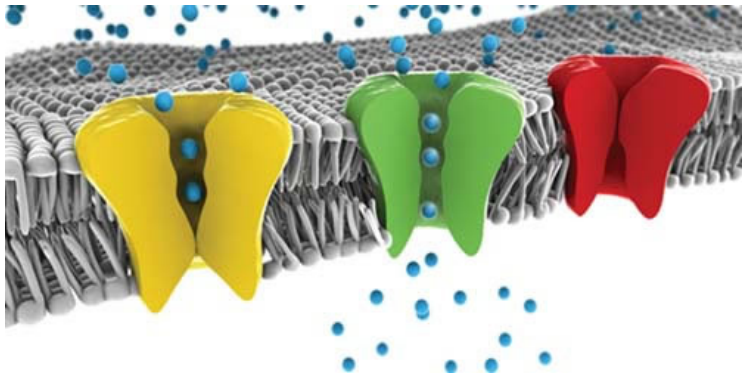
# CELL MEMBRANE, ION CHANNELS, PASSIVE AND ACTIVE PROPERTIES OF THE NEURON



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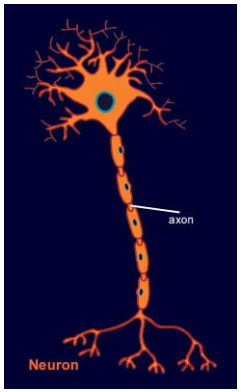
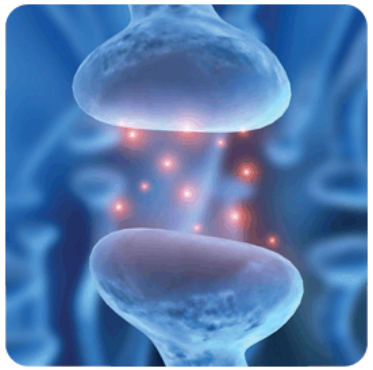
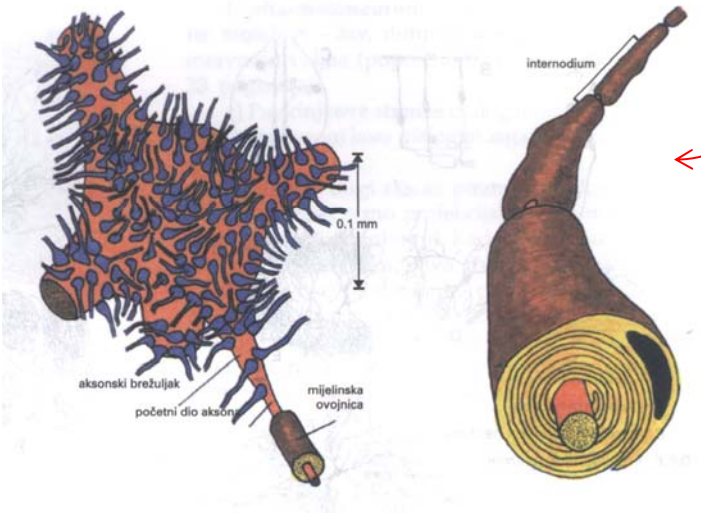
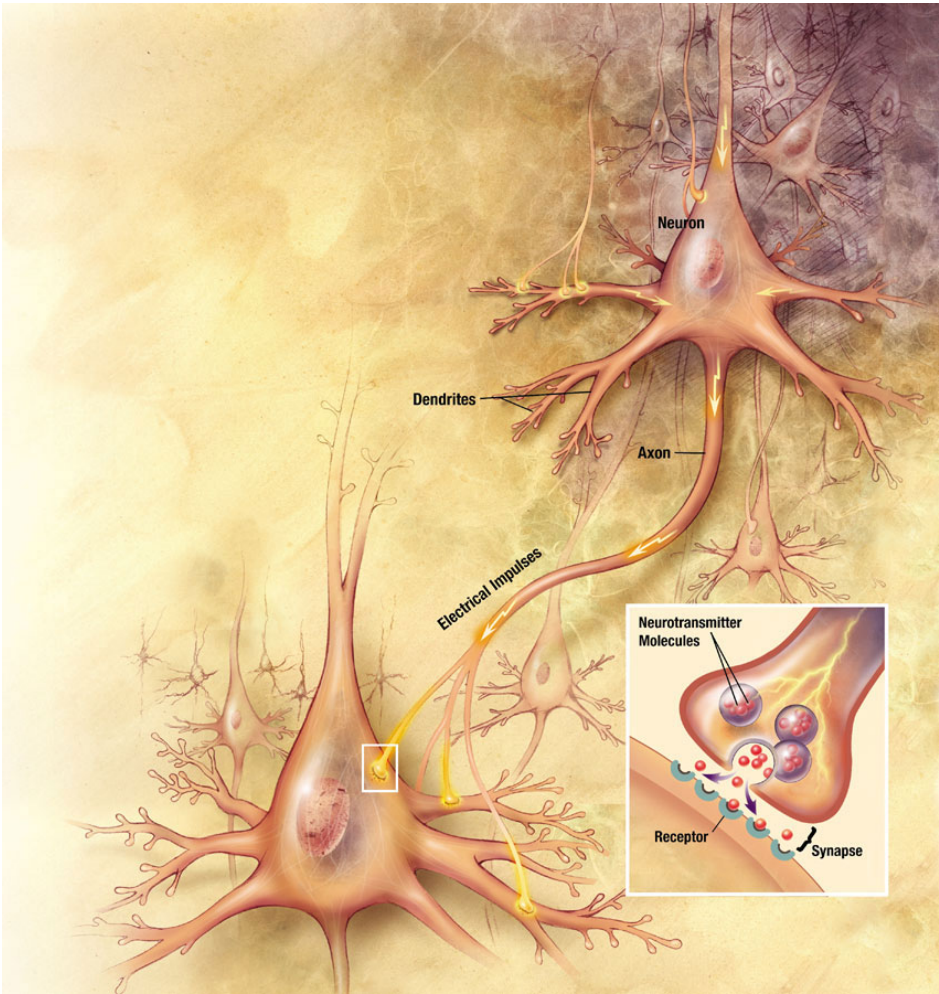


- Literature:
- Siegel and Sapru, Chapter 6
- Kandel, Chapter 6 (part)



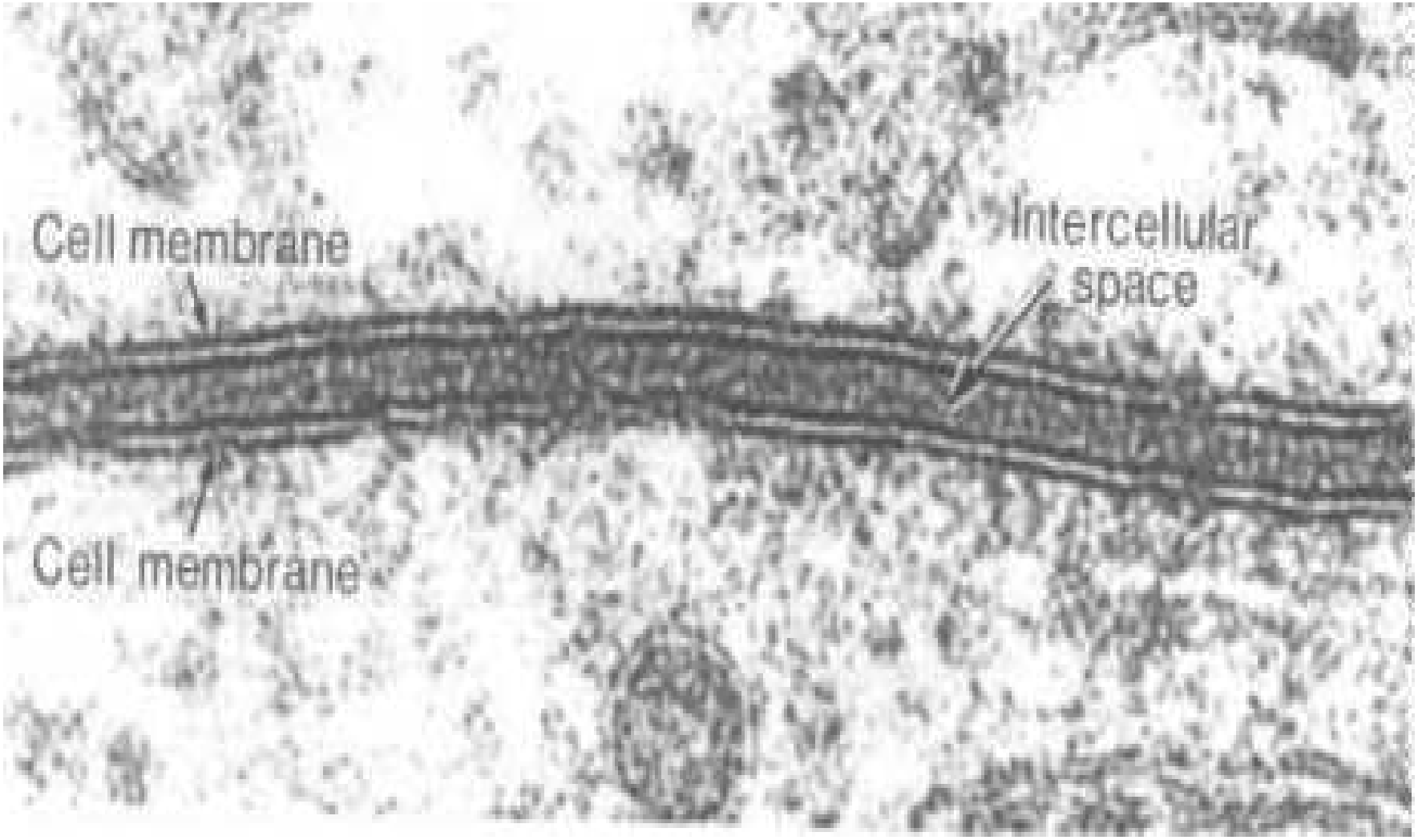
- The most important role of the neuronal membrane is synaptic transmission!

# Synaptic transmission

- Exocytosis of the neurotransmitter
- Receptors
- Ion channel
- Changes in the excitability of the neuron
- Resistance of the cell membrane
- Capacity (Phospholipid  bilayer)
- Conductance (Ion channel)

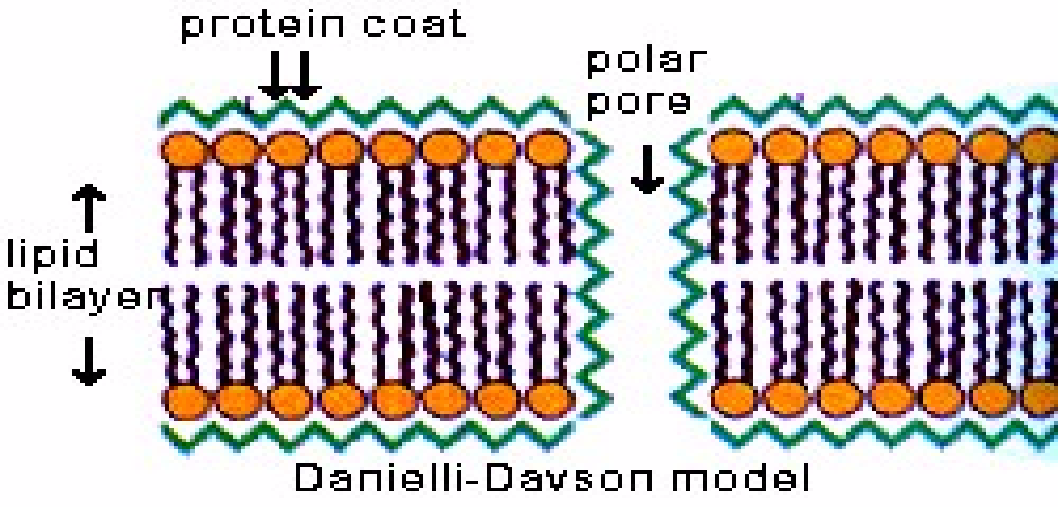
# The role of the cell membrane:

- Protective
- Maintance of the shape
- Regulation of the transport
- Intercellular signalization
- Cellular recognition
- Gap junction – neuronal communication

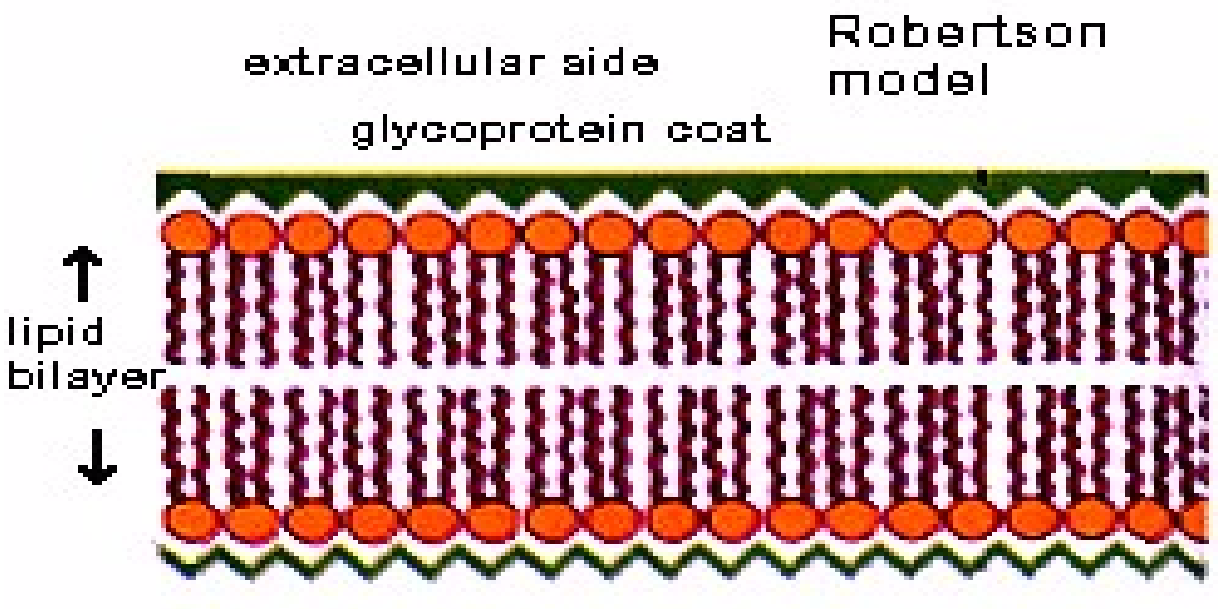

Cell membrane
Intercellular space
Cell membrane

# History:

- 1930-1940; Danielli and  Davson- lipid bilayer



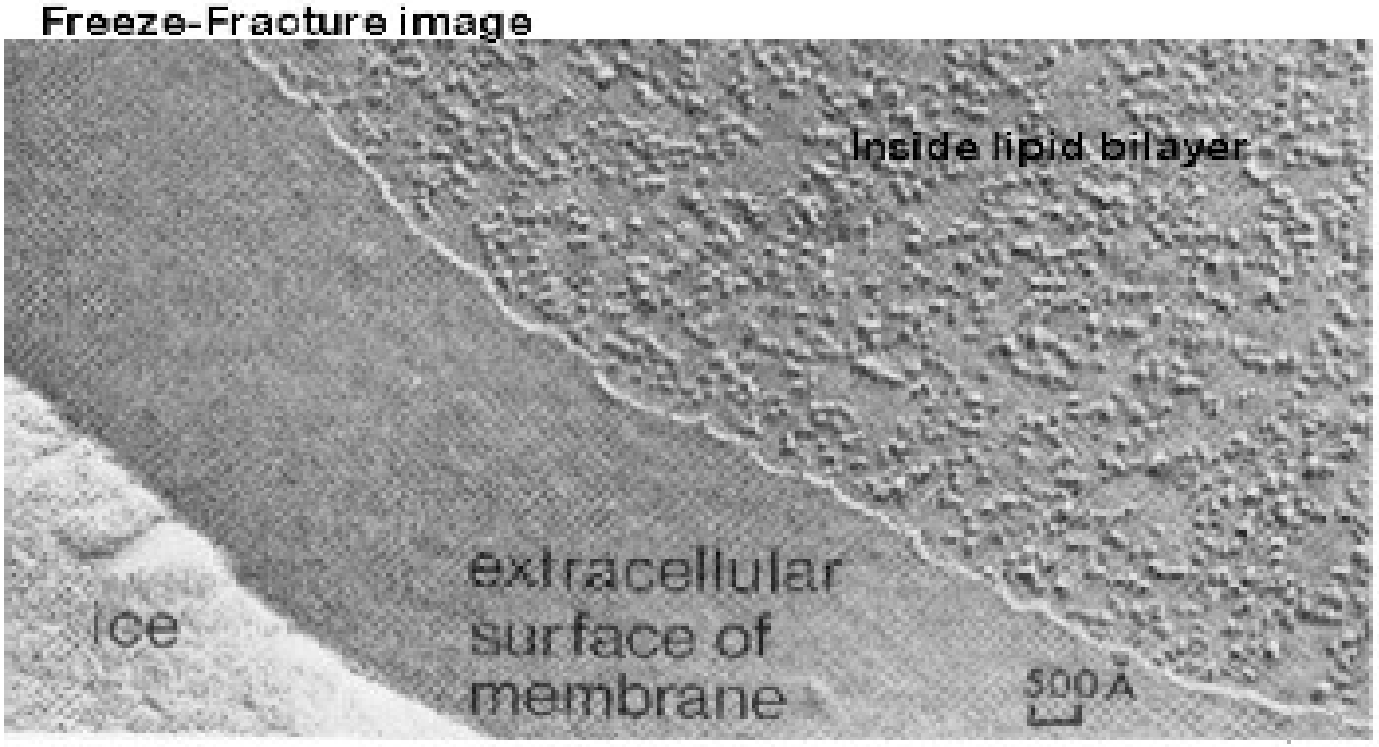
- 1950; Robertson



- 1966; Lenard and Singer

-membran protein, α helix

- freeze fracture

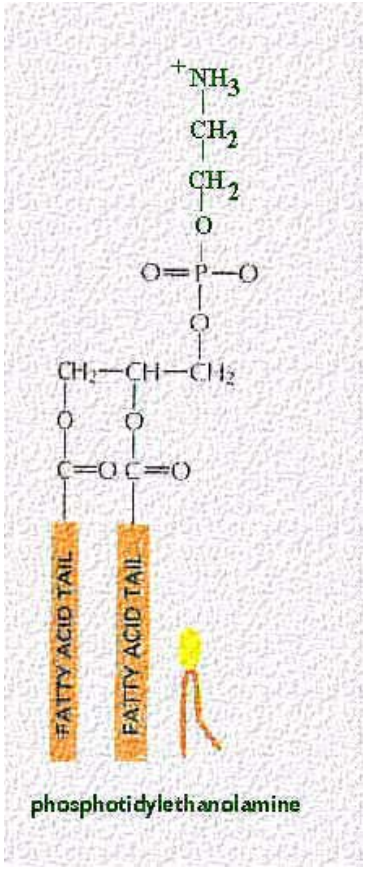


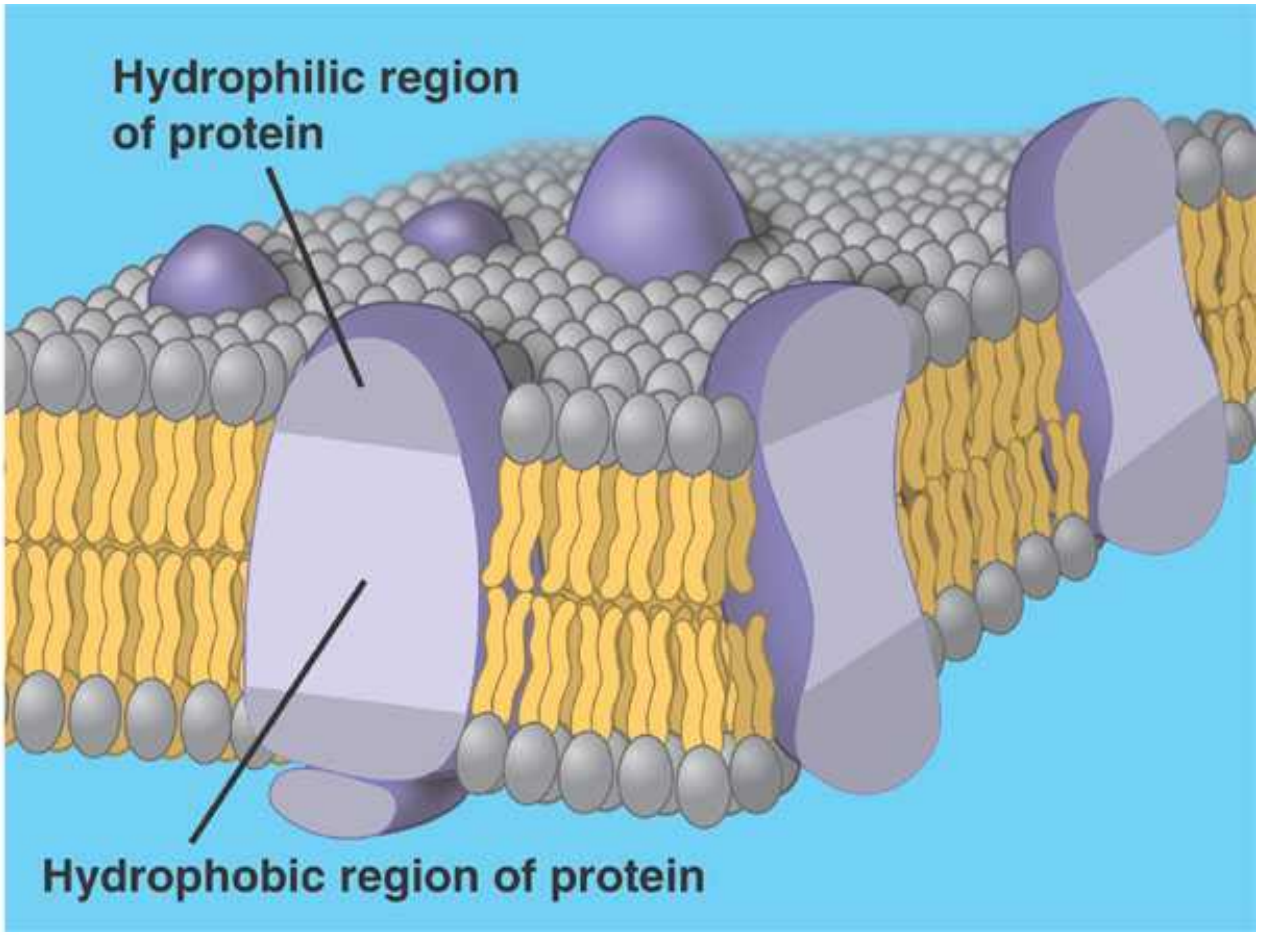
# Structure of the neuronal membrane:

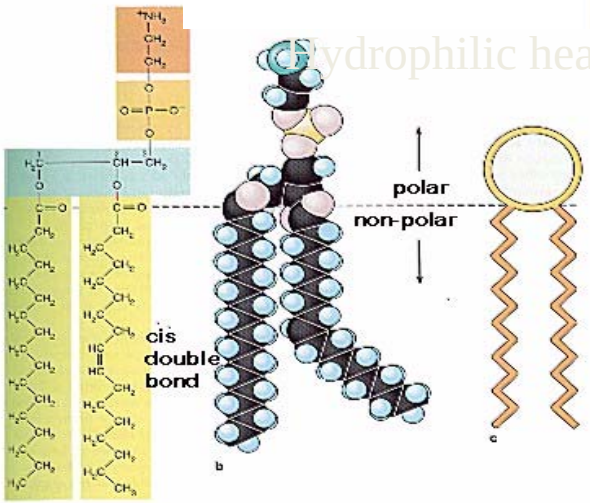
- Lipid bilayer (phospholipids, glycolipids and cholesterol)
- proteins (transmembrane i peripheral)
- glycocalyx (glicolipids, proteoglicans, glicoproteins)

# Phospholipids

- 4 types: phosphatidilcolin, sphingomyelin, phosphatidilserin, phosphatidiletanolamin
- Hydrophilic head, two hydrophobic tails
- Positively or negatively charged
- Inside or outside of the membrane

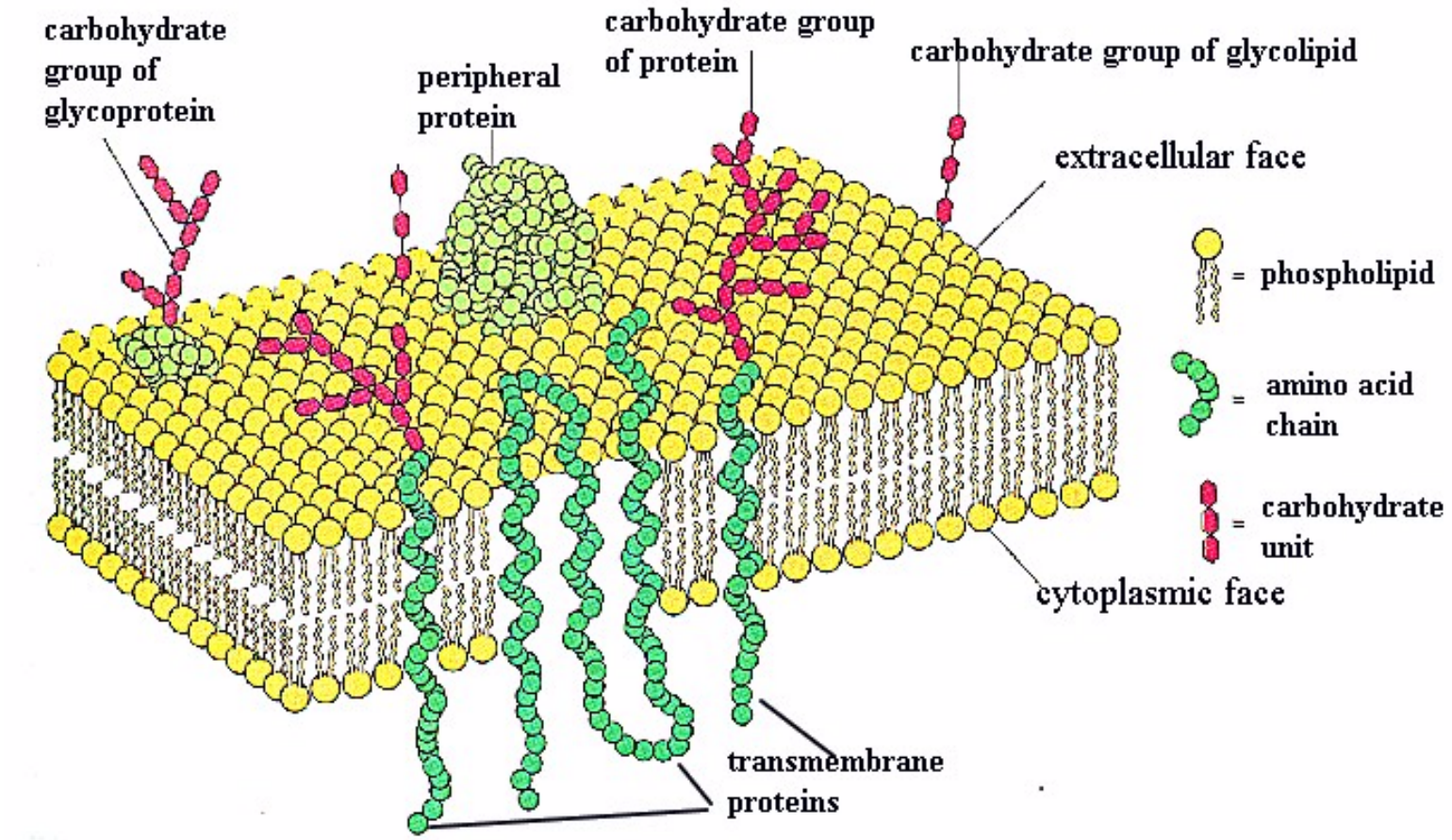



Hydrophilic region of protein
Phospholipid bilayer
Hydrophobic region of protein

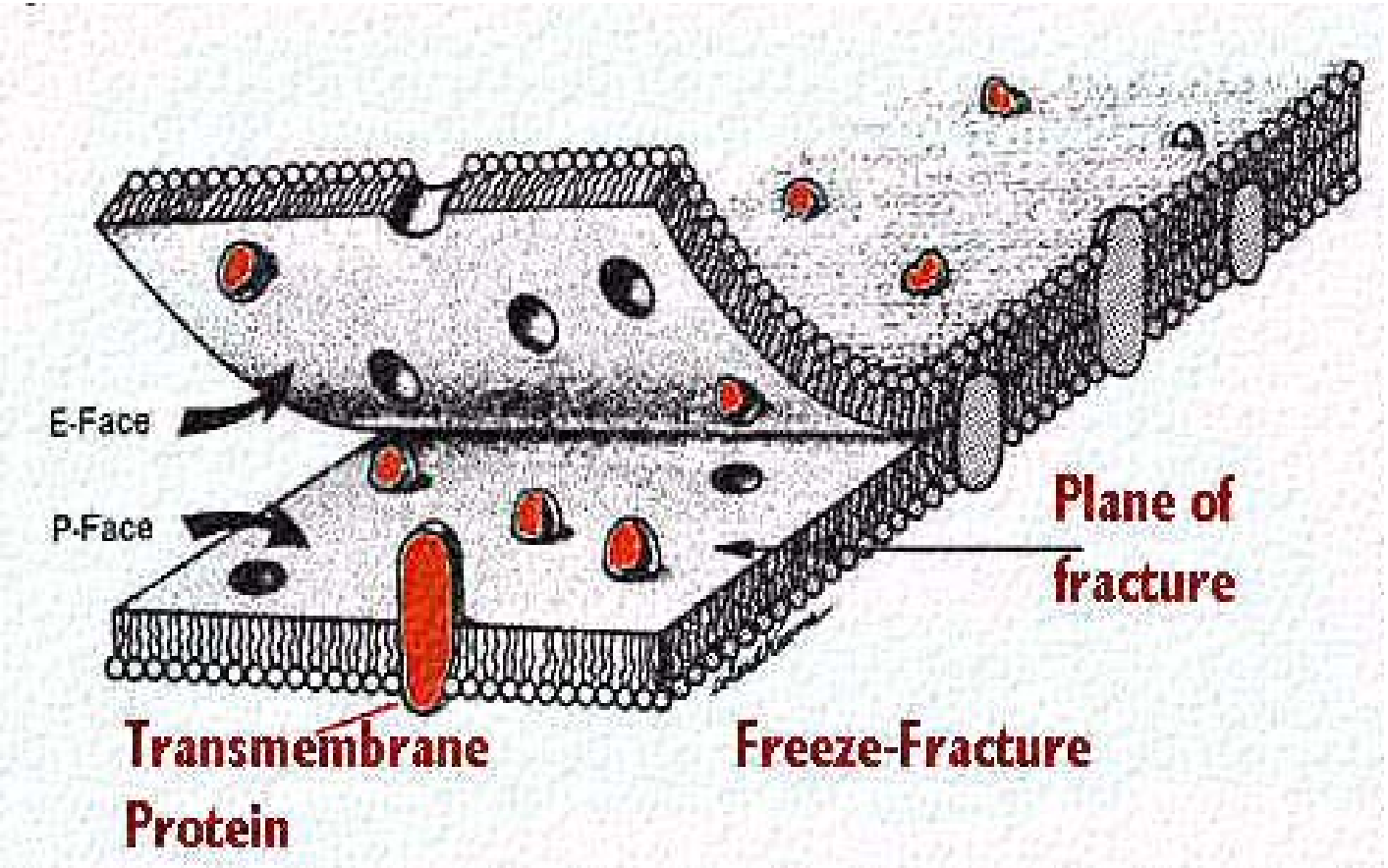

Hydrophilic head
polar
non-polar
cis double bond
b
c

# Proteins:

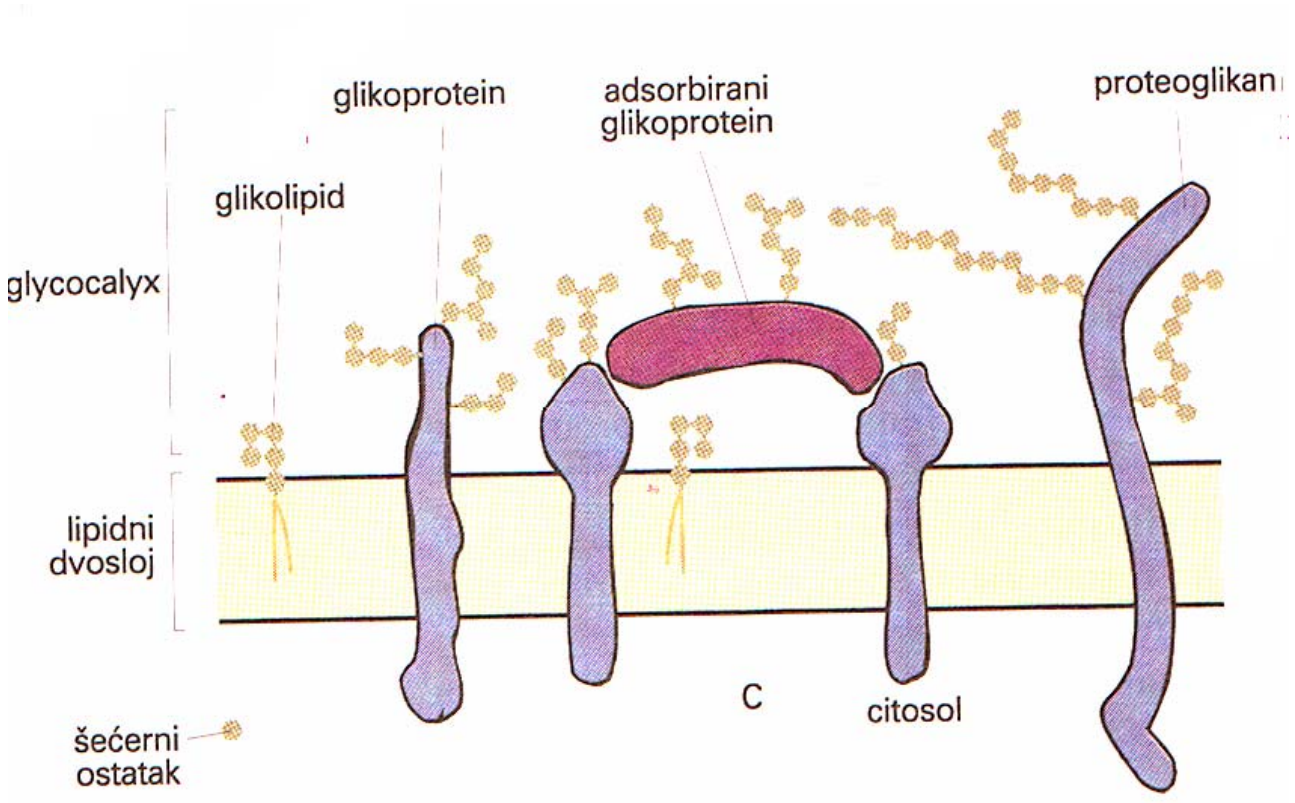
- transmembrane proteins
- peripheral proteins



# Proteins - freeze fracture

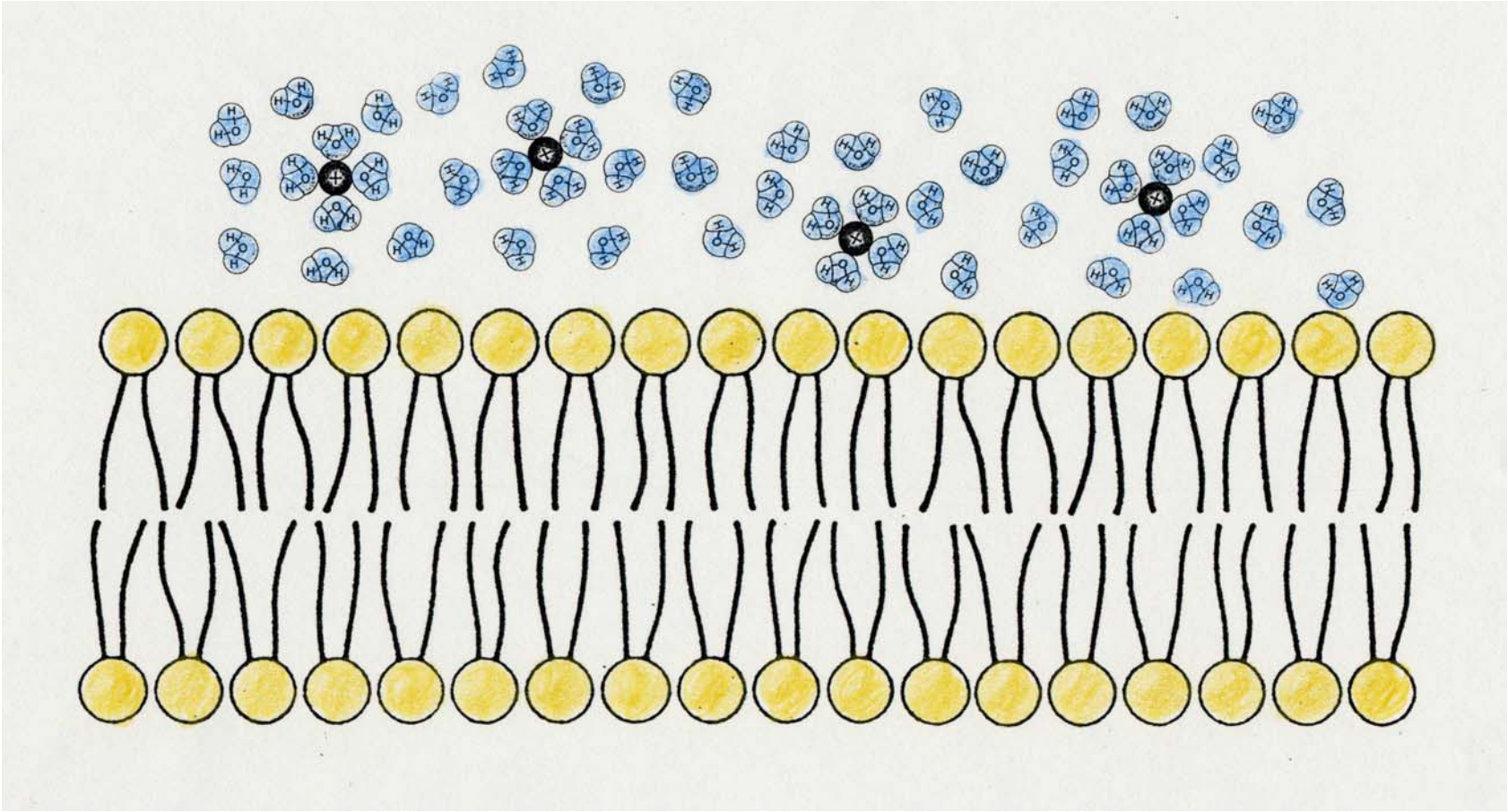


# Glycocalyx-”sugar coat” on the outside of the membrane

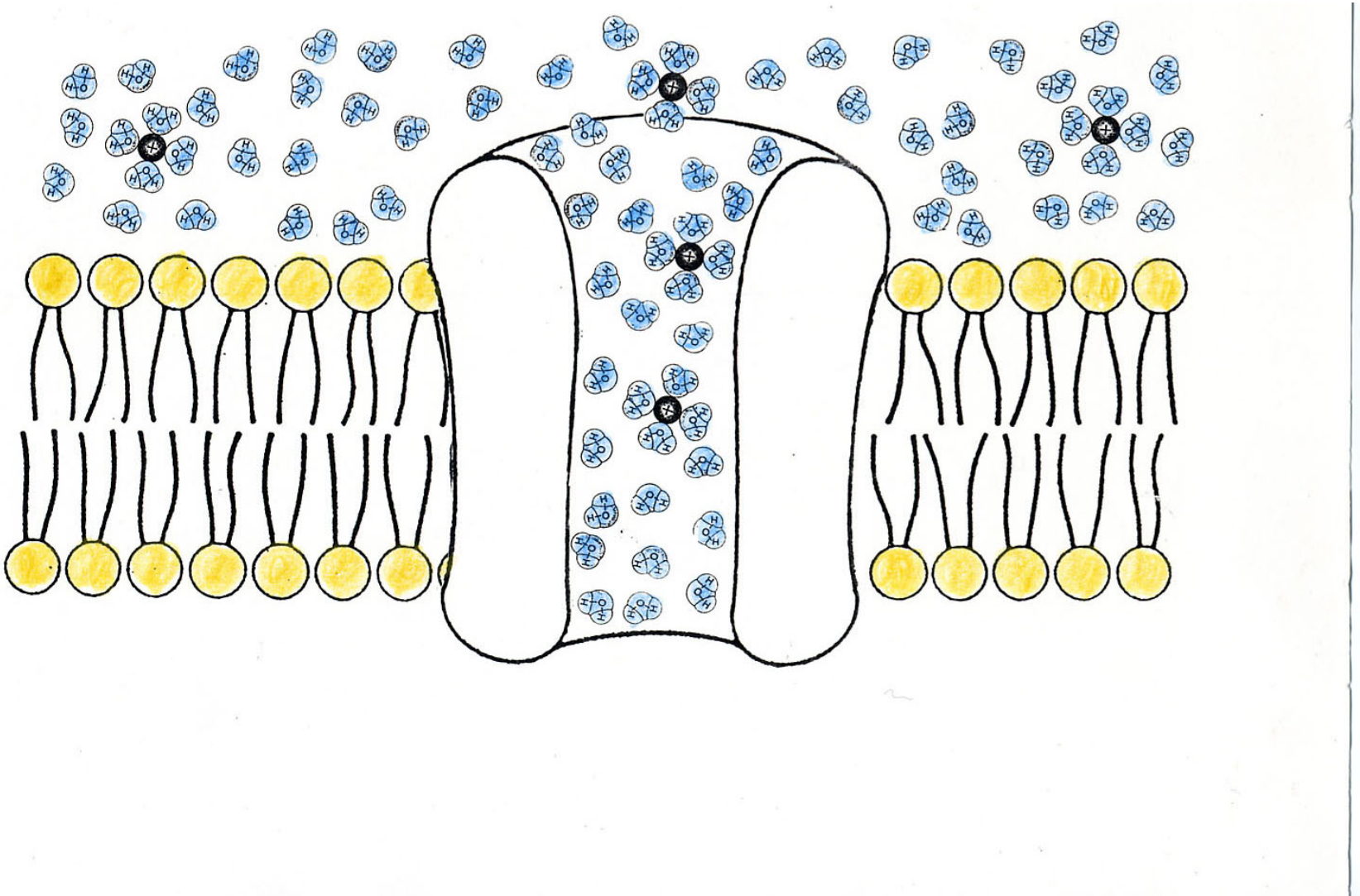



Important role in cellular recognition during development,
and in adhesion of the neurons during migration in fetal development.

# Ions Cannot Diffuse Across the Hydrophobic Barrier of the Lipid Bilayer

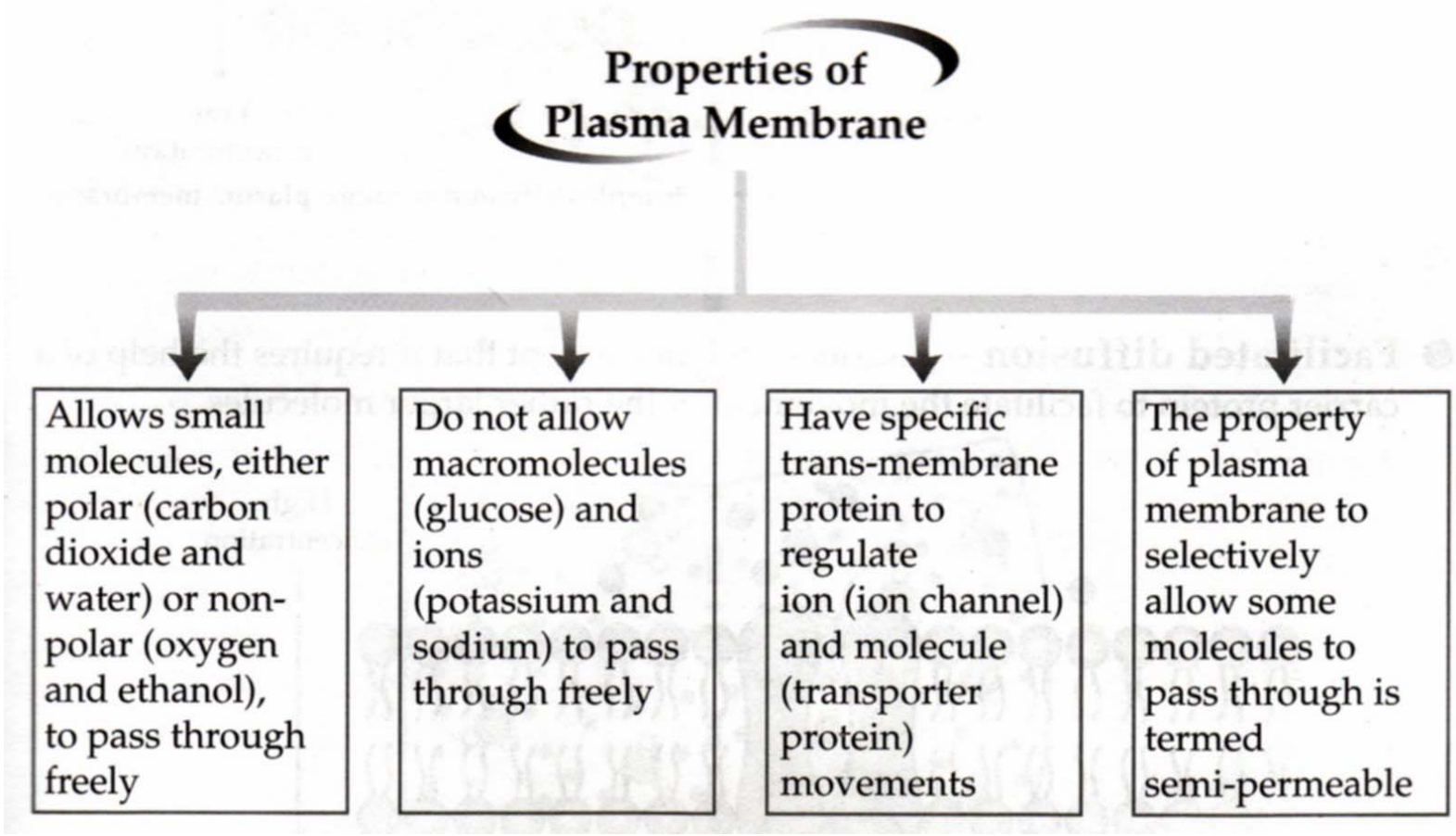


# Ion Channels Provide a Polar Environment for Diffusion of Ions Across the Membrane

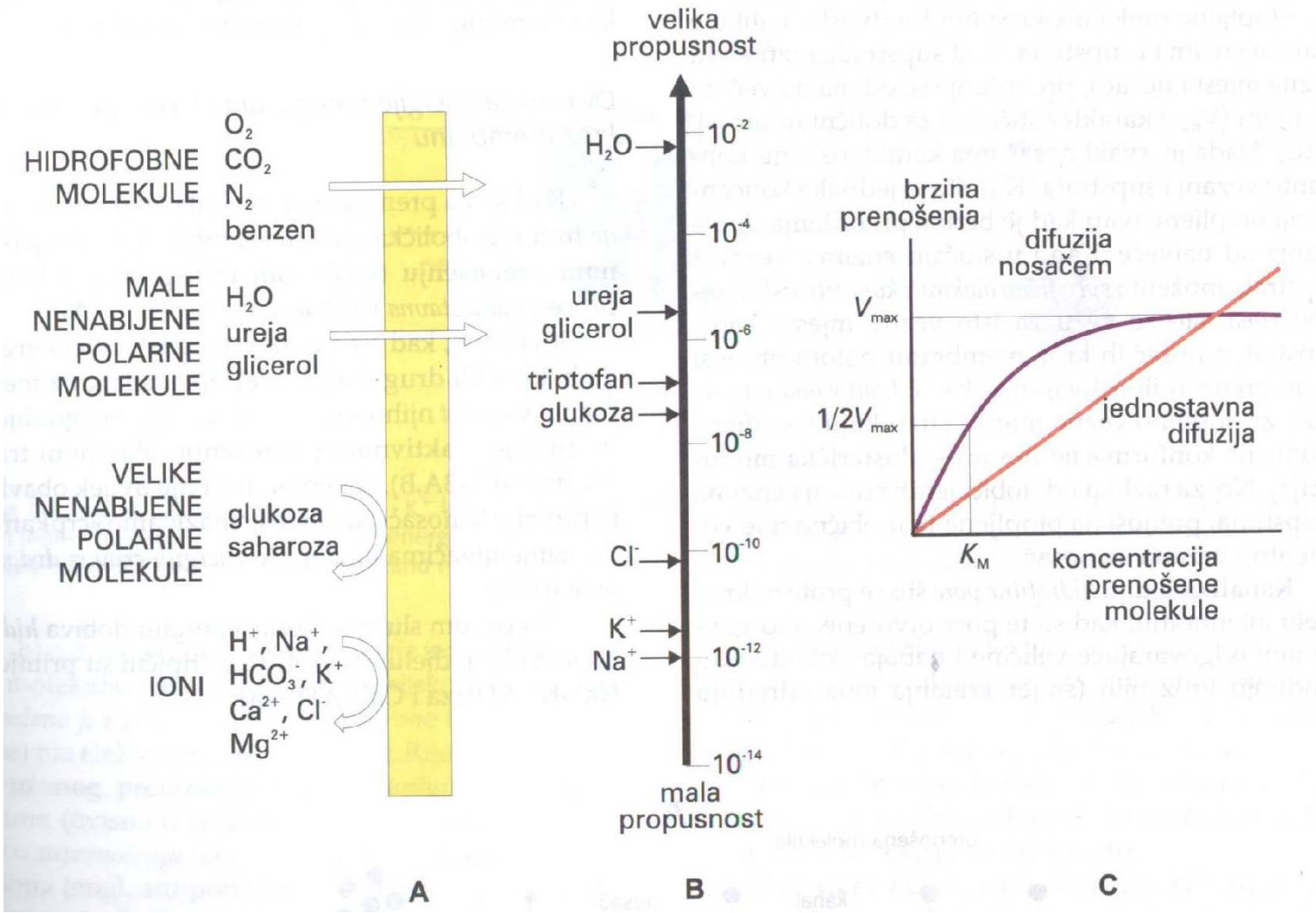


# Selective permeability of the cell membrane

- Enables constant internal milleu
- 1. Macromoleculs (neurotransmitters, neuropeptides, neurohormons) pass through the membrane = Endocytosis and egzocytosis
- 2. simple diffusion: hydrophobic ($O_2$, $CO_2$) and small neutral molecules ($H_2O$)
- 3. glucose, amino-acids, ATP, anorganic ions are transported via carriers and channels


Properties of Plasma Membrane
Allows small molecules, either polar (carbon dioxide and water) or non-polar (oxygen and ethanol), to pass through freely
Do not allow macromolecules (glucose) and ions (potassium and sodium) to pass through freely
Have specific trans-membrane protein to regulate ion (ion channel) and molecule (transporter protein) movements
The property of plasma membrane to selectively allow some molecules to pass through is termed semi-permeable

# Selective permeability of the cell membrane



# A) PASSIVE TRANSPORT

- *diffusion:* ➤down the concentration gradient

***Facilitated Diffusion:*** ➤transport through the protein channels and carriers (ions)

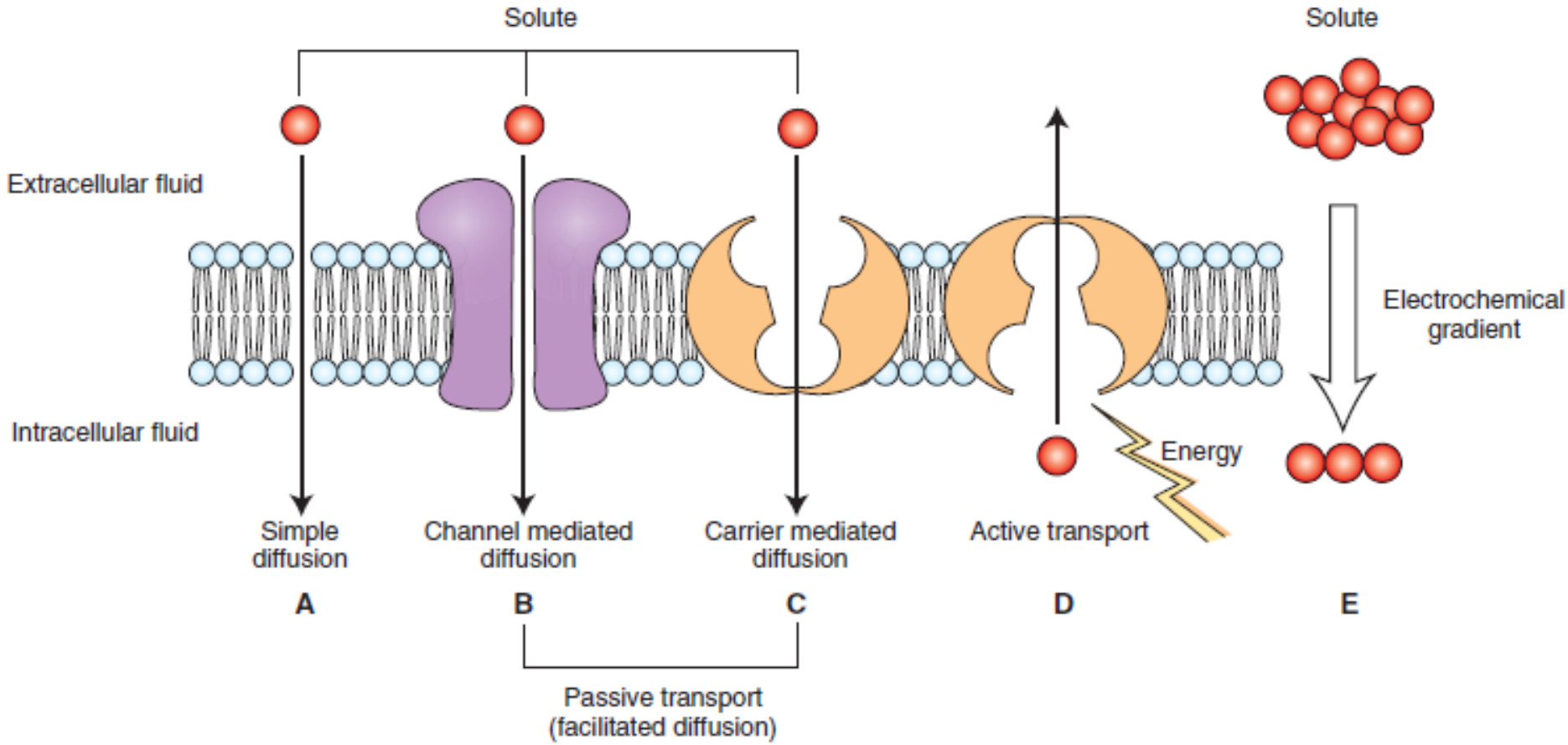
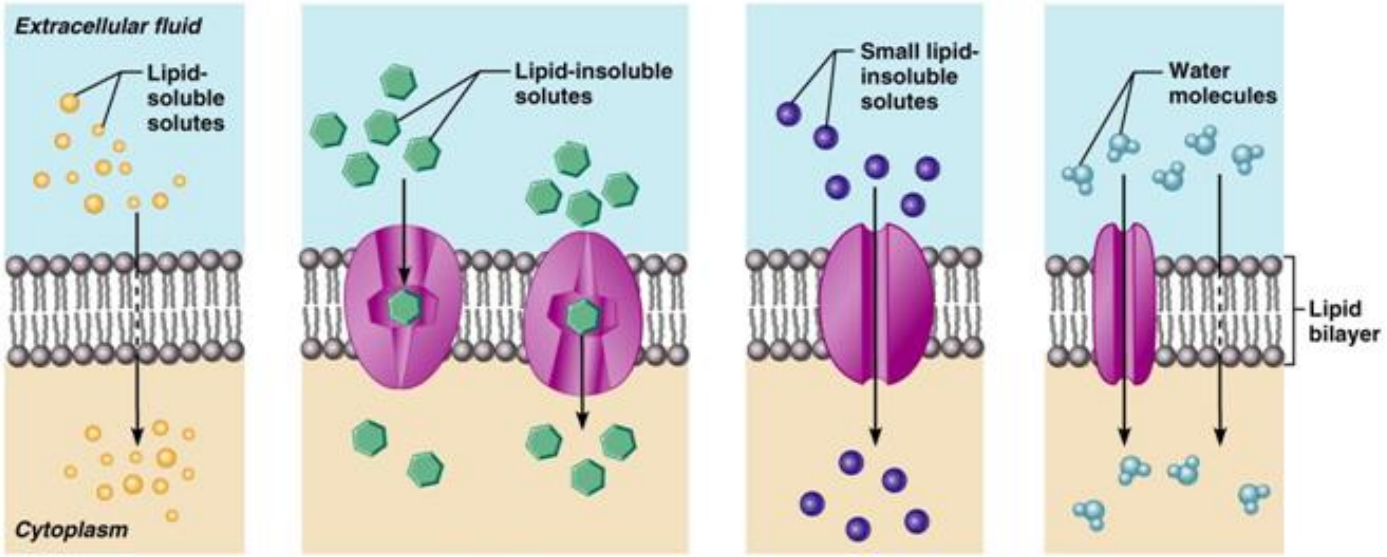



FIGURE 6–2 Transport of solutes across the neuronal membrane. **(A)** Simple diffusion. **(B, C)** Passive transport (facilitated diffusion) occurs either by channel-mediated **(B)** or carrier-mediated **(C)** diffusion. **(D)** Active transport occurs by specific carrier proteins, against the **(E)** electrochemical gradient. Active transport requires coupling of a carrier protein to a source of metabolic energy (e.g., hydrolysis of adenosine triphosphate).

***Facilitated Diffusion:*** ➤transport through the protein channels and carriers (ions)




(a) **Simple diffusion** directly through the phospholipid bilayer

(b) **Carrier-mediated facilitated diffusion** via protein carrier specific for one chemical; binding of substrate causes shape change in transport protein

(c) **Channel-mediated facilitated diffusion** through a channel protein; mostly ions selected on basis of size and charge

(d) **Osmosis**, diffusion through a specific channel protein (aquaporin) or through the lipid bilayer



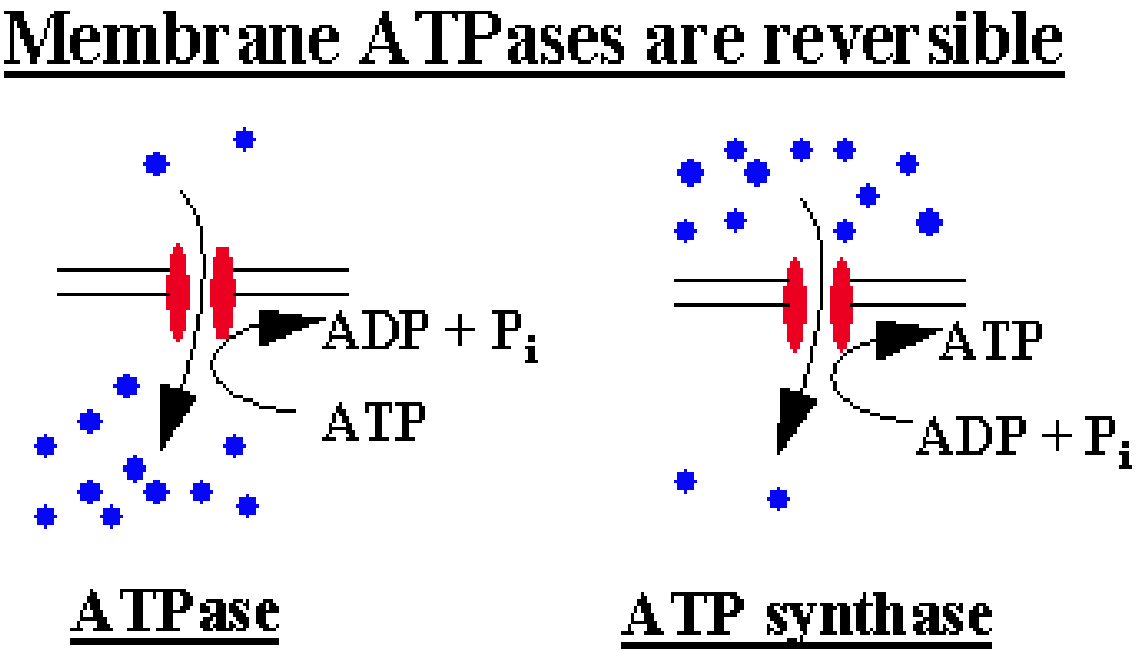
Figure 3.7

# Ion channels

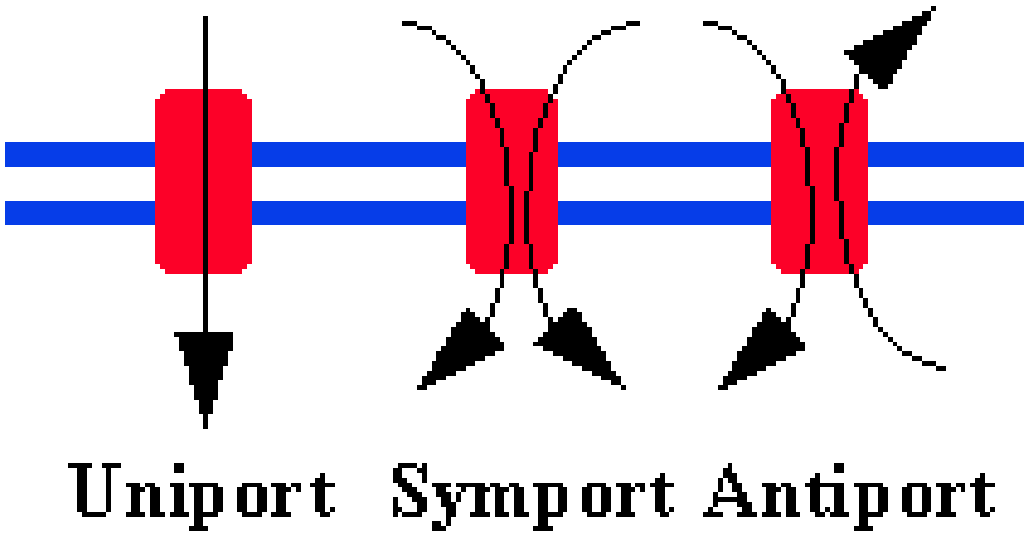
- They form hydrophilic pores through the membrane.
- Transport is faster then via carriers.
- The flow of ions through the channels does not require metabolic energy; the flow is passive.
- The electrochemical driving force across the membrane, but not the channel itself, determines the direction and eventual equilibrium of this flow.

# B) ACTIVE TRANSPORT

- *primar active transport:* ➤ usage of energy (usually hydrolise of ATP)



- coupled transport



# Ion ballance

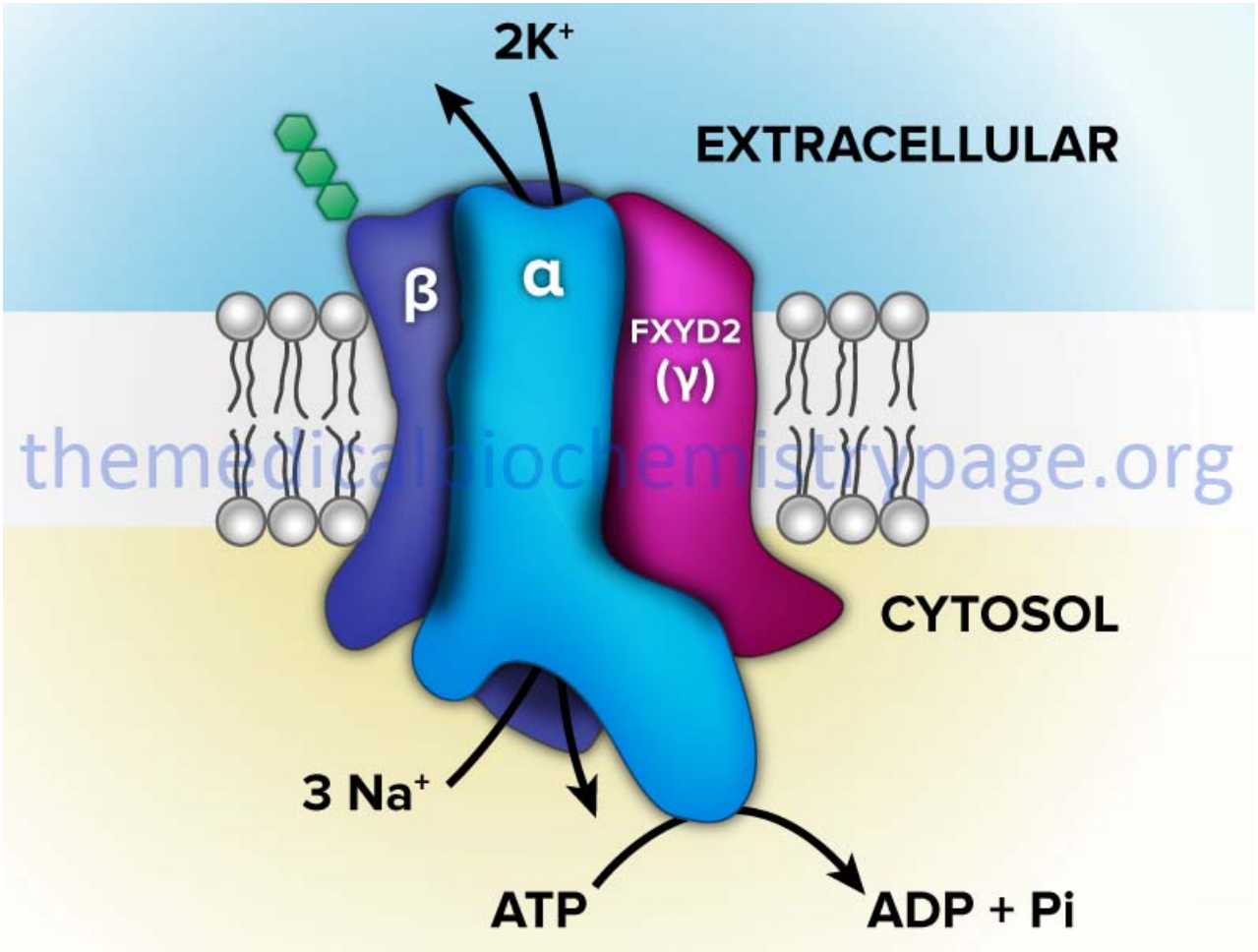
- $Na^{+}$
- $K^{+}$
- $Ca^{2+}$
- $Cl^{-}$

**TABLE 6–1** Approximate Neuronal Intracellular and Extracellular Concentrations of Some Important Ions

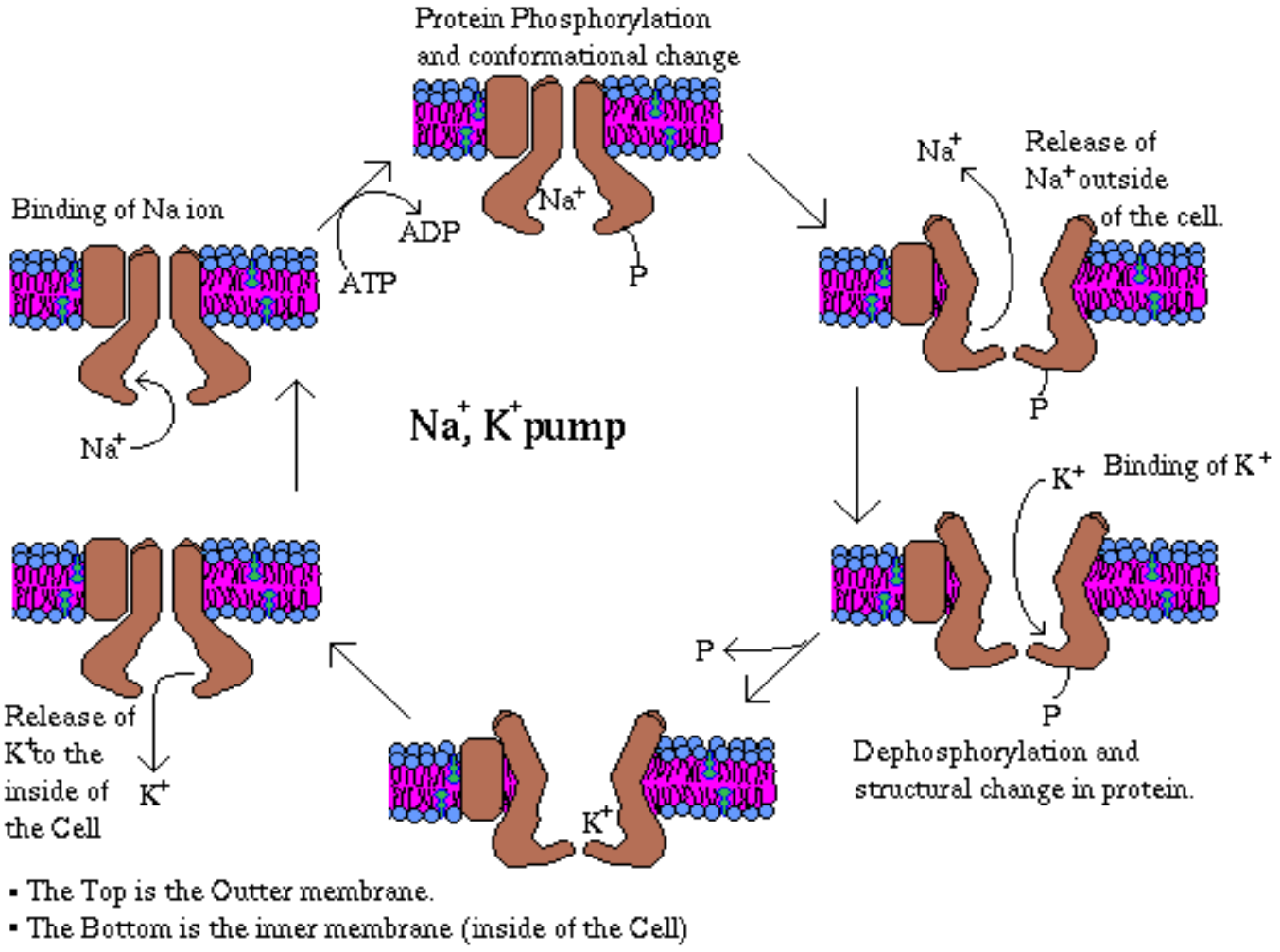
| Ion | Extracellular Concentration (mM) | Intracellular Concentration (mM) |
|---|---|---|
| *Cations* | | |
| $Na^+$ | 150 | 15 |
| $K^+$ | 5 | 100 |
| $Ca^{2+}$ | 2 | 0.0002 |
| *Anions* | | |
| $Cl^-$ | 150 | 13 |
| $A^-$ (fixed anions; organic acids and proteins) | — | 385 |

mM = millimolar concentration.

# $Na^+$-$K^+$ ATPase


2K+
EXTRACELLULAR
β
α
FXYD2
(γ)
themedicalbiochemistrypage.org
CYTOSOL
3 Na+
ATP
ADP + Pi

# ➤$Na^+$-$K^+$ ATPase




CATALITIC SUBUNIT:
- bigger
- more transmembrane domains
- Na & ATP bind on the citosol side
- K binds on the outer side

GLIKOZILITED SUBUNIT:
- smaller
- one transmembrane domain

# $Na^+$-$K^+$ ATP-ase

- Works on the principle of antiport
- ATP is allways hydrolised
- $Na^+$ & ATP have to be in the cell, and $K^+$ outside of the cell
- *ouabain* inhibits Na-K ATP-ase binding itself on the $K^+$ binding spot
- One molecule of ATP: 3 $Na^+$ get out, and 2 $K^+$ get in.

# $Na^+$-$K^+$ ATP-ase

- Contributes to the negative potential inside the neuron
- Contributes to the osmotic balance
- Stabilizes cell volume

# Concentracion of $Ca^{2+}$

- $Ca^{2+}$ inside the cell $10^{-7}$ M
- $Ca^{2+}$ outside the cell $10^{-3}$ M
- concentracion gradient towards inside
- Role of:
- $Ca^{2+}$ ATP-ase
- $Ca^{2+}$ - $Na^{+}$ transporter

# Maintanence of pH value

- Inside the neuron pH=7,2
- $Na^+$ - $H^+$ pump (gets $H^+$ out the cell)
- $Cl^-$ - $HCO_3^-$ pump (HCl gets out, $NaHCO_3$ gets in)

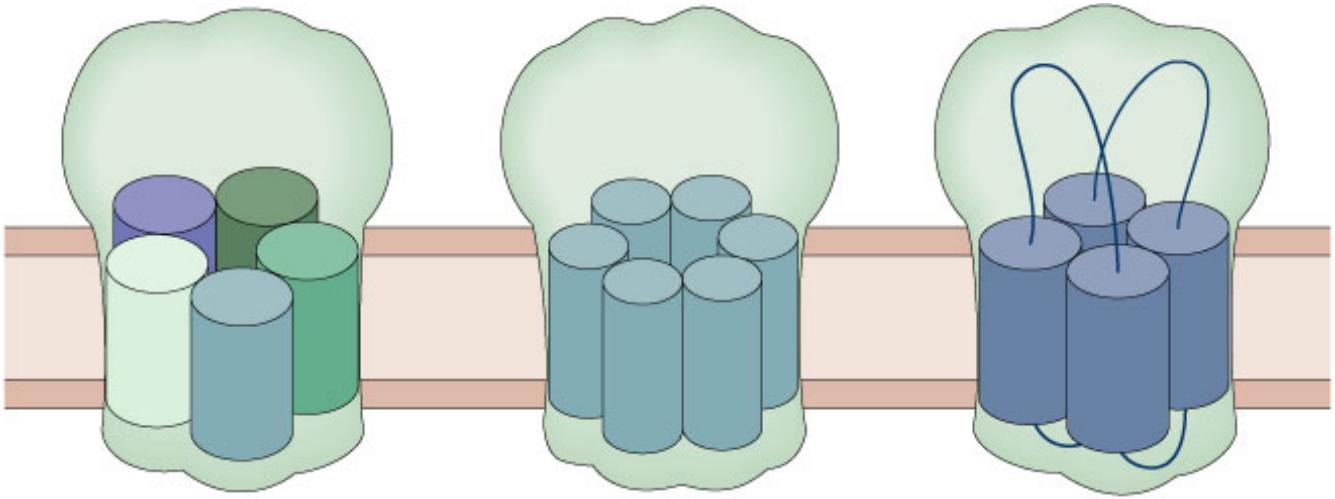
# Ion channels:

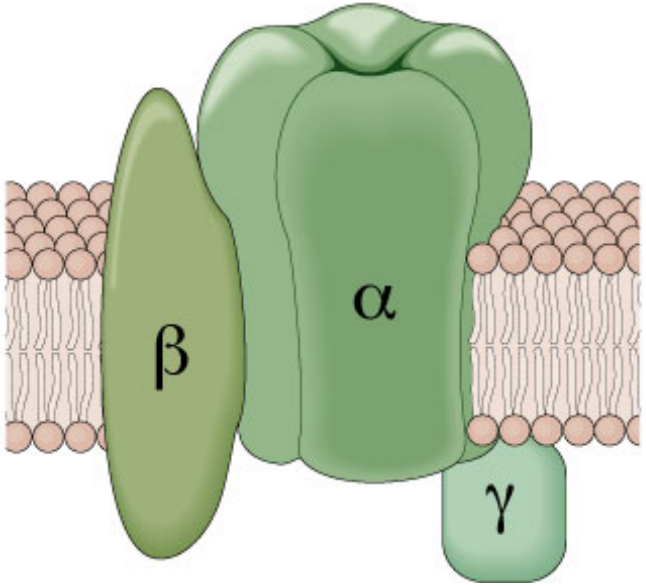
- Transmembrane protiens, hidrophilic protein pores
- Connect citosol to the extracelullar fluid
- Enable passive transport throught the membrane
  - 100 000 000 ions/sec
  - 1000x faster than carriers – bioelectric current is developed
- Properties:

➤ **selectivity**: particular ions can go through, saturation can occur

➤ **voltage-dependent gating**

# Channels are Made Up of Subunits





## *Classification of Ion Channels*:

- Non-gated (leak channels)
- Voltage gated channels
- Ligand gated channels
  - neurotransmitter gated channels
  - ion gated channels (intracellular ions)
  - nucleotide gated channels (intracellular nucleotid)
- Mechanically gated channels

# Nongated Channels

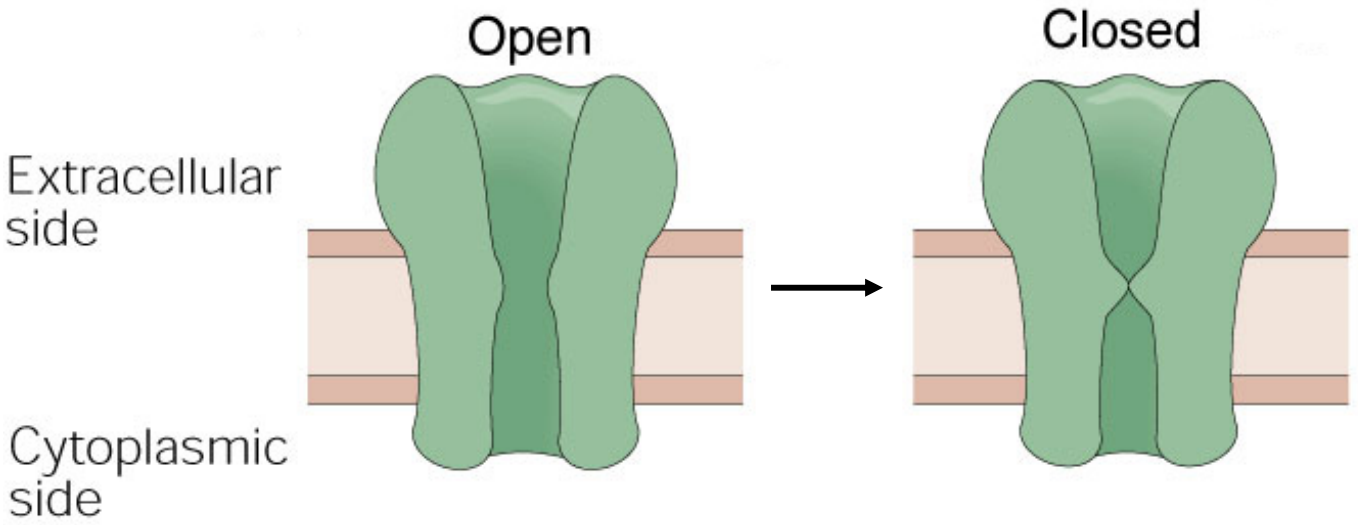
- most of the time they are open
- control the flow of ions during the resting membrane potential
- known as **leak channels**
- nongated Na+ and K+ channels contribute to the resting membrane potential

# Gated Channels

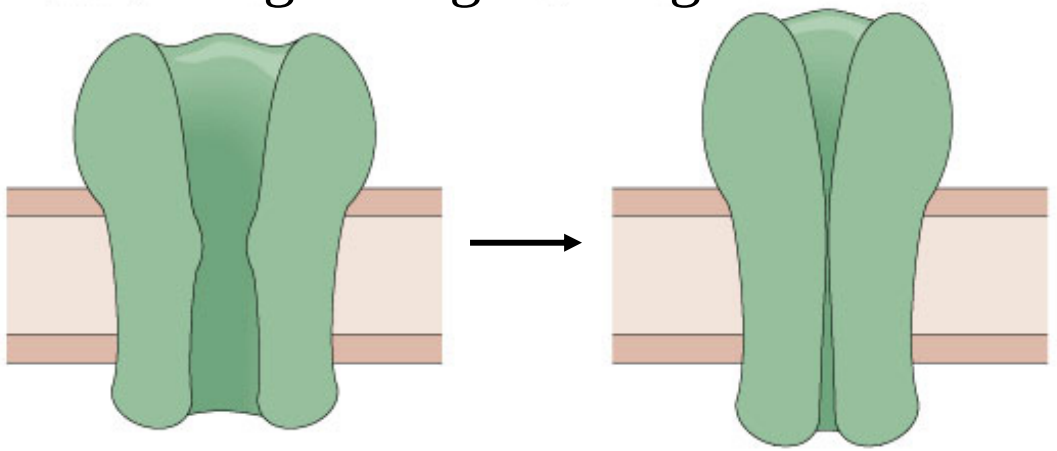
- Allosteric proteins - i.e., they exist in more than one conformation, and their function is altered when they shift from one conformation to another.
- The transition of a channel between the open and closed states is called **gating.**
- At rest, these channels are mostly closed.
- They open in response to different stimuli.

# Gating Can Involve Conformational Changes Along the Channel Walls

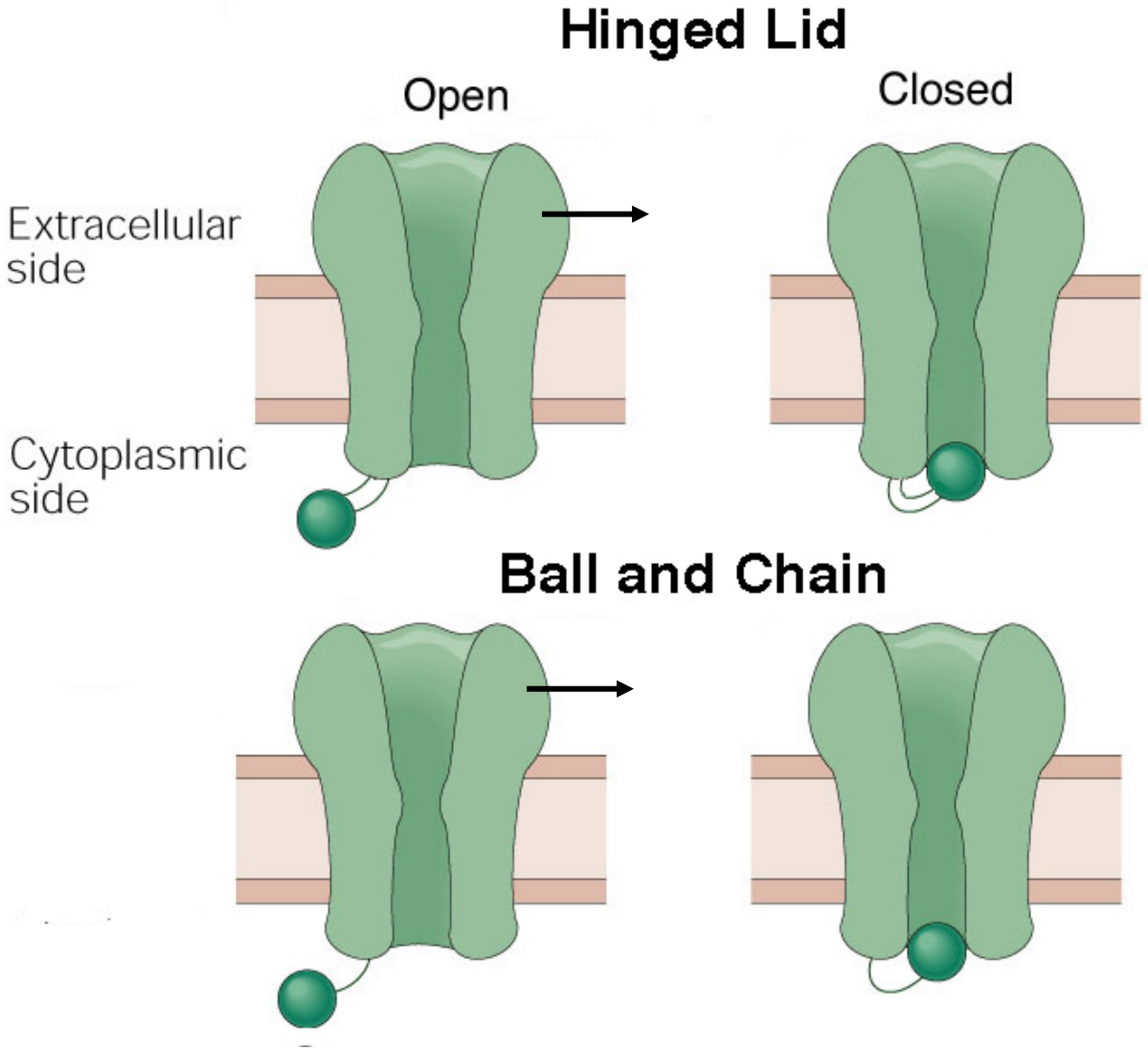
Conformational change in one region



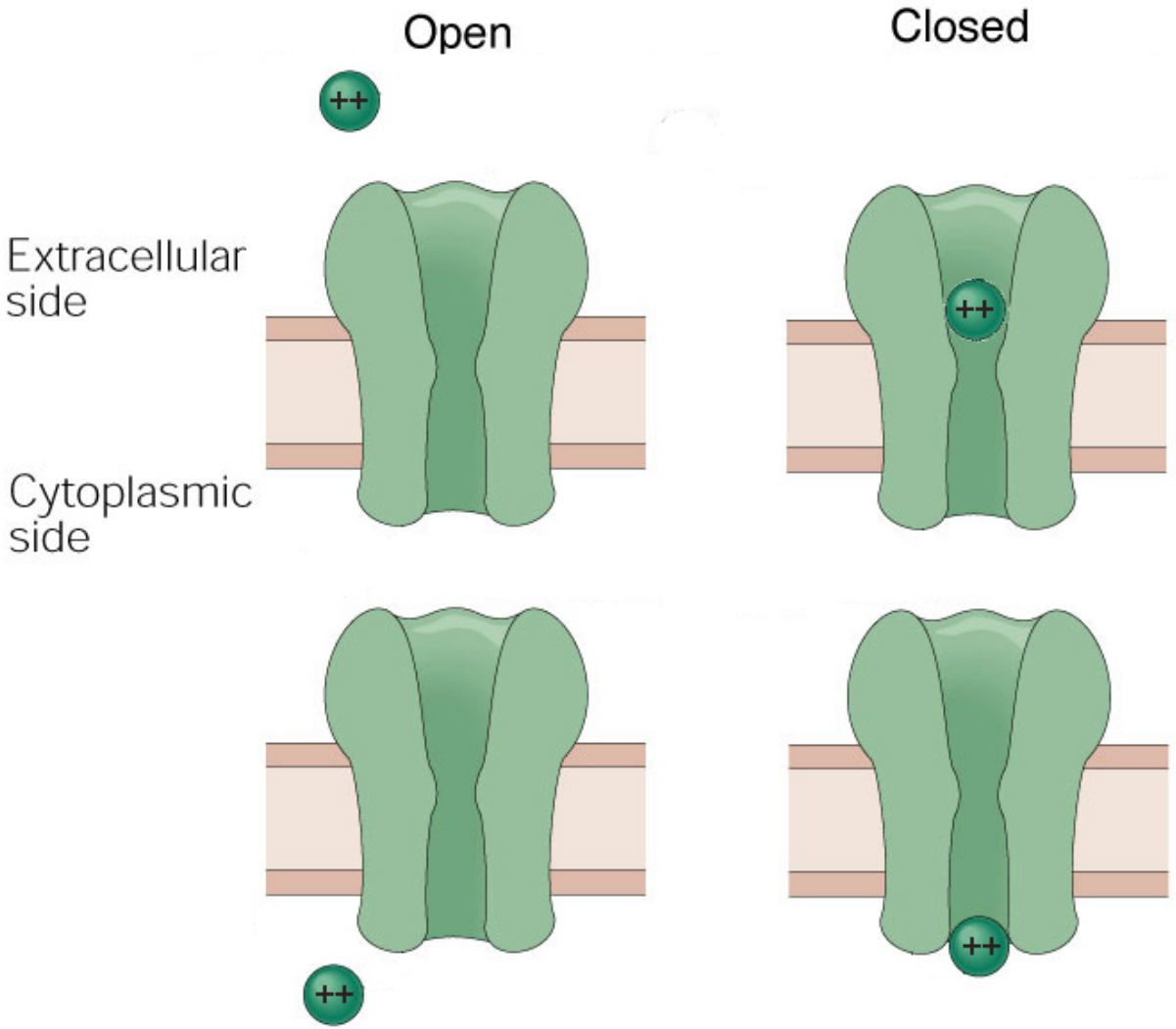

Conformational change along the lenght of the channel



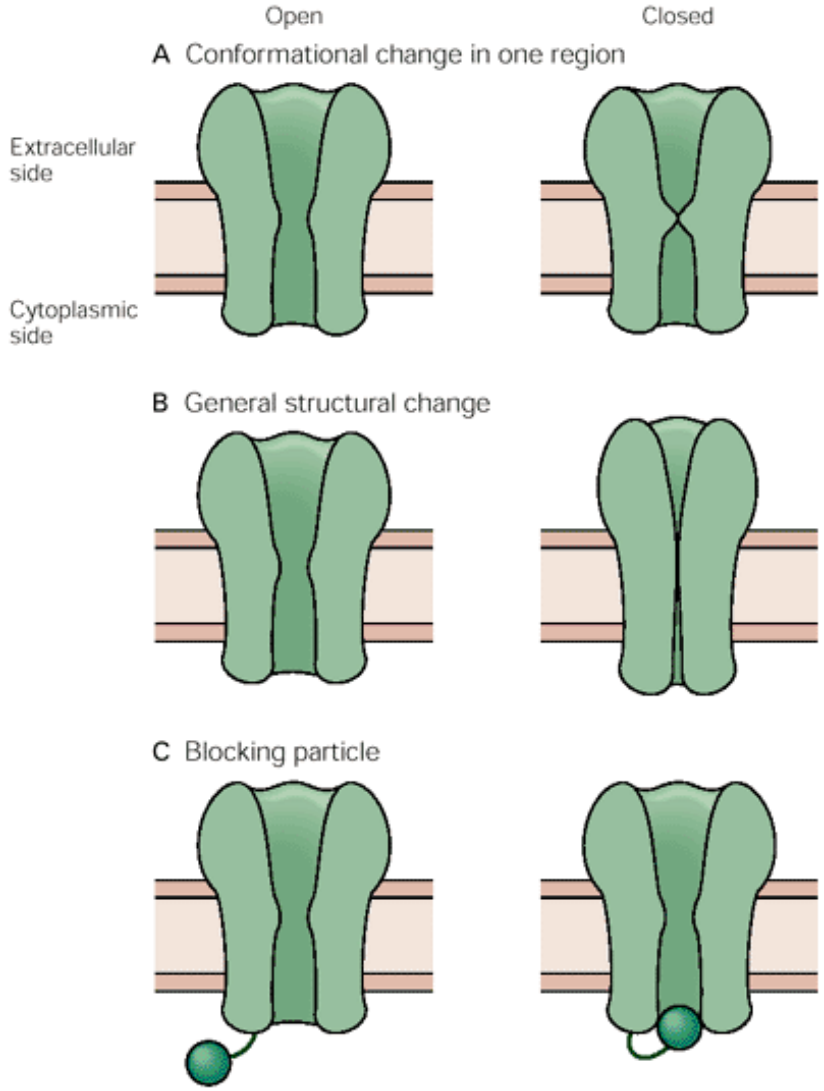
# Gating Can Involve Plugging the Channel



# Gating Can Result from Plugging by Cytoplasmic or Extracellular Gating Particles

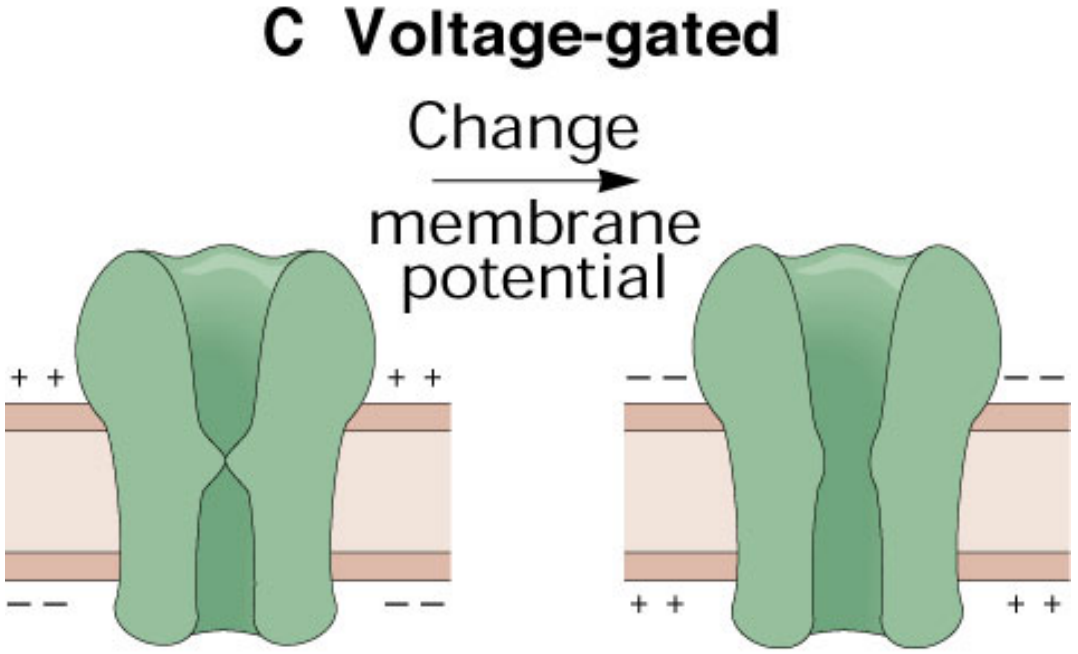


# Three physical models for the opening and closing of ion channels



# Voltage-gated channels

- - are opened or closed by a change in the membrane potential
- exist in three states:
- (1) *resting state, in which the channel is closed but can be* activated;
- (2) *active state, in which the channel is open;*
- (3) *refractory state, in which the channel is inactivated*


C Voltage-gated
Change
membrane
potential
+ +
+ +
– –
– –
– –
– –
+ +
+ +

- **voltage-gated Na+ channel**
- single long polypeptide that has four domains
- Channel is more permeable to $Na^+$ than to $K^+$
- The S4 segment undergoes a conformational change when the membrane potential changes
- When the membrane is depolarized beyond the threshold potential, a sufficient number of voltage-gated $Na^+$ channels open
- action potentials are generated

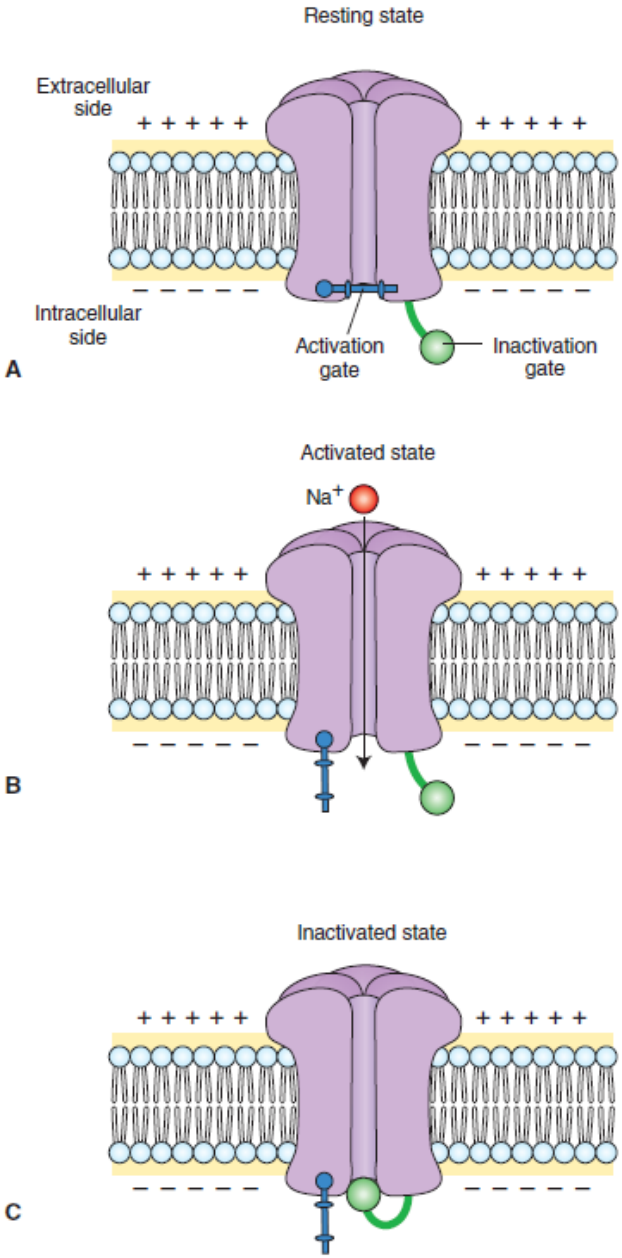
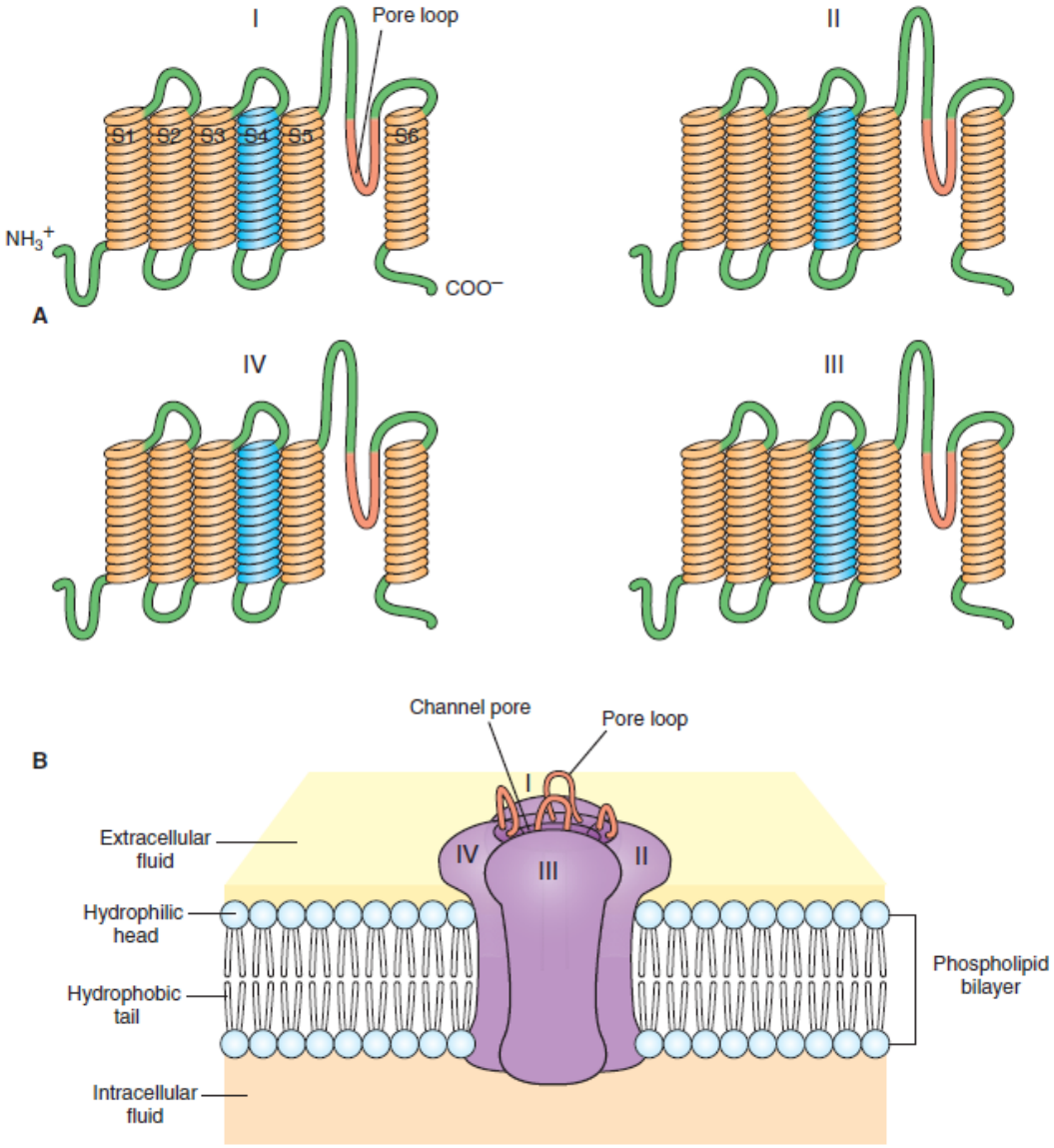
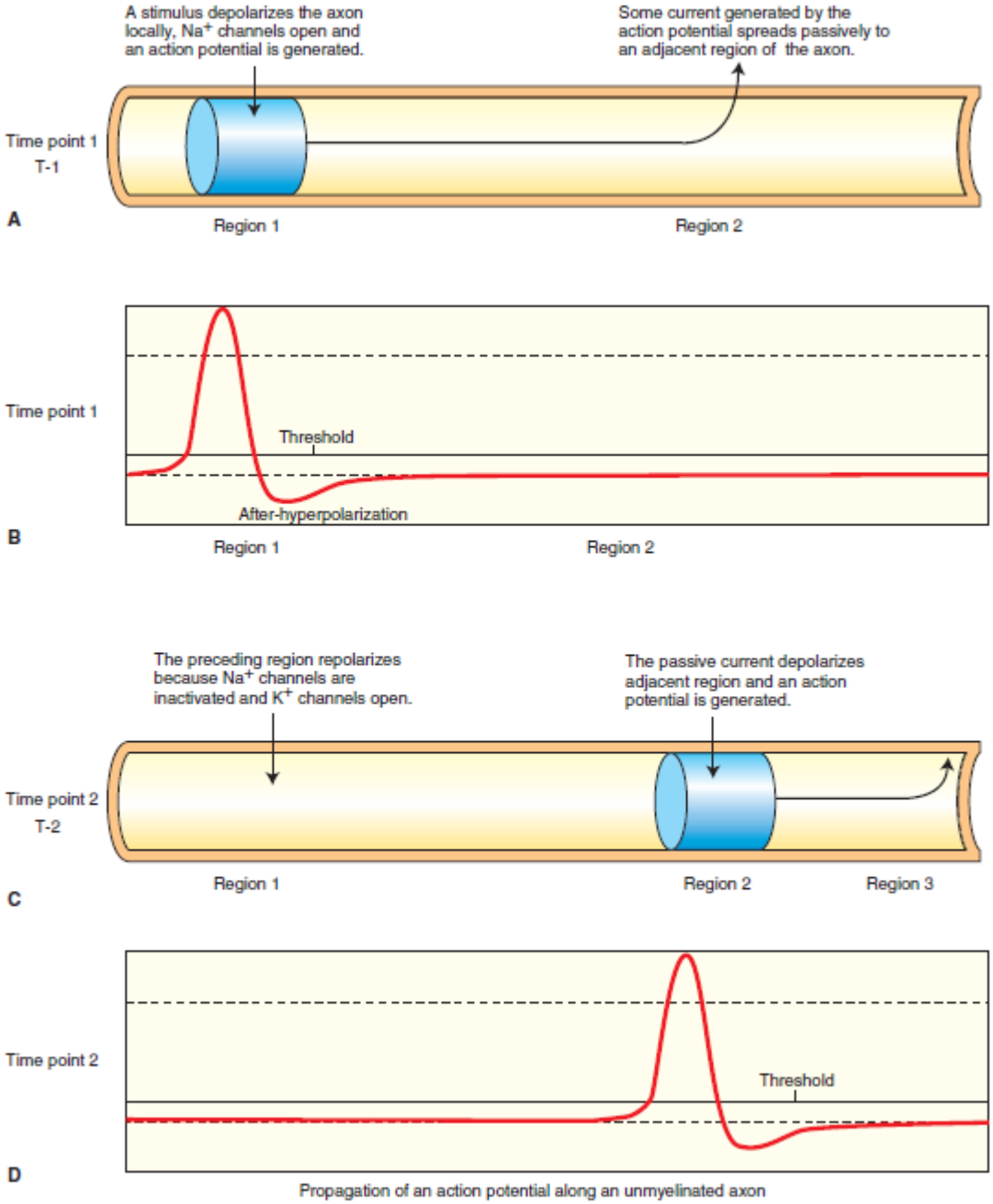



FIGURE 6–5 Different states of voltage-gated $Na^+$ (sodium) channel. **(A)** Resting state. **(B)** Activated state. **(C)** Inactivated state. Note: the "bar" is the activation gate and the "circle" represents the inactivation gate. (See text for descriptions.)




Model of voltage-gated Na+ channel

FIGURE 6–3 Voltage-gated $Na^+$ (sodium) channel. **(A)** The channel is formed by a single long polypeptide that has four domains (I–IV). S1–S5 are hydrophobic alpha helices that span across the membrane. Note also the hydrophobic pore loop. The $NH_3^+$ (hydrogen carbonate) and $COO^-$ (carboxyl group) terminals are exposed on the cytoplasmic side of the membrane. **(B)** The four domains clump together to form a channel with a pore. The wall of the channel pore is formed by the pore loops. The domains are shown in clockwise fashion in **A** and **B** to facilitate orientation.




Propagation of an action potential along an unmyelinated axon

FIGURE 6–6 Propagation of action potentials in unmyelinated axons, as described in the text. (A) Time point 1: Region 1 of an unmyelinated axonal membrane is depolarized. (B) Depolarization in region 1 results in an action potential at time point 1. (C) Time point 2: Passive spread of current by the action potential generated at time point 1 causes depolarization in region 2. (D) Depolarization in region 2 results in an action potential at time point 2. $Na^+$ = sodium; $K^+$ = potassium.

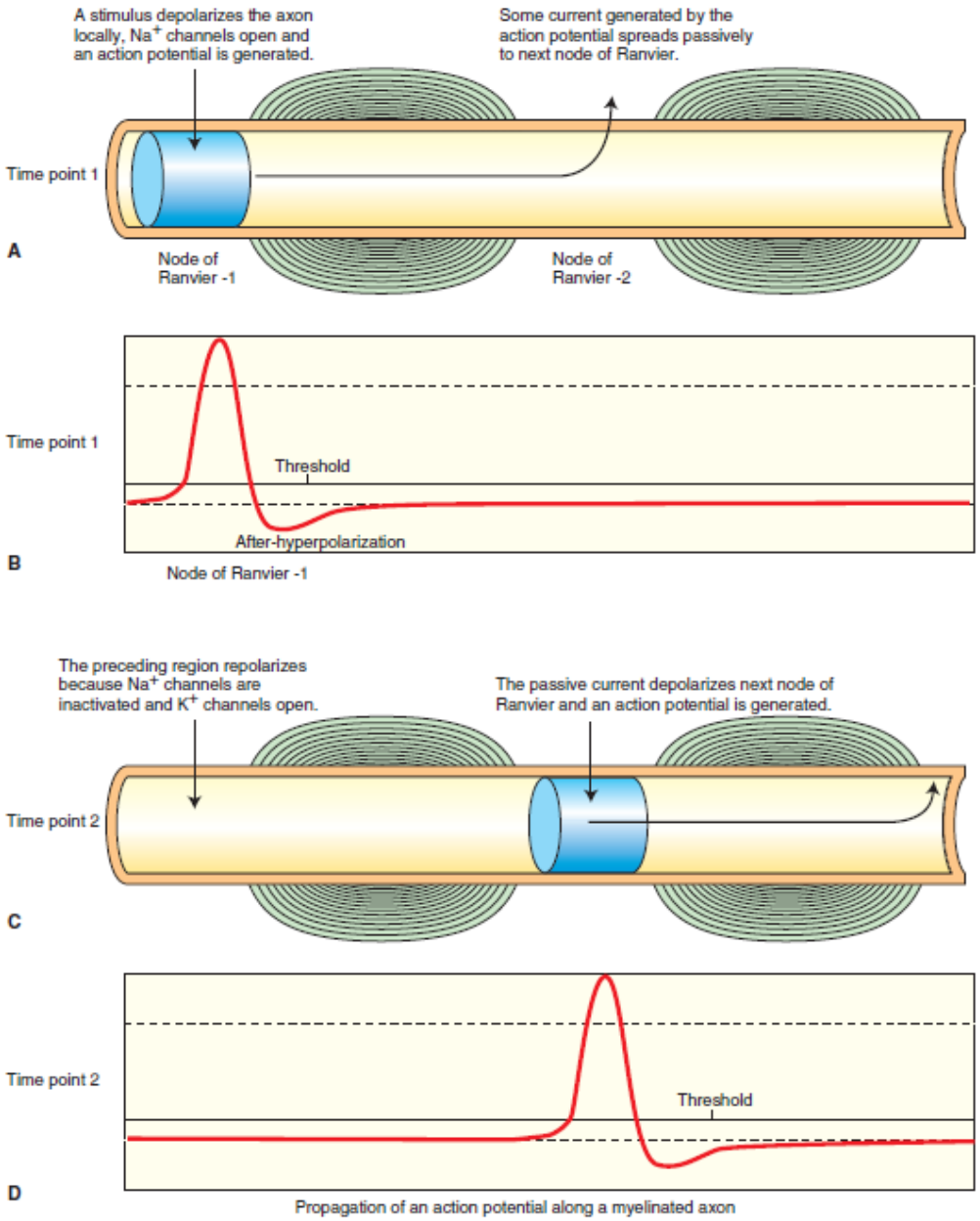


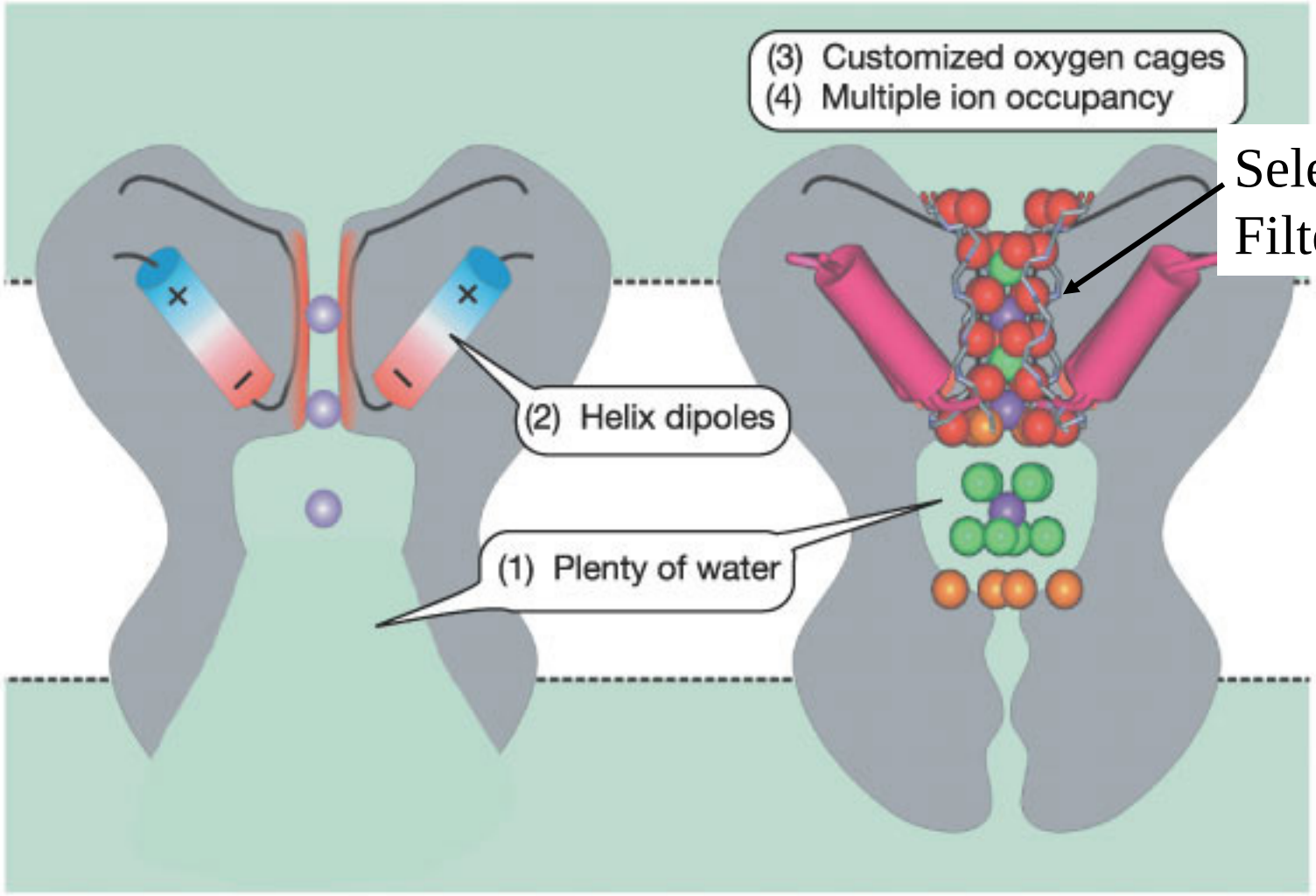

FIGURE 6–7 Propagation of action potentials in myelinated axons. (A) Time point 1: A stimulus depolarizes node of Ranvier 1. (B) An action potential is generated at node 1 at time point 1. (C) Time point 2: Passive spread of current due to action potential generated at time point 1 depolarizes node 2. (D) An action potential is generated at node 2 at time point 2. $Na^+$ = sodium; $K^+$ = potassium.

- There are some cases in which $Na^+$ permeability is blocked.
- Tetrodotoxin (TTX) - a toxin isolated from the ovaries of Japanese puffer fish
- binds to the sodium channel on the outside and blocks the sodium permeability pore
- Neurons are not able to generate action potentials after the application of TTX
- Lidocaine also blocks these channels

- **voltage-gated $Ca^{2+}$ channel**
- $Ca^{2+}$ ions enter the postsynaptic neurons through these channels
- $Ca^{2+}$ ions activate enzymes
- Depolarization of presynaptic nerve terminals results in entry of $Ca^{2+}$ ions into the terminal via these channels
- An increase in the levels of intracellular $Ca^{2+}$ results in the release of transmitters from presynaptic nerve terminals.

- **voltage-gated K+ channels**
- K+ channels are generally blocked by chemicals, such as tetraethylammonium or 4-aminopyridine
- The opening of voltage- gated K+ channels is also caused by depolarization of the neuronal membrane
- Because these voltage-gated K+ channels open with a delay (about 1 msec) after the membrane depolarization, they are called **delayed rectifier K+** channels.
- **Repolarization** of the neuron
- **after-hyperpolarization** or **undershoot**
- **TEA –** blocks these channels

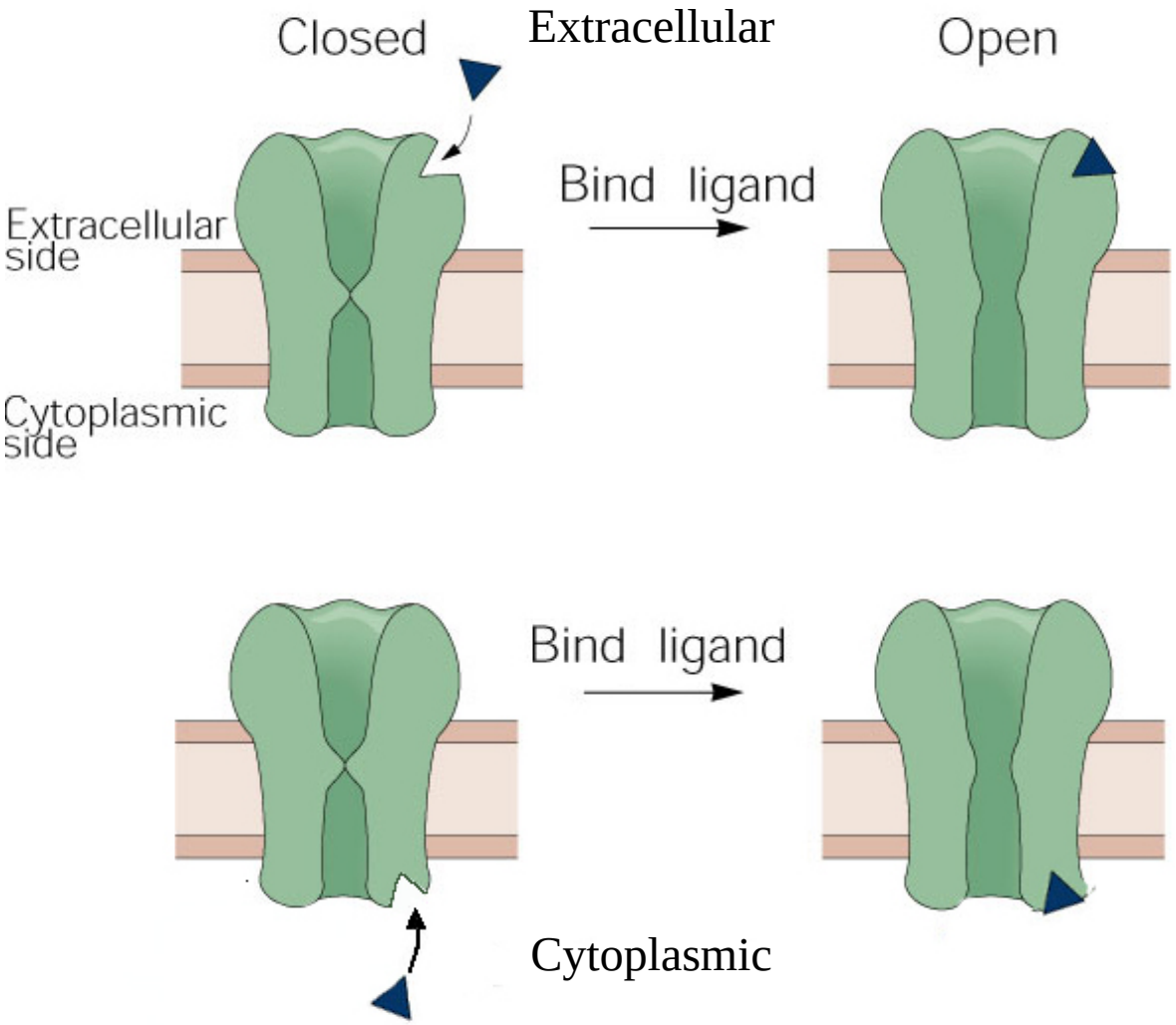
# Structure of $K^+$ Channel Has Multiple Functional Adaptations



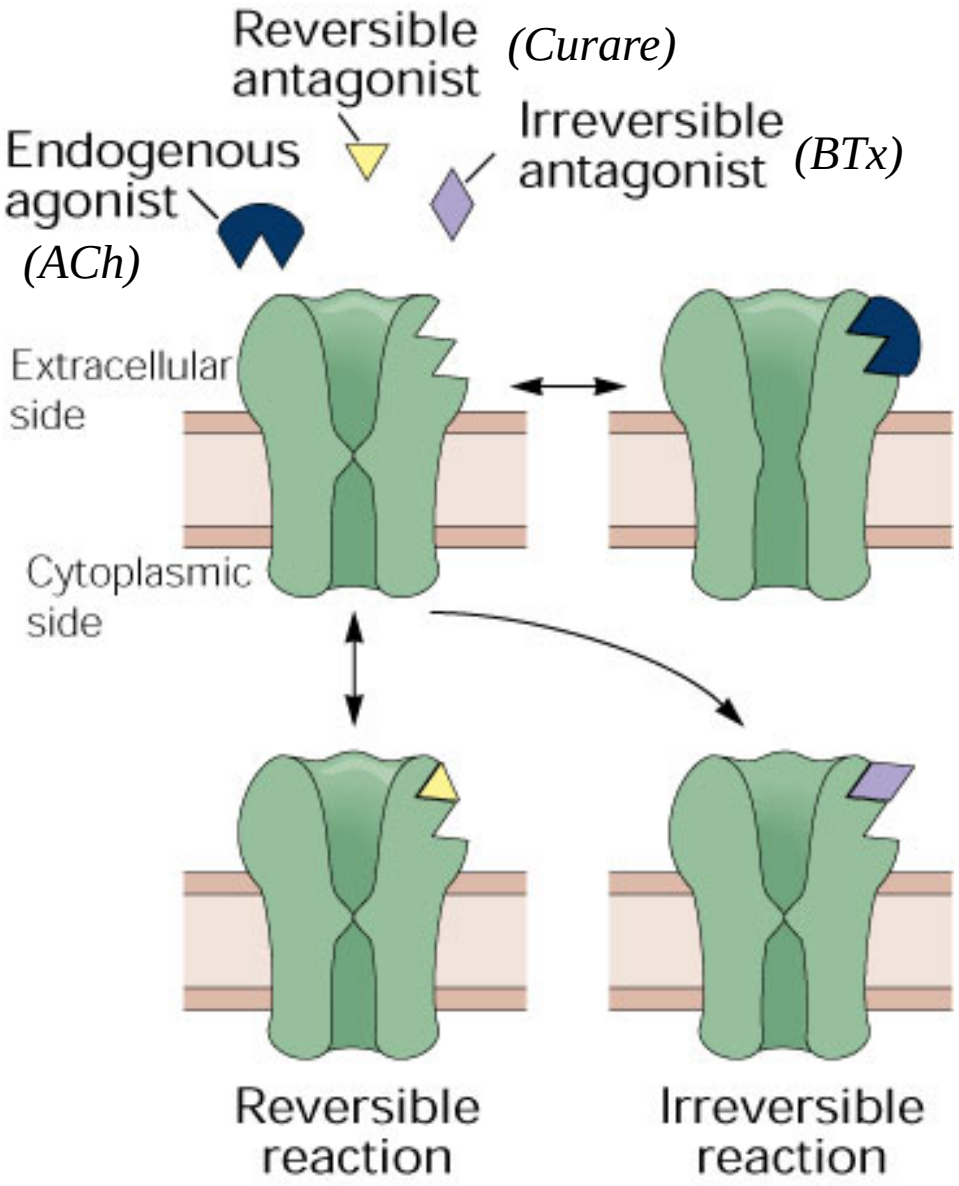
# Ligand-gated channels

- are opened by noncovalent binding of chemical substances with their receptors on the neuronal membrane
- transmitters or hormones present in the extracellular fluid (acetylcholine, γ-aminobutyric acid [GABA], or glycine)
- an intracellular second messenger (e.g., cyclic adenosine monophosphate, which is activated by a transmitter such as norepinephrine

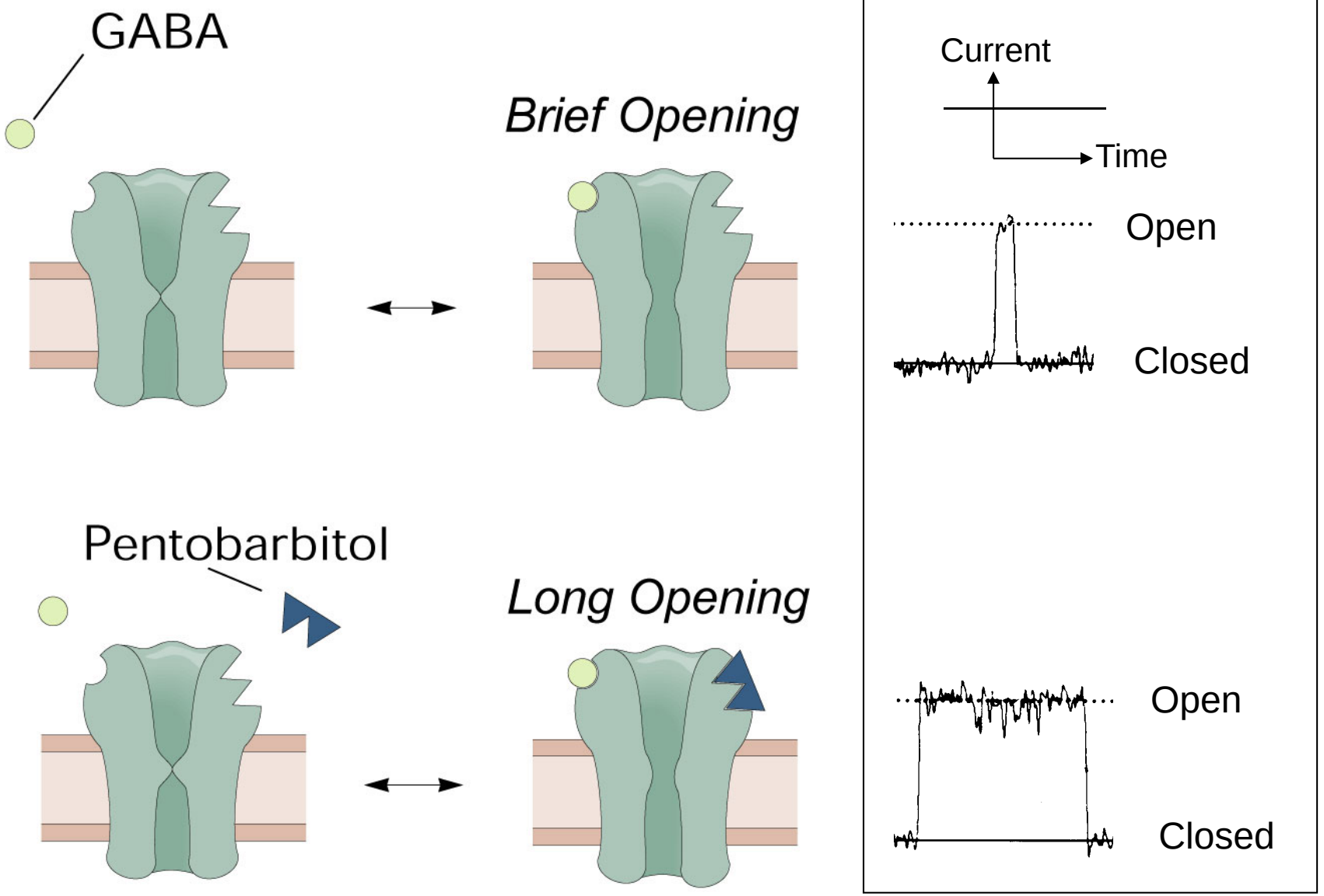
# Ligand Binding



# Binding of Exogenous Ligands Can Block Gating



# Exogenous Modulators Can Modify the Action of Endogenous Regulators

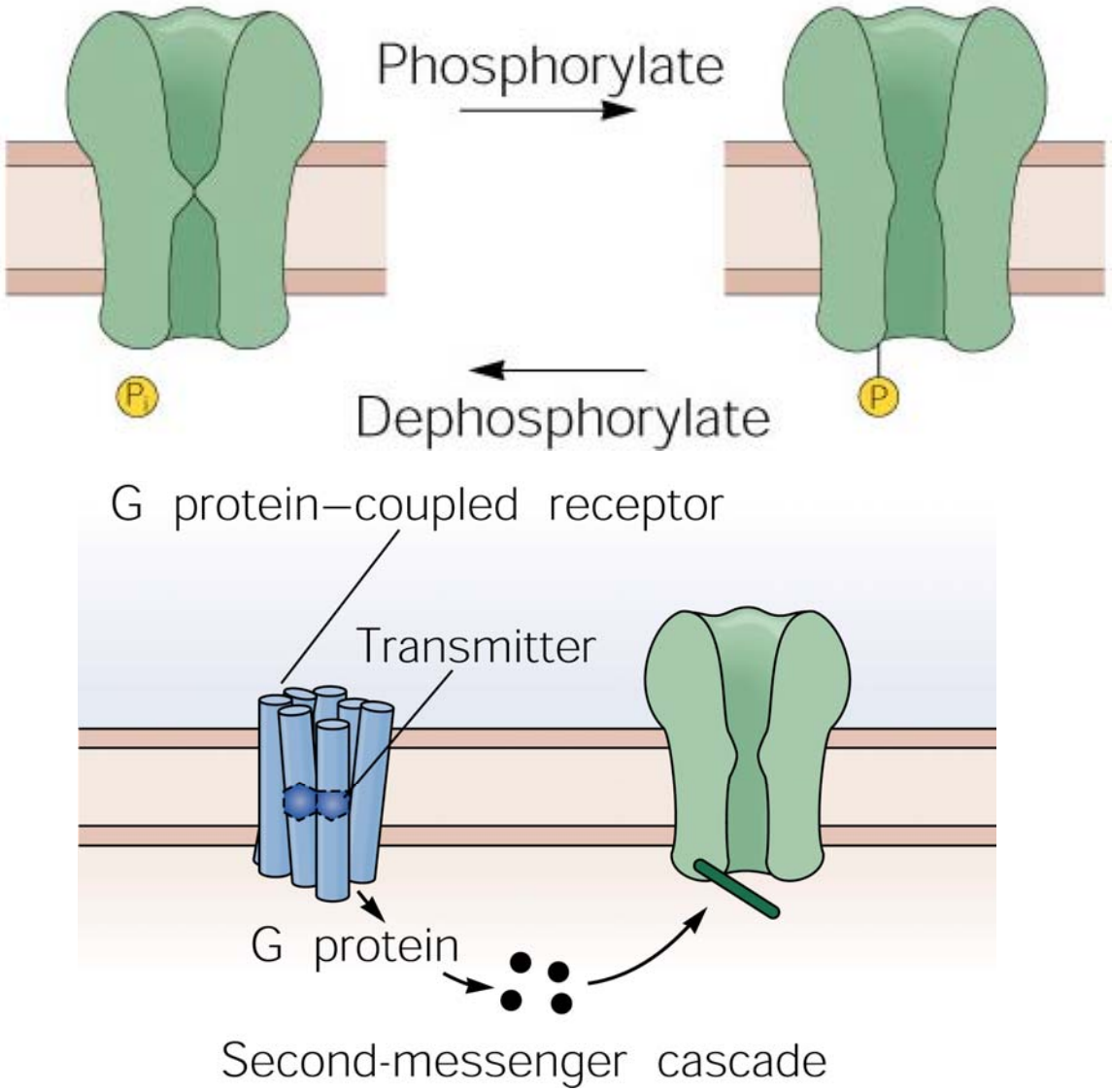


- ***Directly gated ligand channel:***
- five protein subunits
- Recognition site for the chemical substance is part of the ion channel
- **Ionotropic receptor**
- A neurotransmitter binds to an ionotropic receptor and brings about a conformational change that results in the opening of the ion channel
- usually bring about fast synaptic responses that last for only a few milliseconds

- ***An indirectly gated*** ligand channel
- the ion channel and the recognition site for the transmitter (receptor) are separate.
- **Metabotropic receptors**
- When a transmitter binds to the metabotropic receptor, a guanosine-5’-triphosphate- binding protein (G-protein) is activated
- It activates a second-messenger system in the neuron

- The second messenger can either act directly on the ion channel to open it, or
- It can activate an enzyme that, opens the channel by phosphorylating the channel protein in the presence of a protein kinase
- Elicits slow, long-lasting synaptic actions

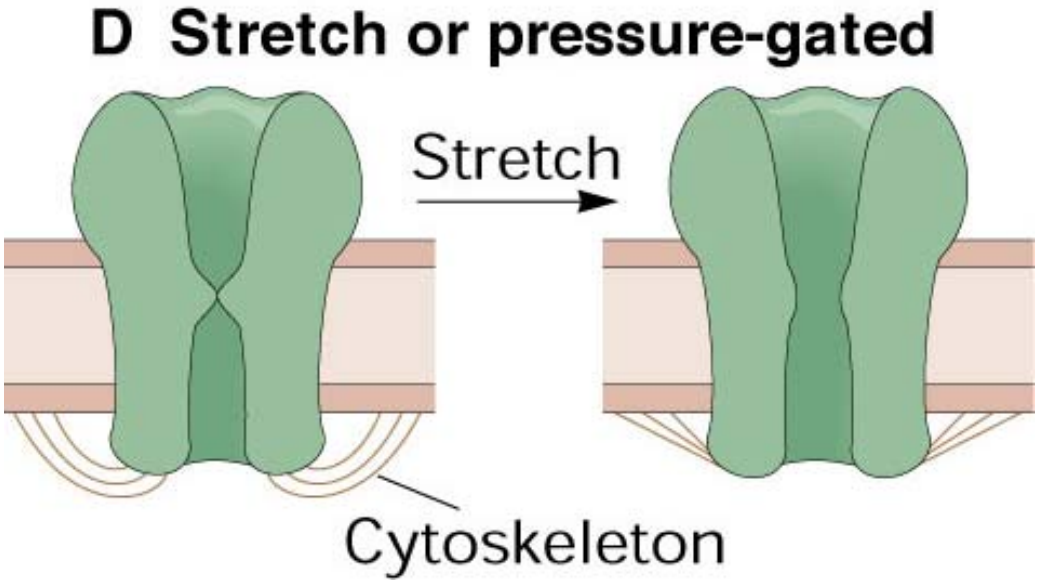
# Phosphorylation

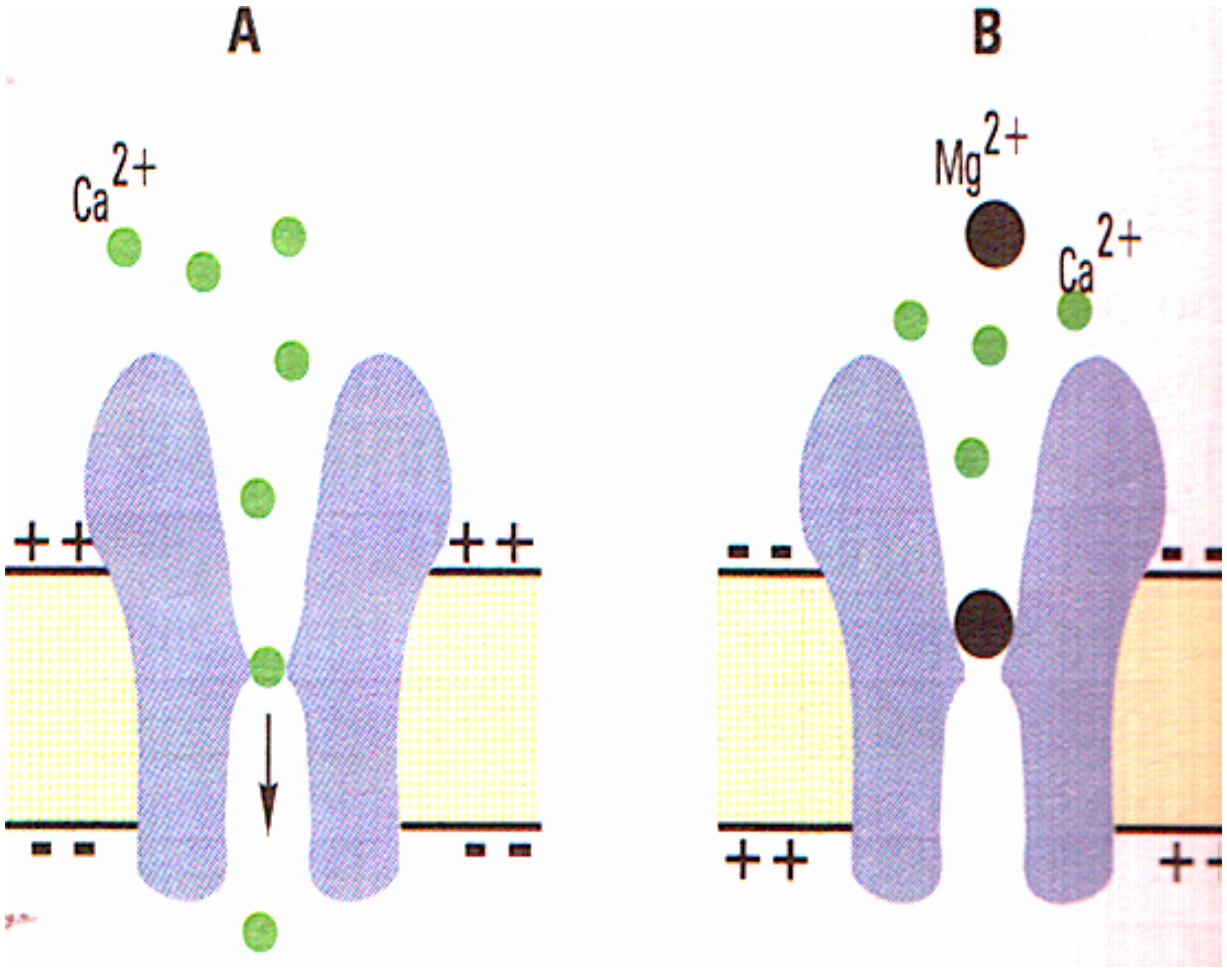


# *Mechanically gated channels*

- open by a mechanical stimulus
- include the channels involved in producing generator potentials of stretch and touch receptors

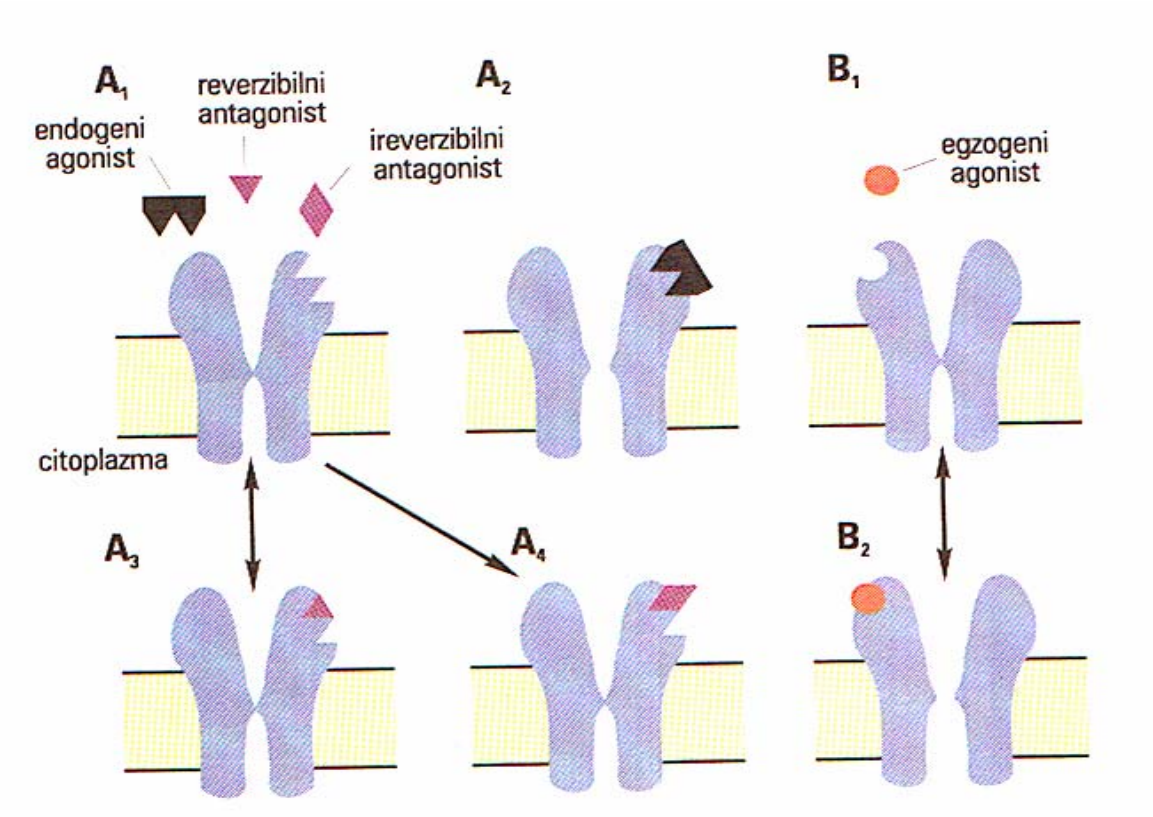
# Mechanically gated ion channels




A
Ca2+
++
++
--
--
B
Mg2+
Ca2+
--
--
++
+-

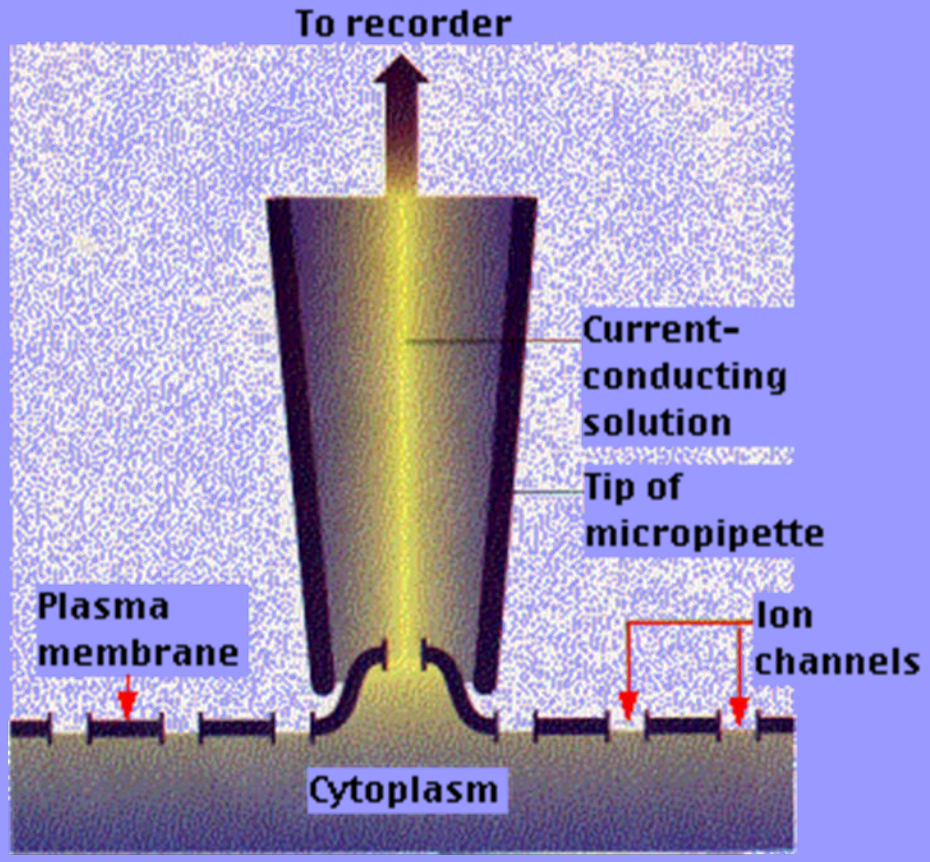
# Ligands:

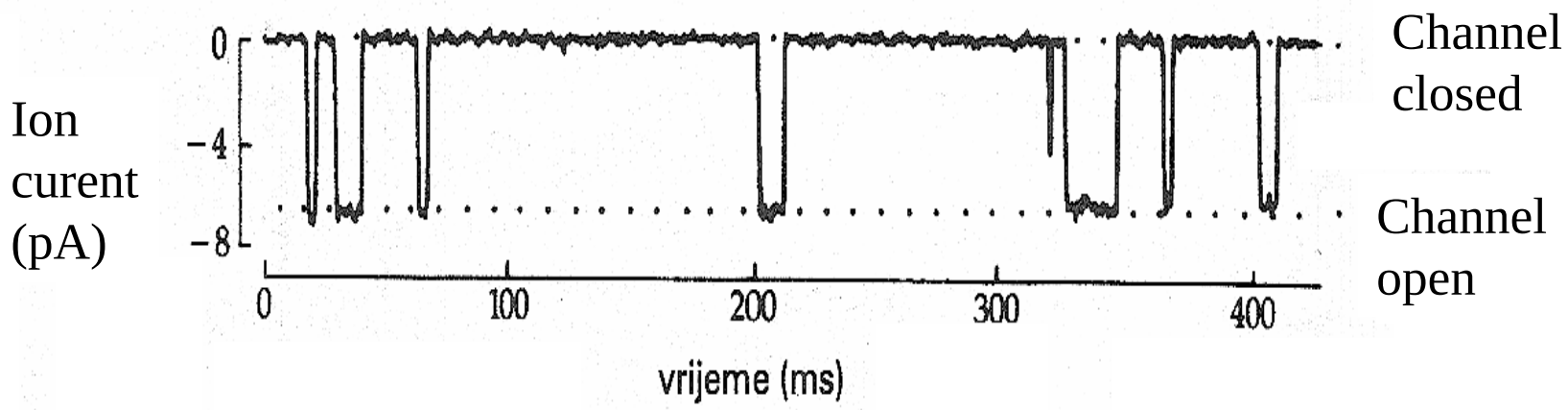
- egzogenic: -competitive
  -non-competitive
- endogenic



# Recording of the ionic currents

- patch-clamp
- voltage-clamp






**Slika 7-5.** Ionski kanal oscilira između dva konformacijska stanja, otvorenog i zatvorenog. Prikazani $Na^+$ kanal (u membrani skeletnog mišića štakora) otvorio se 8 puta tijekom 400 milisekundi u odgovoru na primijenjeni neurotransmiter acetilkolin, što je zabilježeno snimanjem ionskih struja pojedinačnog ionskog kanala posebnom metodom priljubljene elektrode (»patch-clamp« metoda). Kad je kanal otvoren, kroz njega protječe oko $4{,}1 \times 10^7$ iona u sekundi, što se na osciloskopu bilježi kao otklon prema dolje (ionska struja u pA). Prema Hille (1984), uz dopuštenje.

# CLINICAL CONSIDERATIONS

- **Lambert-Eaton (Eaton-Lambert) Syndrome**
- associated with small-cell carcinoma of the lung
- cells expressing voltage-gated Ca2+ channels
- An antibody is produced in the body against these Ca2+ channels,
- its presence results in a loss of voltage-gated Ca2+ channels in the presynaptic terminals
- less Ca2+ enters the presynaptic terminal during depolarization and, consequently, there is a reduction in the release of the transmitter (Ach)
- results in muscle weakness, dry mouth, constipation, reduced sweating, orthostatic hypotension (dizziness while standing or walking), and impotence

- **Multiple Sclerosis**
- an autoimmune disease with infl ammatory features that affect the CNS
- demyelination occurs in the axons of the CNS
- Antibodies attack myelin, which then swells and detaches.
- A scar (sclerosis) develops on the nerve fibers, which delays or blocks nerve impulses
- nerve fibers degenerate
- commonly affects young people, especially women, in the 20-year-old to 40-year-old age group.

- Symptoms:
- numbness in one or more limbs, typically on one side.
- tingling or pain in parts of the body.
- Visual pathways are affected, resulting in vision disturbances
- disturbances in speech
- weakness in one or more limbs on one side
- tremor, lack of coordination, wide steps, and an unsteady gait
- Neurological examination: hyper-reflexia and Babinski's sign
- Magnetic resonance imaging shows scarring in the CNS.

# Management of MS patients:

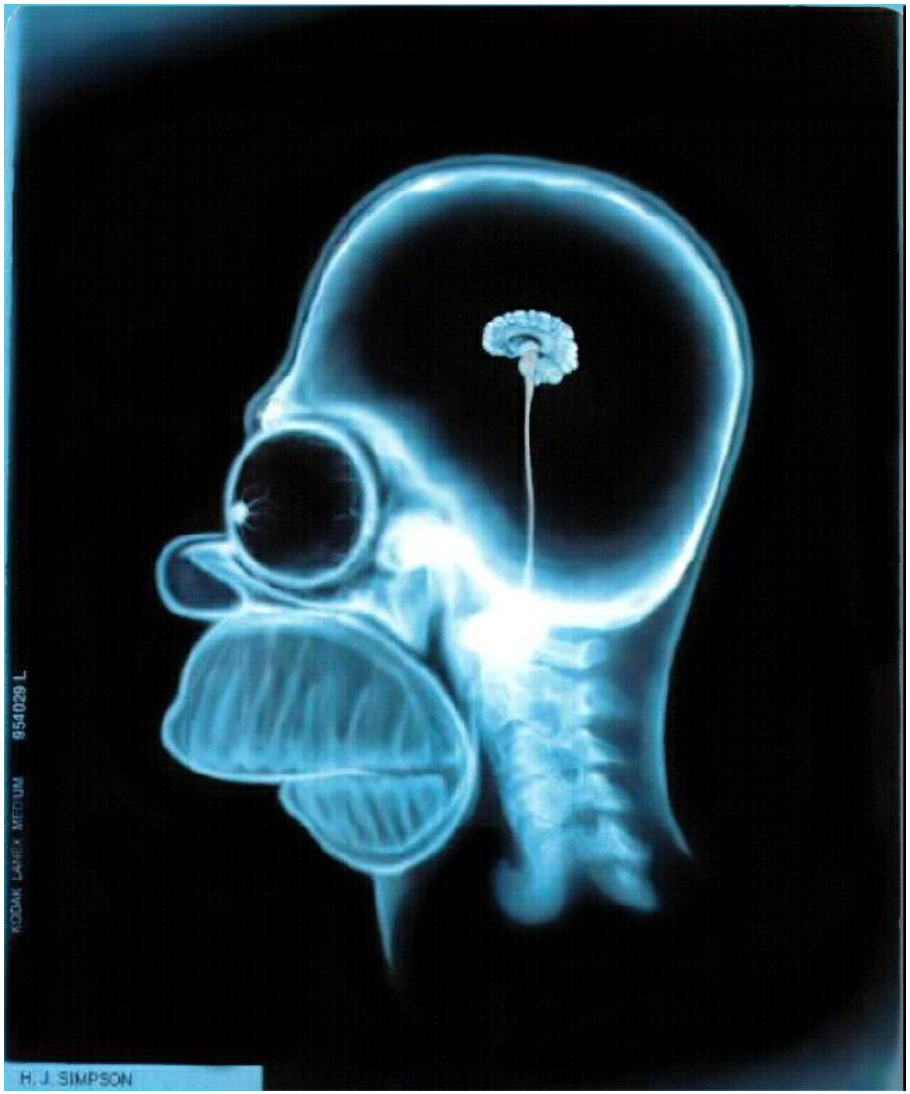
**TABLE 6–3 Drugs or Procedures Used to Treat Multiple Sclerosis**

| Drug or Procedure | Effects |
|---|---|
| Corticosteroids | Reduce inflammation |
| Interferon beta-Ia and interferon beta-Ib | Reduce frequency and severity of relapses; help to fight viral infection and regulate immune system |
| Glatiramer (Copaxone) | Polymer consisting of four amino acids present in myelin; shifts the population of pro-inflammatory T cells (Th1 cells) to regulatory T cells (Th2 cells) that suppress the inflammatory response; should not be used with interferon beta |
| Natalizumab (Tysabril) | Blocks attachment of immune cells to blood vessels of the brain (necessary step for these cells to enter brain tissue) |
| Mitoxantrone (Novantrone) | Used for cancer chemotherapy; useful in some patients in reducing severity of relapses but has serious side effects; potent immunosuppressive agent targeting proliferating immune cells; inhibits proliferation of macrophages, B lymphocytes, and T lymphocytes |
| Plasma exchange (plasmapheresis) | Involves removal of some blood, filtering it to remove plasma, and replacing it with albumin; this preparation is then reinfused into the patient. Procedure may reduce antibodies against myelin; used only in patients not responding to steroid therapy and experiencing sudden and severe attacks |

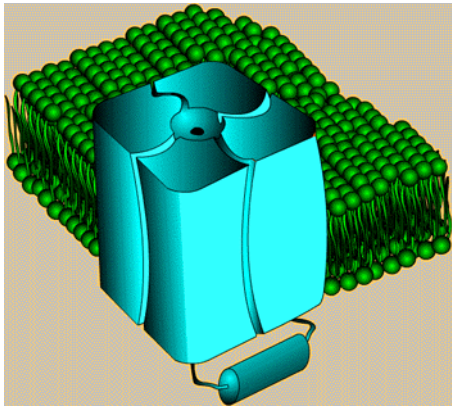
- **Cystic Fibrosis**
- an inherited disease that affects primarily the respiratory system
- Ion channel through which Cl− ions are transported into the cells is affected
- movement of water into the tissues is controlled to maintain the fluidity of mucus is changed

- **Guillain-Barré Syndrome**
- demyelination of axons in the peripheral nerves occurs

# Summary



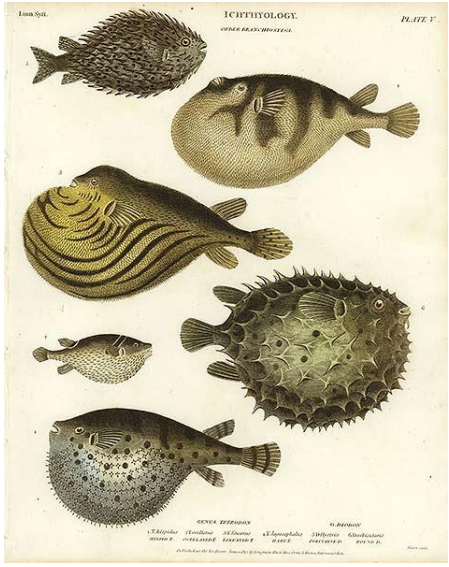
# Voltage-gated Na+ channel



- structure
- function – generation of the action potential (Nernst (equlibrium potential for Na+)
- Characteristics of activation (fast opening and closing in just a few miliseconds)
- pharmacology (TTX, STX, veratridine)

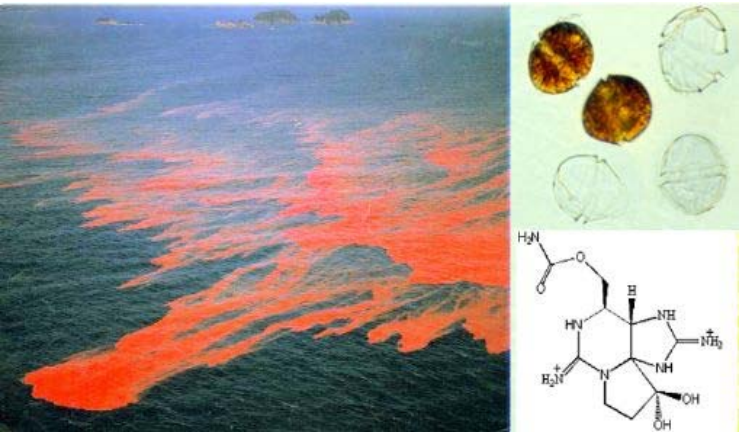
# Voltage-gated Na+ channel

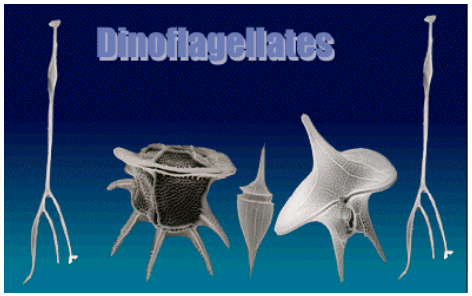
- Blokatori, djeluju na citoplazmatskom ušću:
- Tetrodotoxin – TTX: blocks action potentials by binding to the binding site located at the pore opening. Its name derives from Tetraodontiformes, an order that includes pufferfish, porcupinefish, ocean sunfish or mola, and triggerfish, several species that carry the toxin *(Japan).*



# Voltage-gated Na+ channel

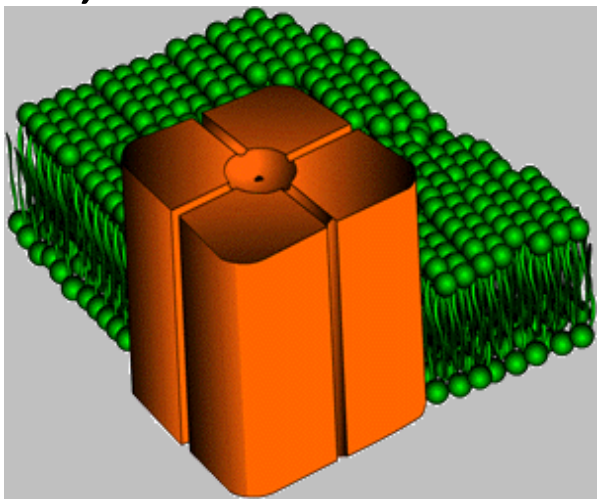
- Saksitoksin – STX: toxin from dinoflagellates red stains, butter clam (*Saxidomus giganteus)*
- Veratridine: functions as a neurotoxin by activating sodium channels






# Voltage-gated K+ channels

- Function – stabilize membrane potential (Nernst (equlibrium potential for K+))
- Types (A-channels, “delayed rectifiers“, “inward rectifiers“, BK-channels, SK-channels)



# Voltage-gated Ca+ channels

- Treshold (low - LT, high - HT)
- Inactivation
- “patch-clamp” (T,L,N)
- P - channels, R - channels

# Task1

- If extracellular concentration of $Na^+$ changes than: :
  - A) If extracellular concentration of $Na^+$ increases than membrane potential doesn't change.
  - B) If extracellular concentration of $Na^+$ increases than membrane potential will be more positive than starting potential.
  - C) ) If extracellular concentration of $Na^+$ decreases than membrane potential will be more positive than starting potential.

# Task 2

- What are the factors to determine resting potential:
  - _____
  - _____
  - _____

# Task 3

- Ion $K^+$ is located in the compartments A and B which are separeted by semipermeabile membrane. Concentration of $K^+$ is 10 times bigger in compartment A than in B. Calculate equlibrium potential.
  - A) 60 mV
  - B) –60 mV
  - C) 0 mV

# Nernst equation

- $E_A - E_B = -60\ mV/z * \log((X_B/X_A))$

# Carriers

- na sebe vežu specifičnu molekulu
- podliježu konformacijskim promjenama
- omogućuju prenošenje te molekule kroz membranu
- nosač ima specifično vezno mjesto
- proces prenošenja nalikuje reakciji enzim-supstrat
- konstanta vezanja supstrata (Km) = koncentracija tvari kad je brzina prenošenja pola od maksimalne
- Vmax – prenošenje najvećom brzinom kad suspstrat zauzme sva vezna mjesta nosača

# Nosači

- Vezanje supstrata može se blokirati
- kompeticijski inhibitori – sprečavaju vezanje supstrata
- nekompeticijski inhibitori – alosterička modulacija