

Ion channels and membrane potential

离子通道和膜电势

Yulong Li

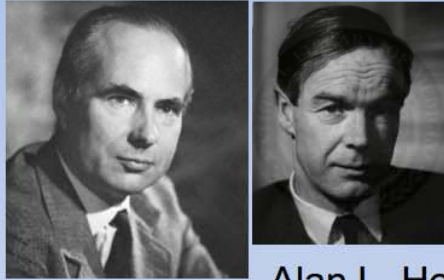
李毓龙

Oct. 8, 2015

2015. 10. 8

Four major breakthroughs in ion channel biology

- 1 Ionic conductances**
Nobel 1963 (Physiol/Medicine)



Andrew F. Huxley Alan L. Hodgkin

- 2 Patch clamp methodology**
Nobel 1991 (Physiol/Medicine)



Erwin Neher Bert Sakmann

- 3 ACh receptor channel cloning/sequencing**

Shosaku Numa
(Kyoto)

- 4 K channel structure**
Nobel 2003 (Chemistry)



Rod MacKinnon

Logics

Illustrate the mechanism of AP generation and propagation.

阐述动作电位产生及扩步的机制
↓

Identification (cloning) of ion channel genes.

离子通道基因的发现
↓

Illustrate the structure basis of ion channel function.

离子通道功能的结构基础

Outlines

- 1. Hodgkin-Huxley model

Hodgkin-Huxley模型

- 2. Ion channel gating (gating current)

门控离子通道

- 3. Ion channel conformation change (inactivation-ball and chain model)

离子通道构象变化

- 4. Structure of ion channels.

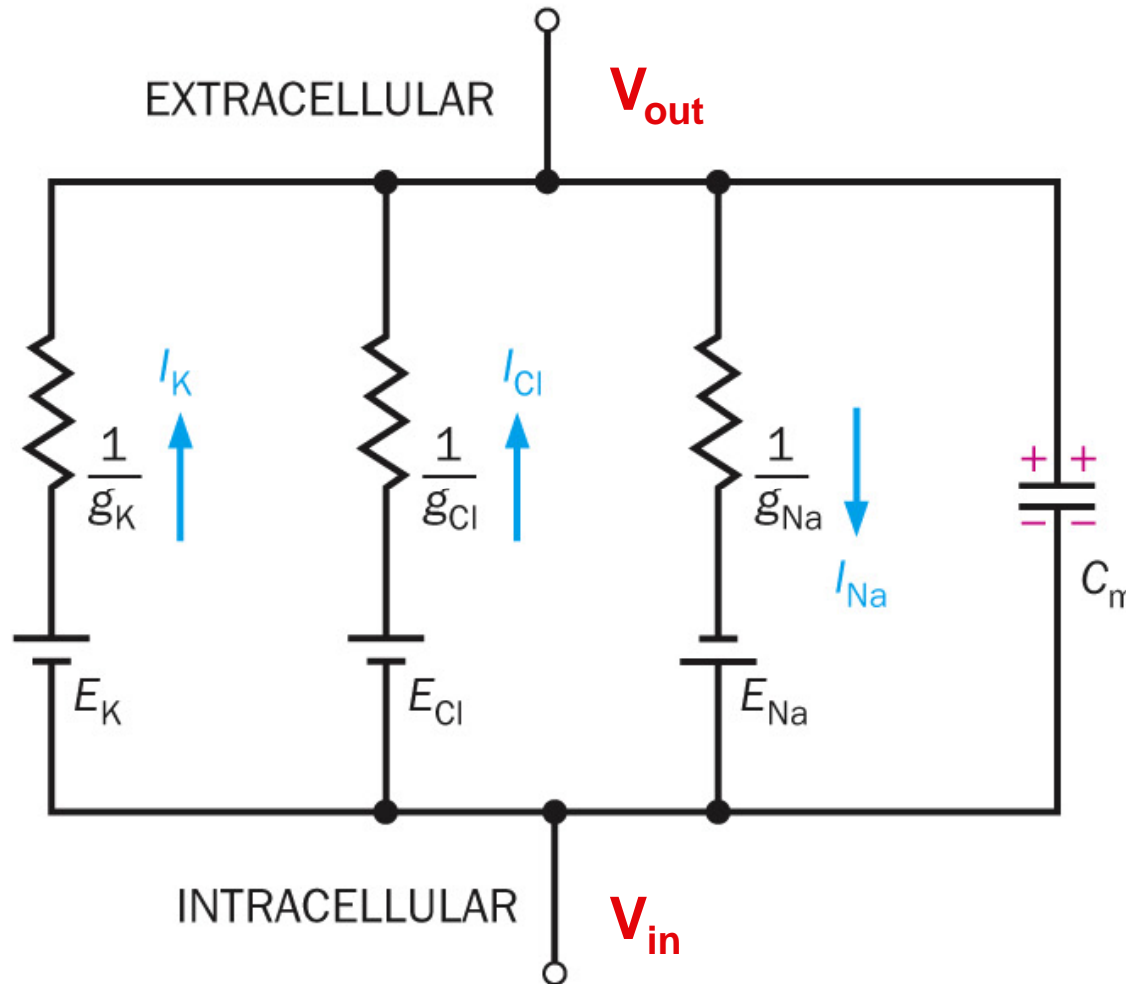
离子通道的结构

Classic papers:

- 1) Hodgkin, and Katz. "The effect of sodium ions on the electrical activity of the giant axon of the squid." In *J. Physiol* 108, (1949): 37–77.
- 2) Hodgkin, Huxley, and Katz. "Measurement of current-voltage relations in the membrane of the giant axon in *Loligo*." In *J. Physiol* 116, (1952): 424–448.
- 3) Hodgkin, and Huxley. "Currents carried by sodium and potassium ions through the membrane of the giant axon of *Loligo*." In *J. Physiol* 116, (1952): 449–472.
- 4) Hodgkin, and Huxley. "The components of membrane conductance in the giant axon of *Loligo*." In *J. Physiol* 116, (1952): 473–496.
- 5) Hodgkin, and Huxley. "The dual effect of membrane potential on sodium conductance in the giant axon of *Loligo*." In *J. Physiol* 116, (1952): 497–506.
- 6) Hodgkin, and Huxley. "A quantitative description of membrane current and its application to conduction and excitation in

The passive equivalent circuit

(B)



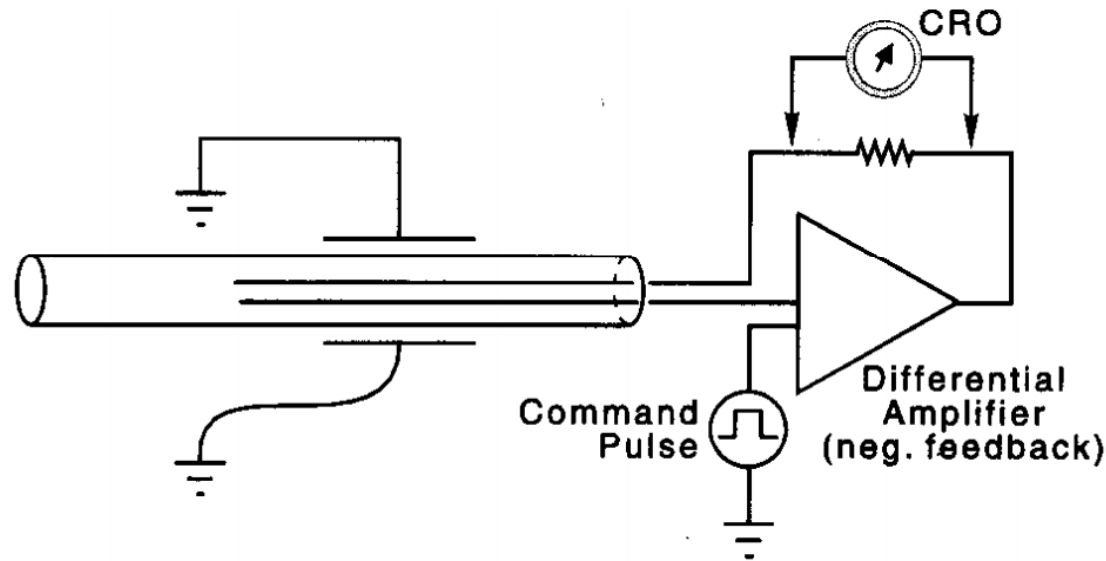
Solving for V_{rest}

1. $I_{Na} + I_K = 0$
2. $V_{in} - V_{out} = E_K + I_K / g_K$
3. $V_m - V_{out} = E_{Na} + I_{Na} / g_{Na}$
4. $I_K = g_K(V_m - E_K)$
5. $I_{Na} = g_{Na}(V_m - E_{Na})$

Finally,

$$V_m = \frac{(E_{Na}g_{Na} + E_Kg_K)}{g_{Na} + g_K}$$

Voltage Clamp

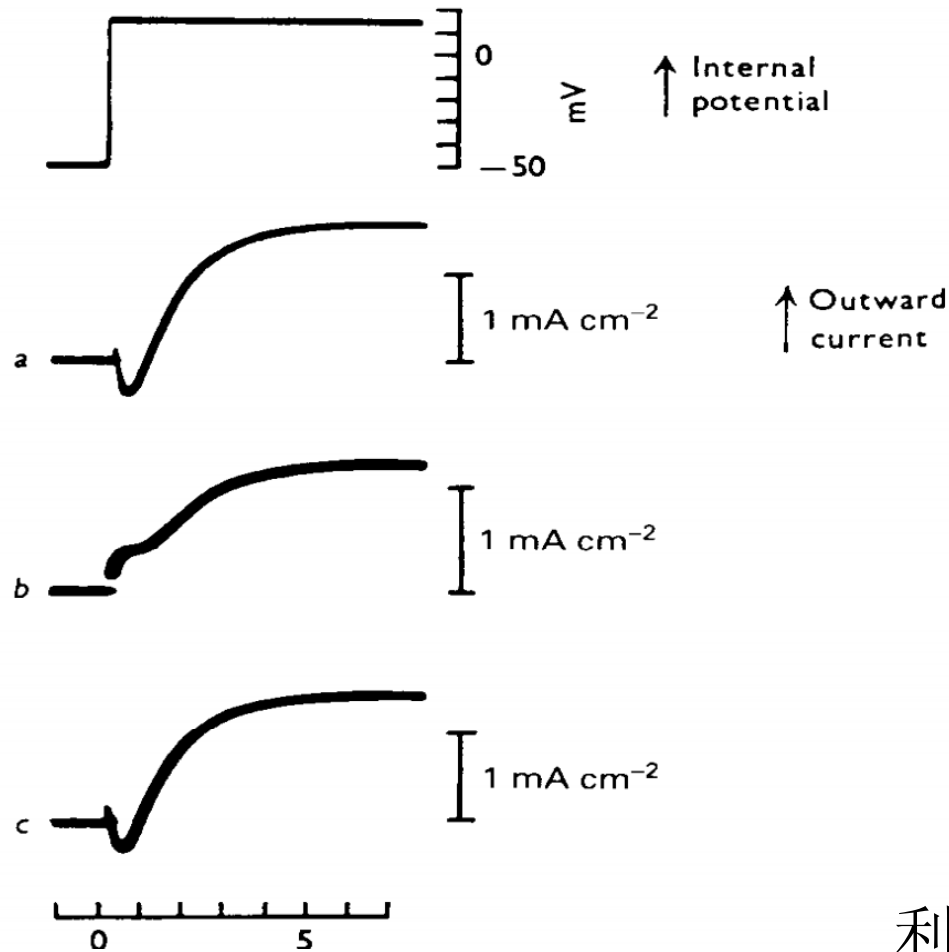


The voltage clamp method used in a squid giant axon. The two wires inserted into the axon are used to measure membrane potential (V) and to pass current (I). The high-gain negative-feedback amplifier compares the command pulse with the membrane potential, and outputs the amount of current necessary to hold the membrane potential constant (or “clamped”). The magnitude of the feedback current can be measured as the IR voltage drop across a resistor and displayed on a cathode-ray oscilloscope (CRO).

以枪乌贼的轴突为实验材料，利用电压钳技术进行实验。将两个电极插入轴突分别测量膜电势和电流。

利用反馈电路，通过向细胞内注入电流的方式，人为的将细胞膜电位钳制在指定电位水平；通过电流检测装置，记录到注入细胞内的电流，这个电流正好相当于离子电流的反向电流。

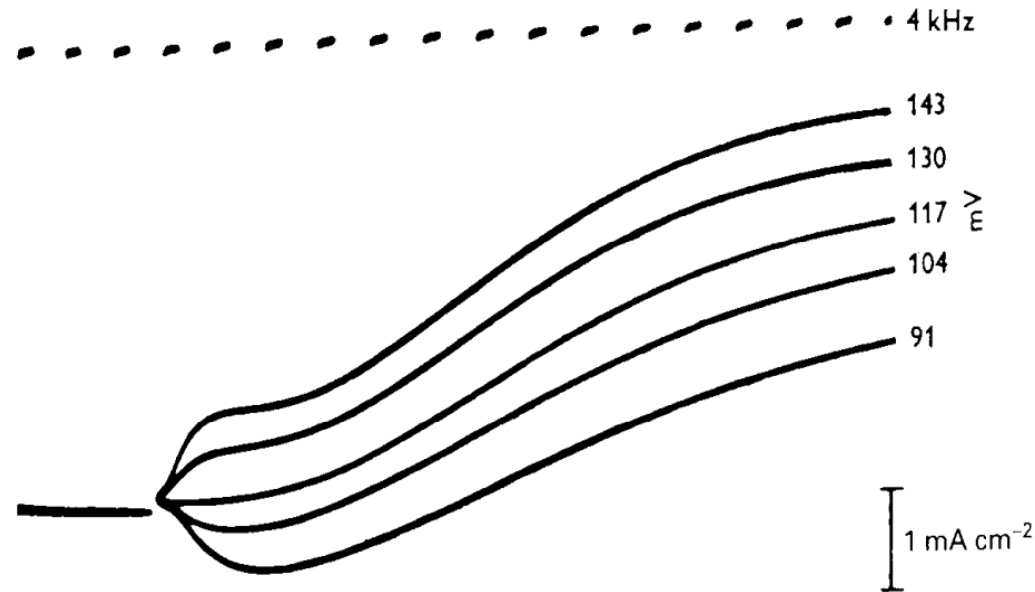
Typical records of the membrane current during a voltage clamp experiment. *a* and *c*: In sea water; *b*: in a sodium-free choline chloride solution. (From Hodgkin, 1958, after Hodgkin and Huxley, 1952*a*.)



利用电压钳记录膜电流实验。

去极化时的膜电流

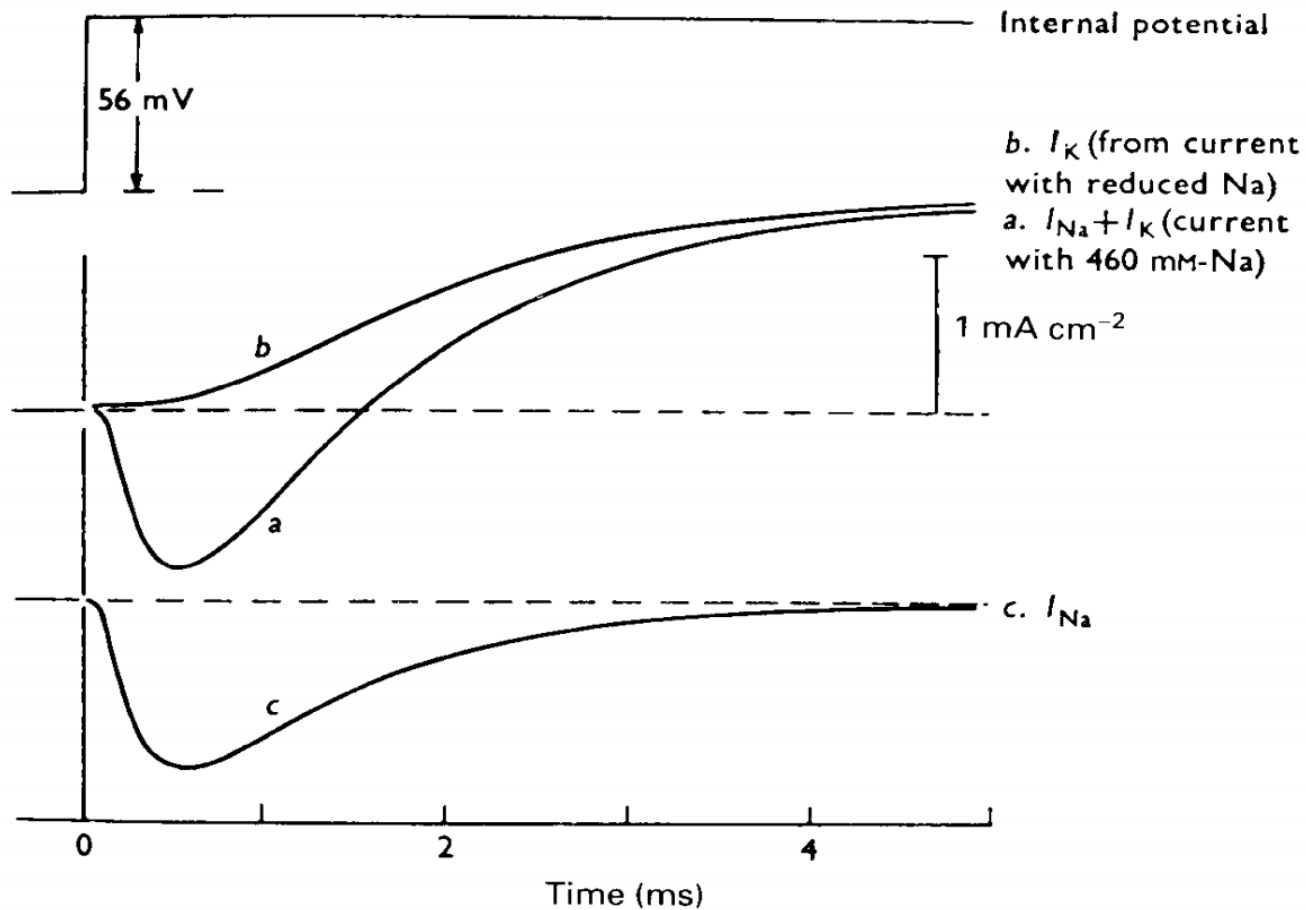
Membrane currents at large depolarizations. Values of V are shown at the right of each record. (From Hodgkin, 1958, after Hodgkin, Huxley and Katz, 1952.)



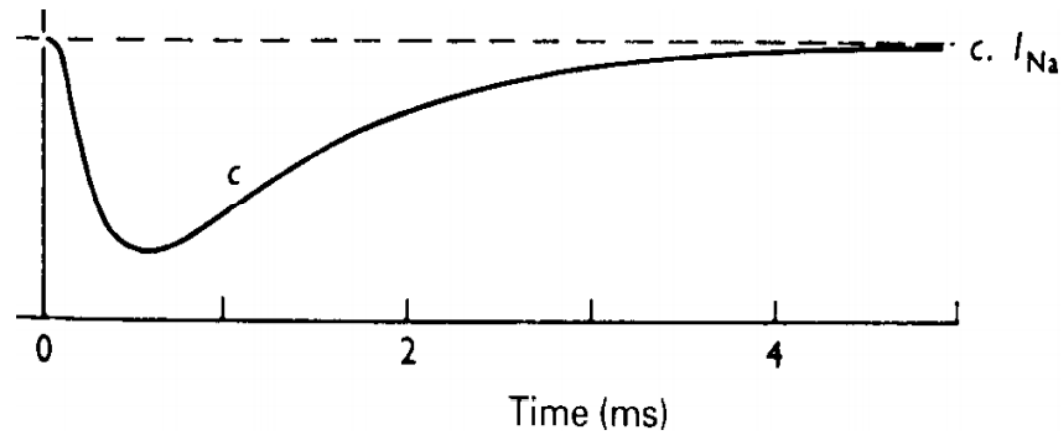
The potential at which the initial (Na current) is neither inward nor outward is the reversal potential E_{Na} for the Na current i.e about 117 mV ?

钠离子通道起始电流既不是正向也不是逆向时的电势是钠离子的逆转电位，117mV

Analysis of the ionic current in a *Loligo* axon during a voltage clamp. Trace *a* shows the response to a depolarization of 56 mV with the axon in sea water. Trace *b* is the response with the axon in a solution comprising



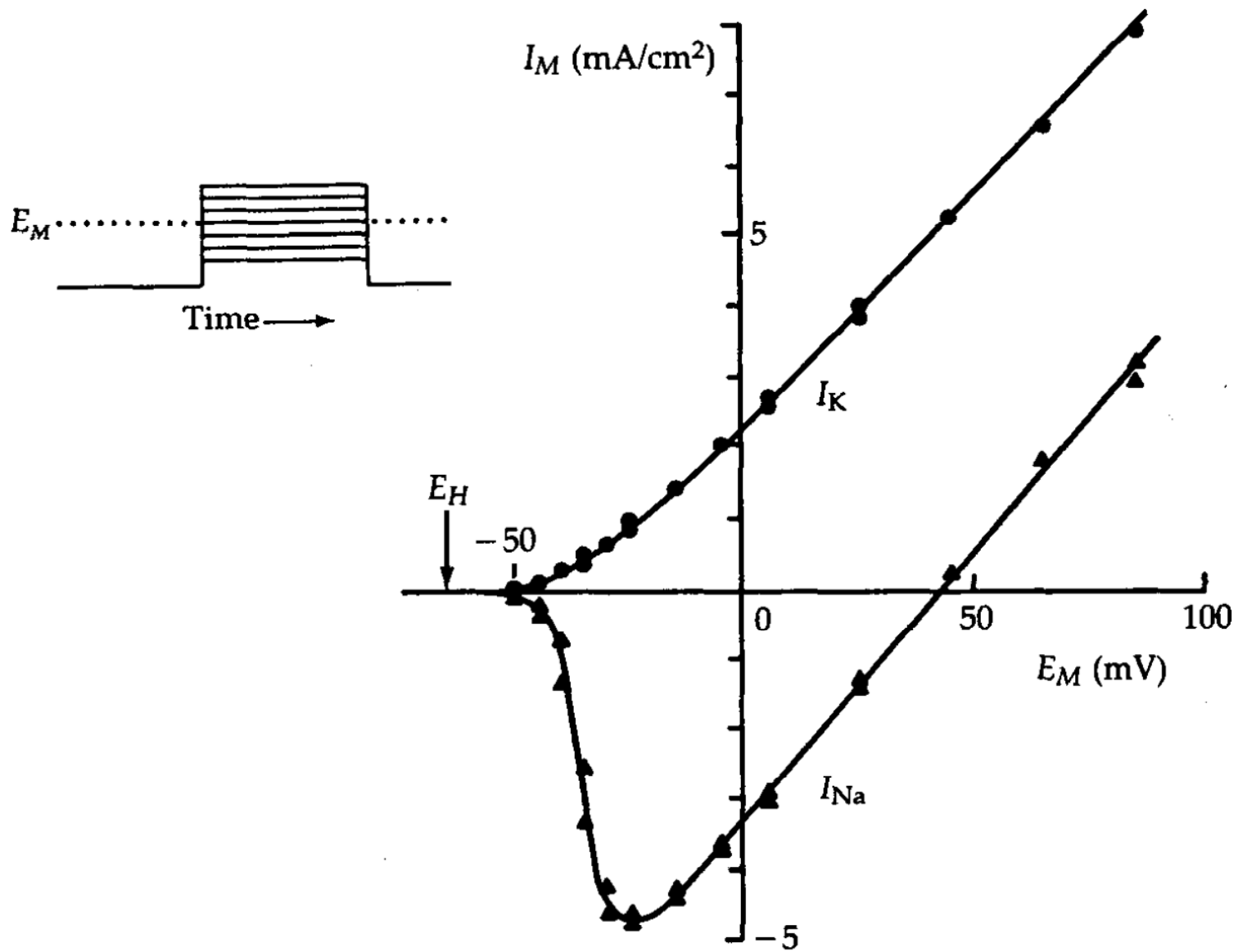
枪乌贼轴突的电压钳离子电流分析图

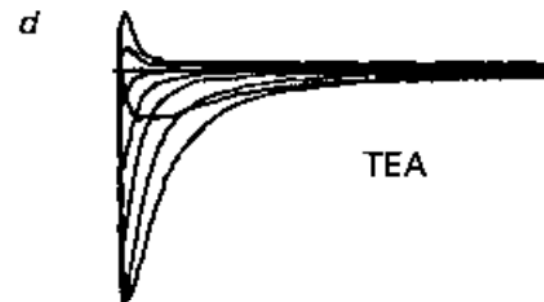
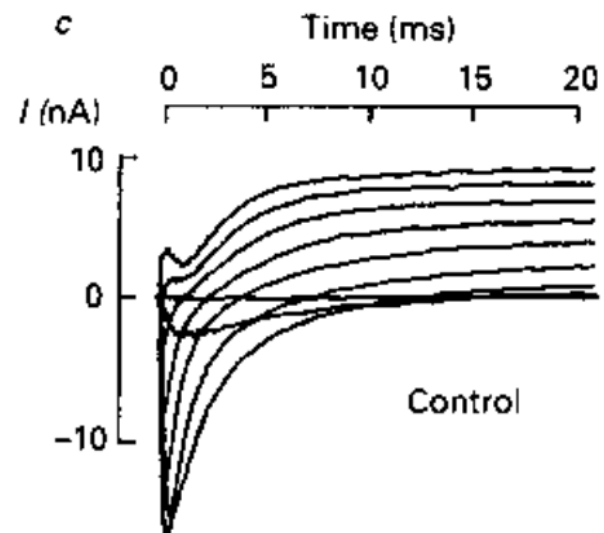
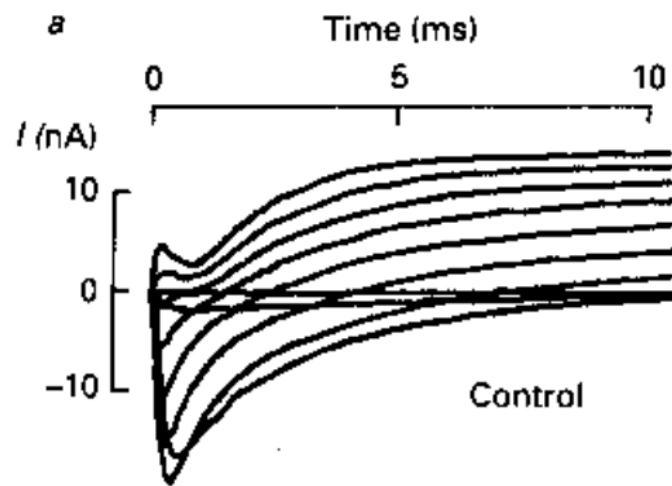


From traces of Na current as a function of time we can obtain g_{Na} by using the equation $I_{Na} = g_{Na}(E - E_{Na})$. E_{Na} is the potential at which the current is nulled.

通过图中获得的钠离子电流及方程 $I_{Na} = g_{Na}(E - E_{Na})$ 可以算出钠离子通道电导。膜电位等于钠平衡电位时，电流为0

可以算





$$g_K = \bar{g}_K n^4 \quad n = \text{probability of 4 charged particles being in the correct configuration for conduction.}$$

N是组成钾离子通道的四个部分处于正确的构象的概率

$$g_{Na} = \bar{g}_{Na} m^3 h \quad n = \text{probability of 3 charged particles being in the correct configuration. } 1-h = \text{probability of inactivation.}$$

M是三个组成钠离子通道的部分处于正确构象的概率，1-h是通道失活的概率
 n is the potassium activation parameter, m and h are the Na activation and inactivation parameters.

$$I_m = C_m dE/dt + I_K + I_{Na} + I_i$$

$$= C_m dV/dt + g_K (E - E_K) + g_{Na} (E - E_{Na}) + g_i (E - E_i)$$

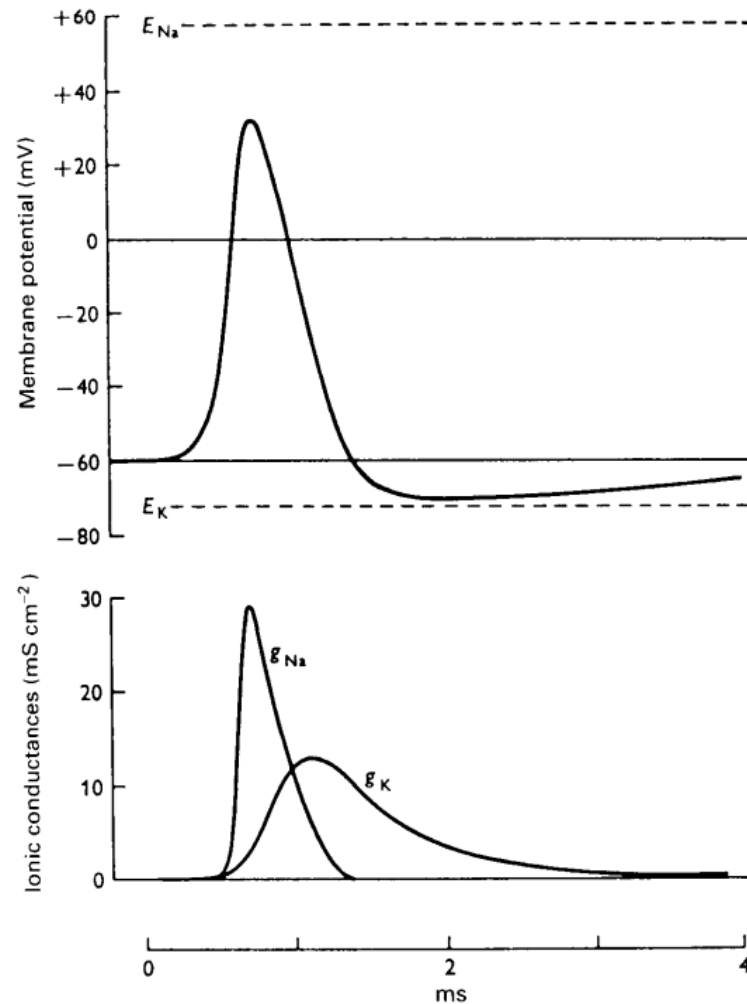
$$= C_m dV/dt + \bar{g}_{Na} n^4 (E - E_K) + \bar{g}_{Na} m^3 h (E - E_{Na}) + g_i (E - E_i)$$

With the voltage and time dependence of m, n and h the Above equation can be solved for V by numerical integration

n、m、h是确定的参数，与钠/钾离子通道的性质相关
 如果知道m\ n\ h三个参数的电压/时间依赖性，就可以通过微积分求解上式

Calculated changes in membrane potential (upper curve) and sodium and potassium conductances (lower curves) during a propagated action potential in a squid giant axon. The scale of the vertical axis is correct, but its position may be slightly inaccurate; it has been drawn here assuming a resting potential of

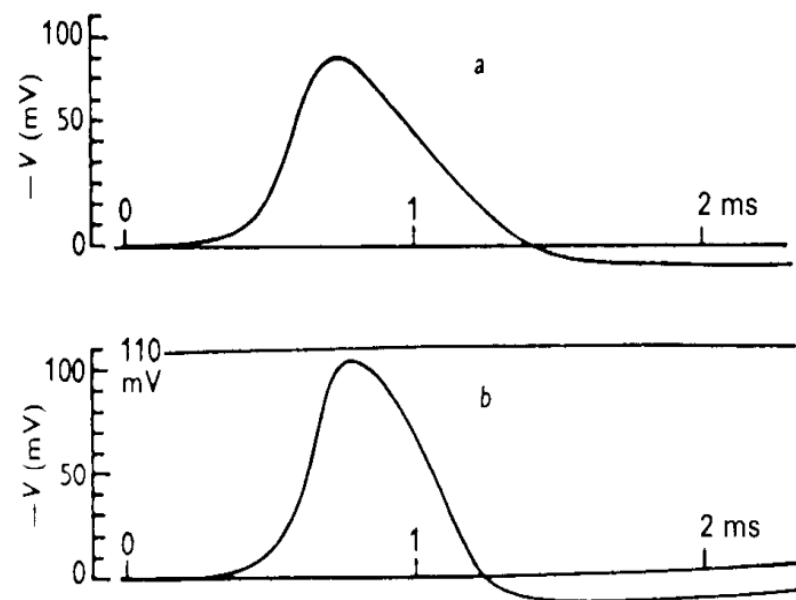
-60 mV. The positions of E_{Na} and E_K are correct with respect to the resting potential. In the original calculations, voltages were measured from the resting potential, as in fig. 5.15. (After Hodgkin and Huxley, 1952d; redrawn.)



上图：动作电位电压-时间曲线

下图：钠离子、钾离子电导与时间的关系

Comparison of computed (*a*) and observed (*b*) propagated action potentials in squid axon at 18.5 °C. The calculated velocity of conduction was 18.8 m s⁻¹; the observed velocity was 21.2 m s⁻¹. (From Hodgkin and Huxley, 1952*d*.)



比较18.5℃时，计算的和实际测得的枪乌贼轴突动作电位

New age after the heroic
era ...

The “Holy Grail – Part I”

(Clay Armstrong)

Primary structure of *Electrophorus electricus* sodium channel deduced from cDNA sequence

Masaharu Noda, Shin Shimizu, Tsutomu Tanabe, Toshiyuki Takai, Toshiaki Kayano, Takayuki Ikeda, Hideo Takahashi, Hitoshi Nakayama*, Yuichi Kanaoka*, Naoto Minamino†, Kenji Kangawa†, Hisayuki Matsuo†, Michael A. Raftery‡, Tadaaki Hirose§, Seiichi Inayama§, Hidenori Hayashida||, Takashi Miyata|| & Shosaku Numa

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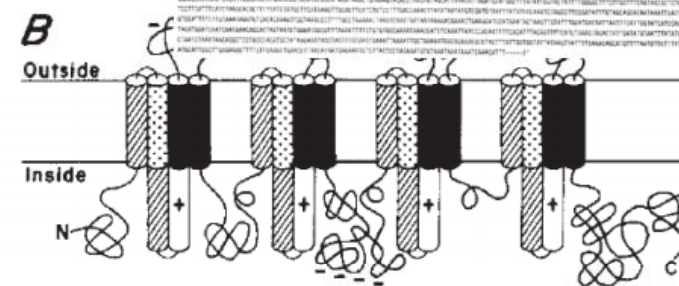
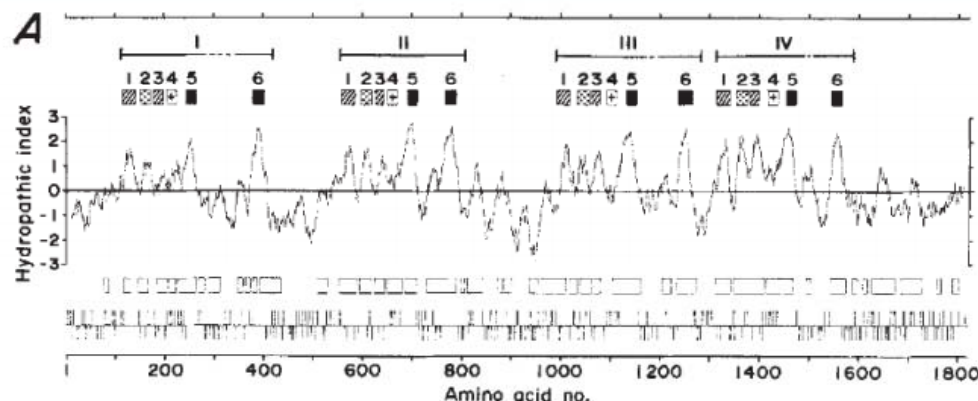
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NATURE VOL. 312 8 NOVEMBER 1984



Molecular Characterization of *Shaker*, a Drosophila Gene That Encodes a Potassium Channel

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and Mark A. Tanouye

Division of Biology 216-76
California Institute of Technology
Pasadena, California 91125

Cell (1987) 50:405

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      15      30      45
GAT CTG AAG TTC CAA GTG CGA GTG GCT TTC DCT TTC CDT ATT CCG GTC CAT
Asp Leu Lys Phe Gln Val Arg Val Ala Phe Ala Phe Arg Ile Arg Val His

      60      75      90      105
TTT CGT TTC GGT TTC GTT GGA AAG CTA GAG CCG TGC TGC CAT CCG CAC AGT TTC
Phe Arg Phe Gly Phe Val Gly Lys Leu Glu Arg Cys Cys His Arg His Ser Phe

      120      135      150      165
TTC GAT CCG AAC CCG ATT TGG GAA ACA GCC GCC AAG ATG ACC ATG TGG CAG AGT
Phe Asp Arg Asn Arg Ile Trp Glu Thr Ala Ala Lys Met Thr Met Trp Gln Ser

      180      195      210      225
GCC GGC ACG AGC GCA TGG CTC CCA TGG ATG AAG CTG ATG GCA TCG TCC ACA ACG
Glu Gly Arg Ser Ala Trp Leu Pro Trp Met Lys Leu Met Ala Ser Ser Thr Arg

      240      255      270      285
AGC GCG CCA CAC GGA GAA CGT TCA GAG TCA GTC CCG TTC CAA CBA CCG CAA CCT
Ser Ala Pro His Gly Glu Arg Ser Glu Ser Val Arg Phe Gln Arg Ala Gln Pro

      300      315      330      345
GAA CCA GTC TTT GCC CAA ATT GAG CAG TCA AGA CBA AGA ACG GCG GCG TGG TCA
Glu Pro Val Phe Ala Gln Ile Glu Gln Ser Arg Arg Arg Arg Gly Gly Trp Ser

      360      375      390      405
TGG CTT TGG TGC GGA CCG CAA CAC TTT GAA CCC ATT CCT CAC GAT GAT GAT TCT
Trp Leu Trp Cys Gly Pro Gln His Phe Glu Pro Ile Pro His Asp Asp Asp Ser

      420      435      450      465
CCG AAA AGA GTC GTT ATA AAT ATA AAT GTA ACG GGA TTA ACG TTT GAG ACA CAA
Ala Lys Arg Val Val Ile Asn Ile Asn Val Ser Gly Leu Arg Phe Glu Thr Gln

      480      495      510      525
CTA CGT ACG TTA AAT CAA TTC CCG BAC ACG CTG CTT GCG BAT CCA GCT CCG AGA
Leu Arg Thr Leu Asn Gln Phe Pro Asp Thr Leu Leu Gly Asp Pro Ala Arg Arg

      540      555      570      585
TTA CCG TAC TTT GAC CCG CTT AGA AAT GAA TAT TTT TTT BAC CBT AGT CBA CCG
Leu Arg Tyr Phe Asp Pro Leu Arg Asn Glu Tyr Phe Phe Asp Arg Ser Arg Pro

      600      615      630      645
AGC TTC GAT CCG ATT TTA TAC TAT TAT CAG AGT GGT GGC CCA CTA CCG AGA CCG
Ser Phe Asp Ala Ile Leu Tyr Tyr Tyr Gln Ser Gly Arg Leu Arg Arg Arg Pro

      660      675      690      705
GAT CAA GCA ATT AAT AAA TTC AGA GAG BAT GAA GGC TTT ATT AAA GAG GAA GAA
Asp Gln Ala Ile Asn Lys Phe Arg Glu Asp Glu Gly Phe Ile Lys Glu Glu Glu

      720      735      750      765
AGA CCA TTA CCG GAT AAT GAG AAA CAG AGA AAA GTC TGG CTG TCC TTC GAG TAT
Arg Pro Leu Pro Asp Asn Glu Lys Gln Arg Lys Val Trp Leu Ser Phe Glu Tyr

      780      795      810      825
CCA GAA AGT TCG CAA GCG GCC AGA GTT GTA GCC ATA ATT AGT GTA TTT GTT ATA
Pro Glu Ser Ser Gln Ala Ala Arg Val Val Ala Ile Ile Ser Val Phe Val Ile

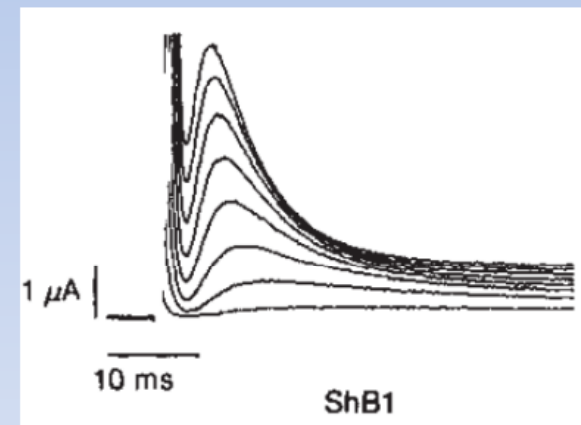
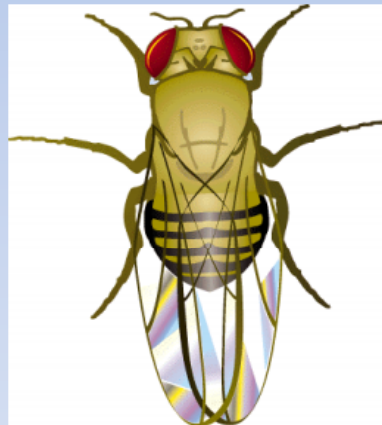
      840      855      870      885
TTG CTA TCA ATT GTT ATA TTT TGT CTA GAA ACA TTA CCG GAA TTT AAG CAT TAC
Leu Leu Ser Ile Val Ile Phe Cys Leu Glu Thr Leu Pro Glu Phe Lys His Tyr

      900      915      930      945
AAG GTG CDT ACG AAT CAA GCG AAA CCT CAG GAC CTC CAA GCG ATA CAA ATC CAT
Lys Val Arg Thr Asn Gln Ala Lys Pro Gln Asp Leu Gln Gly Ile Gln Ile His

      960      975      990      1005
ATT TTC CTT TCC TTT TCT TTT TCC TGT GTT TCT CCG TGG CAT ACT TTC ACG TGT
Ile Phe Leu Ser Phe Ser Phe Ser Cys Val Ser Val Trp His Thr Phe Arg Cys

      1020      1035      1050      1065
TCA ATA CAA CAA CAA ATG GCA CAA AAA TCC CCG AAG CCG GAG TGG CCT GAC ATC
Ser Ile Gln Gln Gln Met Ala Gln Lys Ser Arg Lys Pro Glu Trp Pro Asp Ile

      1080      1095      1110      1125
CAG ATC CTT TCC TTC CTT ATA GAA ACG TTA TGT ATT ATT TGG TTT CAT TTG AAC
Gln Ile Leu Ser Phe Leu Ile Glu Thr Leu Cys Ile Ile Trp Phe His Leu Asn
    
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Timpe et al (1988) *Nature* 331:143

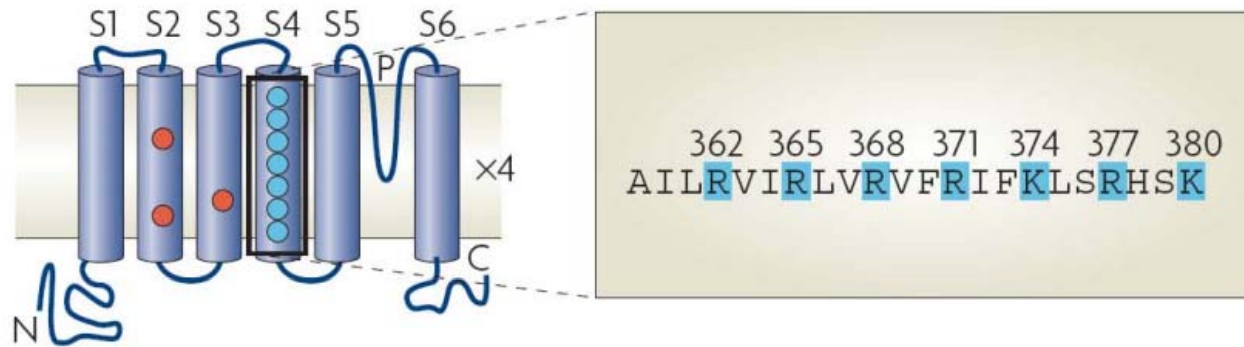
Voltage sensing

VSD: the voltage sensor domain

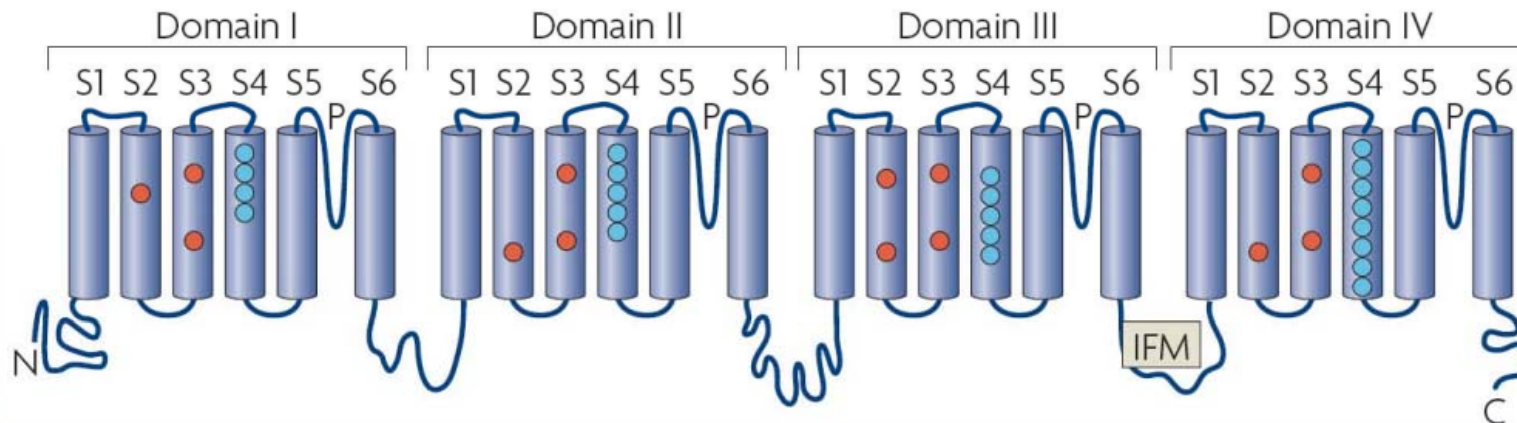
VSD: 电压敏感结构域

VSD = S2/S3/S4

a Shaker B

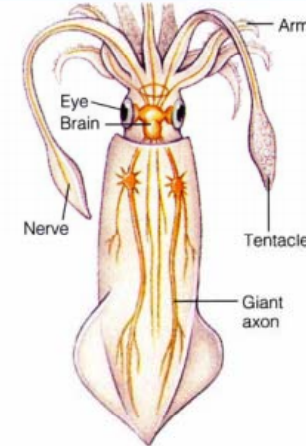


b Nav1.4

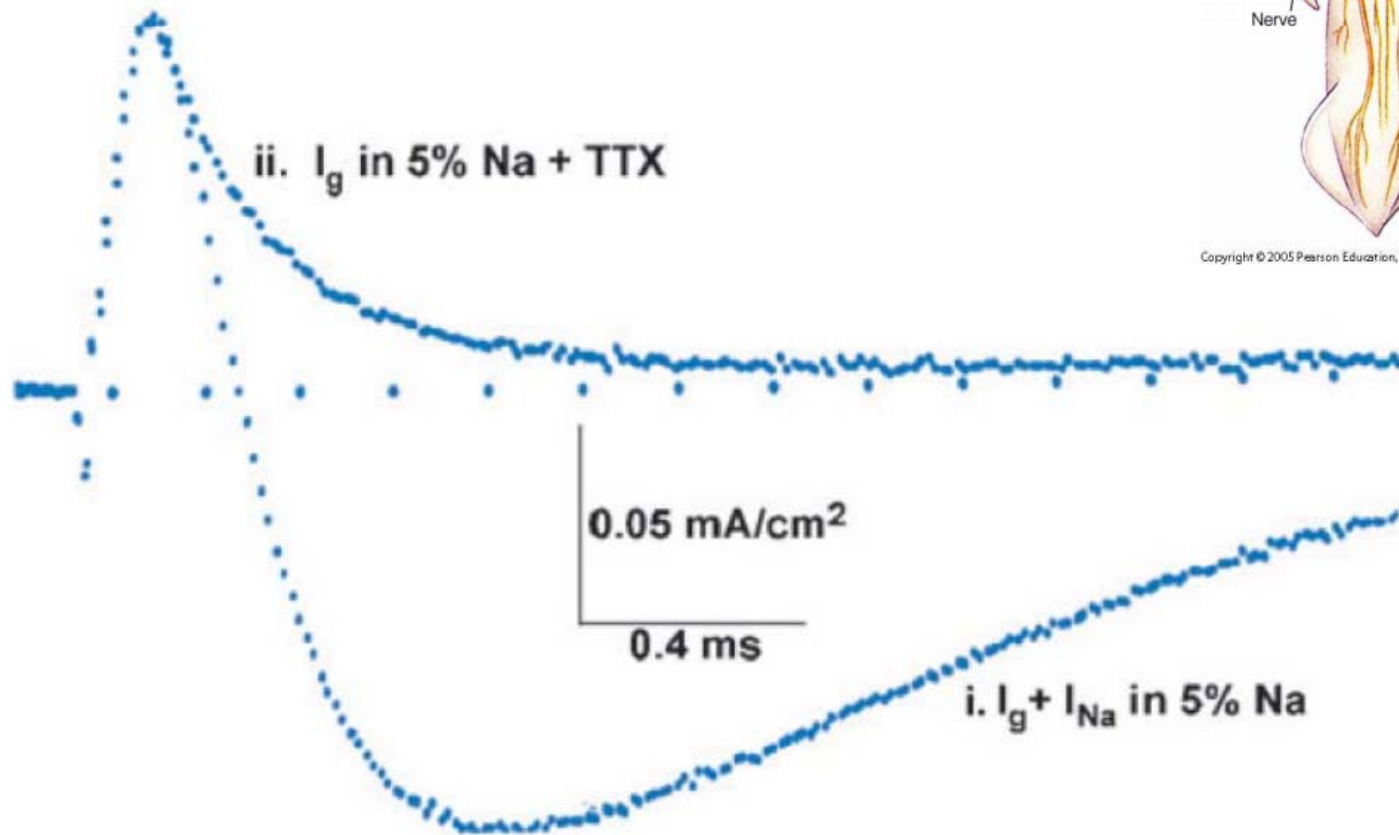


- Salt bridge forms between acidic residues in S2/S3 (red spheres) and basic residues of S4 (blue spheres)
- Consistent with helical screw motion

First recording of gating current (I_g) for Na channels in a squid giant axon



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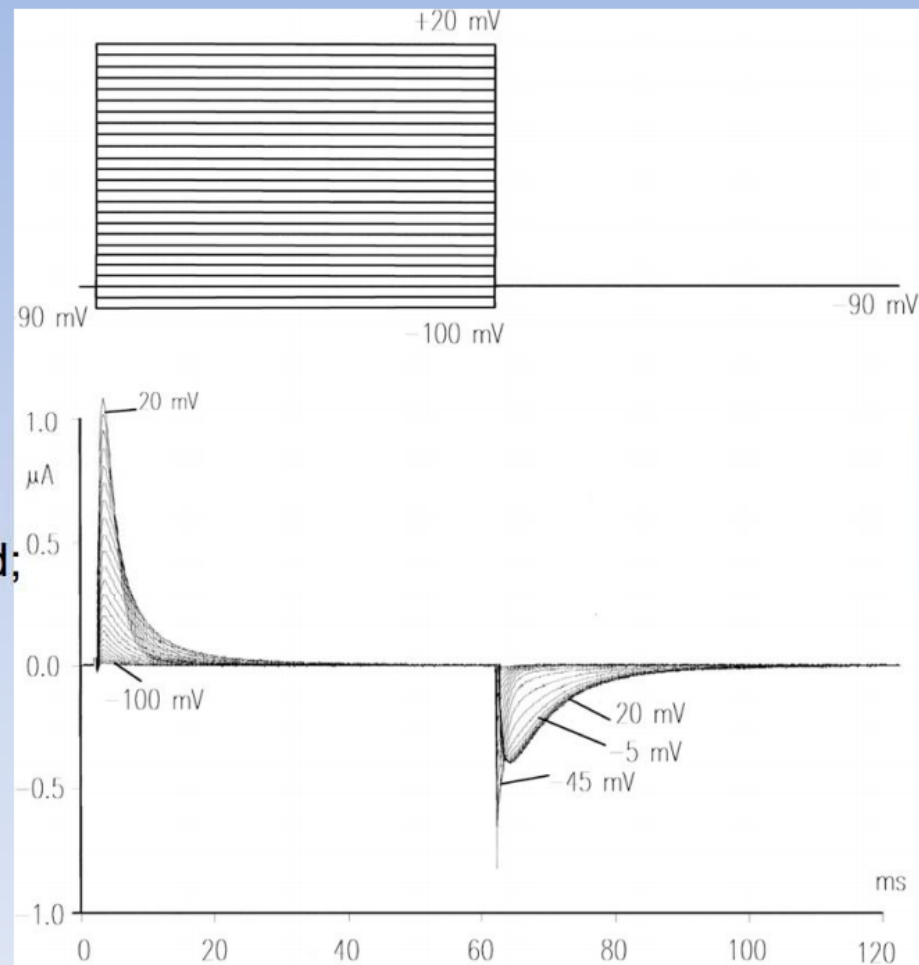
Armstrong CM, Bezanilla F. 1973. Currents related to movement of the gating particles of the sodium channels. *Nature* 242:459–61

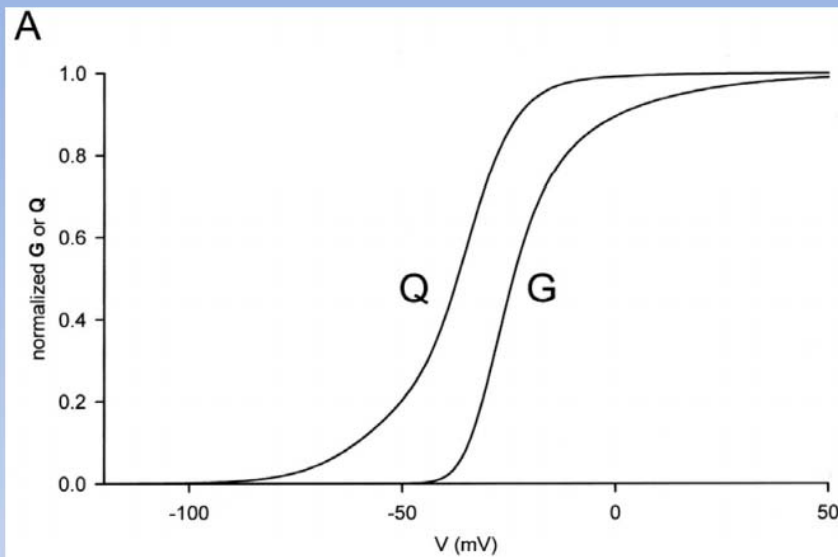
I_K was eliminated by removing all K^+ , I_{Na} was reduced by lowering $[Na^+]$.
 I_{cap} was removed by subtraction, then eliminated with tetrodotoxin (TTX)

Gating currents of cloned *Shaker* K channel

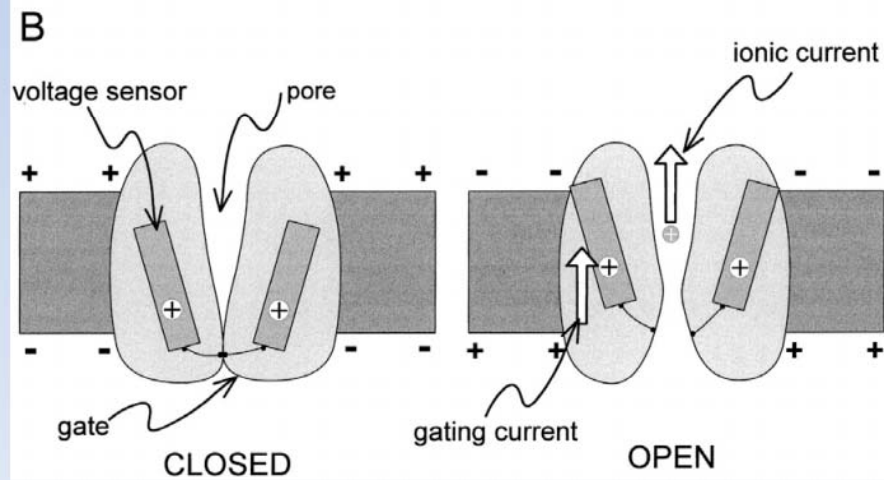
Voltage pulse protocol

Gating currents
(Ionic currents blocked;
or use nonconducting
channels)





Q = charge
(gating current)
 G = conductance
(ionic current)



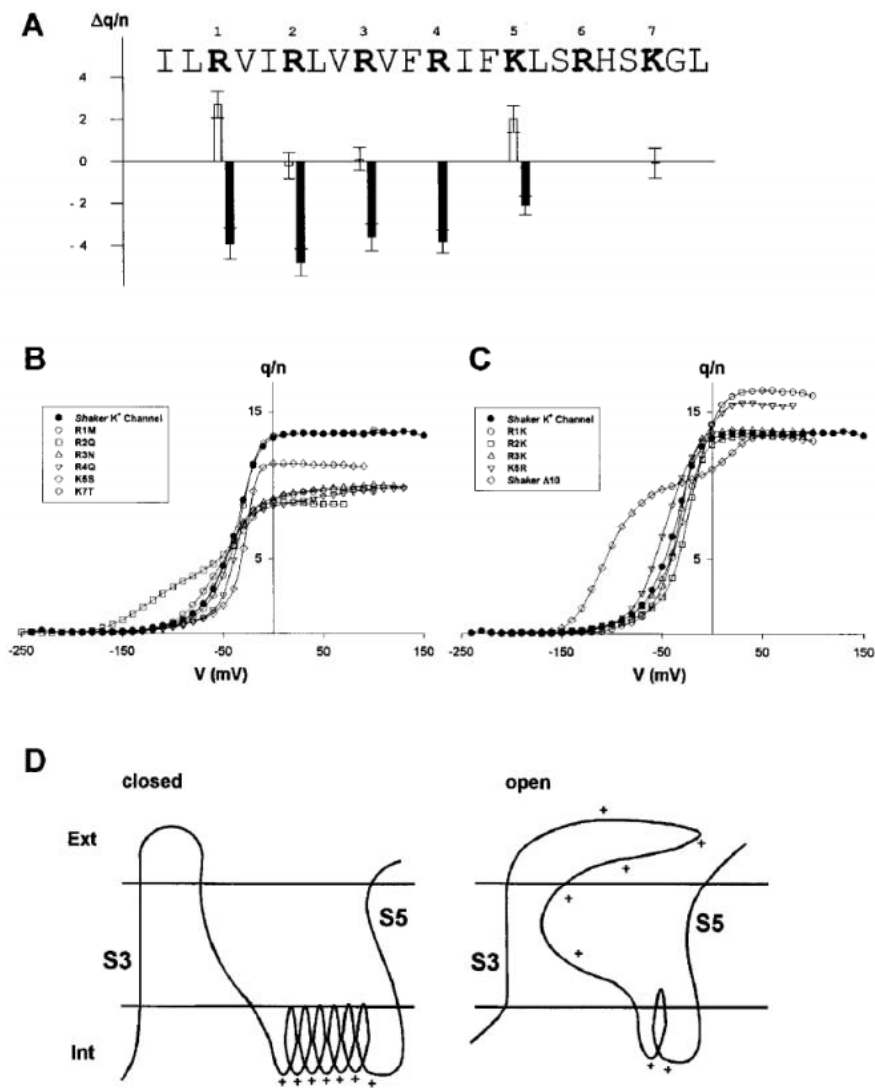
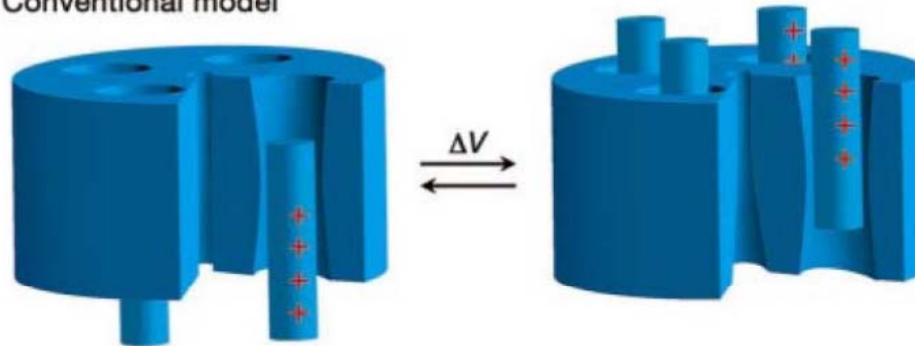


Figure 7. Summary

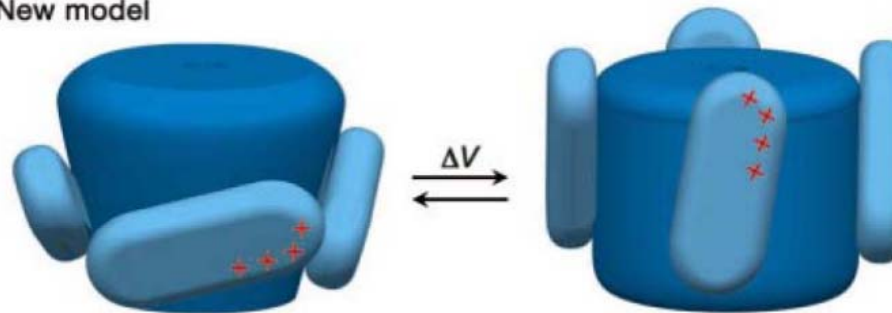
controversy

a Conventional model



Shaker

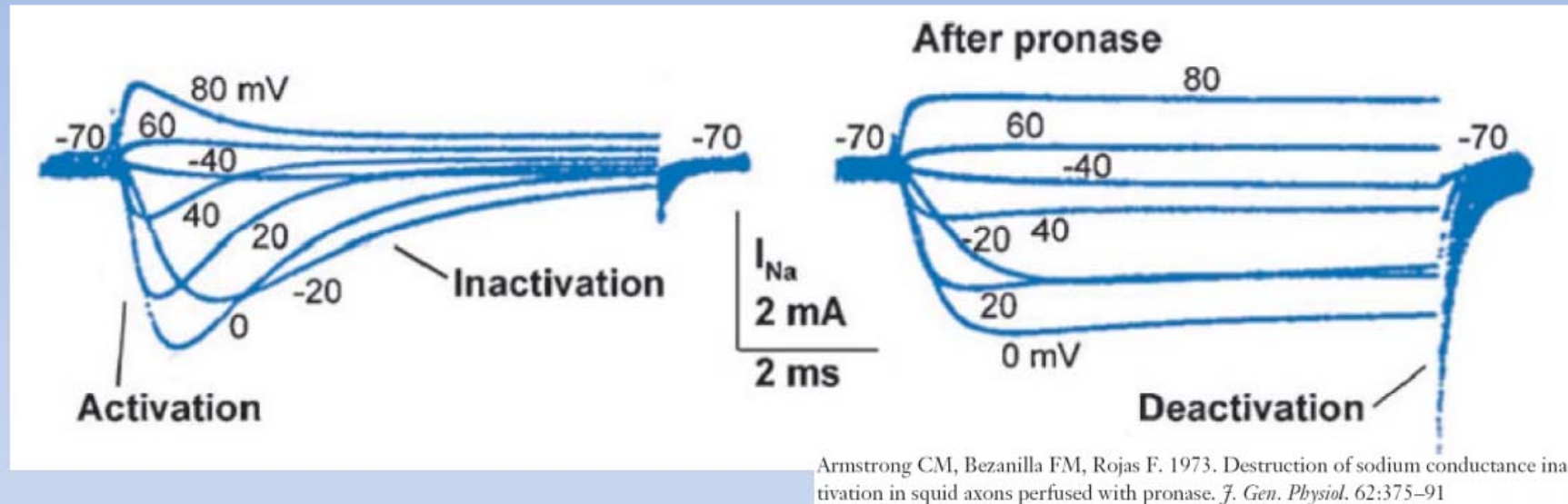
b New model



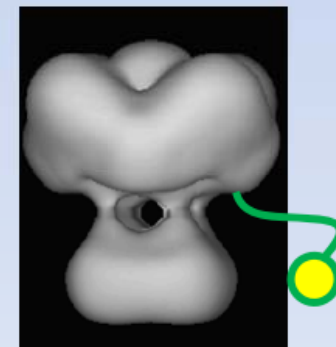
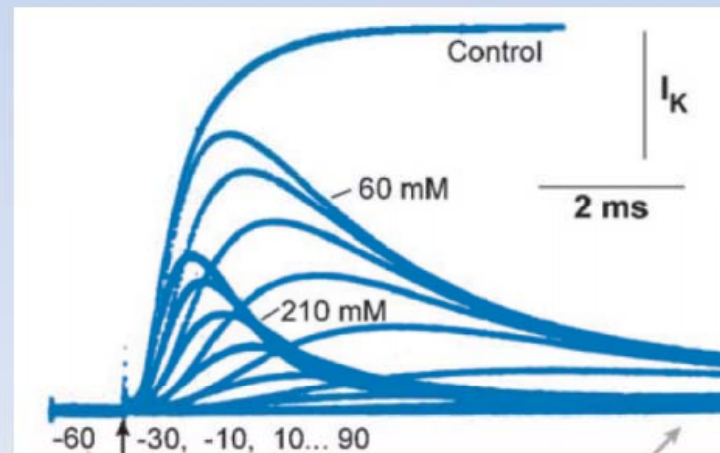
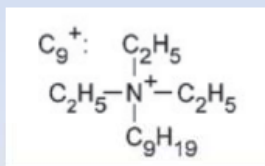
KvAP

Inactivation gates

Pronase, a proteolytic enzyme applied internally to squid giant axons eliminates inactivation of Na channels

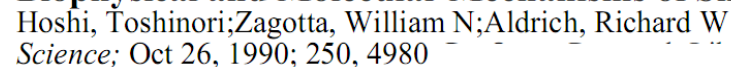


Looks similar to block of K channels by internal C9:

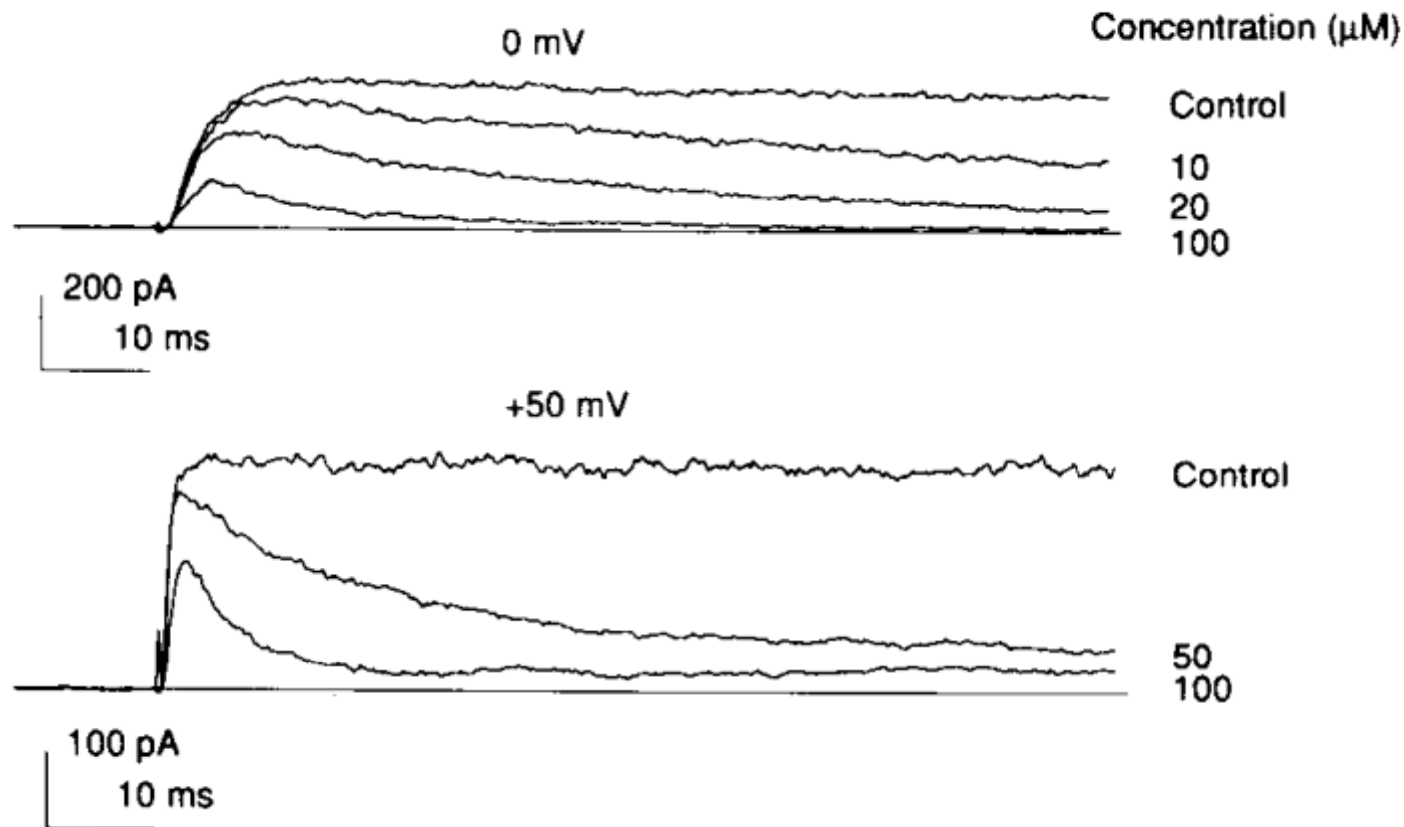


"ball and chain"

1. 1 1



Add peptide (ball) to restore inactivation



Restoration of Inactivation in Mutants of Shaker Potassium Channels by a Peptide Derived from ShB
Zagotta, William N;Hoshi, Toshinori;Aldrich, Richard W
Science; Oct 26, 1990; 250, 4980