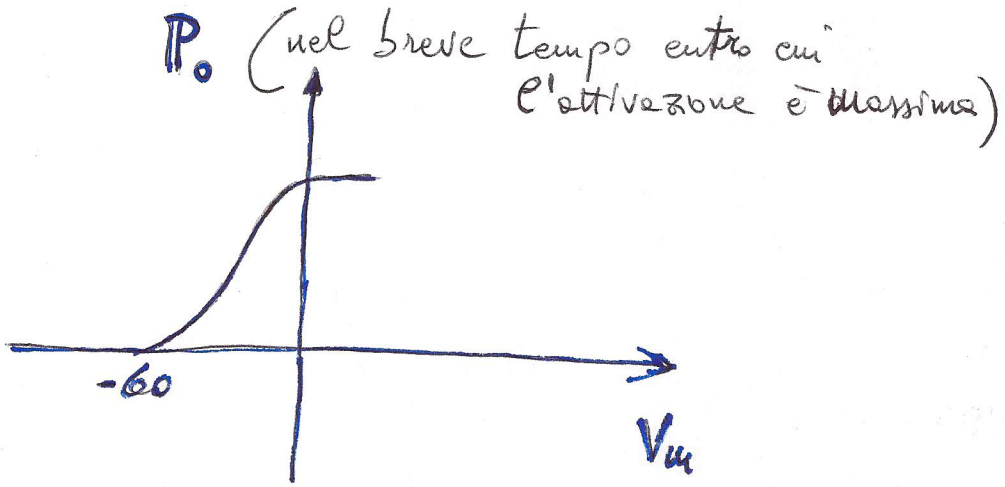
$$I_{NaV} = G_{NaV}(V,t)(V_m - E_{Na})$$

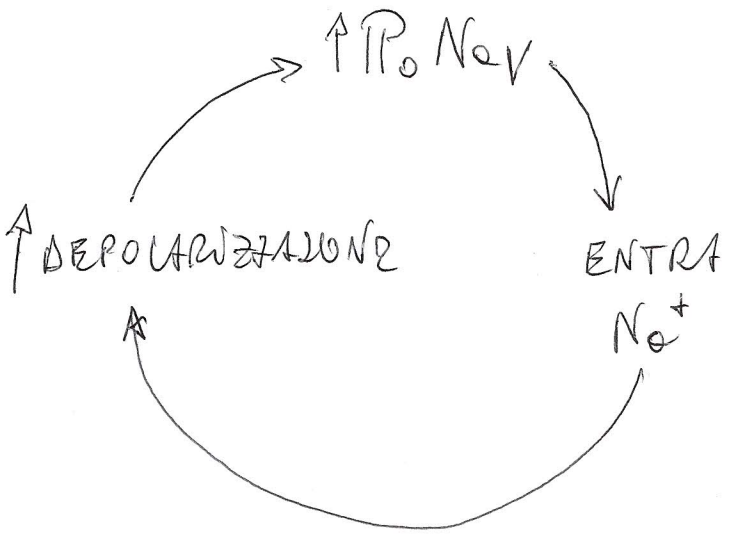
$$I_{KV} = G_{KV}(V,t)(V_m - E_K)$$



ATTIVAZIONE DI UN CANALE IONICO VOLTAGGIO-DIPENDENTE

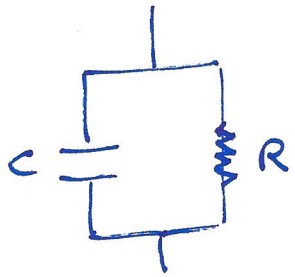
$$I_i = \gamma N P_o(V,t)(V_m - E_i)$$

CICLO DI HODGKIN (RETROAZIONE POSITIVA)



LA SOGLIA È PIÙ BASSA SE LA DENSITÀ DI CANALI DEL Na^+ È PIÙ ALTA, PERCHÉ LO STESSO V_m FA APRIRE PIÙ CANALI (A PARITÀ DI P_{Na}) SE IL NUMERO N DI CANALI È PIÙ GRANDE.

IL METODO DEL VOLTAGE-CLAMP



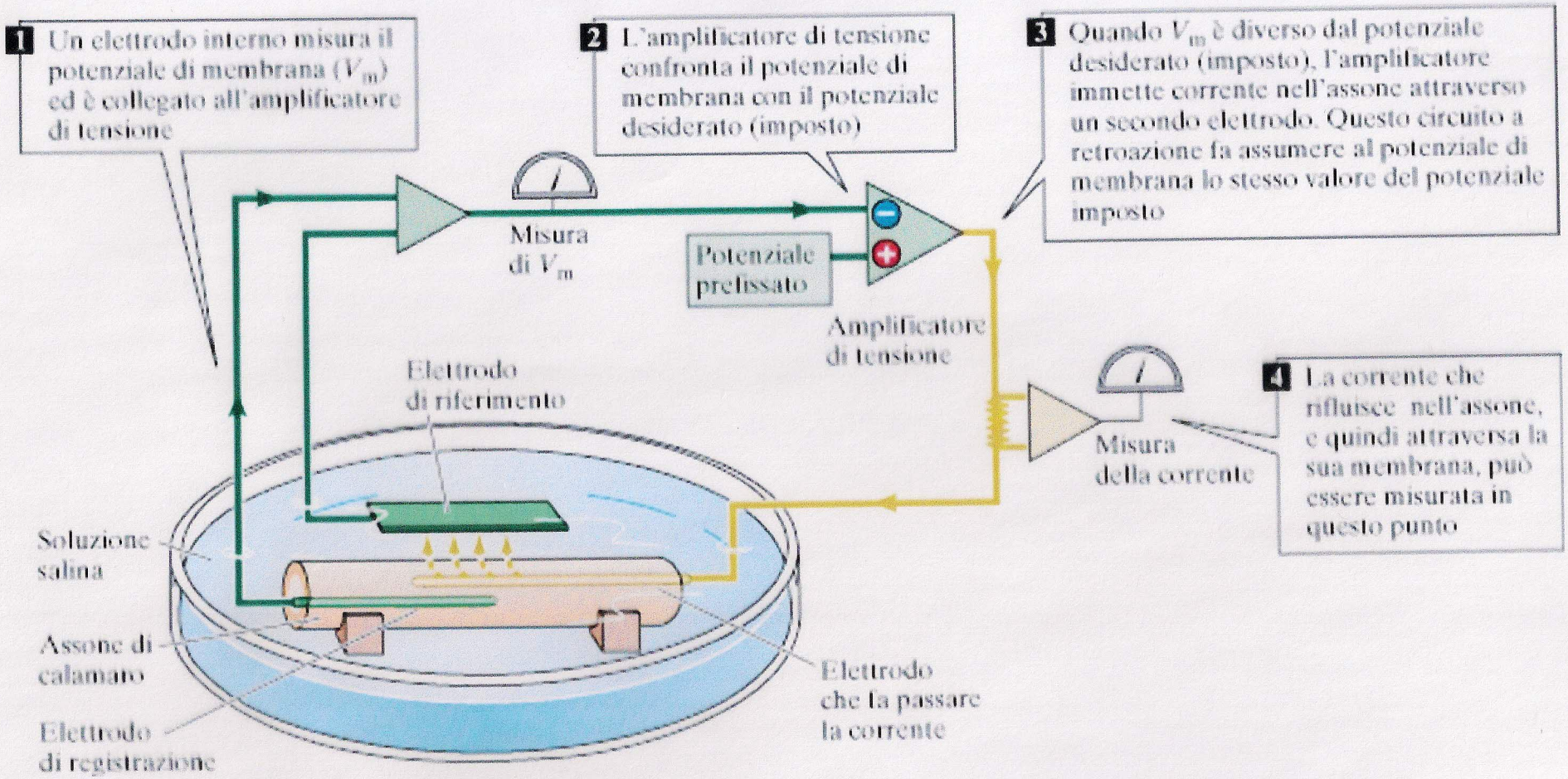
$$Q = CV \qquad \frac{dQ}{dt} = C\frac{dV}{dt} = I_C$$

$$\frac{V}{R} = GV = I_R$$

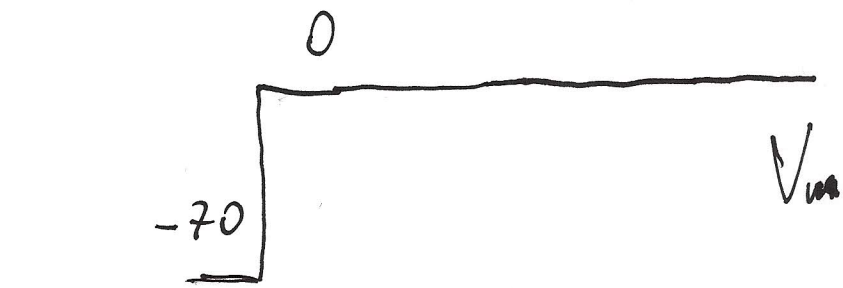
$$I_{TOTALE} = I_R + I_C$$

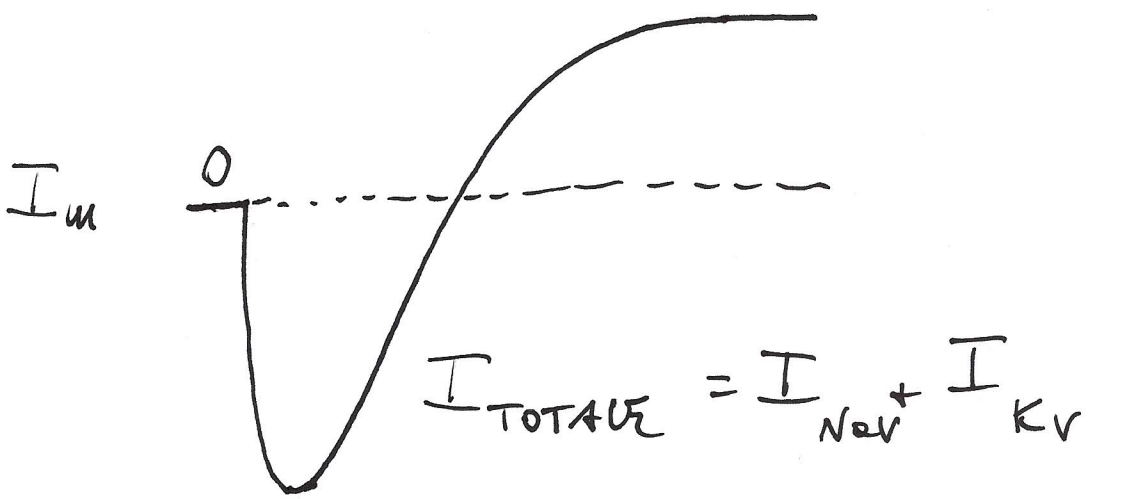
Se V = COSTANTE $\Longrightarrow$ $C\frac{dV}{dt} = 0$

Quindi $I_{TOT} = I_R$ (= I_i sono portate dai diversi ioni i permeanti)

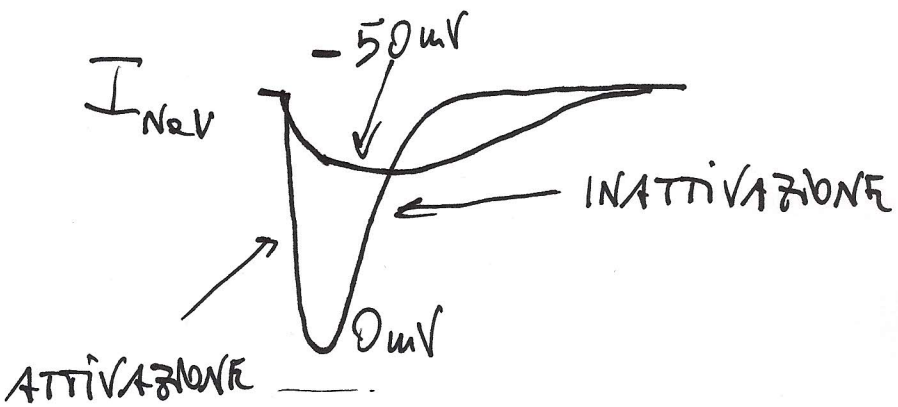

1 Un elettrodo interno misura il potenziale di membrana (V_m) ed è collegato all'amplificatore di tensione
2 L'amplificatore di tensione confronta il potenziale di membrana con il potenziale desiderato (imposto)
3 Quando V_m è diverso dal potenziale desiderato (imposto), l'amplificatore immette corrente nell'assone attraverso un secondo elettrodo. Questo circuito a retroazione fa assumere al potenziale di membrana lo stesso valore del potenziale imposto
4 La corrente che rifluisce nell'assone, e quindi attraversa la sua membrana, può essere misurata in questo punto
Misura di V_m
Potenziale prefissato
Amplificatore di tensione
Misura della corrente
Elettrodo di riferimento
Soluzione salina
Assone di calamaro
Elettrodo di registrazione
Elettrodo che fa passare la corrente

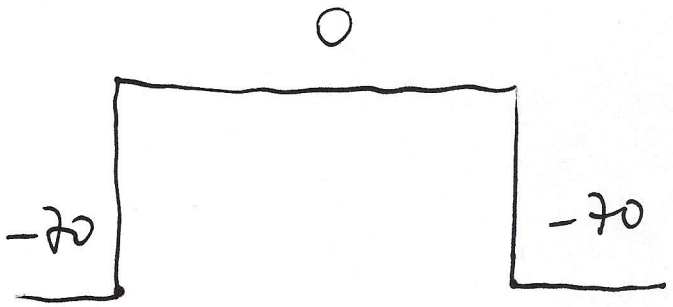
VOLTAGE-CLAMP



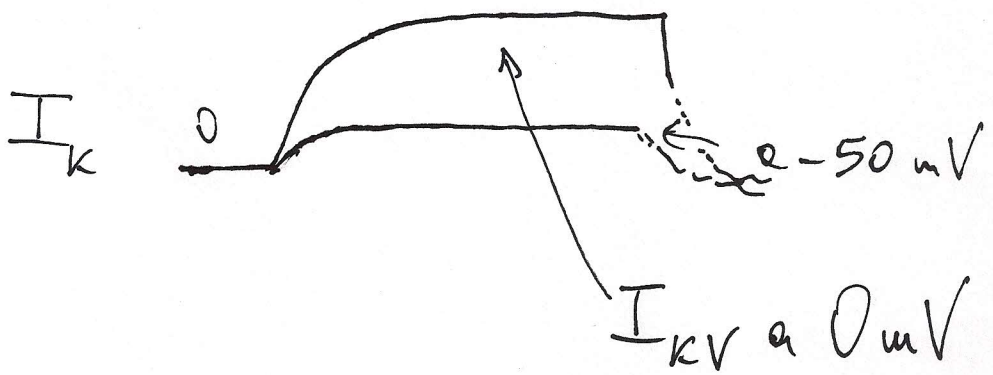


↓ + bloccante I_{KV} (TEA)





MISURA IN PRESENZA DI UN BLOCCANTE
delle I_{NaV} (p.es. TTX)



I_{KV} si chiudono a Vm negativi
(da V_R in giù)

SVILUPPO STORICO

Voltage-clamp ed ISOLAMENTO FUNZIONALE DELLE DIVERSE CORRENTI (p.es. INIBITORI)
(anni '60 - '70)

ANNI '80

PATCH-CLAMP

STUDIO DI CELLULE DI MAMMIFERO anche non eccitabili

BIOFISICA RAFFINATA

PURIFICAZIONE E CLONAGGIO

(p.es. 1983 NaV e AChR)

CRISTALLI dopo il 1998

UNIONE DI METODI FISIOLOGICI e biomolecolari, produzione di modelli animali transgenici.

JOHNSTON, WU

FOUNDATIONS OF CELL. NEUROPHYSIOLOGY

MIT Press 1996

HILLE IONIC CHANNELS OF EXCITABLE MEMBRANES

SINAUER 2001

WAXMAN MOLECULAR NEUROLOGY 2007

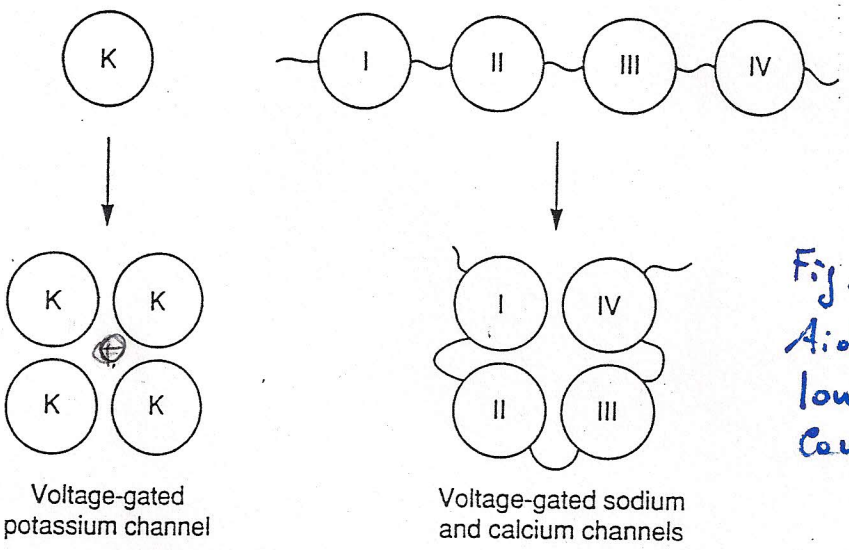


Fig. 4.15 in Aidley and Stanfield Ion Channels Camb. Univ. Press 1996

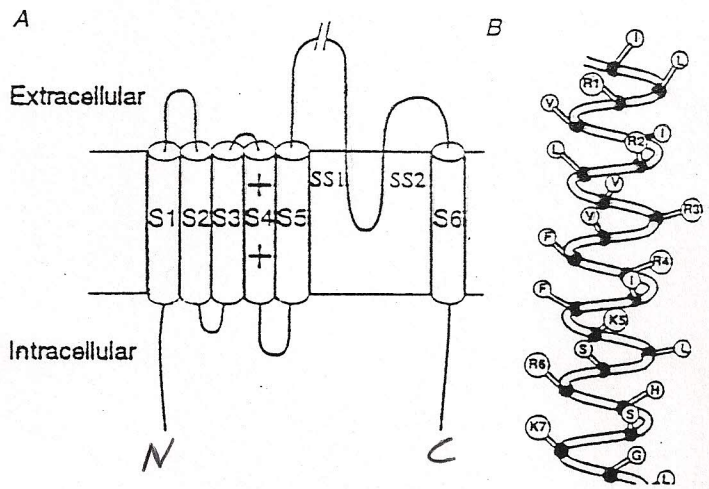


Fig. 4.16 (originally from Catterall 1993, Trends in Neurosci)

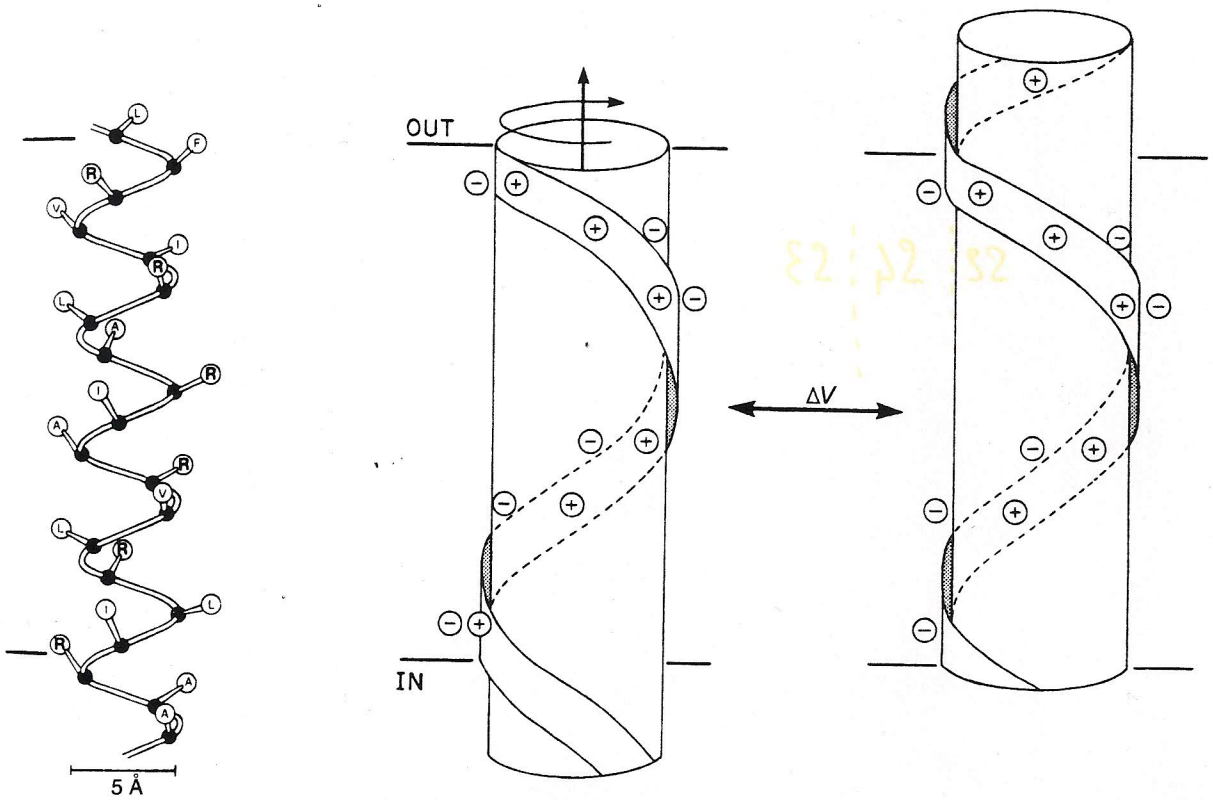
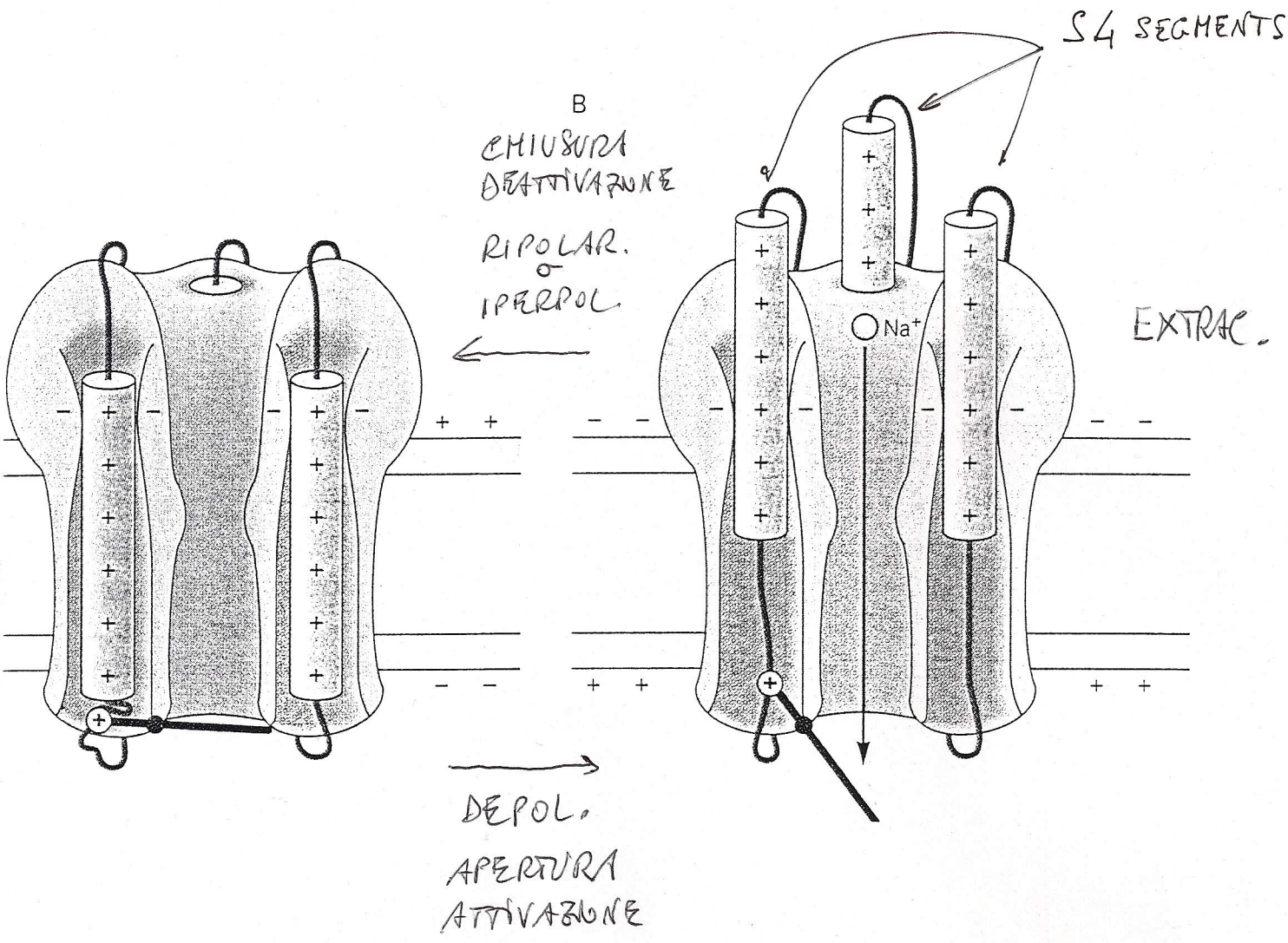


Figure 6.12. Catterall's sliding helix model of gating charge movement in the sodium channel. Segment S4 is presumed to form an α-helix crossing the membrane as shown on the left. Here the black circles represent the α-carbon atoms of the different amino acid residues and the white circles represent their side-chains. Residues are indicated by the single-letter code, with R showing the positively charged arginine; the rest are non-polar. The arginine residues thus form a helix of positive charges, shown on the right. The model proposes that these form ion-pairs with an array of negative charges on other segments, and that depolarization allows an outward movement of the S4 segment by one step along this array. (From Catterall, 1986.)

Aidley, The Physiology of Excitable Cells, Camb. Univ. Press 1998


S4 SEGMENTS
B
CHIUSURA
DEATTIVAZIONE
RIPOLAR.
O
IPERPOL.
EXTRAC.
Na+
DEPOL.
APERTURA
ATTIVAZIONE
C
A
(O)

J Physiol 600.14 (2022) pp 3227–3247

The Journal of Physiology

TOPICAL REVIEW

The 70-year search for the voltage-sensing mechanism of ion channels

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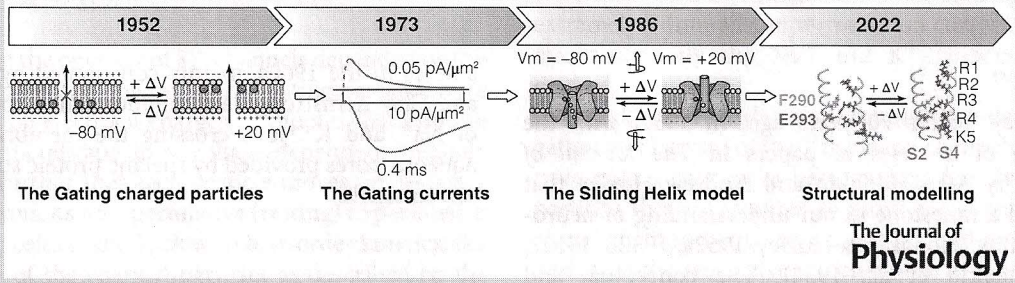
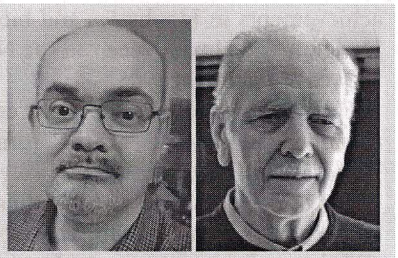
Edited by: Ian Forsythe & Thomas DeCoursey

The peer review history is available in the Supporting Information section of this article (https://doi.org/10.1113/JP282780#support-information-section).



Abstract This retrospective on the voltage-sensing mechanisms and gating models of ion channels begins in 1952 with the charged gating particles postulated by Hodgkin and Huxley, viewed as charges moving across the membrane and controlling its permeability to Na^+ and K^+ ions. Hodgkin and Huxley postulated that their movement should generate small and fast capacitive currents, which were recorded 20 years later as gating currents. In the early 1980s, several voltage-dependent channels were cloned and found to share a common architecture: four homologous domains or subunits, each displaying six transmembrane α-helical segments, with the fourth segment (S4) displaying four to seven positive charges invariably separated by two non-charged residues. This immediately suggested that this segment was serving as the voltage sensor of the channel (the molecular counterpart of the charged gating particle postulated by Hodgkin and Huxley) and led to the development of the sliding helix model. Twenty years later, the X-ray crystallographic structures of many voltage-dependent channels allowed investigation of their gating by molecular dynamics. Further understanding of how channels gate will benefit greatly from the acquisition of high-resolution structures of each of their relevant



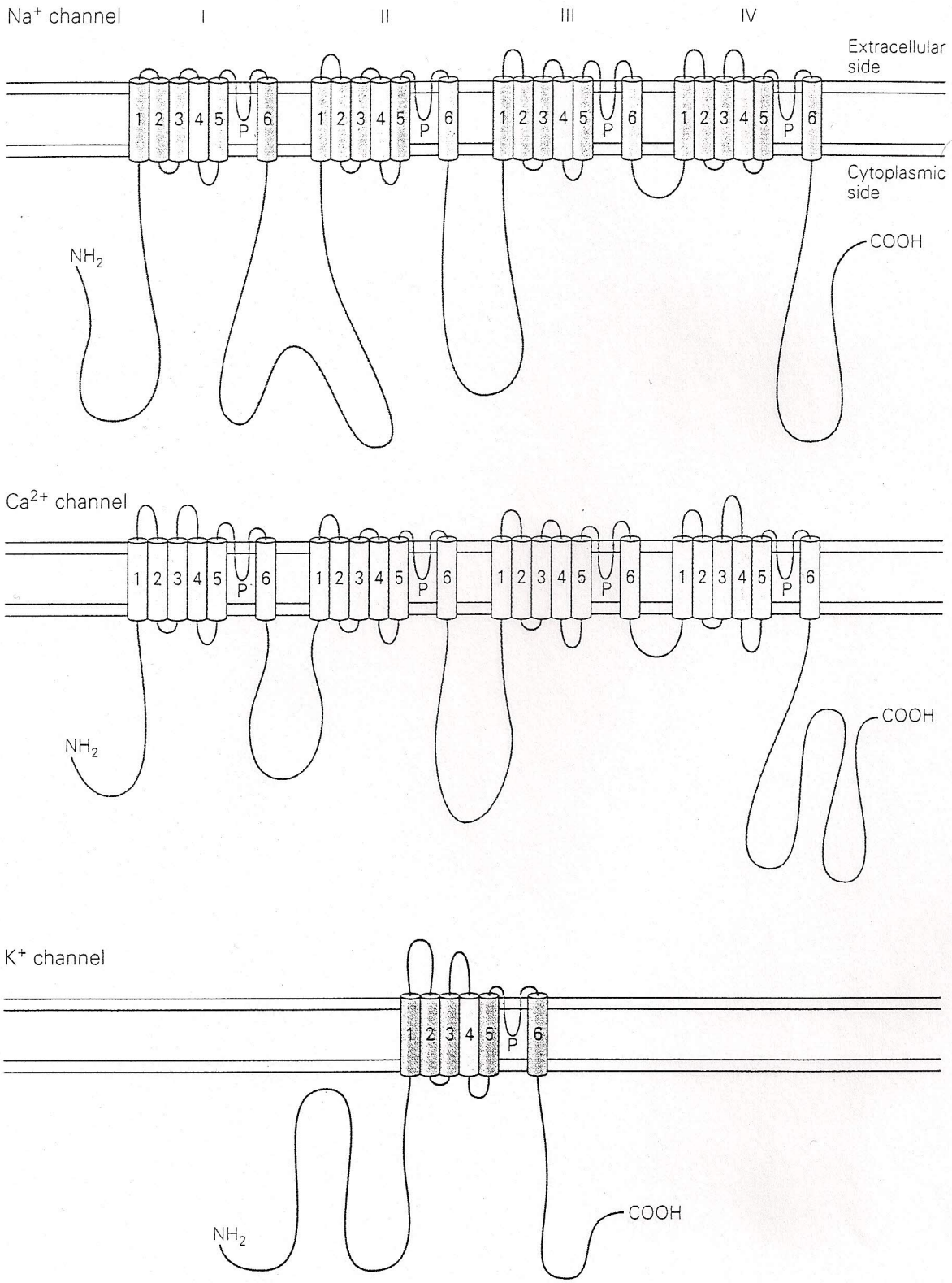
Luigi Catacuzzeno received his PhD degree in Cellular and Molecular Biology at the University of Perugia in 2006. He improved his background in theoretical biophysics on membrane ion channels as a PhD fellow at the Department of Physiology and Biophysics at the University of Miami. He is currently an Associate Professor at the Department of Chemistry, Biology and Biotechnology at the University of Perugia. His research interests include cell electrophysiology and ion channel gating and permeation/selectivity mechanisms. His activity includes both experimental and theoretical approaches. **Fabio Franciolini** graduated in Biological Sciences at the University of Perugia. He spent 2 years in Dr Chris Ashley's laboratory at the Department of Physiology, Oxford University, followed by 5 years in Dr Wolfgang Nonner's laboratory at the Department of Physiology and Biophysics, University of Miami. He then returned to the University of Perugia, where he established his own electrophysiology laboratory and continues to research the physiology and biophysics of ion channels.

DOI: 10.1113/JP282780

CANALI DEL Na^+ VOLTAGG.-DIPENDENTI

	Gene	Subunità
Na_v 1.1	SCN1A	α
1.2	2A	α
1.3	3A	α
1.4	4A	α
1.5	5A	α
1.6	8A	α
1.7	9A	α
1.8	10A	α
1.9	11A	α
	1B	$\beta 1$
	2B	$\beta 2$
	3B	$\beta 3$
	4B	$\beta 4$

PERSIST.
TTX-RESIST.


Na+ channel
I
II
III
IV
Extracellular side
Cytoplasmic side
1 2 3 4 5 P 6
NH2
COOH
Ca2+ channel
1 2 3 4 5 P 6
NH2
COOH
K+ channel
1 2 3 4 5 P 6
NH2
COOH

maggiore espressione
e attiv - inatt. più veloce

PORZIONE EXTRAC.: omologa regione IGG de Cys e'sulfone

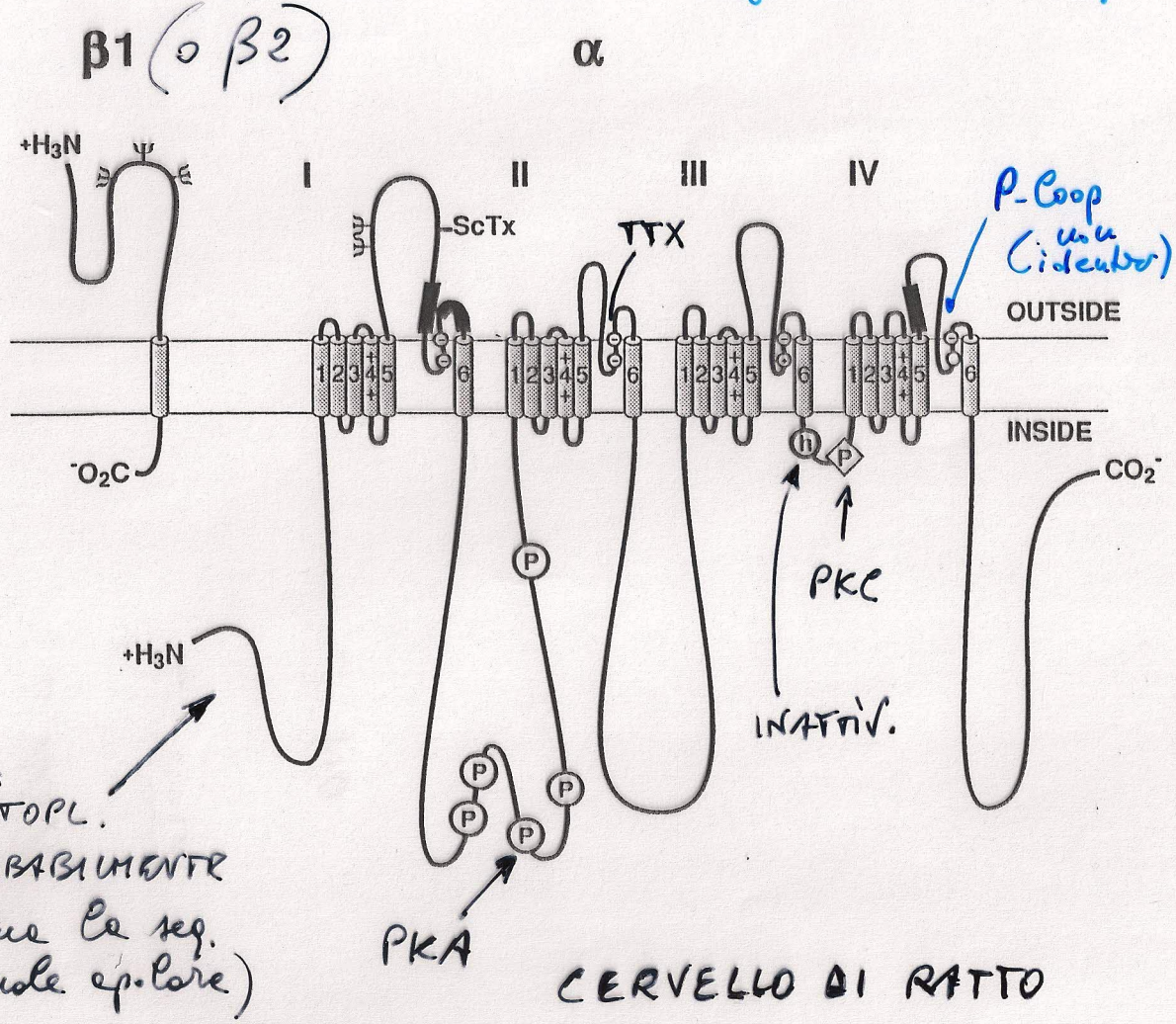


FIG. 4. Primary structures of α- and β1-subunits of sodium channel illustrated as transmembrane folding diagrams. Bold line, polypeptide chains of α- and β1-subunits with length of each segment approximately proportional to its true length in rat brain sodium channel. Cylinders represent probable transmembrane α-helices. Other probable membrane associated segments are drawn as loops in extended conformation like remainder of sequence. Sites of experimentally demonstrated glycosylation (ψ), cAMP-dependent phosphorylation (P in a circle), protein kinase C phosphorylation (P in a diamond), amino acid residues required for tetrodotoxin binding (small circles with +, −, or open fields depict positively charged [Lys^{1422}], negatively charged, or neutral [Ala^{1714}] residues, respectively), and amino acid residues that form inactivation particle (h in a circle).

W.A. CATTERALL
Physiol. Rev.
72 : S15 October 1992

S5-S6
H5
P-LOOP
] SINONIMI

STRUTTURA E PERMEAZIONE CANALI IONICI

BIOCHEMISTRY

BERG, TYMOCZKO, STRYER

↓

BIOCHIMICA (ZANICHELLI)

CAPITOLO 13

FIGURE 13.16
13.18
13.19
13.20
13.21
13.22

TABELLA 13.1

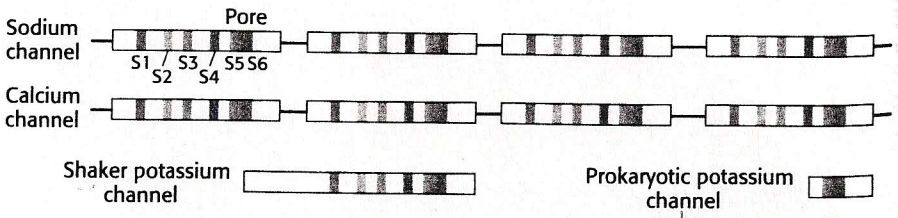
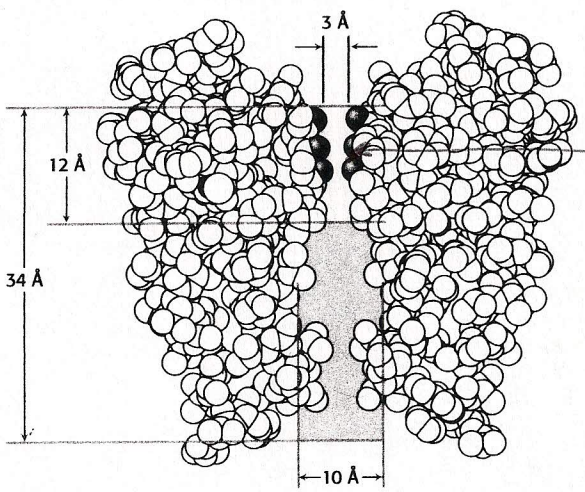


Figure 13.16 Sequence relations of ion channels. Like colors indicate structurally similar regions of the sodium, calcium, and potassium channels. Each of these channels exhibits approximate fourfold symmetry, either within one chain (sodium, calcium channels) or by forming tetramers (potassium channels).

Streptomyces Lividans



TVGYG
(P-LOOP)

Figure 13.18 Path through a channel. A potassium ion entering the K^+ channel can pass a distance of 22 Å into the membrane while remaining solvated with water (blue). At this point, the pore diameter narrows to 3 Å (yellow), and potassium ions must shed their water and interact with carbonyl groups (red) of the pore amino acids.

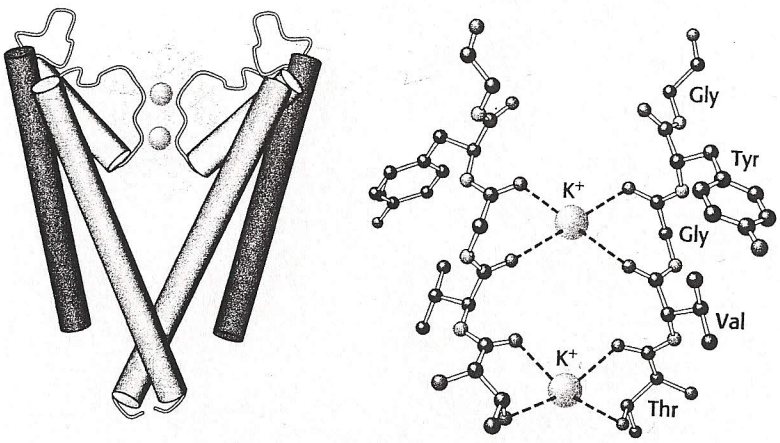


Figure 13.19 Selectivity filter of the potassium ion channel. Potassium ions interact with the carbonyl groups of the TVGYG sequence of the selectivity filter, located at the 3-Å-diameter pore of the K^+ channel. Only two of the four channel subunits are shown.

TABLE 13.1 Properties of alkali cations

Ion	Ionic radius (Å)	Hydration free energy in kJ mol^{-1} (kcal mol^{-1})
Li^+	0.60	−410 (−98)
Na^+	0.95	−301 (−72)
K^+	1.33	−230 (−55)
Rb^+	1.48	−213 (−51)
Cs^+	1.69	−197 (−47)

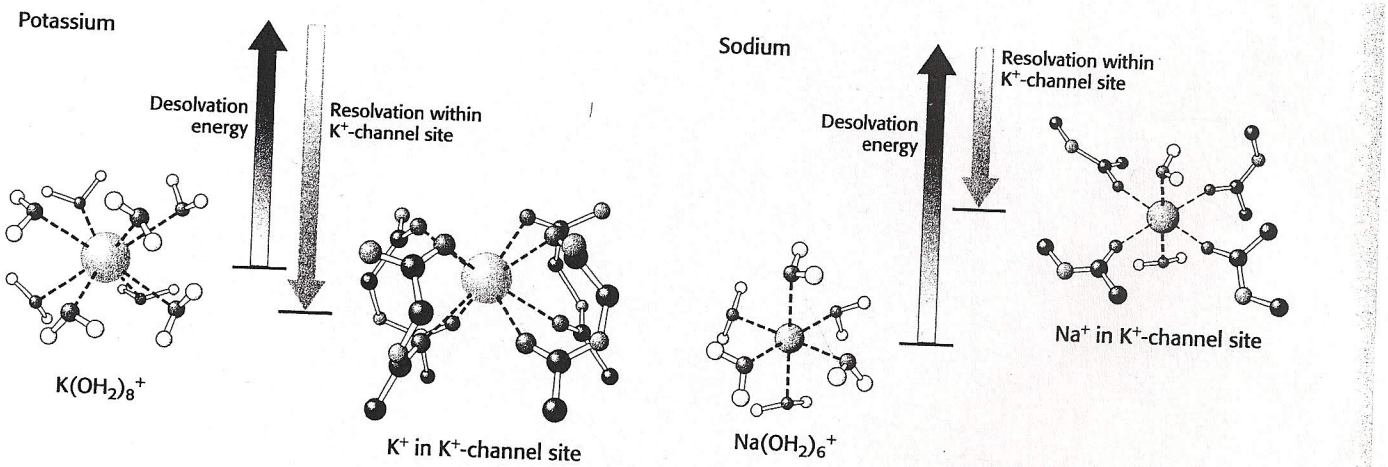
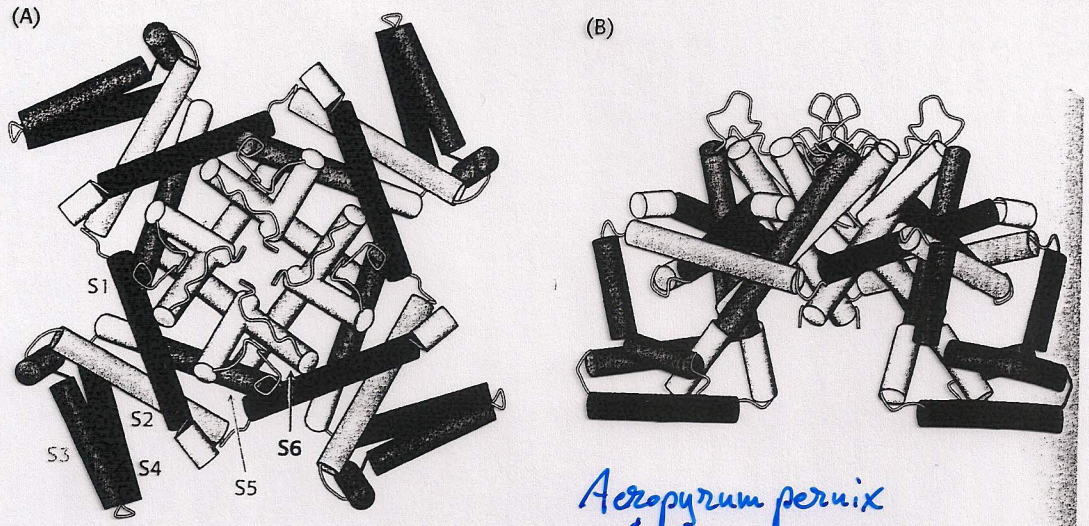


Figure 13.20 Energetic basis of ion selectivity. The energy cost of dehydrating a potassium ion is compensated by favorable interactions with the selectivity filter. Because a sodium ion is too small to interact favorably with the selectivity filter, the free energy of desolvation cannot be compensated and the sodium ion does not pass through the channel.



Aeropyrum pernix

Figure 13.22 Structure of a voltage-gated potassium channel. (A) A view looking down through the pore. (B) A side view. *Notice* that the positively charged S4 region (red) lies on the outside of the structure at the bottom of the pore. [Drawn from 1ORQ.pdb.]

PARADOSSO APPARENTE: ALTA SELETTIVITÀ (forte interazione ione-poro) e ALTO FLUSSO

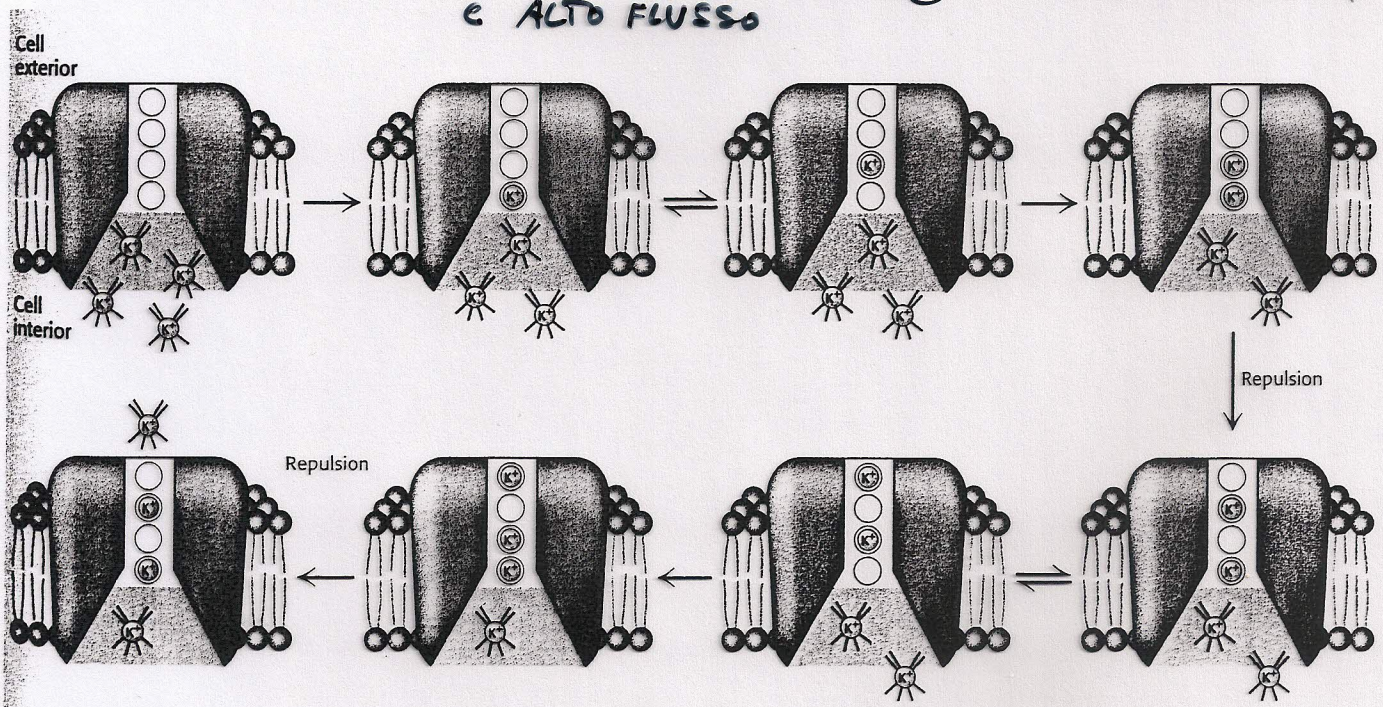


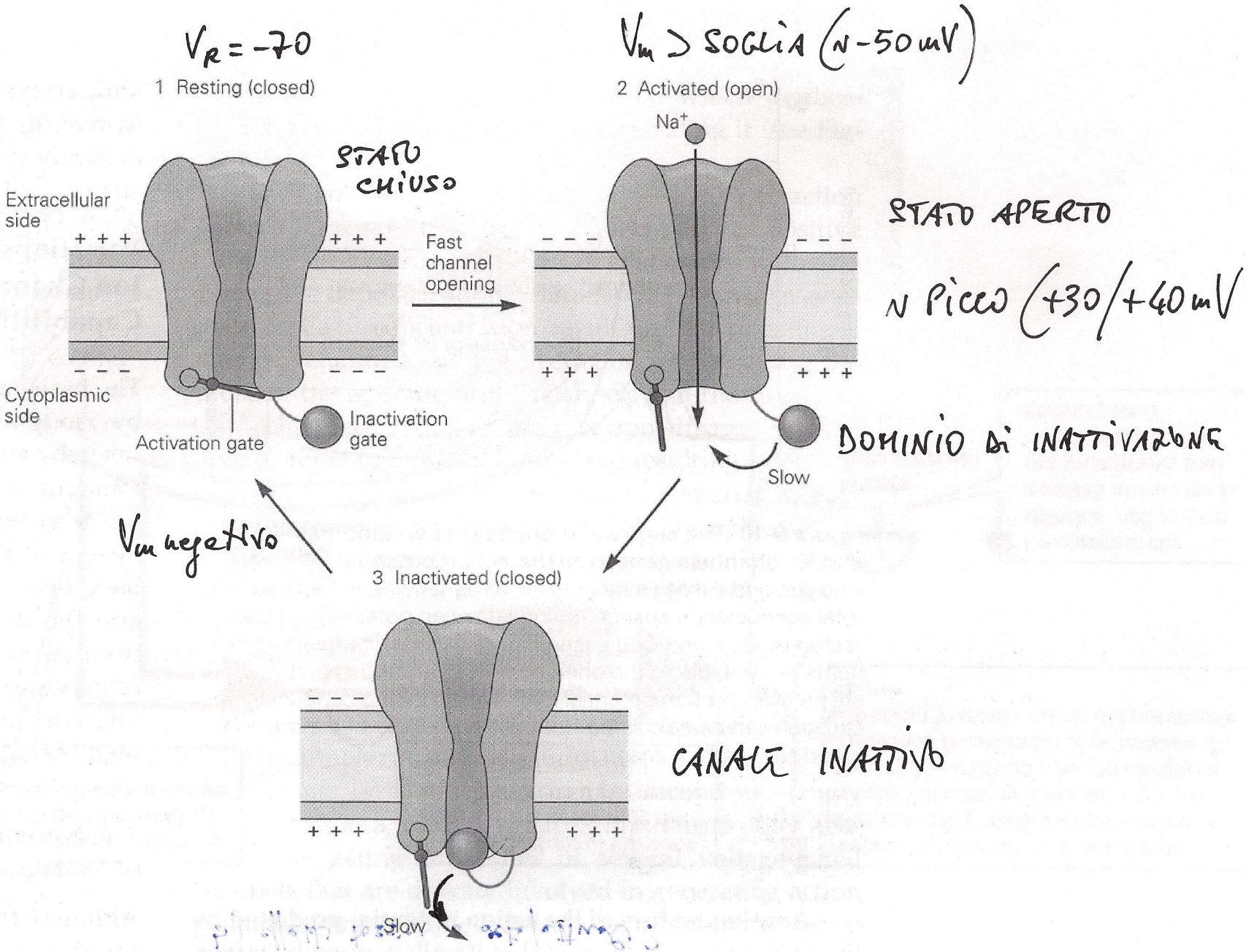
Figure 13.21 Model for K^+-channel ion transport. The selectivity filter has four binding sites. Hydrated potassium ions can enter these sites, one at a time, losing their hydration shells. When two ions occupy adjacent sites, electrostatic repulsion forces them apart. Thus, as ions enter the channel from one side, other ions are pushed out the other side.

Na: DEKA
Asp-Glu-Lys-Ala

Ca^{2+}: EEEE

$V_R = -70$

$V_m >$ SOGLIA (~ -50 mV)



STATO CHIUSO

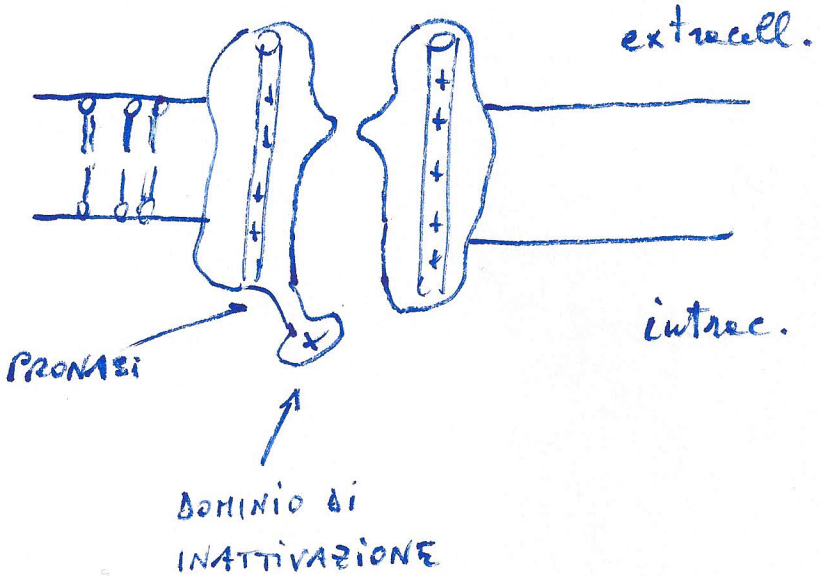
STATO APERTO

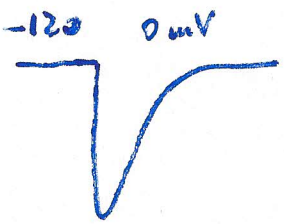
$\sim$ PICCO (+30/+40 mV

DOMINIO DI INATTIVAZIONE

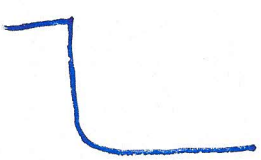
V_m negativo

CANALE INATTIVO

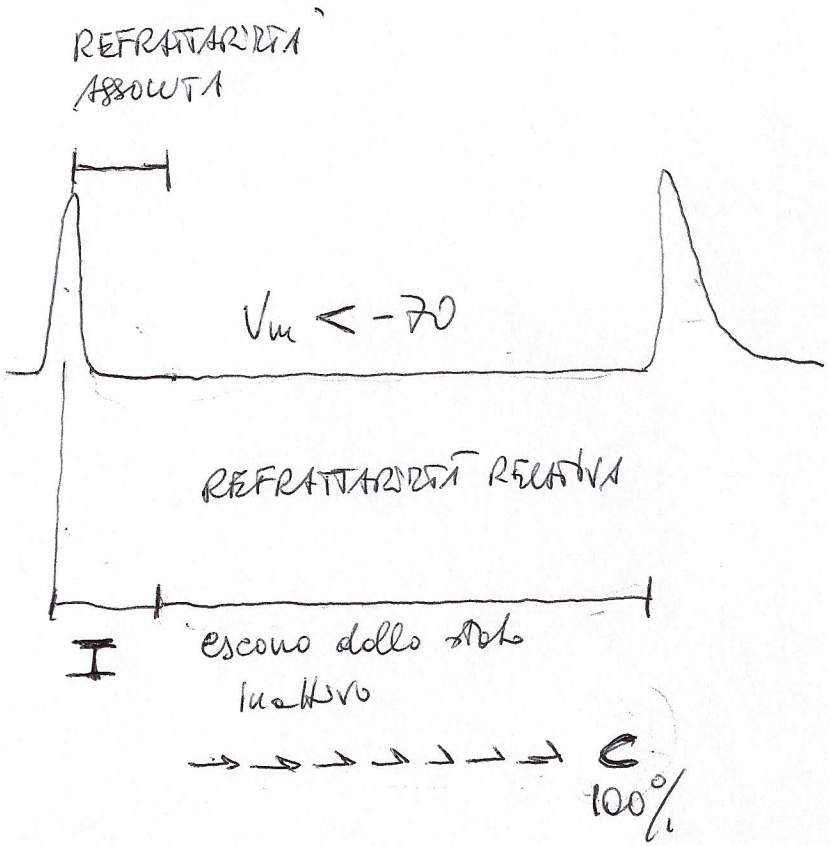




I_{Na}

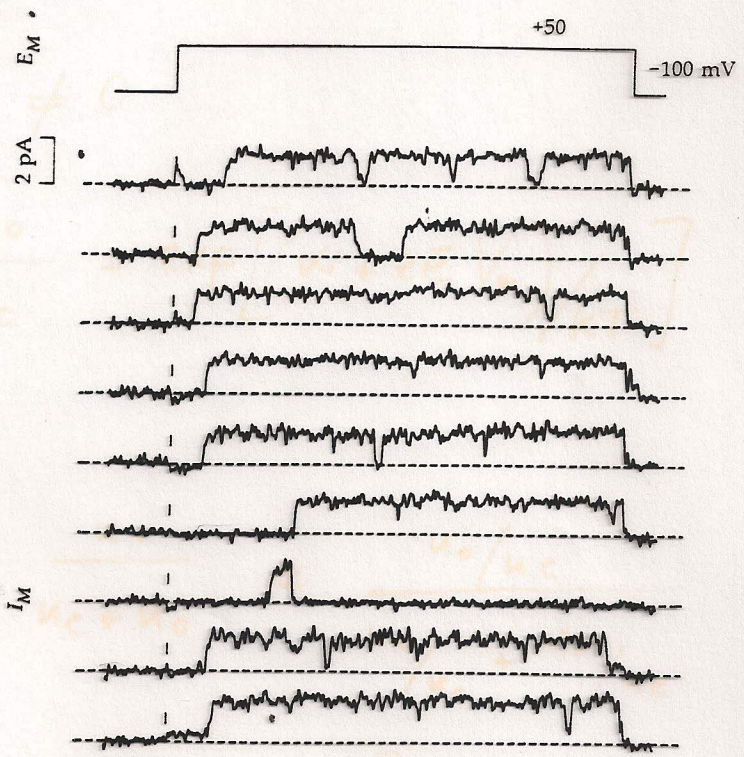


I_{Na} in PRONASI (intracell.)

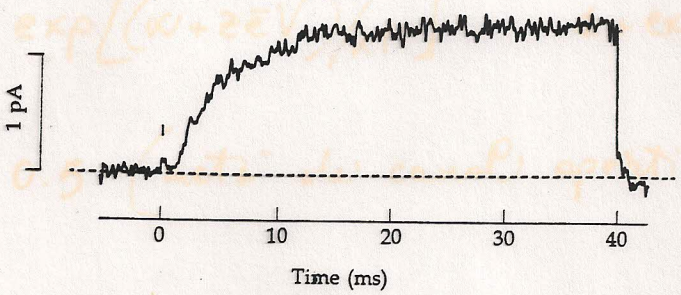

REFRATTARIETÀ ASSOLUTA
Vm < -70
REFRATTARIETÀ RELATIVA
I
escono dallo stato inattivo
100%

From: Hille, Ionic Channels of Excitable Membranes
2nd Ed. Sinauer 1992

(A) UNITARY K CURRENTS



(B) ENSEMBLE AVERAGE



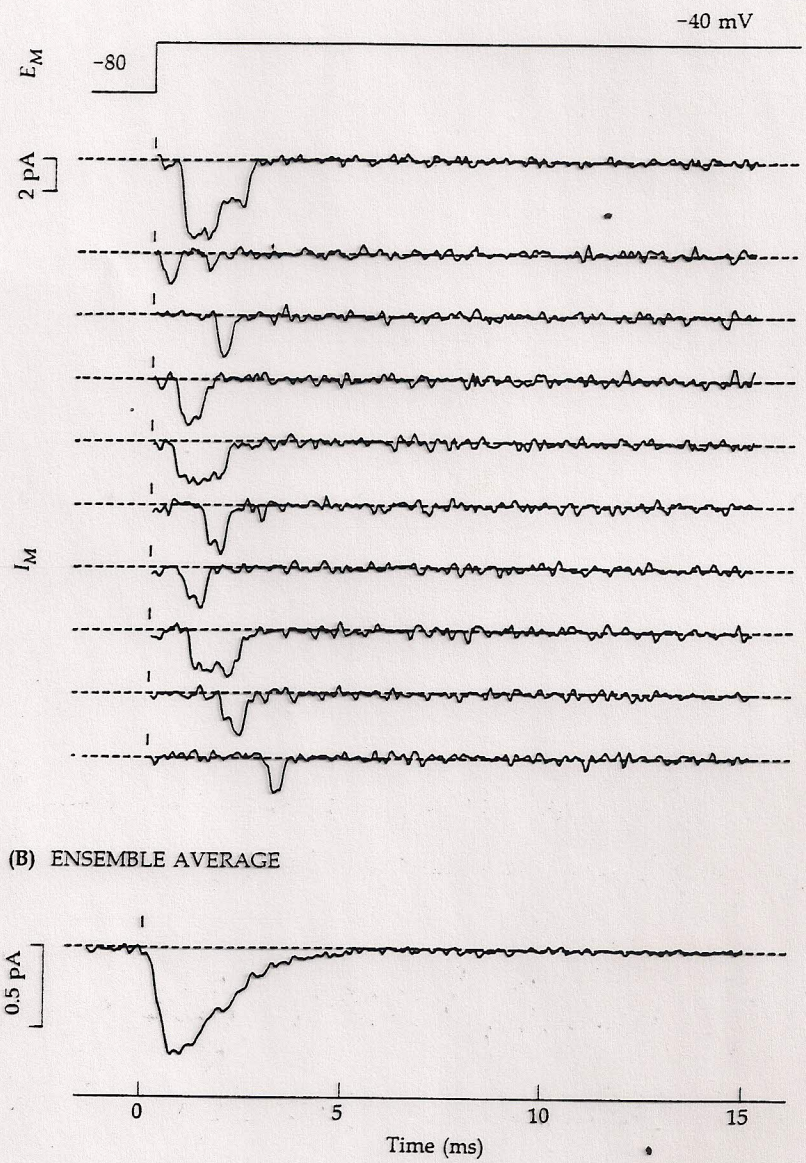
7 GATING IN SINGLE K CHANNELS

Patch-clamp recording of unitary K currents in a squid giant axon during voltage steps from −100 to +50 mV. To avoid the overlying Schwann cells, the axon was cut open and the patch electrode sealed against the *cytoplasmic* face of the membrane. (A) Nine consecutive trials showing channels of 20-pS conductance filtered at 2-kHz bandwidth. (B) Ensemble mean of 40 repeats. T = 20°C. [Kindly provided by F. Bezanilla and C.K. Augustine; see Llano et al., 1988.]

FREQUENZE CAMPIONAMENTO : N 10 KHz

MA CON METODI OTTICI 2 MOLTO MENO

From: Hille, Ionic channels of Excitable Membranes 2nd ed. Sinauer 1992.



6 GATING IN SINGLE Na CHANNELS

Patch-clamp recording of unitary Na currents in a toe muscle of adult mouse during a voltage step from −80 to −40 mV. Cell-attached recording from a Cs-depolarized fiber. (A) Ten consecutive trials filtered at 3-kHz bandwidth. Two channel openings are superimposed in the first record but not in any of the others. This patch may contain >10 Na channels. Dashed line indicates the current level when Na channels are closed. (B) The ensemble mean of 352 repeats of the same protocol. T = 15°C. [Kindly provided by J.B. Patlak; see Patlak and Ortiz, 1986.]

$$\gamma_{Na} = \frac{i_{Na}}{V_m - E_{Na}} = \frac{-1.6\,pA}{-90\,mV} = 18\,pS \quad (a\ V_m \simeq -30)$$

$$\frac{ioni}{s} = \frac{i \times N_A}{F} = \frac{1.6 \times 10^{-12} \times 6 \cdot 10^{23}}{9.6 \times 10^4} = 10^7\ \frac{ioni}{s} \quad (\text{si può considerare il N di turnover})$$

$\bar{t}_o = 0.7\,ms$ quindi N 7000 ioni entrano in media ad ogni apertura