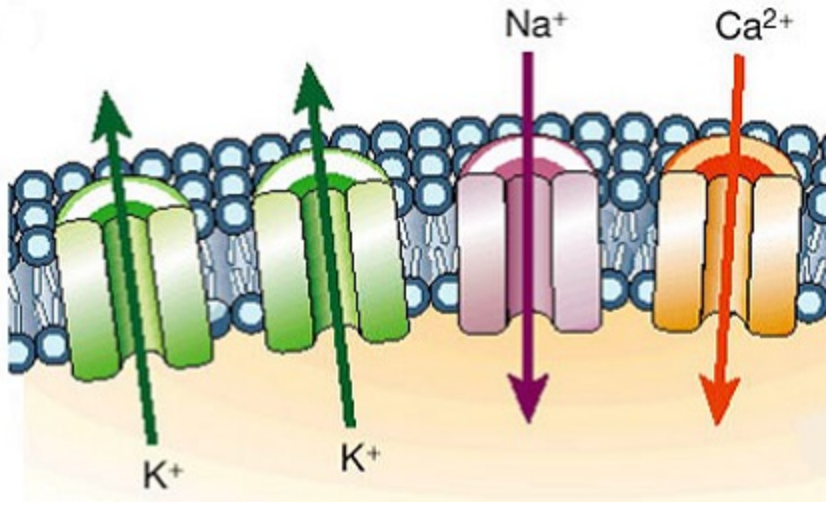
Voltage Gated Ion Channels

Which force moves the ions?

The electrochemical gradient

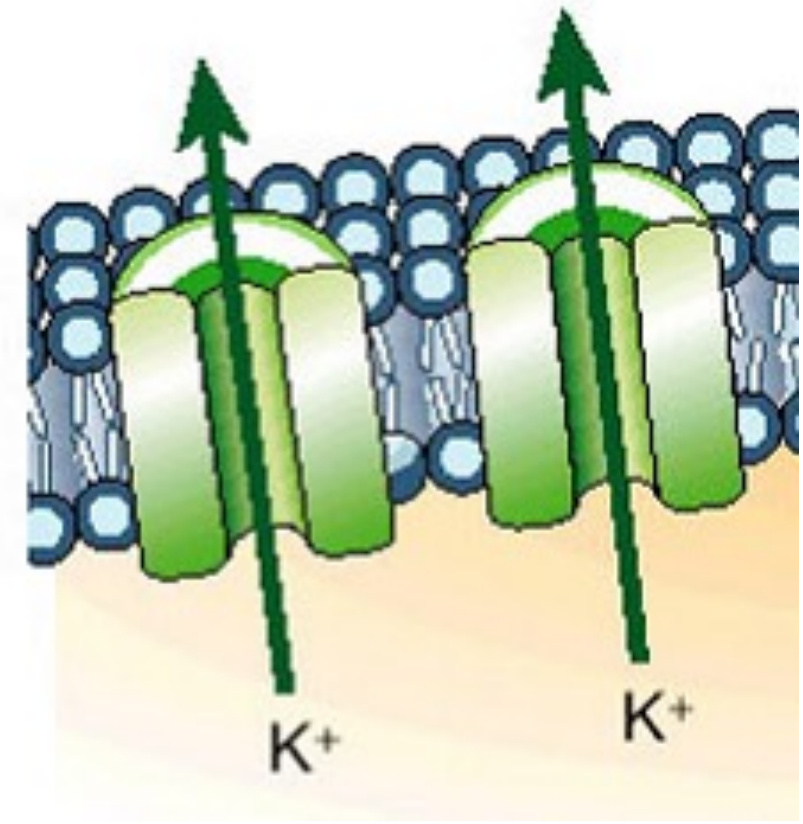


0 mV

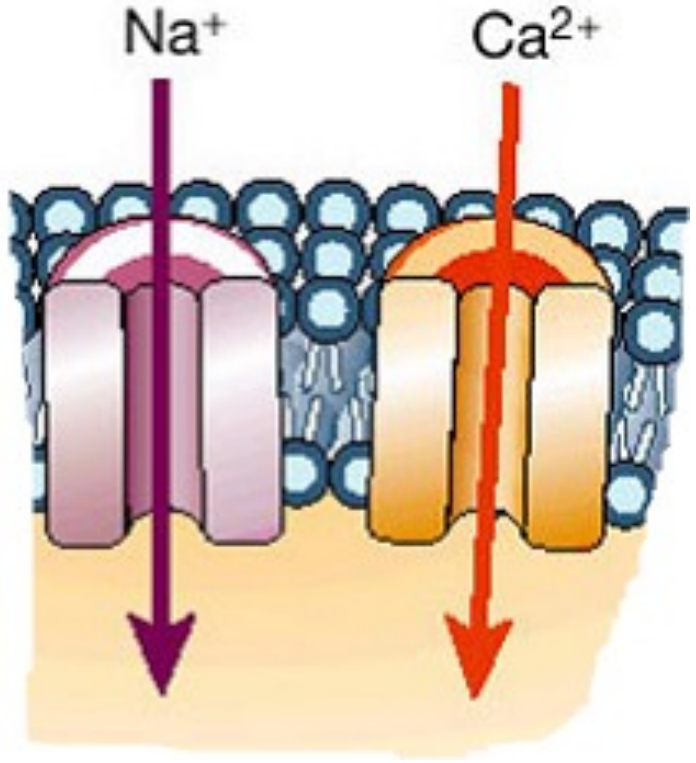
-90 mV

	muscolo di mammifero	
	m Eq	m Eq
Na^+	145	12
K^+	4	155
Ca^{++}	3,4	0,02
Mg^{++}	1,3	34*

What happens when ions move?



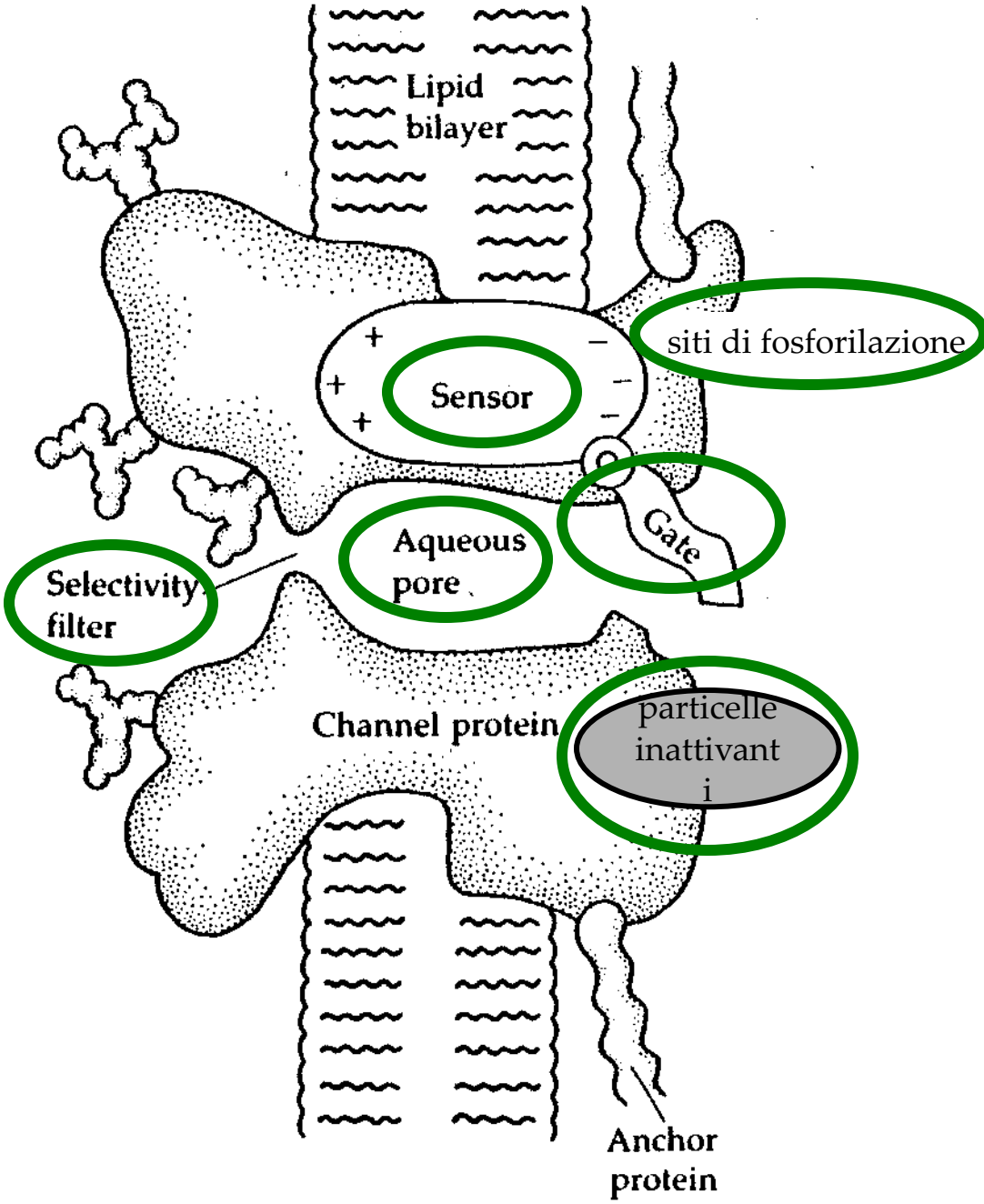
Vm -> -90 mV



Vm -> +50 mV

Structural motifs

ie: what's in all the channels?



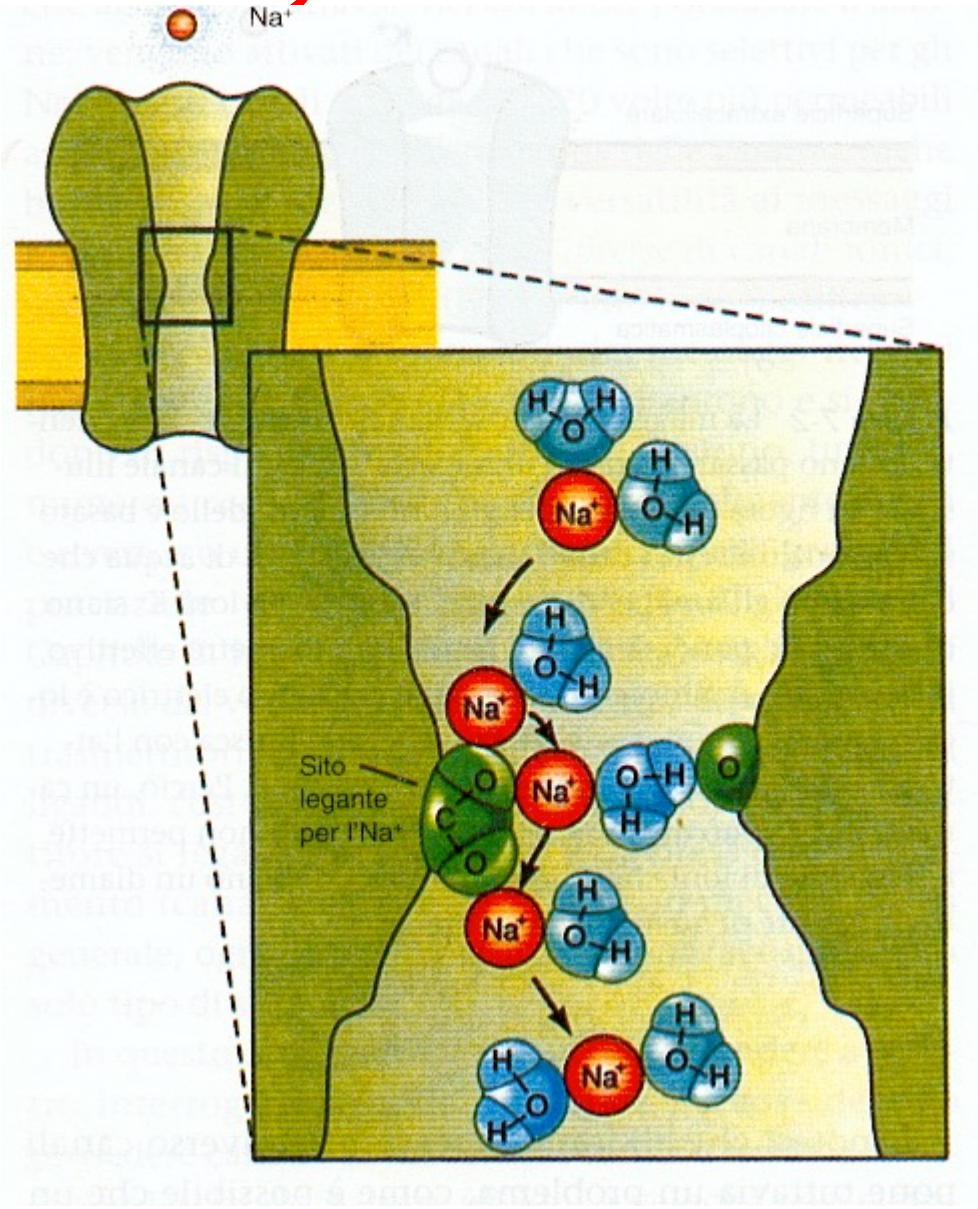
Selectivity filter (3/4 aa)

Ions interact with charged aa

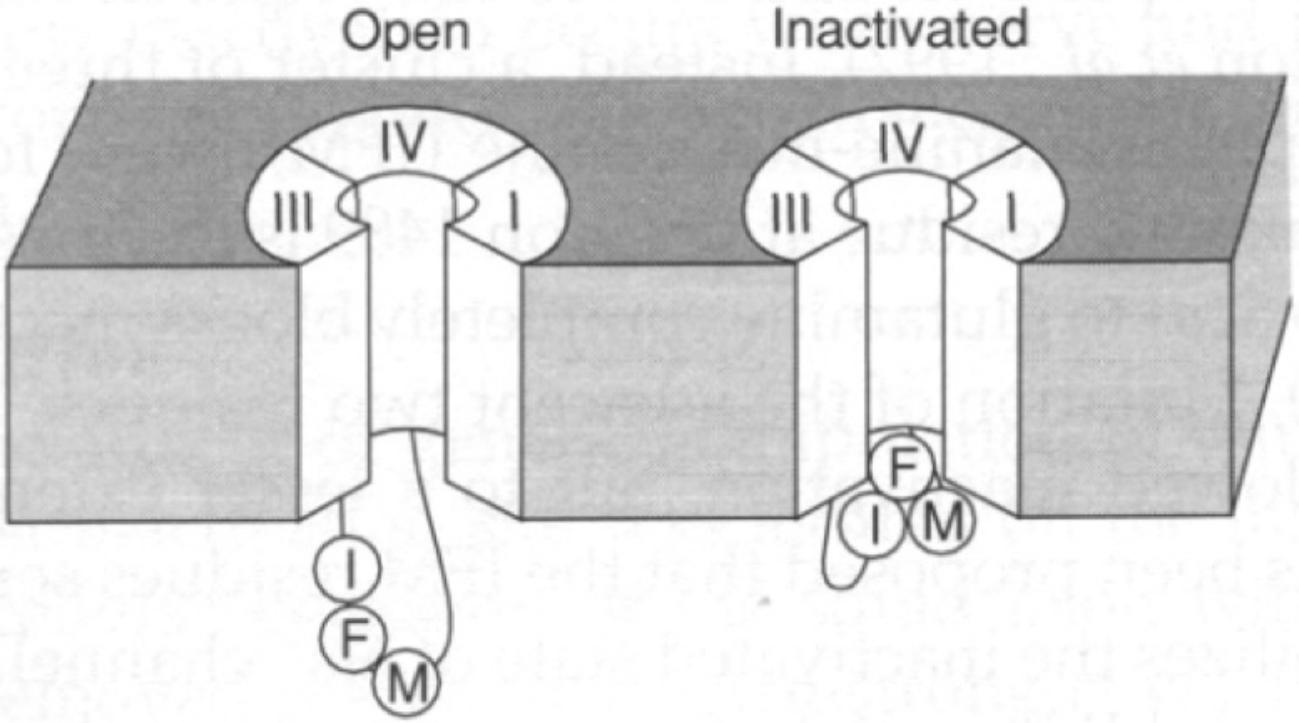
1) Each channel excludes ions of dimensions> characteristic value. Es. K 3A°, Ca 5A°…

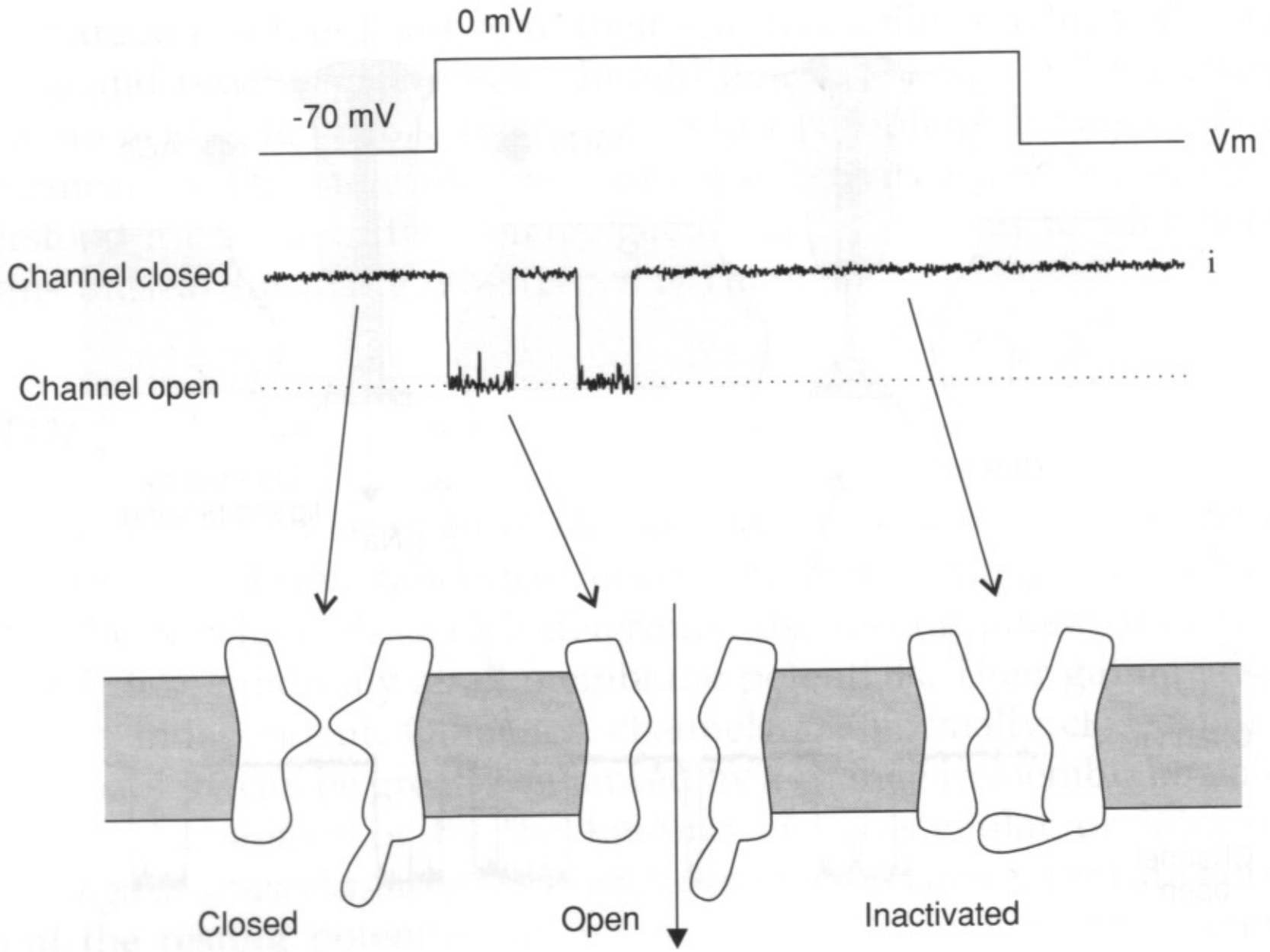
2) Important charges aa are cations binding sites - and exclude ion water hydration molecules

3) Due to the reduced size of the filter important steric effects occur, more ions occupy - greater repulsion (2 Ca or 4 Na neutralize 4 + charges)



Inactivating particles



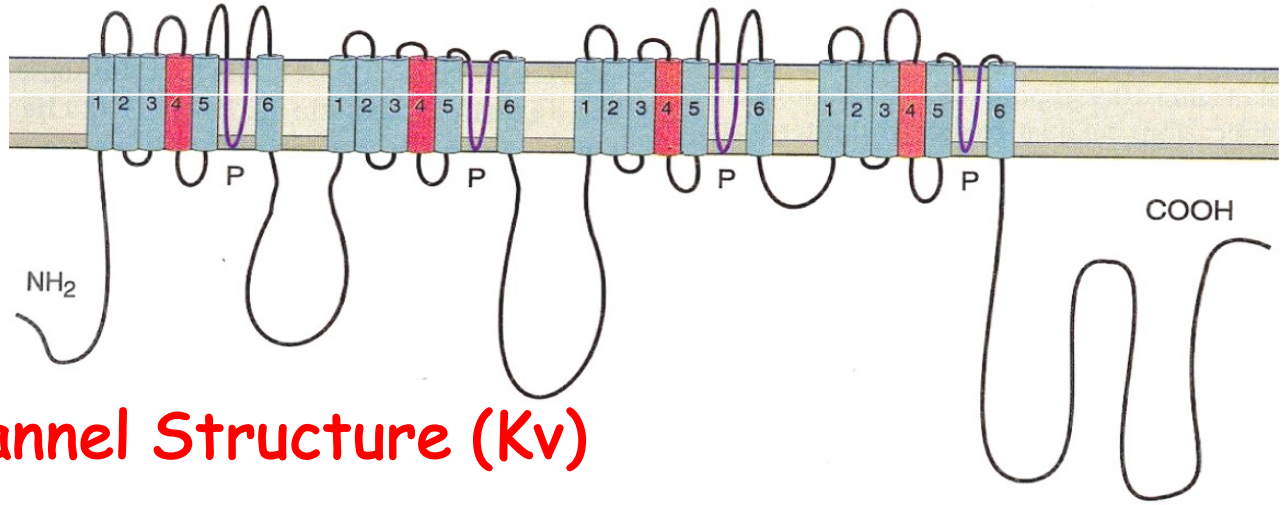

0 mV
-70 mV
Vm
Channel closed
i
Channel open
Closed
Open
Inactivated

Voltage-gated

- sodium: I, II, III, μ1, H1, PN3
- potassium: K_A, K_v (1-5), K_v(r), K_v(s),K_{SR}, BK_{Ca}, IK_{Ca}, SK_{Ca}, K_M, K_{ACh}
- calcium: L, N, P, Q, T
- chloride: ClC-0 - ClC-8

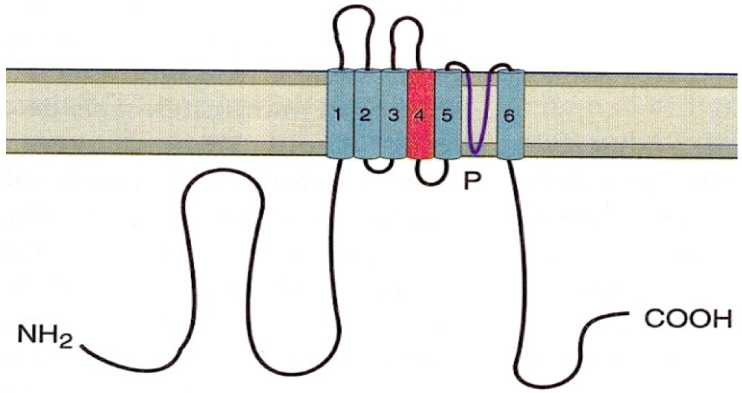
Sodium and calcium channel structure

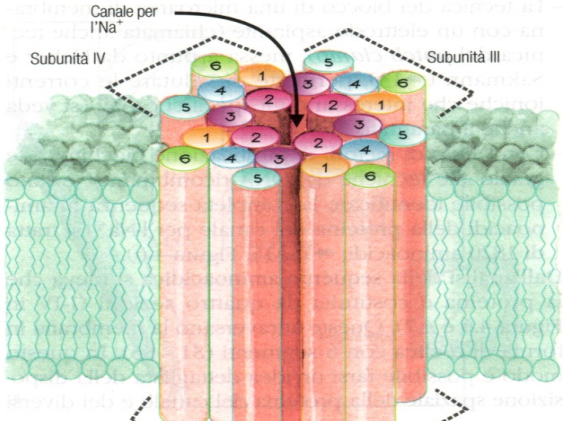
Large protein, a polypetide chain with 4 homologous domains (I-IV), each with 6 hydrophobic segments (S1-S6), P-handle between S5-S6.S4 aa basic loads = voltage sensor 6TM

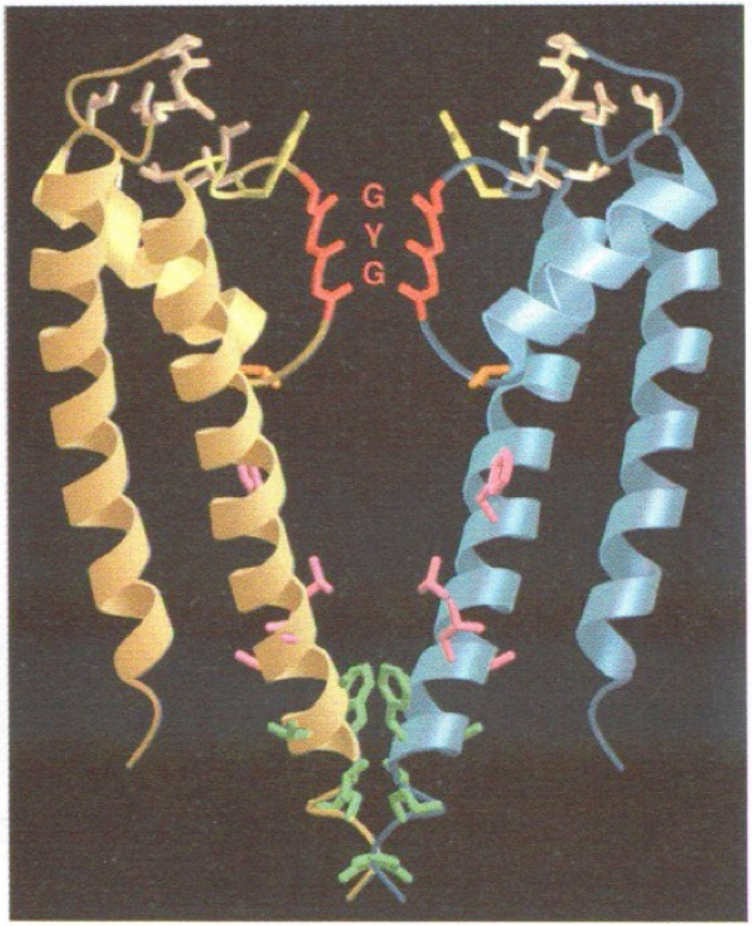


Potassium Channel Structure (Kv)

4 polypetide chains aggregated in tetrameric structure. Each with a similar domain to sodium and potassium 6TM (S1-S6)

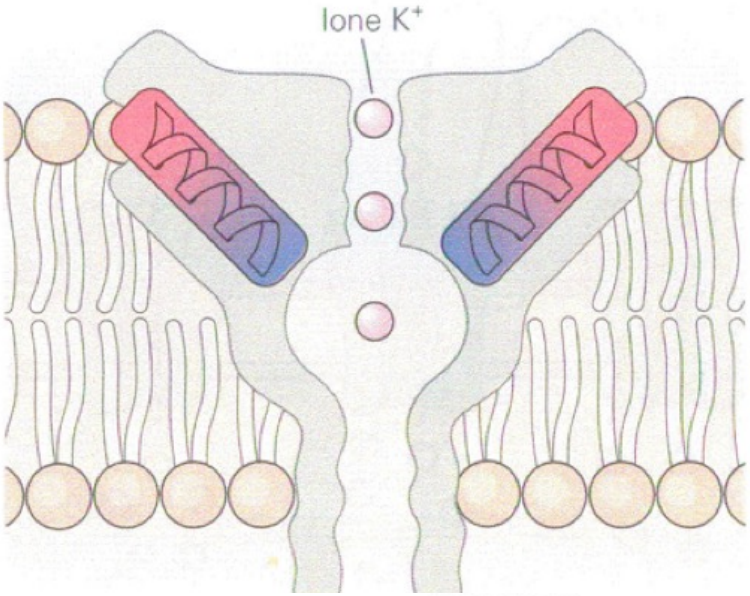


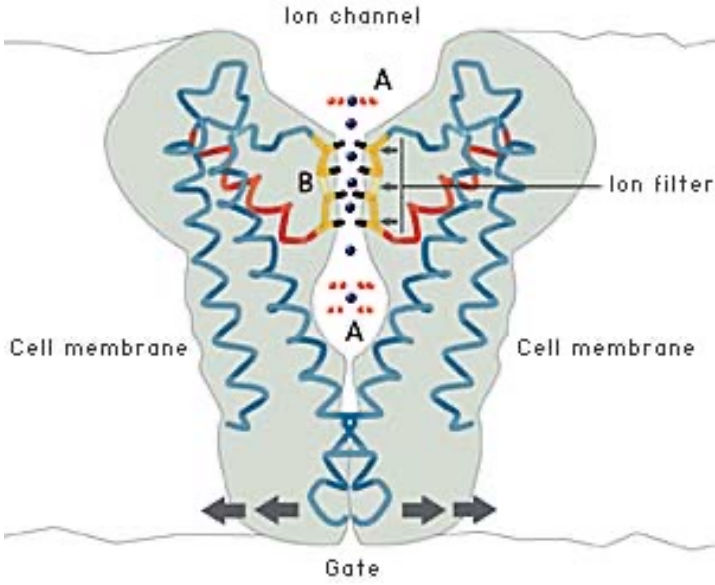




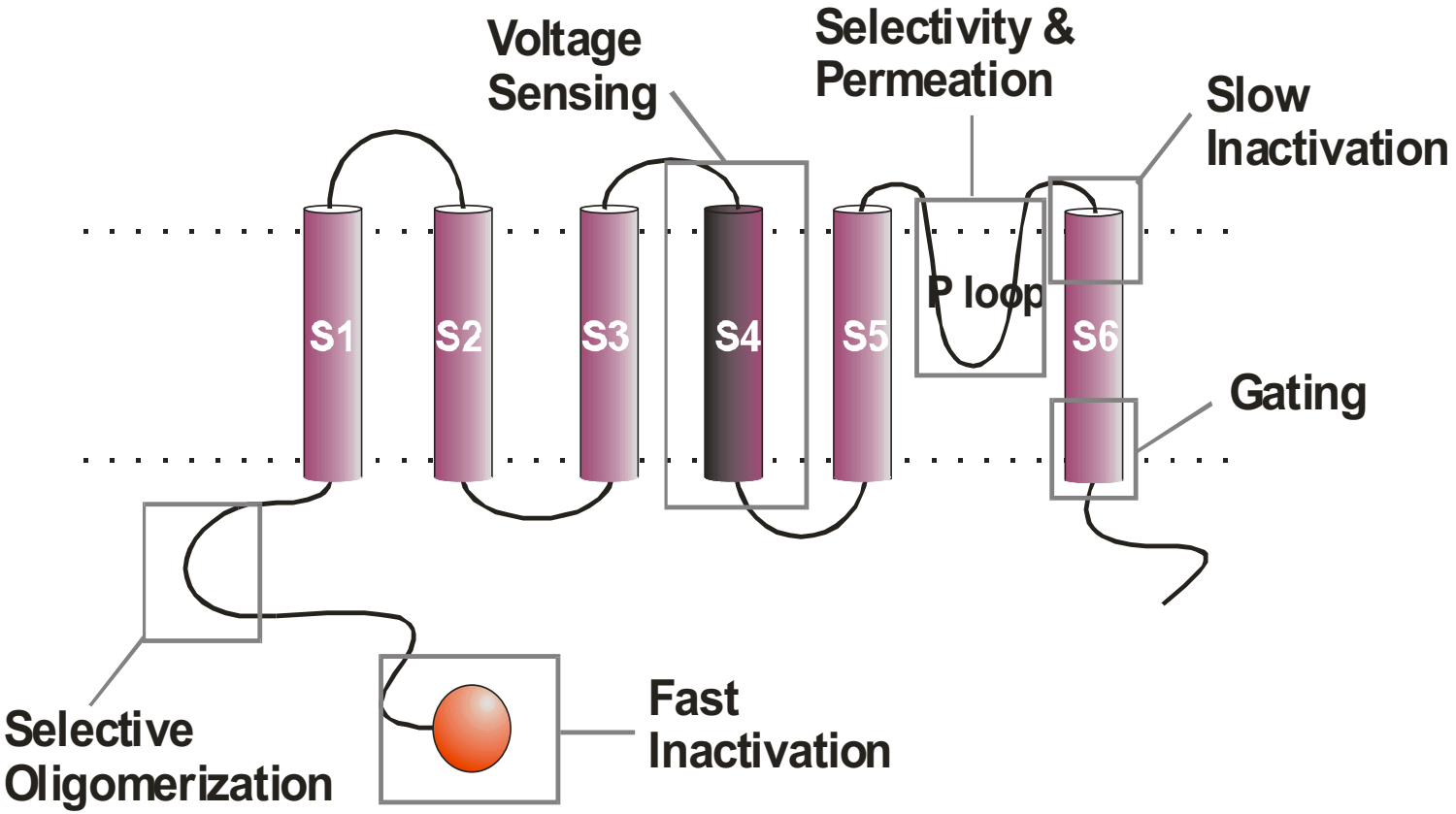
Bacterial K+ channel KcsA

MacKinnon, 1998





(4 subunits with two transmembrane segments connected by loop P, pore channel)

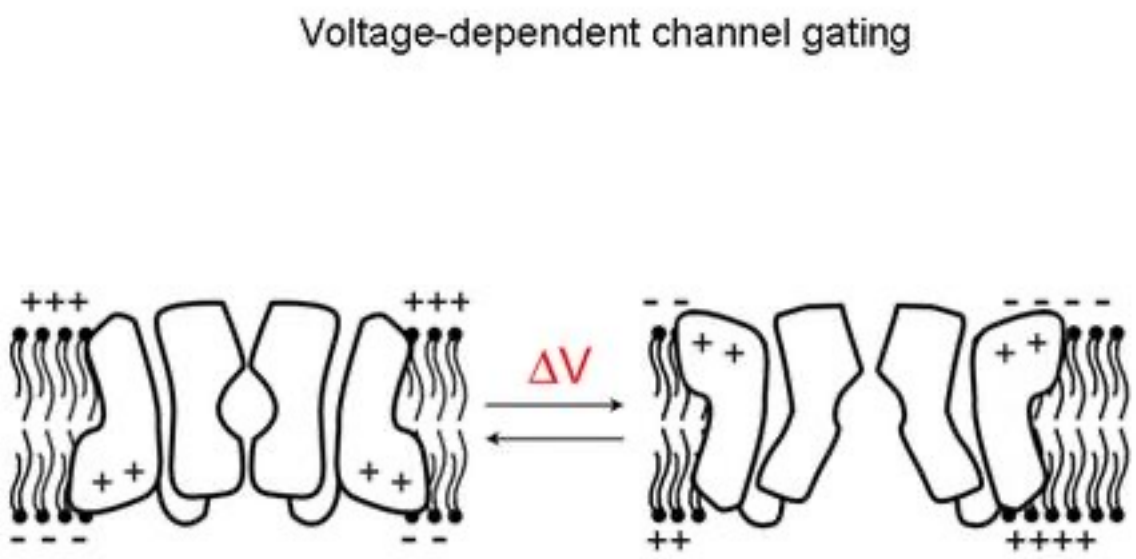

Voltage
Sensing
Selectivity &
Permeation
Slow
Inactivation
S1
S2
S3
S4
S5
P loop
S6
Gating
Selective
Oligomerization
Fast
Inactivation

Two classes of Voltage-dependent Ion Channels

channels open by depolarization

channels open by hyperpolarization

channels open by depolarization

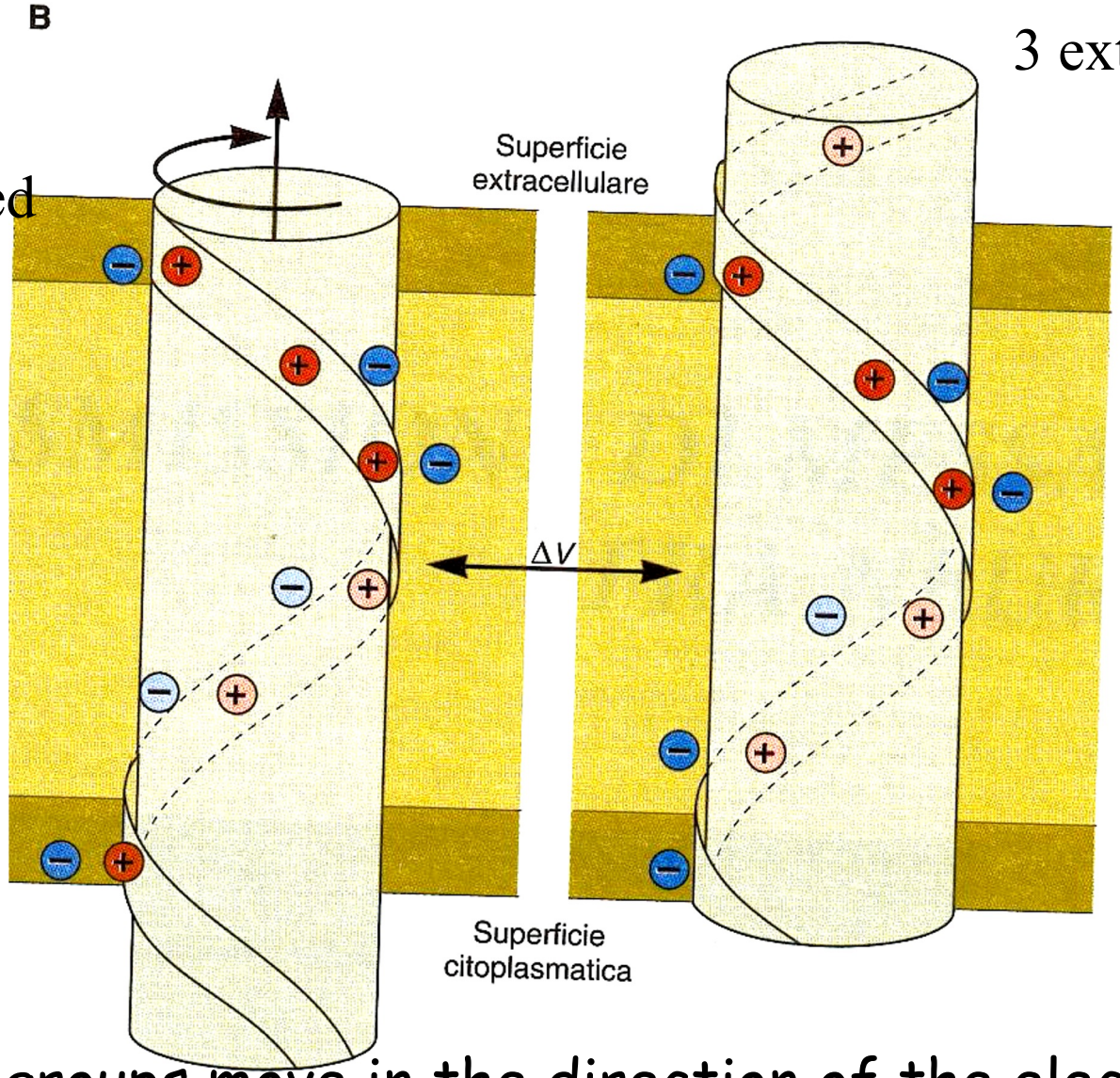


They are a family of selectively permeable channels for Na^+, K^+, Ca^{2+}

Voltage sensor

S4

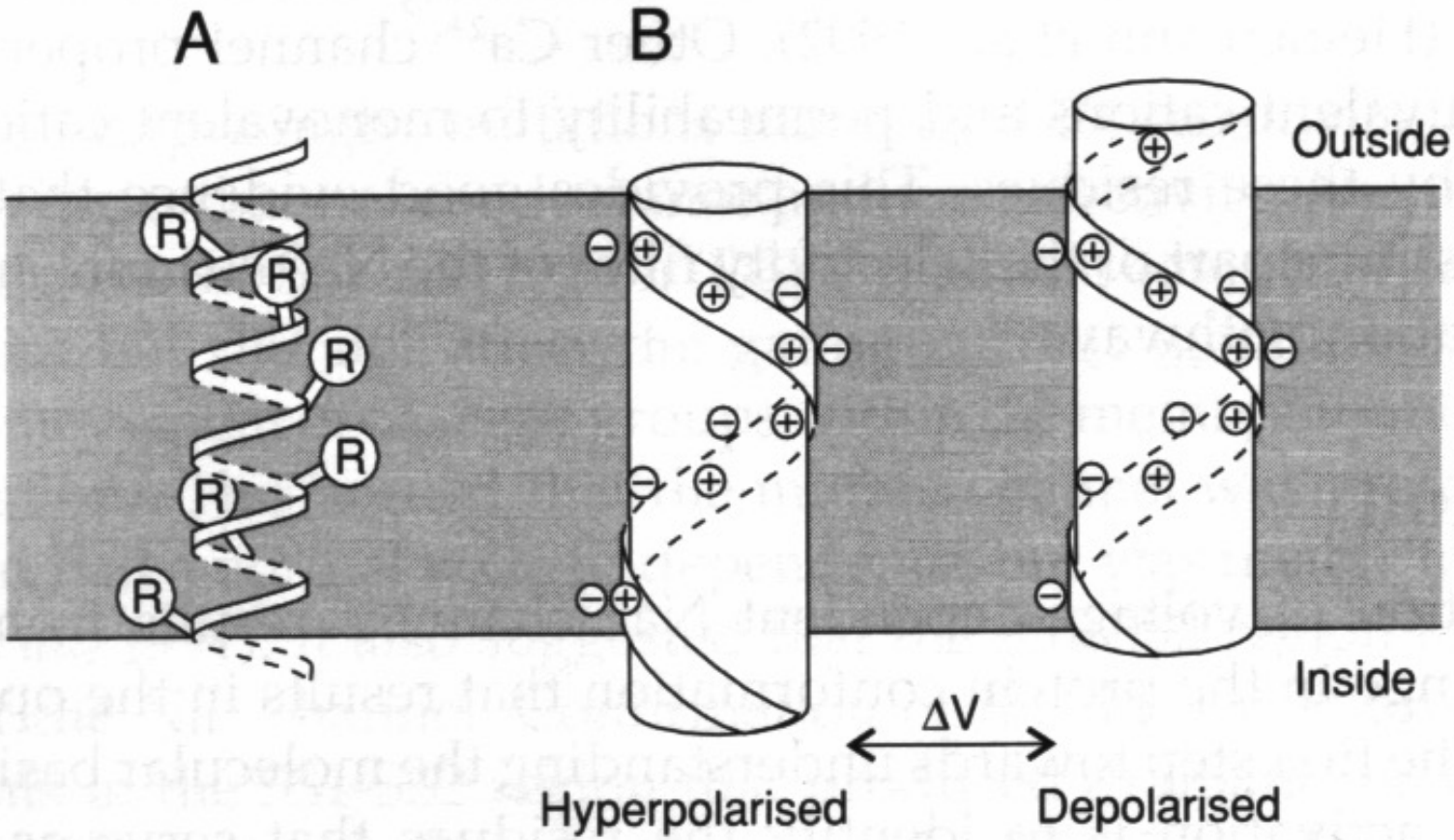
4-8 aa charged



3 external charges

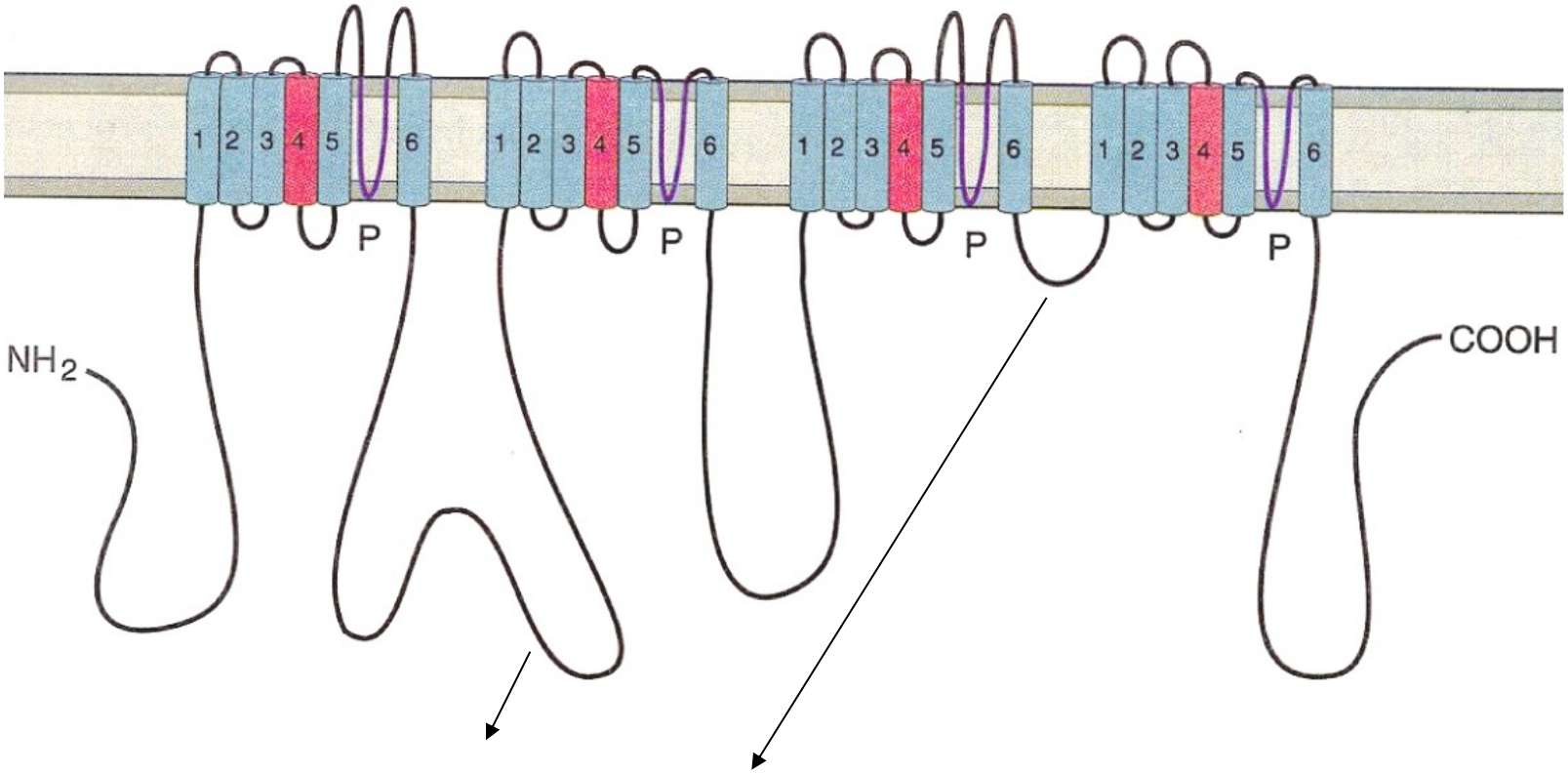
charged groups move in the direction of the electric field
(shown with transient currents)

gating

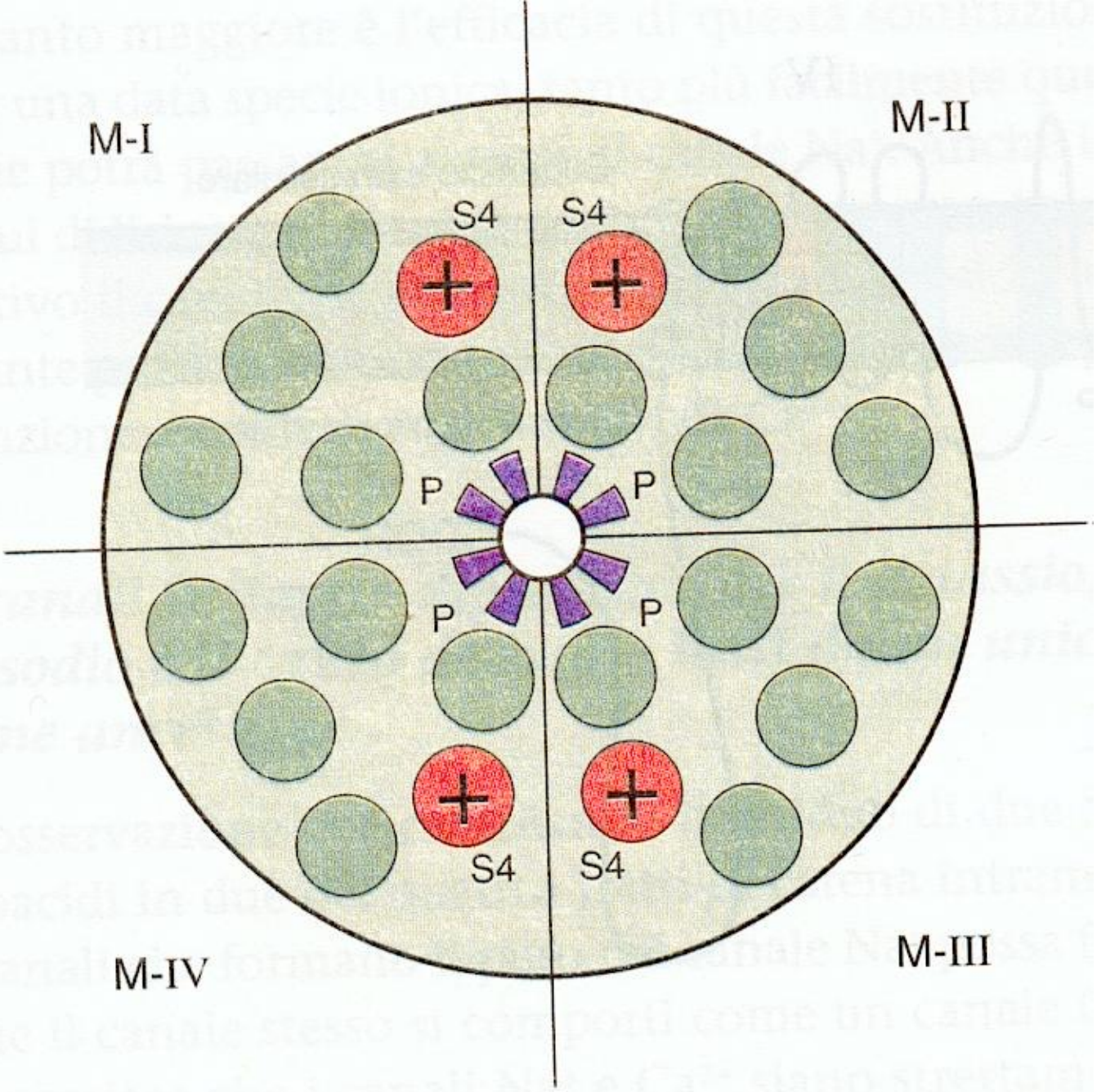


Voltage gated Na^+ channels

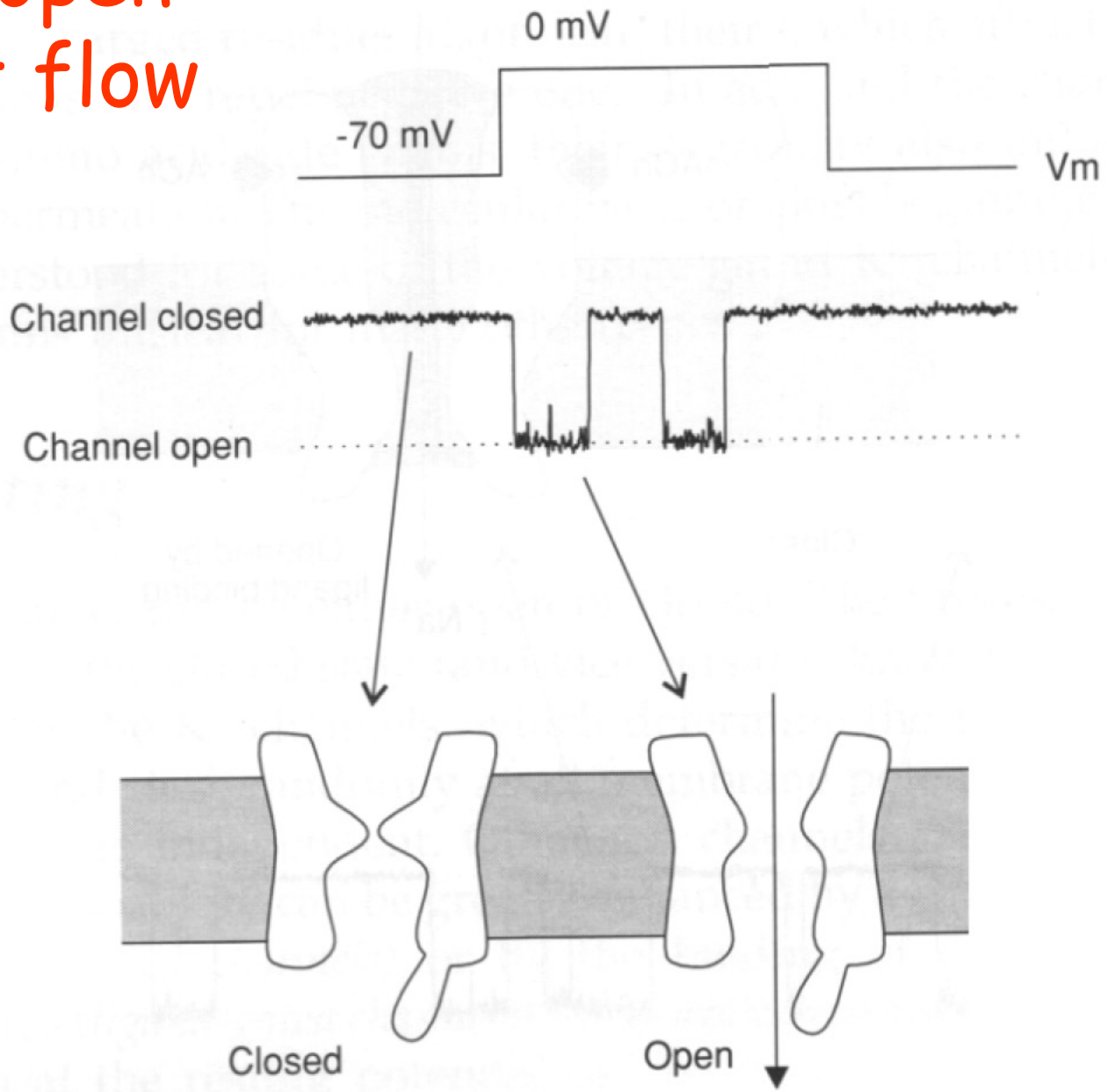
alpha subunit
(+ 1 o 2 beta accessorial subunits)



PKA and PKC phosphorylation sites

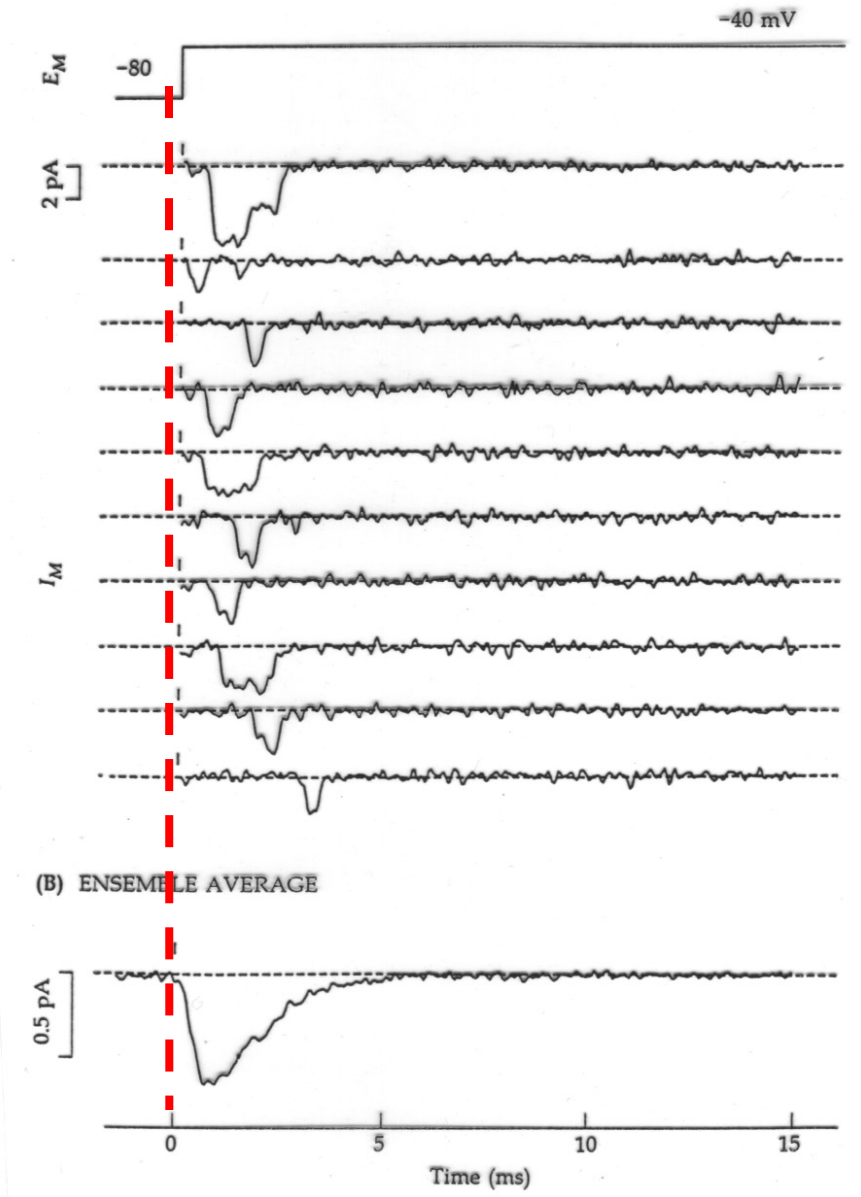

M-I
M-II
M-III
M-IV
S4
S4
S4
S4
P
P
P
P

Channel open = current flow



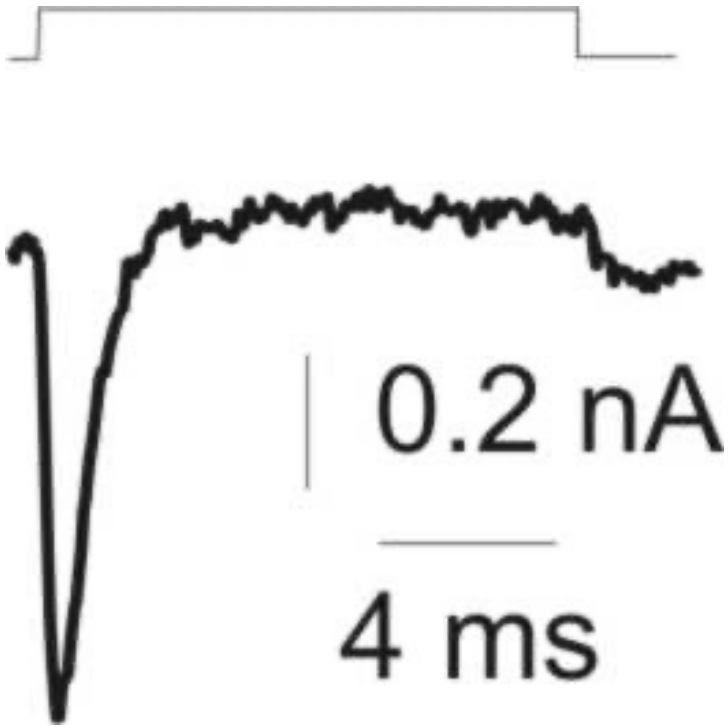
Channels selectively permeable to Na^+

- Rapid activation
- Rapid inactivation

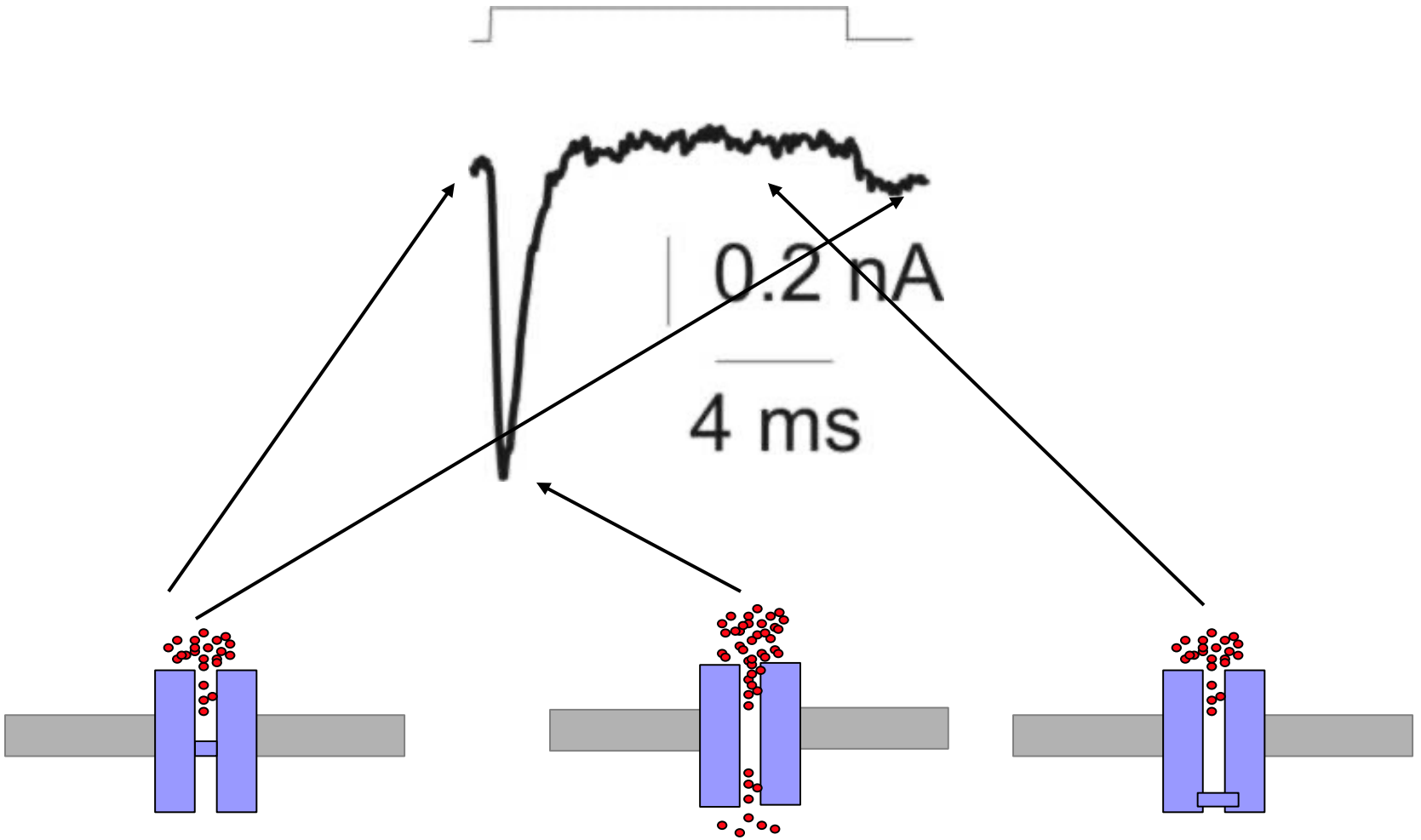


Channels selectively permeable to Na^+

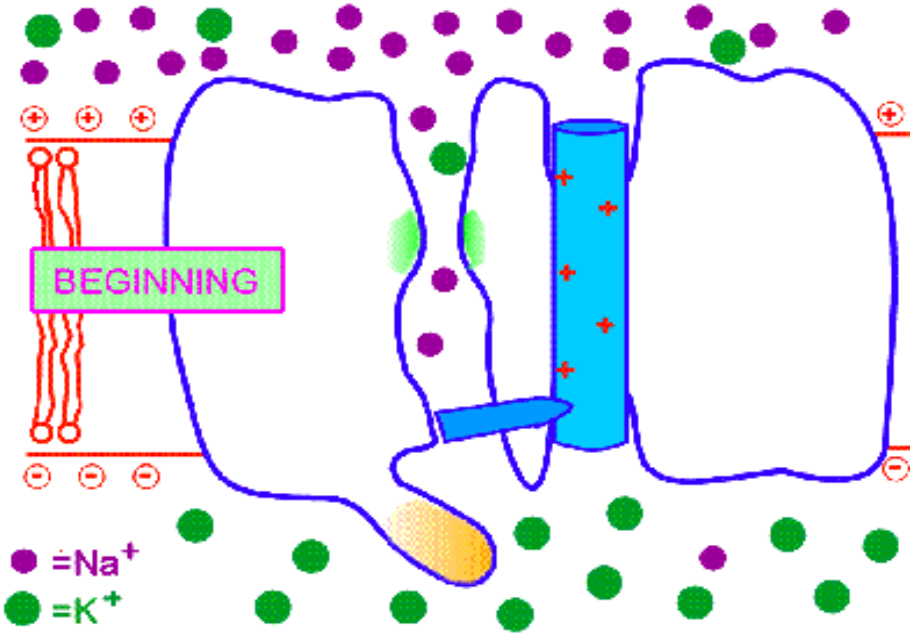
- Rapid activation
- Rapid inactivation

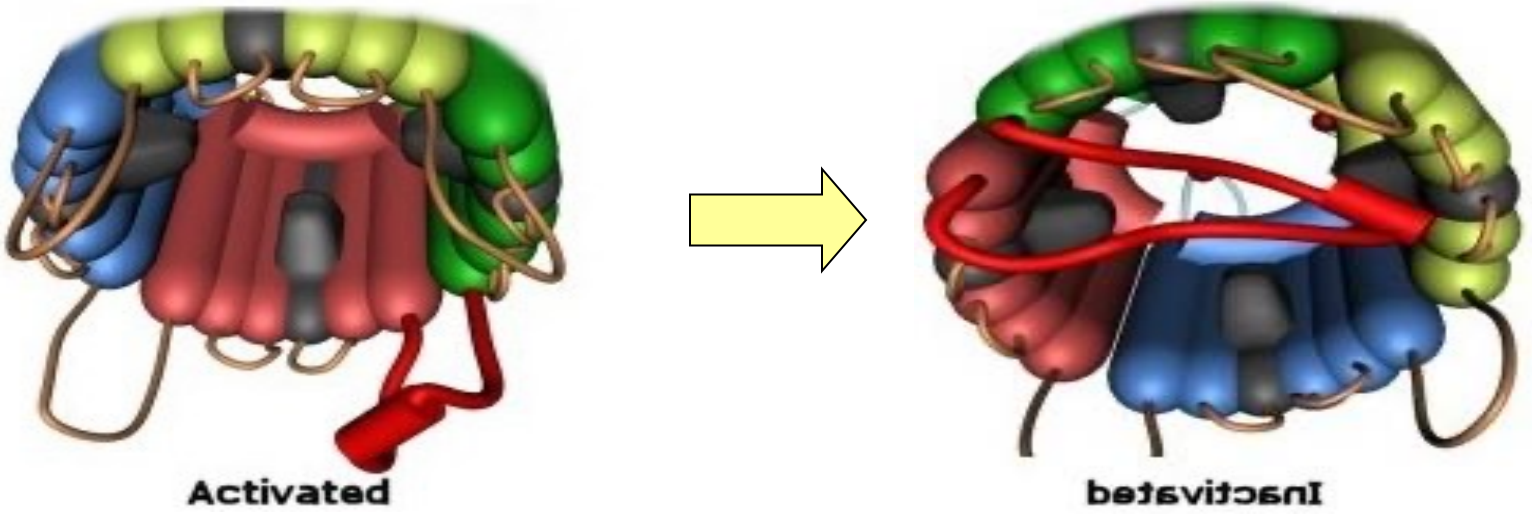


Inactivation - Na^+



Voltage gated Na^+ channels





Voltage gated Na^+ channels blockers

- Tetrodotoxin (TTX)
- Amioderone
- Lidocaine
- Procainamide
- Mexilitine
- Ketamine
- Many, many others

Opening of voltage gated Na^+ channels $\Rightarrow$ depolarization (E_{Na}=+55 mV)

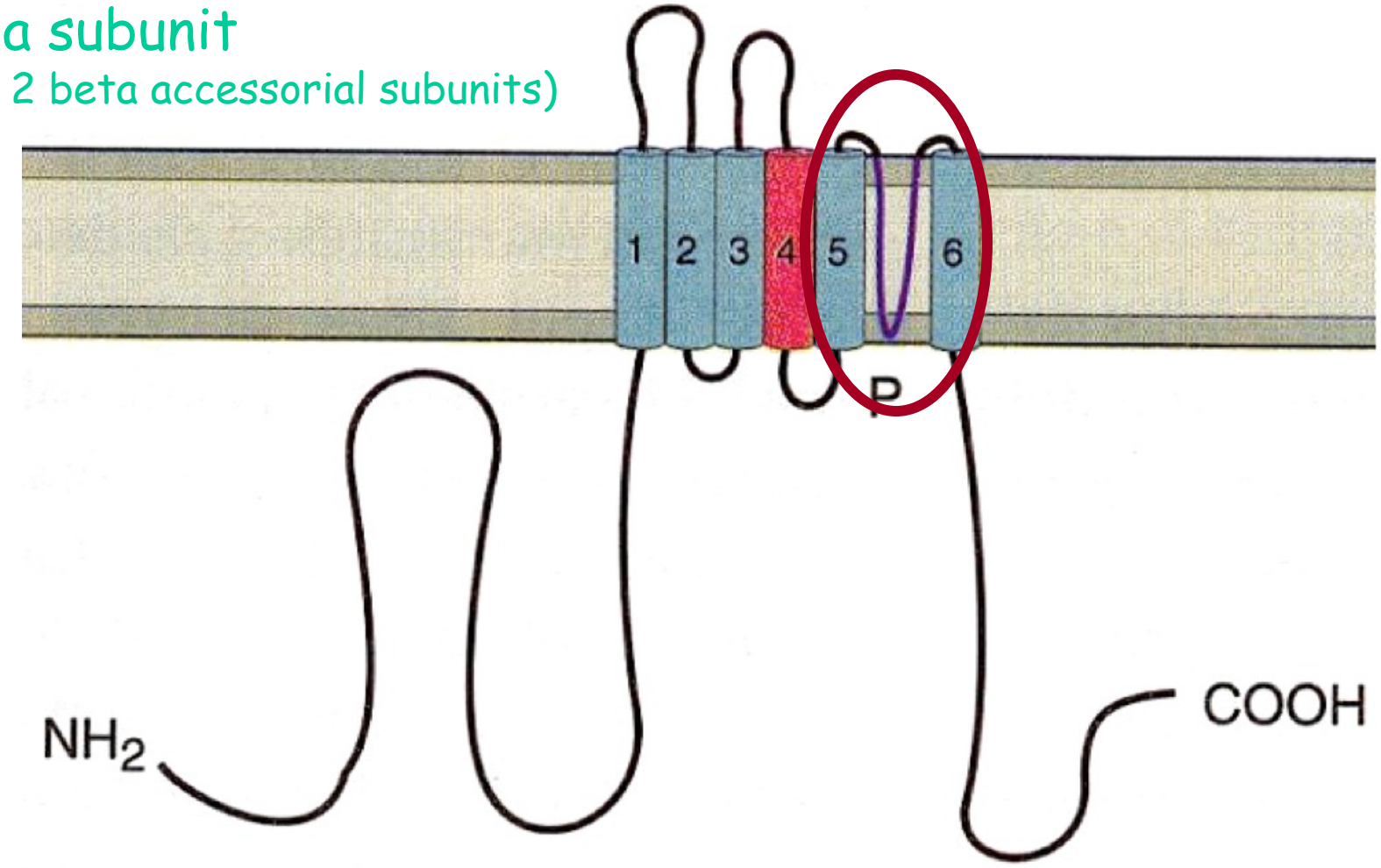
Na^+ channels in:

- skeletal muscle
- working myocardium
- Axons

Important in
1)Generation and propagation of acton potential
2)Depolarization post action potential
3)Discharge frequency regulation

Voltage gated K+ channels

alpha subunit
(+ 1 o 2 beta accessorial subunits)



Selectively permeable to K^+

There are various channel families for K +

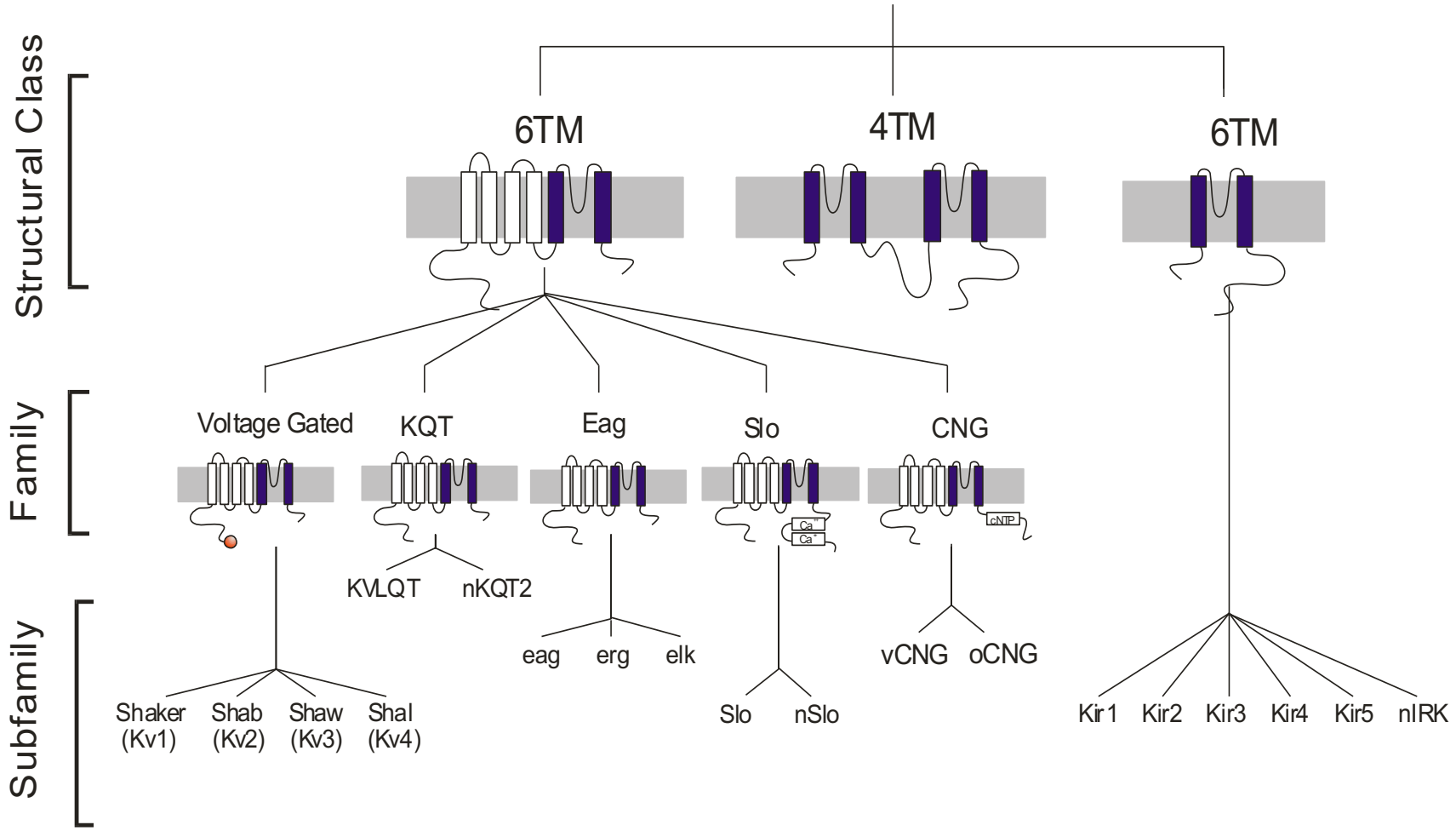
they are found in all cell types

Channel opening $K^+ \Rightarrow$
repolarization (E_K=-90 mV)

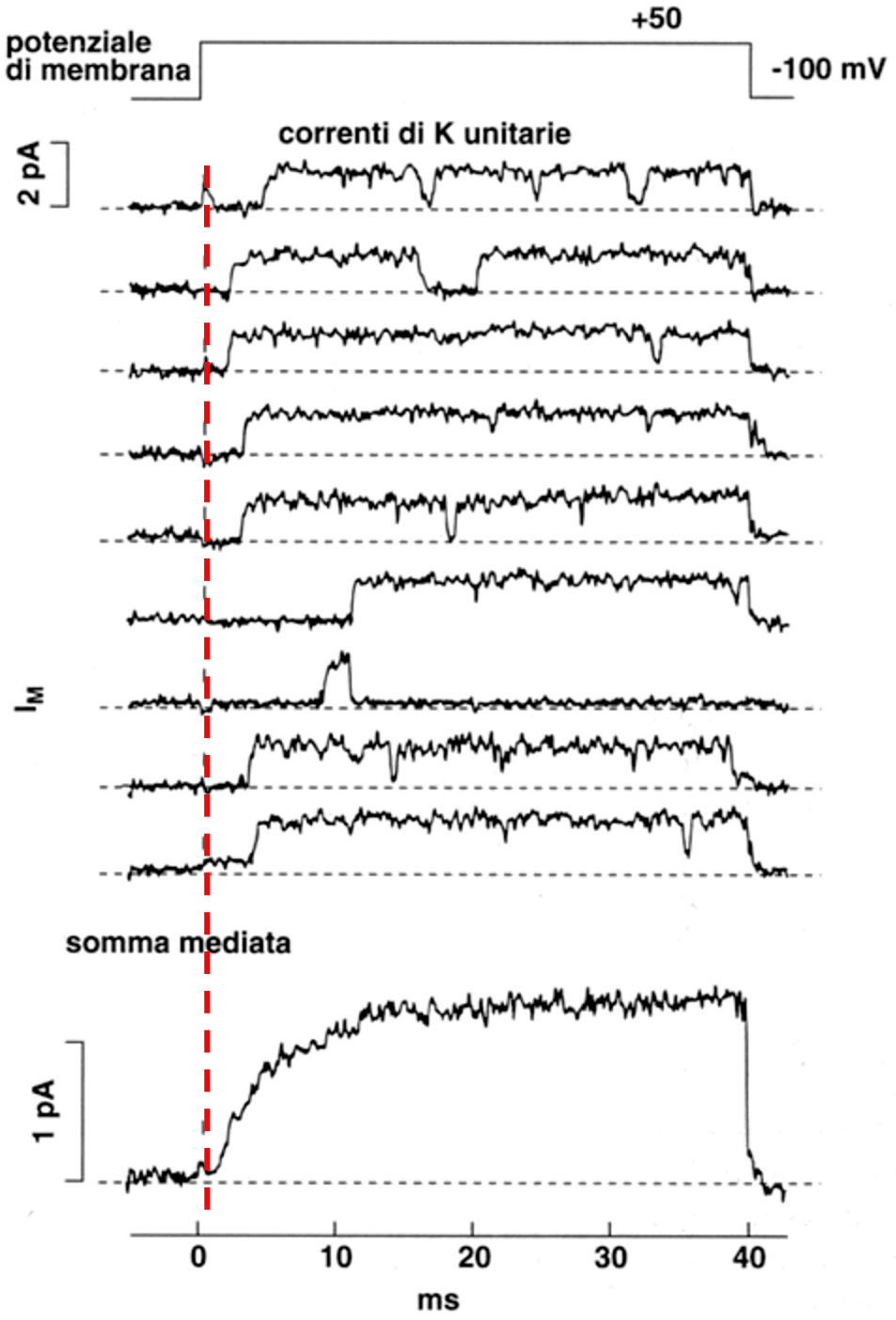
Important in excitable cells for:
1) They contribute to the resting potential
2) They support the repolarizing phase of the action potential
3) Inhibitively contribute to synaptic integration

canali K^+ voltaggio-dipendenti

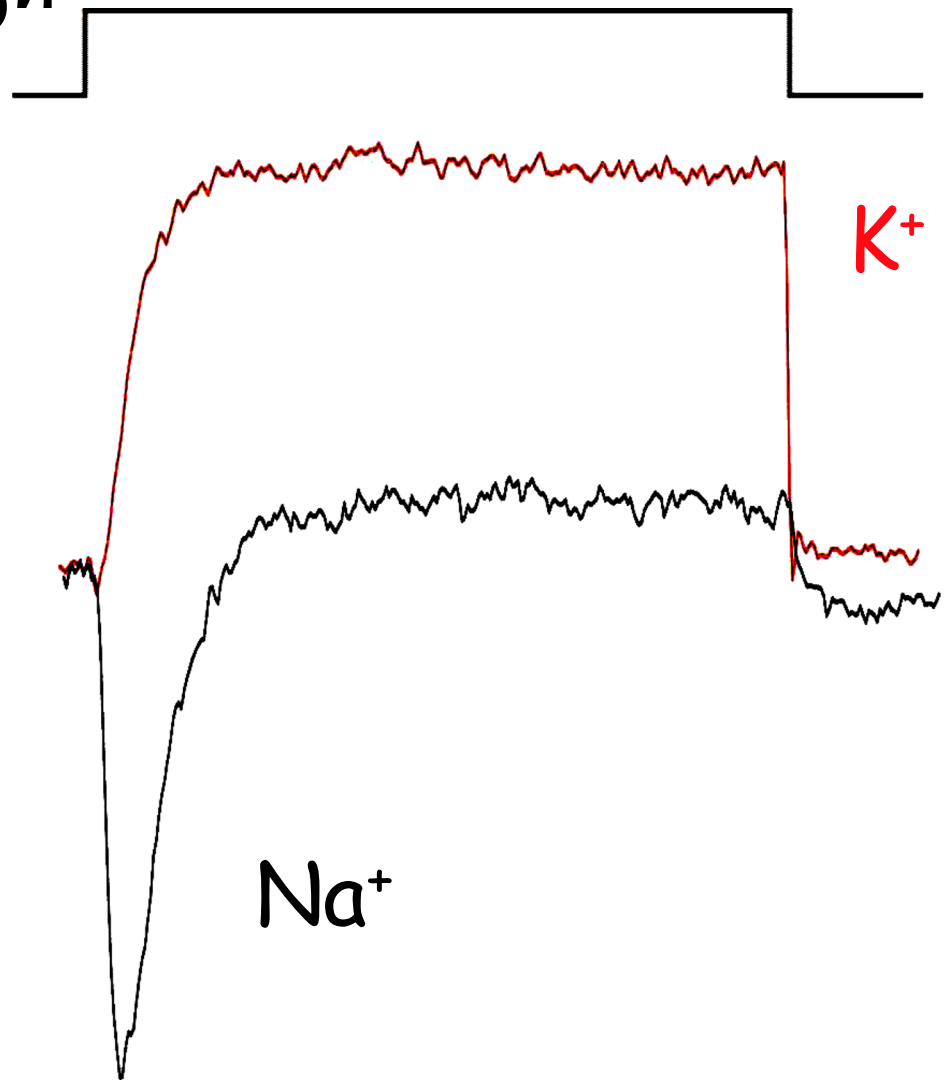
(more than 100 genes!!!

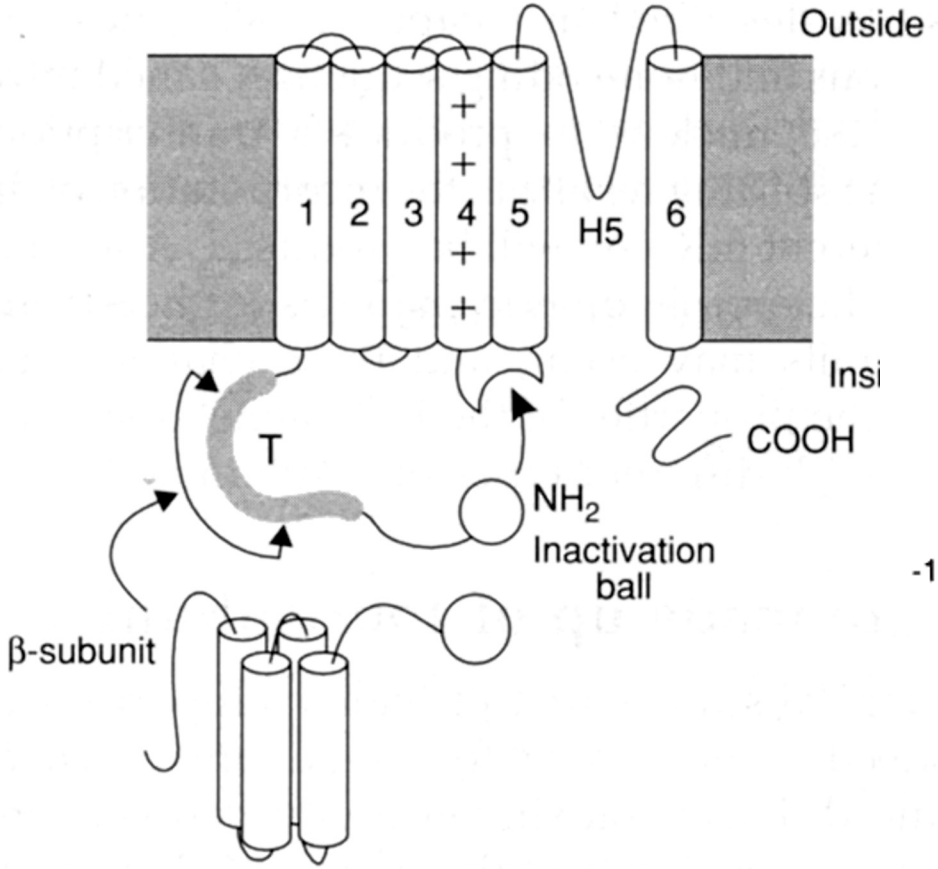


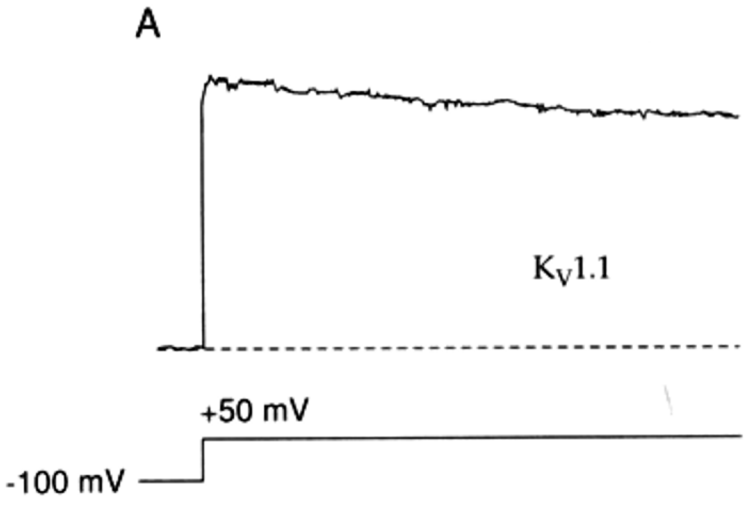
Channels in the axons

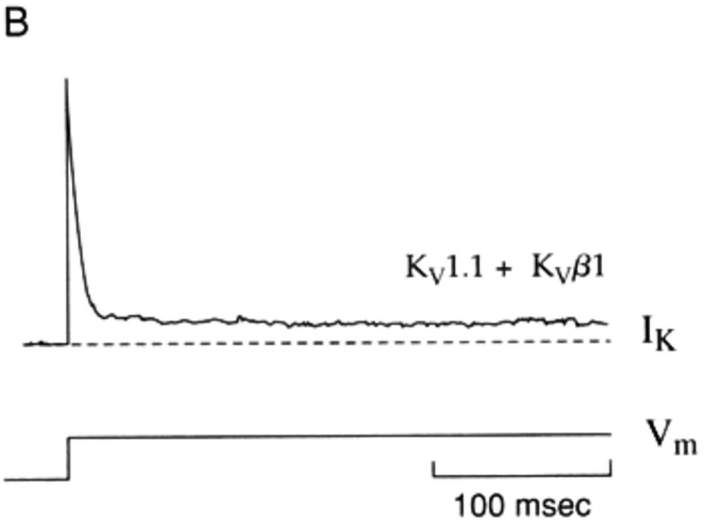


comparison







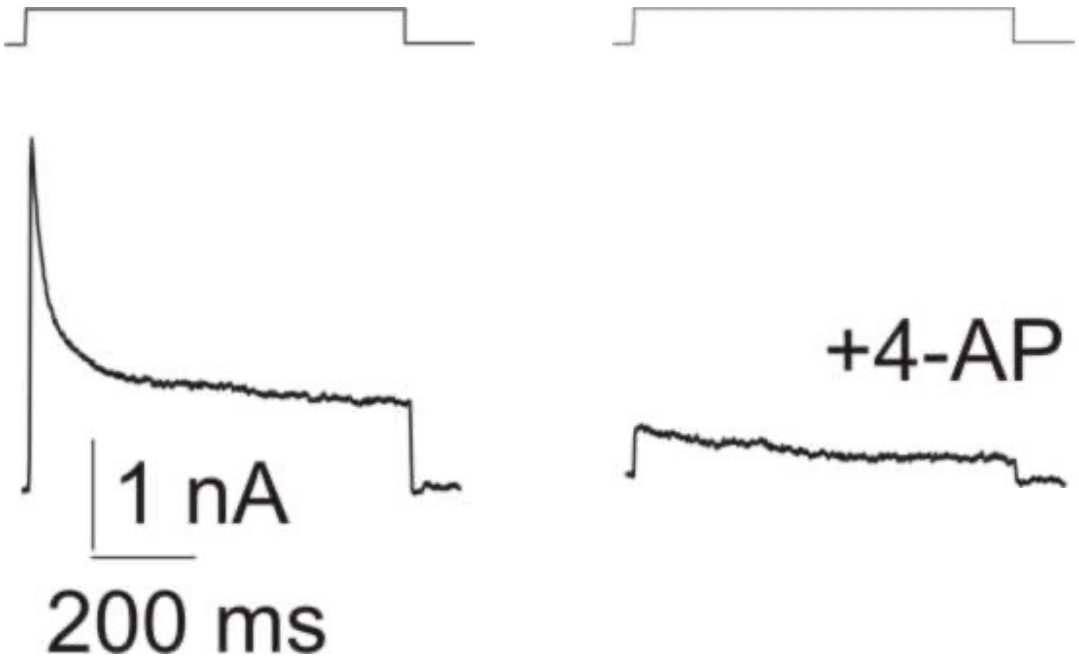


Omomerici

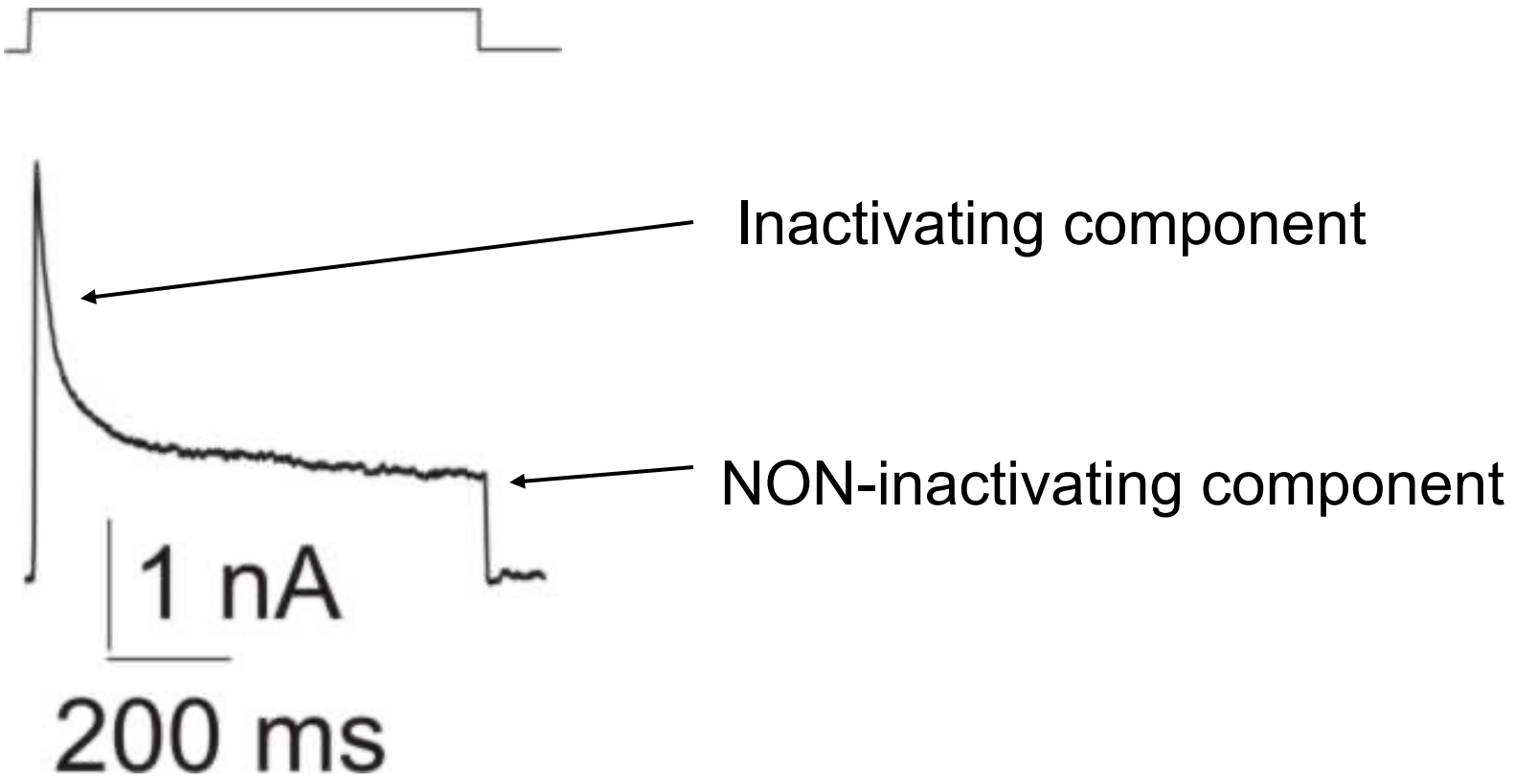
Eteromerici

Channels selectively permeable to K^+

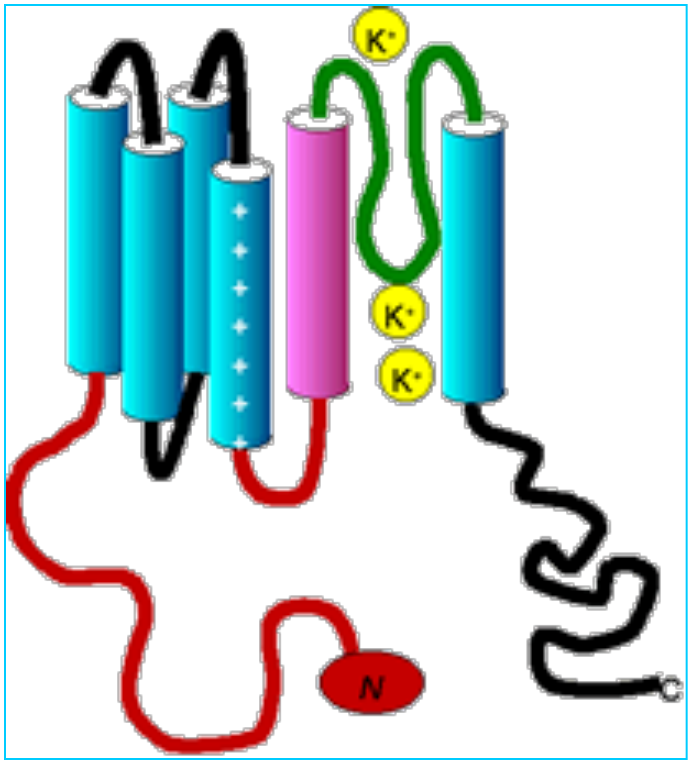
Many types of K + channels are blocked by 4 aminopyridine

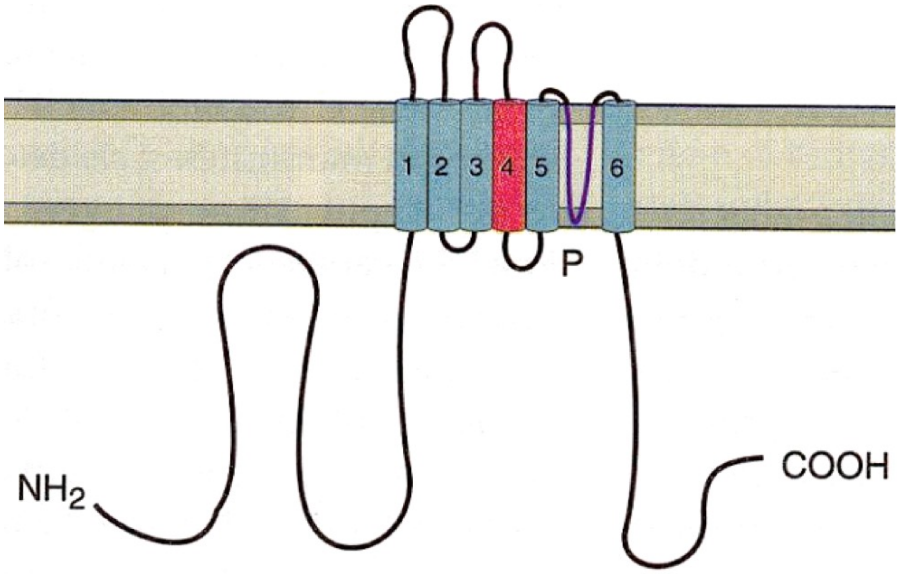


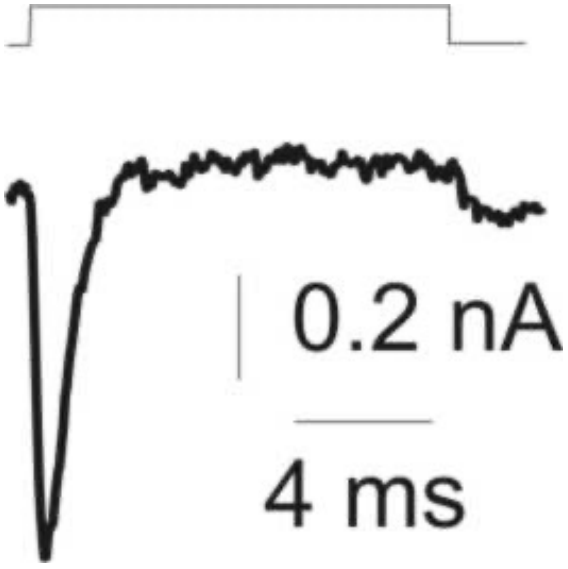
Different inactivation profile



There are different combinations of subunits


K+
K+
K+
N
C

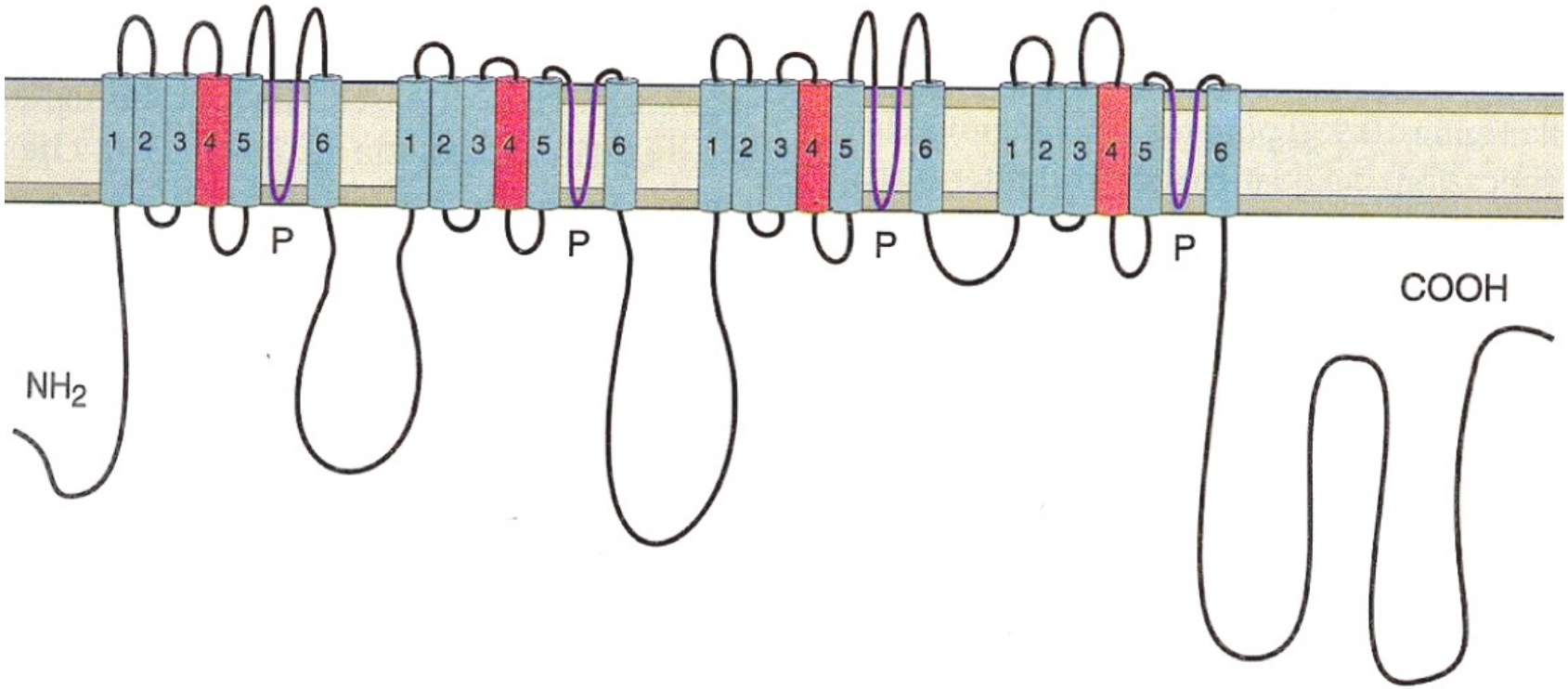

1
2
3
4
5
6
P
NH2
COOH


0.2 nA
4 ms

Voltage gated Ca^{2+} channels

Alpha Subunit

(+ 1 beta, alpha2, delta and gamma)

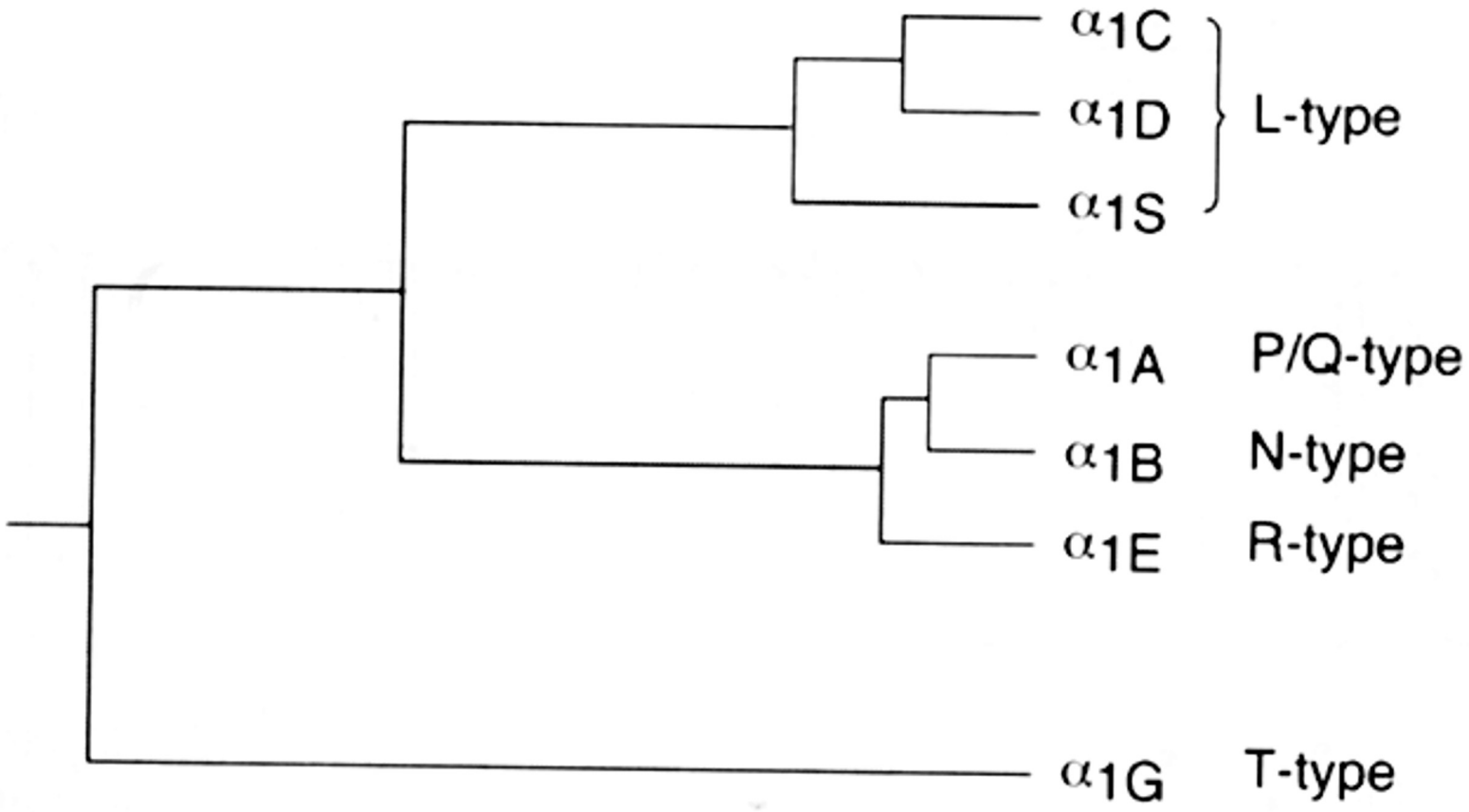


They transduce electrical signals into metabolic signals

$[Ca^{2+}]_{rest}$ = 100 nM

$[Ca^{2+}]_{stim}$ = 1000 nM

(1 μM)

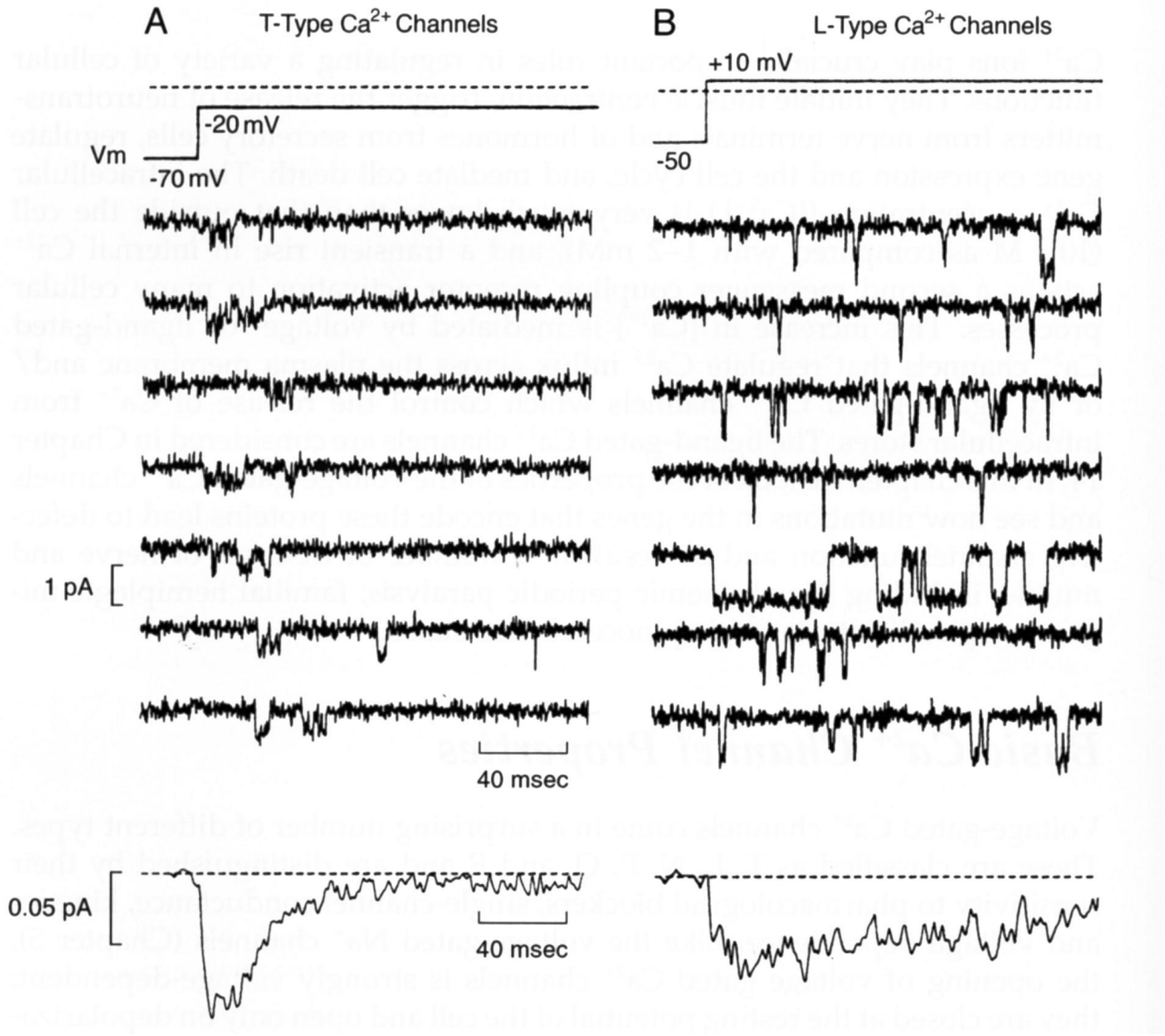

α1C
α1D
L-type
α1S
α1A
P/Q-type
α1B
N-type
α1E
R-type
α1G
T-type

There are 2 types of voltage-dependent channels for Ca2+:

HIGH threshold
(open for large depolarizations)

LOW threshold
(open with slight depolarizations)

Low threshold and High threshold



Low threshold and High threshold

Low threshold

In posthumous hyperpolarization
Repeated discharge control
Generation of action potentials independent of Na

Inhibite: amiloride, low nickel conc

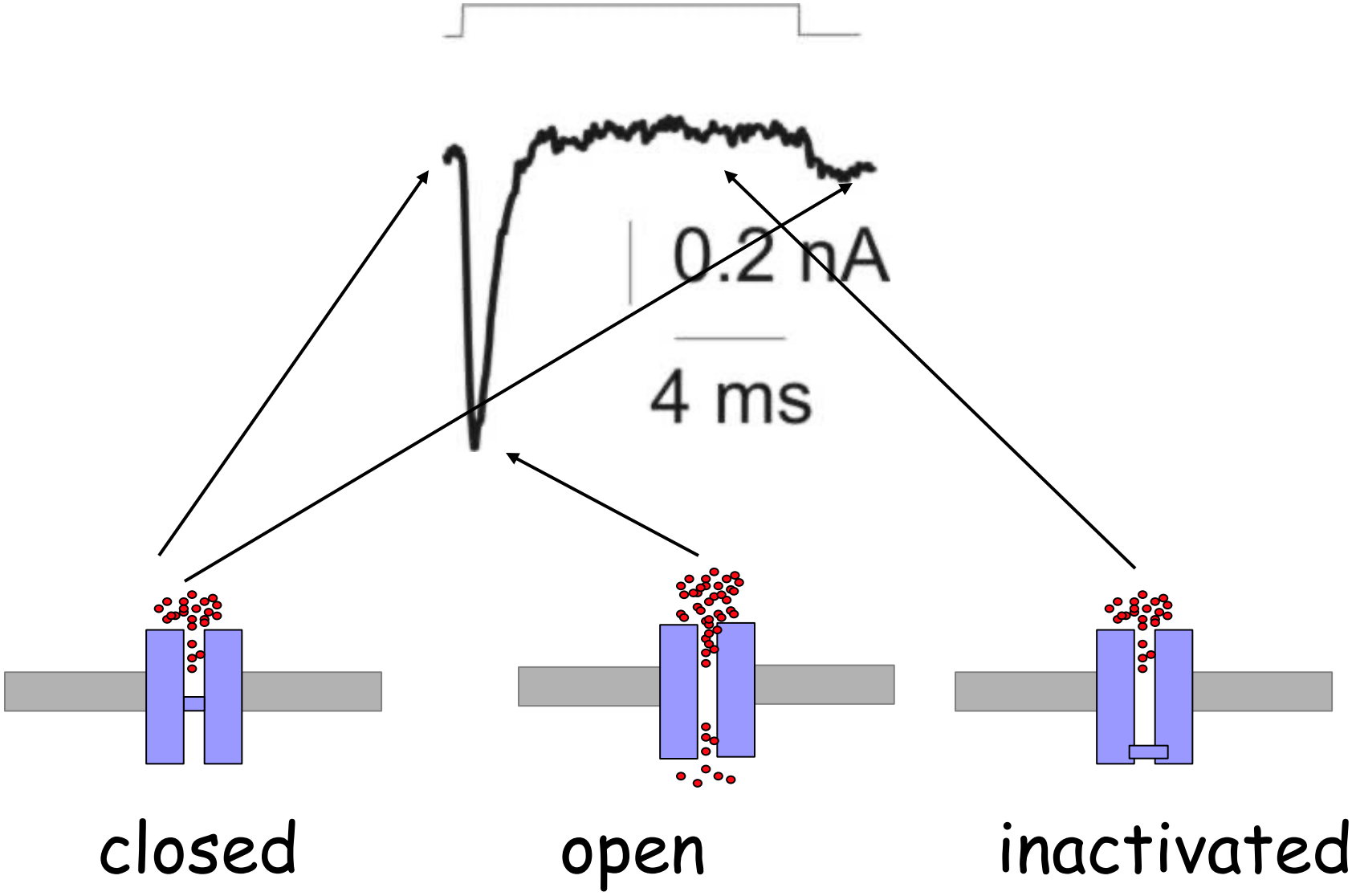
High threshold

Activate Ca-dip potassium channels
Vesicular exocytosis

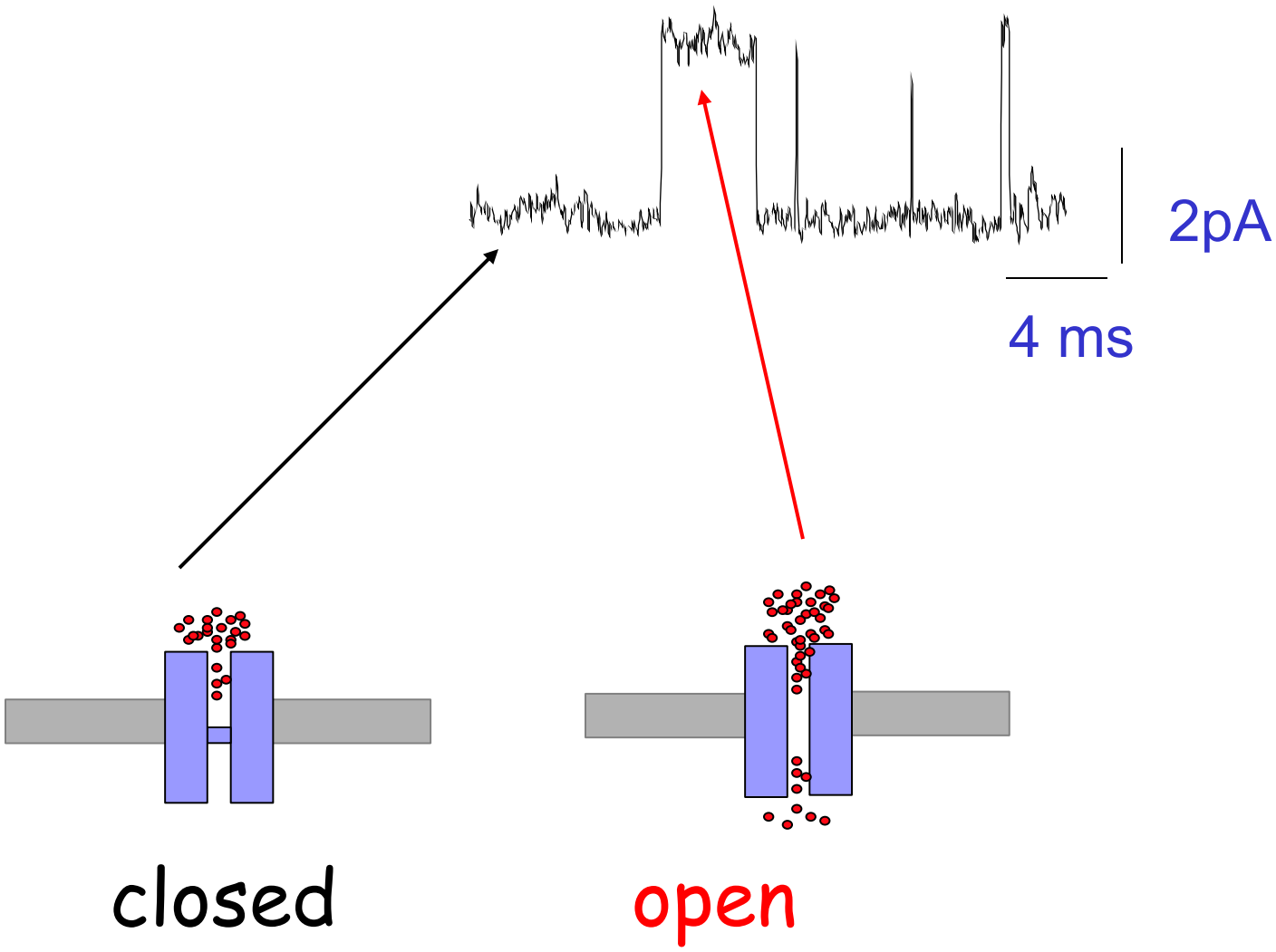
Dihydropyridine inhibitors: L channels
Not inhibited by dihydropyridines: non-L channels (N, R, P/Q)

Parameters that characterize the ion channels

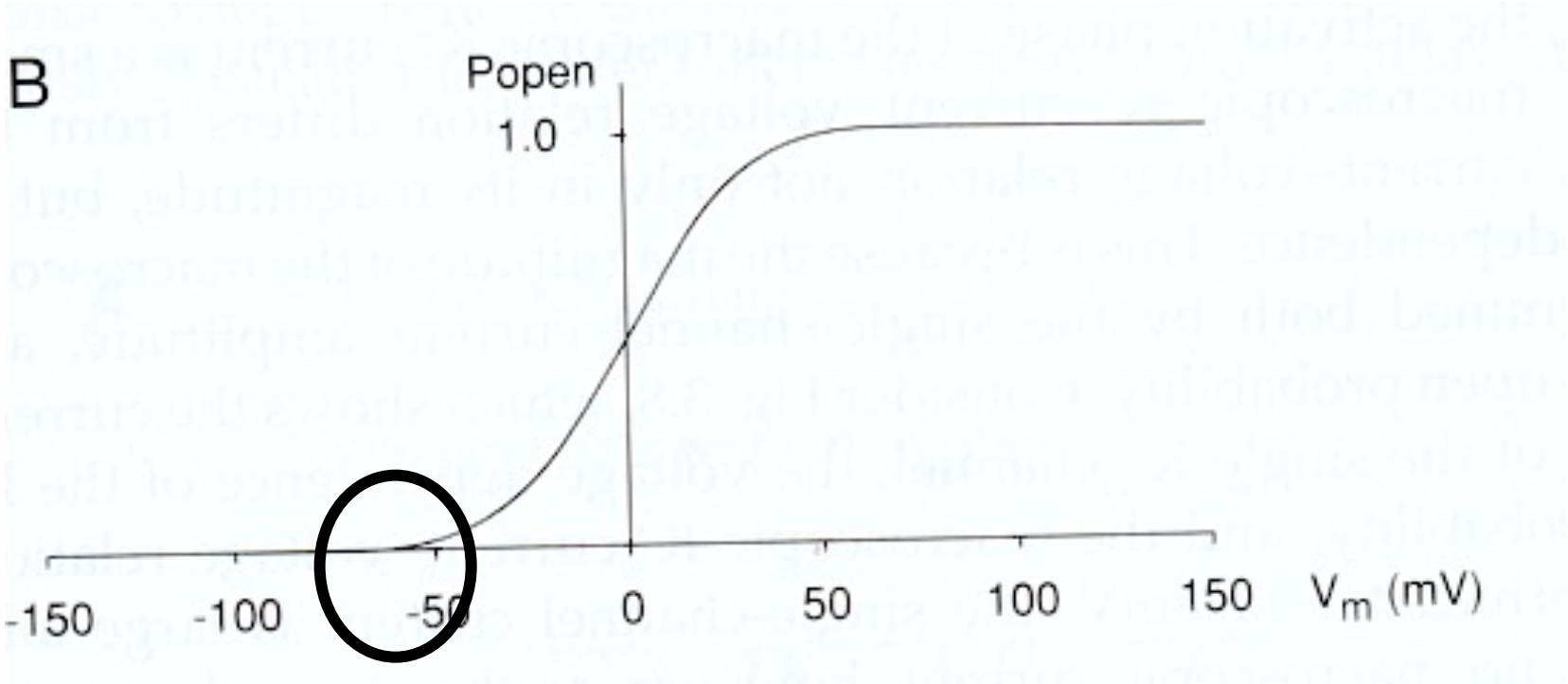
Macroscopic and microscopic



Conductance

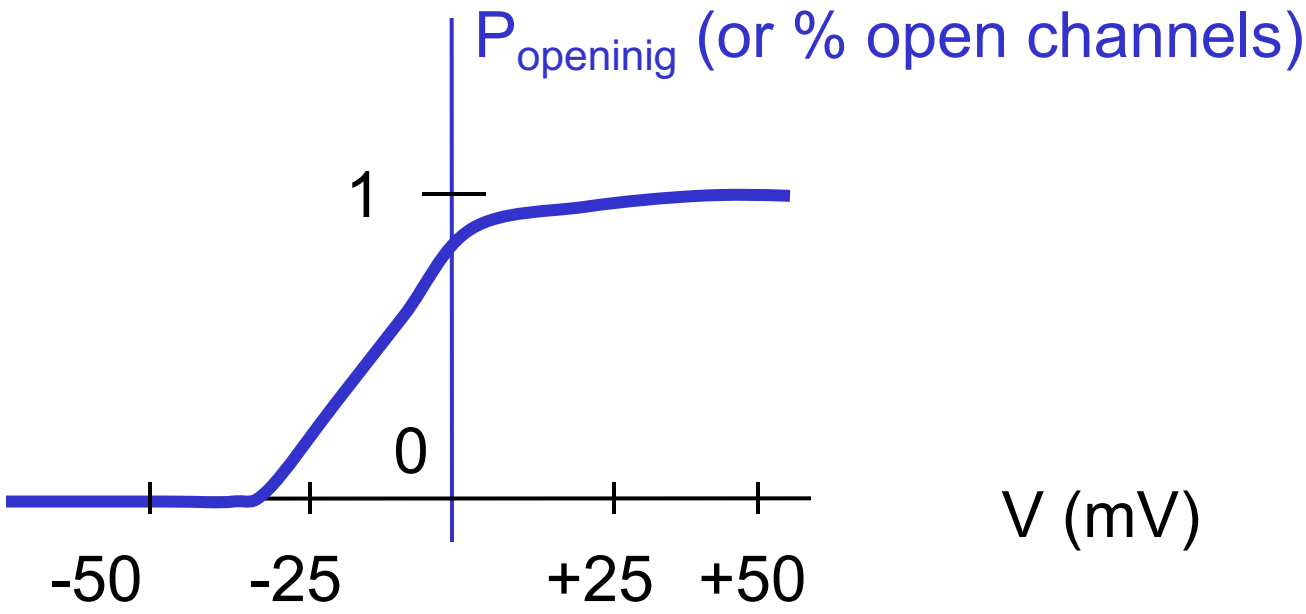


"threshold"
potential



Current-voltage

Opening probability



Electrochemical gradient

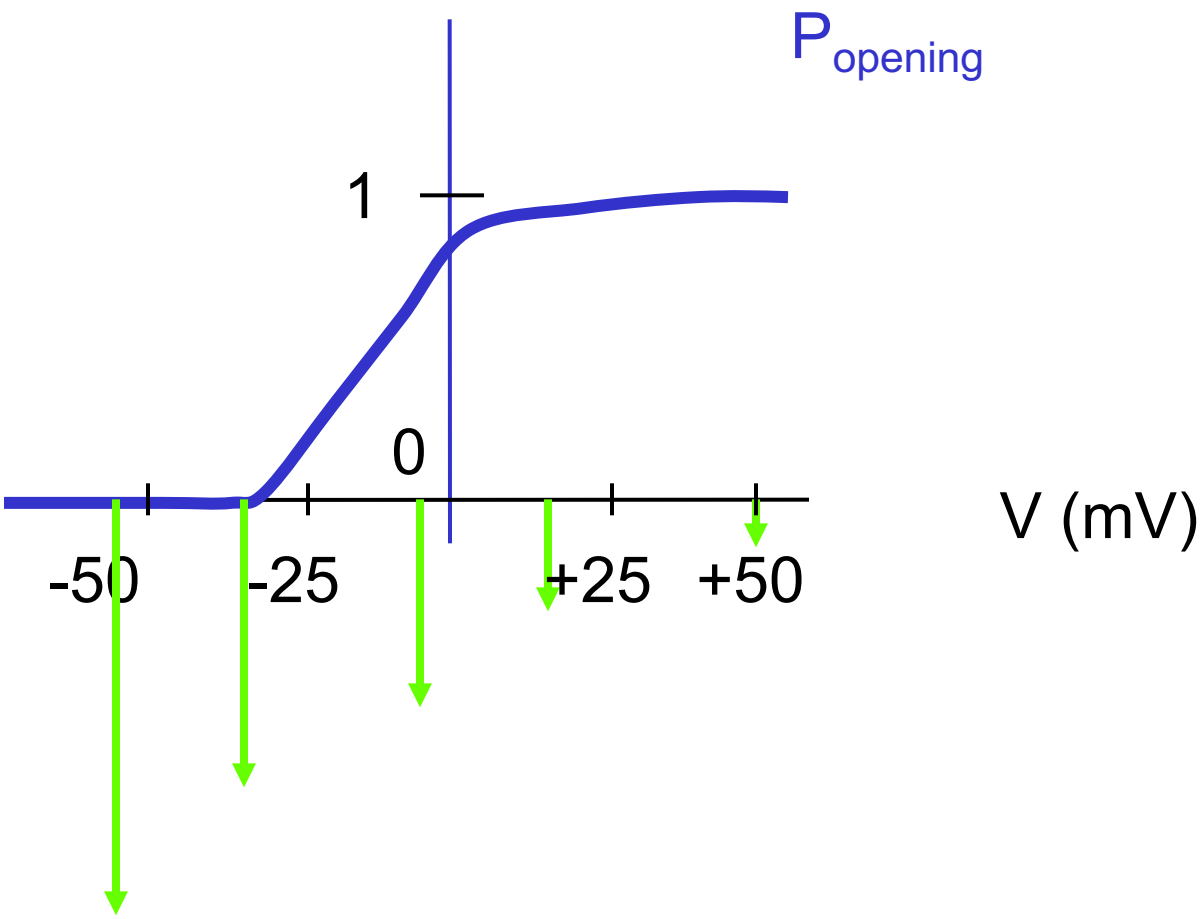
$I = 1/R \times V$

$I = \text{conductance} \times df$

current = No. of open channels x (channel conductance) x df

current = No. of open channels x (channel conductance) x (Vm -ENa)

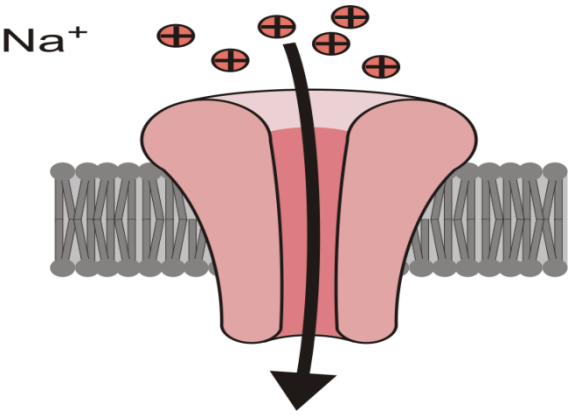
Na^+



The incoming flows of Na +

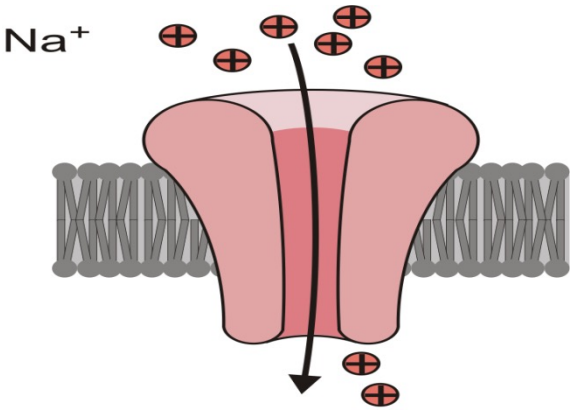
f.e.m. = V_m – E_{Na} con **E_{Na} = + 63mV**

The total current of Na + which enters the cell depends not only on f.e.m. also from the number of channels open to a certain potential, or from the conductance (gNa):



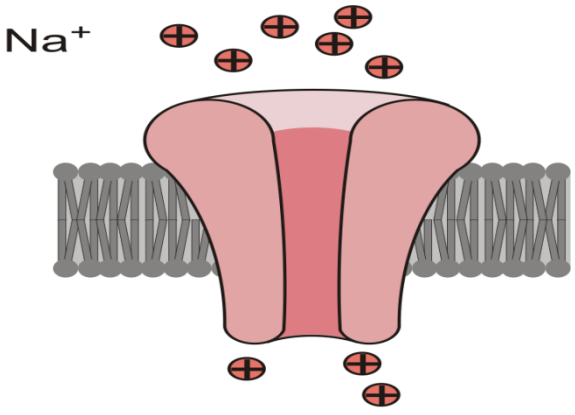
--35 mV

10% di canali aperti
f.e.m. **elevata**
flussi di Na+ **elevati** (s.c.)
I_{Na} totale **bassa**



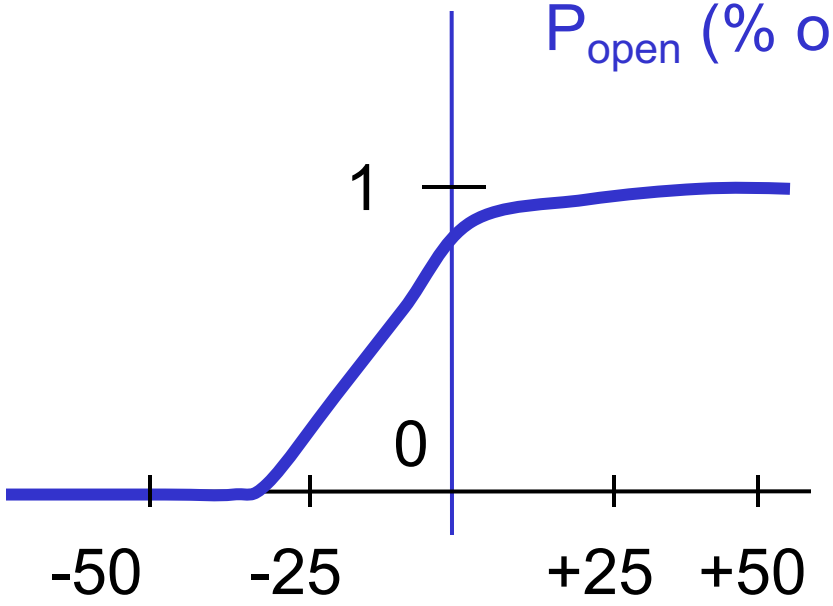
0 mV

80% di canali aperti
f.e.m. **media**
flussi di Na+ **medi** (s.c.)
I_{Na} totale **massima**

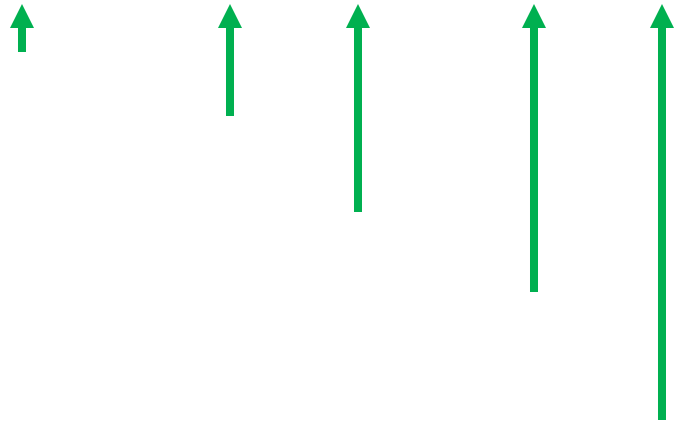


+63 mV

100% di canali aperti
f.e.m. **zero**
flussi di Na+ **nulli** (s.c.)
I_{Na} totale **zero**



K^+

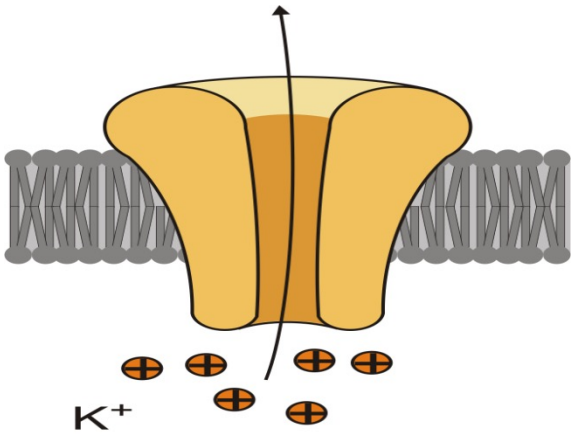


Electrochemical gradient

Current = N. open channels x (V_m -E_K) x (conduttanza canale)

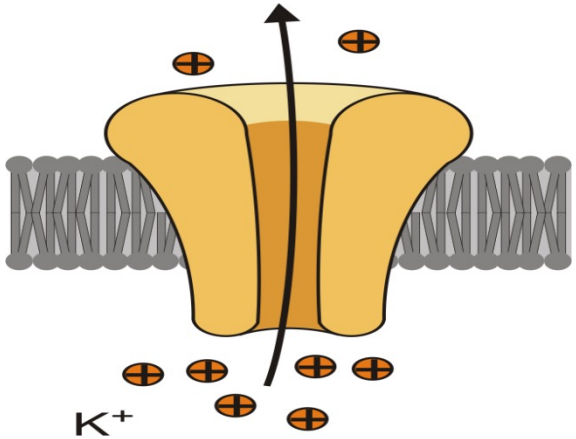
The outgoing flows of K +

Nel caso del K^+: **f.e.m. = $V_m - E_K$** con **E_K = – 75 mV**



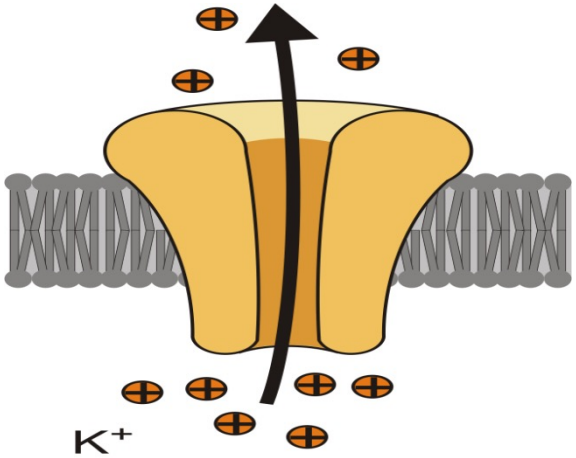
--35 mV

4% di canali aperti
f.e.m. **bassa**
flussi di K^+ **bassi** (s.c.)
I_K totale **bassa**



0 mV

70% di canali aperti
f.e.m. **media**
flussi di K^+ **medi** (s.c.)
I_K totale **media**



+63 mV

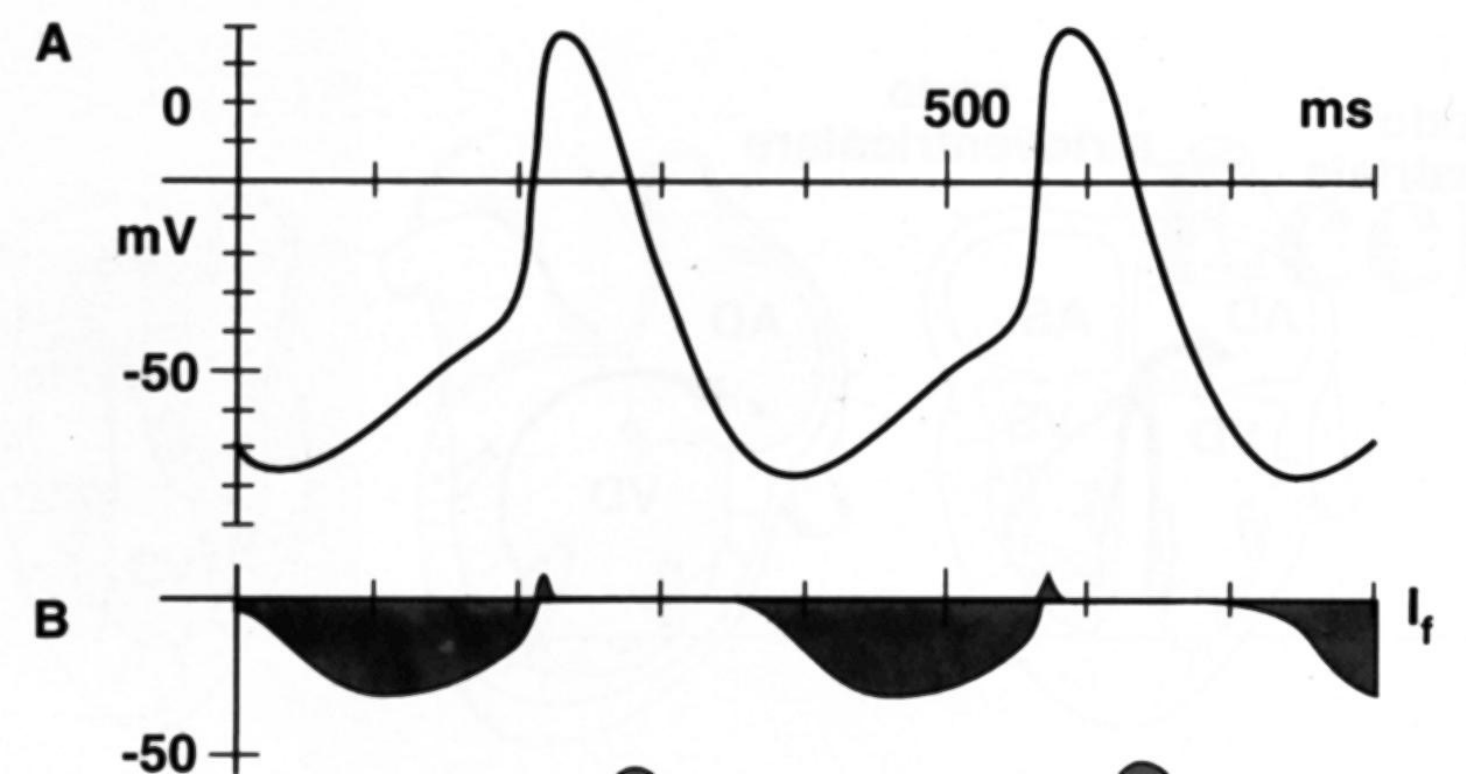
100% di canali aperti
f.e.m. **alta**
flussi di K^+ **alti** (s.c.)
I_K totale **alta**

channels open by hyperpolarization

I_{funny}

ey are cationic channels permeable to Na + and K

They determine rhythmic variations of Vm



The current If has singular characteristics

1) It is a voltage dependent current that is activated in hyperpolarization: the channel opens when the others close

2) The opening of the channels induce a very slow current, entering "inward" that begins at the end of the action potential after the cell has reached its negative potential

3) Both sodium and potassium flow in the channel, it has a mixed conductance

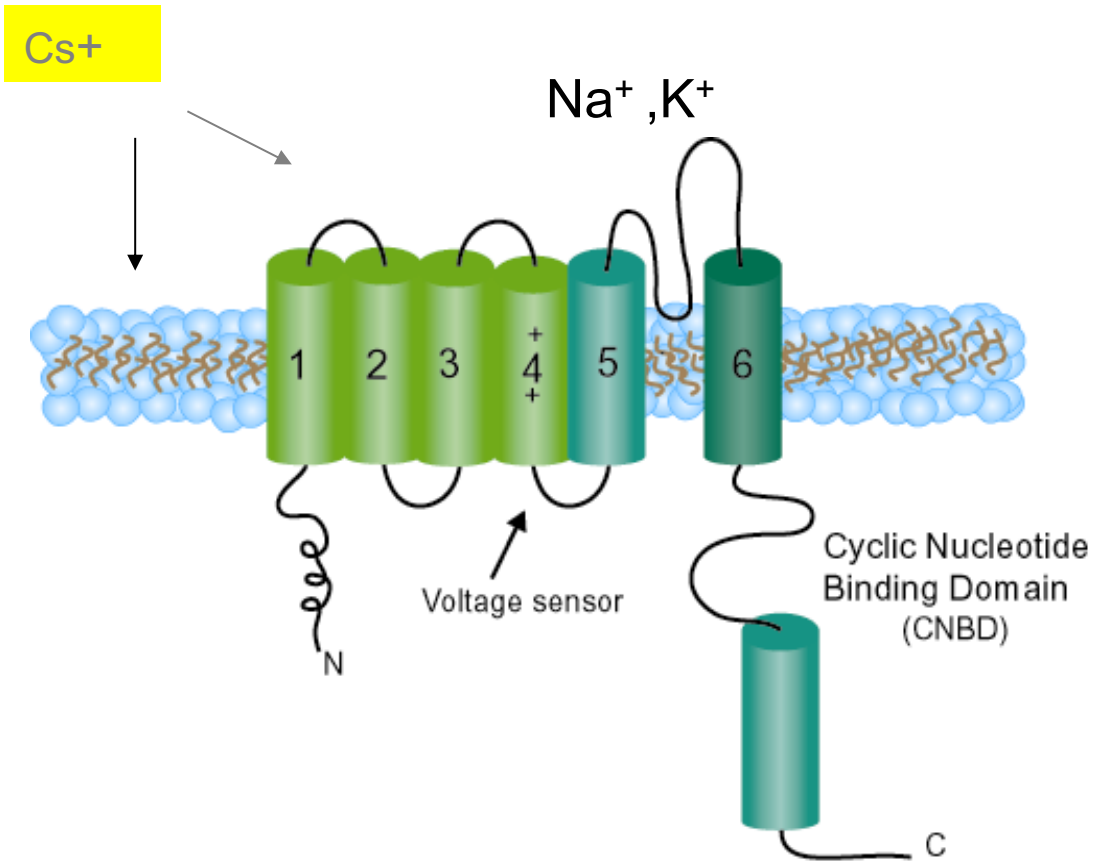
4) The channel is modulated directly by the cyclic nucleotides and in particular by cAMP and blocked by Cesium

If has helped to found a new family of channels whose exact definition is "activated by hyperpolarization and modulated by nucleotides (HCN)" whose recognition occurred in 1998 with the identification of the genes coding for these proteins.

To this family belong similar currents to If discovered in neurons and photoreceptors.

Structure of HCN channels

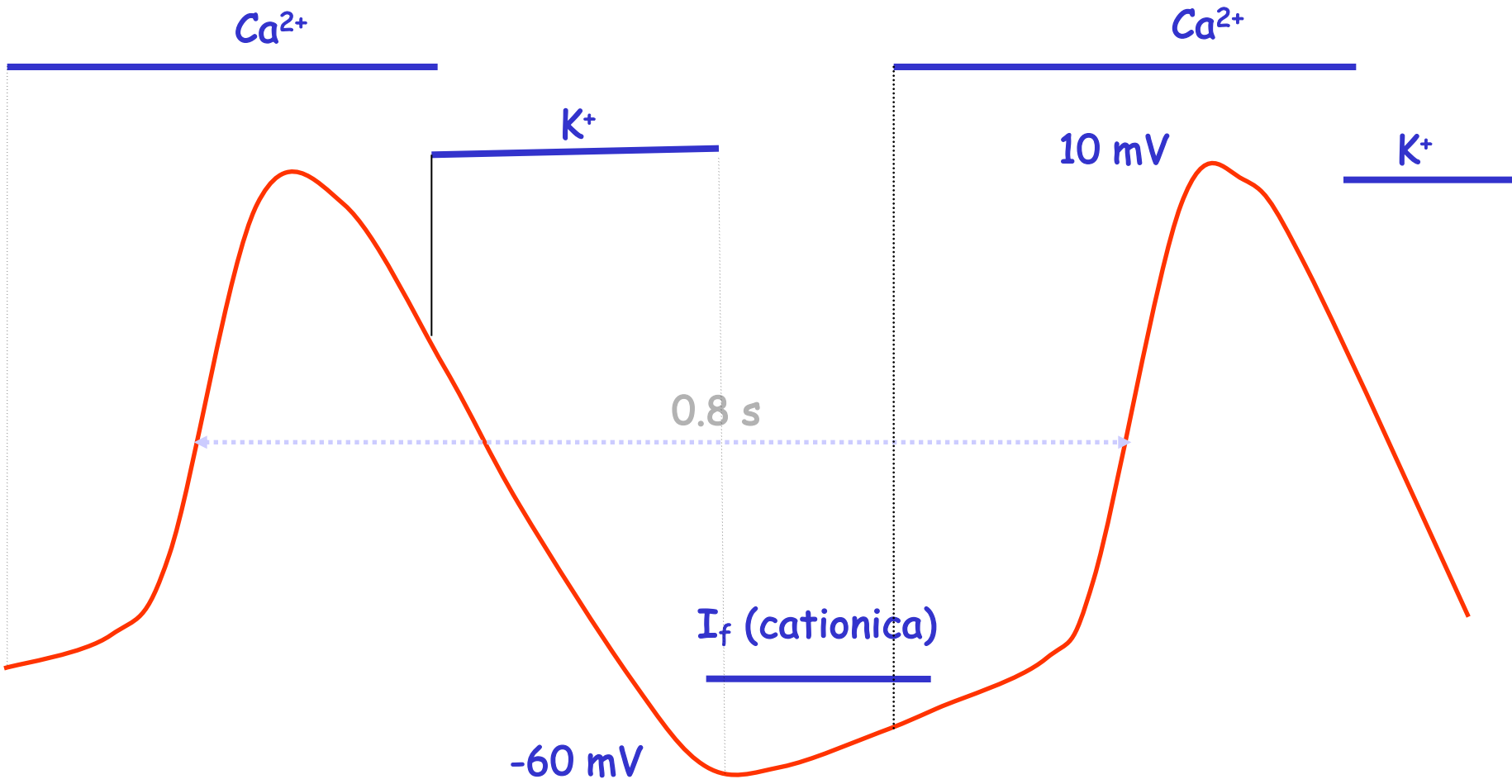
similar to V gated K channels



Action potential in the Seno-atrial node

1) Starter tissue cells (pacemakers) have a low membrane potential of -60 mV
2) To this Vm is active the cationic current depolarizing If permeable to Na + and to K +.
3) After a first depolarization due to If follows a current of voltage-dependent Ca2 + which at a certain point becomes strongly regenerative. Depolarization inhibits If.
4) Depolarization activates a current to K + that repolarizes the cell.
5) Repolarization closes the channels to the Ca2 + and activates the If

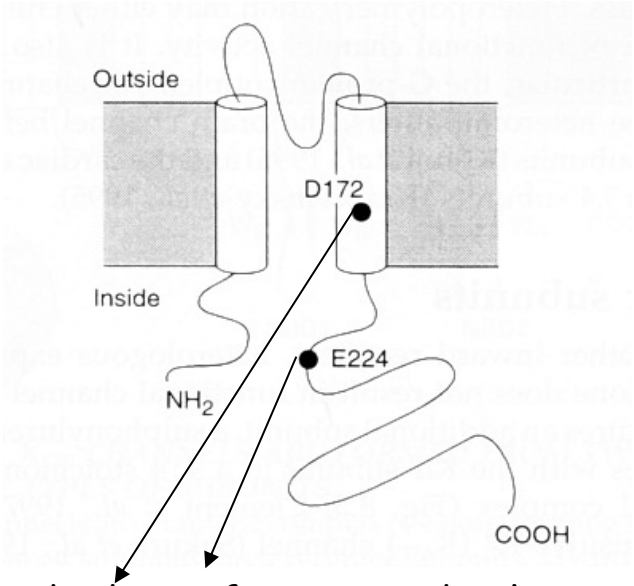
- I_f : activated by repolarization
- I_{Ca2+}: activated by depolarization, transient current
- I_{k+} : activated by depolarization



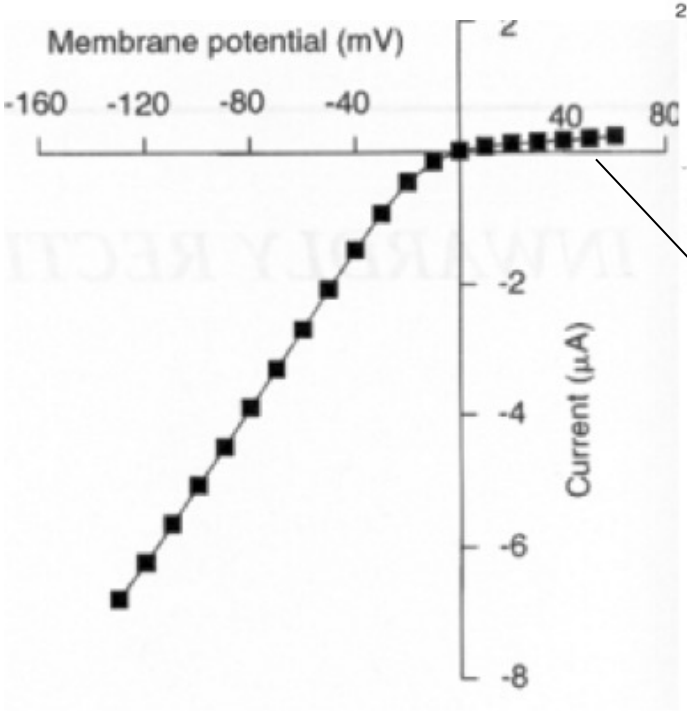
Kir channels

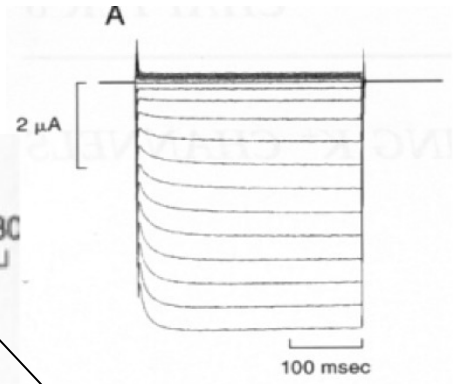
2TM

Omo- and hetero –tetramers



bindind site for Mg and polyamines





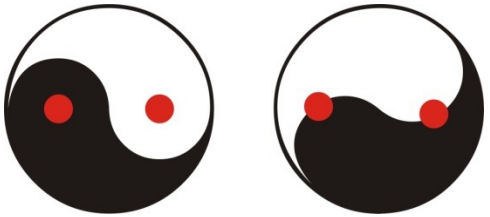
Volt-dip block for organic cations(Mg and polyamines)

stabilize the membrane potential (important in glia cells for reabsorption K)

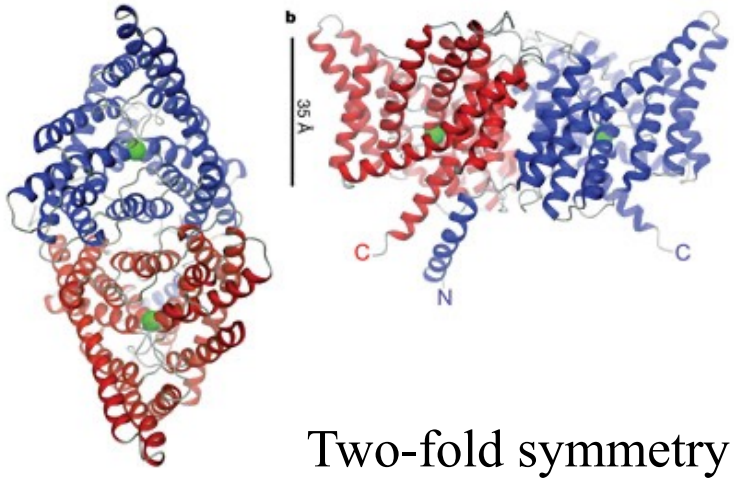
Insensitive to the potential ????

The Chloride Channel breaks the Rules!

ClC single channel behavior suggests a double barrel arrangement:

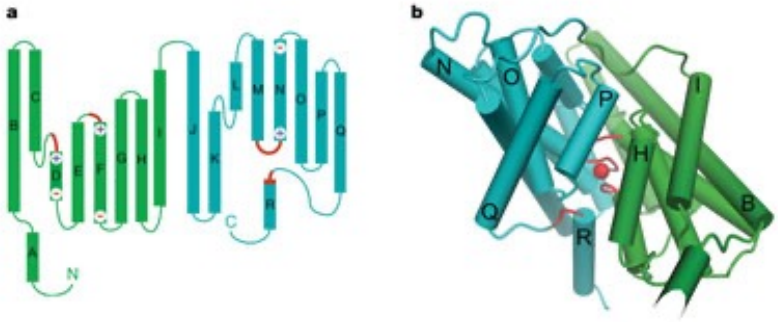


The structure of the ClC chloride channel deviates from "classical" membrane protein architectures



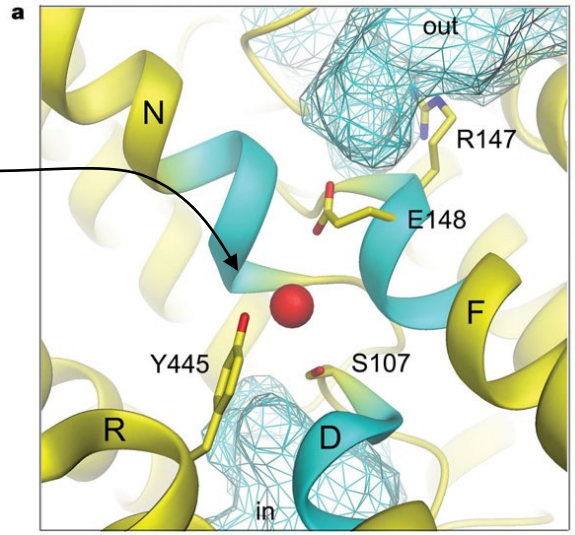
Two-fold symmetry

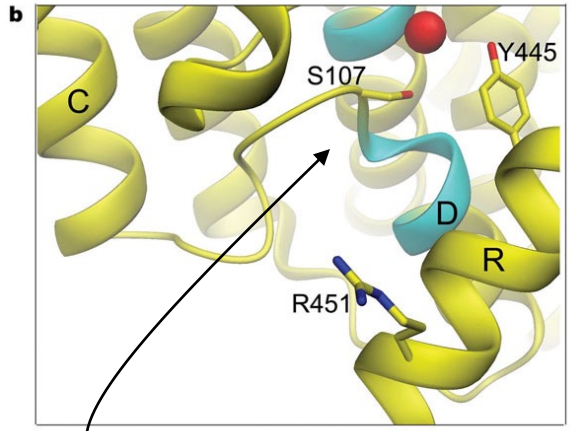
Helix packing is very complex



Anionic Selectivity Appears to be Based on Ion Stabilization by Helix Dipoles

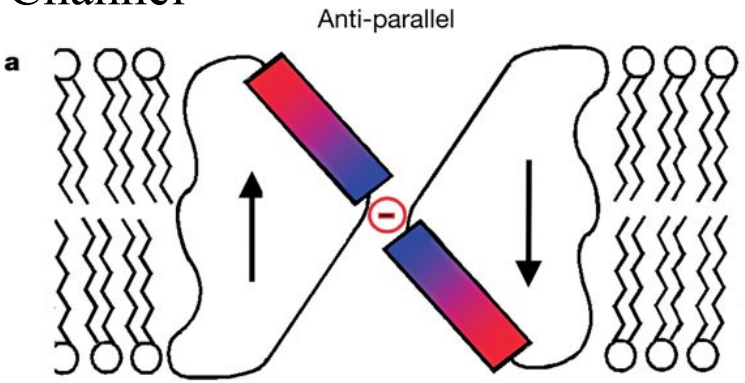
Cl^- coordination site



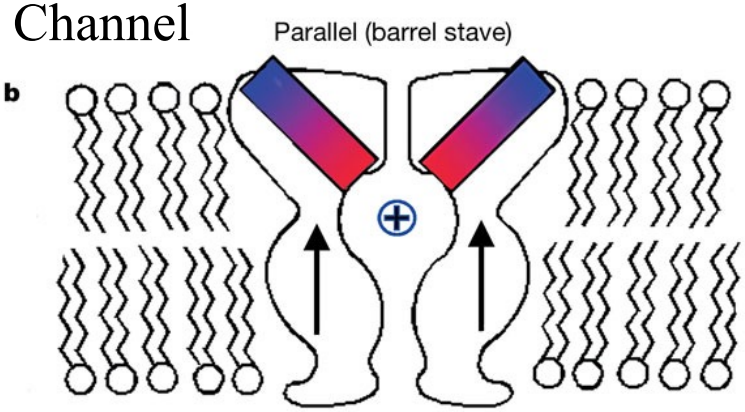


Channel entry

Cl Channel

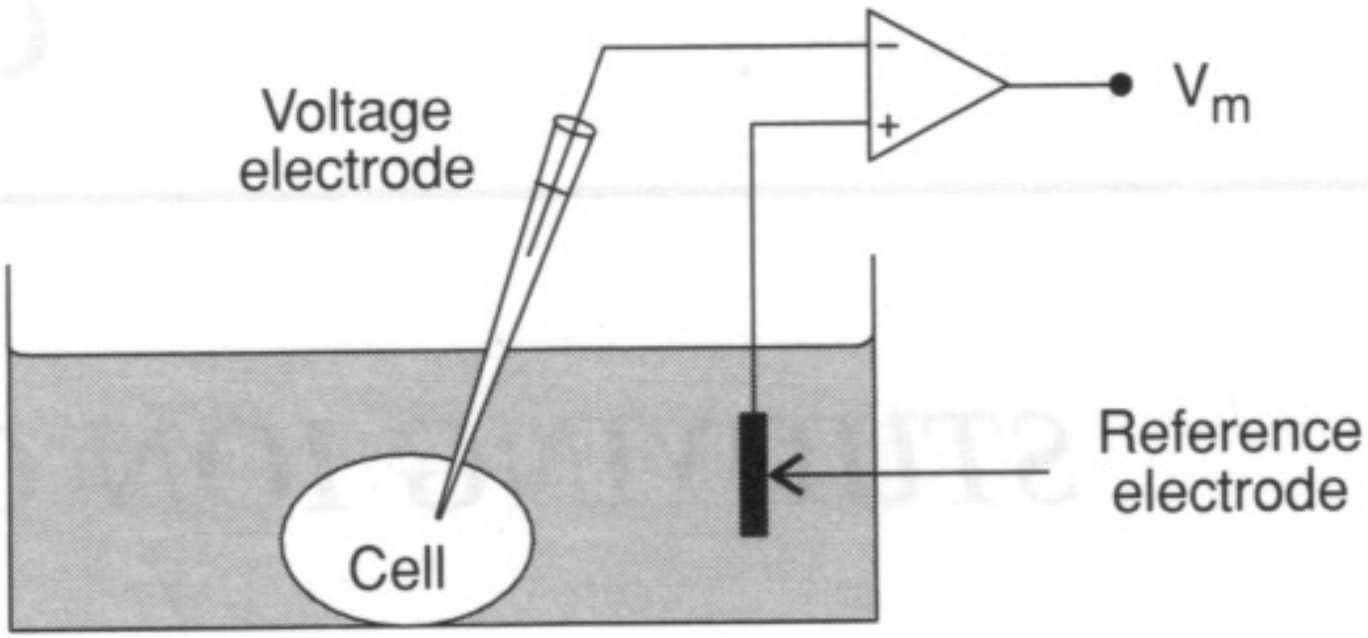


K Channel

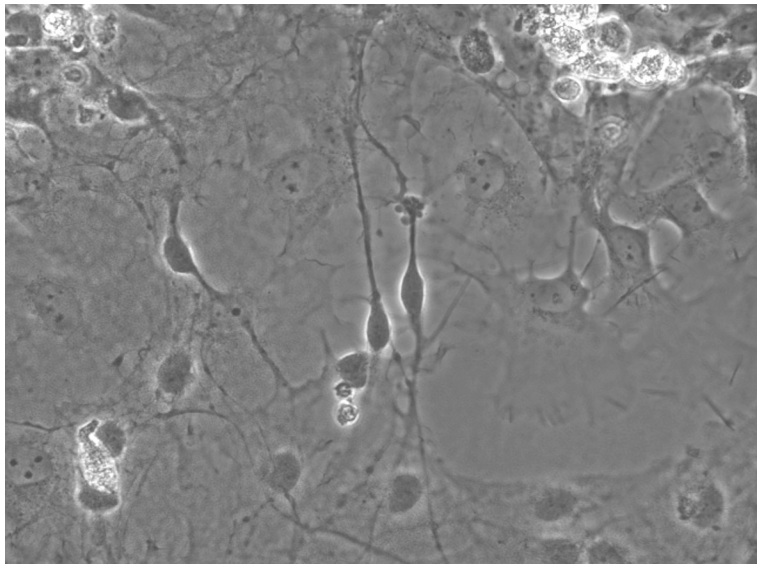


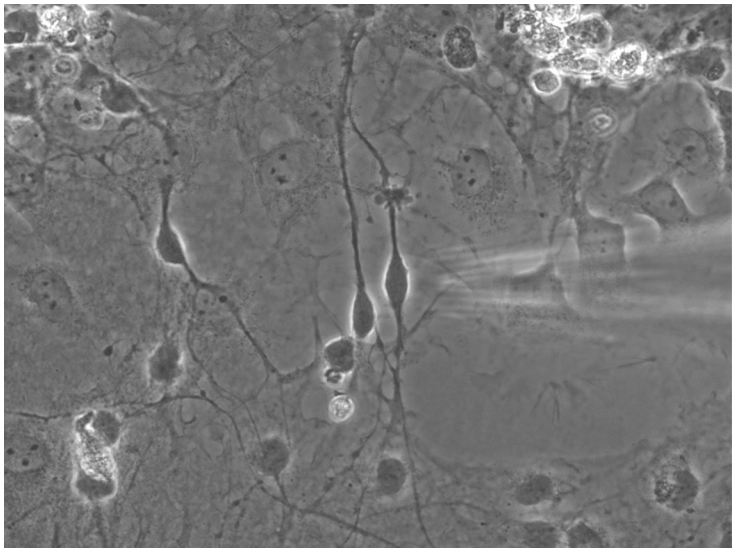
How to study ion channels?

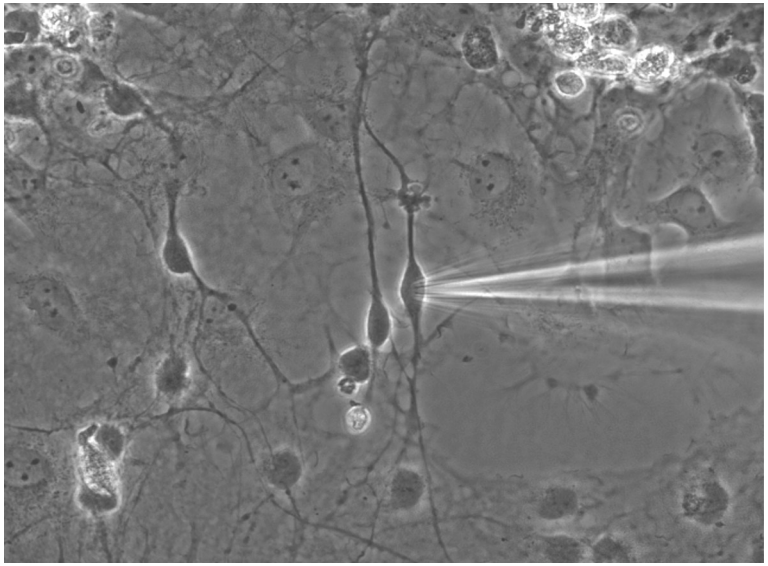
How to study ion channel function?

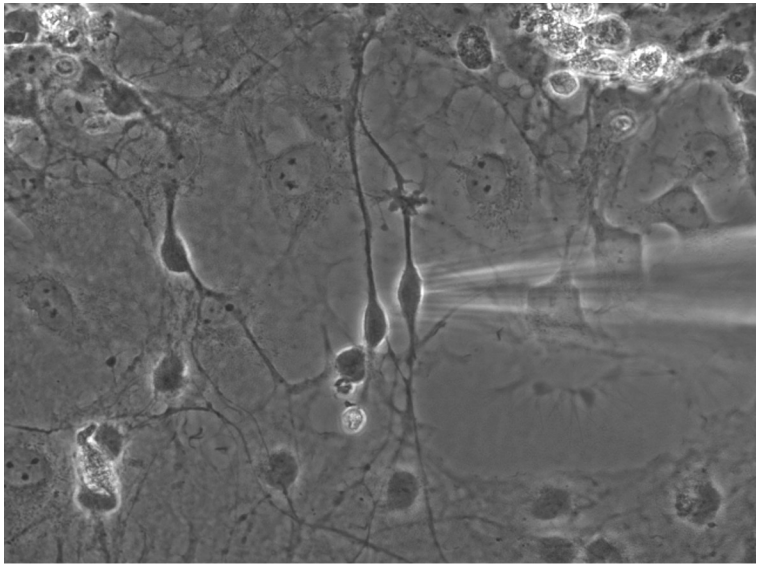


Electrophysiological recordings







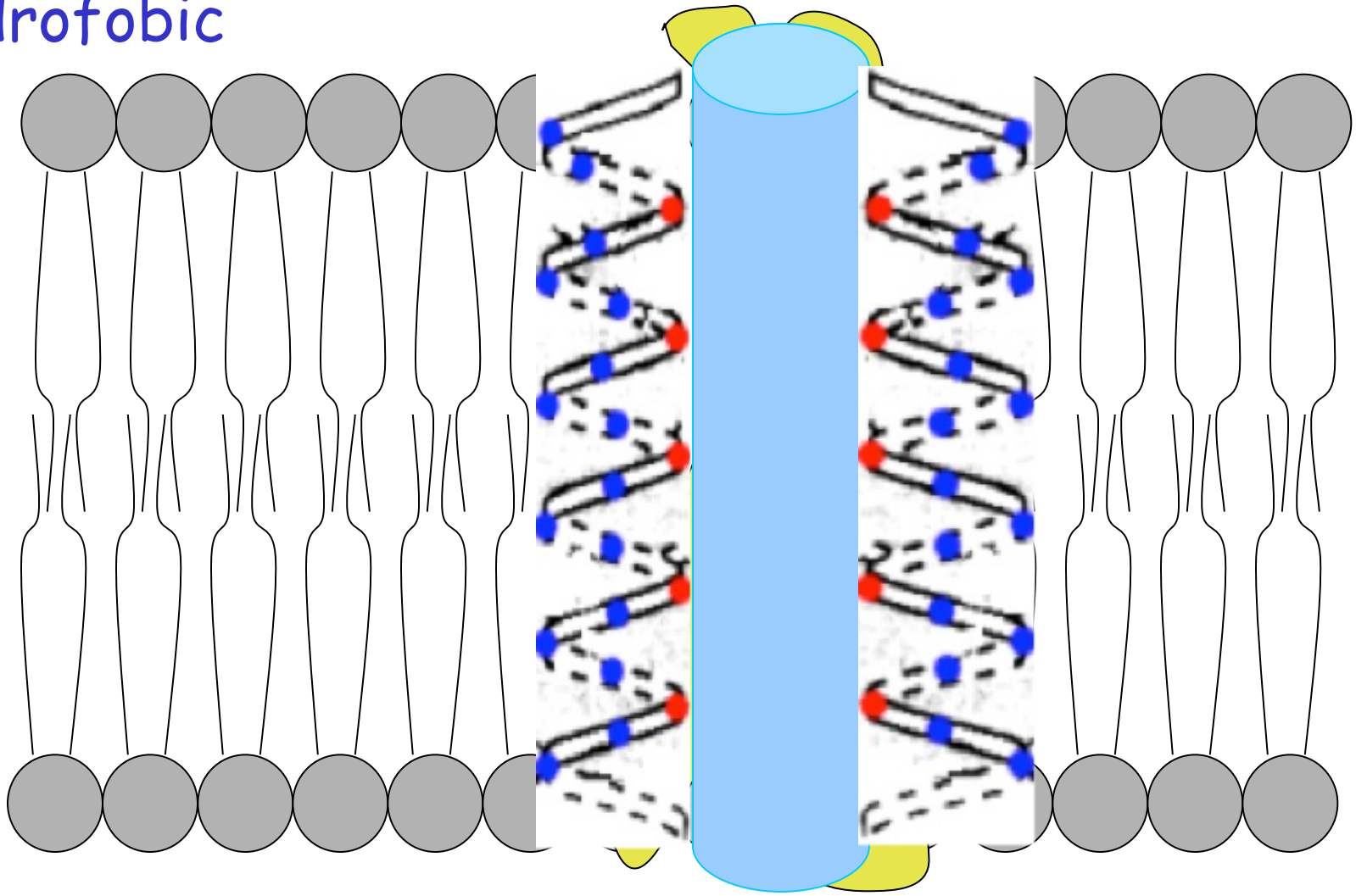


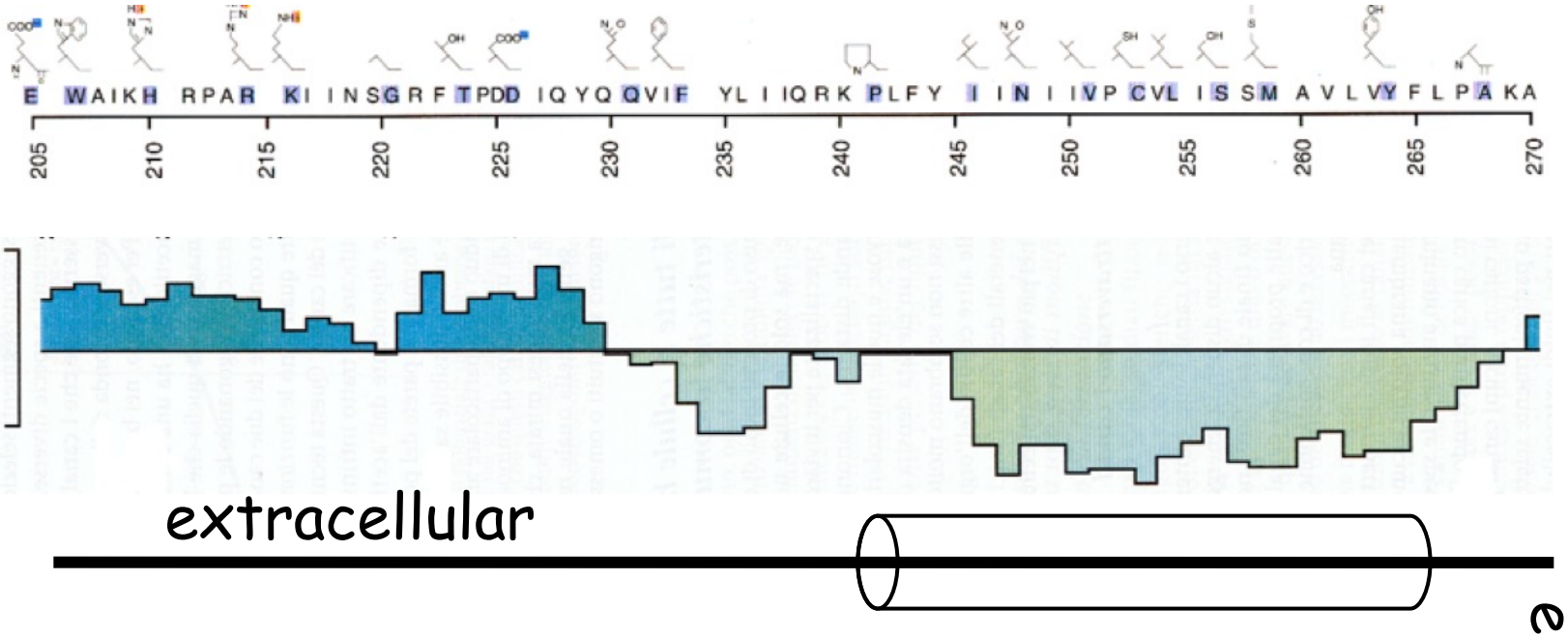
The Patch Clamp Method

Hydrophilic
Hydrofobic

Predictions…

Hydrophilic
Hydrofobic





Idrofilia

Idrofobicità

Transmembrane segment

(α-elix or β-sheet)

Structure