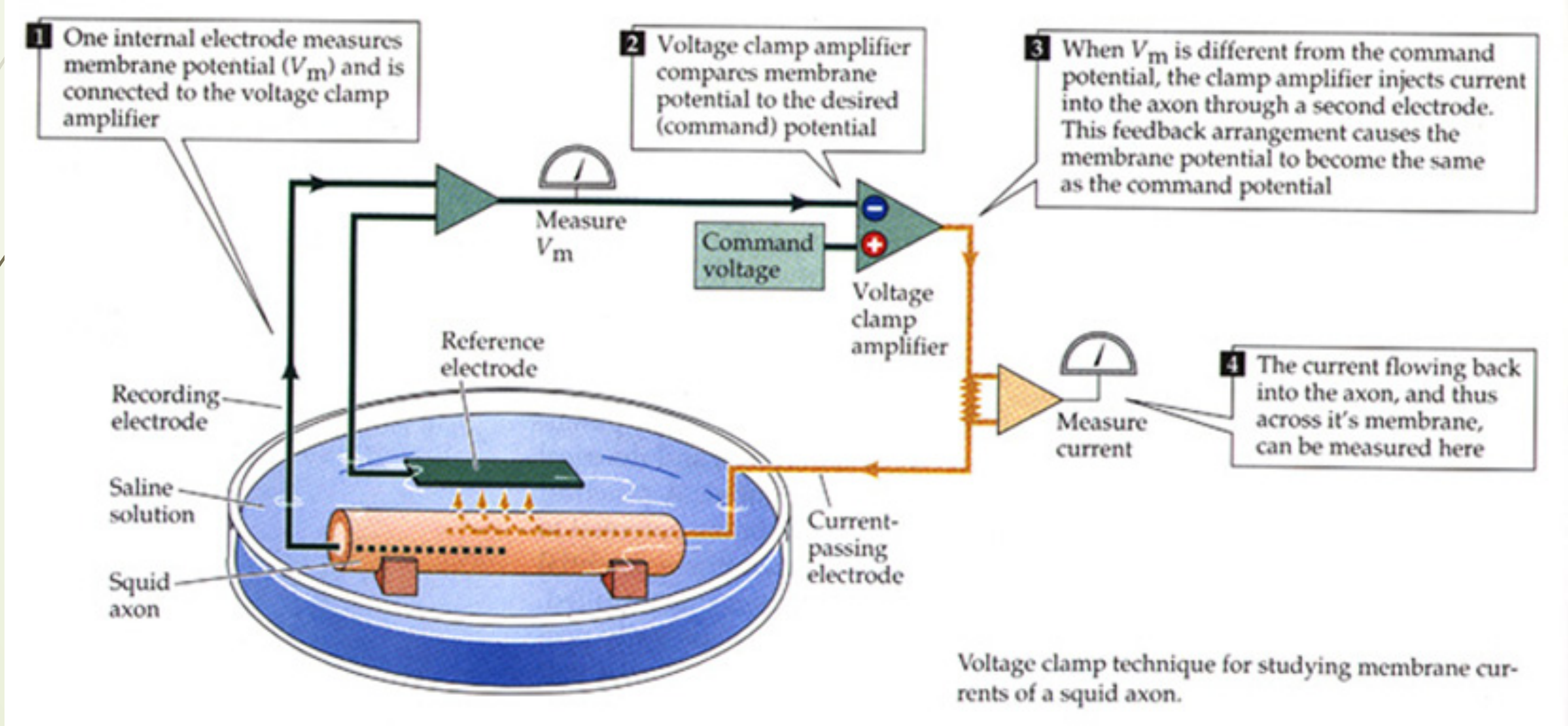
The Nobel Prize in Physiology or Medicine 1963 was awarded jointly to Sir John Carew Eccles, Alan Lloyd Hodgkin and Andrew Fielding Huxley "for their discoveries concerning the ionic mechanisms involved in excitation and inhibition in the peripheral and central portions of the nerve cell membrane."



Cole ('47)

VOLTAGE CLAMP technique

Quantitative analysis of ionic currents 'blocking' membrane voltage at a given value.



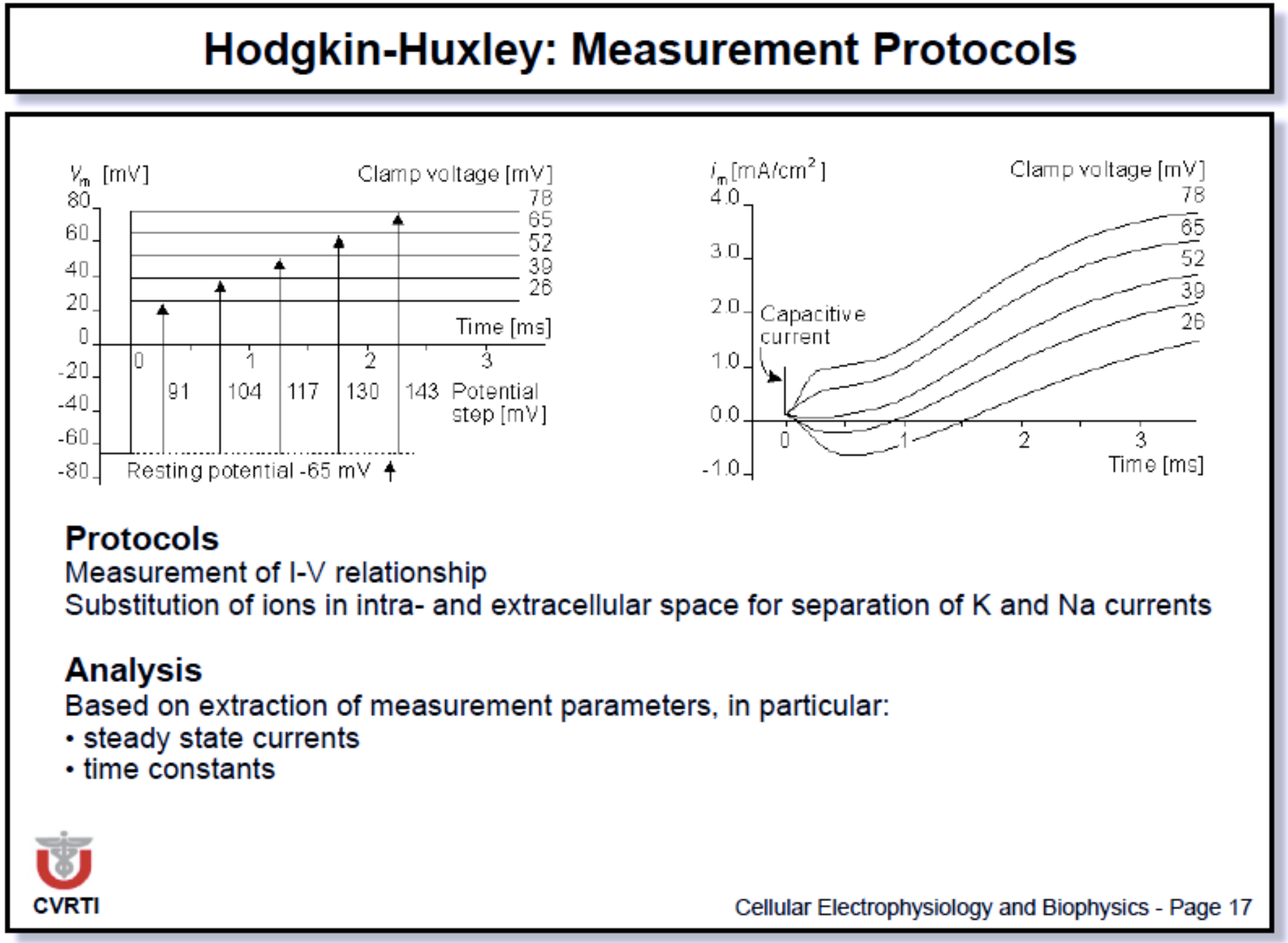
1 One internal electrode measures membrane potential (V_m) and is connected to the voltage clamp amplifier

2 Voltage clamp amplifier compares membrane potential to the desired (command) potential

3 When V_m is different from the command potential, the clamp amplifier injects current into the axon through a second electrode. This feedback arrangement causes the membrane potential to become the same as the command potential

4 The current flowing back into the axon, and thus across it's membrane, can be measured here

Voltage clamp technique for studying membrane currents of a squid axon.



Hodgkin-Huxley: Measurement Protocols

Protocols

Measurement of I-V relationship

Substitution of ions in intra- and extracellular space for separation of K and Na currents

Analysis

Based on extraction of measurement parameters, in particular:

- steady state currents
- time constants

CVRTI

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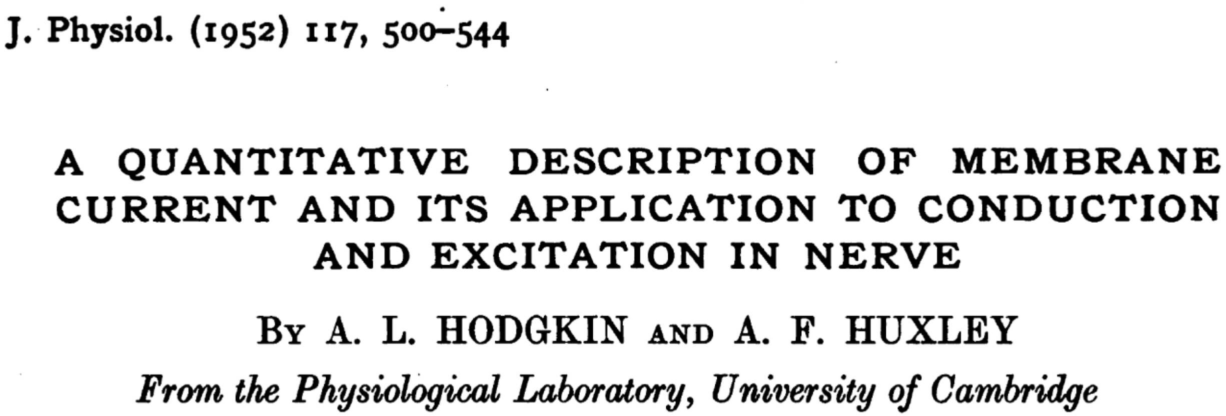
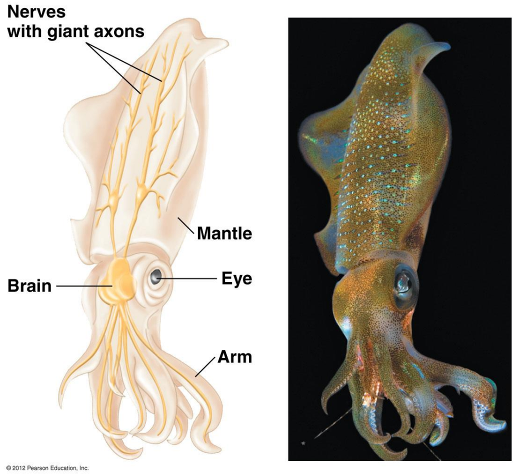


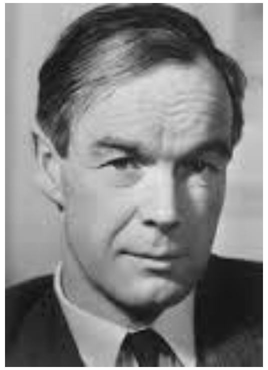
J. Physiol. (1952) 117, 500–544

A QUANTITATIVE DESCRIPTION OF MEMBRANE CURRENT AND ITS APPLICATION TO CONDUCTION AND EXCITATION IN NERVE

BY A. L. HODGKIN AND A. F. HUXLEY

From the Physiological Laboratory, University of Cambridge





Hodgkin

Huxley

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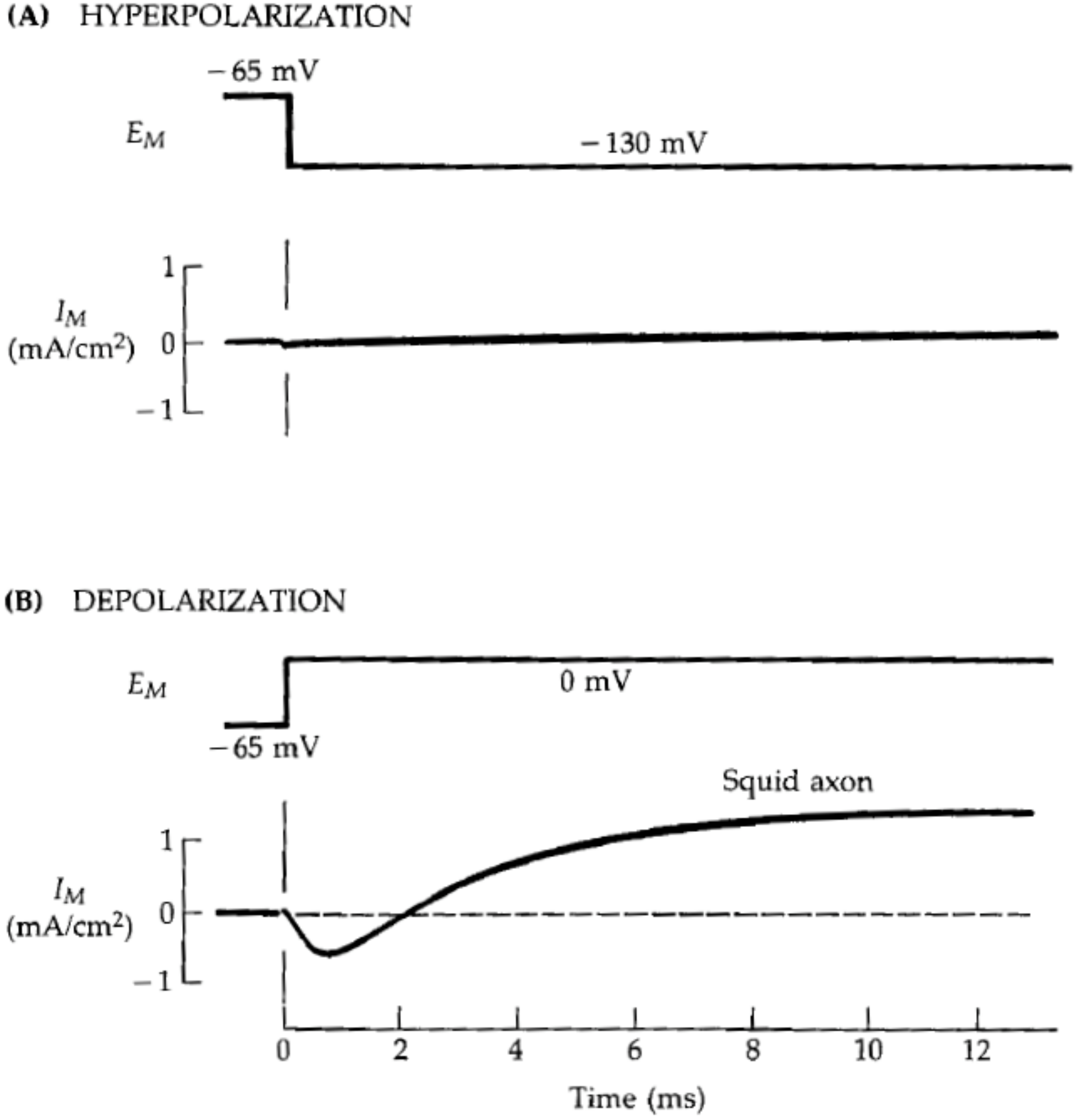


voltage-clamp

experiments

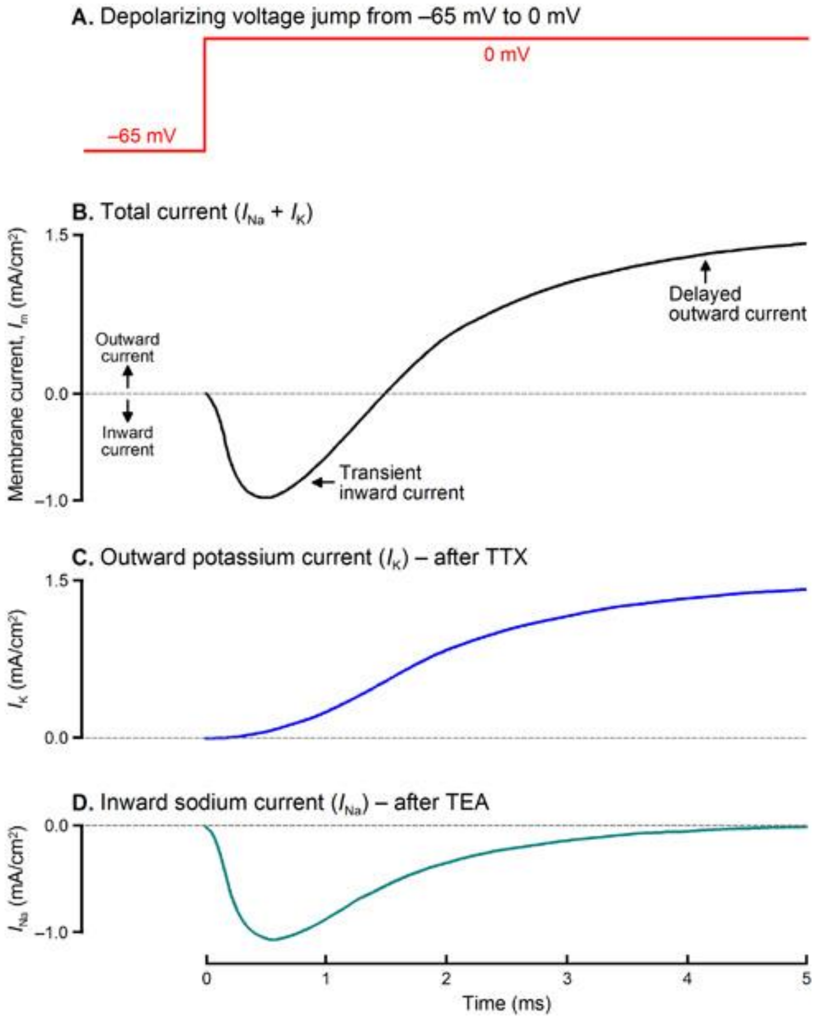
Na and K currents can be separated:

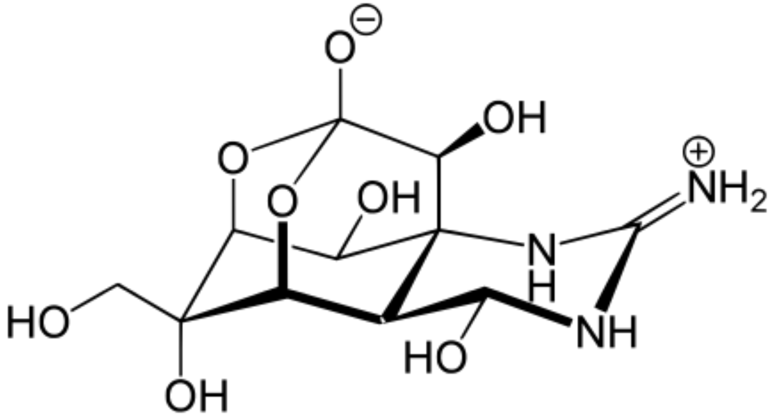
1. pharmacologically

(TTX blocks Na current

TEA blocks K current)

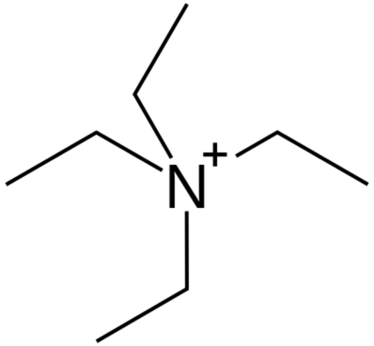
2. **by ion substitution** (extracellular non permeable choline subs Na+)

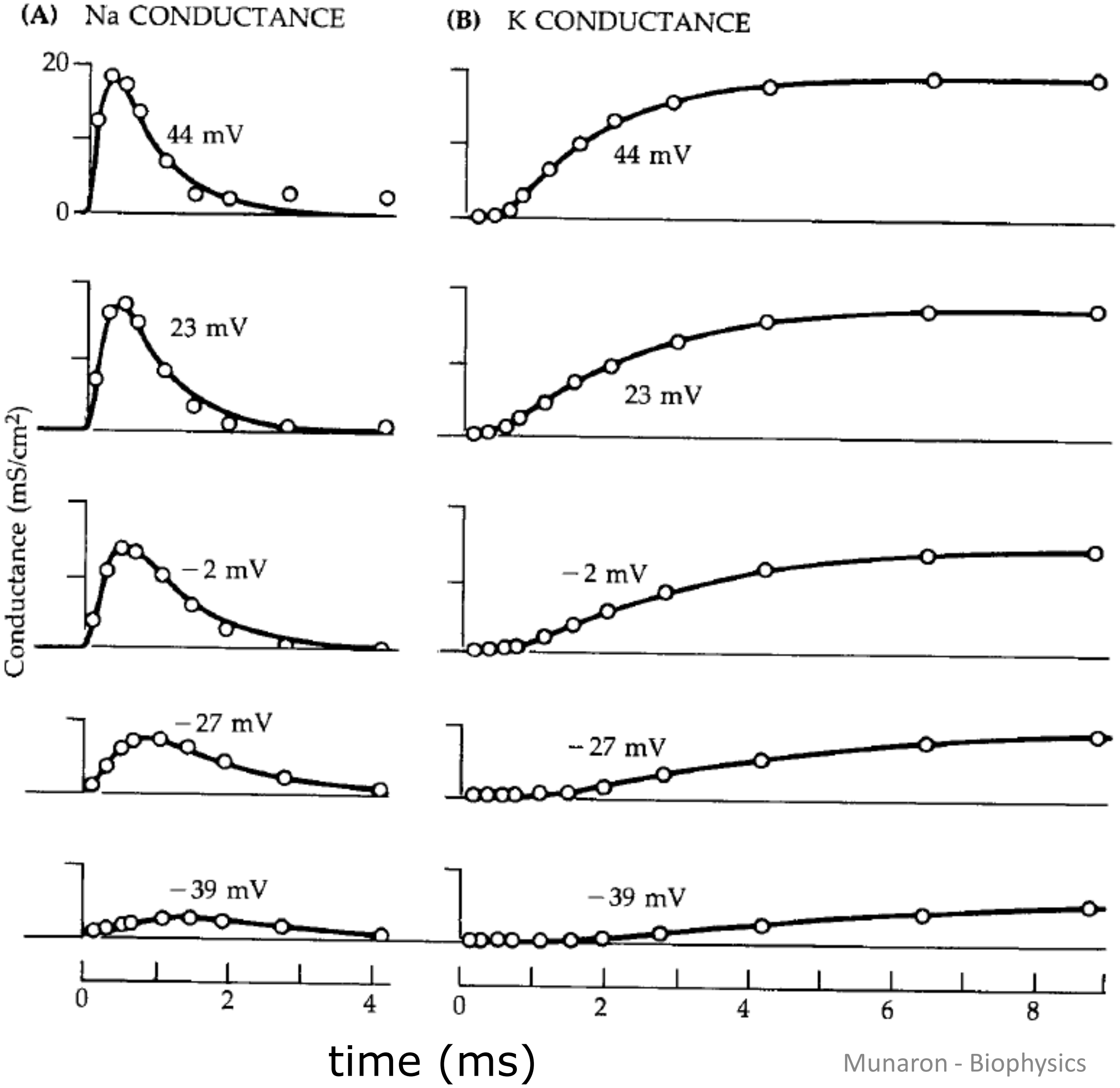




TTX

TEA





currents at each voltage

↓

conductance

Analysis:

extraction of 2 parameters

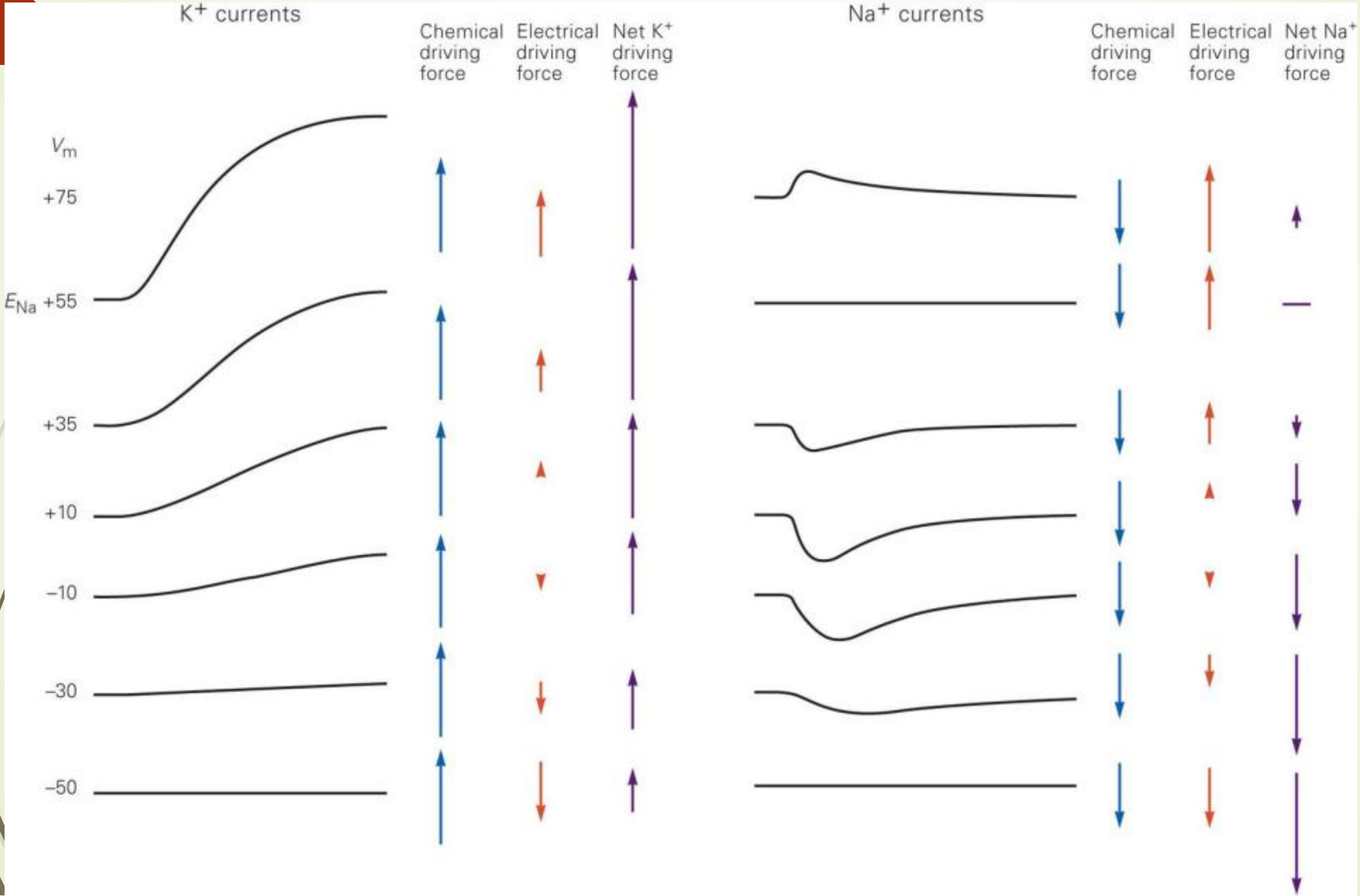
1. steady state currents
2. time constants

The size of Na^+ and K^+ currents depends on two factors:

1. The magnitude of the Na^+ or K^+ conductances g_{Na} or g_K, which reflect the number of Na^+ or K^+ channels open at any instant.
2. Electrochemical driving force of Na^+ ions ($V_m – E_{Na}$) or K^+ ions ($Vm – E_K$)

$$I_{Na} = g_{Na} \ * (V_m \ - E_{Na})$$
$$I_K = g_K \ * (V_m \ - E_K)$$

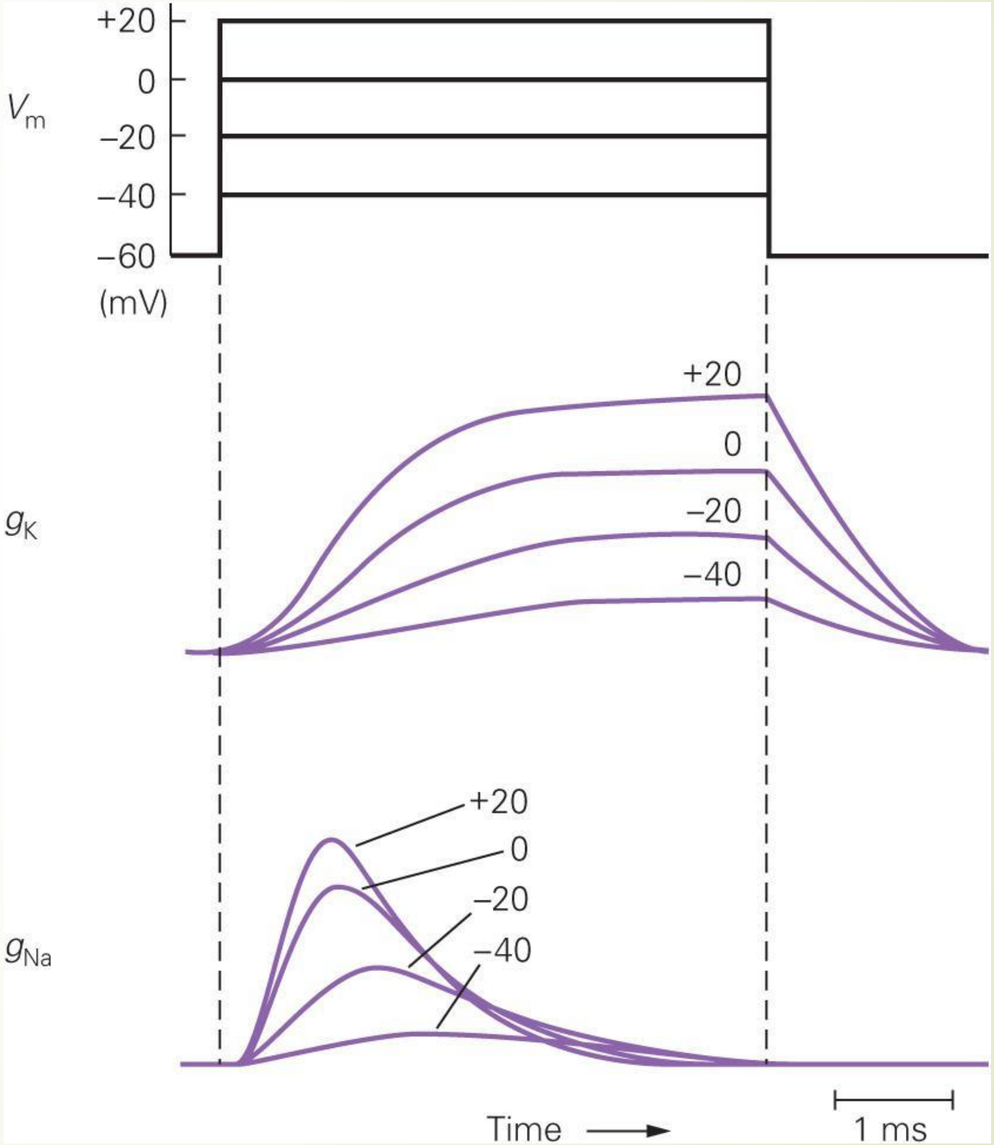
Na+ and K+ currents (I) calculated at different V



Na+ and K+ conductances (g) are calculated from their currents

From the I values obtained Hodgkin and Huxley were able to obtain g_{Na} and g_K by the following equation

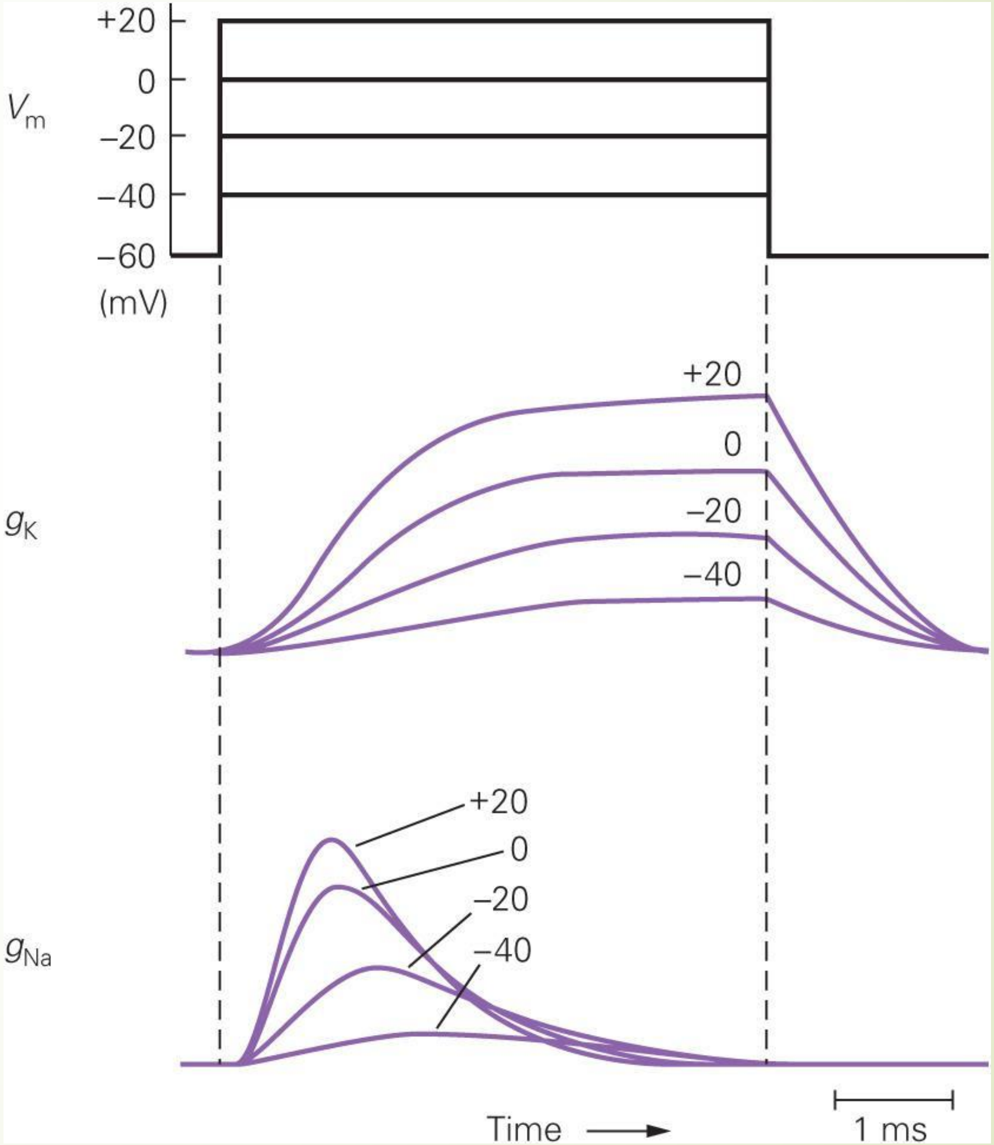
$$g_{Na} = \frac{I_{Na}}{V - V_{Na}} \qquad g_k = \frac{I_K}{V - V_K}$$



Na+ and K+ conductances (g) are calculated from their currents

Two **common features**:

- Both g increase in response to depolarization
- As the size of depolarization increases, the g increases



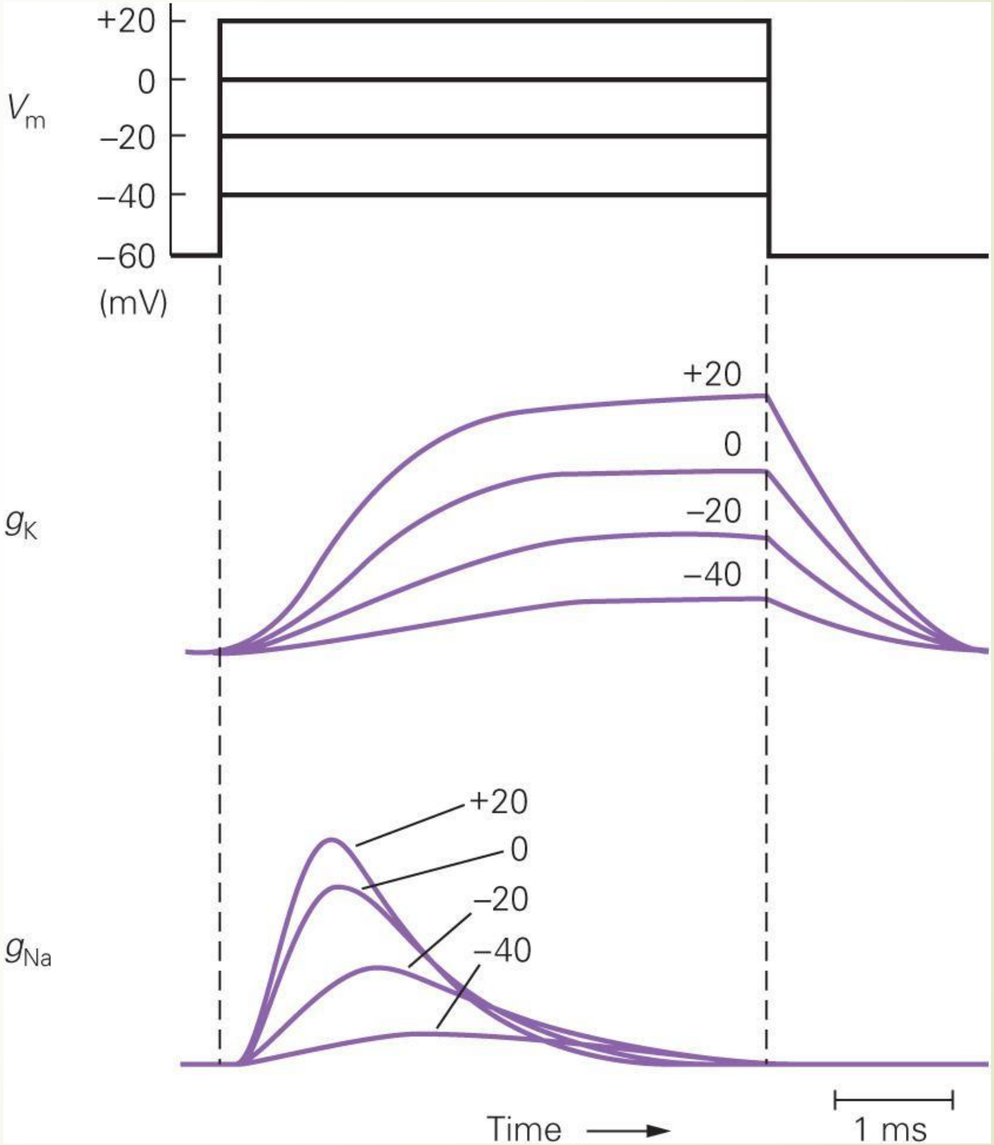
Na+ and K+ conductances (g) are calculated from their currents

Differences:

- The g differs in the rate at which they open :

gNa is developing more rapid at every Vm as comapred to gK

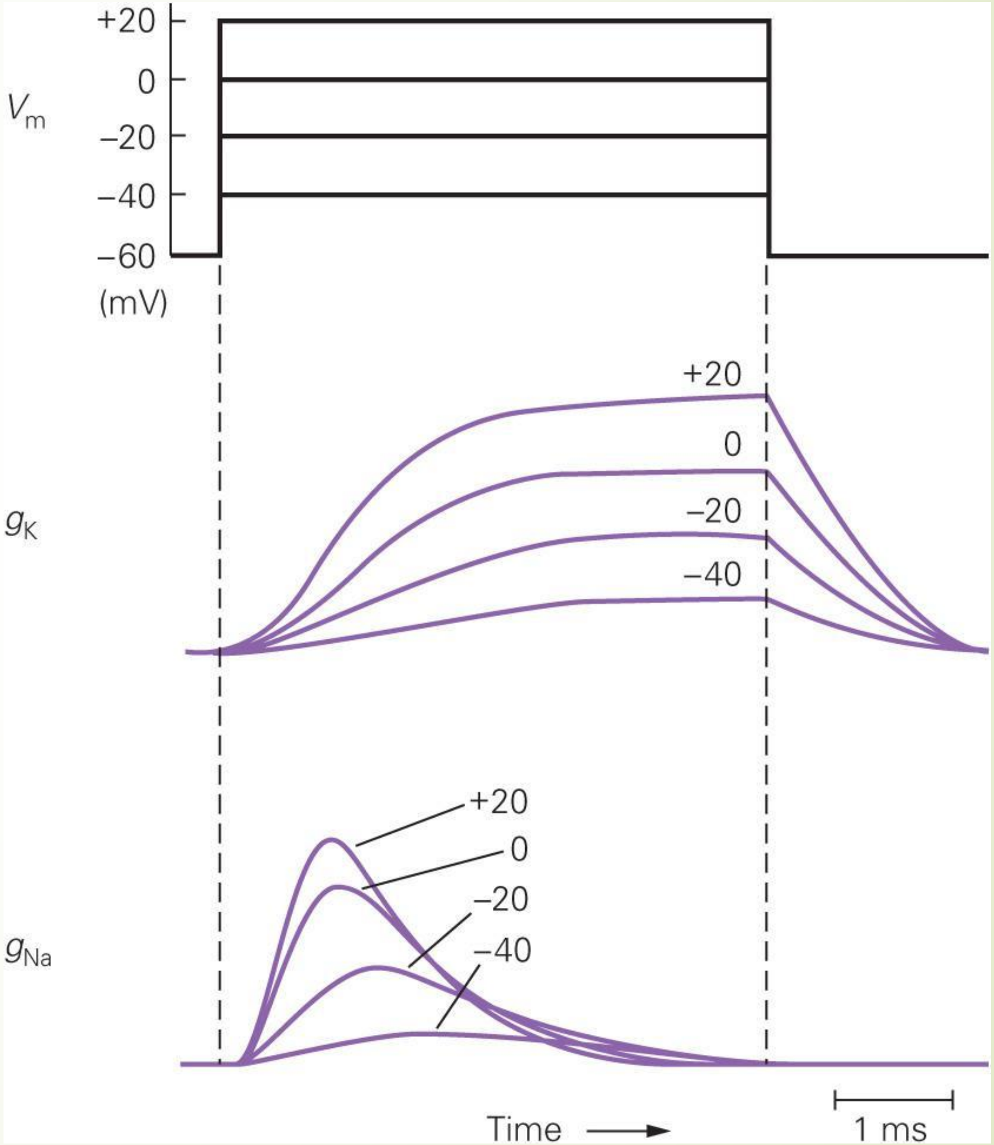
- When depolarization is maintanied for some times gNa decrease leading to a decrease of inward current = INACTIVATION Na+ channels
- gK (of the squid axon)remains stable as lomg as the membrane is depolarizaed (at least for depolarizations lasting 10ms)



Na+ and K+ conductances (g) are calculated from their currents

Time-dependent effect of depolarization on gNa are determined by the kinetics of two gating mechanisms in Na+ channels.

- Activation gate closed while the membrane is at resting potential and opened by depolarization.
- Inactivation gate open at resting potential and closes after the channel opens in response to depolarization. The channel conducts Na+ only when both gates are open.



Action potential can be reconstructed from the properties of Na+ and k+ channels

Hodgkin and Huxley were able to fit their measurements of membrane g to a set of empirical equations that completely describe Na+ and K+ conductances as a function of membrane potential and time.
Using these equations and measured values for the passive properties of the axon, they computed the shape and conduction velocity of the action potential.

The calculated waveform of action potential matched the waveform of unclamped action potential almost perfectly indicating that the model developed by Hodgkin and Huxley accurately described the properties of the channels that are essential for generating and propagating the the Action potential. **This is still the most SUCCESSFUL QUANTITATIVE MODEL IN NEURAL SCIENCES (at least) if not in all biology**

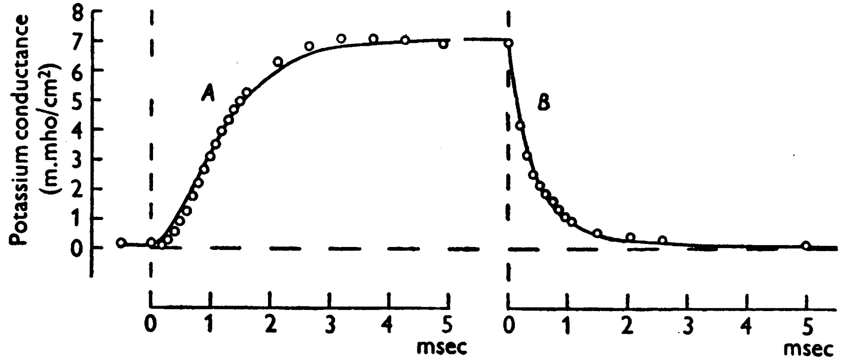
The size of Na^+ and K^+ currents depends on two factors:

1. The magnitude of the Na^+ or K^+ conductances g_{Na} or g_K, which reflect the number of Na^+ or K^+ channels open at any instant.
2. Electrochemical driving force of Na^+ ions (V_m – E_{Na}) or K^+ ions (Vm – E_K)

$$I_{Na} = g_{Na} * (V_m - ENa)$$
$$I_K = g_K * (V_m - EK)$$

gK+ kinetic HH MODEL

«Our object here is to find equations which describe the conductances with reasonable accuracy and are sufficiently simple for theoretical calculation of the action potential and refractory period.» *HH, JPhysiol, 1952*

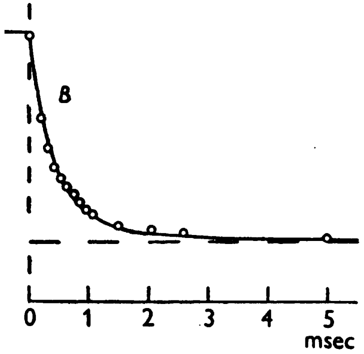


A, rise of potassium conductance associated with **depolarization of 25mV**;

B, fall of potassium conductance associated with **repolarization** to the resting potential.

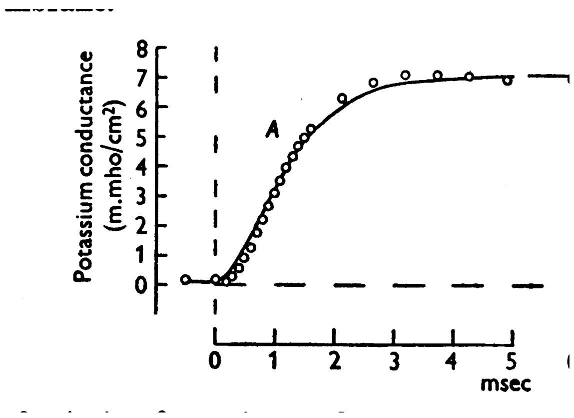
gK+ kinetic HH MODEL

Our object here is to find equations which describe the conductances with reasonable accuracy and are sufficiently simple for theoretical calculation of the action potential and refractory period.



fall curve (ripolarization) is fitted by a simple exponential.

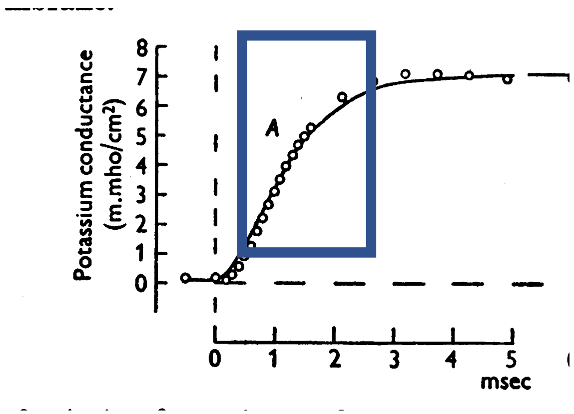
gK+ kinetic HH MODEL



This part of the curve in general can be described with and exponential

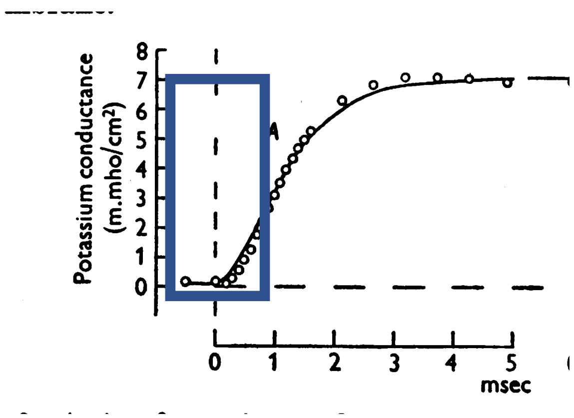
1-exp(-t)^4

gK+ kinetic HH MODEL



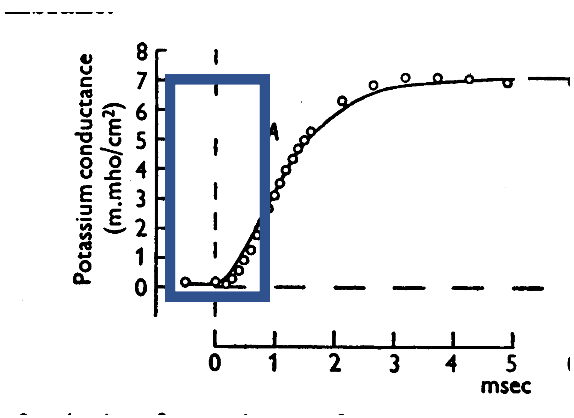
This part of the curve can be fitted with an first order equation

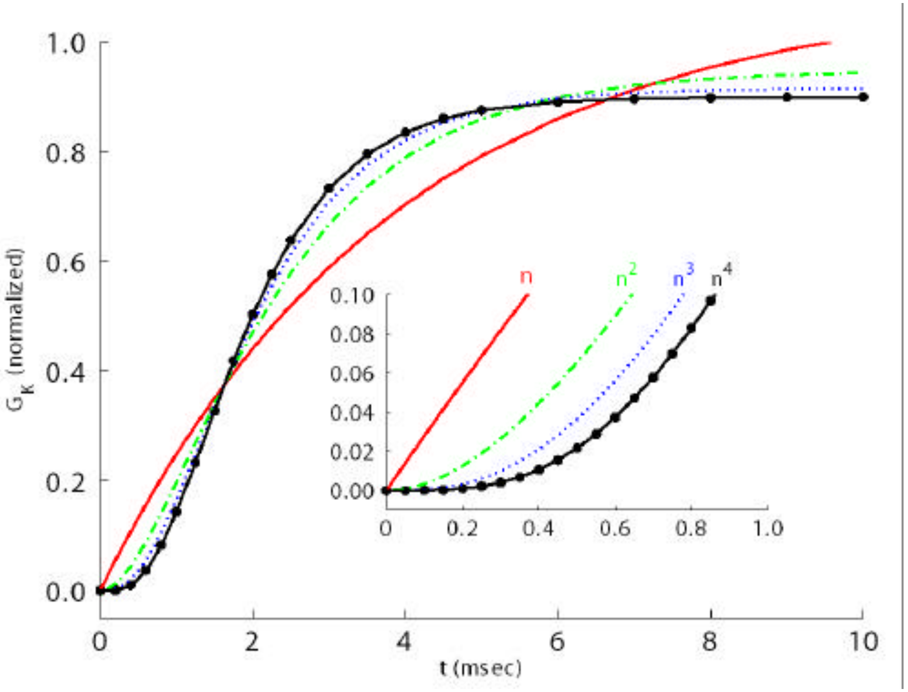
gK+ kinetic HH MODEL



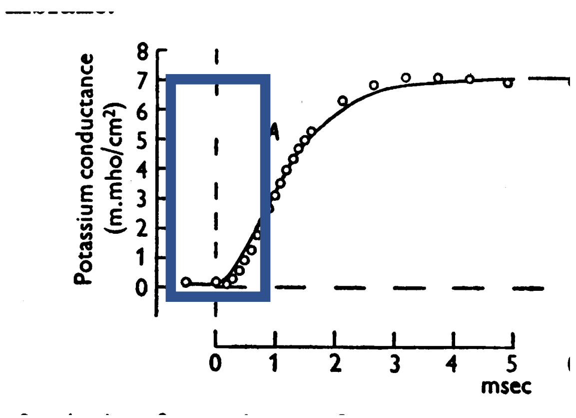
This first part «S-shaped» curve part of the curve can be fitted with an fourth order equation

gK+ kinetic HH MODEL





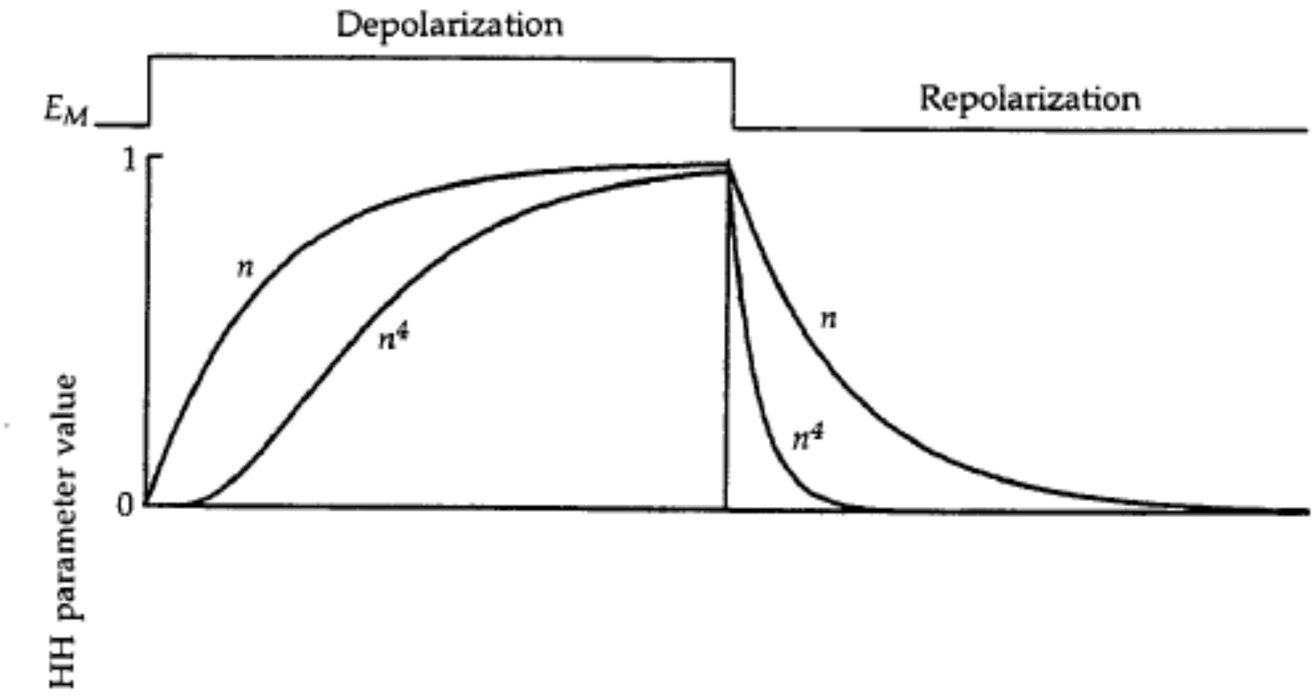
gK+ kinetic HH MODEL



This first part «S-shaped» curve part of the curve can be fitted with an fourth order equation

Useful simplification: supposing that gK+ is proportional to the fourth power of a variable (n) which obeys a first-order equation.

HH supposed that the kinetics of gK+ is controlled by 4 independent membrane bound particles, each with a probability n of being in the current position to set the opening of the channel. The probability that all 4 of them are placed in the correct position is n^4. Since the gK is V dependent these n are assumed to have same charge and move within the membrane = **Gating charges**



$$g_{\mathbf{K}} = \bar{g}_{\mathbf{K}} n^4,$$

$$\frac{\mathrm{d}n}{\mathrm{d}t} = \alpha_n \,(1-n) - \beta_n n,$$

gK+ kinetic HH MODEL

If we put this in a mathematical form

$$gK = n^4 \bar{g}K$$

$\bar{g}$K is a constant with the dimensions of conductance/cm2

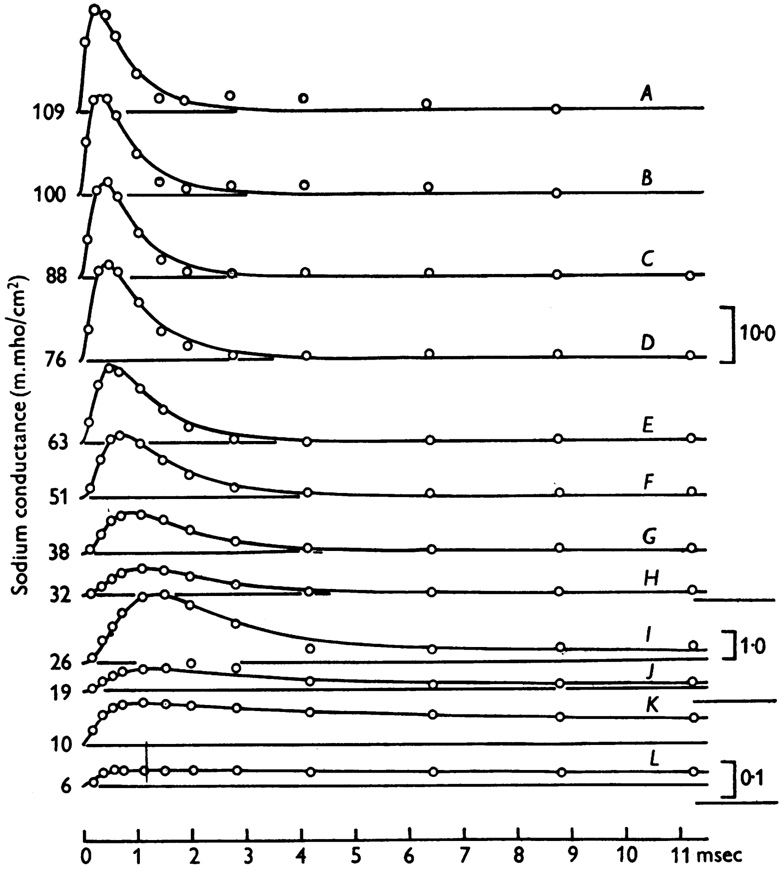
The voltage and time-dependent changes of n are given by a first order reaction

$$(1-n) \underset{\beta}{\overset{\alpha}{\rightleftharpoons}} n$$

α and β are rate constant. If the initial value of n is known, the subsequent values can be calculated by solving this difeffential equation:

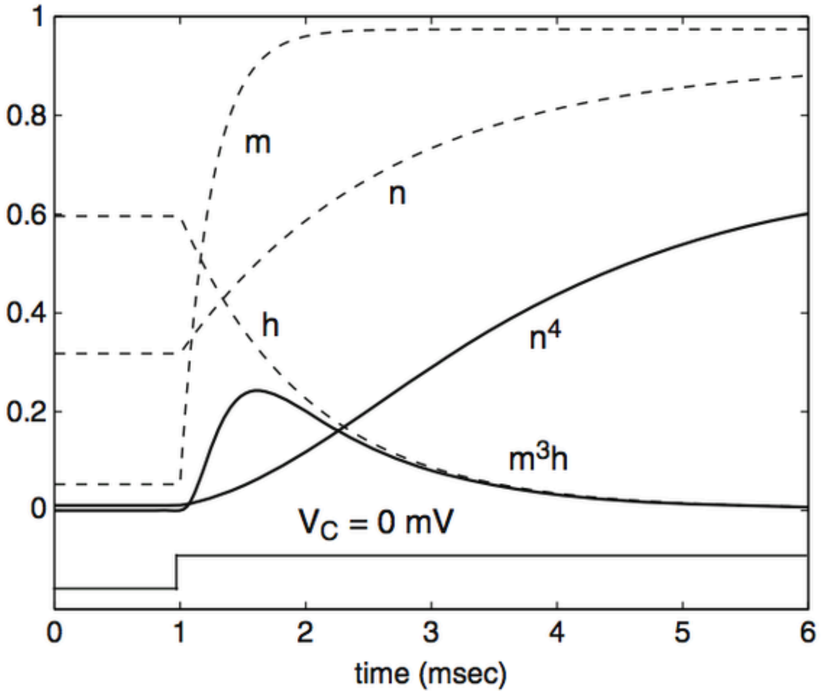
$$\frac{dn}{dt} = \alpha_n(1-n) - \beta_n n$$

gNa+ kinetic HH MODEL



HH model uses a similar formalism to describe gNa+ with 4 hypothetical particles making independent first order transitions between permissive and non permissive positions to control the channel

gNa+ kinetic HH MODEL



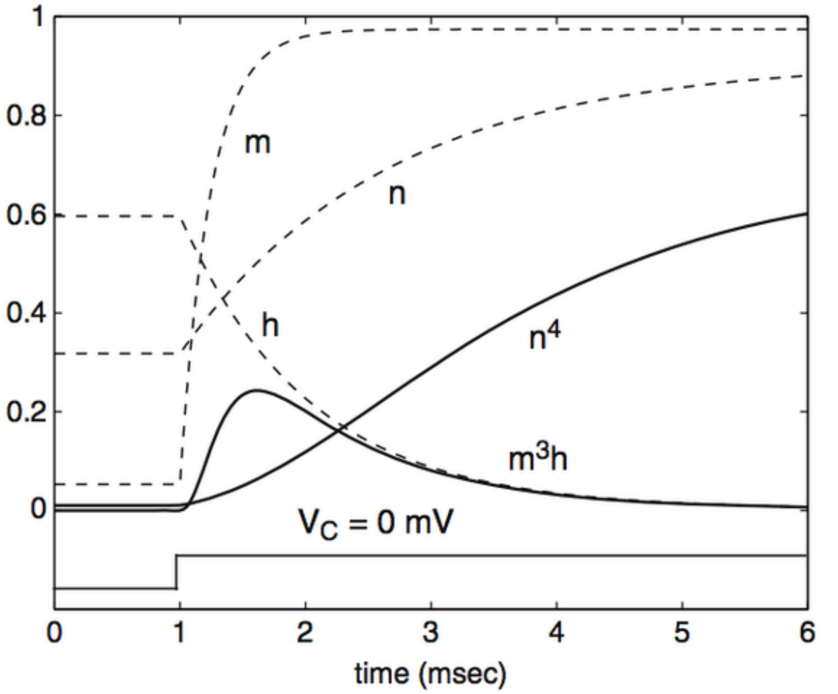
In this case here are two opposing gating processes, activation and inactivation = there are 2 different kind of gating particles

m

h

3m control the activation and 1h control inactivation

gNa+ kinetic HH MODEL



The probability that they are all in the permissive state is m^3h and the

$$gNa = m^3 h\bar{g}Na$$

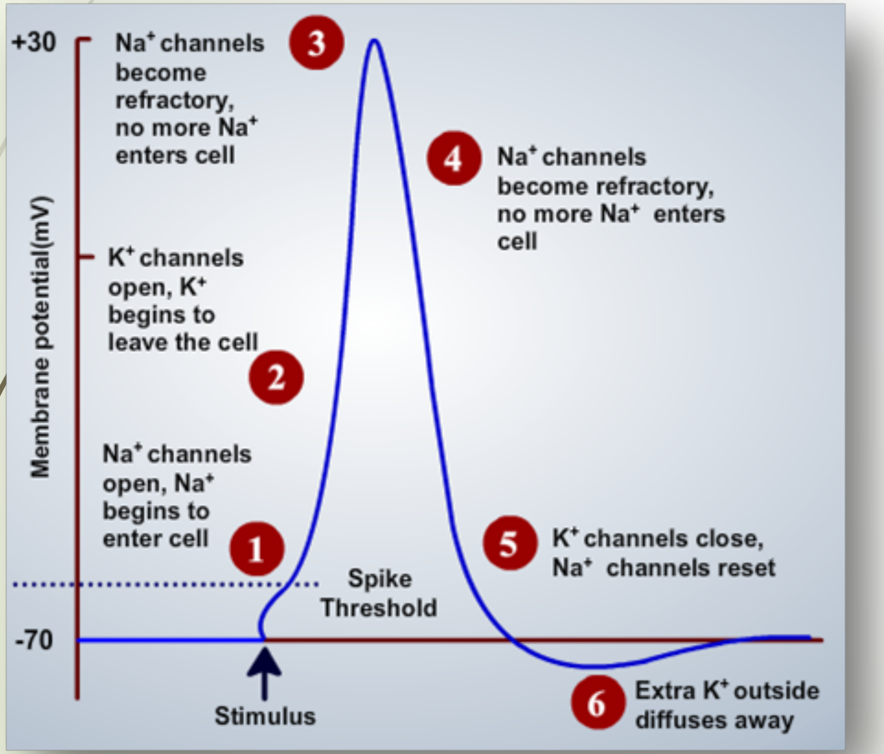
$$INa = m^3 hgNa\ (Vm - VNa)$$

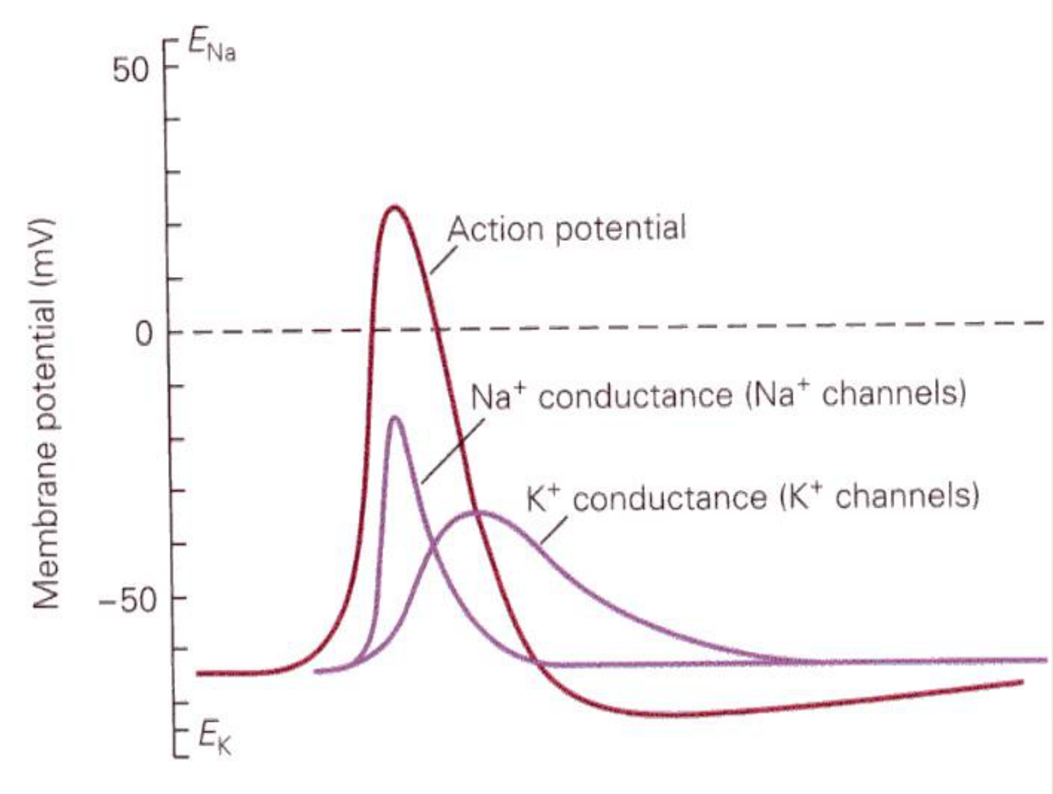
$$(1-m) \underset{\beta_m}{\overset{\alpha_m}{\rightleftharpoons}} m$$

$$(1-h) \underset{\beta_h}{\overset{\alpha_h}{\rightleftharpoons}} h$$

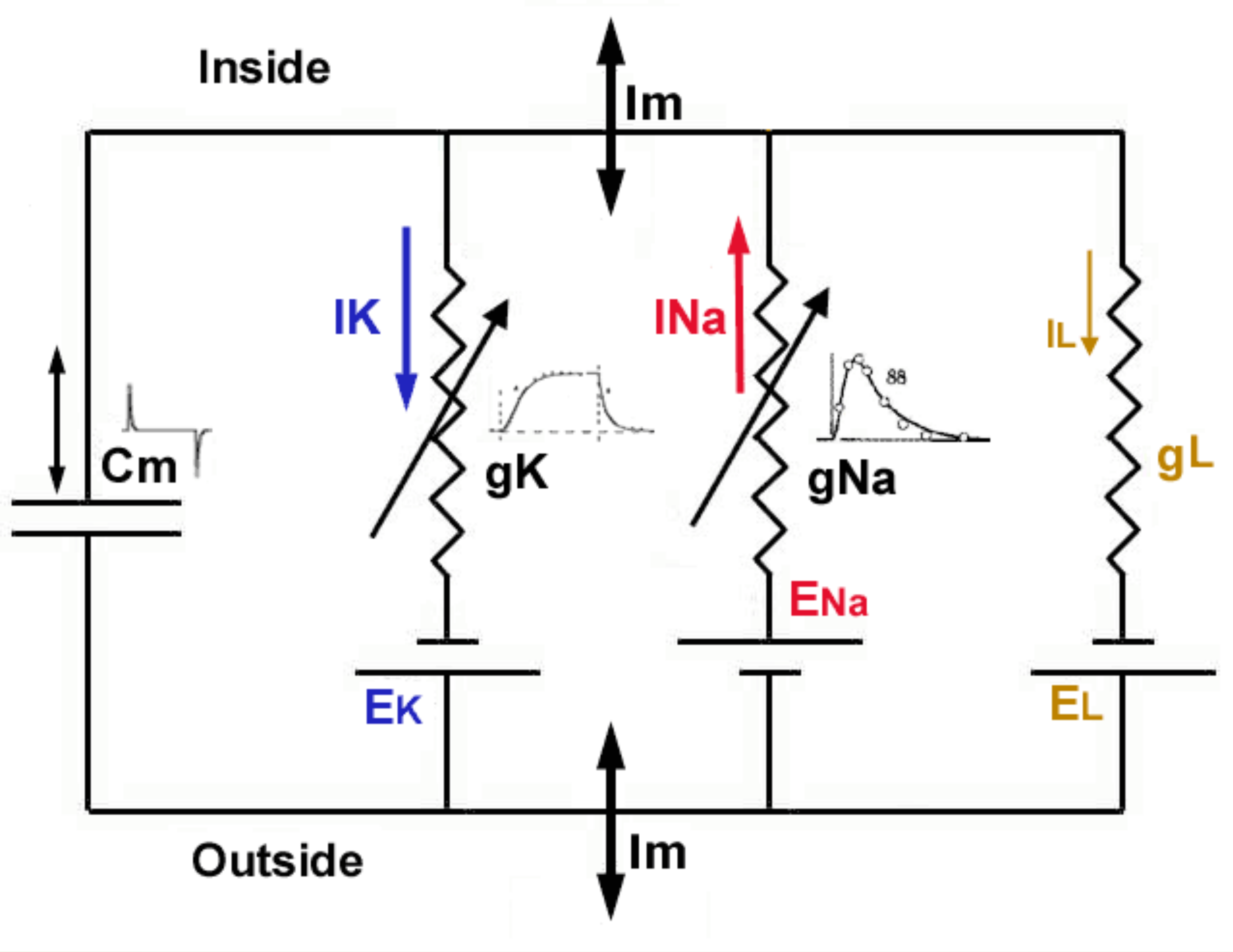
Action potential can be reconstructed from the properties of Na+ and k+ channels

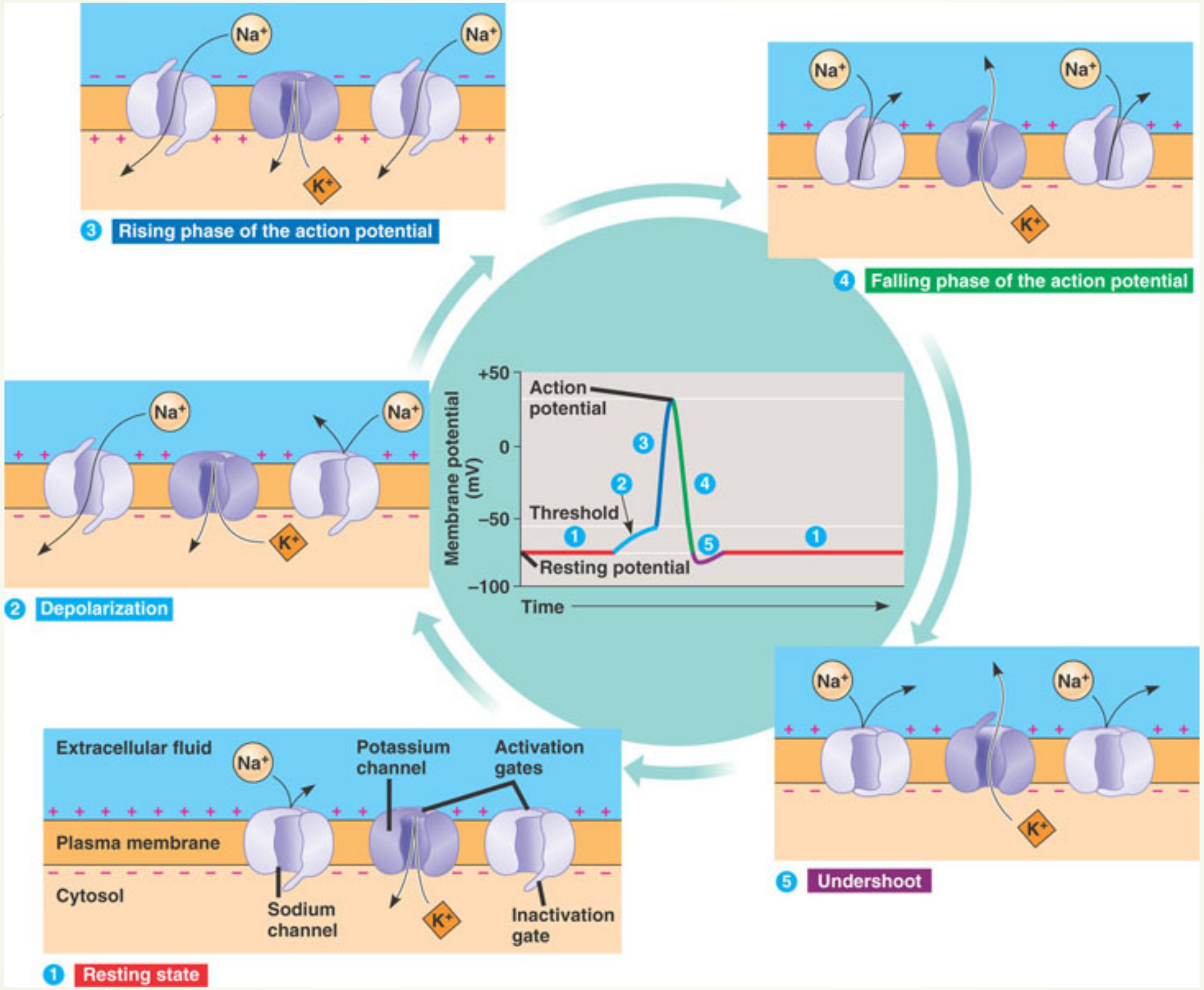
The model decribe action potential as a process involving several steps





Hodgkin and Huxley model

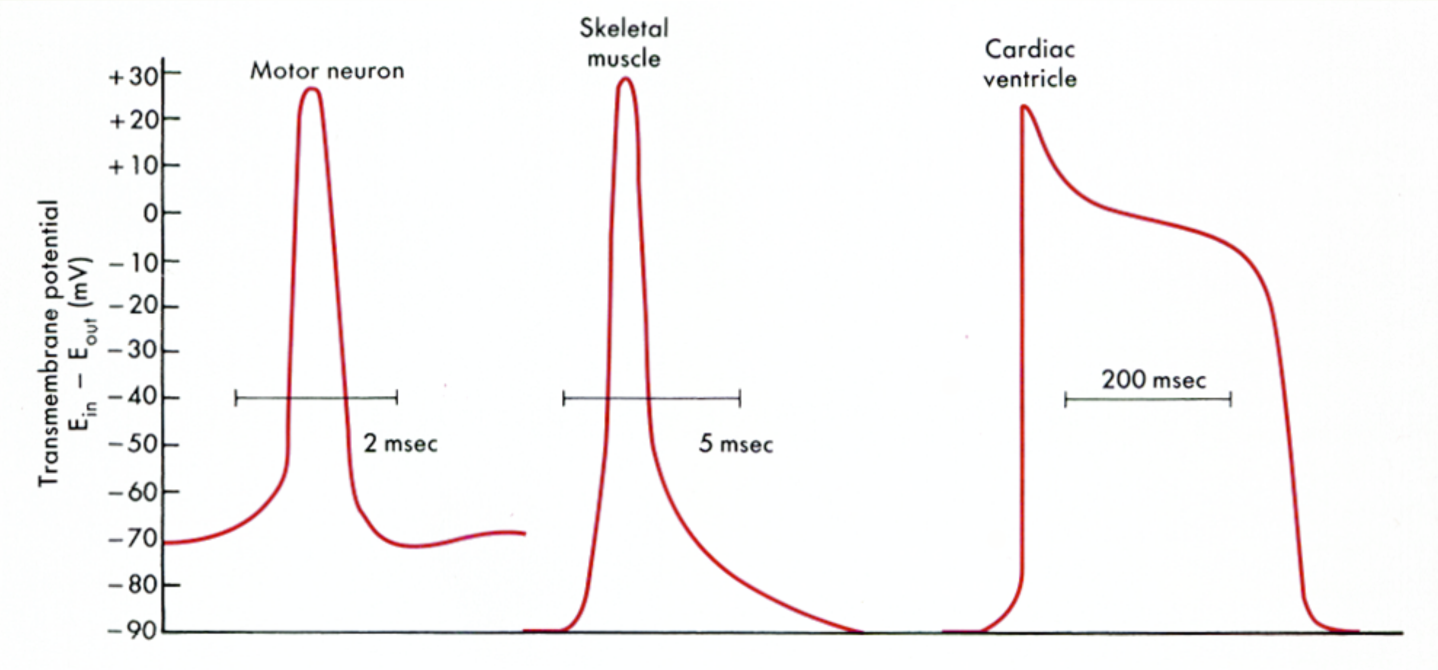




Na+
K+
③ Rising phase of the action potential
④ Falling phase of the action potential
② Depolarization
⑤ Undershoot
① Resting state
Extracellular fluid
Plasma membrane
Cytosol
Potassium channel
Activation gates
Sodium channel
Inactivation gate
Membrane potential (mV)
+50
0
−50
−100
Action potential
Threshold
Resting potential
Time

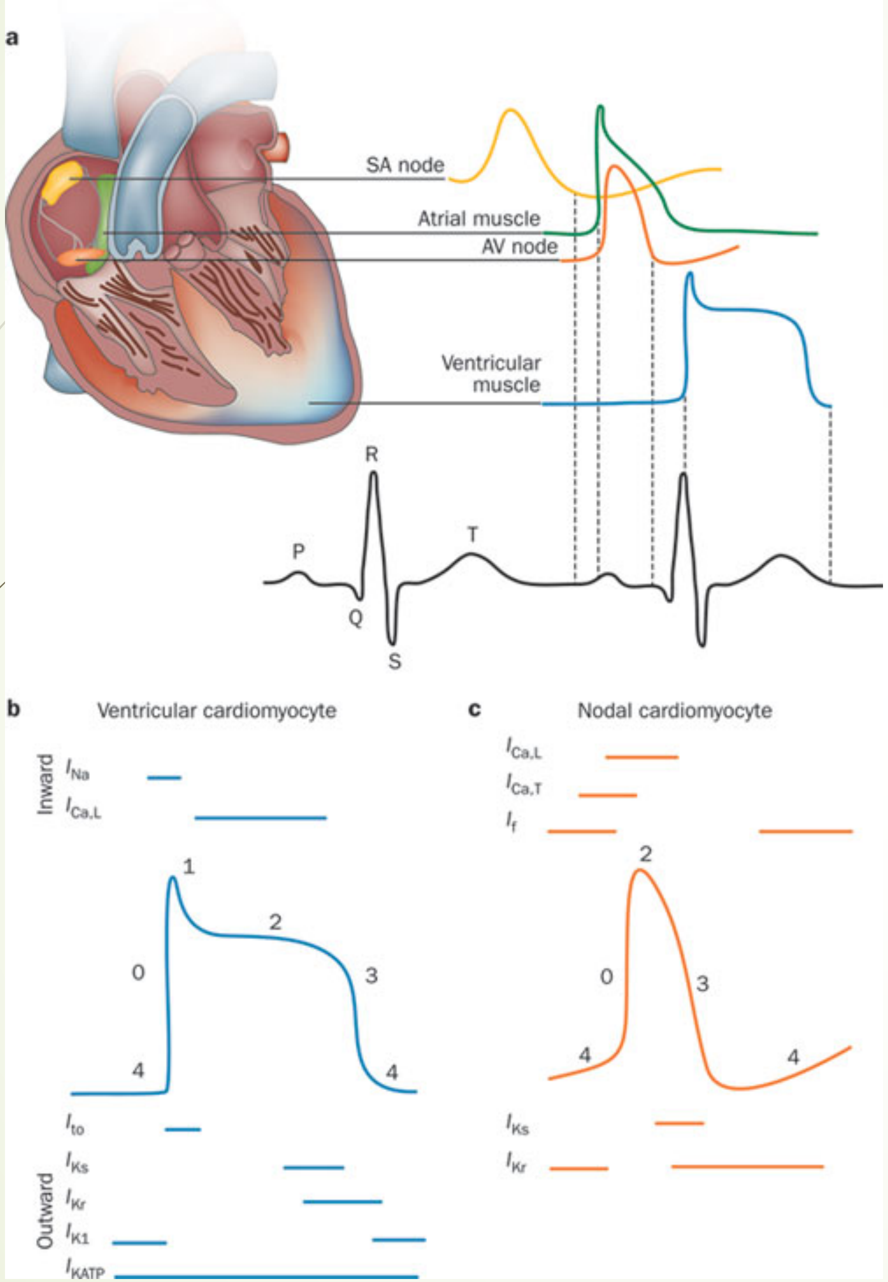
Excitable cells express high densities of VOCs and fire action potentials

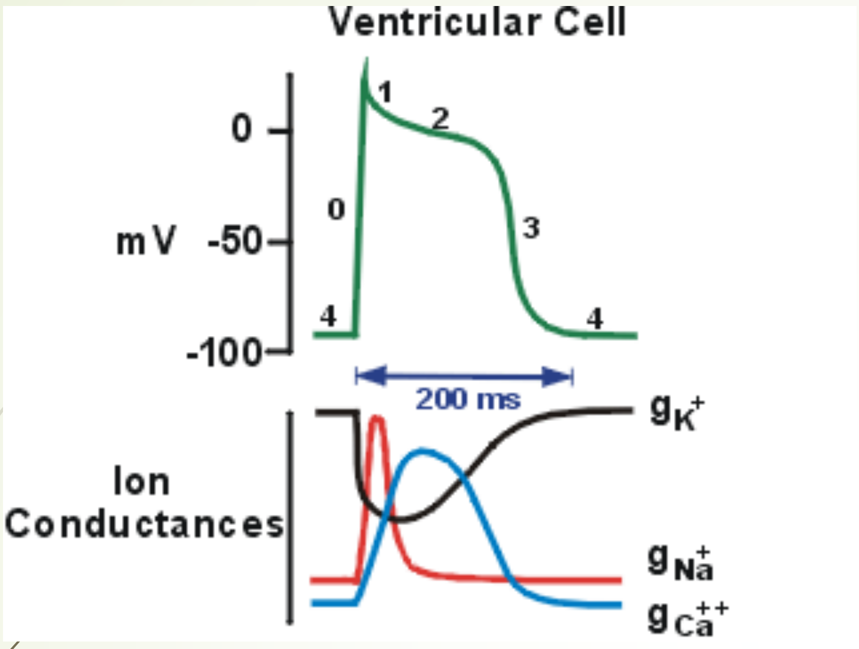
neurons
muscle cells
secretory cells

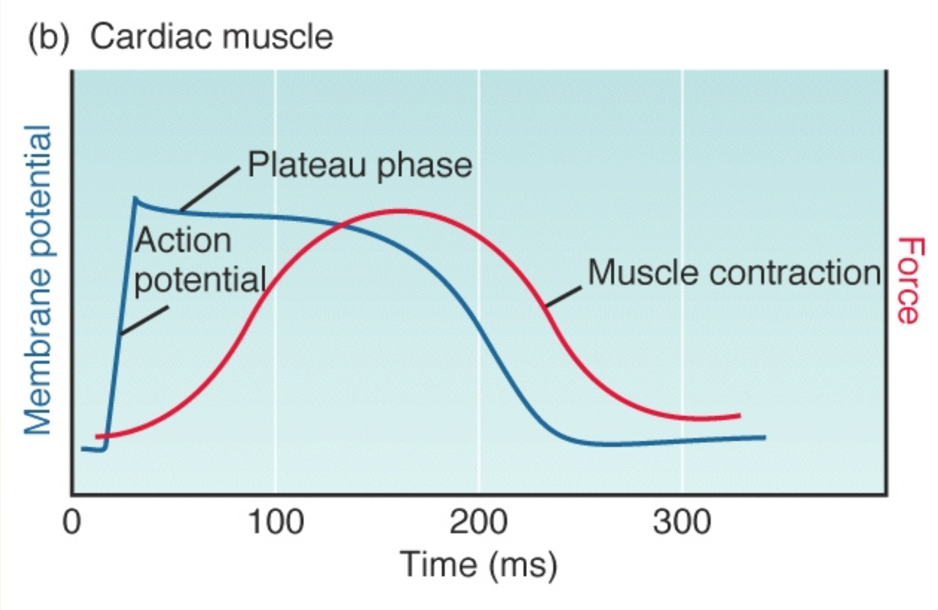


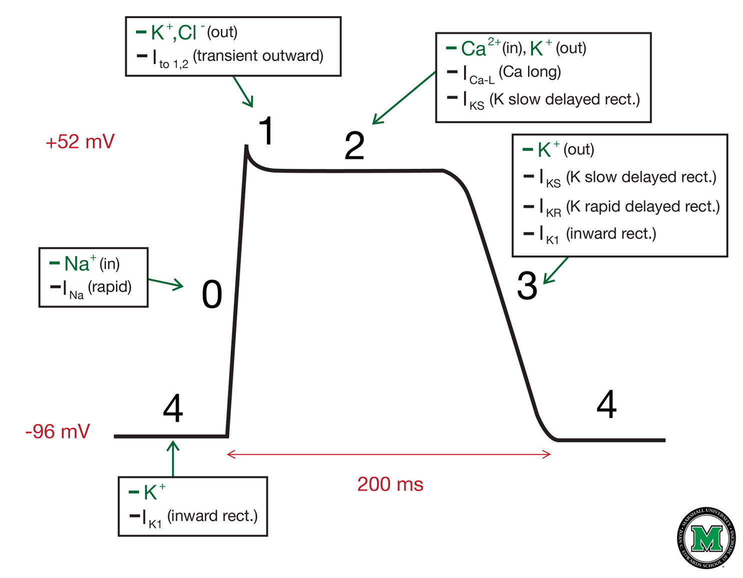
PdA with very different kinetics!

Cardiac Action Potentials









Long-lasting ventricular PdA

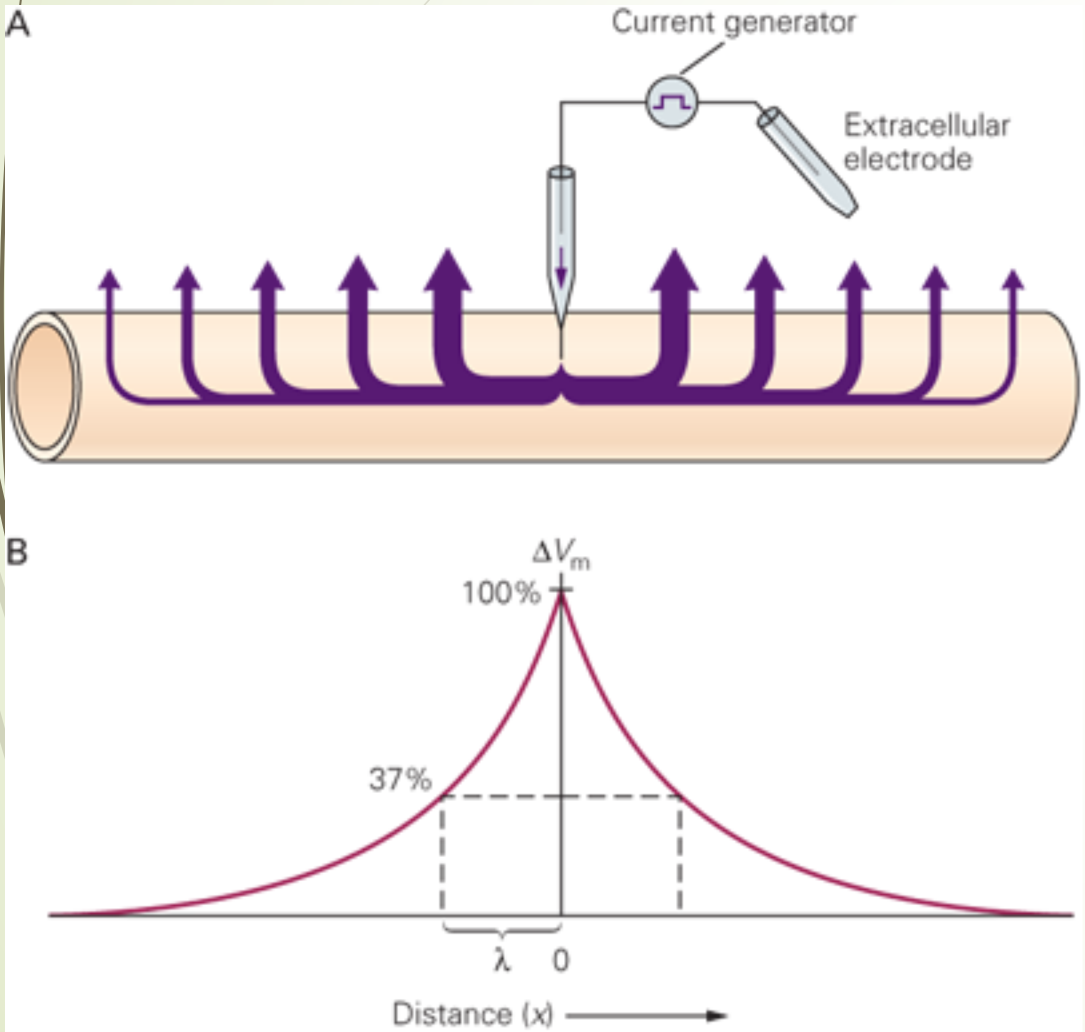
Propagation of signal conduction

Distance is not a relevant factor in the propagation of a signal in neuron's soma because the cell body can be approximated to a tiny sphere whose membranes voltage is uniform.

However when considering the signal travelling along extended structures such as dendrites, axons and muscle fibers, the signal decrease in amplitude with distance from the site of initiation.

Propagation of signal conduction

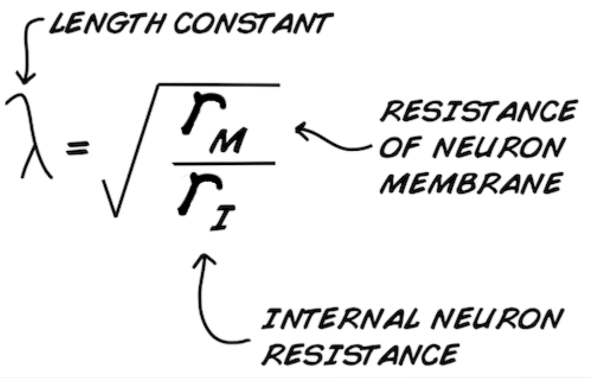
How geometry influences the distribution of current



The variation of the Vm with distance depends on the relative value of the **membrane resistance** in a unit length of dendrite, **r_m** (units Ω * cm) and internal neuron resistance per unit length of the dendrite, **r_i** (units Ω/cm).

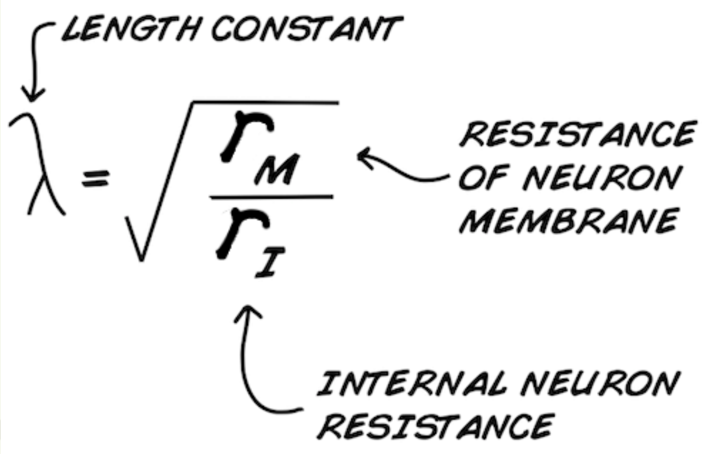
The change in Vm becomes smaller with distance along the dendrite away from the electrode. The decay with distanceis exponential:

$$V_x = V_0 e^{\frac{-x}{\lambda}}$$



Propagation of signal conduction

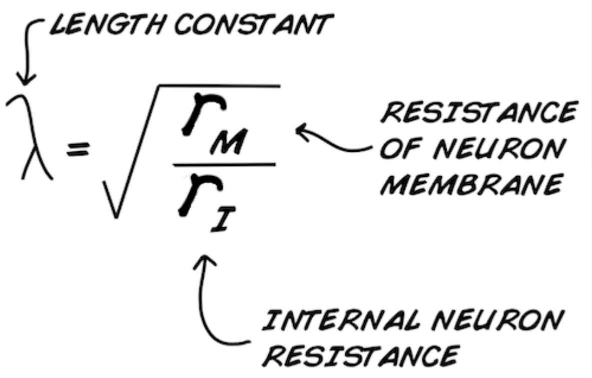
How geometry influences the distribution of current



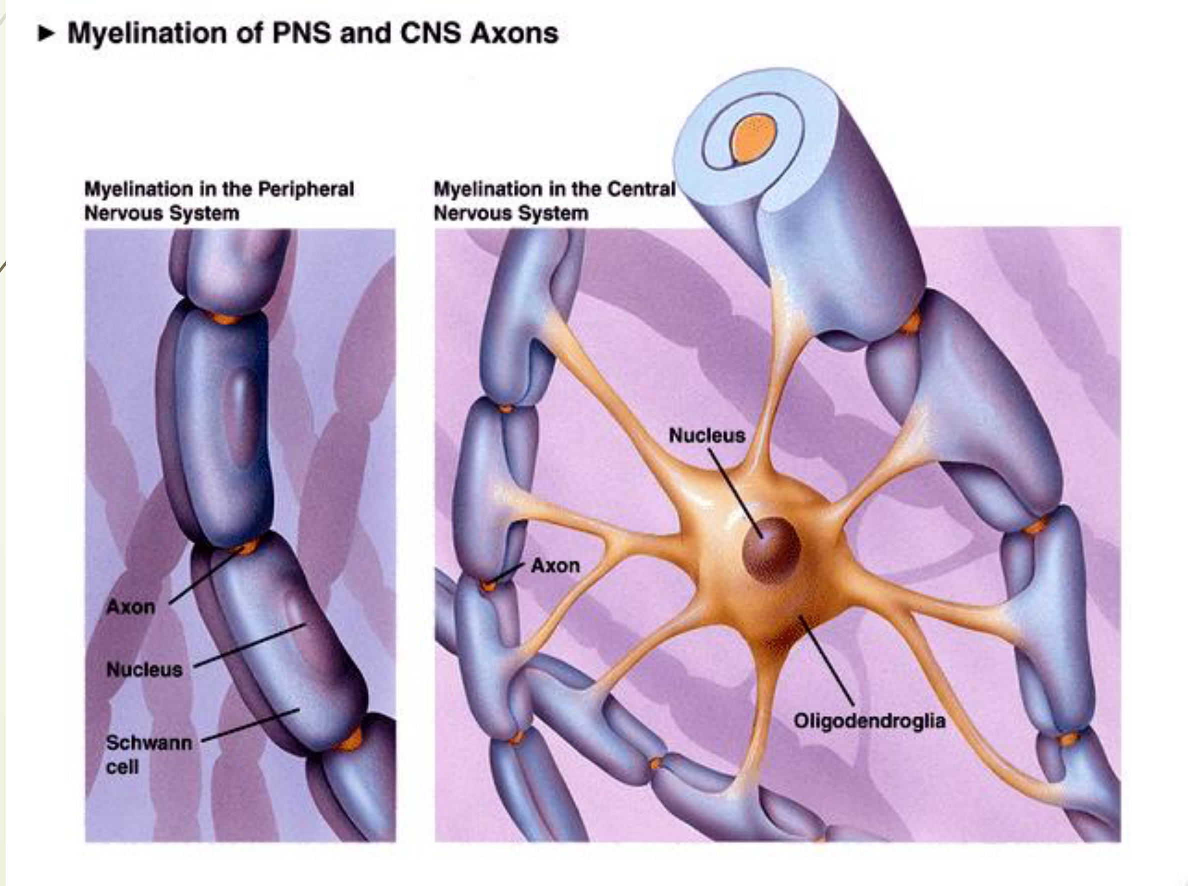
$$\lambda = \sqrt{\frac{r_M}{r_I}}$$

The better the insulation of the membrane (the greater $\mathbf{r_m}$), the better the conducting properties of the inner core (the lower $\mathbf{r_i}$), the greater the length constant of the dendrite

Myelination changes PdA propagation:
it increases resistance of neuron membrane (r_m)



$$\lambda = \sqrt{\frac{r_M}{r_I}}$$


► Myelination of PNS and CNS Axons

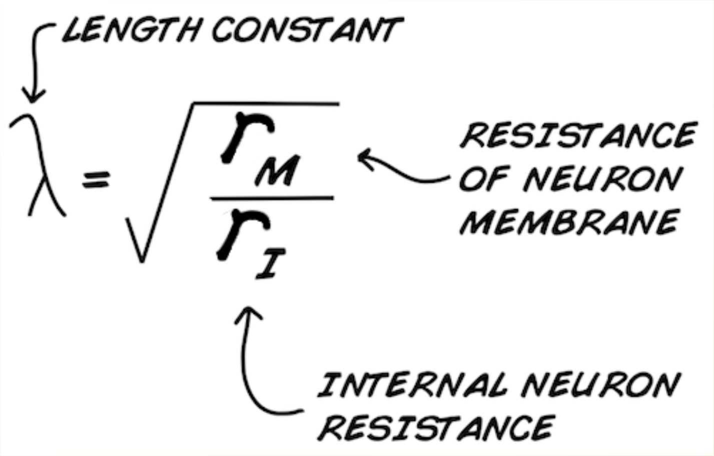
Propagation of signal conduction

The length constant is also a function of the diameter of the neuronal process

For neuronal processes with similar ion channels density and cytoplasmic composition, the larger the diameter, the longer is the length constant.

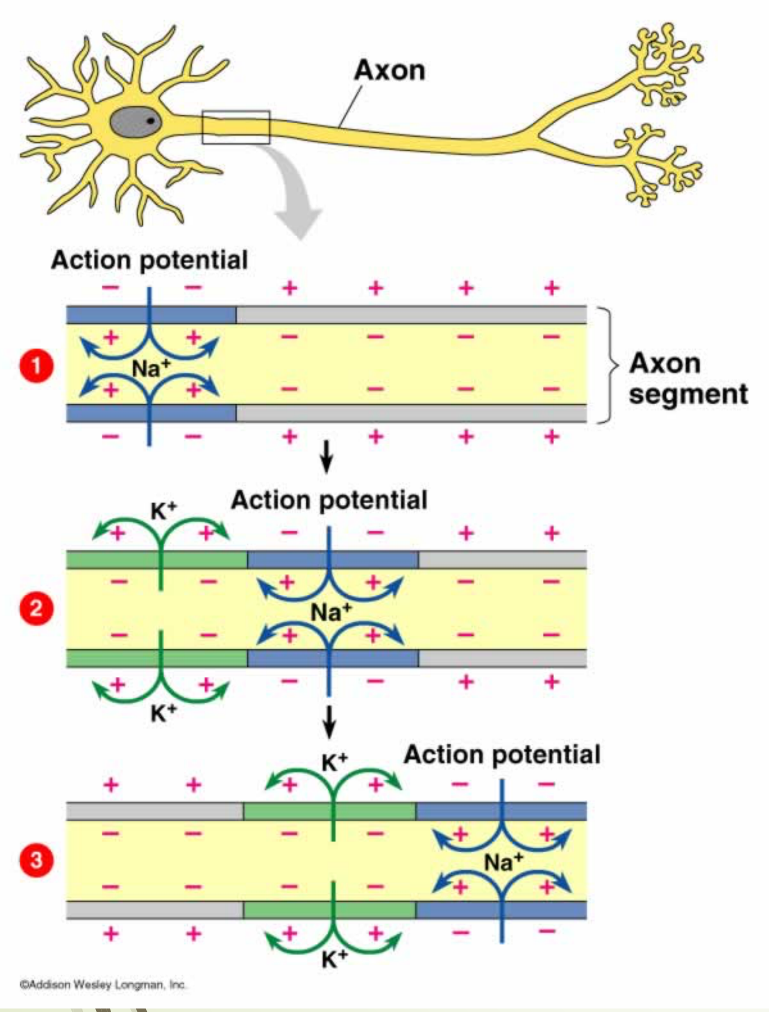
Thicker axons and dendrites have longer length constant than do narrower processes
Can **transmit signals for greater distances**

r_m (units Ω * cm)
r_i (units Ω/cm)



$$\lambda = \sqrt{\frac{r_M}{r_I}}$$

Propagation of signal conduction: electrotonic conduction



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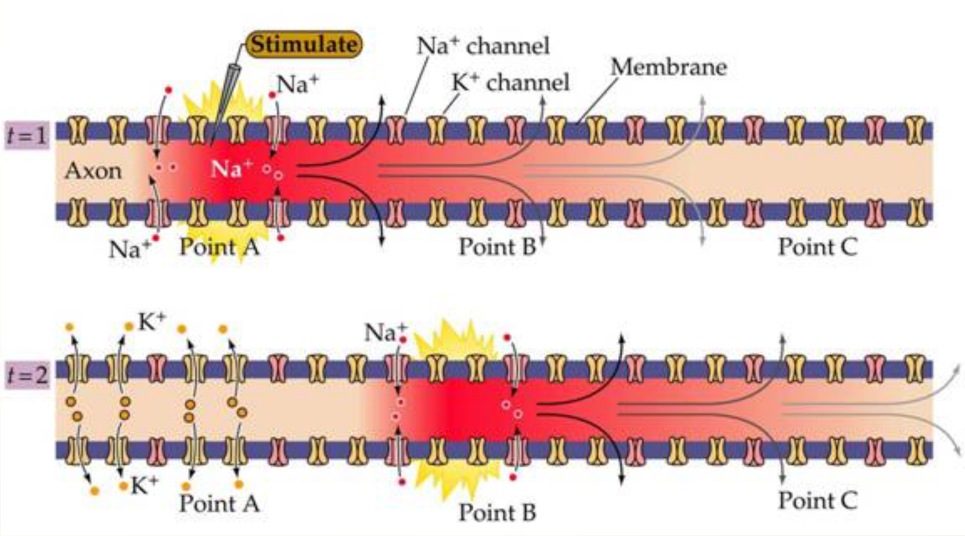
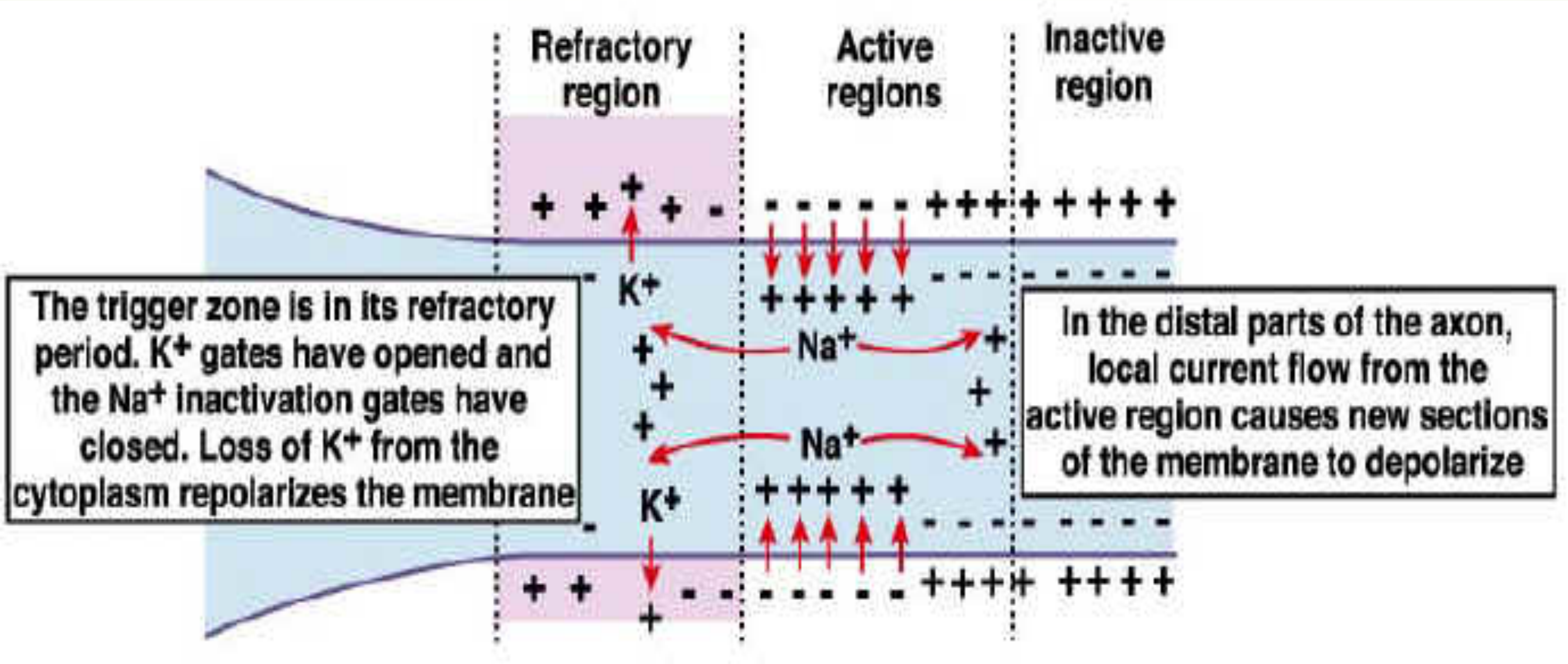
The electrotonic conduction is a factor in the propagation of action potential.

Once the membrane at any point along the axon has been depolarized beyond threshold, an action potential is generated in that region. This local depolarization spreads passively down the axon, causing a successive adjacent regions of the membrane to reach the threshold for generating an action potential



Propagation of signal conduction: electrotonic conduction

The electrotonic conduction is a factor in the propagation of action potential. Once the membrane at any point along the axon has been depolarized beyond threshold, an action potential is generated in that region. This local depolarization spreads passively down the axon, causing a successive adjacent regions of the membrane to reach the threshold for generating an action potential



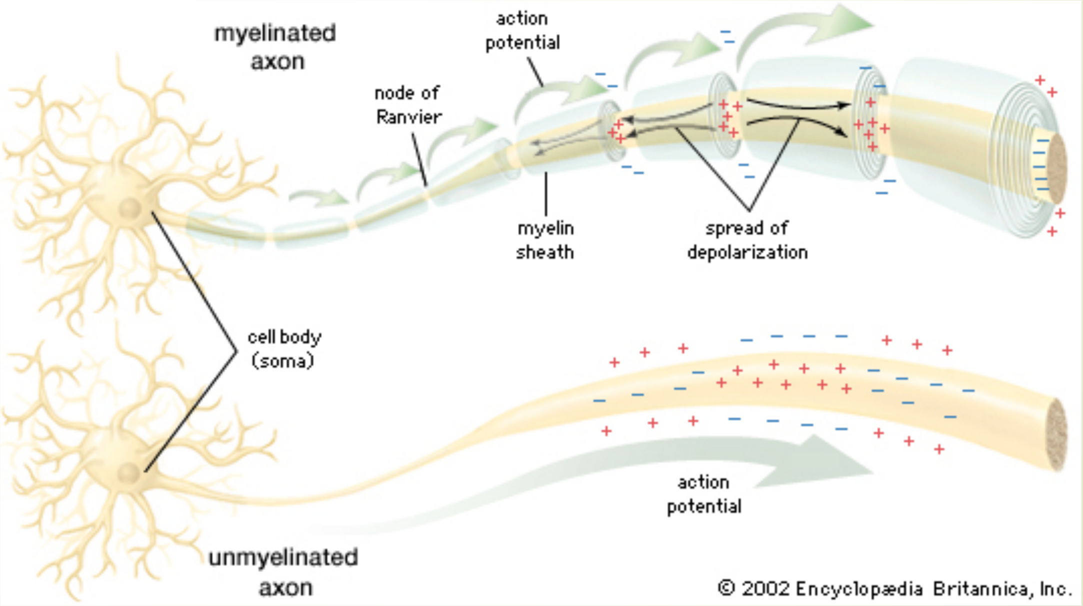
The trigger zone is in its refractory period. K+ gates have opened and the Na+ inactivation gates have closed. Loss of K+ from the cytoplasm repolarizes the membrane

In the distal parts of the axon, local current flow from the active region causes new sections of the membrane to depolarize

Rapid Propagation of signal conduction:

Neurons have adopted an adaptive strategy to allow a rapid conduction propagation by wrapping a myelin sheath around the axonal membrane.
On the other hand the PdA is triggered in a non myelinated initial segment of membrane just distal to the axon hillock.
Even though the capacitance of the axon is quite small(because of the myelin insulation), the amount of current down the core of the axon from the trigger zone is not enough to discharge the capacitance along the entire length of the myelinated axon

Saltatory conduction: **nodes of Ranvier**.
The myelin sheath is interrupted every 1 or 2 mm by bare patches of axon membrane approximately 1μm in length



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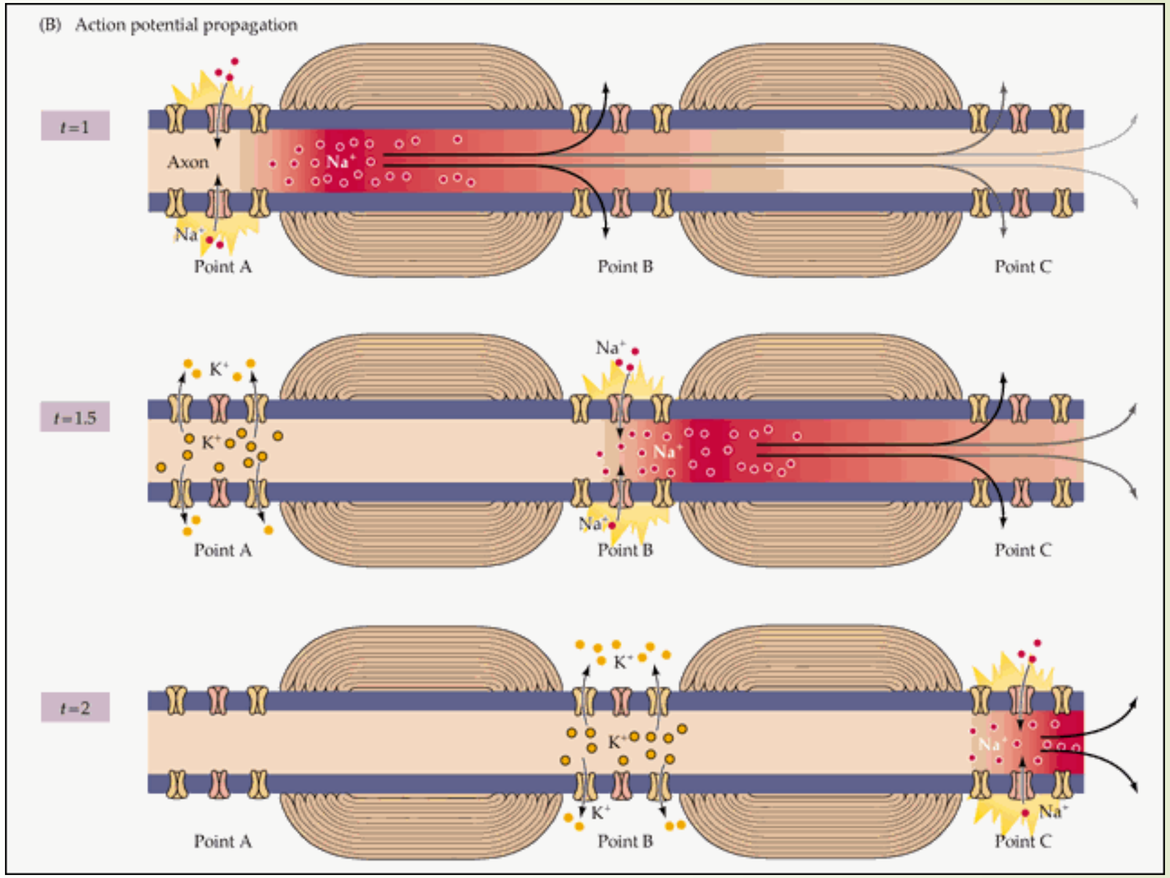
Rapid Propagation of signal conduction:

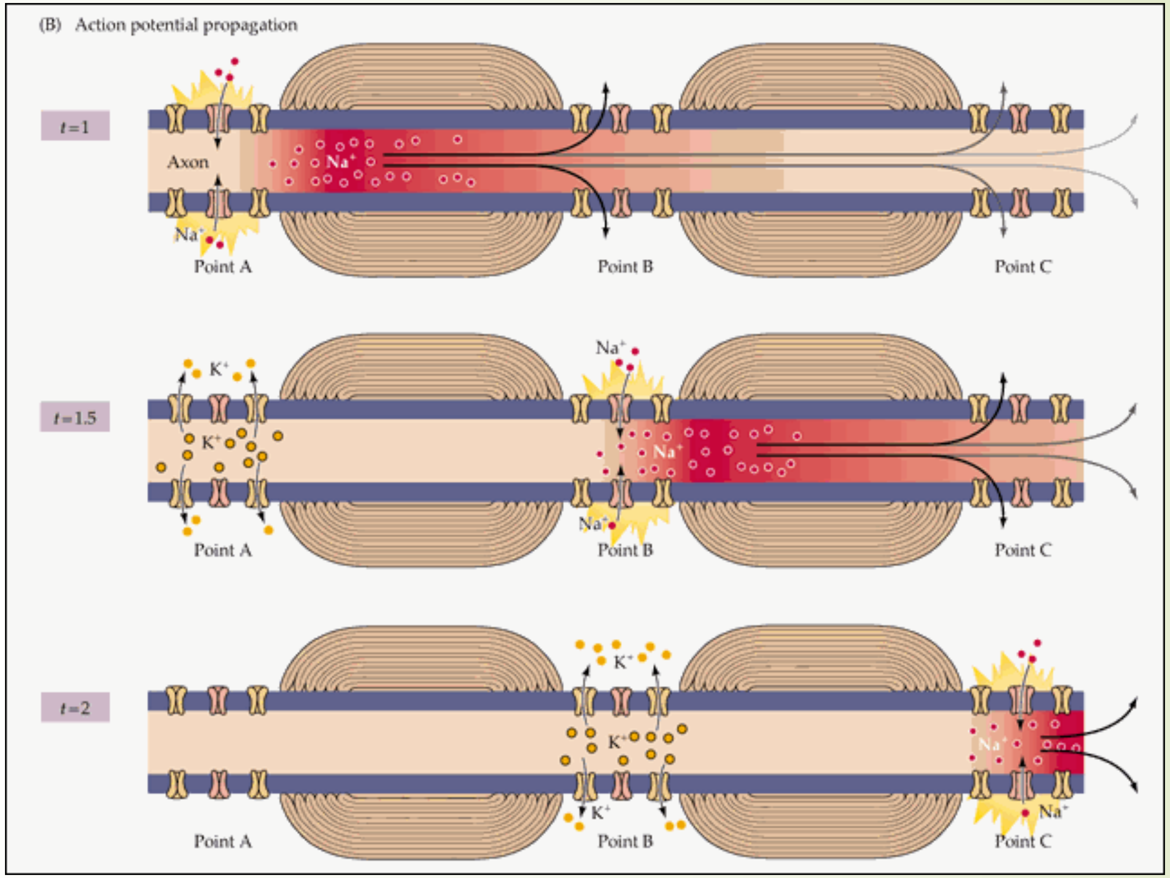
Although the area of the nodal membrane at each node is quite small, the nodal membrane is rich of voltage-gated Na+ and K+ channels and thus can generate an intense depolarizing inward Na+ current in response to the passive spread of depolarization down along the axon



The Ranvier nodes Boost the amplitude of the depolarization periodically, preventing it from decaying with distance

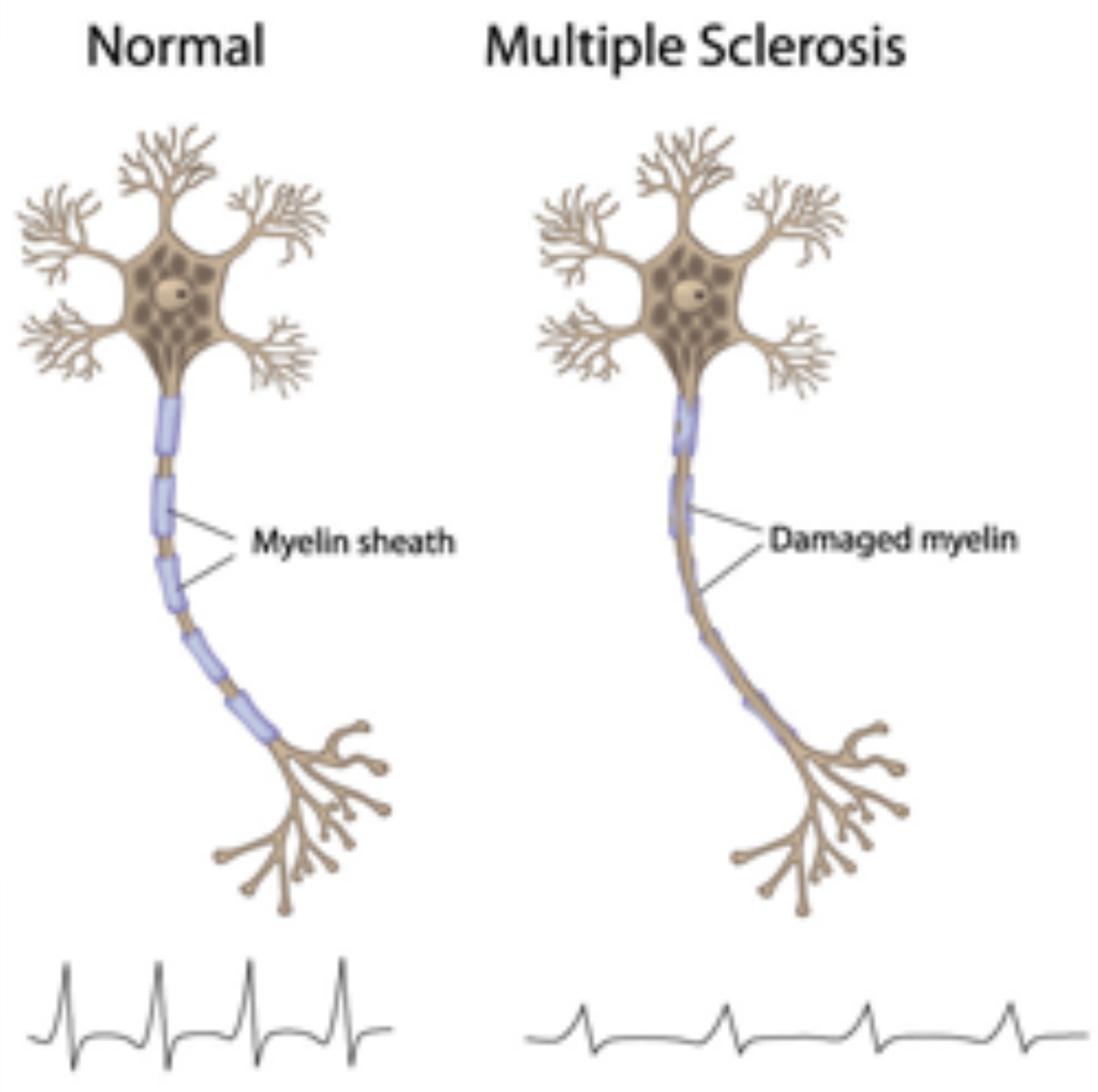
Rapid Propagation of signal conduction:

Because ionic membrane current flows only at the nodes in myelinated fibers, saltatory conduction is also favorable from the metabolic standpoint. Less energy must be expected by the Na+-K+ pump in restoring the Na+ and K+ concentration gradients, which tend to run down as the Action potential is propagated


(B) Action potential propagation

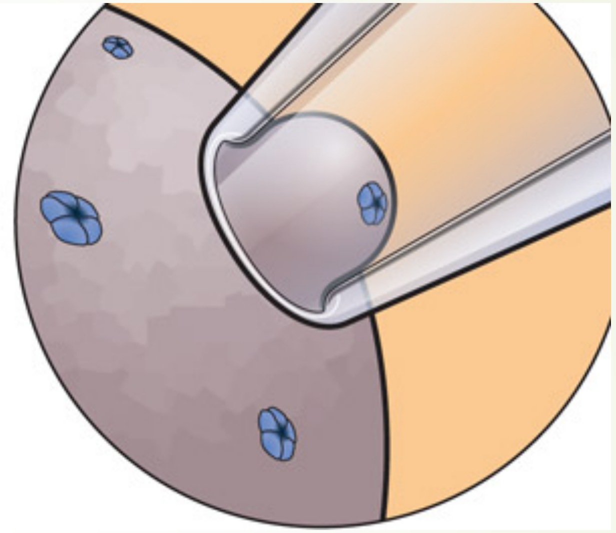
Rapid Propagation of signal conduction:

Various diseases are caused by demyelination, such as multiple sclerosis and Guillain-Barré syndrome.



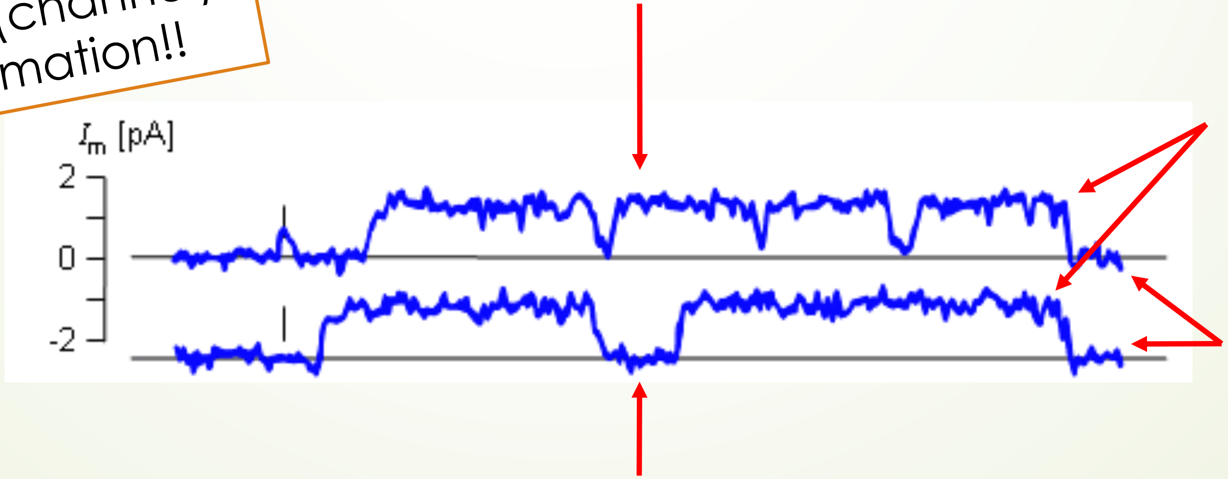
PATCH CLAMP technique

•**Erwin Neher and Bert Sakmann** developed the patch clamp in the late 1970s and early 1980s. They **received the Nobel Prize in Physiology or Medicine in 1991 for this work.**



Measurement of ionic currents flowing through the entire plasma membrane of a cell or a SINGLE CHANNEL:
high resistance seal

A single protein (channel) changes conformation!!

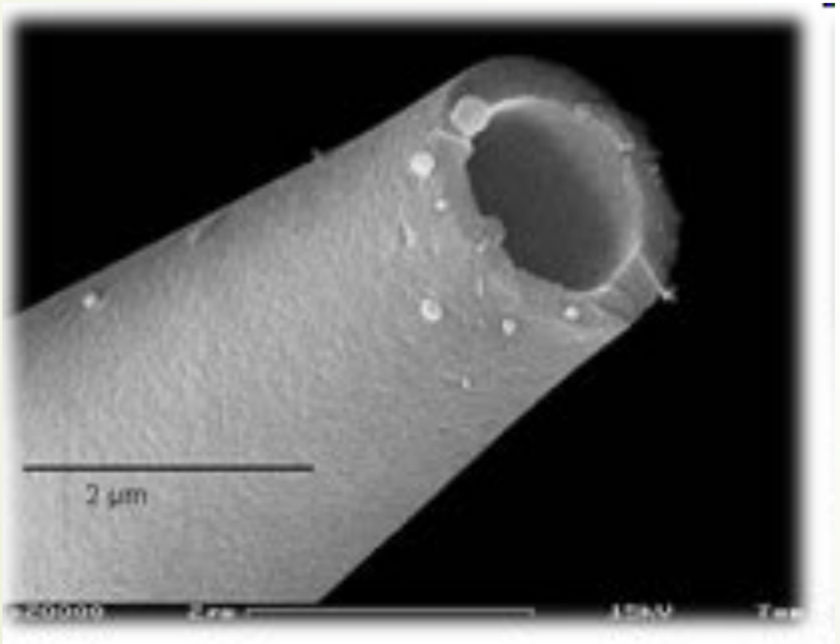


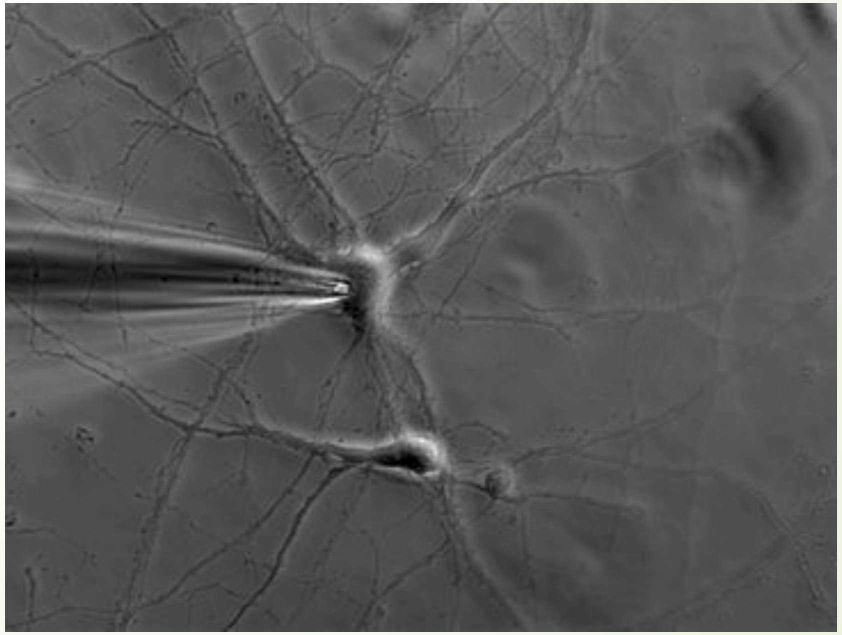
Open state

Closed state

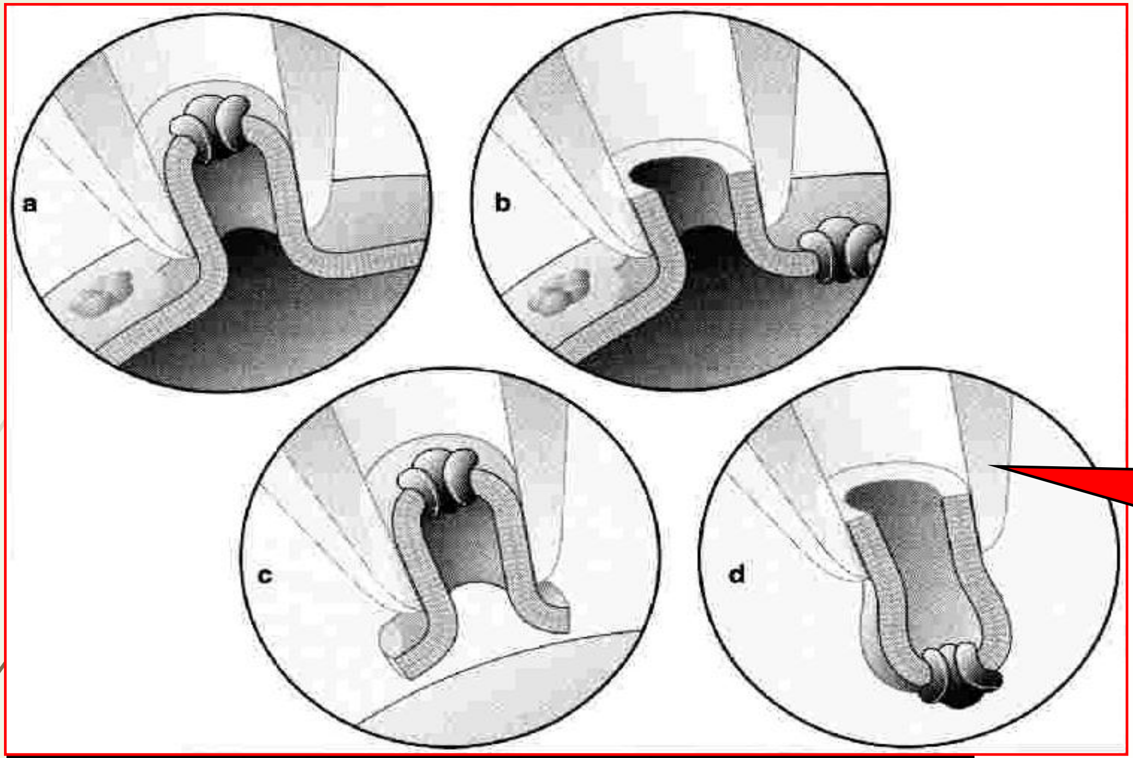
Patch-Clamp

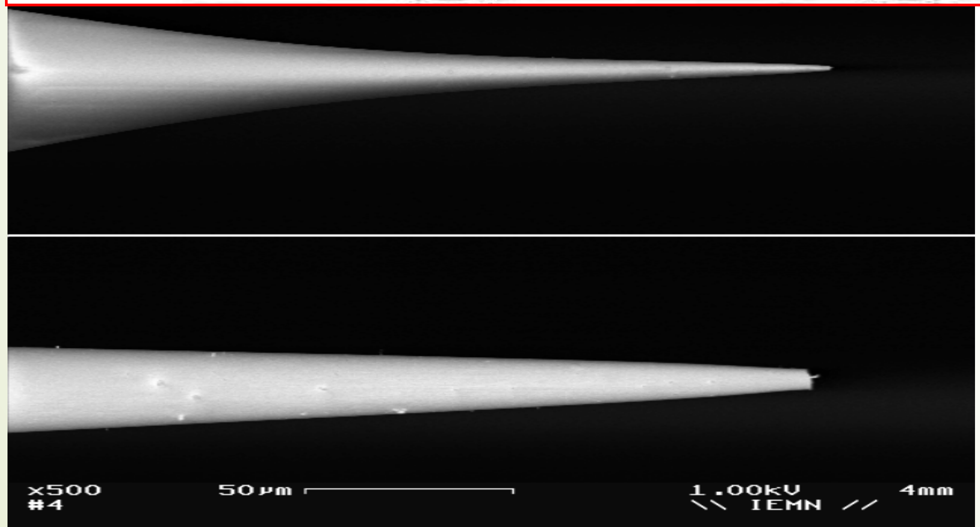
- The diameter of the capillary tip is about 0,5 uM
- The tip is filled with a saline solution (extra or intracellular depending on the configuration)

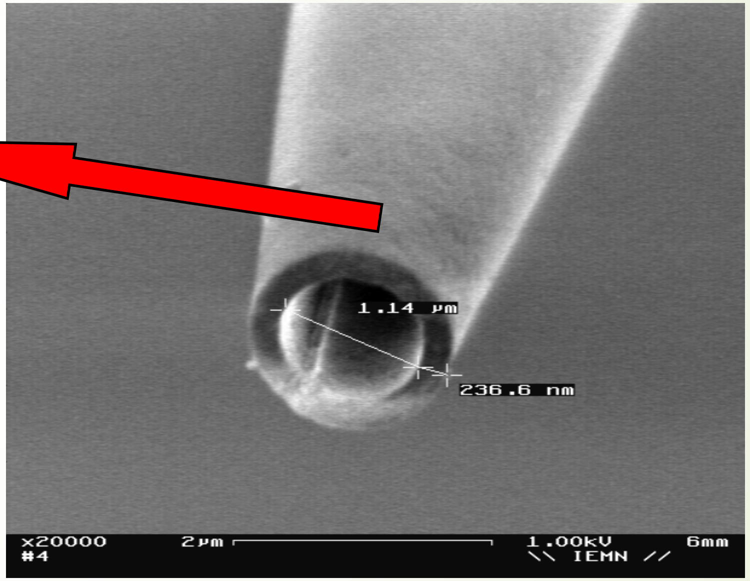


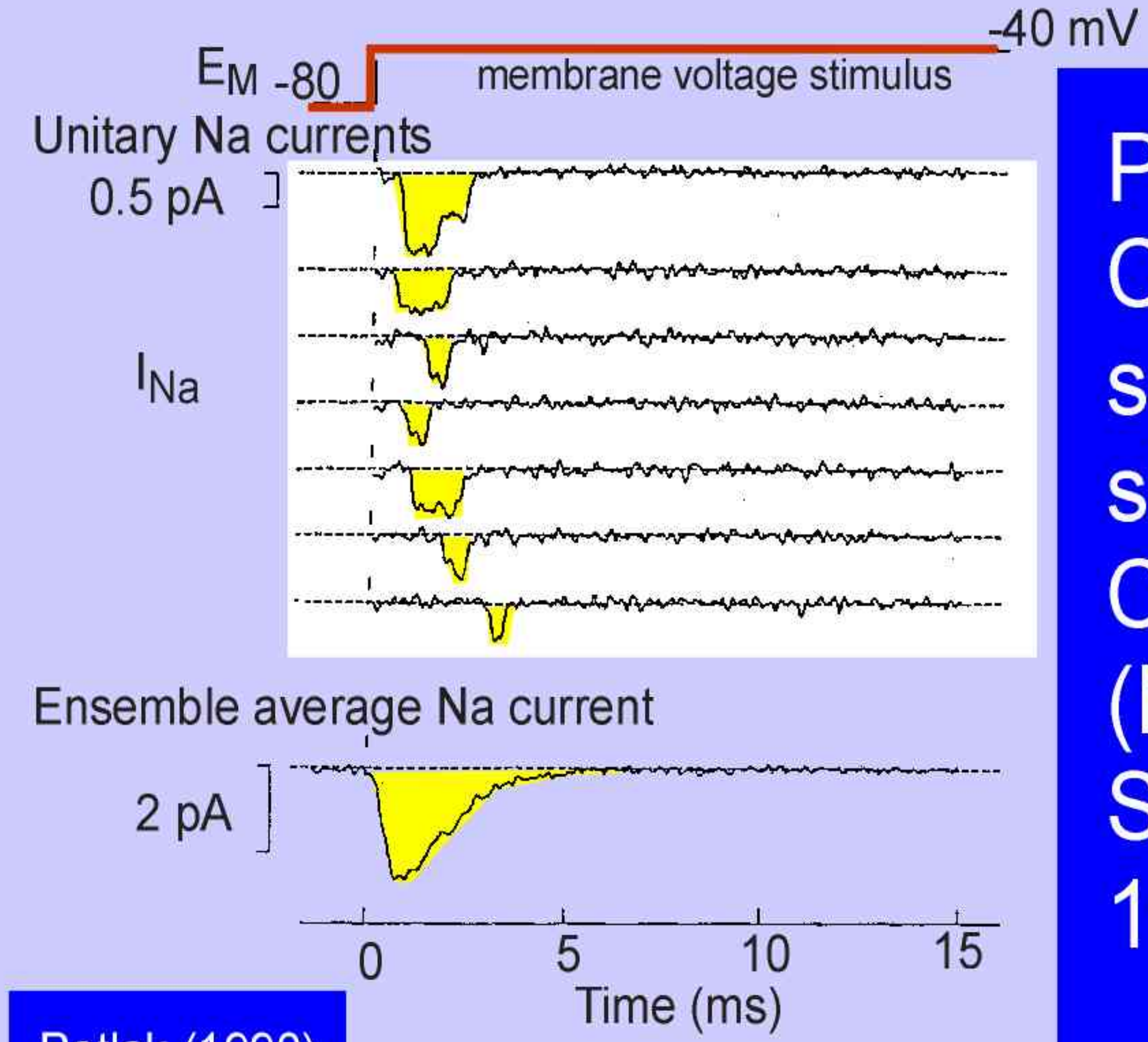


Patch-Clamp


a
b
c
d

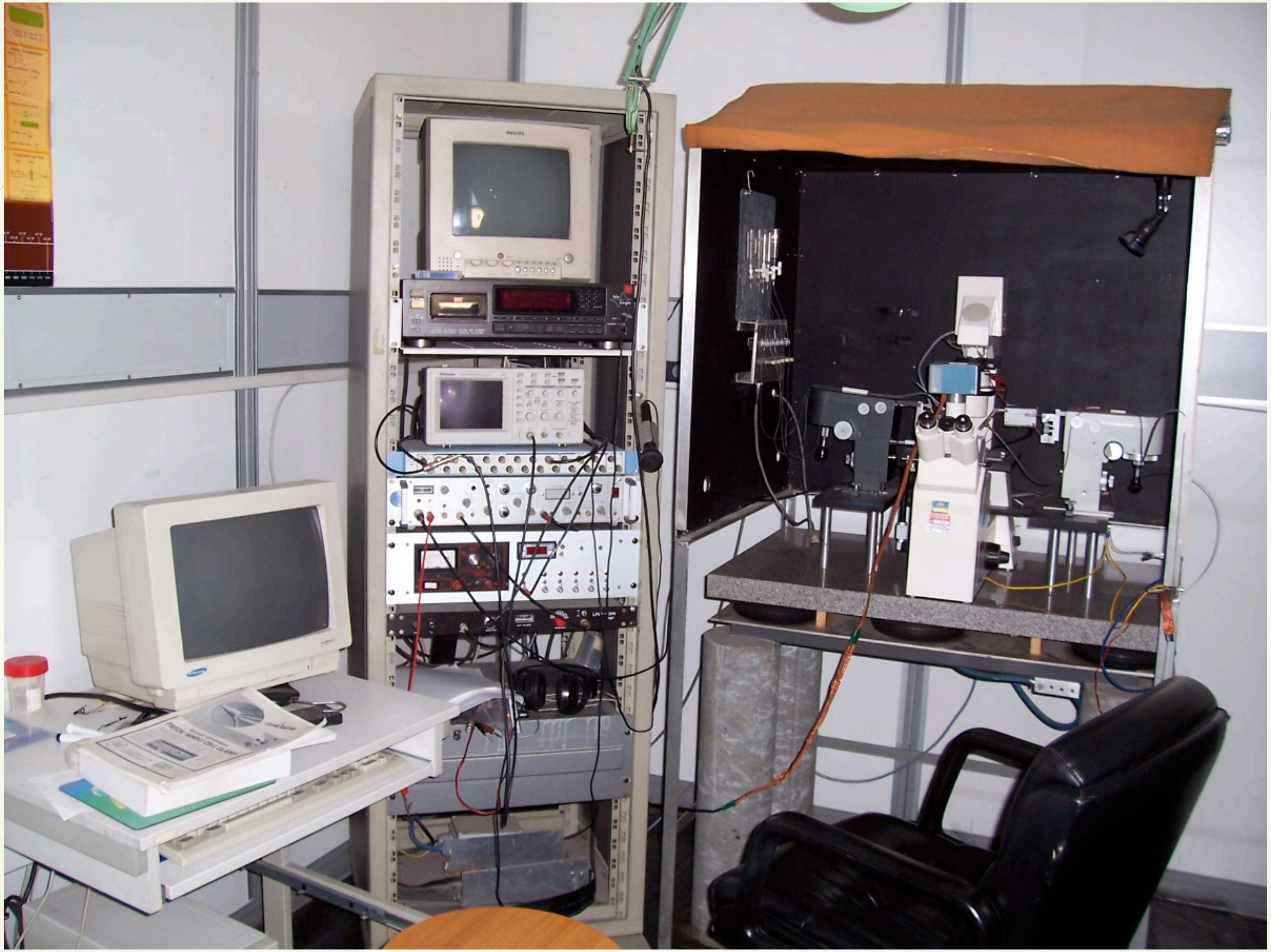

x500
#4
50 µm
1.00kV
4mm
\\ IEMN //

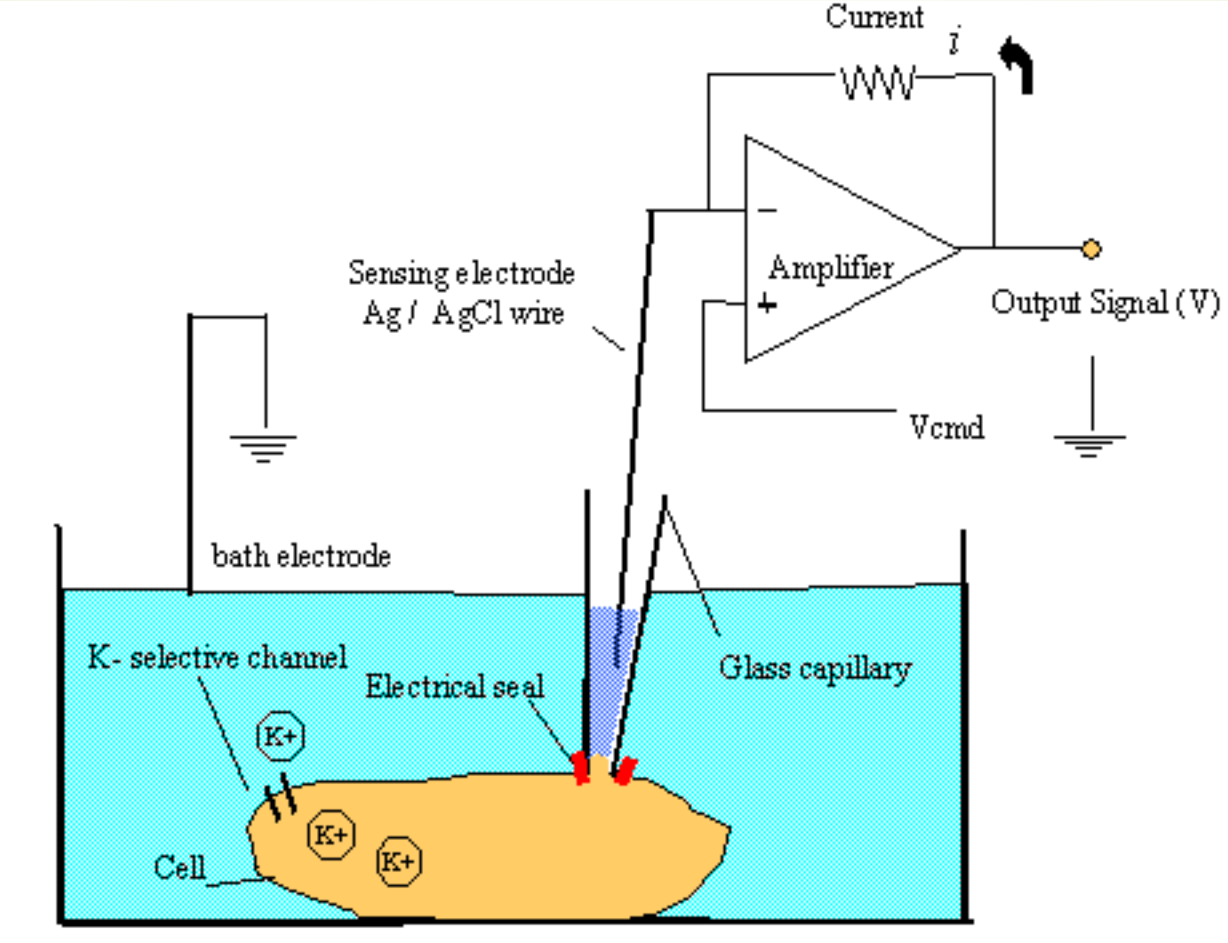

1.14 µm
236.6 nm
x20000
#4
2µm
1.00kV
6mm
\\ IEMN //



Patlak (1990)

Patch Clamp sees single Ion Channels (Neher & Sakmann, 1981)





Functional depiction of classical patch-clamp electrophysiology

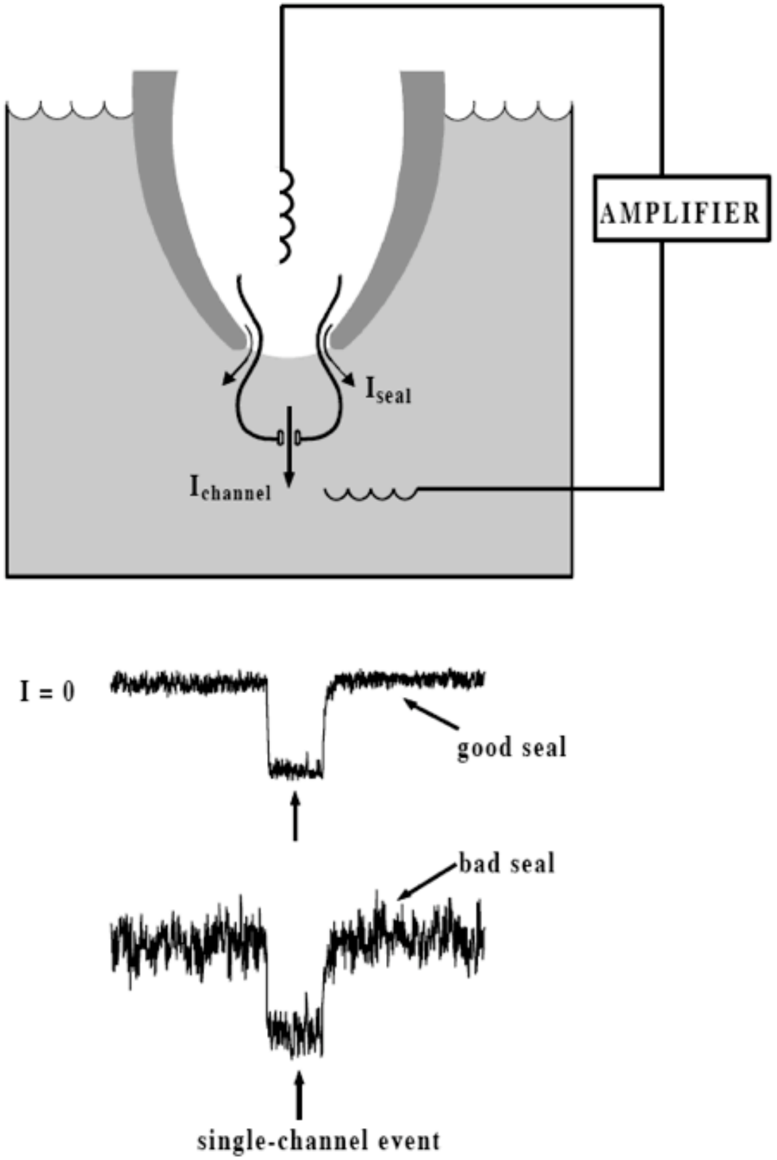
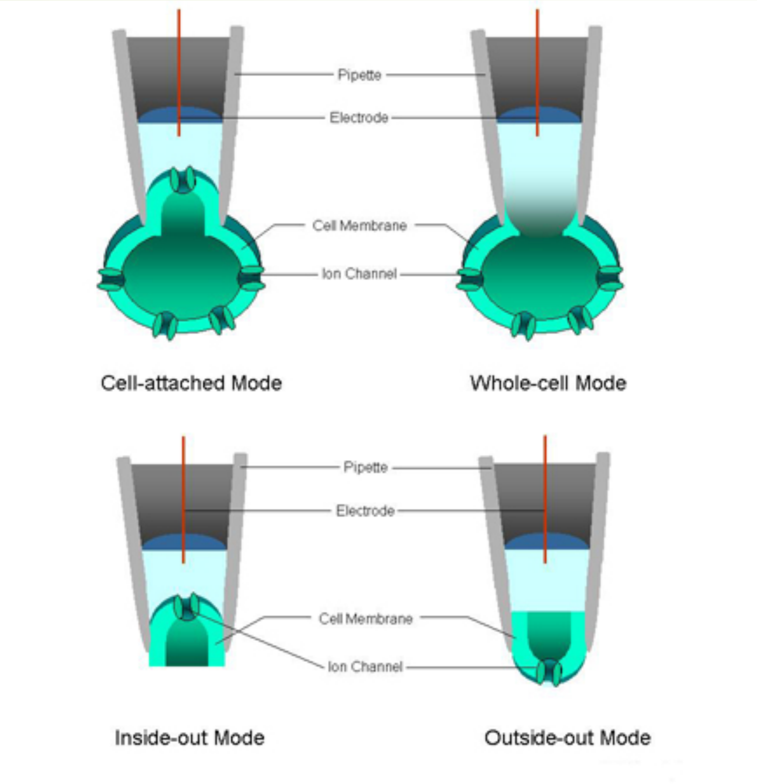
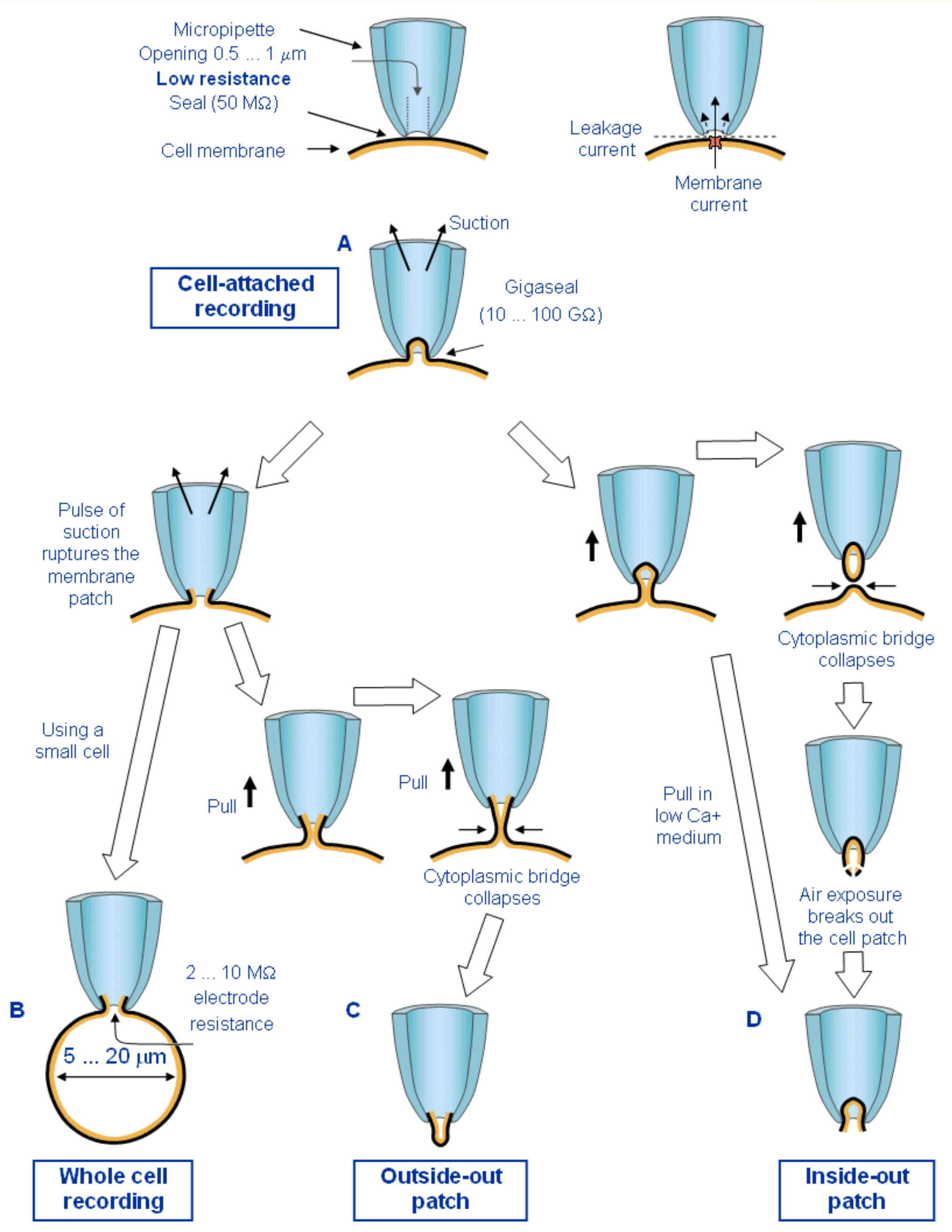
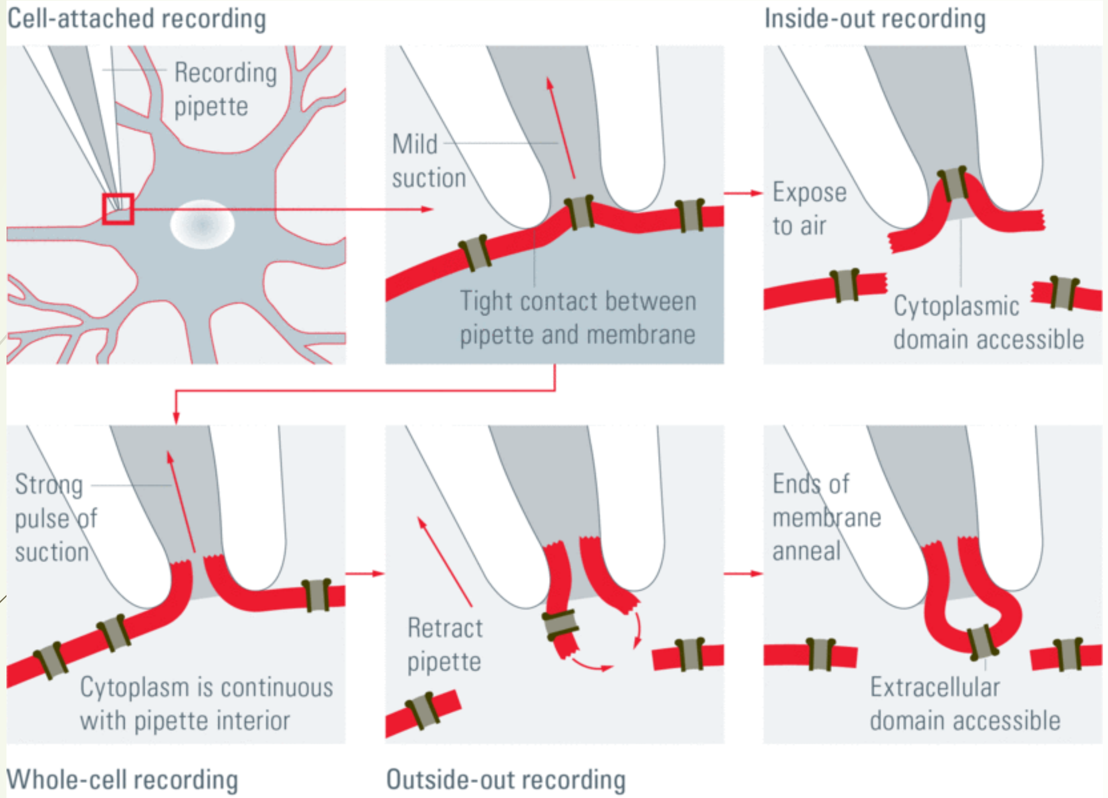


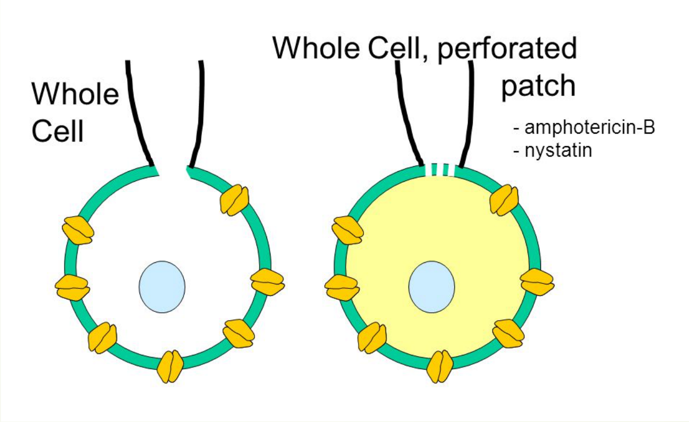
Figure 1-17. Good and Bad Seals

In a patch recording, currents through the seal also flow through the measuring circuit, increasing the noise on the measured current.


Pipette
Electrode
Cell Membrane
Ion Channel
Cell-attached Mode
Whole-cell Mode
Pipette
Electrode
Cell Membrane
Ion Channel
Inside-out Mode
Outside-out Mode


Micropipette
Opening 0.5 ... 1 µm
Low resistance
Seal (50 MΩ)
Cell membrane
Leakage current
Membrane current
A
Suction
Cell-attached recording
Gigaseal (10 ... 100 GΩ)
Pulse of suction ruptures the membrane patch
Using a small cell
Pull
Pull
Cytoplasmic bridge collapses
Cytoplasmic bridge collapses
Pull in low Ca+ medium
Air exposure breaks out the cell patch
B
2 ... 10 MΩ electrode resistance
5 ... 20 µm
C
D
Whole cell recording
Outside-out patch
Inside-out patch


Cell-attached recording
Recording pipette
Mild suction
Tight contact between pipette and membrane
Inside-out recording
Expose to air
Cytoplasmic domain accessible
Strong pulse of suction
Cytoplasm is continuous with pipette interior
Whole-cell recording
Retract pipette
Outside-out recording
Ends of membrane anneal
Extracellular domain accessible


Whole Cell
Whole Cell, perforated patch
- amphotericin-B
- nystatin

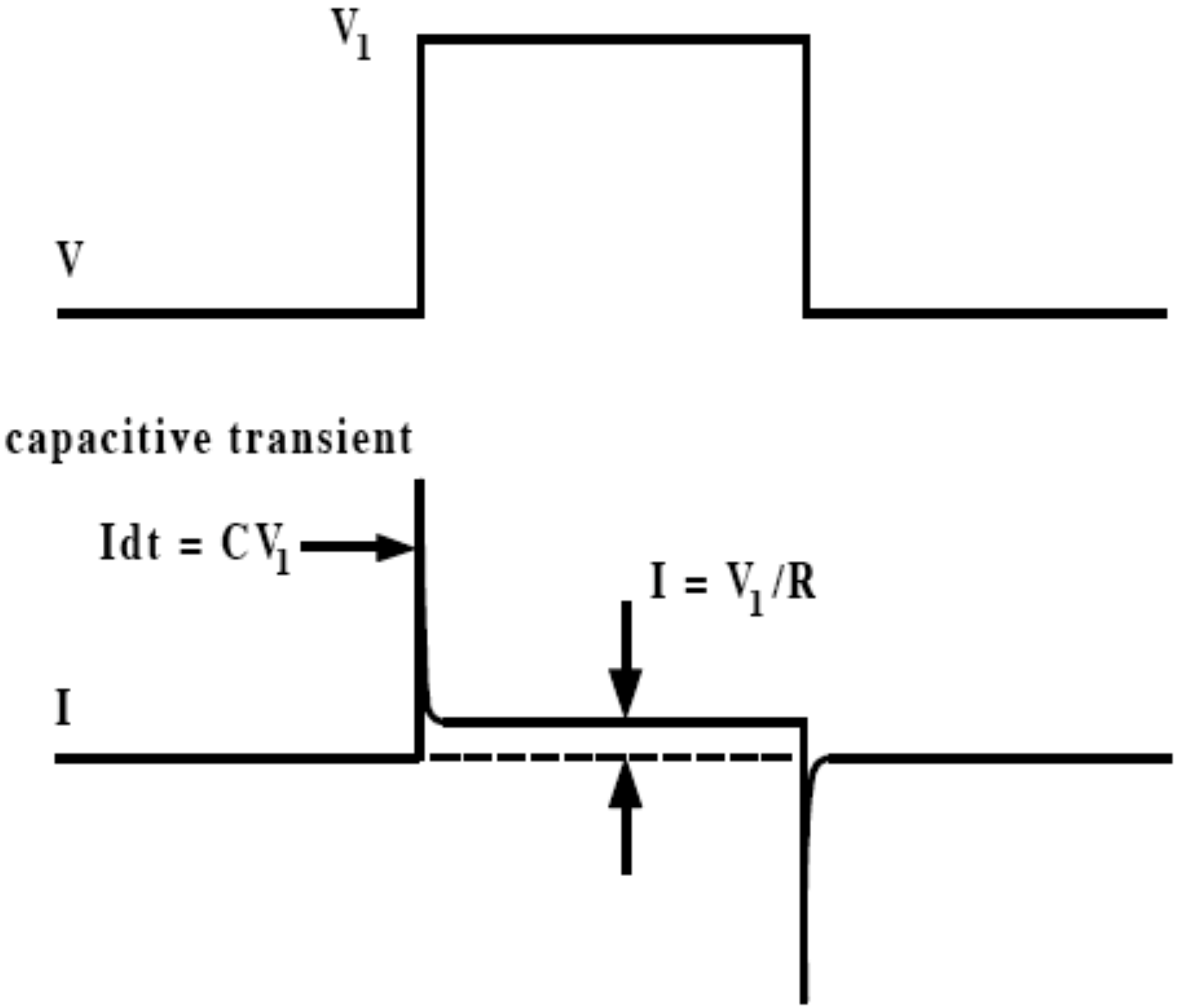
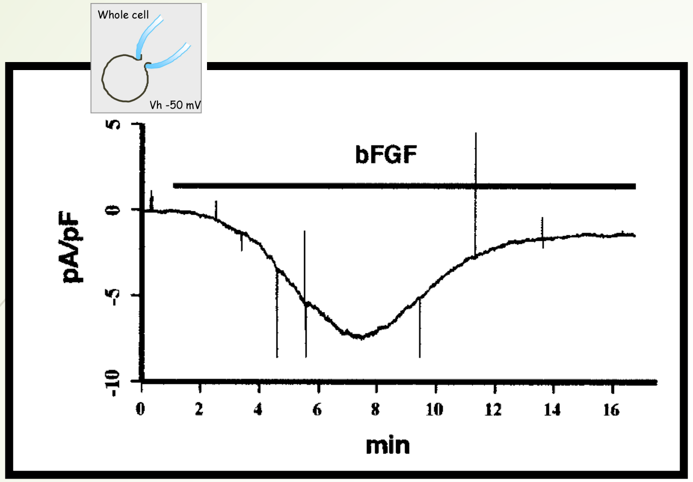
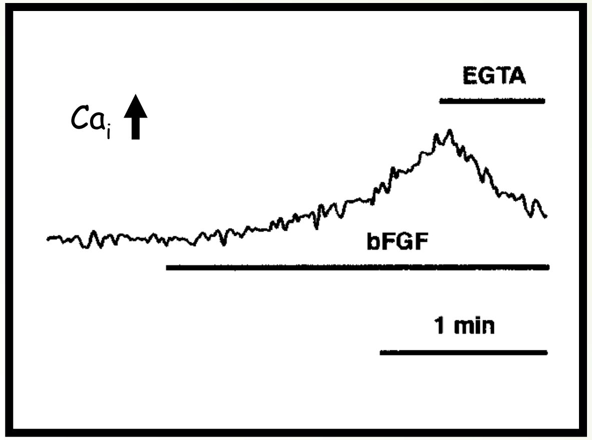
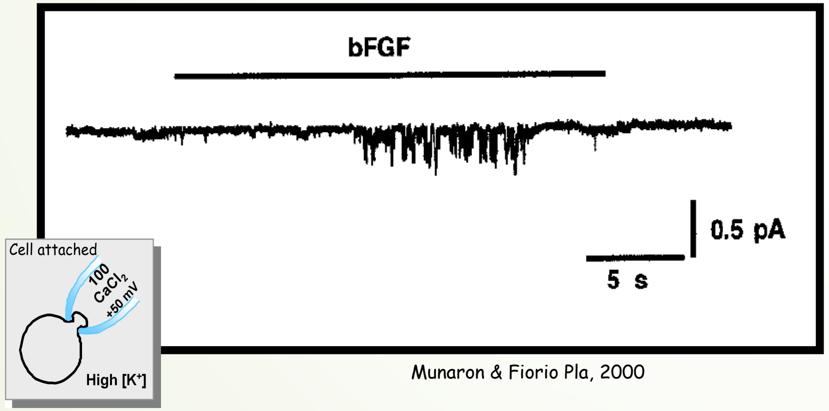


Figure 1-15. Typical Voltage-Clamp Experiment

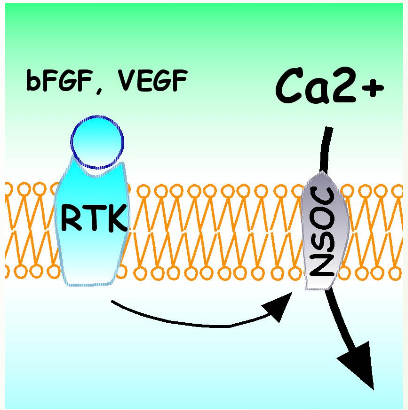
A voltage-clamp experiment on the circuit of Figure 1-13.



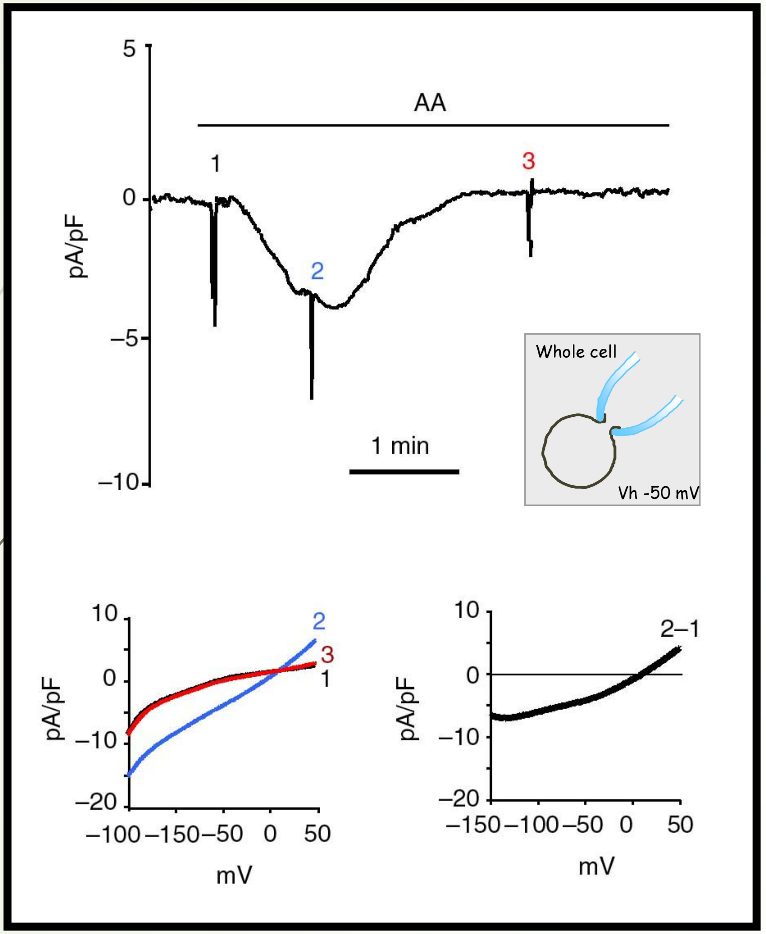


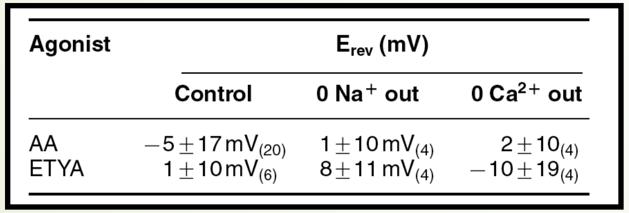


Munaron & Fiorio Pla, 2000



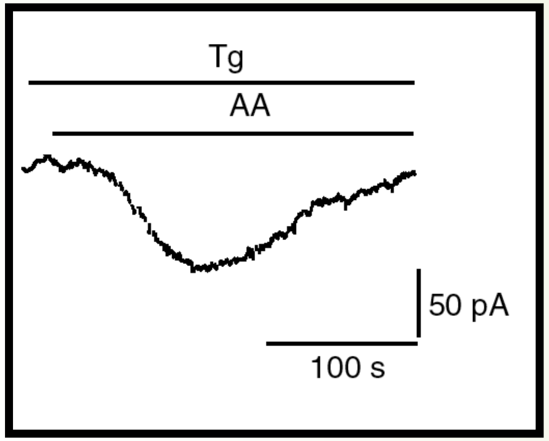
AA is able to activate NSOCs in BAECs

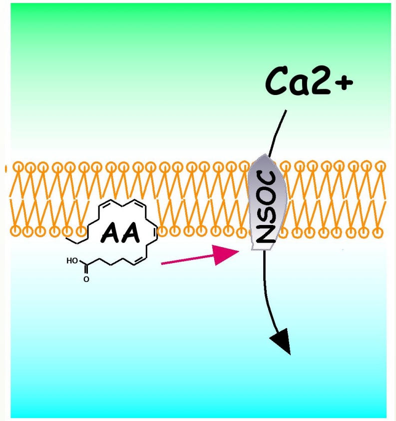




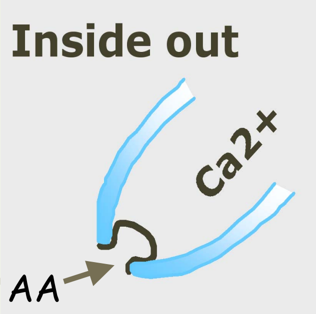
Agonist	E_{rev} (mV)		
	Control	0 Na^+ out	0 Ca^{2+} out
AA	$-5 \pm 17\,\mathrm{mV}_{(20)}$	$1 \pm 10\,\mathrm{mV}_{(4)}$	$2 \pm 10_{(4)}$
ETYA	$1 \pm 10\,\mathrm{mV}_{(6)}$	$8 \pm 11\,\mathrm{mV}_{(4)}$	$-10 \pm 19_{(4)}$

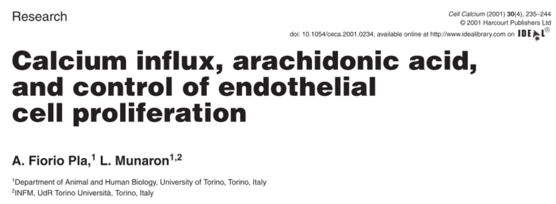
Fiorio Pla & Munaron, 2001





Single channel analysis revealed that arachidonic acid activates 3 different calcium channels in endothelial cells




Research

Cell Calcium (2001) **30**(4), 235–244
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Calcium influx, arachidonic acid, and control of endothelial cell proliferation

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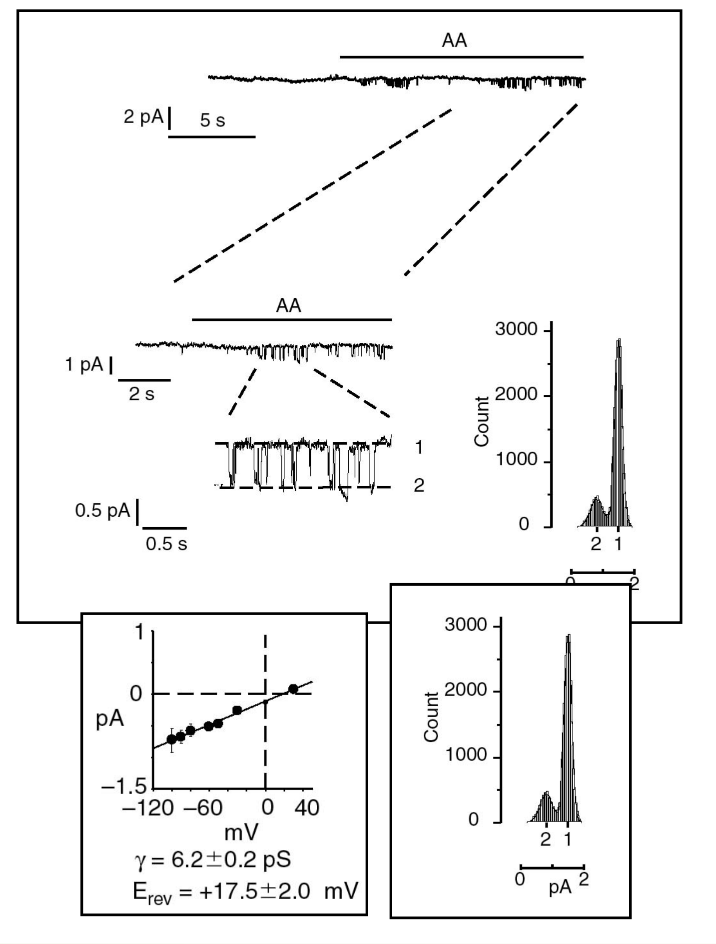

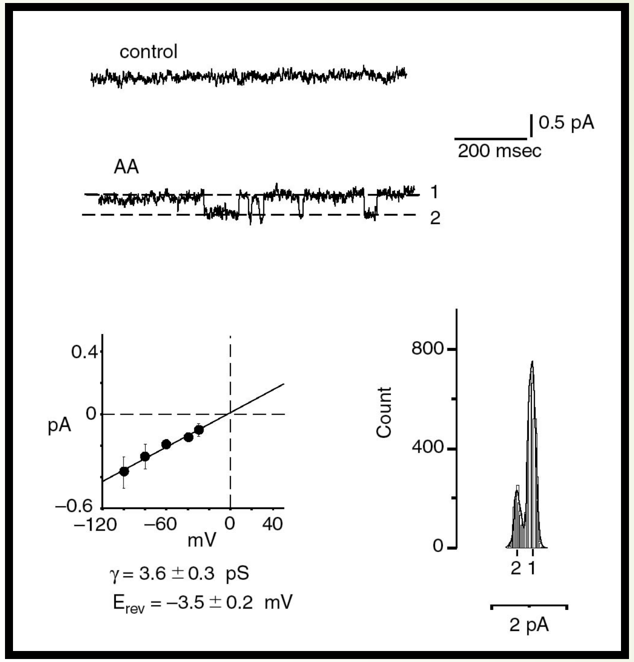

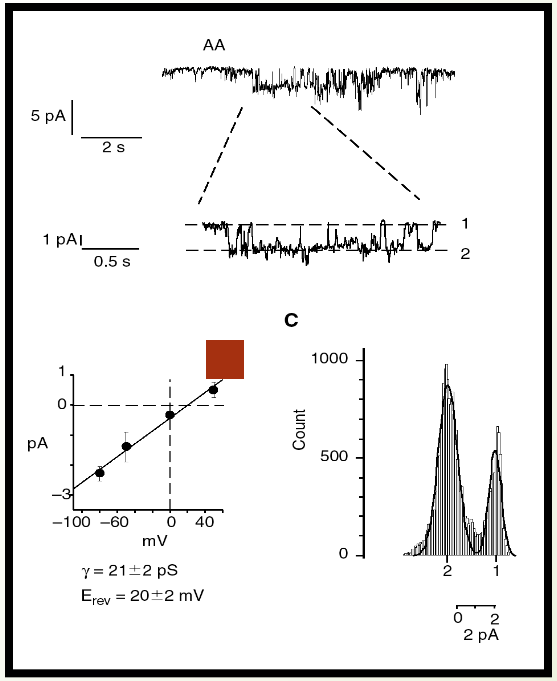

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