



☒ Sheet

☐ Slides

Number

7

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Brief introduction about membrane potential

- ✓ The term membrane potential refers to a separation of opposite charges across the membrane or to a difference in the relative number of cations and anions in the ICF and ECF.
- ✓ There are three factors that affect the resting membrane potential:
 - a- activity of K^+ channels.
 - b- activity of Na^+ channels.
 - c- Na^+ / K^+ pump.
- ✓ At resting, the membrane potential is closer to the equilibrium potential for K^+ ,why?

Because the membrane at rest is 25 to 30 times more permeable to K^+ than to Na^+ (the membrane has many more leak channels always open for passive K^+ traffic than channels open for passive Na^+ traffic). So, K^+ passes through more easily than Na^+ . Thus, K^+ influences the resting membrane potential to a much greater extent than Na^+ does.

- ❖ Effect of the Movement of K^+ across the membrane & Na^+ on Membrane Potential
 - what would happen if more K^+ channels / Na^+ channels are activated?
 - K^+ ions would flow from inside to outside (higher to lower concentration), carrying their positive charge with them, so more positive charges would be on the outside. (more negative potential).
 - Na^+ ions would flow from outside to inside (higher to lower concentration). So, more positive charges would be inside (less negative potential).

conductance of plasma membrane (Ohm's law):

Ohm's law is a formula used to calculate the relationship between voltage, current and resistance in an electrical circuit. Since the plasma membrane has electrical properties, we can measure the current across the membrane using ohm's law $I = \Delta V / R$

Where: I : current ΔV : potential at the membrane R : resistance of that membrane

So, if we took the relationship between R and G (which is the conductance) We would find that G is equal to 1 over R ($G = 1/R$).

And if we replaced $1/R$ in the first equation with G , then current would be equal to:

$$G \cdot \Delta V$$

✓ Ohm's law is very similar to Fick's law ($J = P \Delta C \cdot S$)

what are the similarities between them?

- J represents the rate of diffusion and I represents the rate of diffusion for ions (current)
- G (conductance) can be replaced with P (permeability)
- Conductance & permeability are almost the same.
- Finally, ΔC & ΔV represents the driving force, which is in Fick's law the concentration, while in Ohm's law is the potential

Calculating the membrane potential:

We can calculate it according to the conductance as well as equilibrium potential values of the membrane potential ions contributing to the membrane potential using the **Goldman-Hodgkin-Katz conductance equation**:

$$V_m = \frac{g_K}{g_{tot}} E_K + \frac{g_{Na}}{g_{tot}} E_{Na} + \frac{g_{Cl}}{g_{tot}} E_{Cl}$$

Where:

V : the voltage for the membrane

G_x : the conductance for the ion x

G_{total} : the conductance for all ions involved

E_x : equilibrium potential for x

- So, according to the equation above, activation of Na^+ channel will increase the current for Na^+ and activation of K^+ channels will increase the current for K^+

patch clamp

- ✓ The patch clamp is an electronic device used mainly to measure ionic currents at specific membrane potential of a cell membrane.
- ✓ The experimenter can either control the voltage across the membrane (resulting currents are recorded) or control the current passing across the membrane (resulting voltage are recorded in the form of action potential).

NOTE

Clamp = fix

So, patch clamp is employed to fix/maintain the membrane potential at a set value, recording the ionic current through individual channels.

✓ After using patch clamp, how can we measure the conductance?

After fixing the **voltage** of the membrane at specific value, we got the **current** records. Therefore, we have to go back to Ohm's law and calculate **G**.

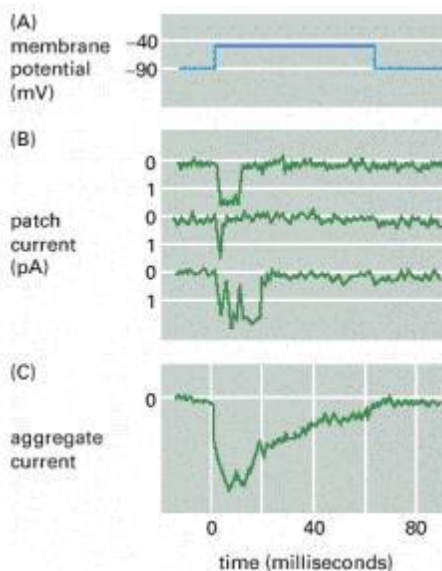
Steps of using patch clamp :

1) Fix the potential at specific voltage (for example, at zero voltage which means at this case we have no potential difference).

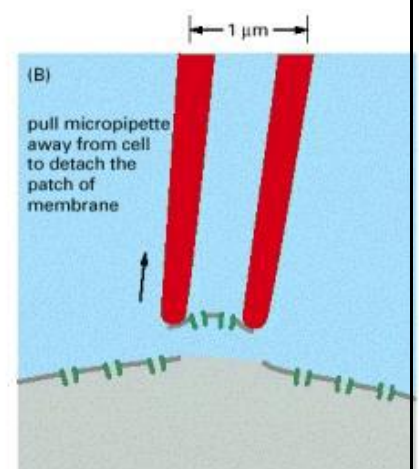
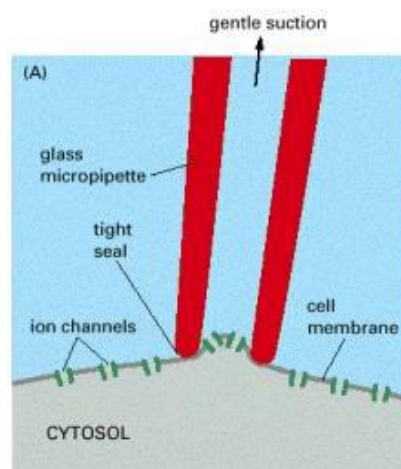
2) Place the membrane into different compartments(how?)

- a) We seal a part of the membrane with a micropipette and either keep it attached to cell membrane or detach it. This sealed part held by the pipette is a solution that is like extracellular fluid.
- b) We then place the micropipette in a free solution similar to intracellular fluid.

3) Measure the current created between intracellular and extracellular parts. Also, we can alter the concentration of the solutions, as well as recording current through individual channels.



- Current records



- Patch still attached to the rest of the cell, as in (A), or detached, as in (B).

NOTE

In patch clamp, if we clamp at +60mV, which is the equilibrium potential for Na⁺, there will not be Na⁺ current at that point, so we can study the other currents like potassium current or Cl⁻.

✓ To conclude, clamping at different voltages (different concentrations of solutions) produces different currents and thus different conductance.

describing changes in membrane potential

1. Polarization:

Charges are separated across the plasma membrane, so the membrane has potential. Any time membrane potential is other than 0 millivolts (mV), in either the positive or the negative direction, the membrane is in a state of polarization.

2. Depolarization:

The membrane becomes less polarized; the inside becomes less negative than at resting potential, with the potential moving closer to 0 mV. (for example, a change from -70 to -60 mV); fewer charges are separated than at resting potential. This term also refers to the inside even becoming positive as it does during an action potential.

- Activating Na^+ channels cause depolarization.

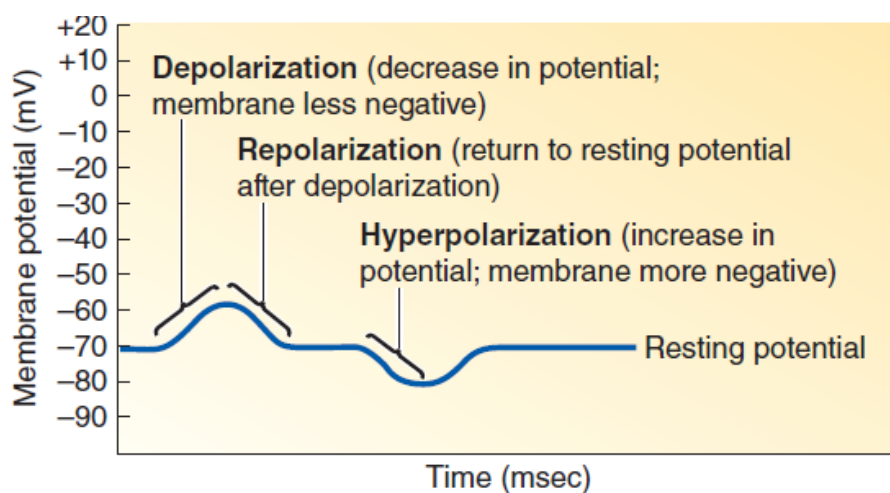
3. Repolarization:

The membrane returns to resting potential after having been depolarized.

4. Hyperpolarization:

The membrane becomes more polarized; the inside becomes more negative than at resting potential, with the potential moving even farther from 0 mV. (for instance, a change from -70 to -80 mV); more charges are separated than at resting potential.

- Activating k^+ channels cause hyperpolarization.



Types of changes in membrane potential.

Electrical signals

- ✓ Changes in membrane potential are brought about by **changes in ion movement** across the membrane (how?).

Changes in ion movement occur when membrane **permeability** changes in response to triggering events, as these triggering events alter membrane permeability and consequently **altering ion flow** across the membrane. So, membrane potential changes.

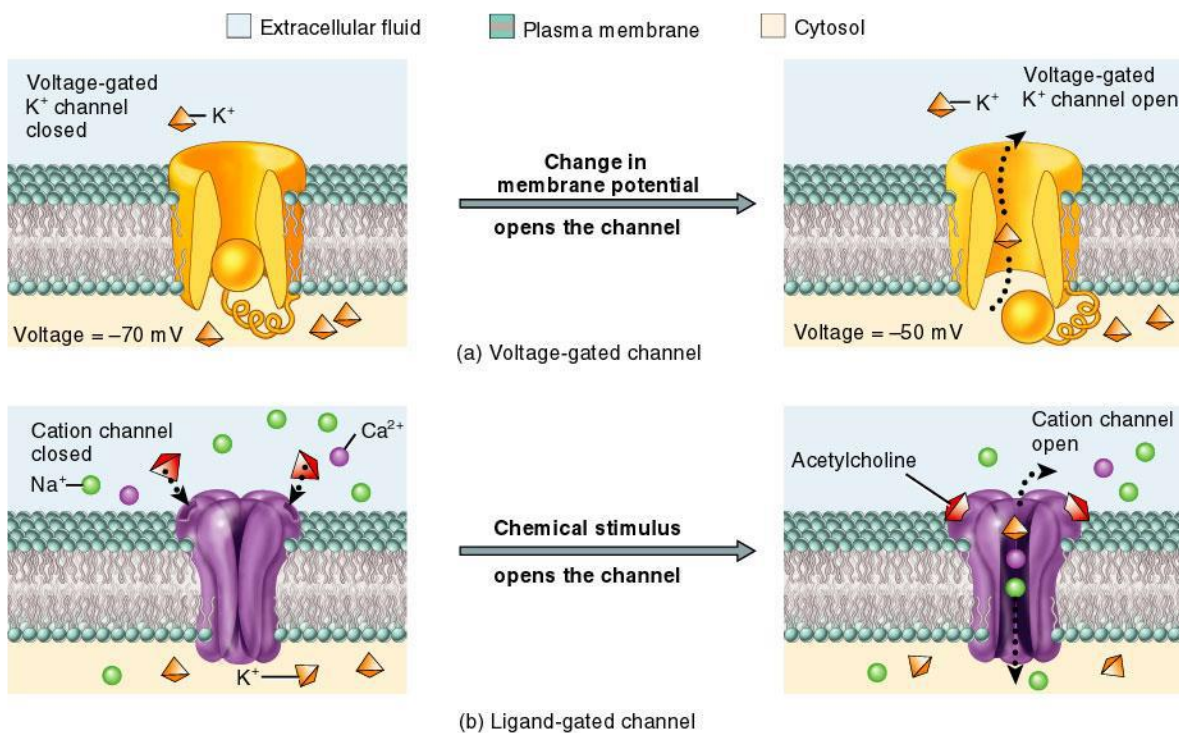
- ✓ Ions move across the membrane through **leakage channels** or **gated channels**. In addition, there are two types of gated channels **depending on the factor** that causes the channels to change their shapes:

a) Voltage gated channels

That open or close in response to changes in membrane potential

b) Chemically gated channels (ligand gated channels)

They change their shapes in response to binding of a specific extracellular chemical messenger (ligand) to a surface membrane receptor.



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- ✓ **Changes in Channels activity result in action potential.**
- ✓ There are two ways of activating these membrane gated channels:
 - By ionic currents that cause depolarization.
 - Chemical way (substances reach the voltage gated channels and activate them)

NOTE

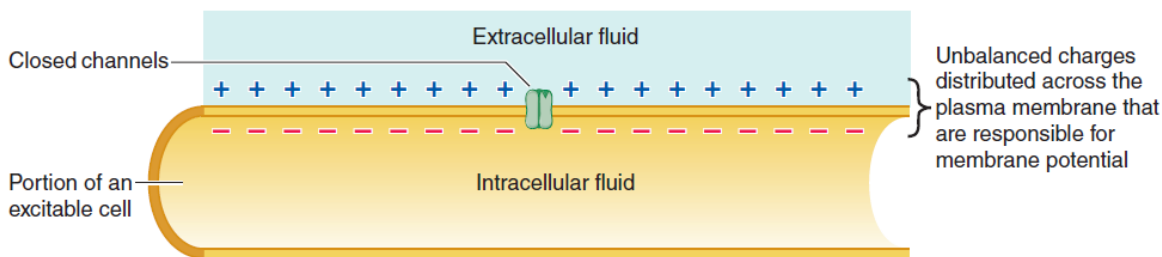
The constant membrane potential present when a cell is electrically at rest, that is, **not** producing electrical signals, is referred to as **the resting membrane potential**

Changing the activity of gated channels by ionic current

- ✓ It is usually produced by a specific triggering event that causes **gated ion channels** to open in a specialized region of the excitable cell membrane. so, ions can move causing the potential and this potential is called Graded Potential.

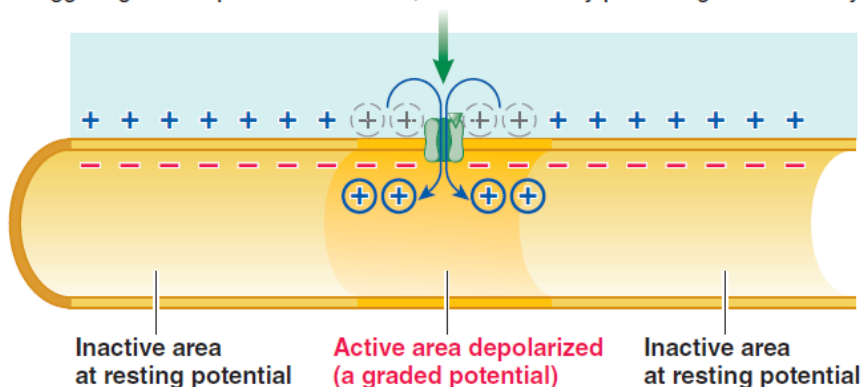
❖ Steps of generating ionic current (graded potential):

- At the beginning, the membrane of an excitable cell will be at **resting potential**.
- A triggering event **opens** ion channels leading to net Na^+ entry that **depolarizes** the membrane at this site (active area), while the adjacent inactive areas are still at **resting potential**.
 - inside the cell, the active area is relatively more **positive** than the neighboring inactive areas that are still at **resting potential**. Outside the cell, the active area is relatively **less positive** than adjacent inactive areas.
- Because of this difference in potential, ions passively flow between the active and the adjacent resting regions on **both** the inside and the outside of the membrane. **And any flow of ions is called current**. so we can say that the current flows between the active and the adjacent inactive areas, resulting in **depolarization** of the previously inactive areas. In this way, the depolarization spreads away from its point of origin.



(a) Entire membrane at resting potential

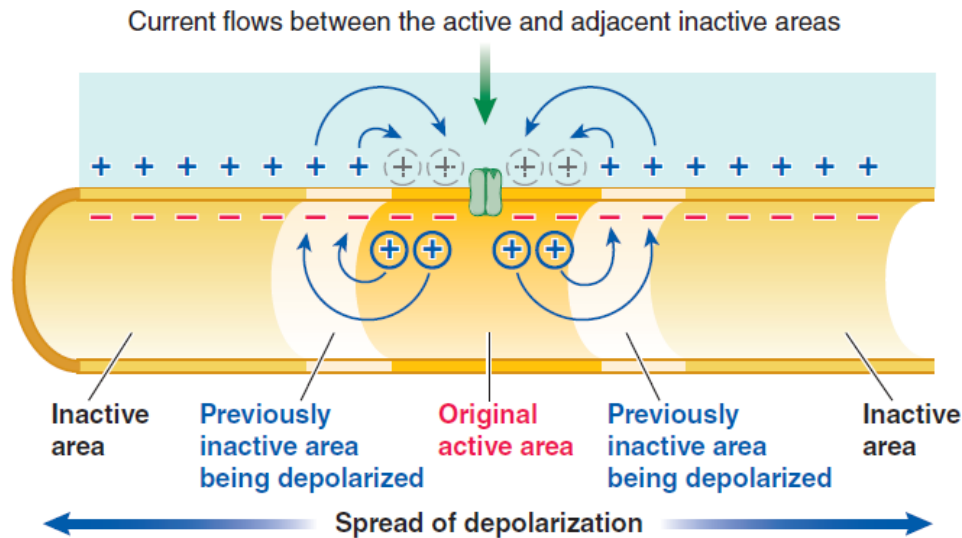
Triggering event opens ion channels, most commonly permitting net Na^+ entry



(b) Inward movement of Na^+ depolarizes membrane, producing a graded potential

NOTE

Remember: current flow is always expressed as the direction in which the positive charges are moving. (+ \rightarrow -)



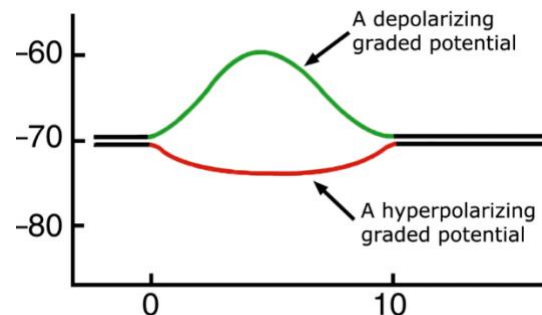
(c) Depolarization spreads by local current flow to adjacent inactive areas, away from point of origin

- If the graded potential is **large** enough, it can initiate **action potentials**, the long-distance signals, in an excitable cell (**graded potentials** die out over short distances).

NOTE

- Excitable cells: cells that can generate action potential by having many voltage gated channels in their membranes. For example, neurons (nerve cells) and muscle cells are excitable cells.
- Epithelial cells are not excitable cells.

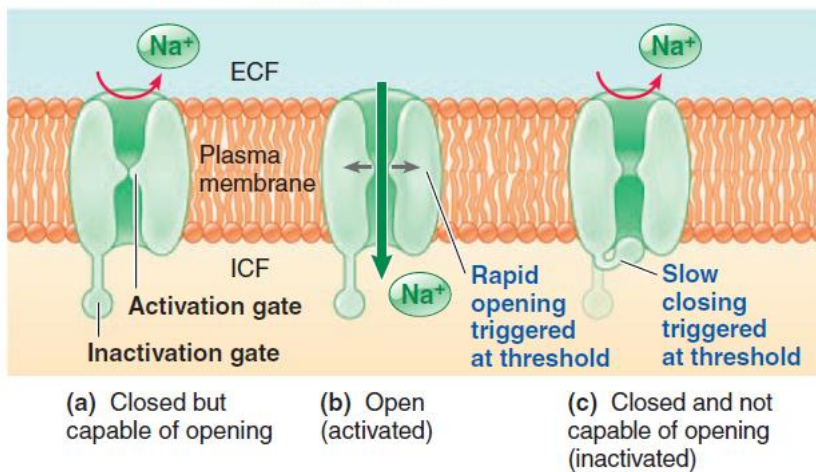
This figure shows the changes in the membrane potential due to a graded potential.



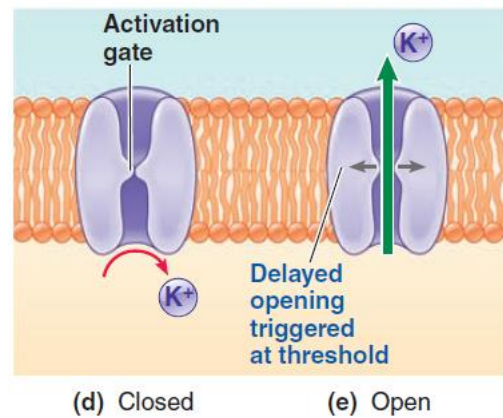
❖ **Conformations of voltage-gated sodium and potassium channels.** (this topic will be discussed in detail in the next sheet, as the doctor didn't mention it in this sheet).

- 1) The voltage-gated K^+ channel has only an activation gate, which can be either closed or opened.
- 2) voltage-gated Na^+ channel can exist in three conformations:
 - a) closed but capable of opening (activation gate is closed; inactivation gate is opened)
 - b) activated, both gates are opened.
 - c) closed and not capable of opening, or inactivated (activation gate open, inactivation gate closed).

VOLTAGE-GATED SODIUM CHANNEL



VOLTAGE-GATED POTASSIUM CHANNEL



Action potential

the phases of action potential:

1) **Resting Phase**: the normal state of membrane potential with no changes in membrane's permeability.

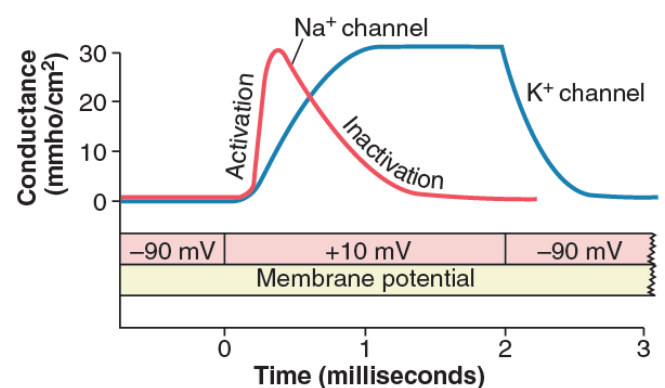
2) **Depolarization**:

a) **Slow depolarization to threshold** (before reaching the threshold) :

When a stimulation occurs, the resting membrane potential is changed by the activation of Na^+ chemical gated channels and some Na^+ voltage gated channels. The flow of Na^+ to the inside of the cell causes the membrane potential to **be less negative**. This situation proceeds slowly until it reaches a critical level known as threshold potential in which all Na^+ voltage gated channels are activated.

- If the initial triggered depolarization does not reach threshold potential, no action potential takes place. Thus, threshold is a critical all-or-none point. Either the membrane is depolarized to threshold and an action potential takes place, or threshold is **not reached** in response to the depolarizing event **and no action potential occurs**.
- At threshold both Na^+ & K^+ voltage channels open (see the figure).

the figure illustrates that typical changes in conductance of sodium and potassium ion channels when the membrane potential is suddenly increased from the normal resting value of -90 mV to a positive value of $+10 \text{ mV}$ for 2 ms . Also, this figure shows that the sodium channels open (activate) and then close (inactivate) before the end of the 2 ms , whereas the potassium channels only open (activate), and the rate of opening **is much slower** than that of the sodium channels.



b) **Firing** (rapid reversal of membrane potential):

at threshold Na^+ flows into the cell, rapidly eliminating the internal negativity and even making the inside of the cell more positive than the outside in an attempt to drive the membrane potential to the Na^+ equilibrium potential which is +60 mV.

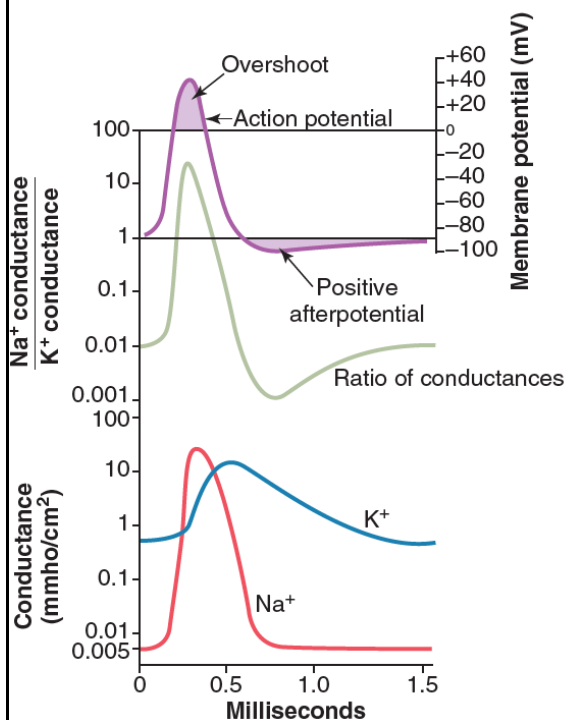
- The potential reaches +30 mV, close to the Na^+ equilibrium potential. The potential **does not** become any more positive, because, at the peak of the action potential, the Na^+ channels start to **close** to the inactivated state and Na^+ permeability starts to **fall** to its low resting value. In addition, the state at which the membrane potential becomes positive is called **overshoot**.

3) Repolarization Phase:

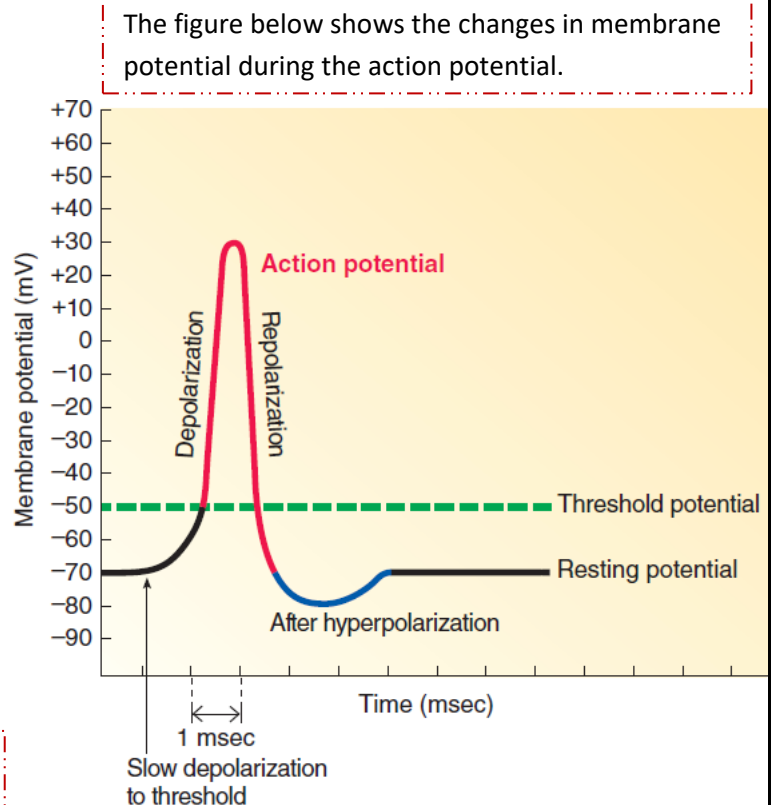
K^+ channels continue opening while Na^+ channels are closing. In addition you can see from the figure in the previous page that all K^+ channels **open** when all Na^+ channels are closed. So, Na^+ **cannot** enter the cell while K^+ channels are completely opened and diffusion of K^+ from inside to outside down its electrochemical gradient is allowed. The outward movement of K^+ rapidly **restores** the negative resting potential

4) After hyperpolarization & returning to resting potential.

the voltage-gated K^+ channels close **slowly**. As a result of this Continuous increased permeability to K^+ , more K^+ may leave than is necessary to bring the potential to resting. This slightly excessive K^+ efflux (flow from inside to outside) makes the interior of the cell transiently even more negative than resting potential, causing the after **hyperpolarization**. When the voltage-gated K^+ channels **all close**, the membrane **returns to resting potential**

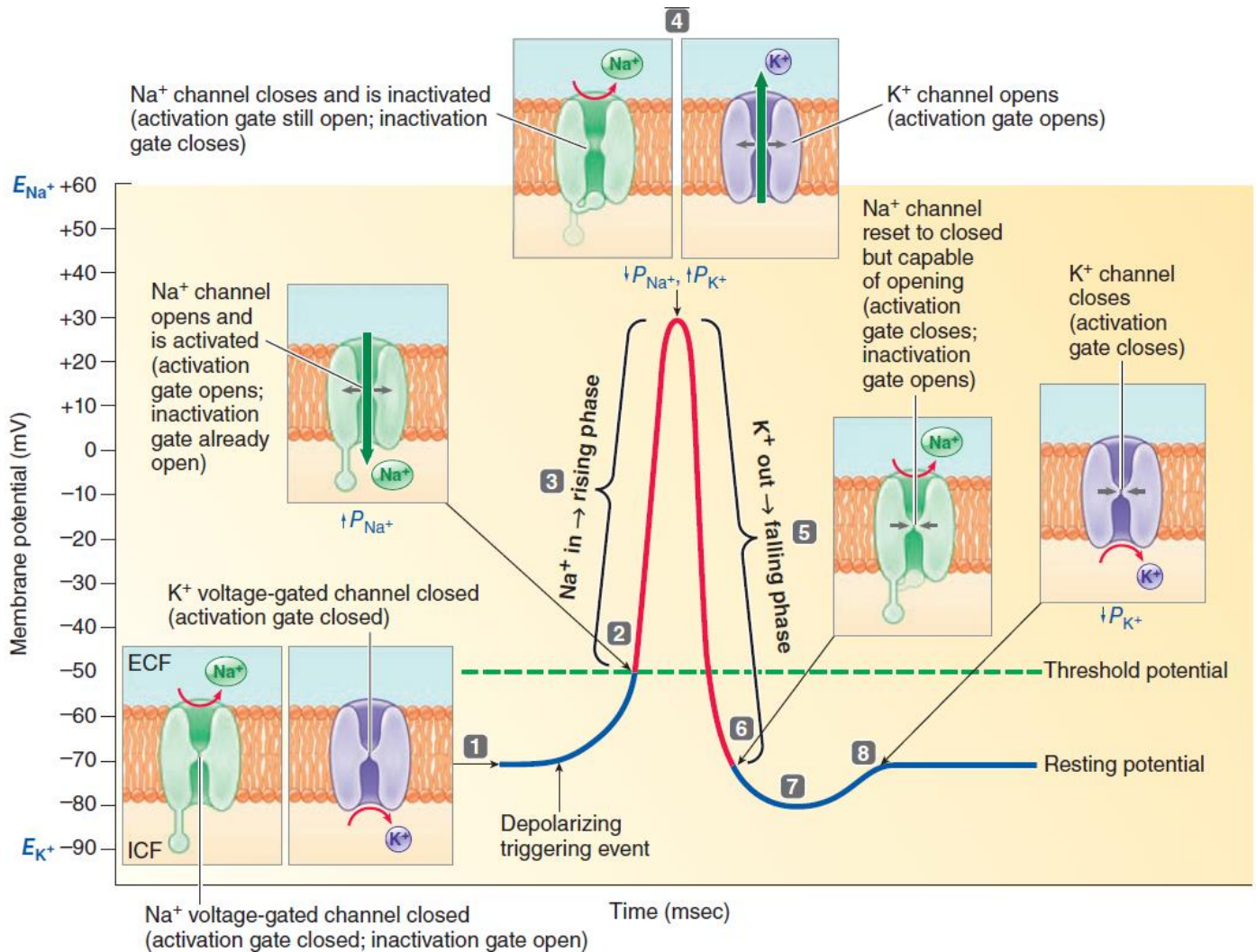


The figure above shows the changes in sodium and potassium conductance during the action potential



The figure below shows the changes in membrane potential during the action potential.

Quick Revision about action potential



- 1 Resting potential: all voltage-gated channels closed.
- 2 At threshold, Na^+ activation gate opens and P_{Na^+} rises.
- 3 Na^+ enters cell, causing explosive depolarization to +30 mV, which generates rising phase of action potential.
- 4 At peak of action potential, Na^+ inactivation gate closes and P_{Na^+} falls, ending net movement of Na^+ into cell. At the same time, K^+ activation gate opens and P_{K^+} rises.
- 5 K^+ leaves cell, causing its repolarization to resting potential, which generates falling phase of action potential.
- 6 On return to resting potential, Na^+ activation gate closes and inactivation gate opens, resetting channel to respond to another depolarizing triggering event.
- 7 Further outward movement of K^+ through still-open K^+ channel briefly hyperpolarizes membrane, which generates after hyperpolarization.
- 8 K^+ activation gate closes, and membrane returns to resting potential.