Bioelectrical Phenomena of the Cell:

1: Resting Membrane Potential

Recap of Basic Concepts
Forces that determine ions movement

- Electrostatic forces
  - Opposite charges attract
  - Identical charges repel
- Concentration forces
  - Diffusion – movement of ions through semipermeable membrane
  - Osmosis – movement of water from region of high concentration to low

Selective Permeability of Membranes

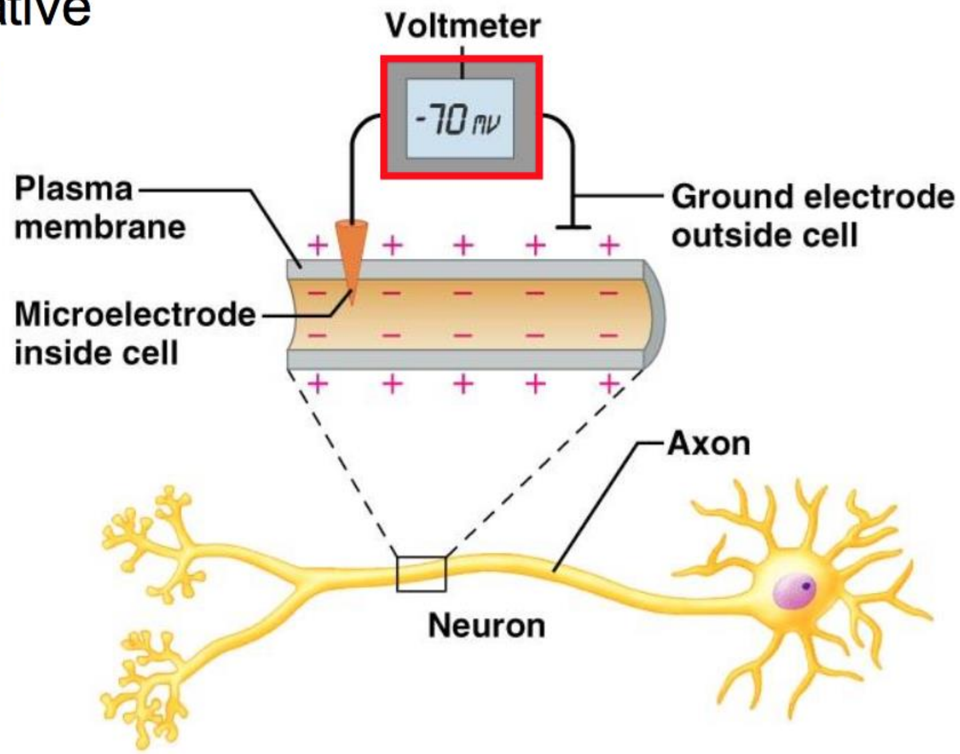
- Some ions permitted to cross more easily than others
- Neuronal membranes contain ion channels
  - Protein tubes that span the membrane
  - Some stay open all the time (nongated)
  - Some open on the occasion of an action potential, causing a change in the permeability of the membrane (gated)

Resting Membrane Potential

- A constant potential difference across the resting cell membrane

The basic signaling properties of neurons are influenced by changes in the resting potential

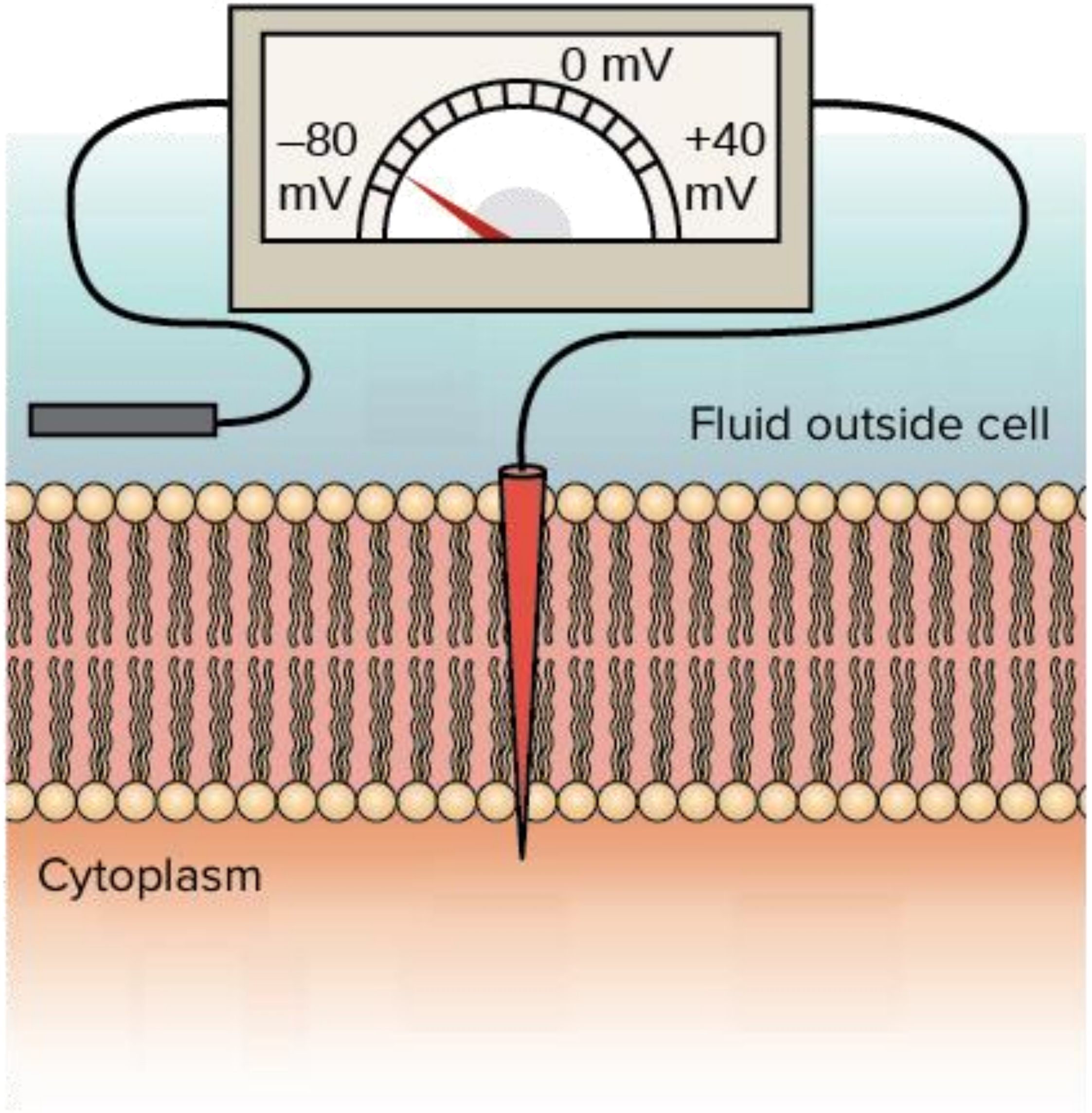
- *Usually* the cytoplasm is negative
  - (-20 to -110 mV; relative to the ECF = 0 mV)
- Depends upon ions present:
  - Permeability
  - Electrochemical gradients



Resting Membrane Potential

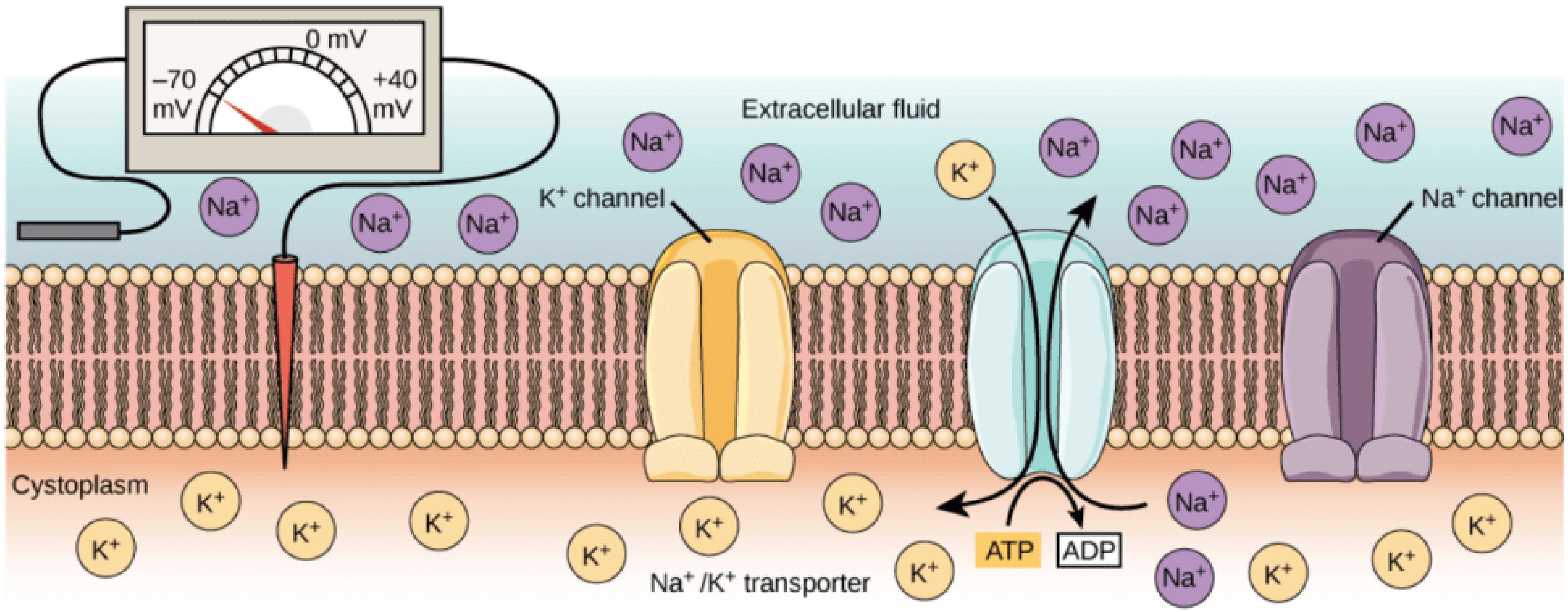
Imagine taking two electrodes and placing one on the outside and the other on the inside of the plasma membrane of a living cell.

If you did this, you would measure an electrical potential difference, or voltage, between the electrodes. This electrical potential difference is called the **membrane potential**.



Resting Membrane Potential

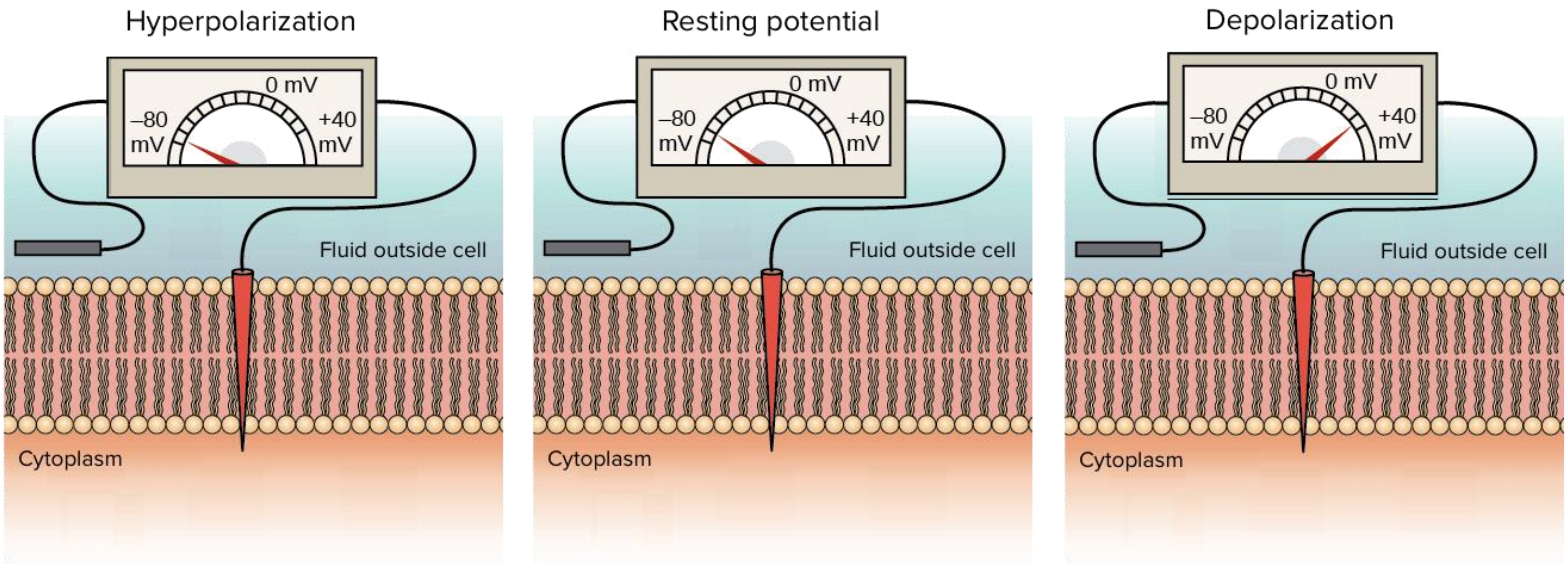
(a) Resting potential



At the resting potential, all voltage-gated Na^+ channels and most voltage-gated K^+ channels are closed. The Na^+/K^+ transporter pumps K^+ ions into the cell and Na^+ ions out.

- Every neuron has a separation of electrical charge across its cell membrane.
- The membrane potential results from a separation of positive and negative charges across the cell membrane.

Membrane Potentials



Because there is a potential difference across the cell membrane, the membrane is said to be **polarized**.

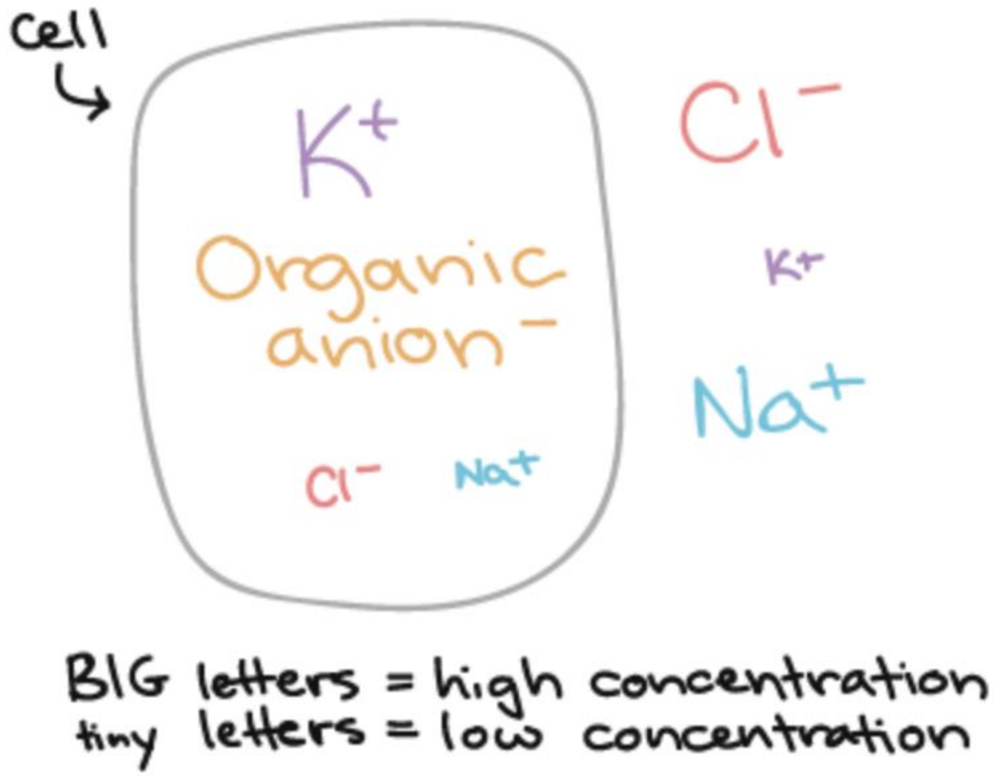
- If the membrane potential becomes more positive than it is at the resting potential, the membrane is said to be **depolarized**.
- If the membrane potential becomes more negative than it is at the resting potential, the membrane is said to be **hyperpolarized**.

Resting Membrane Potential

- A potential difference across the cell membrane at the rest stage or when the cell is not stimulated.
- Property:
  - It is constant or stable
  - It is negative inside relative to the outside
  - Resting potentials are different in different cells.

Where does the resting membrane potential come from?

The resting membrane potential is determined by the uneven distribution of **ions** (charged particles) between the inside and the outside of the cell, and by the different permeability of the membrane to different types of ions.



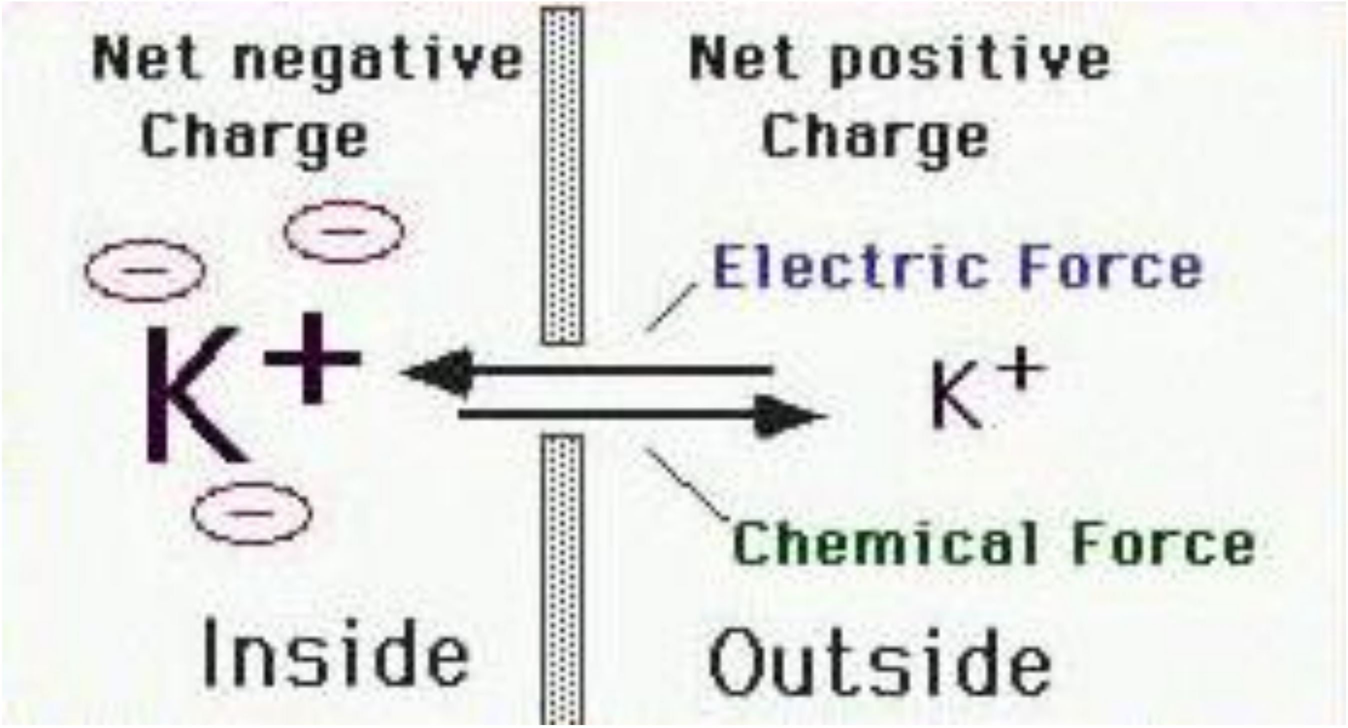
We always have to take in mind that….

Ion	Intracellular concentration (mM)	Extracellular concentration (mM)
K+	140	4
Na+	15	145
Cl-	4	110
Ca2+	0.0001	5

We have an additional information here…

Ion	Intracellular concentration (mM)	Extracellular concentration (mM)	Membrane permeability at rest
K+	140	4	1
Na+	15	145	0.05
Cl-	4	110	0.1
Ca2+	0.0001	5	0

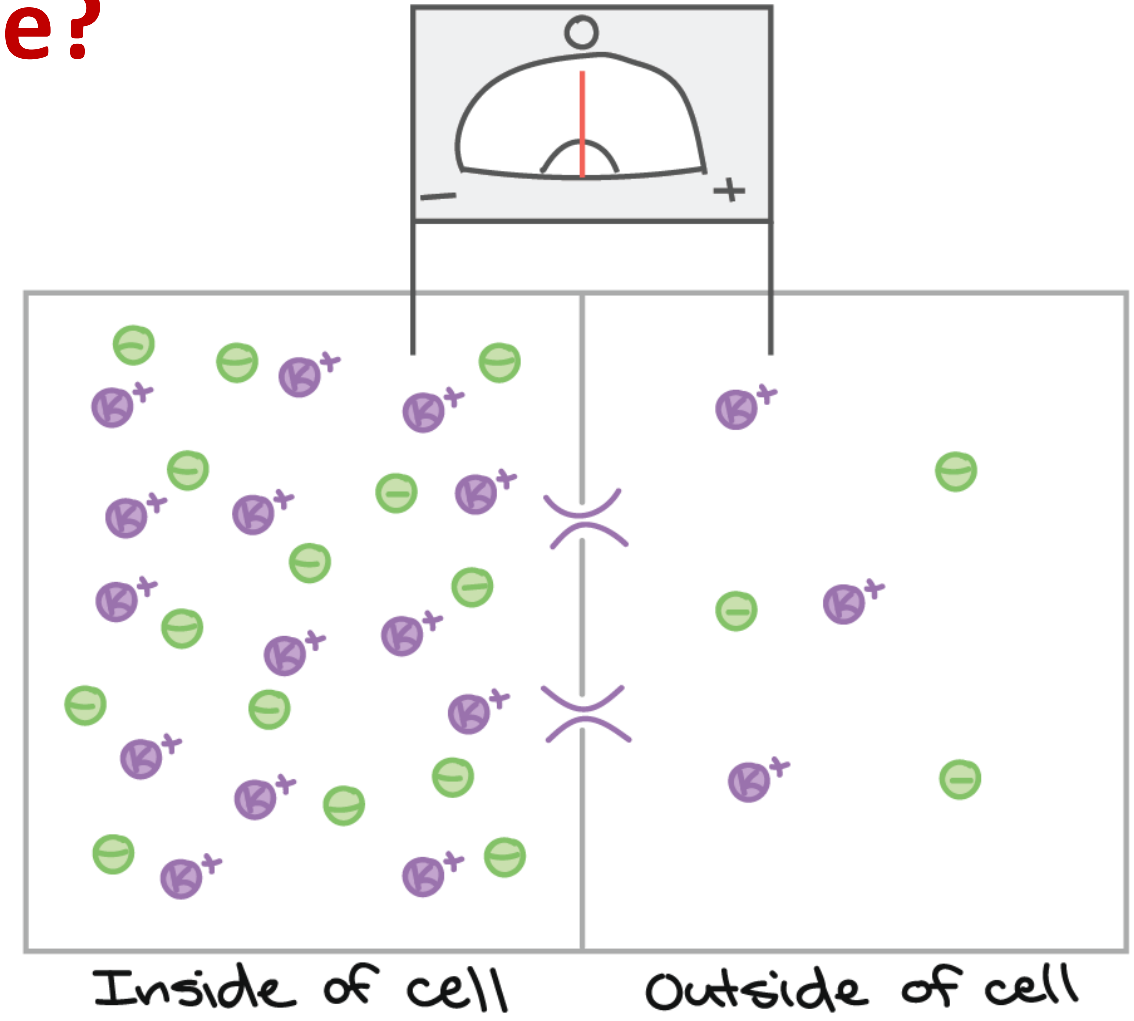
Resting Membrane Potential



- Potassium ions, concentrated inside the cell tend to move outward down their concentration gradient through non-gated potassium channels
- But the relative excess of negative charge inside the membrane tend to push potassium ions out of the cell

What happens if only K+ can cross the membrane?

1



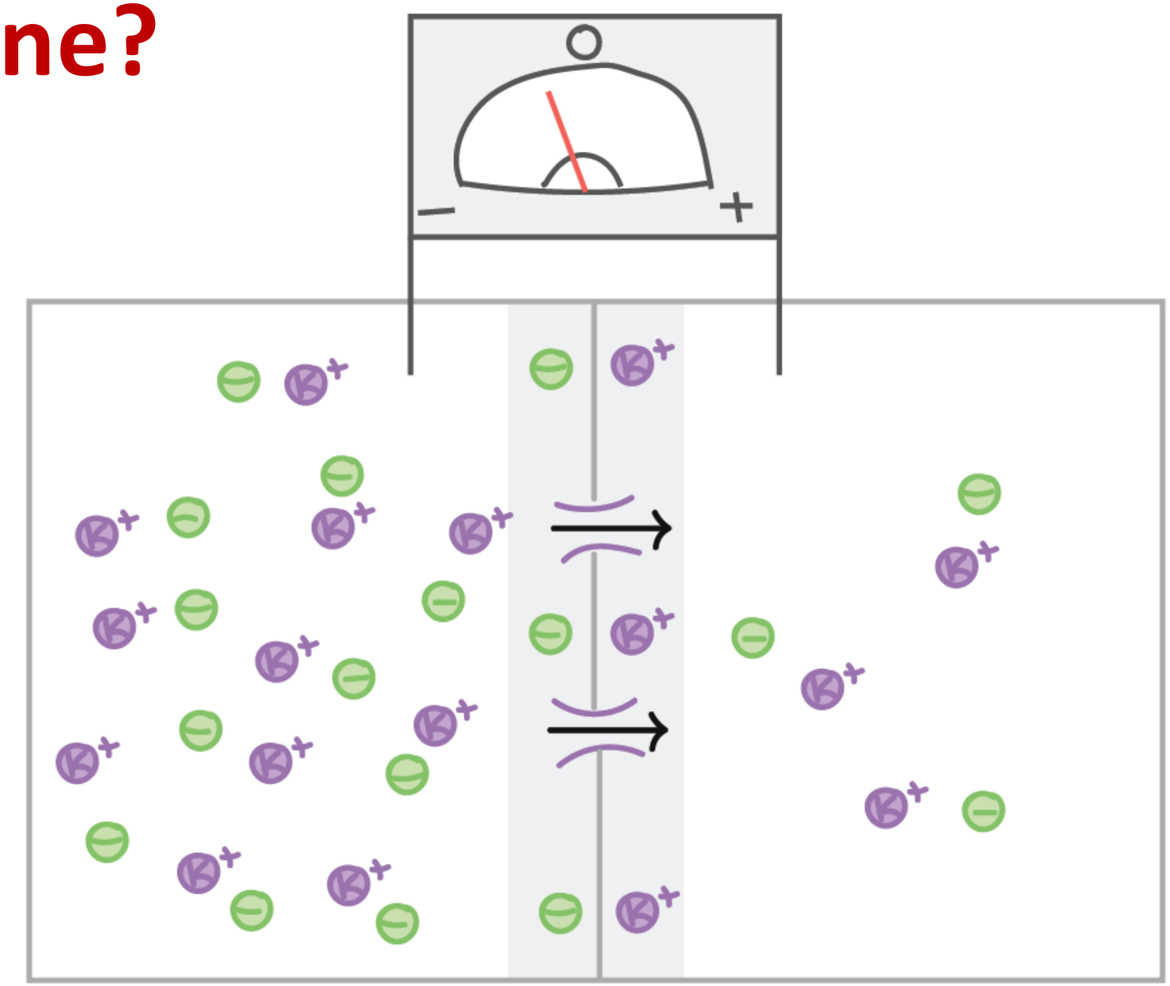
STARTING STATE:

There are potassium (K+) ions and other ions (including anions) inside and outside the cell.

K+ is more concentrated on the inside and less concentrated outside of the cell.

What happens if only K+ can cross the membrane?

2



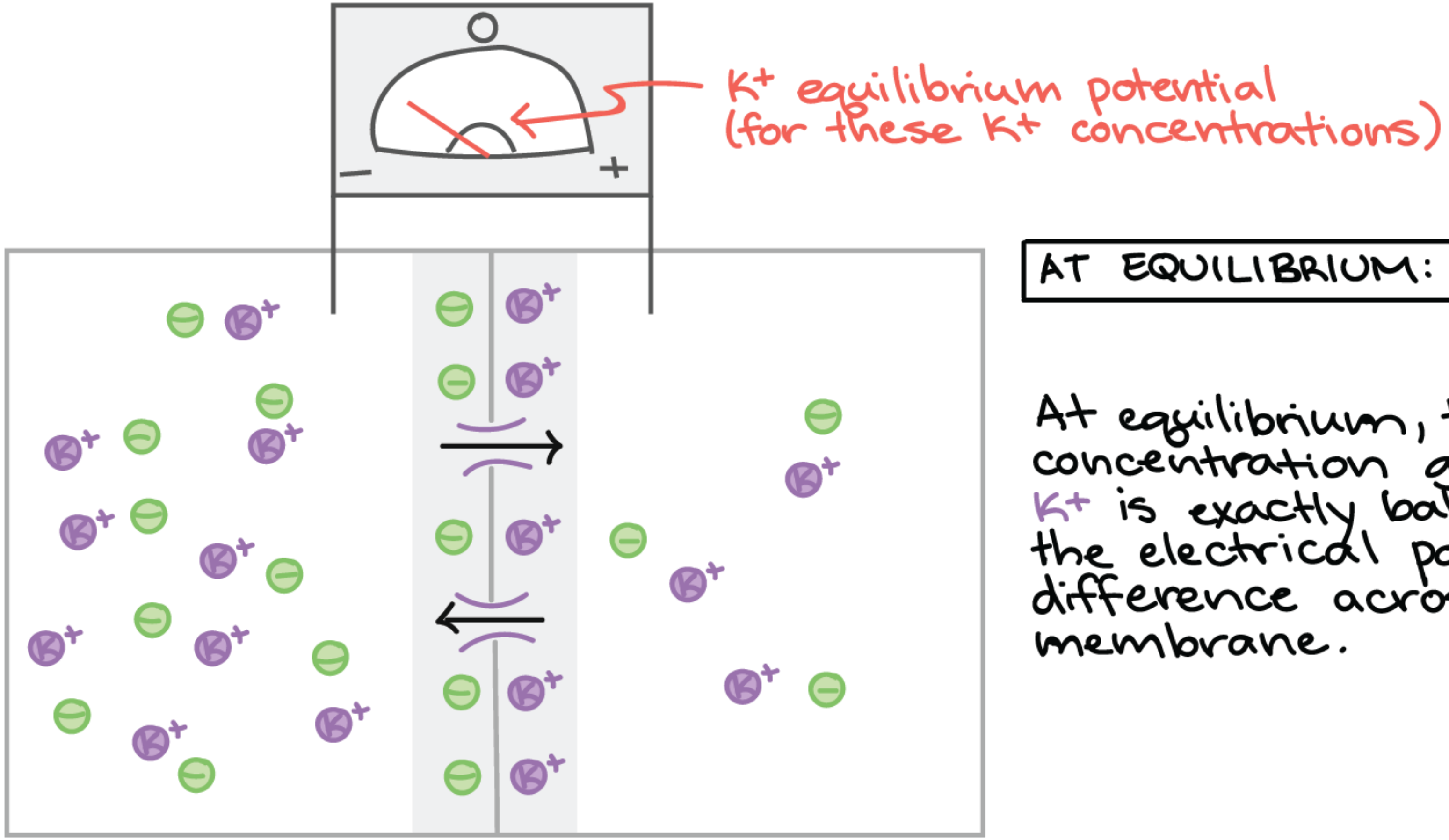
If K+ can cross via channels, it will begin to move down its concentration gradient and out of the cell.

The movement of K+ ions down their concentration gradient creates a charge imbalance across the membrane.

The charge imbalance opposes the flow of K+ down the concentration gradient.

What happens if only K+ can cross the membrane?

3



AT EQUILIBRIUM:

At equilibrium, the concentration gradient of K^+ is exactly balanced by the electrical potential difference across the membrane.

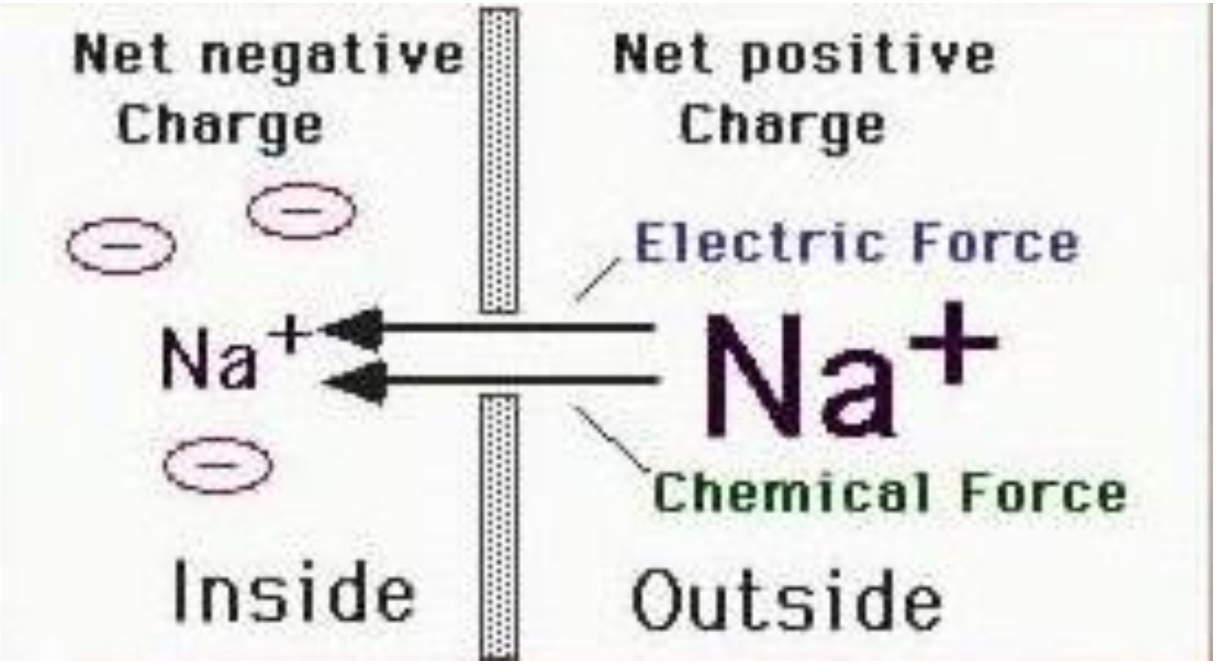
Does membrane potential equal K^+ equilibrium potential?

In glial cells the resting membrane potential is equal to the K equilibrium potential.
In neurons, however, the resting membrane potential is close but not identical to K equilibrium potential.

In physiological conditions neuron resting membrane potentials are slightly less negative than K equilibrium potential.

What does that mean? In a neuron, other types of ions besides K must contribute significantly to the resting membrane potential.

Resting Membrane Potential

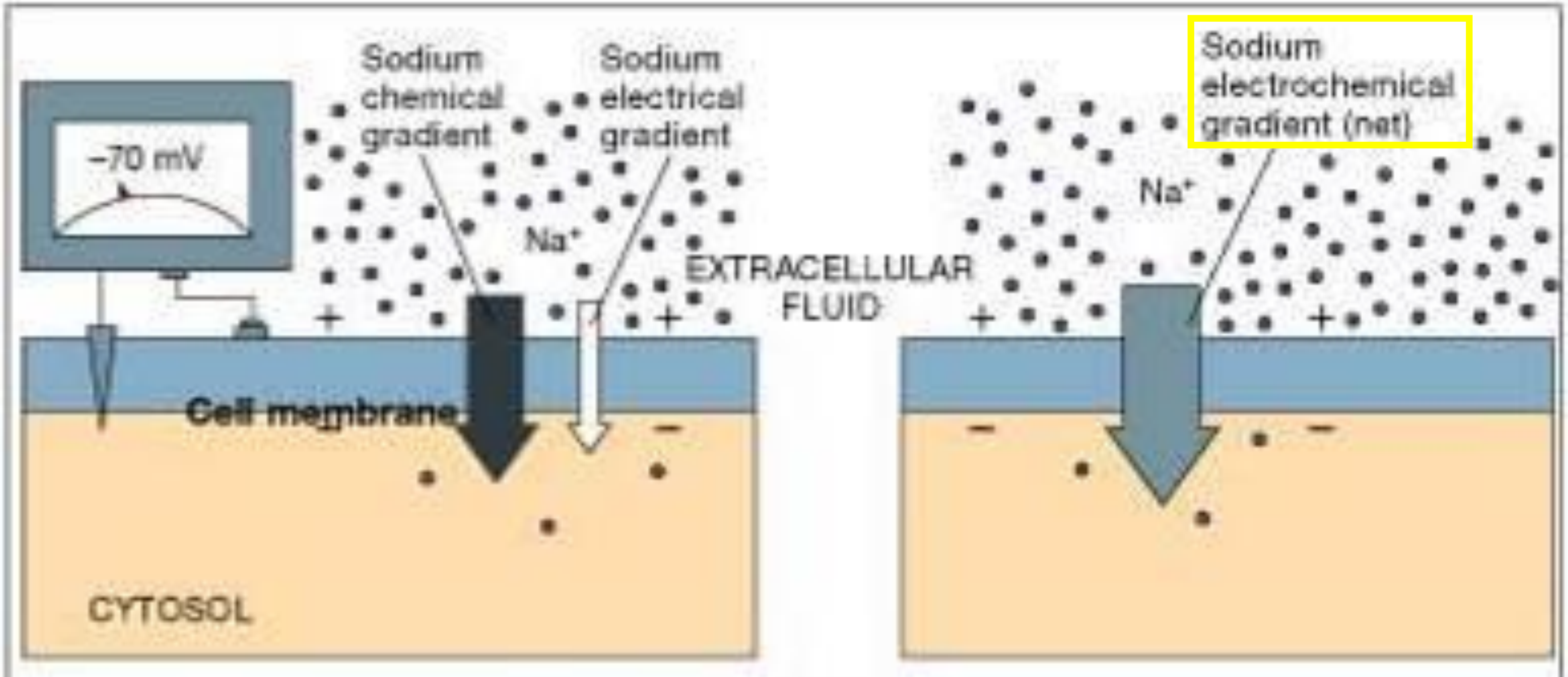


Na^+ is more concentrated outside than inside and therefore tends to flow into the cell down its concentration gradient

Na^+ is driven into the cell by the electrical potential difference across the membrane.

- *But what about sodium?*
- Electrostatic and Chemical forces act together on Na^+ ions to drive them into the cell
- The Na^+ channel close during the resting state

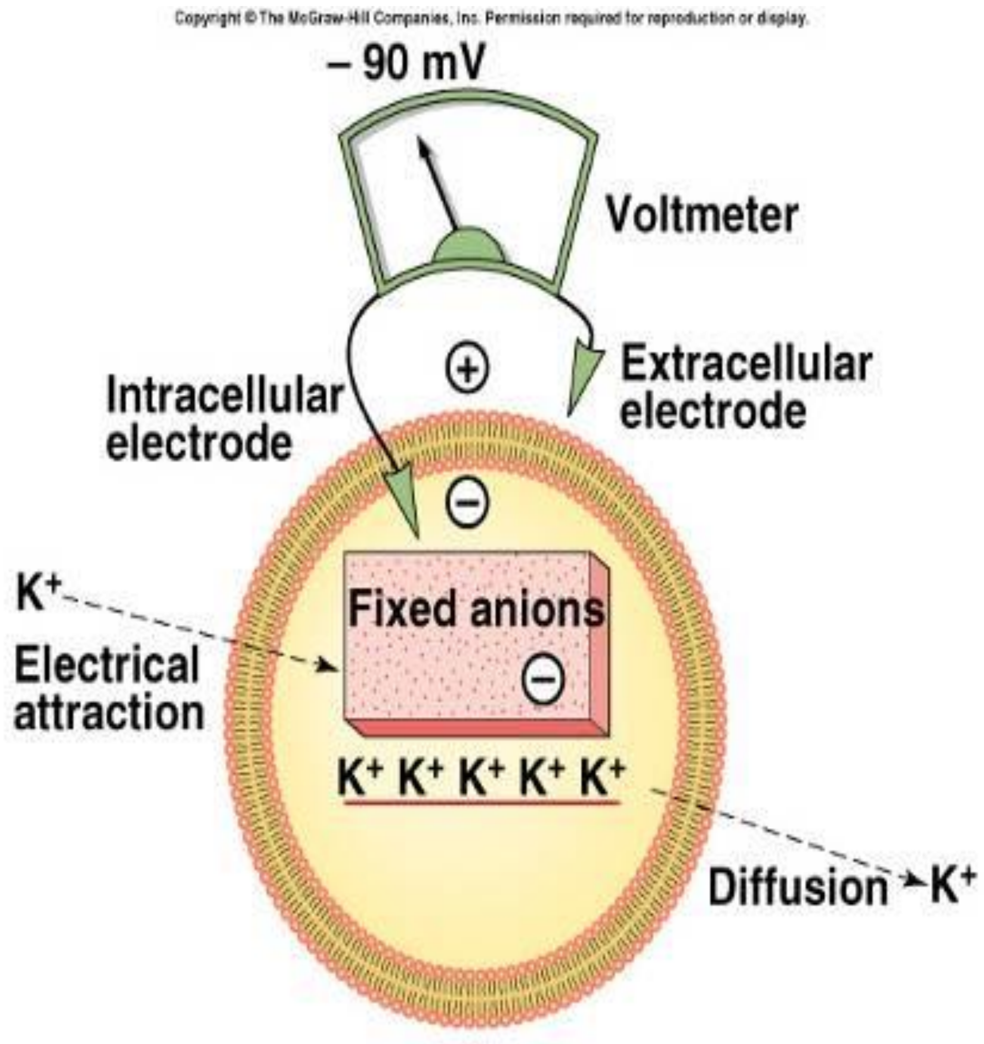
Na^+ electrochemical gradient



(a)

Equilibrium Potentials

- Theoretical voltage produced across the membrane
  - if only one kind of ion could diffuse through the membrane.
  - If membrane only permeable to K^+, K^+ diffuses until $[K^+]$ is at equilibrium.
- Force of electrical attraction and diffusion are = opposite.



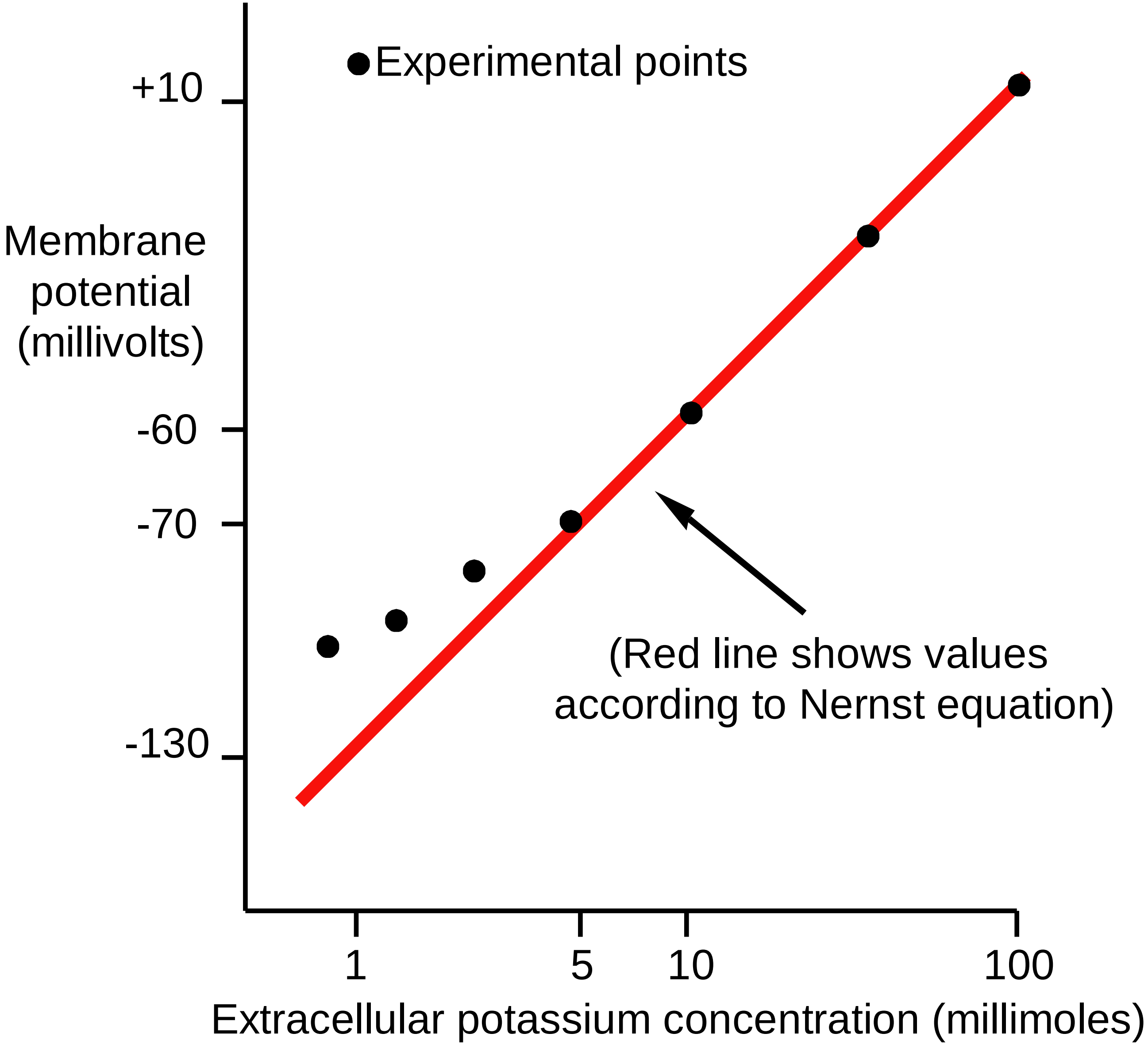
Calculating equilibrium potential
Nernst Equation

- Allows theoretical membrane potential to be calculated for particular ion.
  - Membrane potential that would exactly balance the diffusion gradient and prevent the net movement of a particular ion.
  - Value depends on the ratio of [ion] on the 2 sides of the membrane.

Calculating equilibrium potential
Nernst Equation

Nernst Equation (at body temp RT/F simplifies to 61.5):
E= membrane potential in mV
R= Ideal Gas Constant 8.314 J/(K*mol)
T= temp in K
z= charge of ion in question ex: for Ca2+ z= +2
F= Faraday's Constant 96,485 C/mol e-

$$E = \frac{RT}{zF} \ln \frac{[ion_{outside}]}{[ion_{inside}]} = \frac{61.5}{z} \log \frac{[ion_{outside}]}{[ion_{inside}]}$$


Experimental points
+10
Membrane
potential
(millivolts)
-60
-70
-130
(Red line shows values
according to Nernst equation)
1
5
10
100
Extracellular potassium concentration (millimoles)

$[K^+]_o = 4\ mmol.l^{-1}$

Resting Membrane Potential

- Resting membrane potential is less than E_k because some Na^+ can also enter the cell.
- The slow rate of Na^+ influx is accompanied by slow rate of K^+ outflux.
- Depends upon 2 factors:
  - Ratio of the concentrations of each ion on the 2 sides of the plasma membrane.
  - Specific permeability of membrane to each different ion.
- Resting membrane potential of most cells ranges from - 65 to – 85 mV.

The Goldman–Hodgkin–Katz voltage equation,

Goldman–Hodgkin–Katz Voltage Equation (derived from Nernst):
Vm= membrane potential in mV
P= permeability for relevant ion

$$V_m = 61.5 \log \left[\frac{P_{Na^+} \times [Na^+]_{outside} + P_{K^+} \times [K^+]_{outside} + P_{Cl^-} \times [Cl^-]_{inside}}{P_{Na^+} \times [Na^+]_{inside} + P_{K^+} \times [K^+]_{inside} + P_{Cl^-} \times [Cl^-]_{outside}} \right]$$

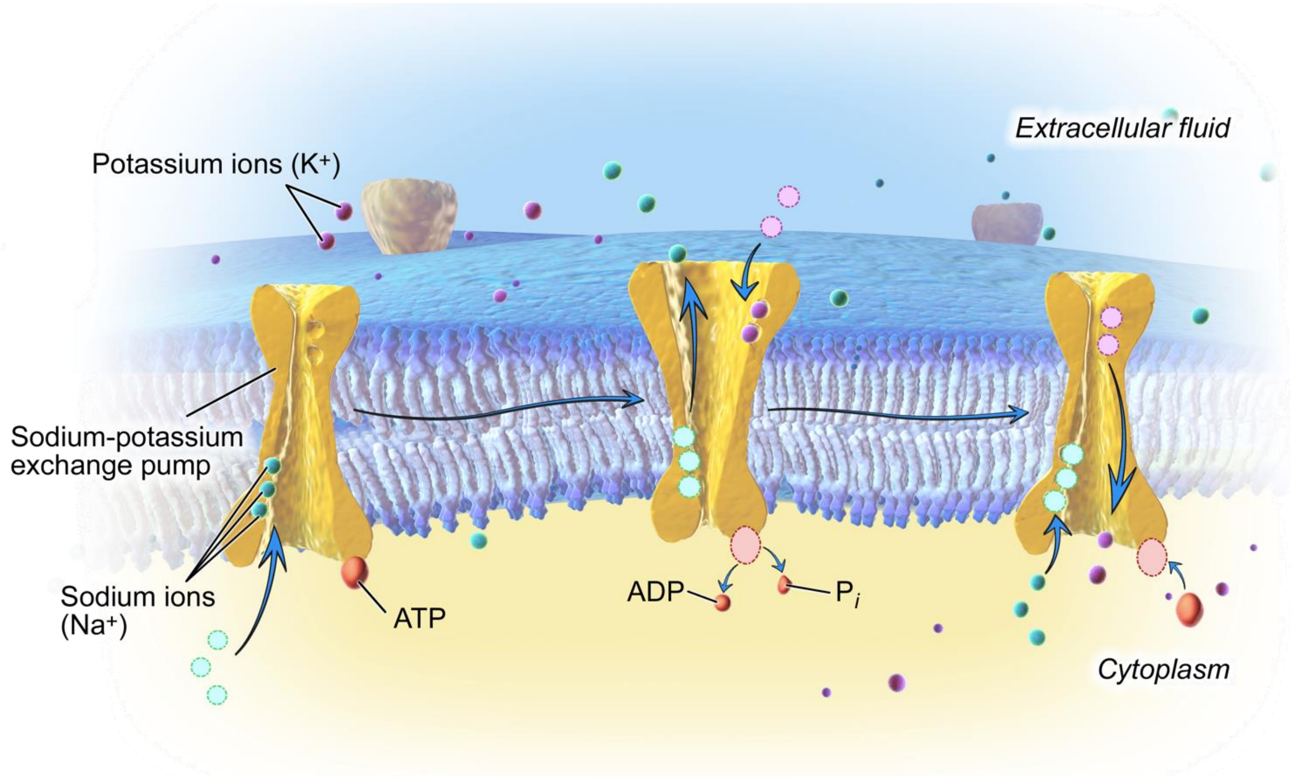
The Goldman–Hodgkin–Katz voltage equation,

The membrane potential is a weighted mean of the equilibrium potentials of the different permeant ions.

If only one permeant ionic species is present, the membrane potential will be determined by that ion's equilibrium potential. It doesn't matter how permeable it is (how readily it can cross the membrane), because there is nothing to counter it.

If multiple permeant ionic species are present (and not at equilibrium), the resting potential will be between the equilibrium potentials for the different permeant ions. The greater the permeability to a given ionic species, the more it will dominate the final membrane potential.

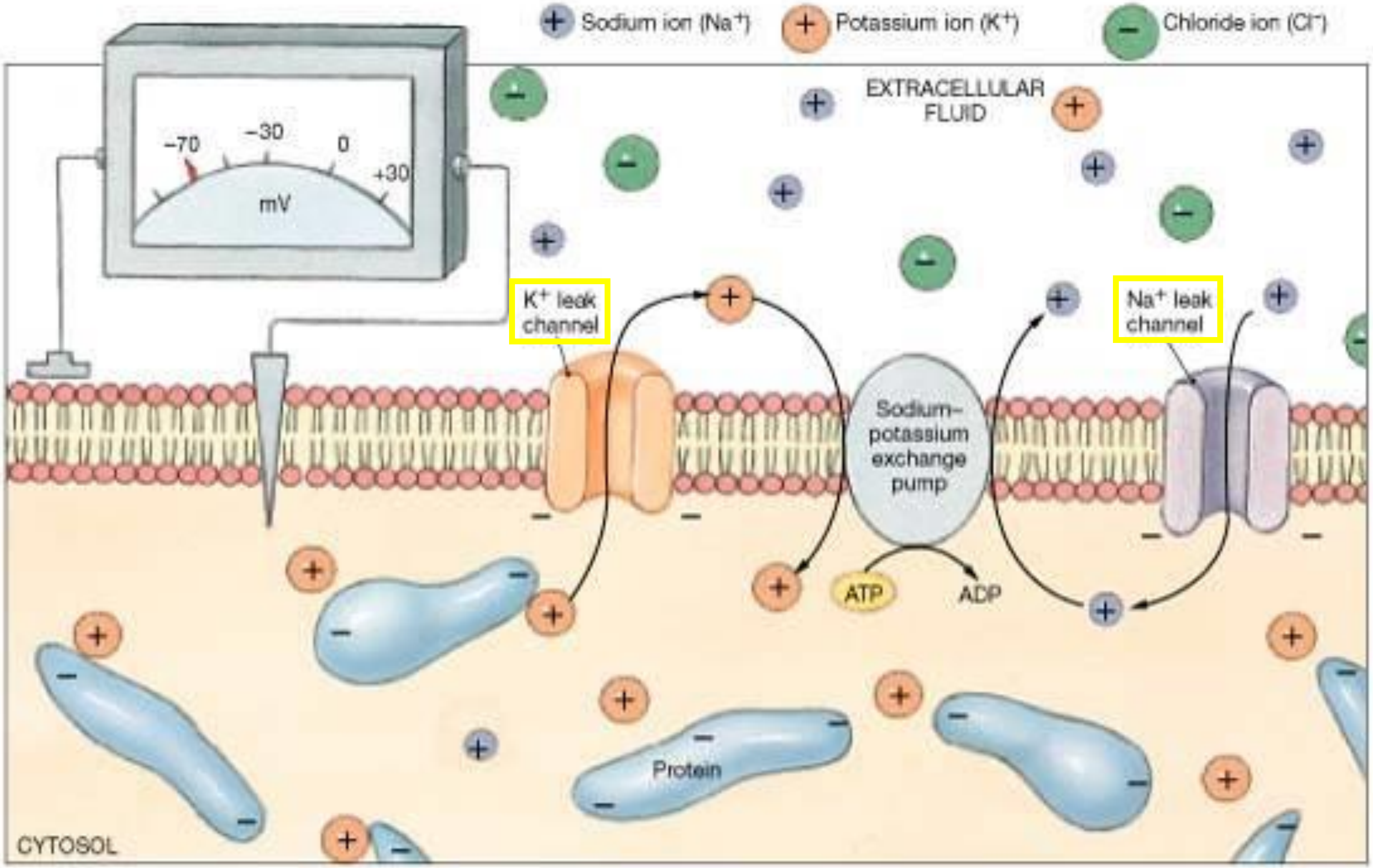
The Sodium-Potassium Pump



extrudes Na^+ from the cell while taking in K^+

- Dissipation of ionic gradients is ultimately prevented by Na^+-K^+ pumps

Resting Membrane Potential

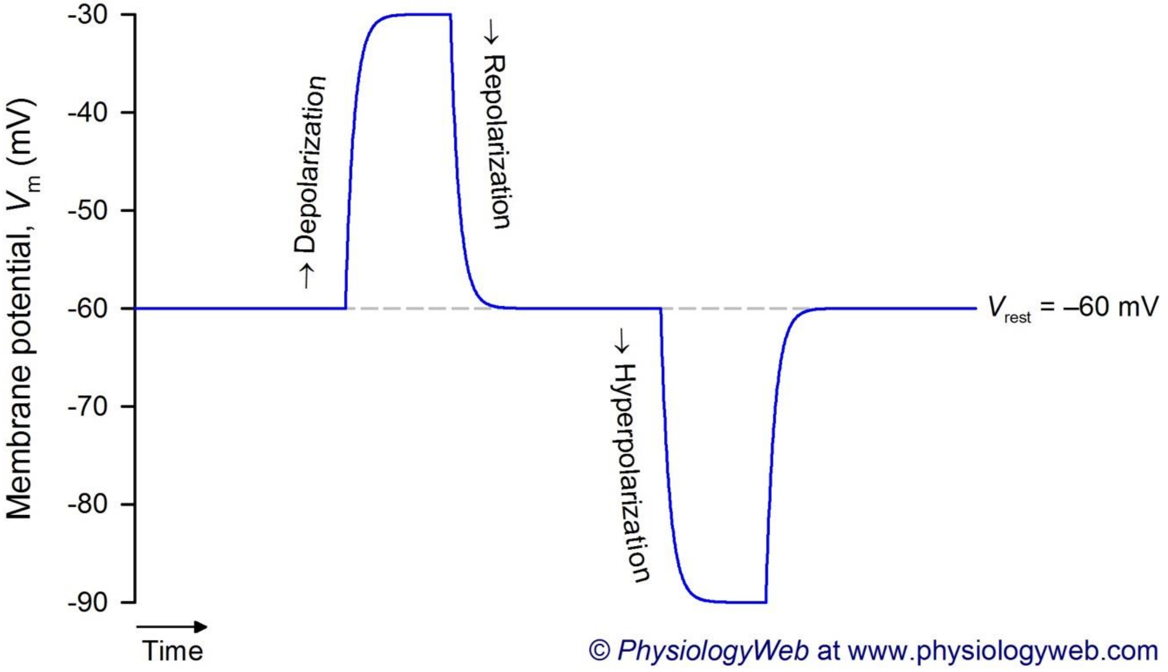


The formation of resting potential depends on:

- Concentration difference of K^+ across the membrane
- Permeability of Na^+ and K^+ during the resting state
- Na^+-K^+ pump

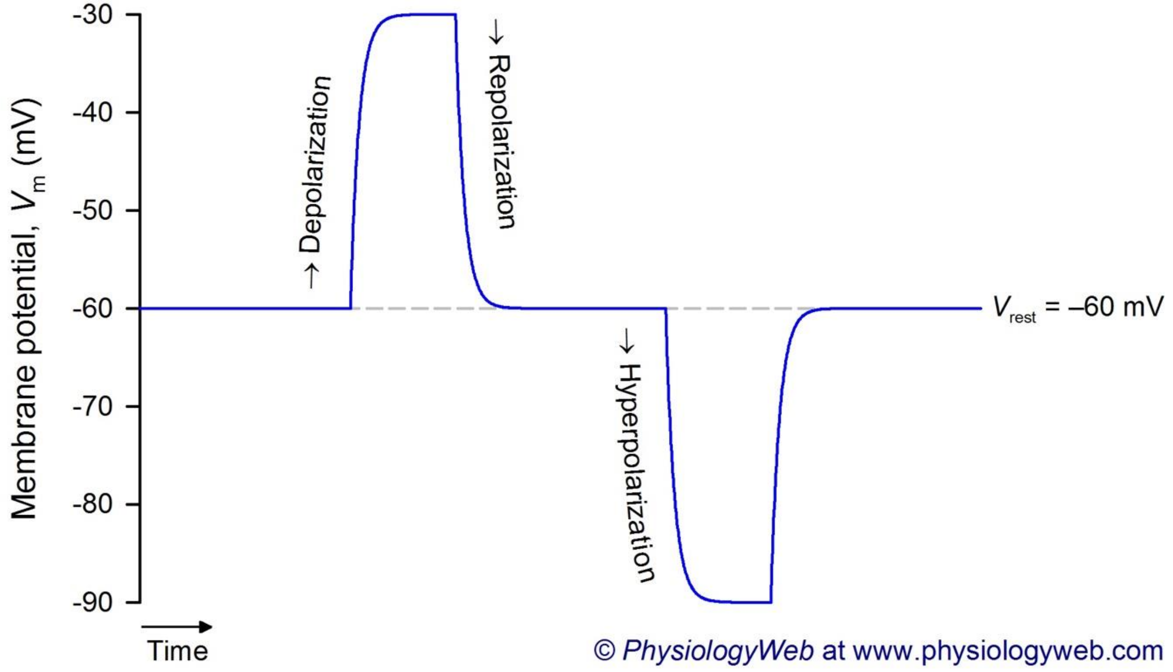
- **Bioelectrical Phenomena of the Cell:**
- **2: Action Potential**

Basic Electrophysiological Terms



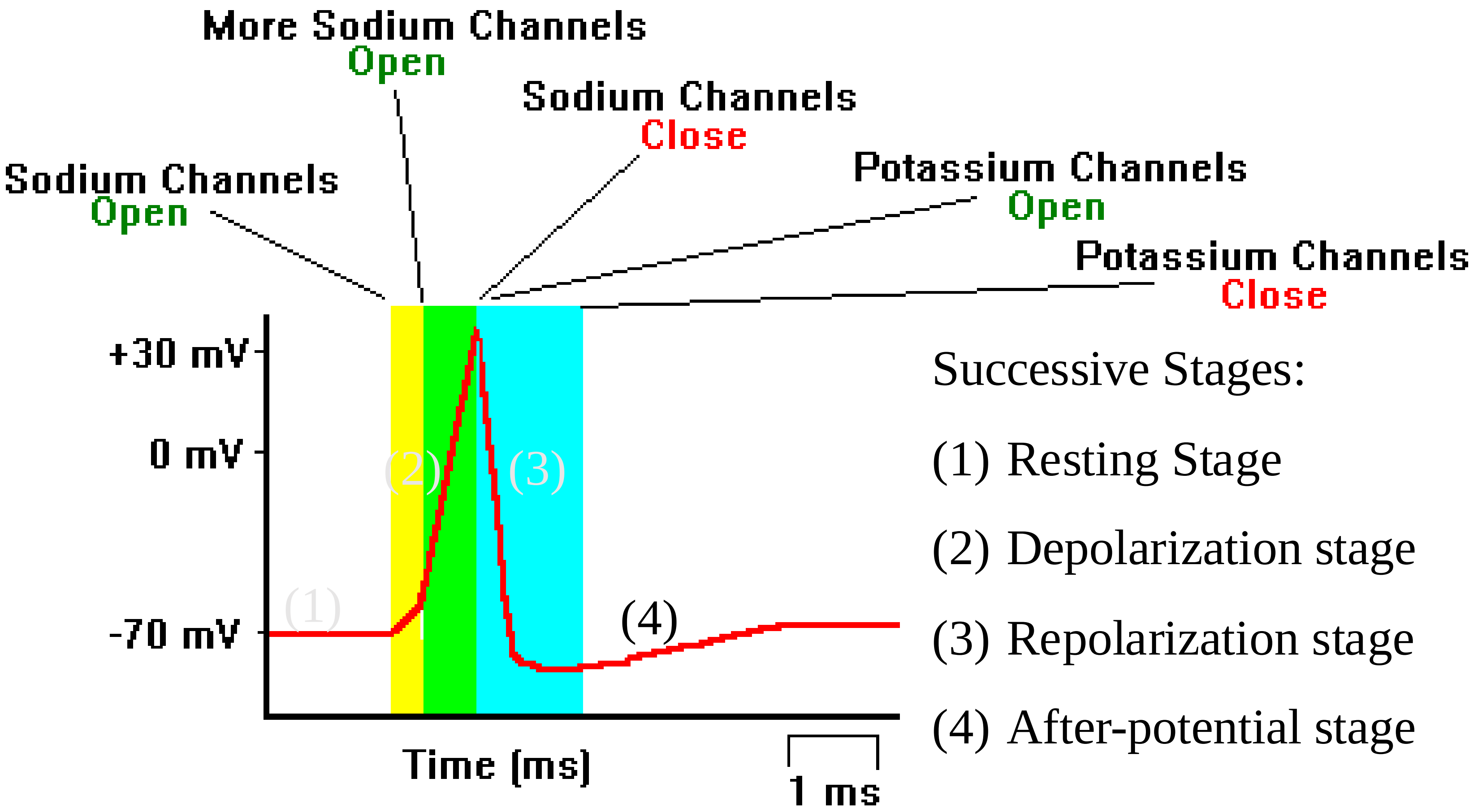
- **Polarization**: a state in which membrane is polarized at rest, negative inside and positive outside.
- **Depolarization**: the membrane potential becomes less negative than the resting potential (close to zero).
- **Hyperpolarization**: the membrane potential is more negative than the resting level.

Basic Electrophysiological Terms



- **Reverspolarization**: a reversal of membrane potential polarity.
  - The inside of a cell becomes positive relative to the outside.
- **Repolarization**: restoration of normal polarization state of membrane.
  - from depolarized level

Action Potential



Successive Stages:

(1) Resting Stage

(2) Depolarization stage

(3) Repolarization stage

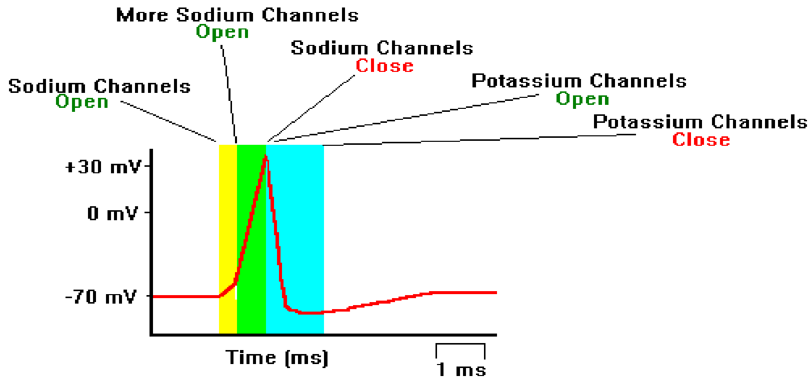
(4) After-potential stage

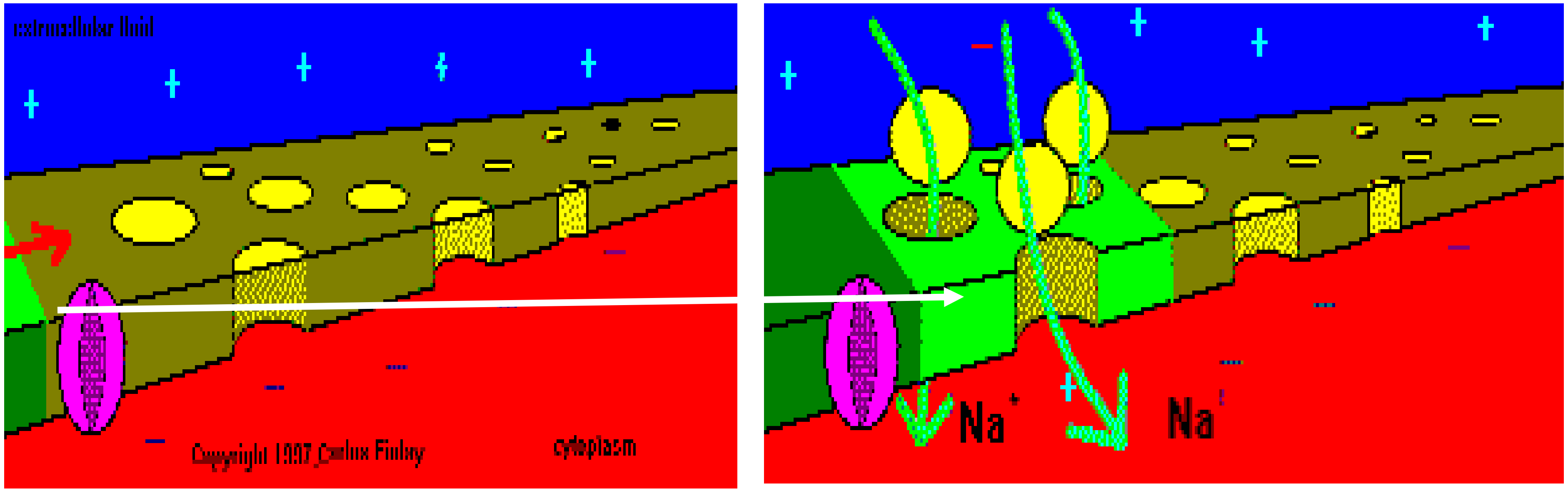
Concept

- Action potential is a **rapid, reversible**, and **conductive** change of the membrane potential after the cell is stimulated.

- Nerve signals are transmitted by action potentials.

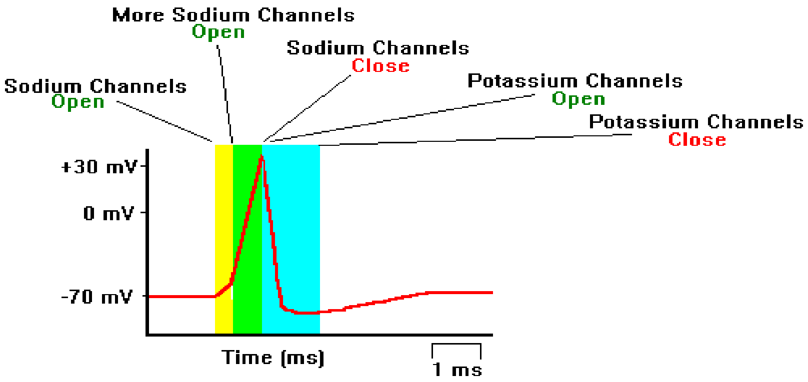
Action Potential Sequence

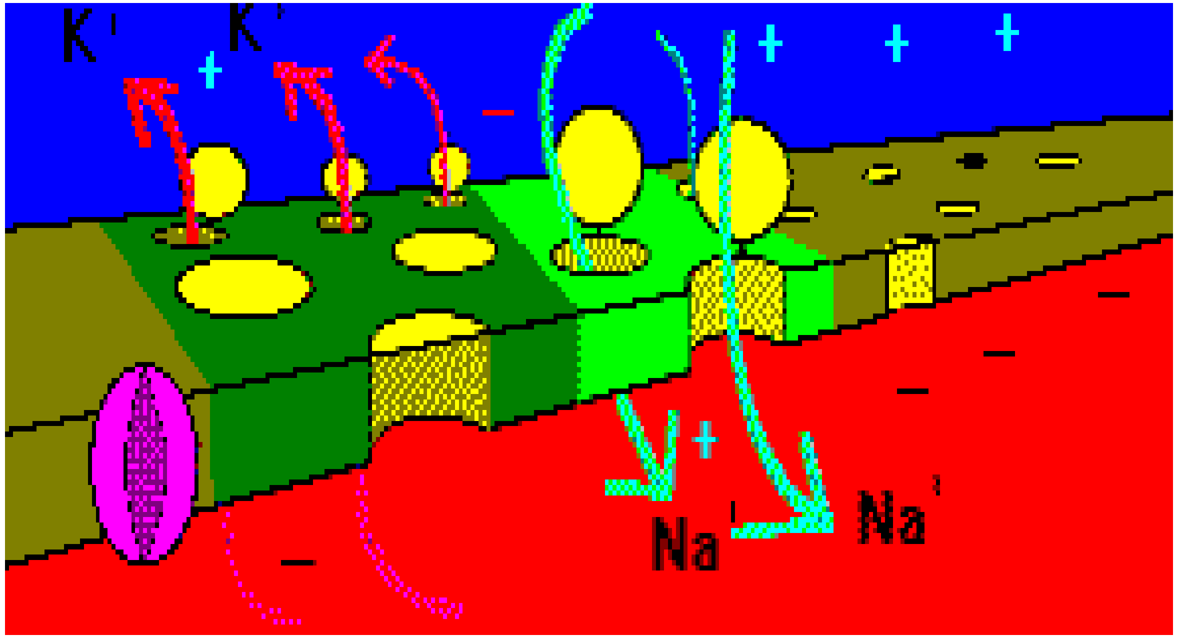




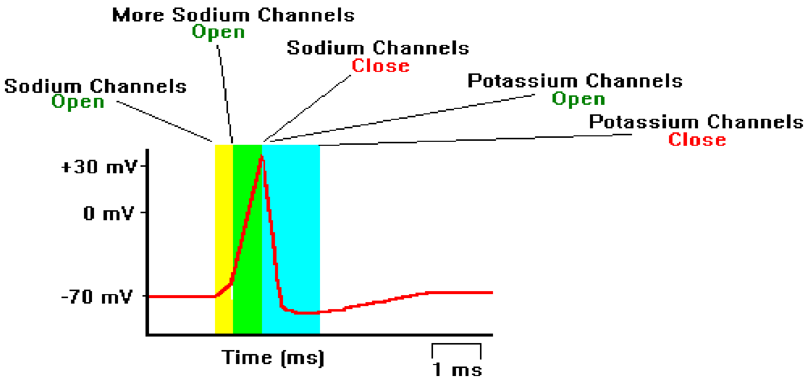
- **Voltage-gated Na^+ Channels open and Na^+ rushes into the cell**

Action Potential Sequence

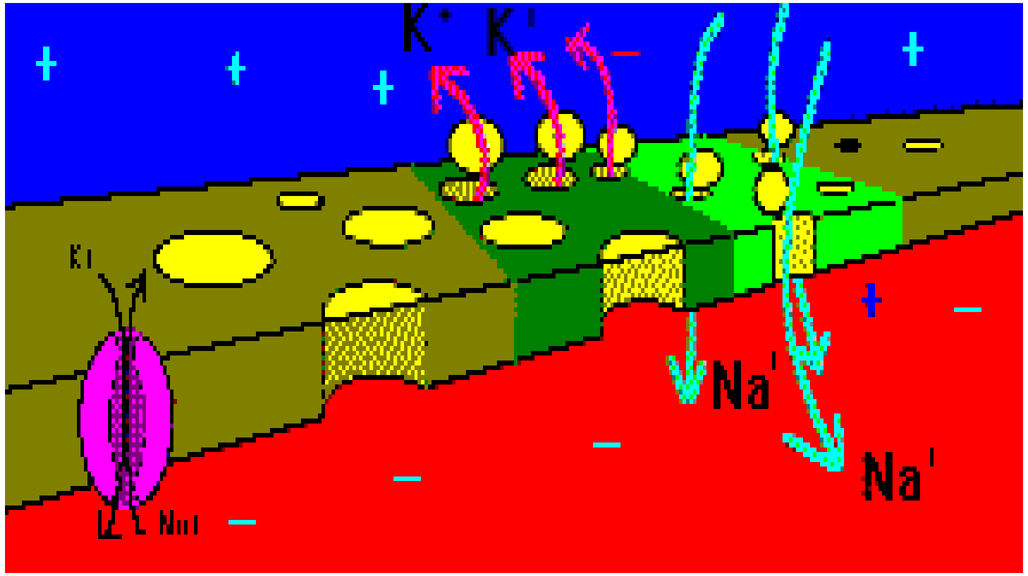




- At about +40 mV, Sodium channels close, but now, voltage-gated potassium channels open, causing an outflow of potassium, down its electrochemical gradient

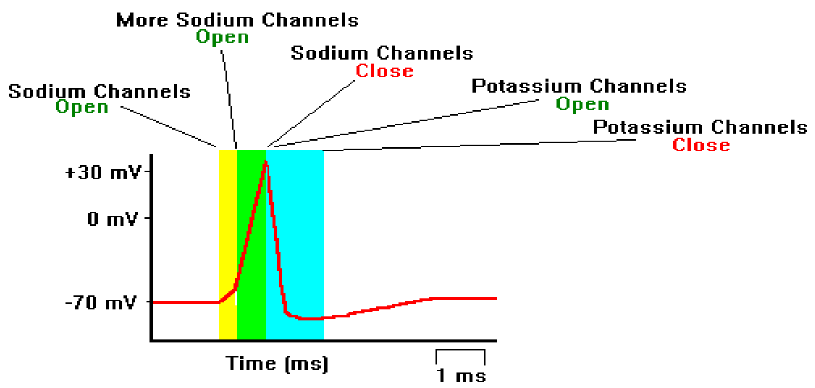


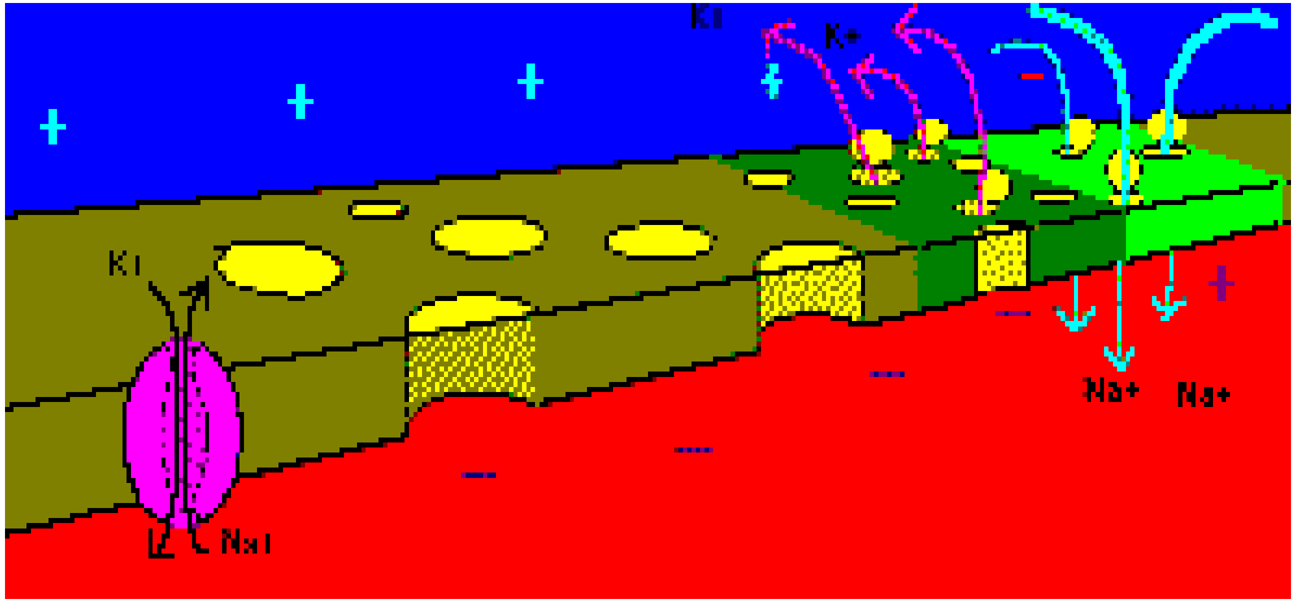
Action Potential Sequence



equilibrium potential of the cell is restored

Action Potential Sequence





- **The Sodium – Potassium Pump is left to clean up the mess…**

Ion Permeability during the AP

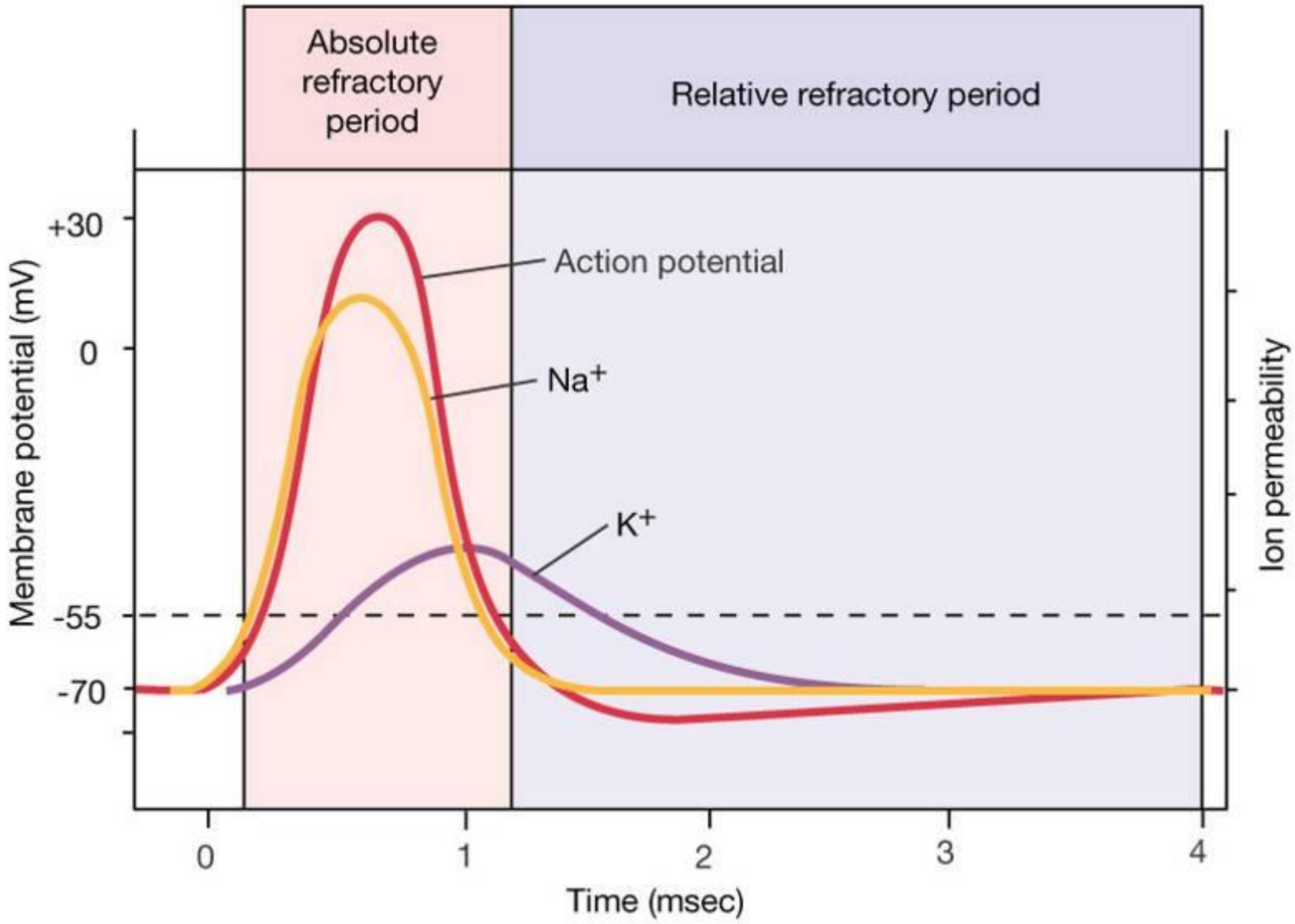


Figure 8-12: Refractory periods

Basic Electrophysiological Terms

- **Excitability**: The ability of the cell to generate the action potential

- **Excitable cells**: Cells that generate action potential during excitation.
    - in excitable cells (muscle, nerve, secretary cells), the action potential is the **marker** of excitation.

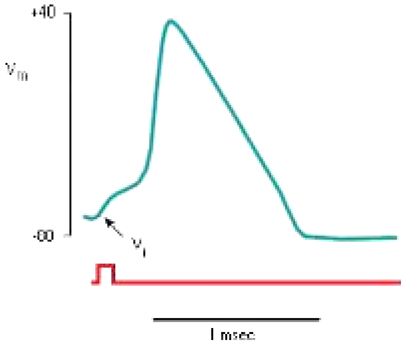
Basic Electrophysiological Terms

- **Stimulus**: a sudden change of the (internal or external) environmental condition of the cell.
  - includes physical and chemical stimulus.
  - The electrical stimulus is often used for the physiological research.
- **Threshold** (intensity): the lowest or minimal intensity of stimulus to elicit an action potential
  - (Three factors of the stimulation: intensity, duration, rate of intensity change)

Basic Electrophysiological Terms

- **Types of stimulus:**
  - **Threshold stimulus**: The stimulus with the intensity equal to threshold
  - **Subthreshold stimulus**: The stimulus with the intensity weaker than the threshold
  - **Suprathreshold stimulus**: The stimulus with the intensity greater than the threshold.

Action Potential Summary



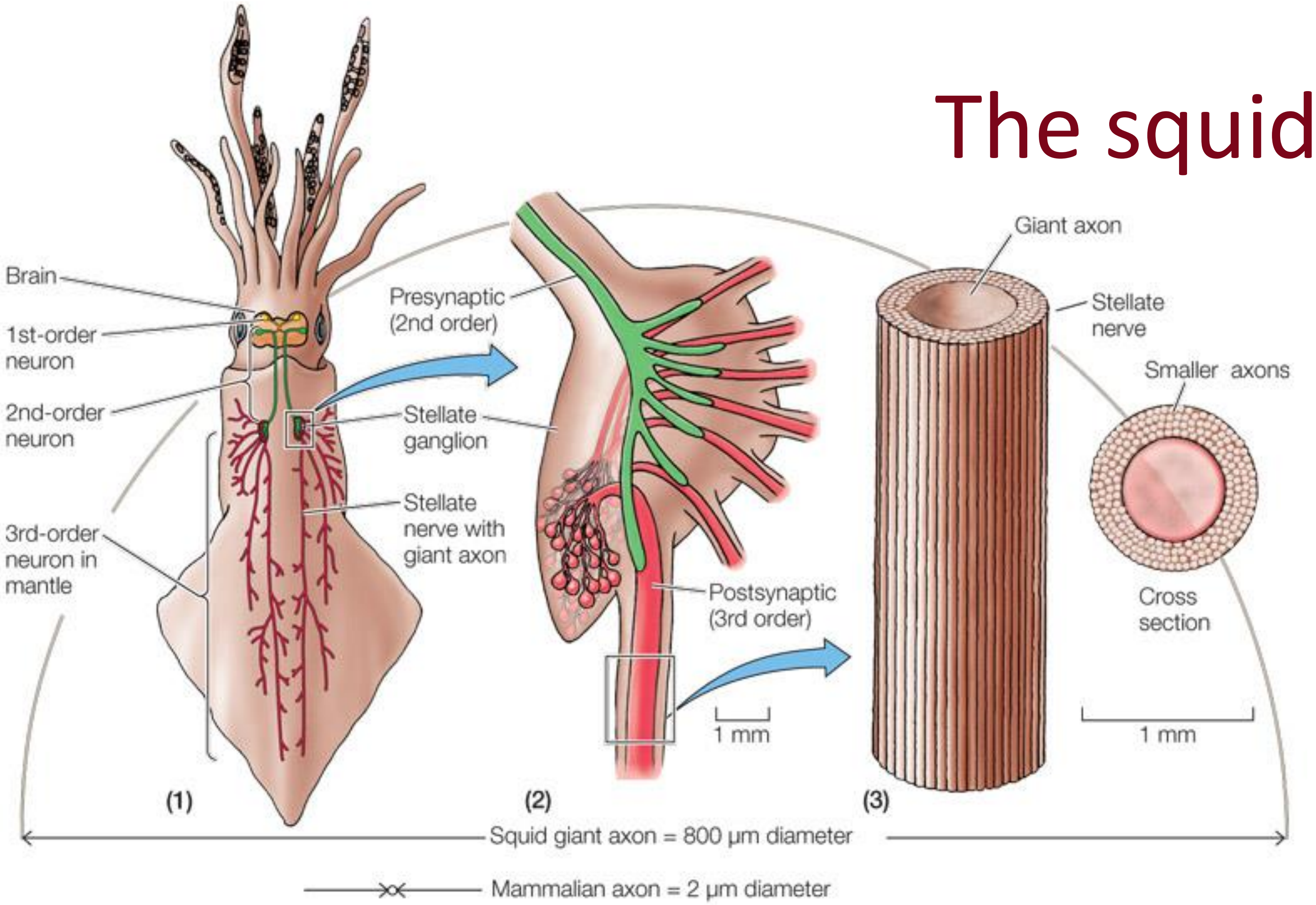
- Reduction in membrane potential (depolarization) to "threshold" level leads to opening of Na^+ channels, allowing Na^+ to enter the cell
- Interior becomes positive
- The Na^+ channels then close automatically followed by a period of inactivation.
- K^+ channels open, K^+ leaves the cell and the interior again becomes negative.
- AP Process lasts about 1ms

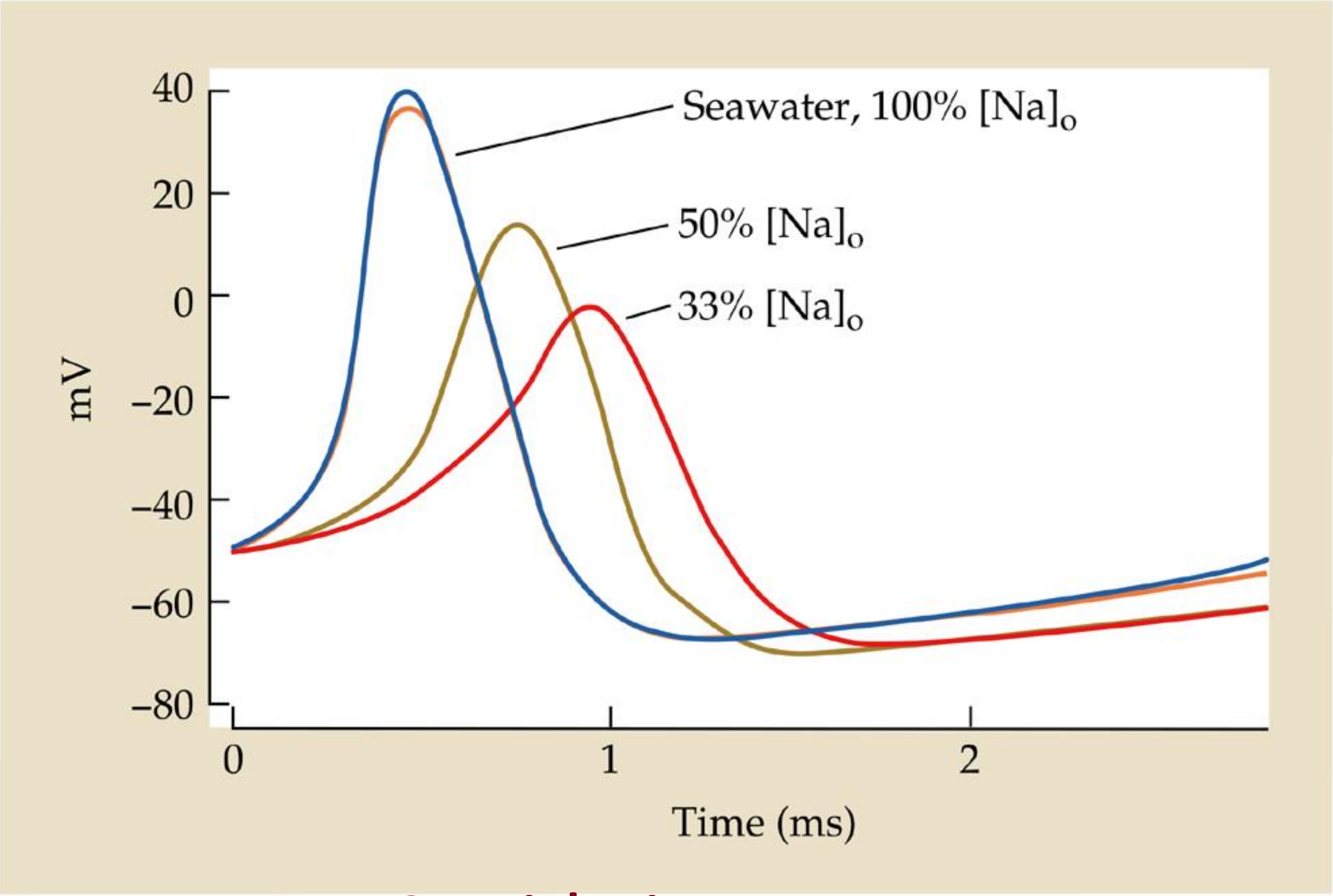
Properties of the Action Potential

- "All or none" phenomenon
  - constant **amplitude**, **time course** and **propagation velocity**.
- Propagation
- Transmitted in both direction in a nerve fiber?

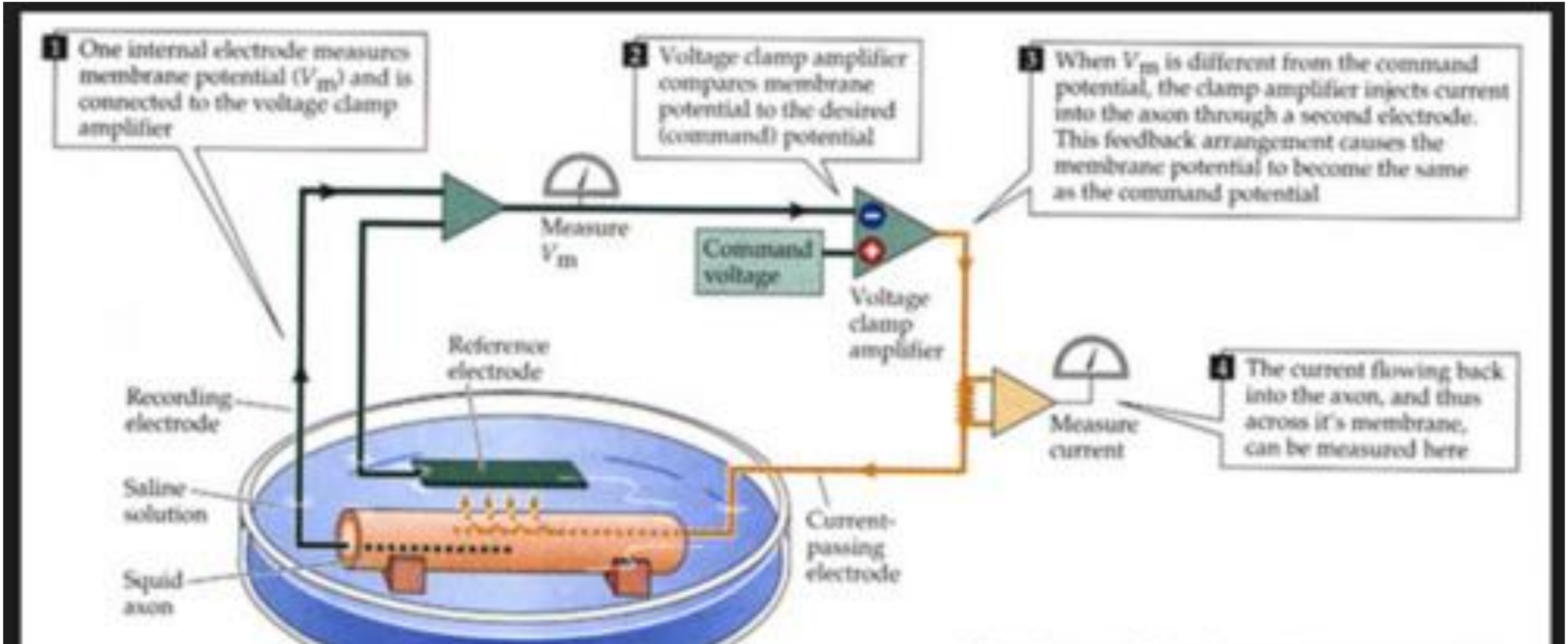
Initiation of Action Potential

The squid giant axon



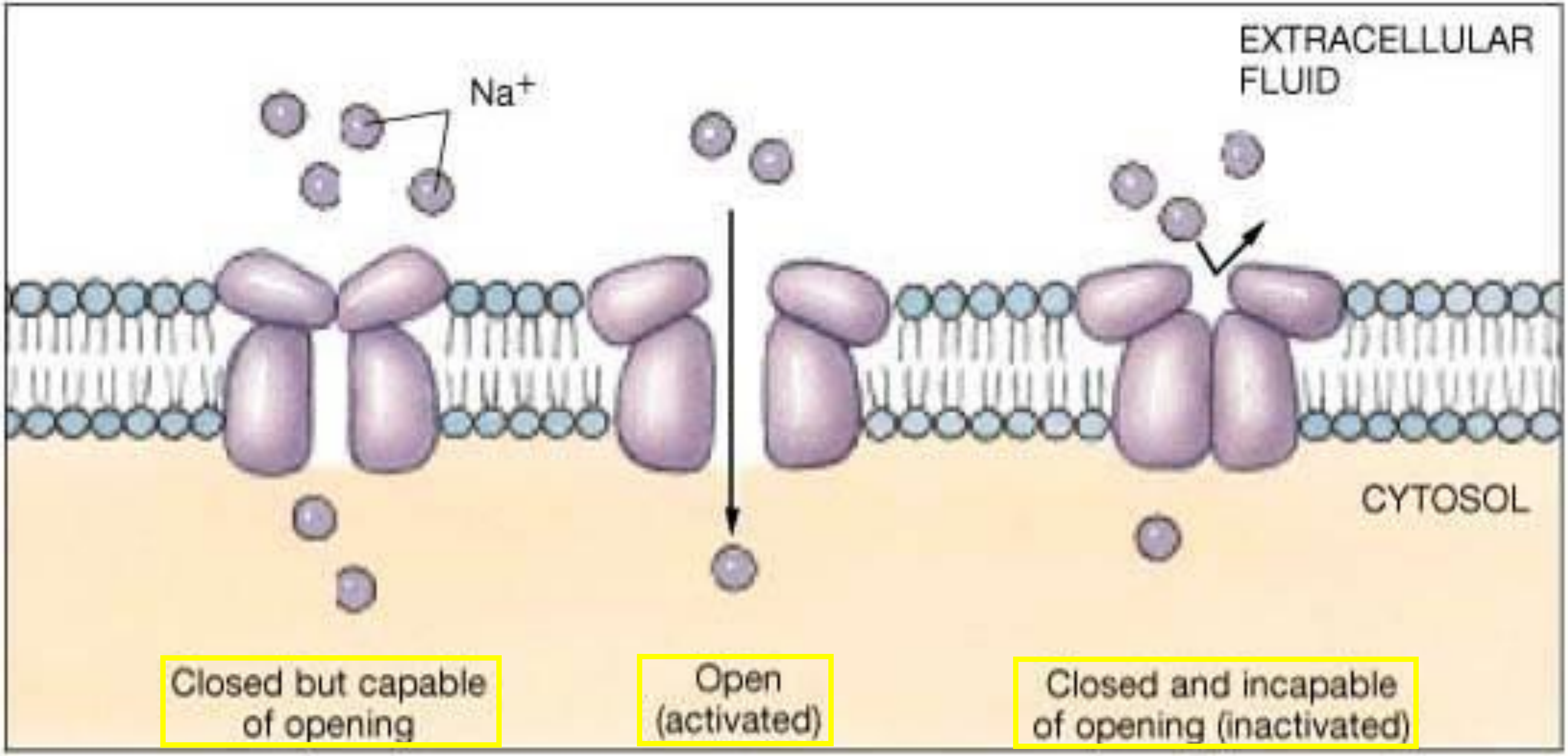


Squid giant axon



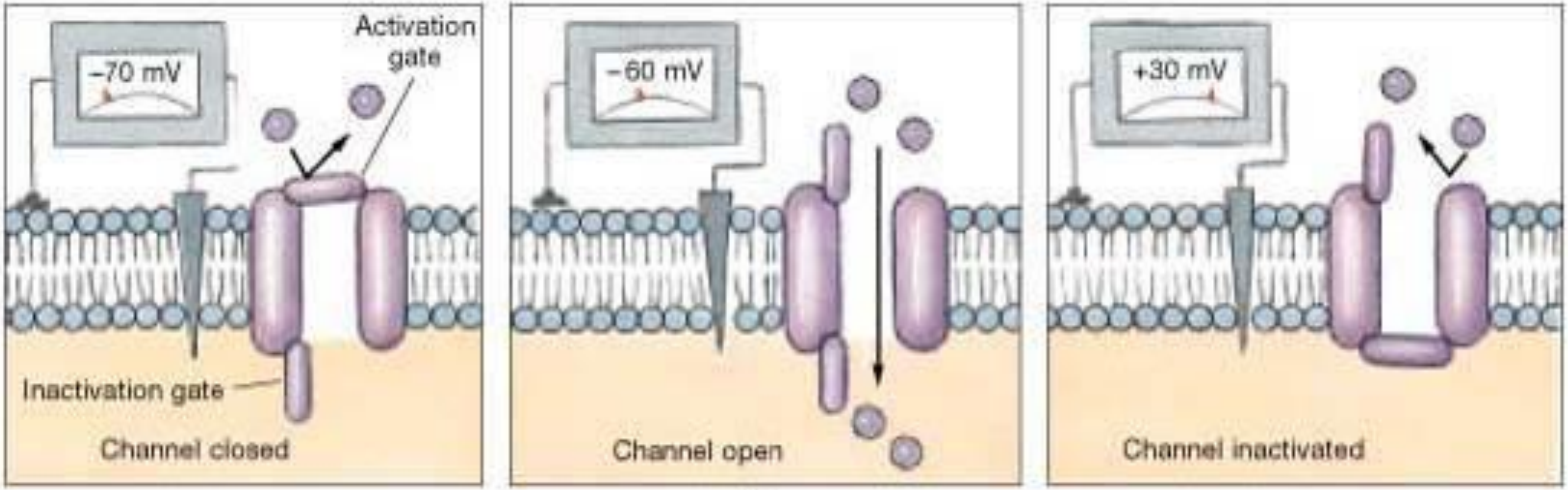
Voltage clamp technique for studying membrane currents of a squid axon.

Voltage Gated Na^+ Channels



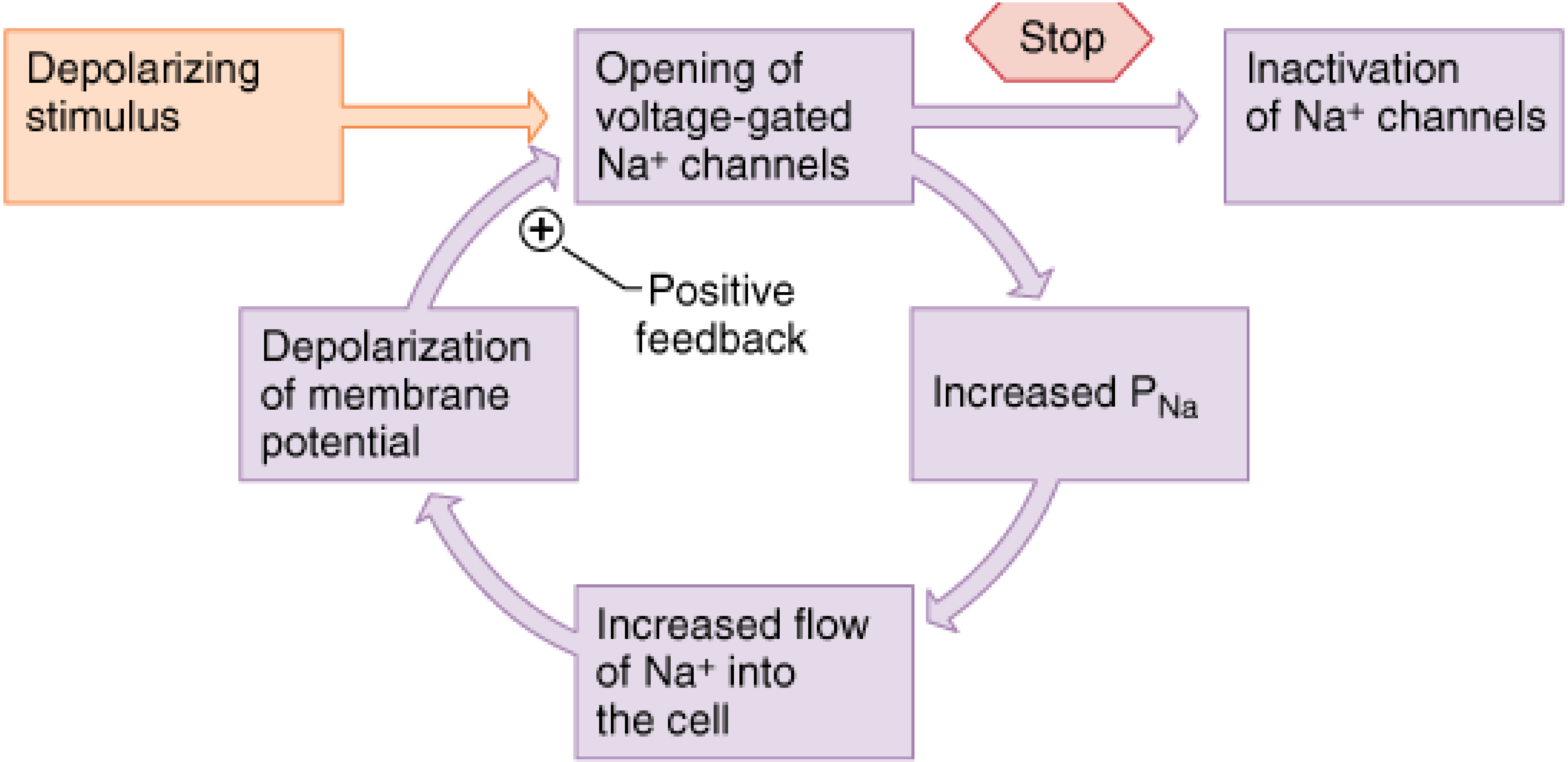
(a)

Voltage gates

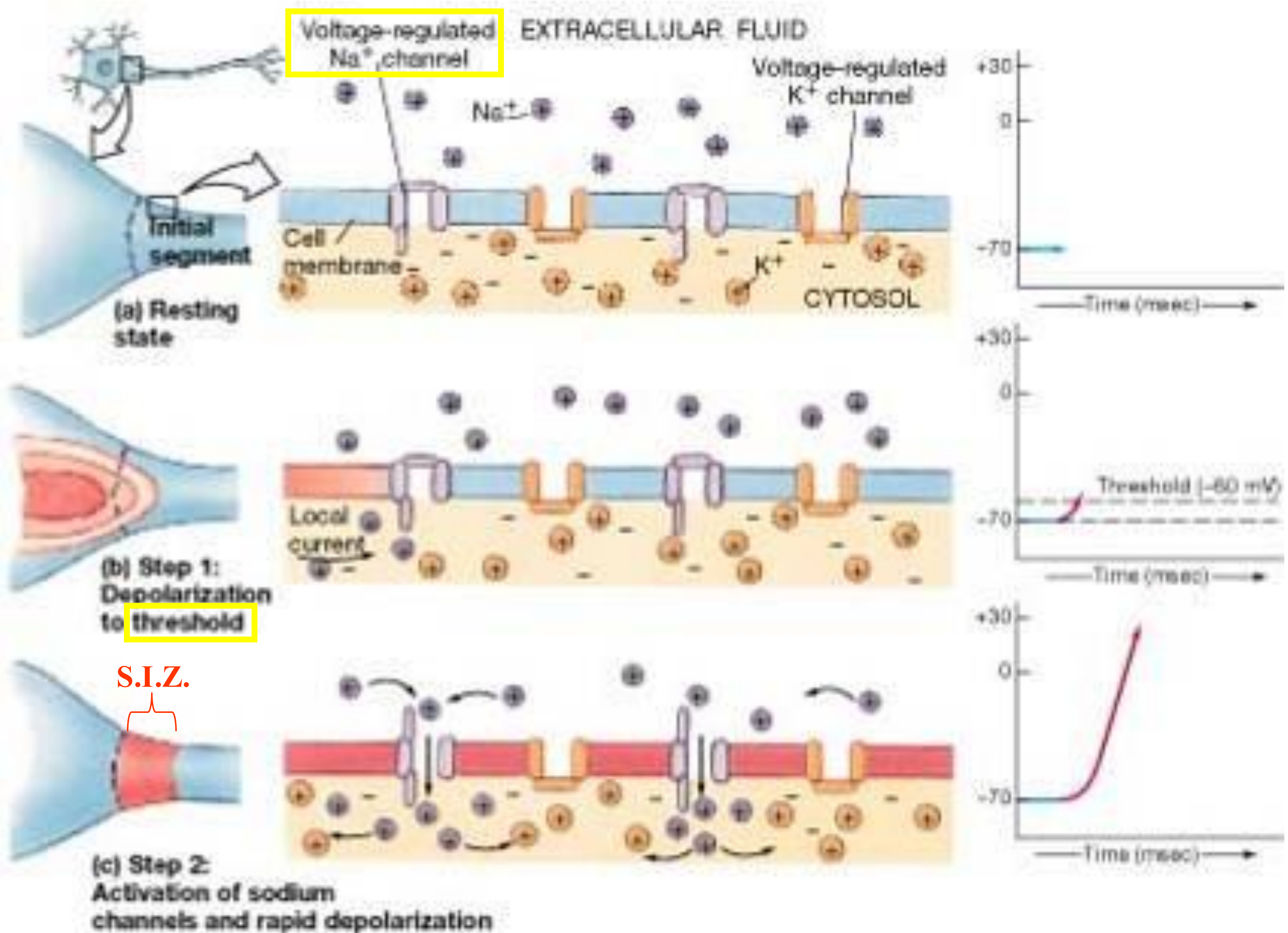


(c)

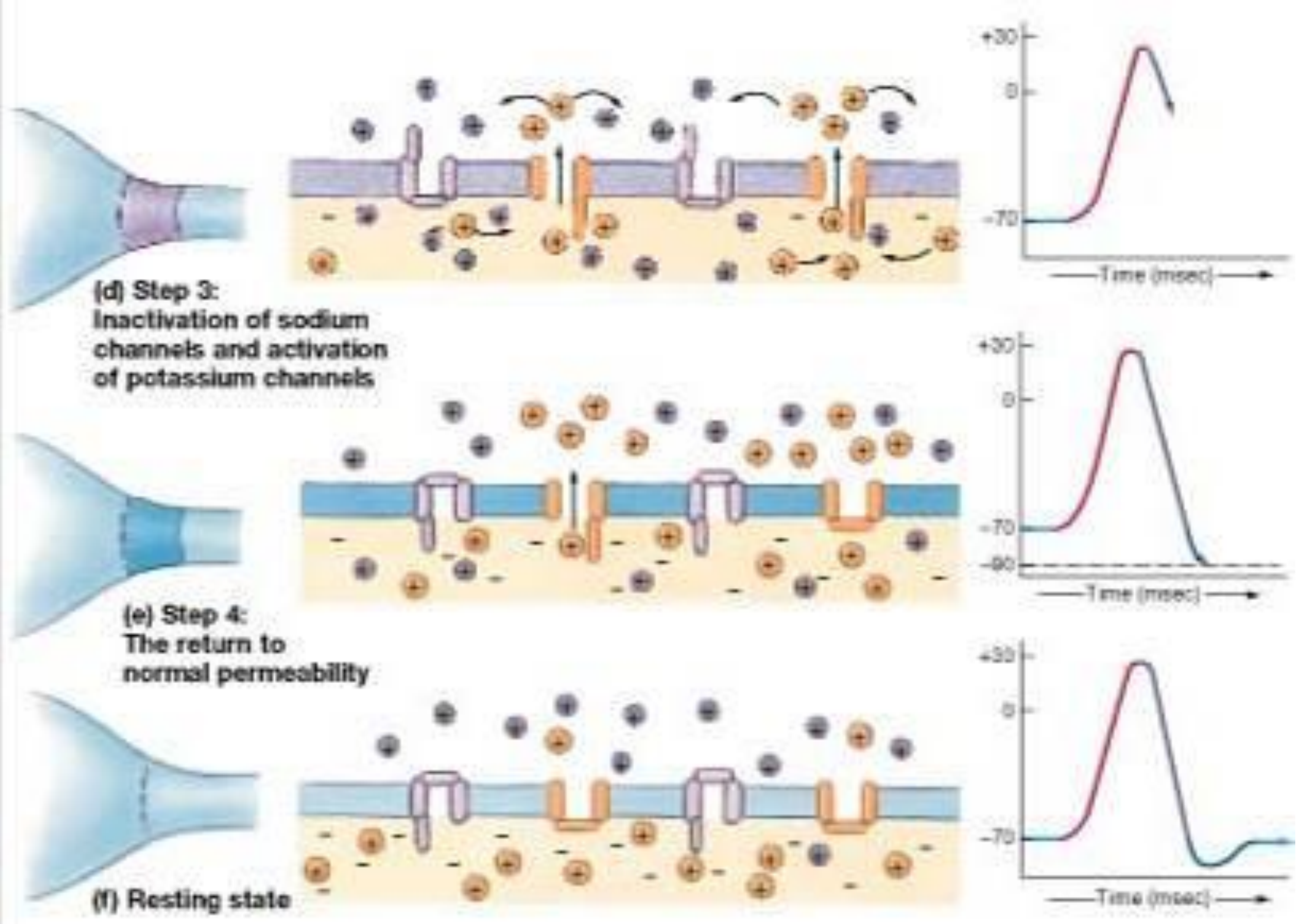
Positive feedback loop



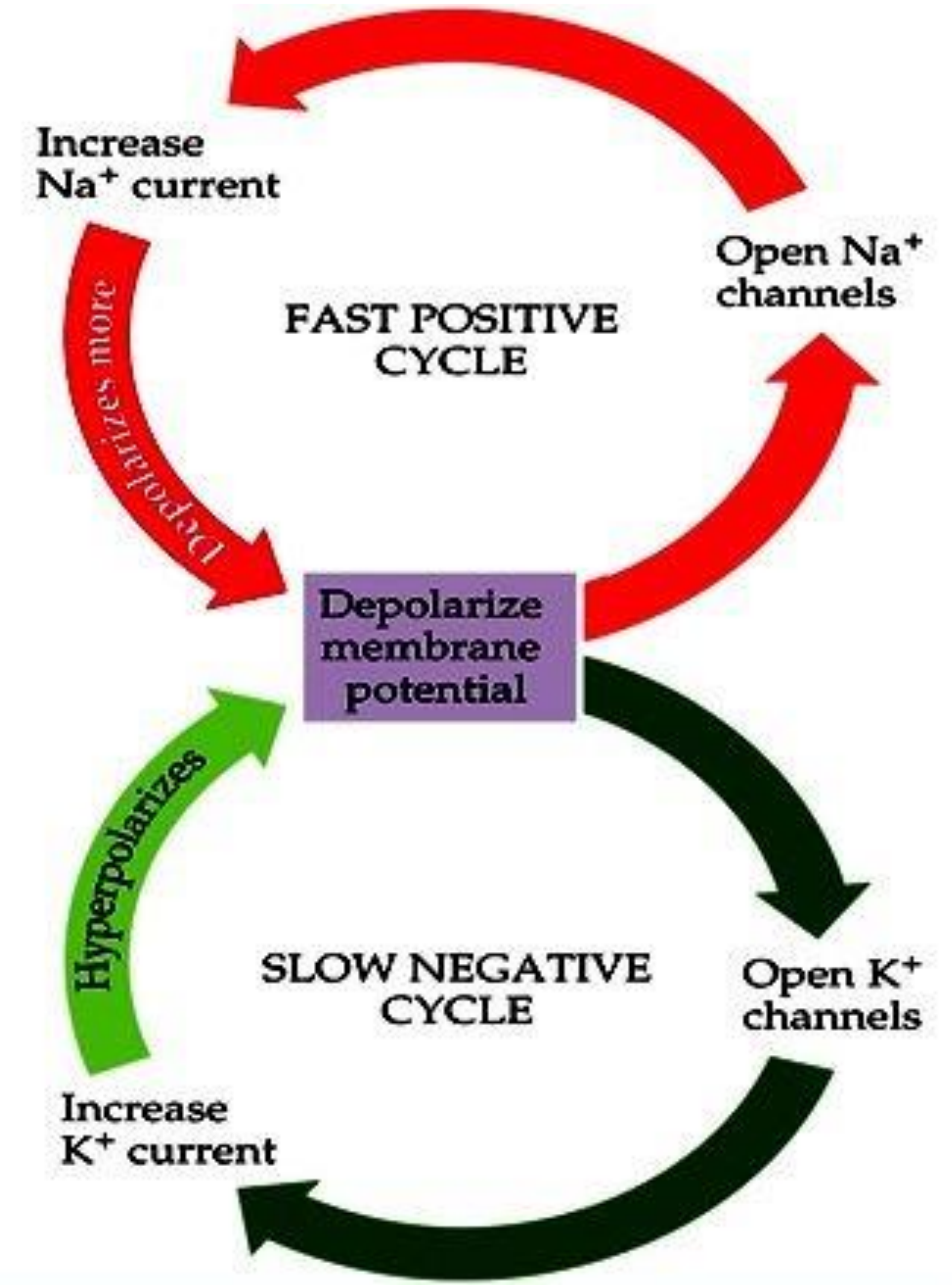
Action potential initiation

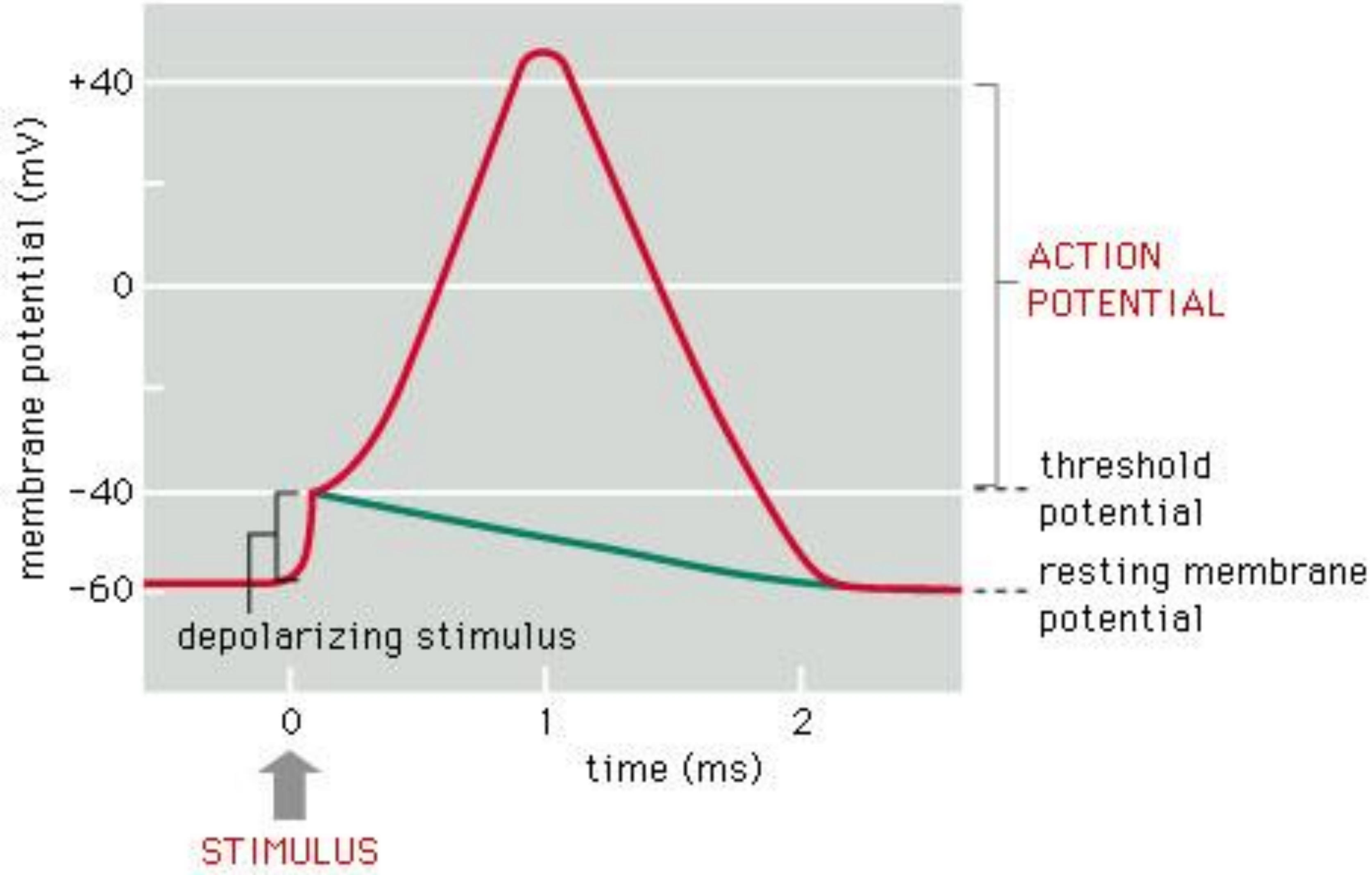


Action potential termination



Double feedback loop





+40
0
-40
-60
membrane potential (mV)
ACTION POTENTIAL
threshold potential
resting membrane potential
depolarizing stimulus
0
1
2
time (ms)
STIMULUS

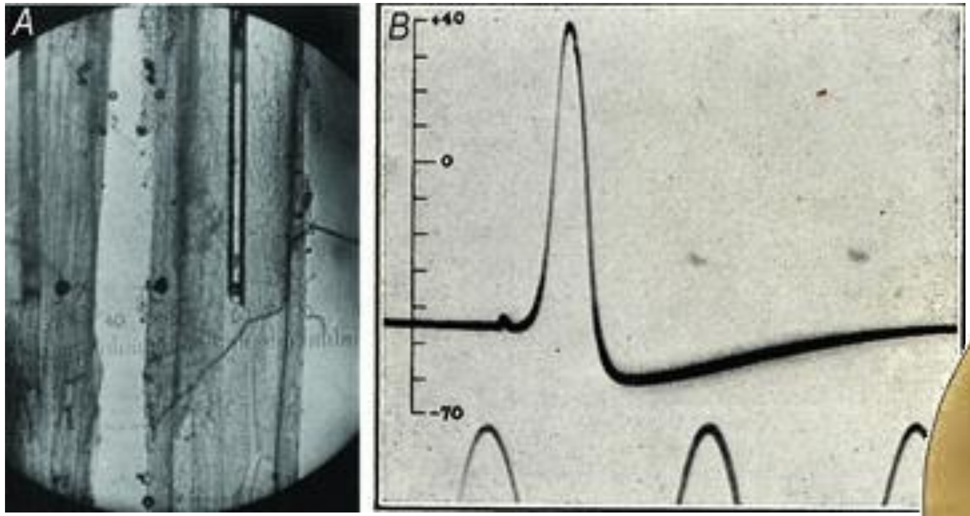
Threshold Potential

- **Threshold potential**
  - a critical membrane potential level at which an action potential can occur.
  - plays a key role in the genesis of action potential.
- **threshold stimulus**
  - Stimulus is just strong enough to depolarize the membrane to the threshold potential level

Electrophysiological Methods to Record Membrane Potential

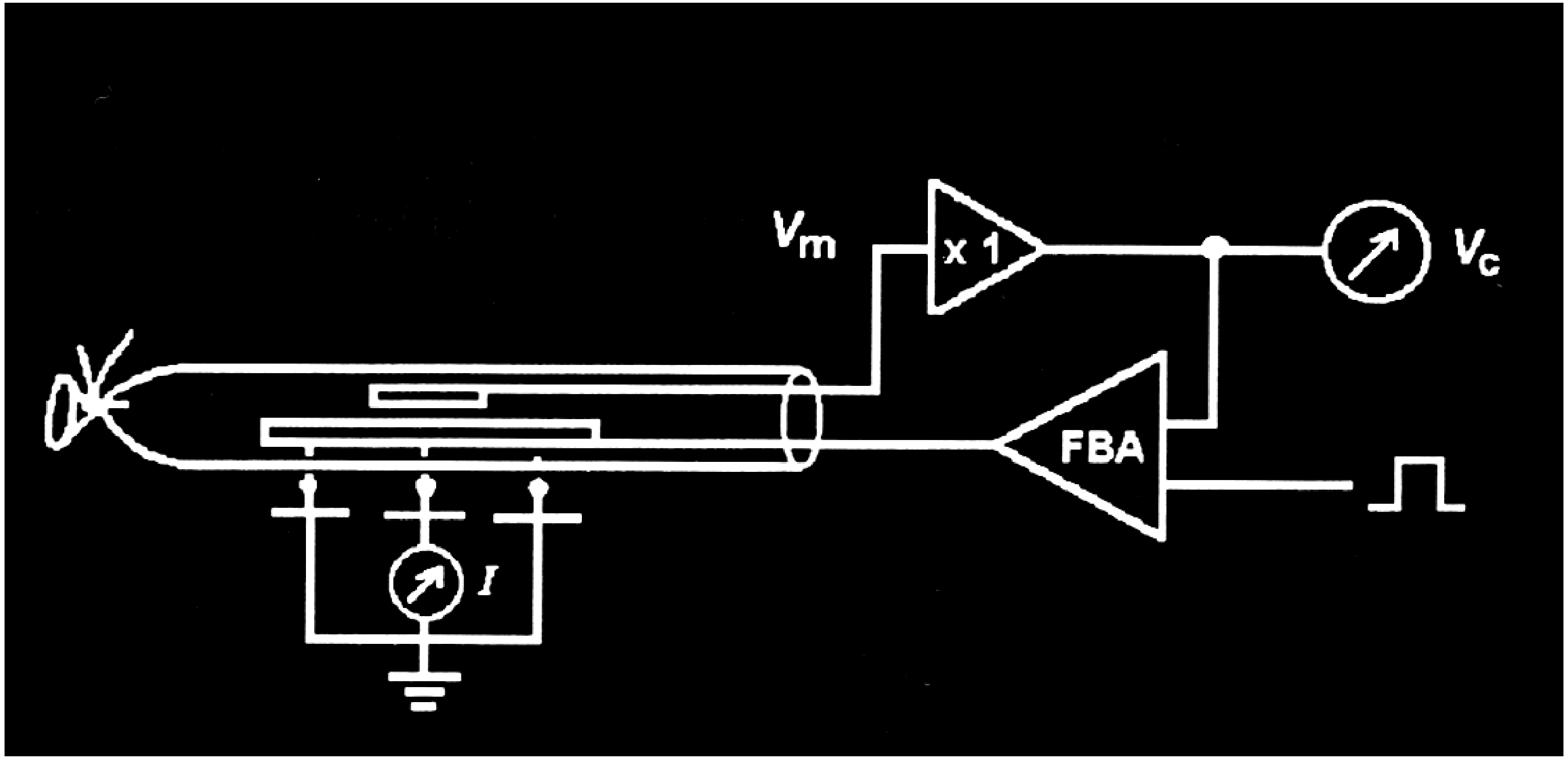
- Two electrodes Voltage Clamp

Hodgkin and Huxley

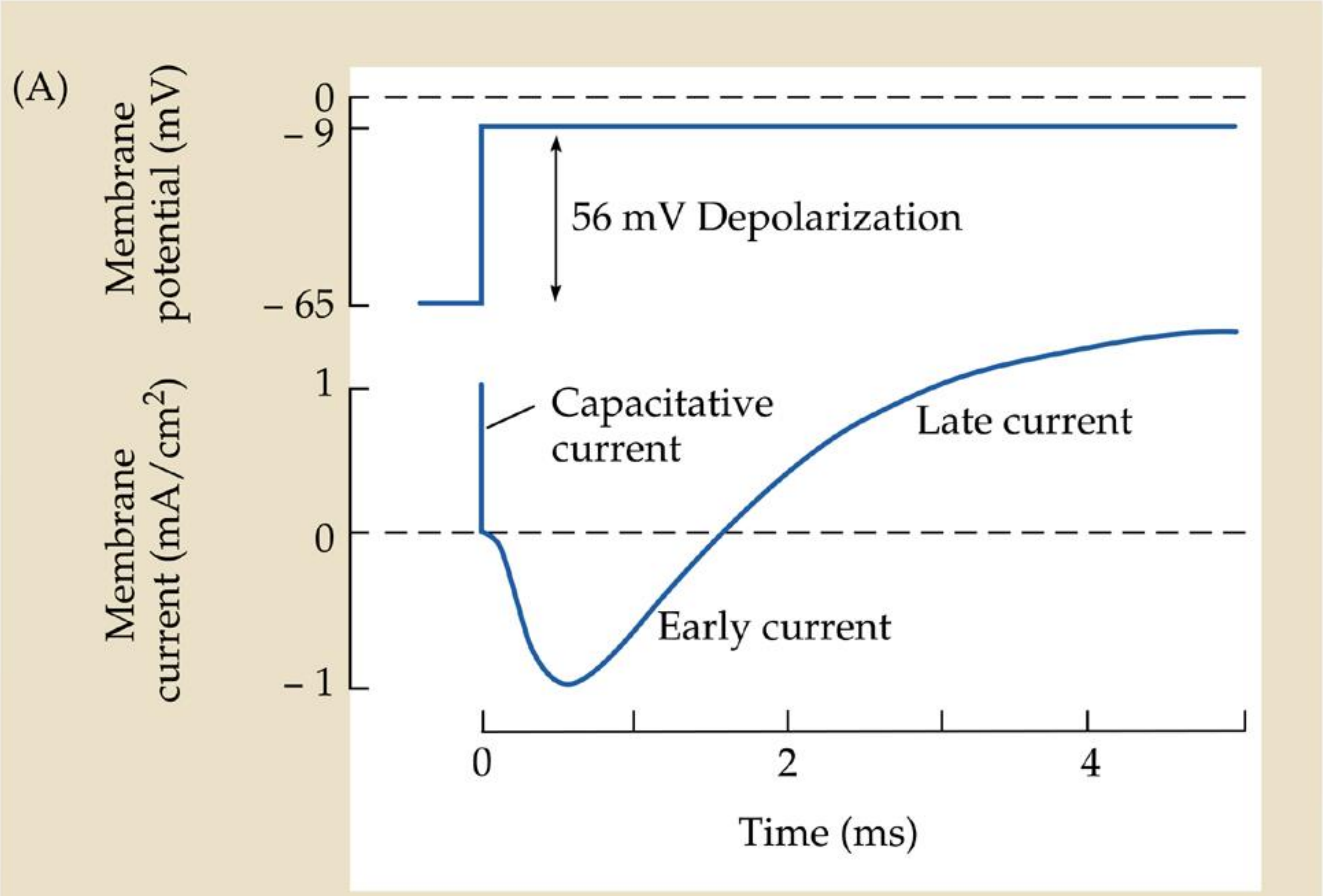


"for their discoveries concerning the ionic mechanisms involved in excitation and inhibition in the peripheral and central portions of the nerve cell membrane"
1963

The voltage clamp

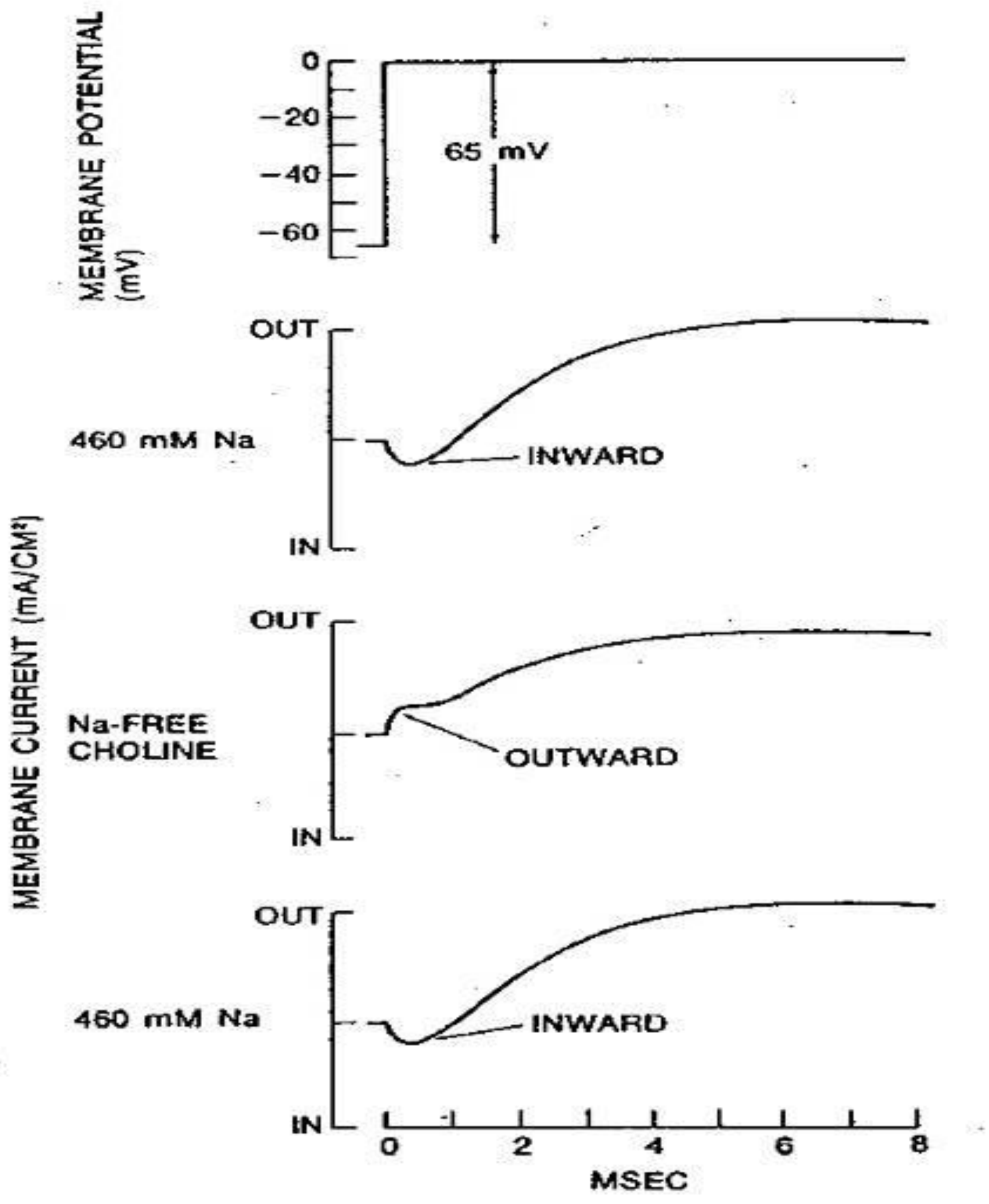


- a method for maintaining V_m at any desired voltage level (FBA, Feedback Amplifier)

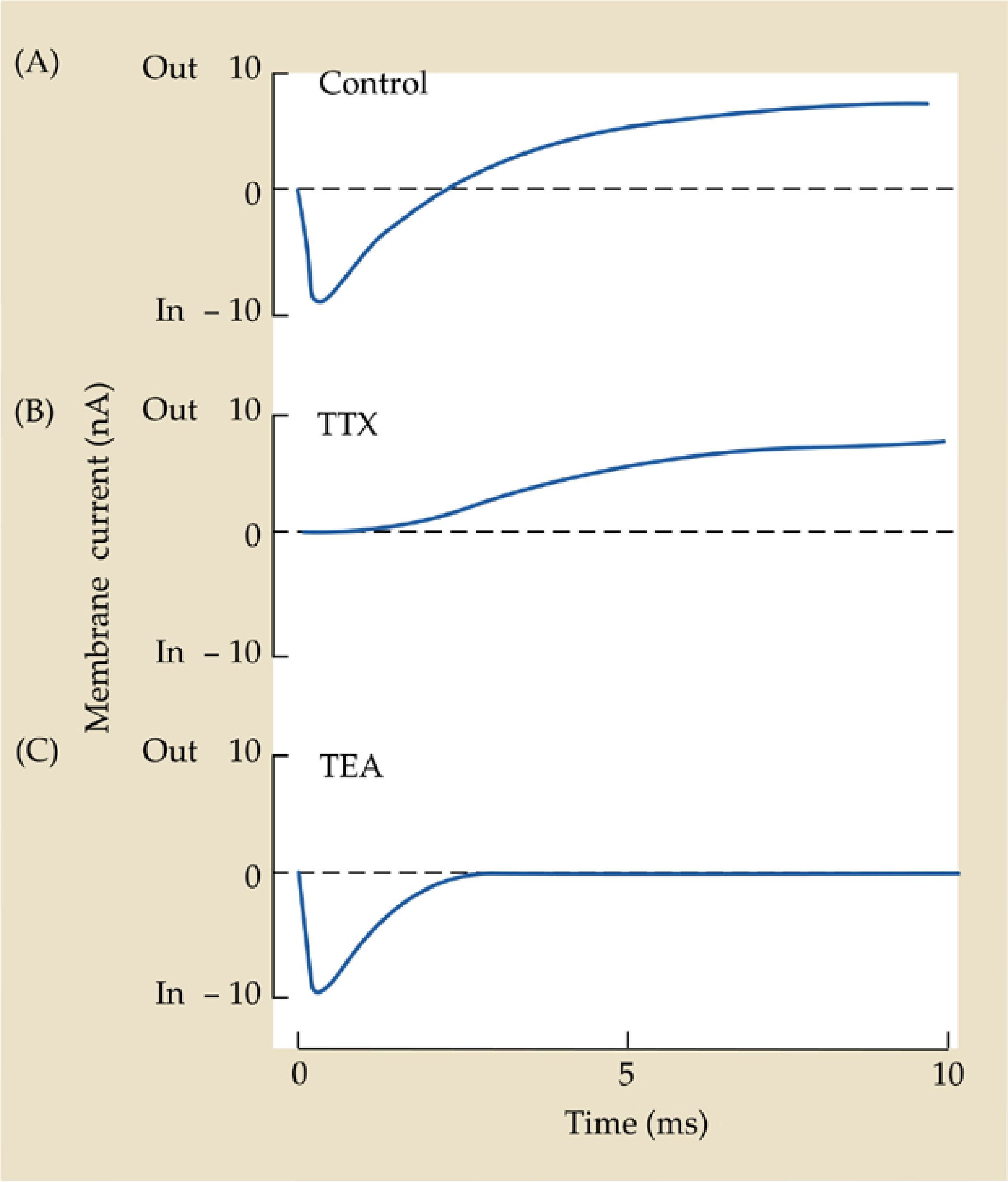


Triphasic response

Evidence for a Sodium Current

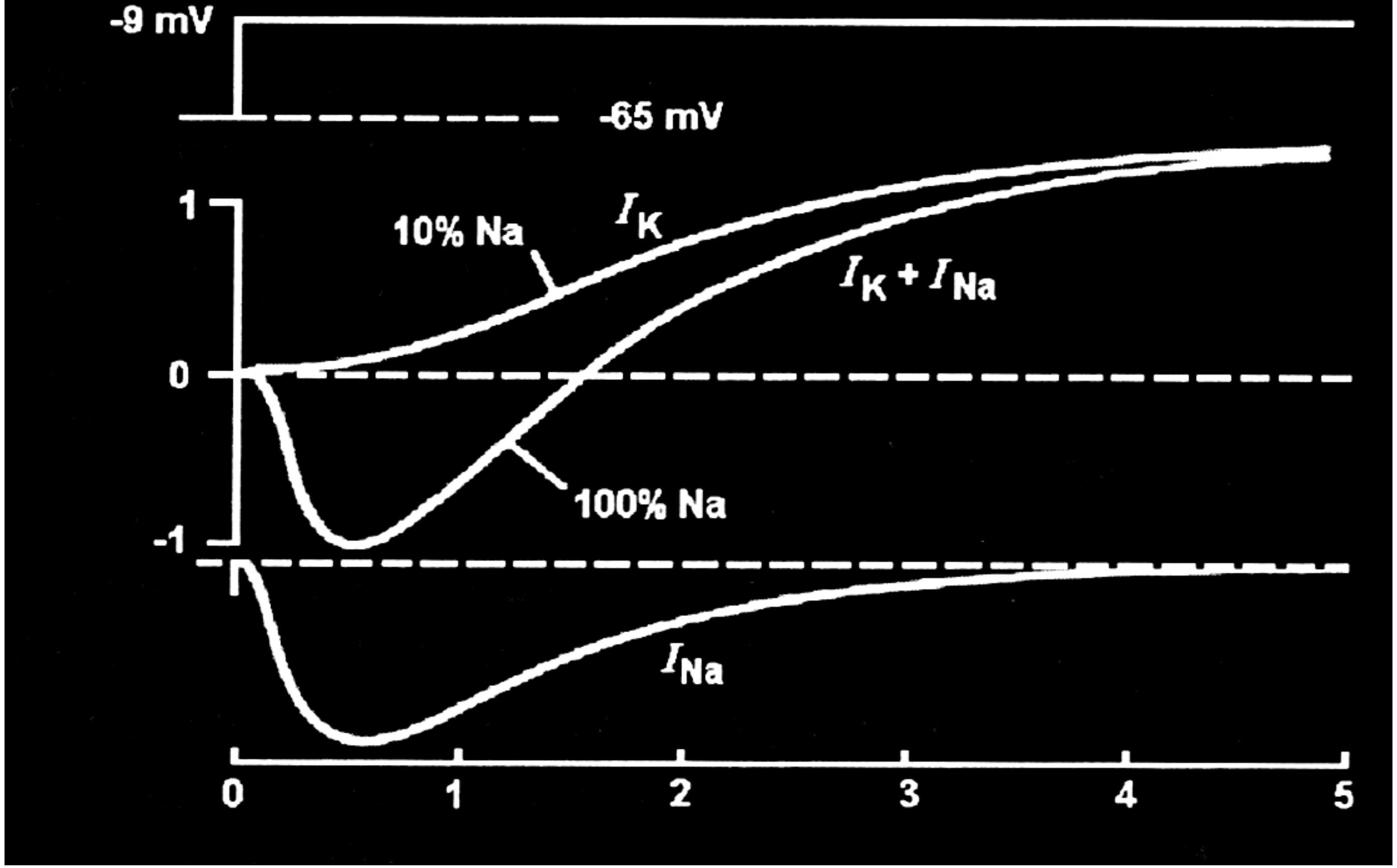


Remove extracellular sodium

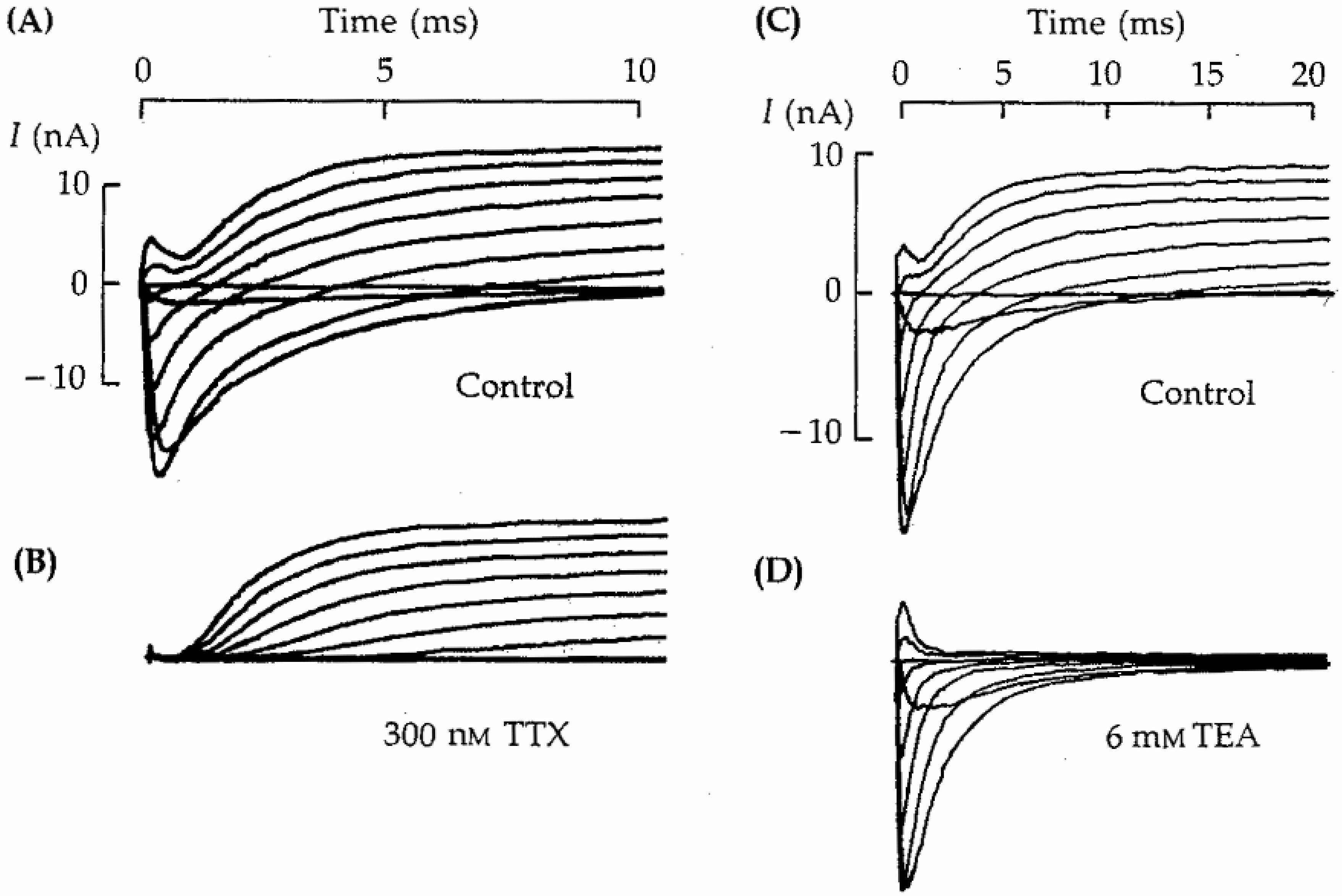


Modern proof of nature of currents

Use ion selective agents

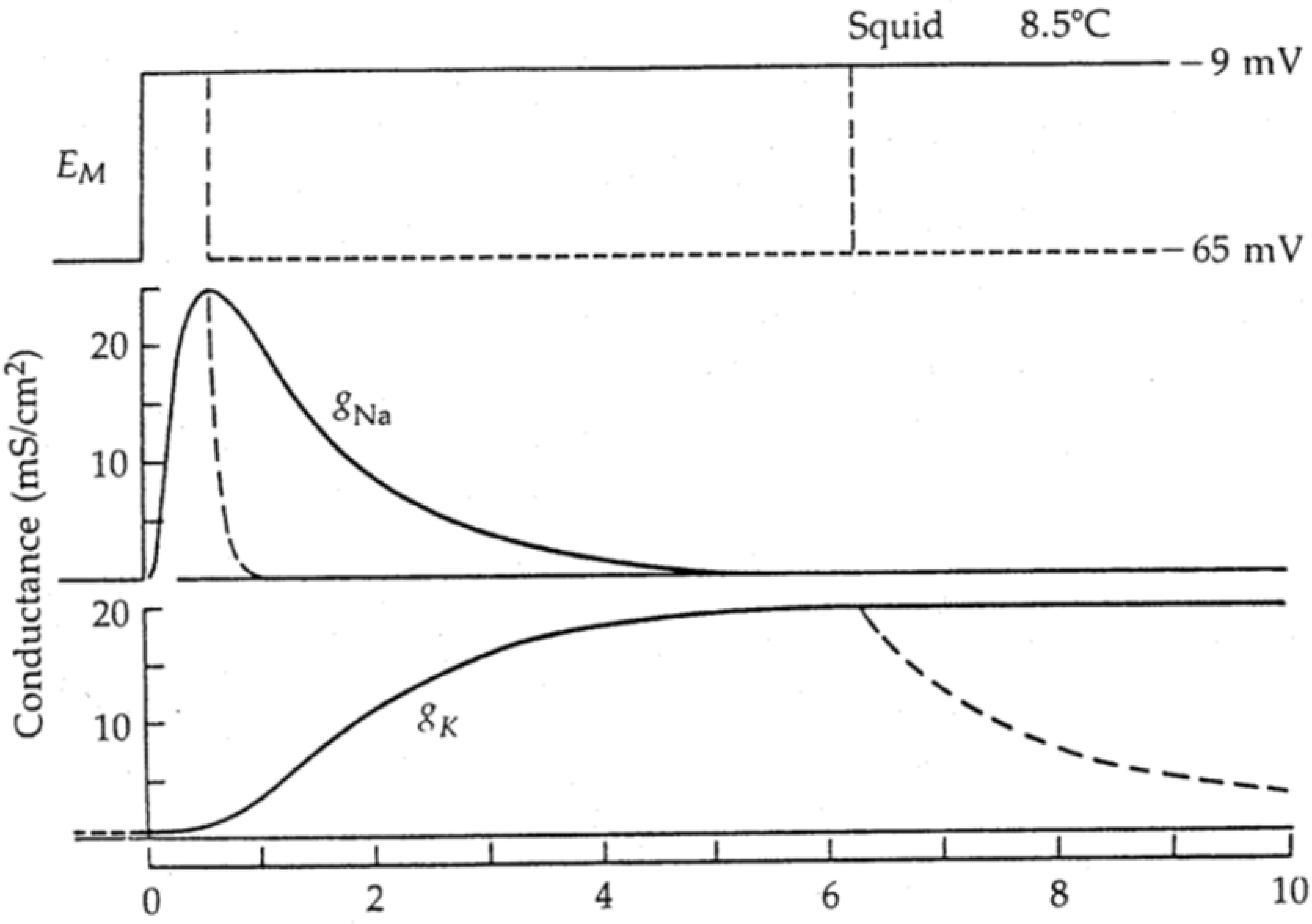


- Removing Na^+ from the bathing medium, I_{Na} becomes negligible so I_K can be measured directly.
- Subtracting this current from the total current yielded I_{Na}.

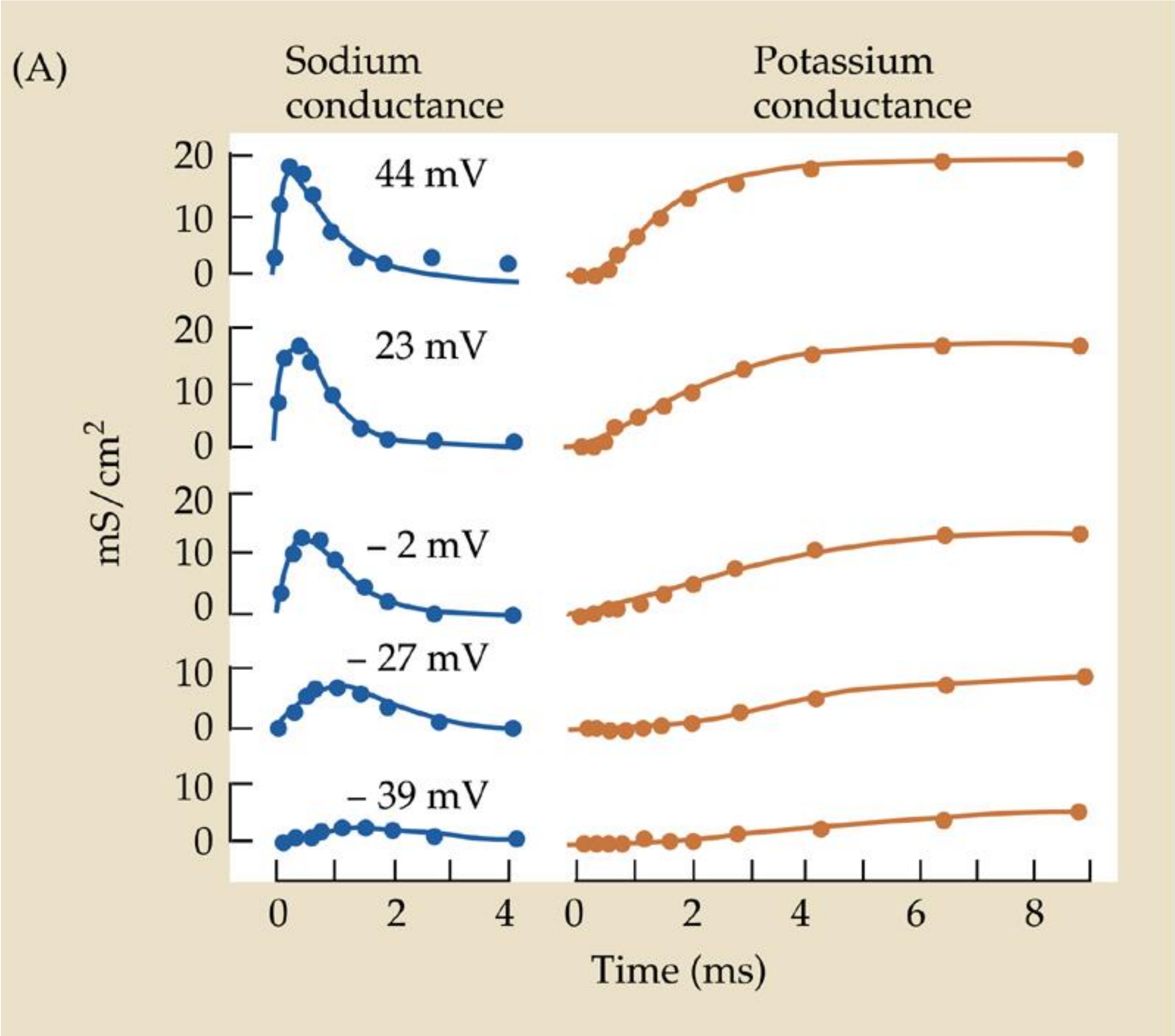


(A)
Time (ms)
0
5
10
I (nA)
10
0
−10
Control
(B)
300 nM TTX
(C)
Time (ms)
0
5
10
15
20
I (nA)
10
0
−10
Control
(D)
6 mM TEA

Conductance of Na^+ and K^+ channels

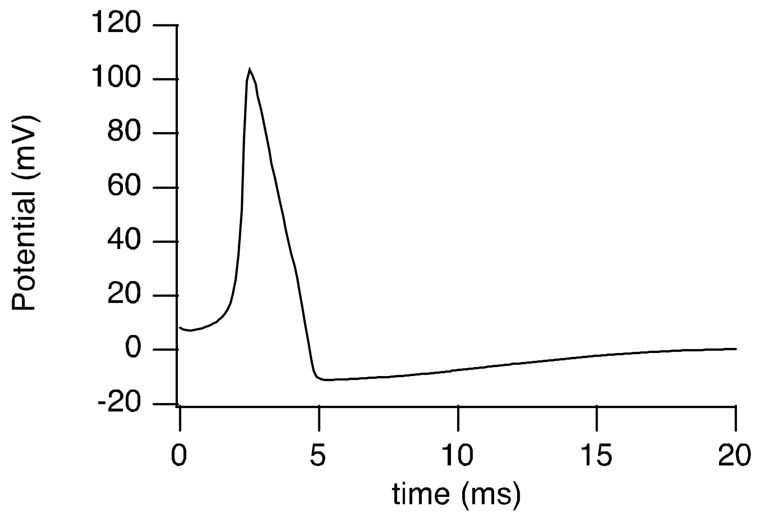


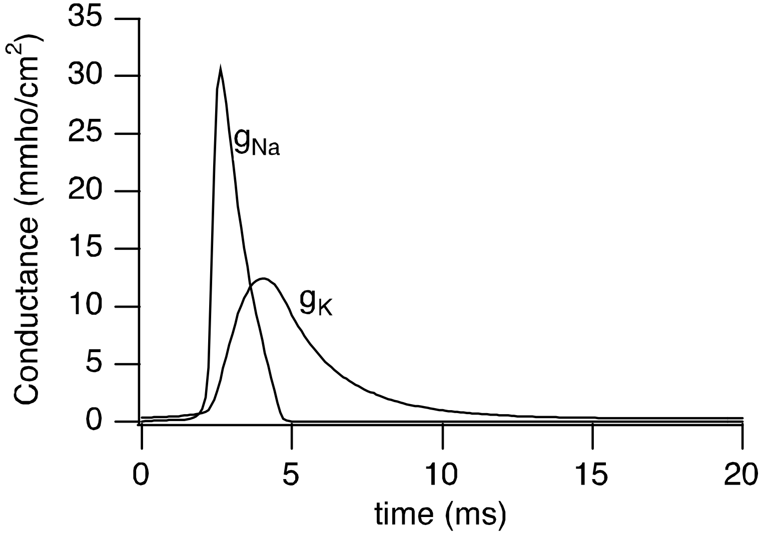
Voltage-Dependence of Conductance



Action potential

- g_{Na} increases quickly, but then inactivation kicks in and it decreases again.
- g_K increases more slowly, and only decreases once the voltage has decreased.
- The Na^+ current is **autocatalytic**. An increase in V increases g_{Na}, which increases the Na^+ current, and increases V, etc.
- Hence, **the threshold for action potential initiation is where the inward Na^+ current exactly balances the outward K^+ current**.

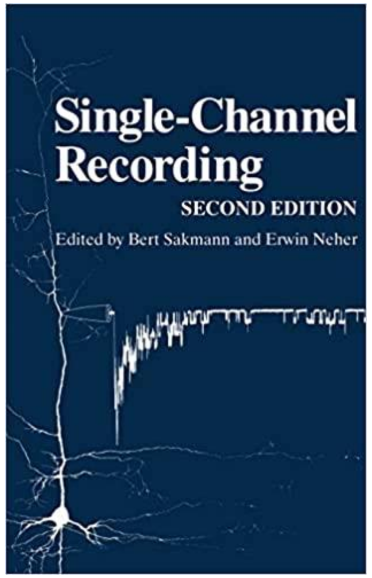


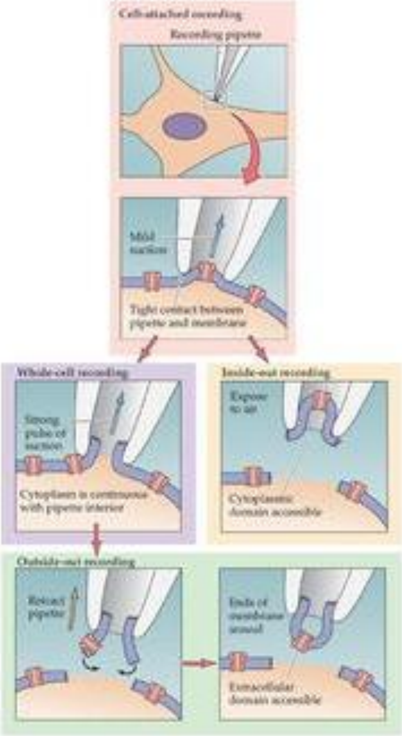


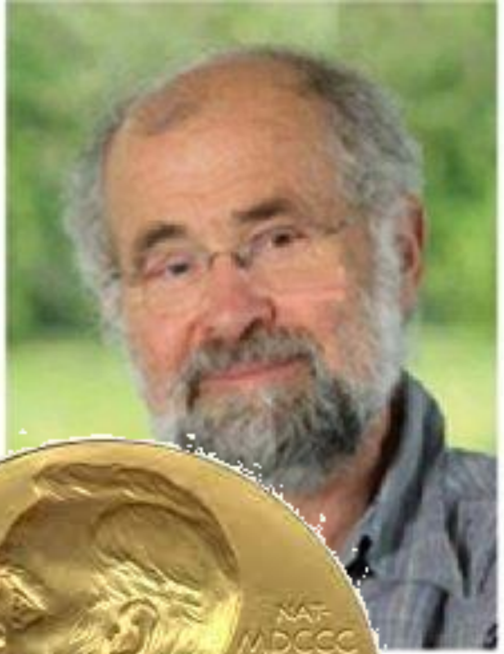
Electrophysiological Methods to Record Membrane Potential

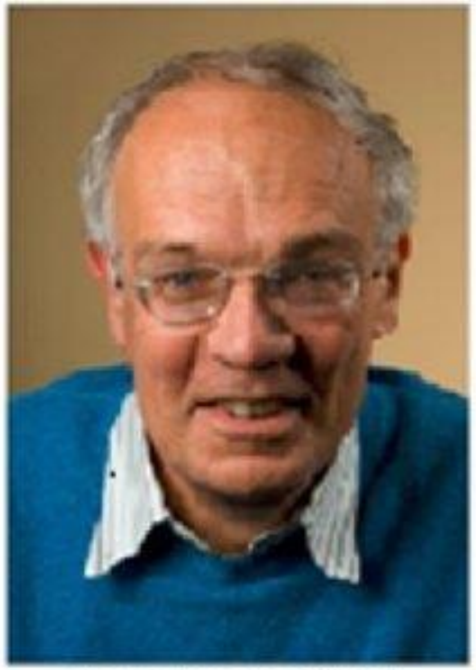
Patch Clamp

Erwin Neher and Bert Sakmann

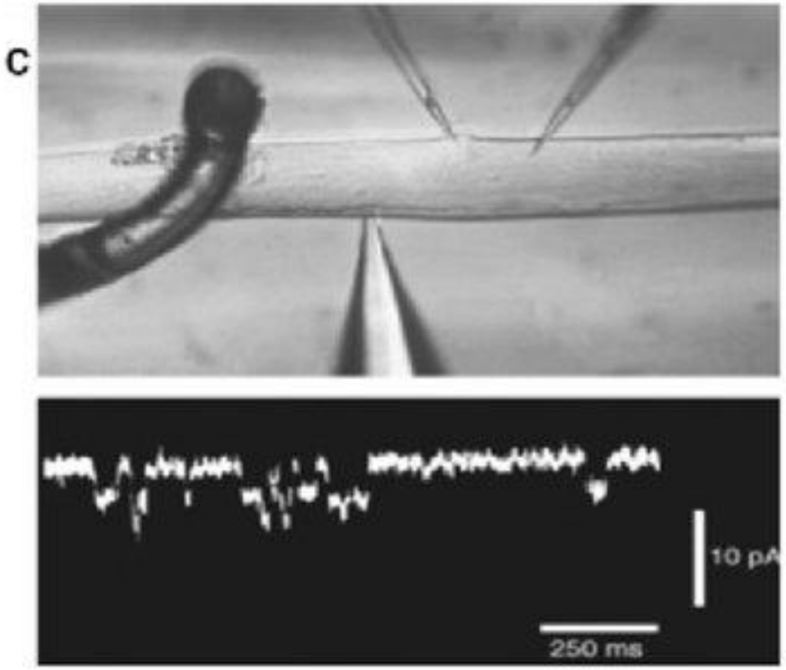








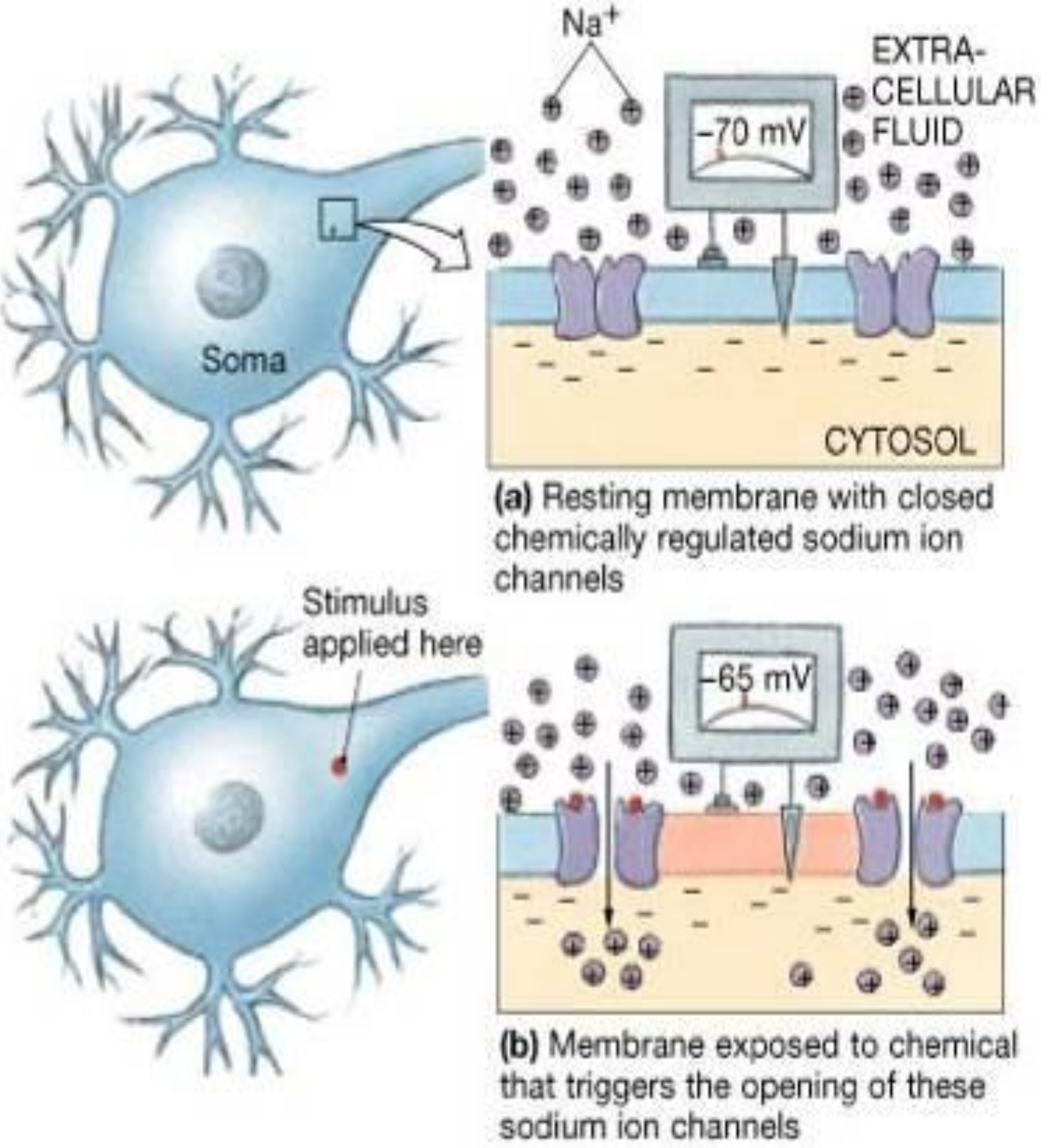
Bert Sakmann (1942)



"for their discoveries concerning the function of single ion channels in cells"
1991

- **Bioelectrical Phenomena of the Cell:**
- **3: Local responses**

Graded (local) potential changes

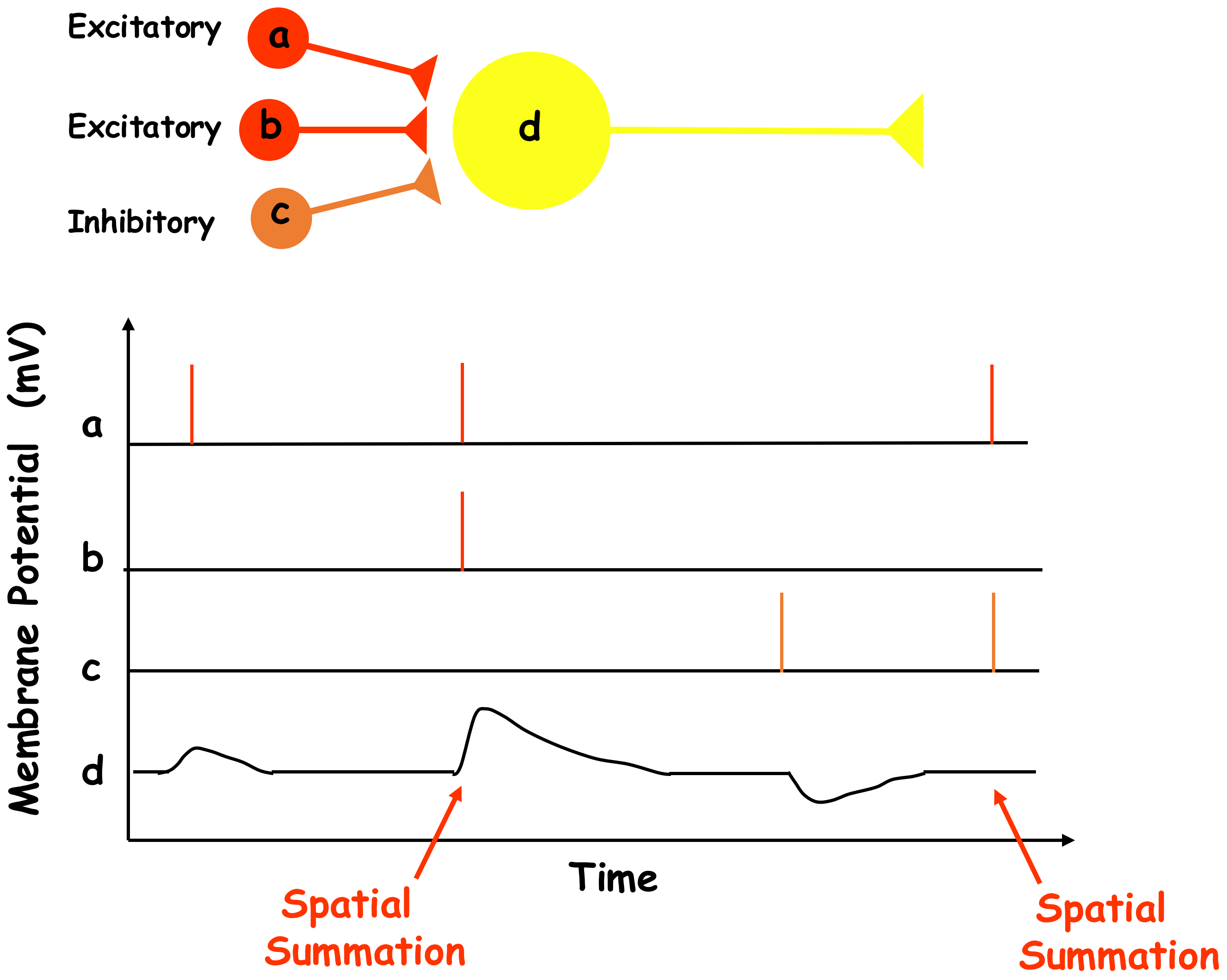


2 x more chemical= 2 x more potential change

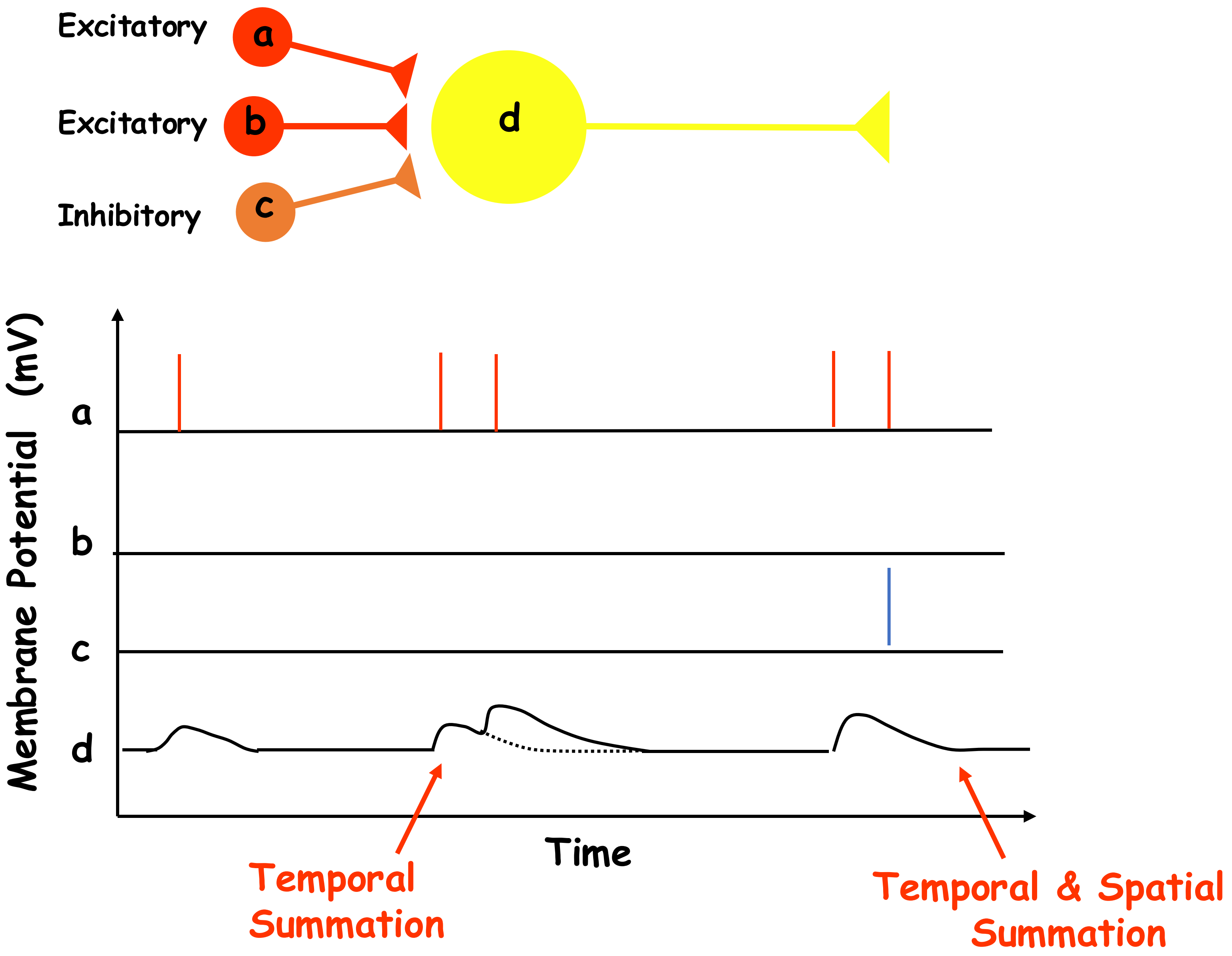
Local Response

- Definition:
  - a small change in membrane potential caused by a subthreshold stimulus
- Properties:
  - graded potential
  - Propagation: electronic conduction
  - can be summed by two ways
    - Spatial summation
    - Temporal summation

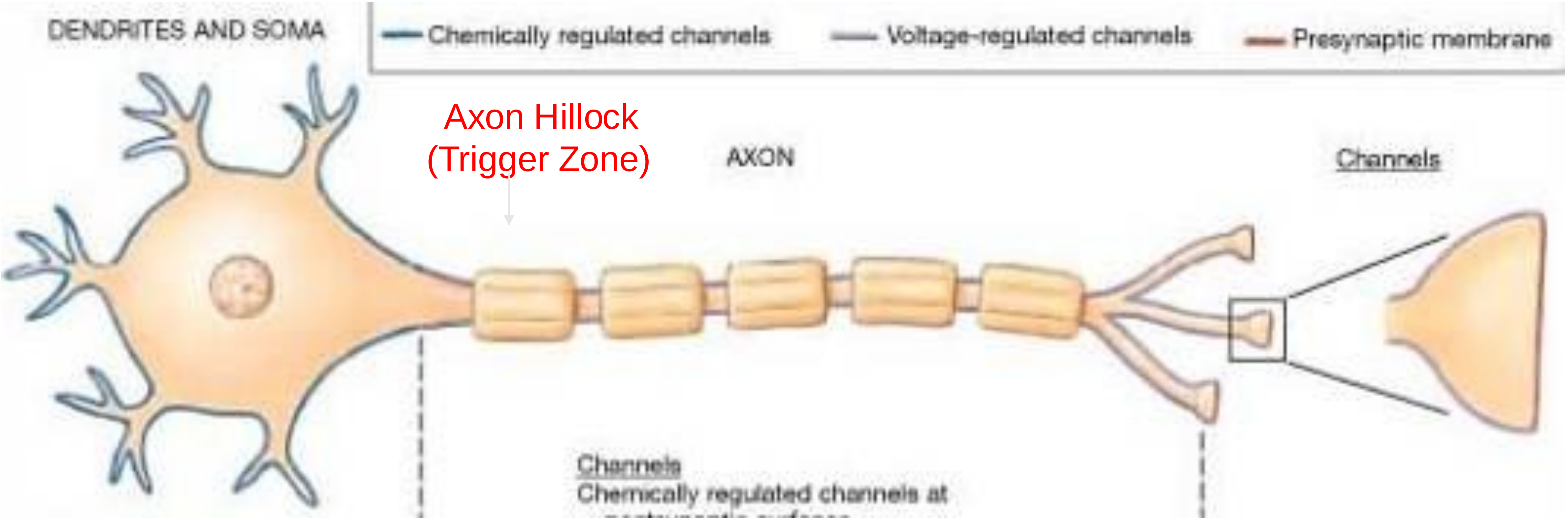
Local Response: spatial summation



Local Response: temporal summation



Distribution of ion channels

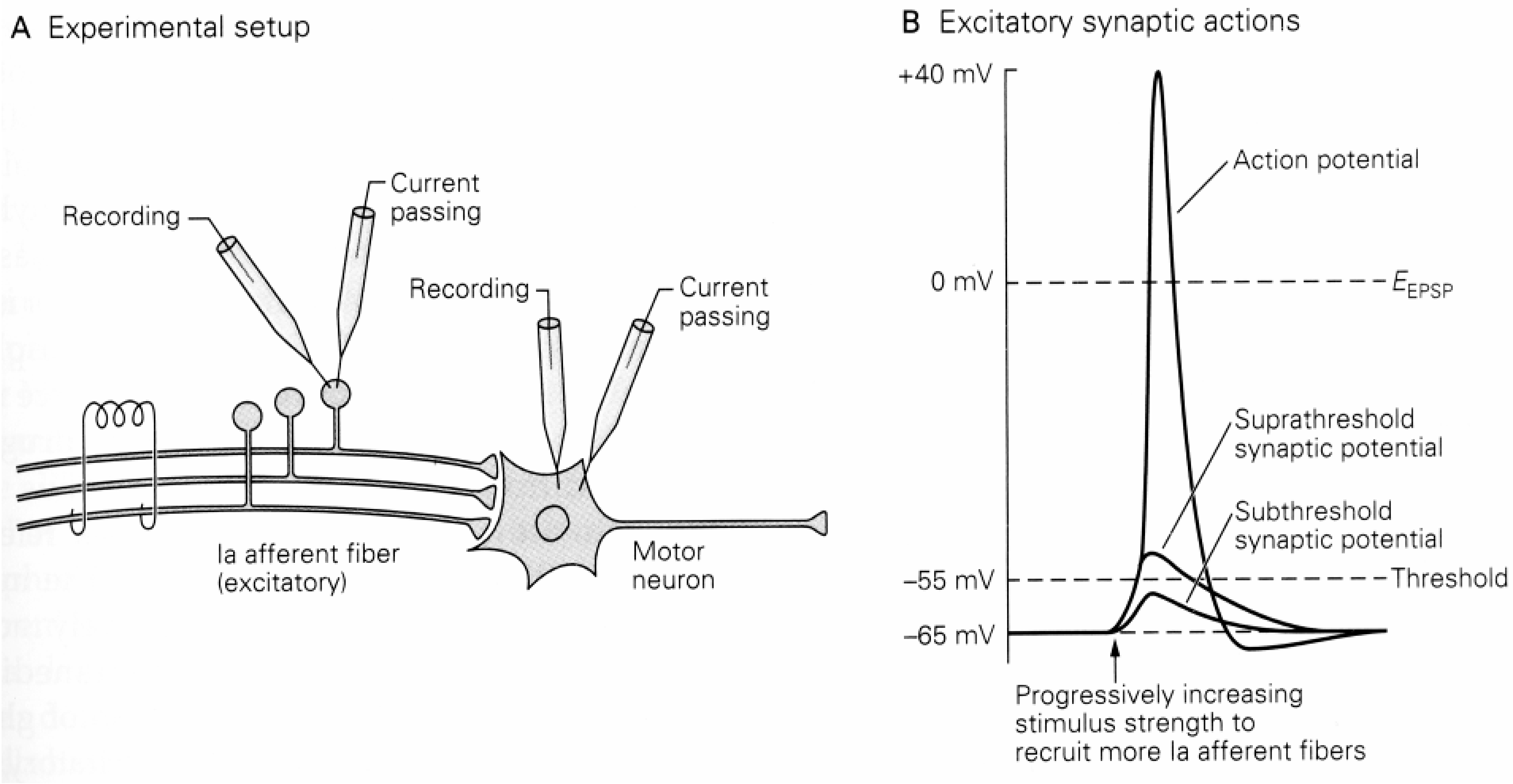


Leak channels everywhere

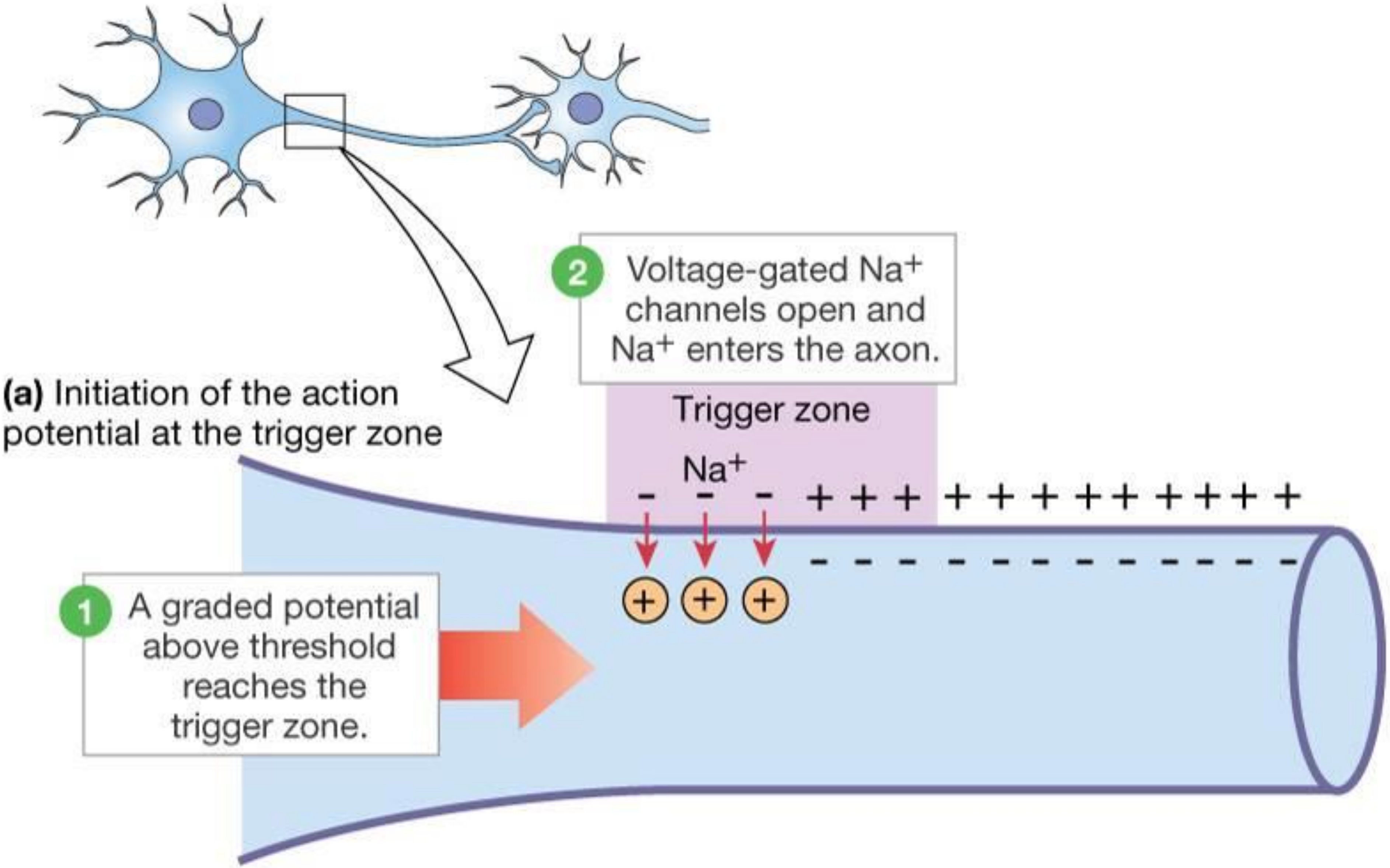
Role of the Local Potential

- Facilitate the cell.
  - This means it increase excitability of the stimulated cell
- Cause the cell to excite once it is summed to reach the threshold potential

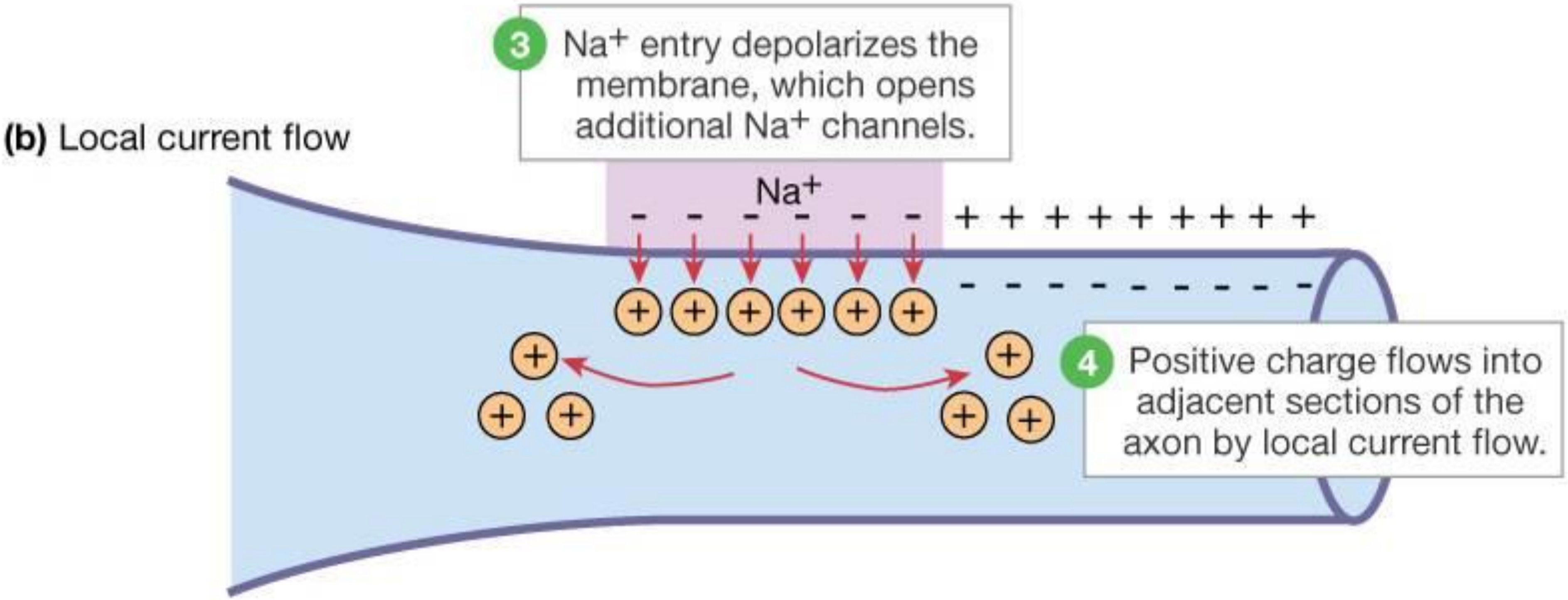
Role of the Local Potential



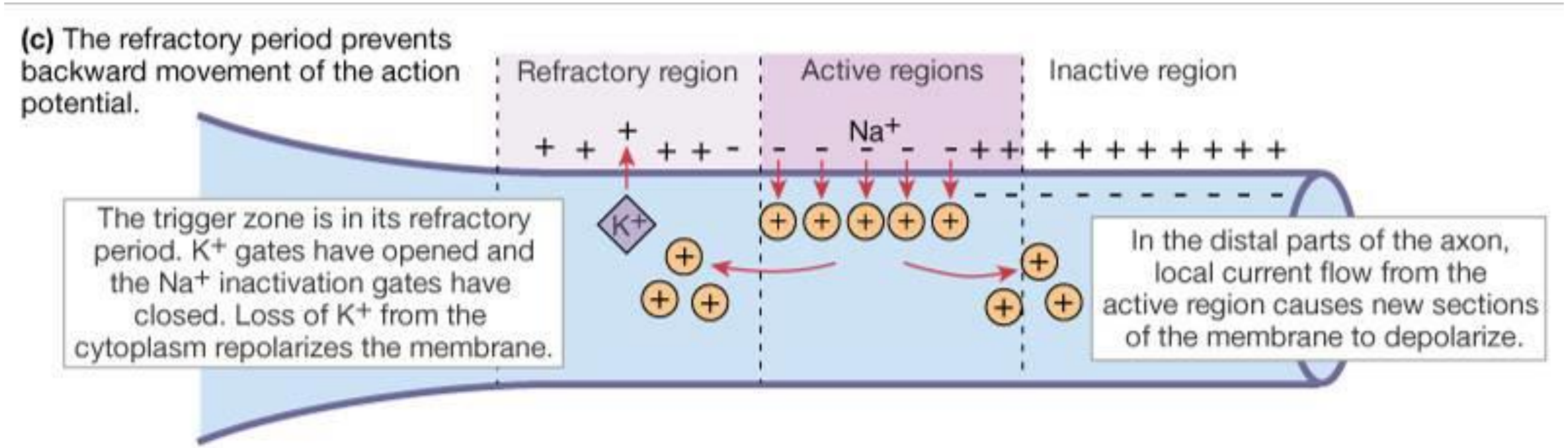
Propagation of the Action Potential



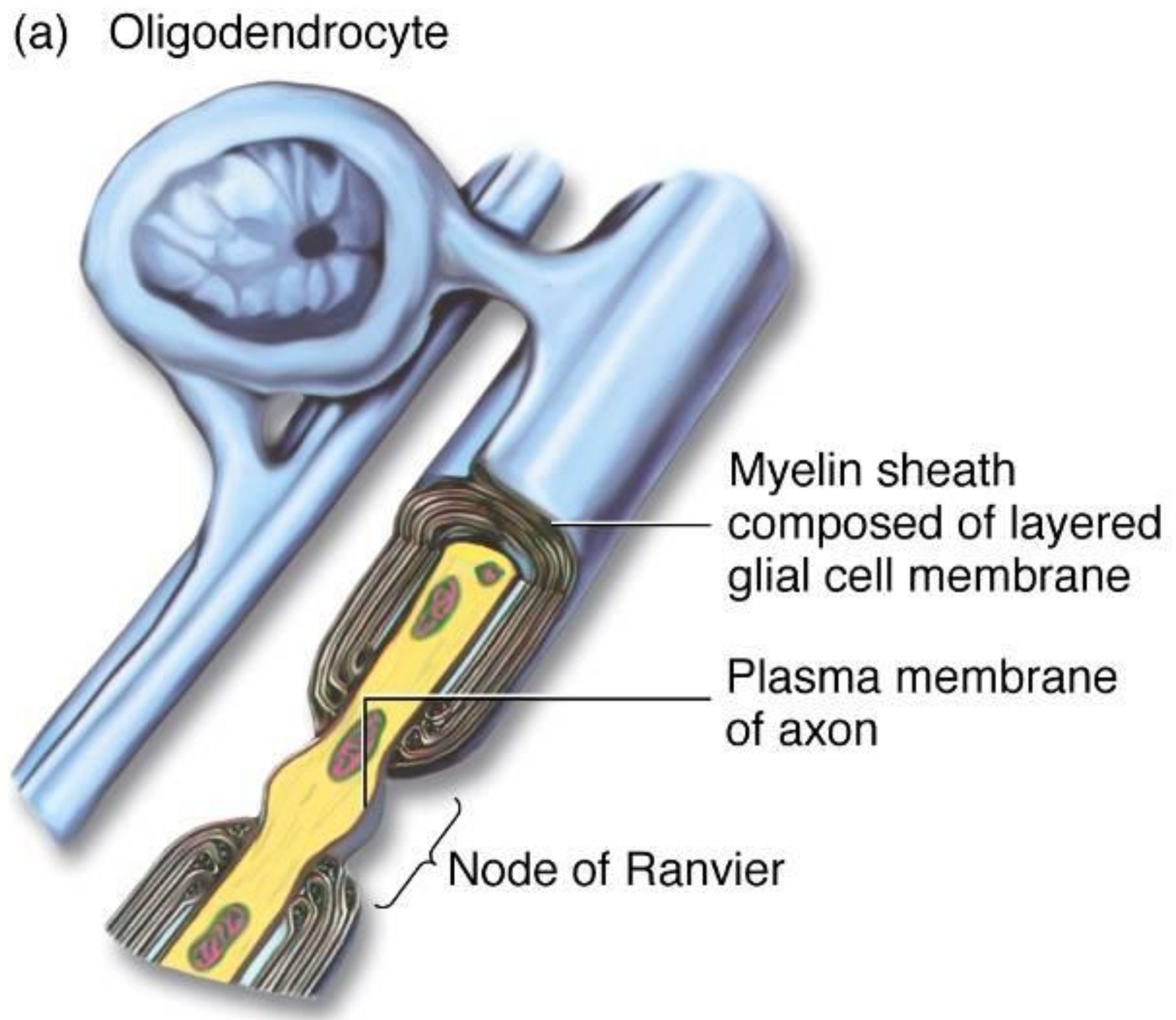
Propagation of the Action Potential



Propagation of the Action Potential

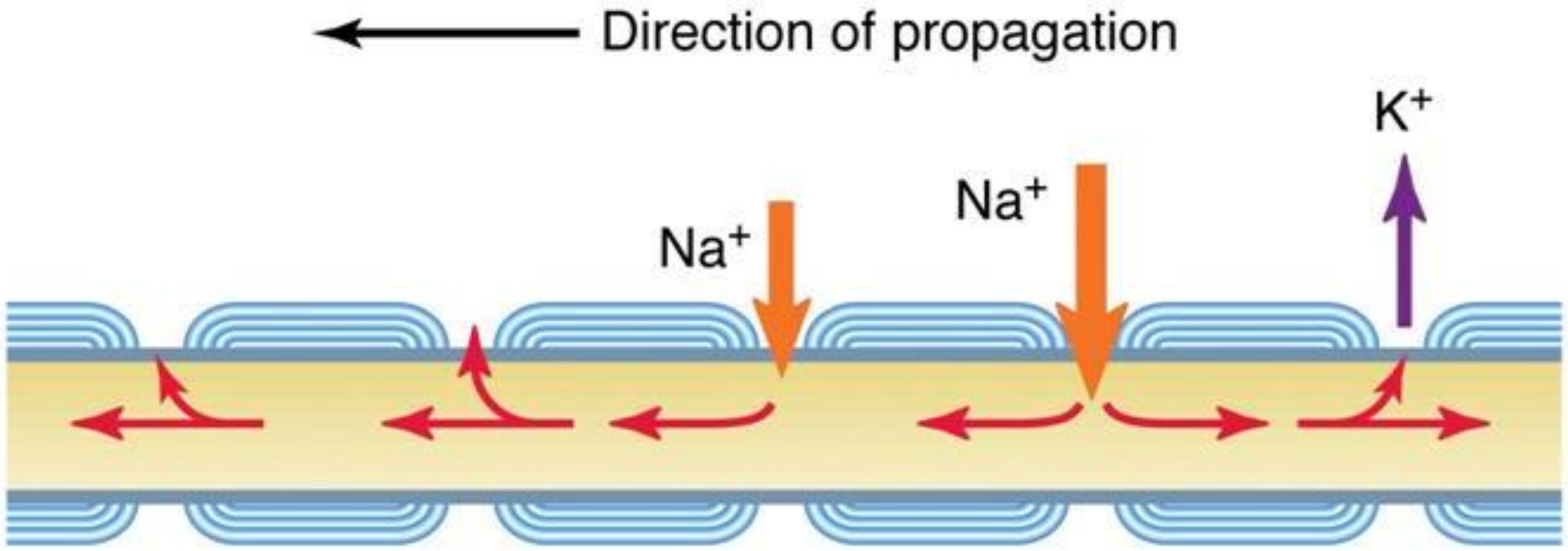


Propagation of the Action Potential: Myelin



Myelinated neuron of the central nervous system

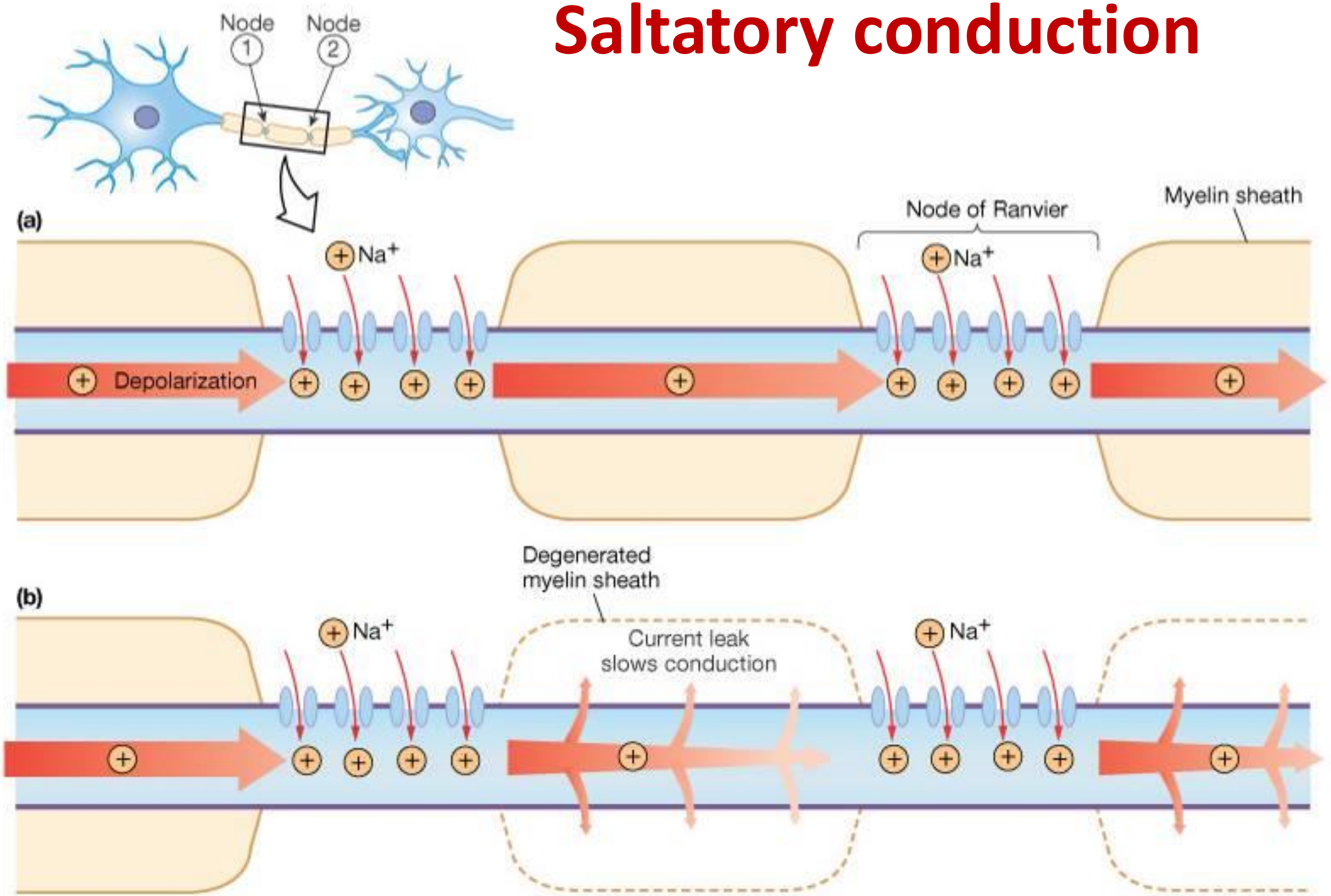
Propagation of the Action Potential: Myelin



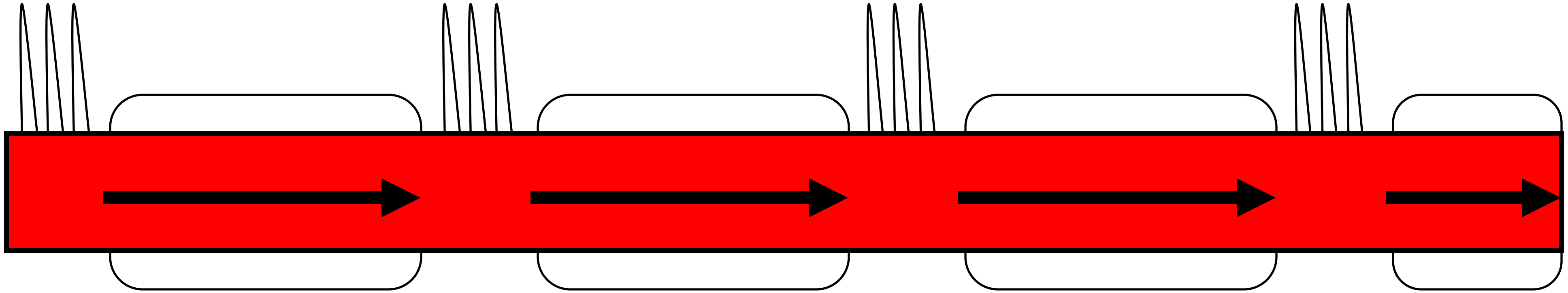
Saltatory conduction:

The action potential jumps from node to node

Saltatory conduction



Saltatory conduction



Saltatory conduction

- The pattern of conduction in the myelinated nerve fiber from node to node
- It is of value for two reasons:
  - very fast
  - preserves energy.

Propagation of the Action Potential

Table 6-1 The diameter of frog axons and the presence or absence of myelination control the conduction velocity.

Fiber type	Average axon diameter (μm)	Conduction velocity ($m \cdot s^{-1}$)
Myelinated fibers		
Aα	18.5	42
Aβ	14.0	25
Aγ	11.0	17
B	Approximately 3.0	4.2
Unmyelinated fibers		
C	2.5	0.4–0.5

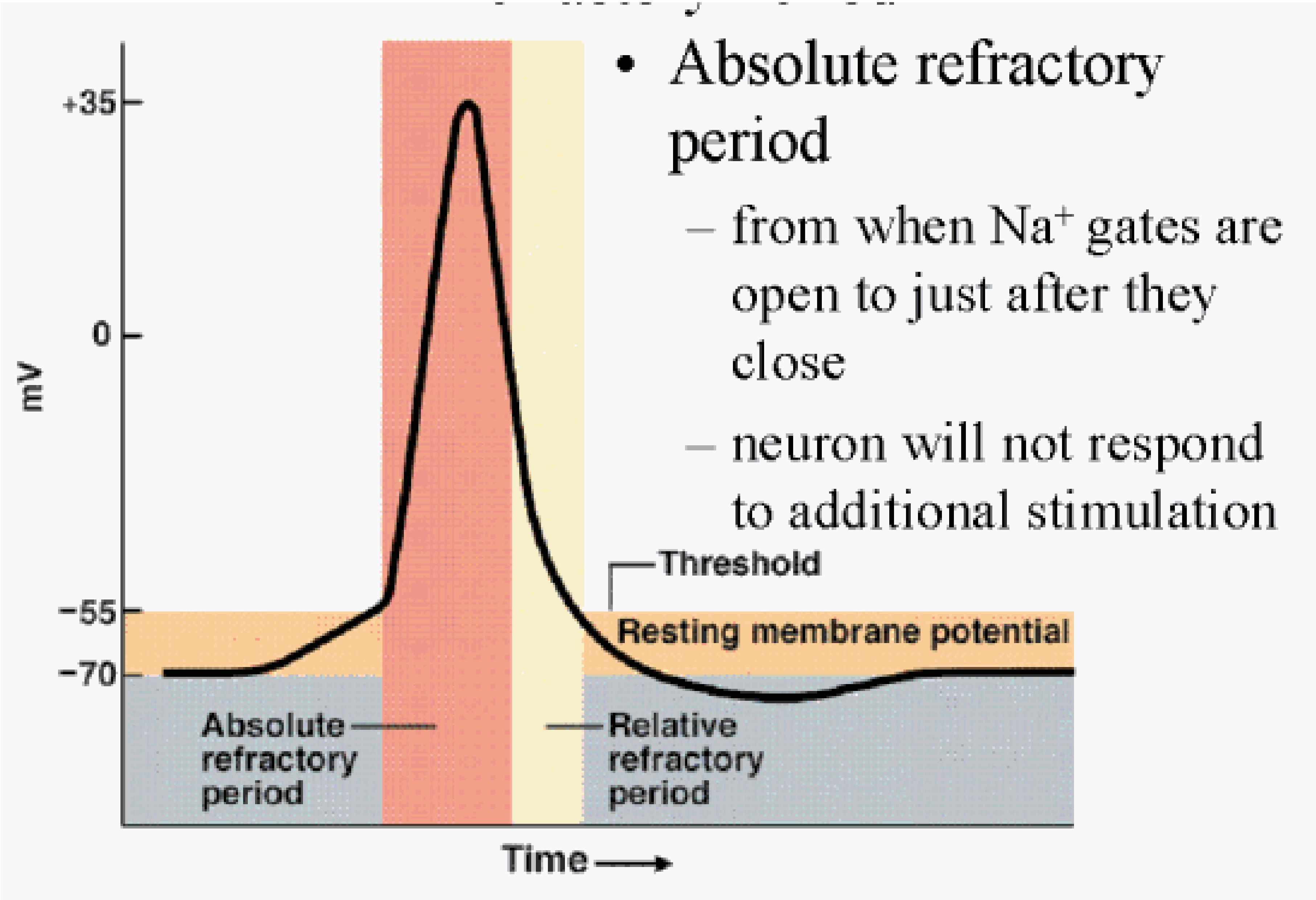
Source: Erlanger and Gasser, 1937.

Factors that affect the propagation

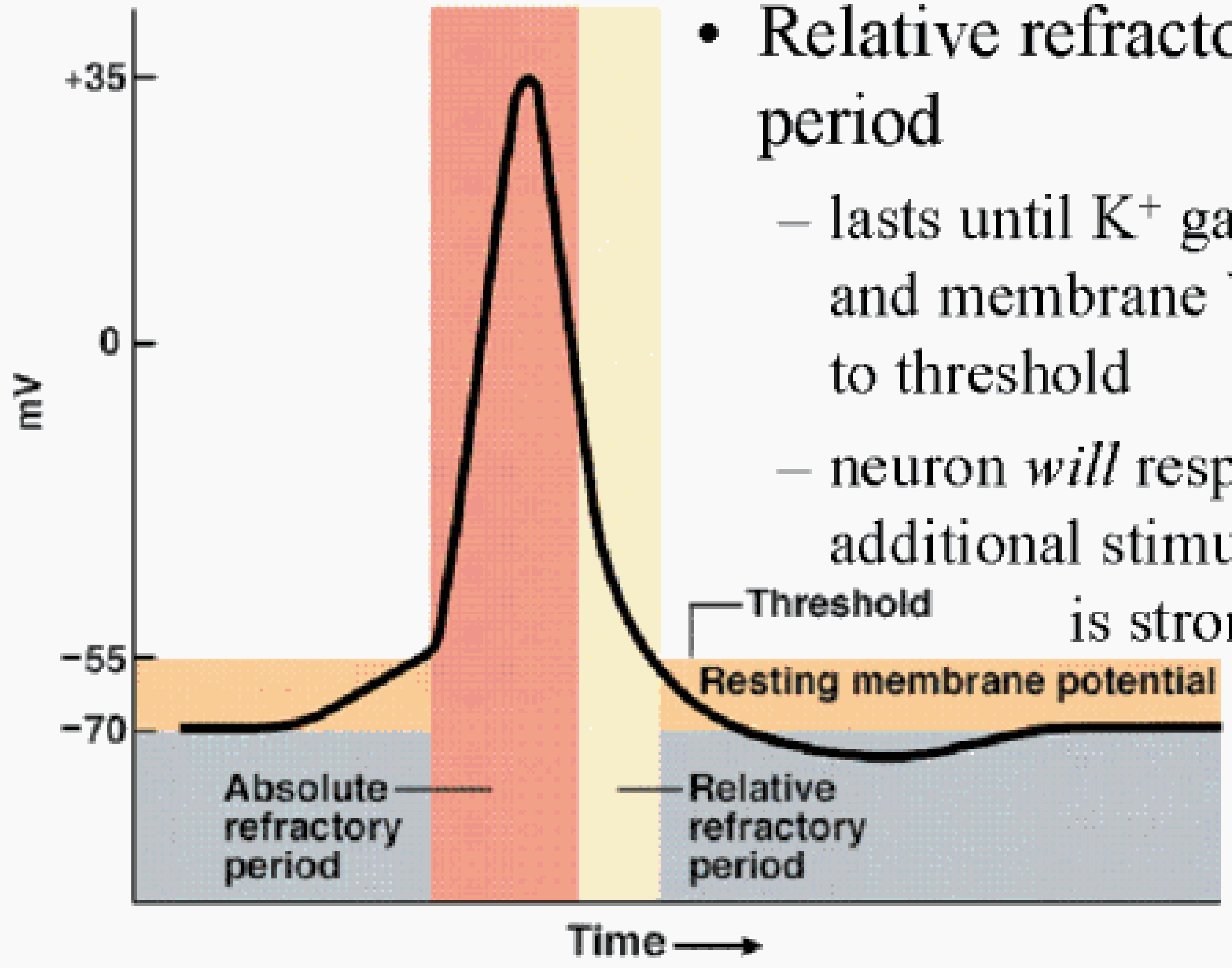
- Bioelectric properties of the membrane
- Velocity and amplitude of membrane depolarization

Refractory periods:

Refractory periods:



Refractory periods:



- Relative refractory period
  - lasts until K^+ gates close and membrane V. returns to threshold
  - neuron *will* respond to additional stimulation if it is strong enough

Debriefing:

Excitation and Excitability

Excitation and Excitability

- Review: Excitation and Excitable Cell
- Review: Threshold Stimulation and Excitability
- Change of Excitability after the Excitation