Ion channels and membrane potential

离子通道和膜电势

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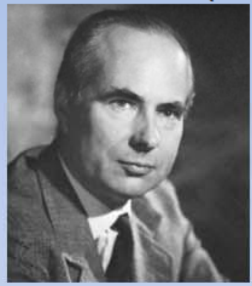
Oct.8, 2015
2015.10.8

Four major breakthroughs in ion channel biology

1 Ionic conductances
Nobel 1963 (Physiol/Medicine)




Alan L. Hodgkin
Andrew F. Huxley

2 Patch clamp methodology
Nobel 1991 (Physiol/Medicine)


Bert Sakmann
Erwin Neher

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 propogation.

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. Hodgin-Huxeley model

  Hodgin-Huxeley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

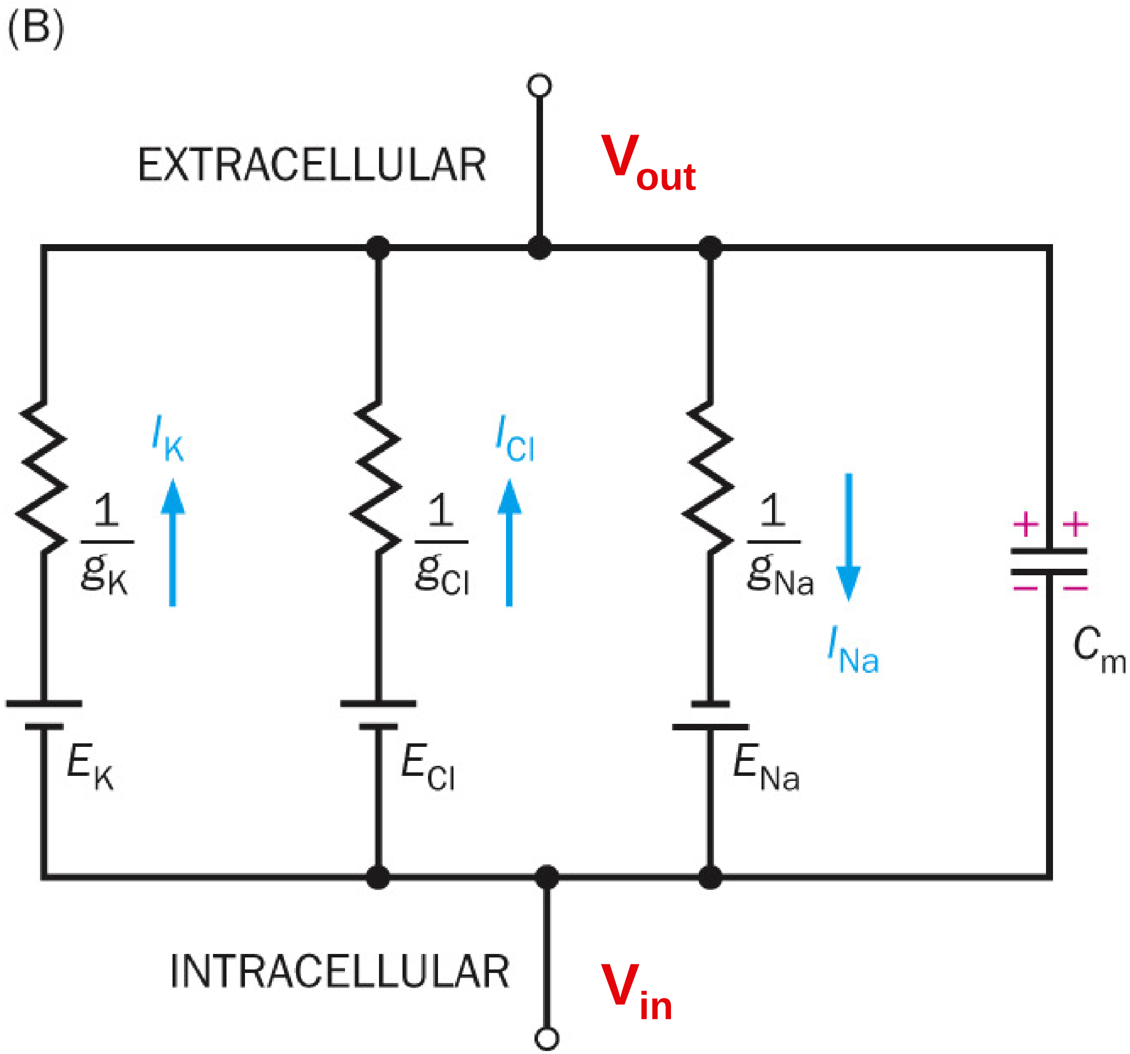
- 4. Structure of ion channels.

  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



Solving for V_{rest}

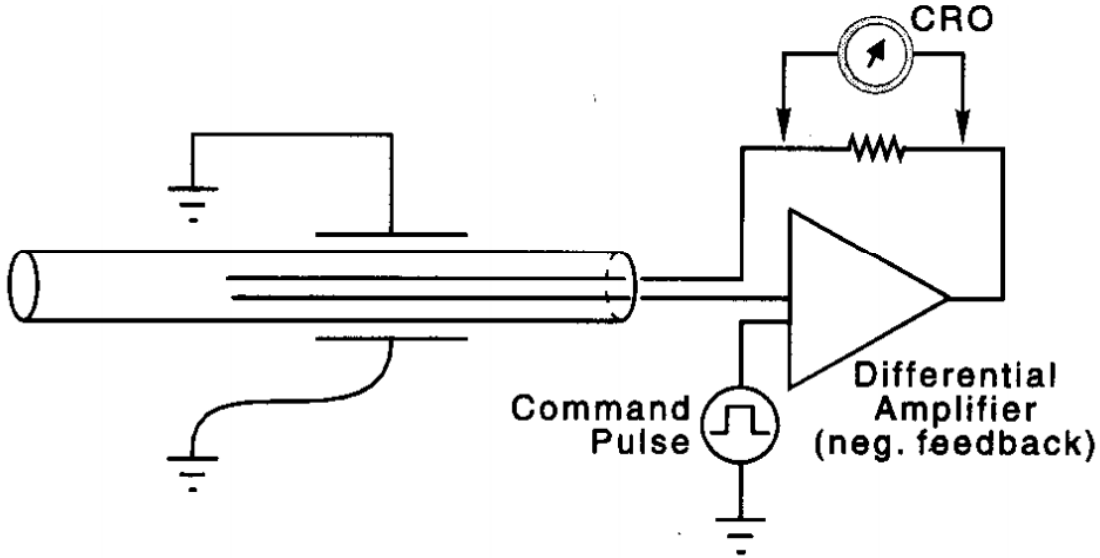
1. $I_{Na} + I_K = 0$
2. $V_{in} - V_{out} = E_K + I_K / g_K$
3. $V_{in} - V_{out} = E_{Na} + I_{Na} / g_{Na}$
4. $\mathbf{I_K = g_K(V_m - E_K)}$
5. $\mathbf{I_{Na} = g_{Na}(V_m - E_{Na})}$

Finally,

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

Hodgkin A L. Series B, Biological Sciences

Voltage Clamp

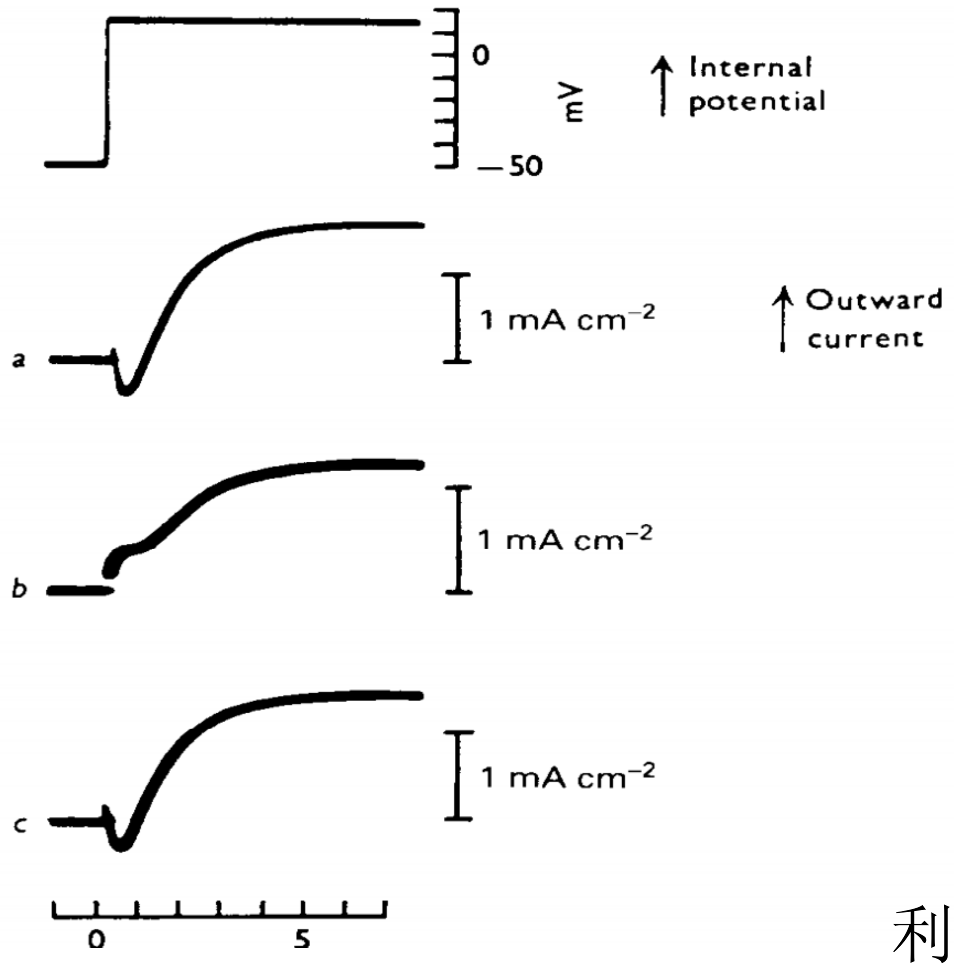


The voltage clamp method used in a squid giant axon. The two wires inserted into the axon are used to measure membrane potential (*V*) and to pass current (*I*). The high-gain negative-feedback amplifier compares the command pulse with the membrane potential, and outputs the amount of current necessary 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*.)

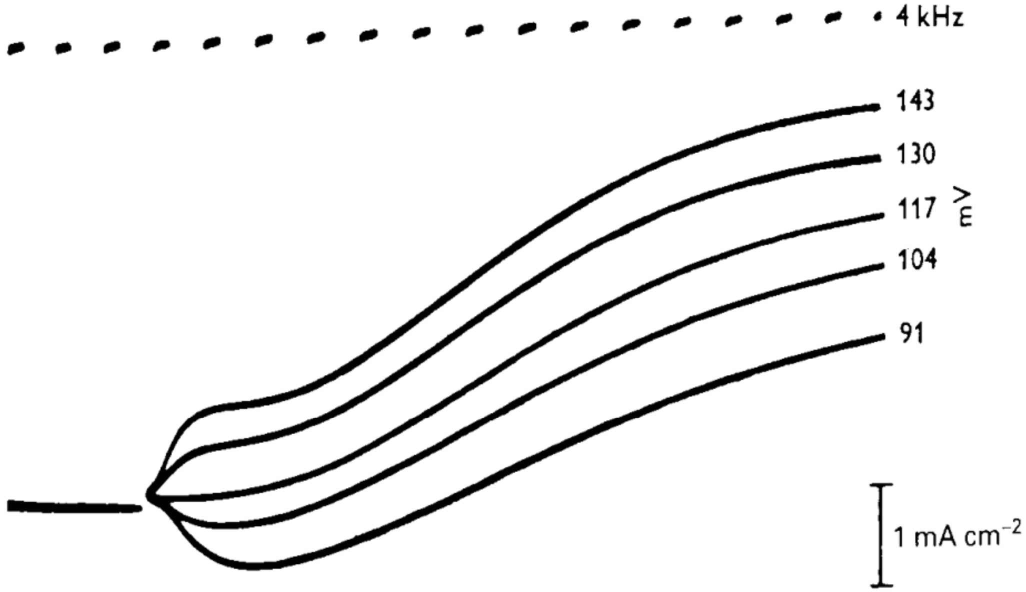


利用电压钳记录膜电流实验。

Hodgkin A L. Proceedings of the Royal Society of London. Series B, Biological Sciences

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.)

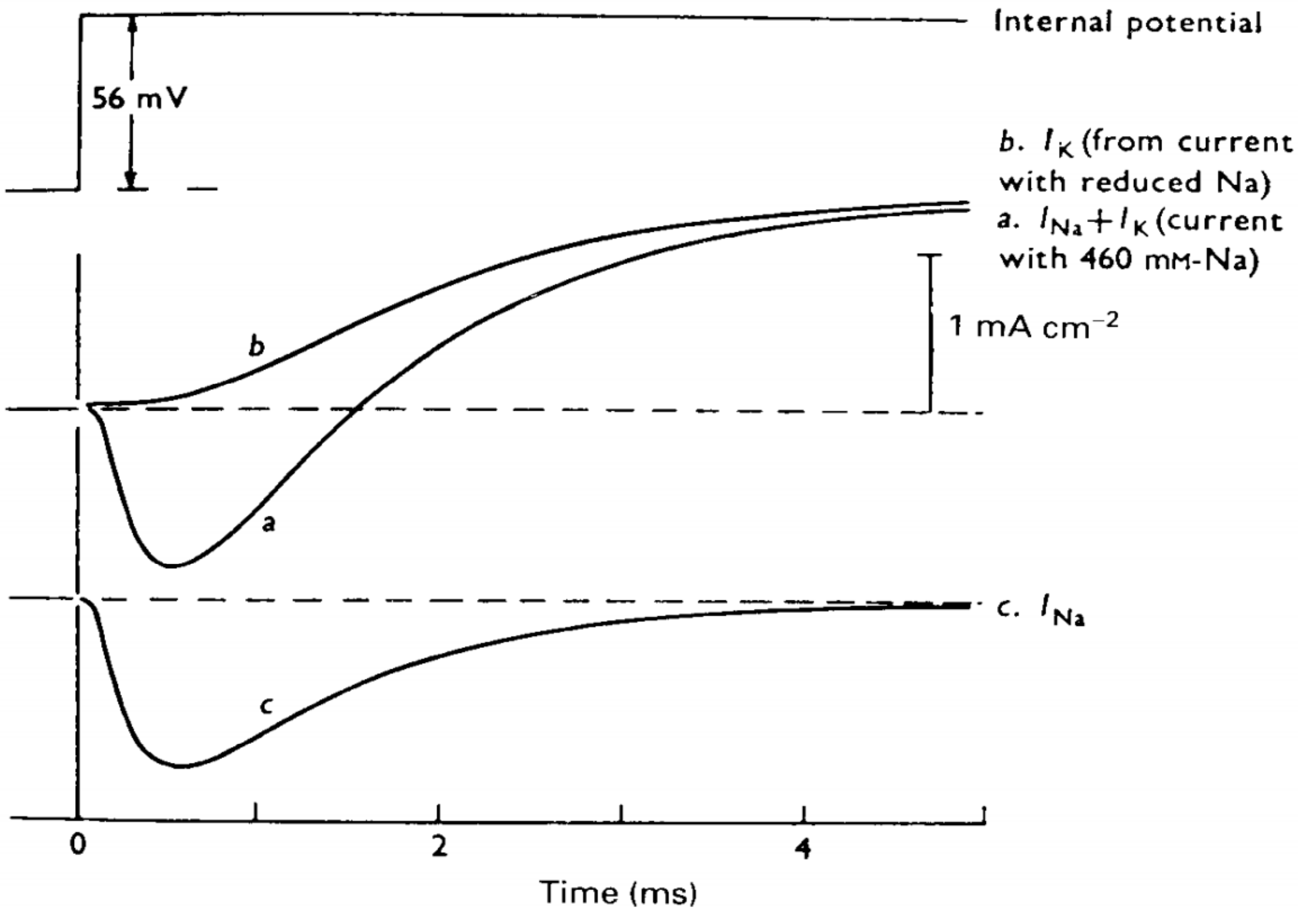


The potential at which the initial (Na current) is neither inward nor outward is the reversal potential E_{Na} for the Na current i.e about 117 mV？

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

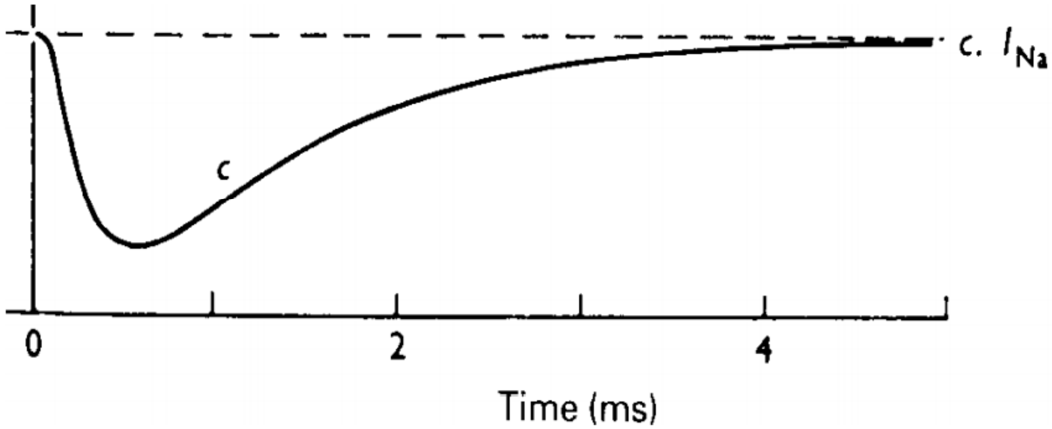
Hodgkin A L Series B, Biological Sciences, 1958.

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

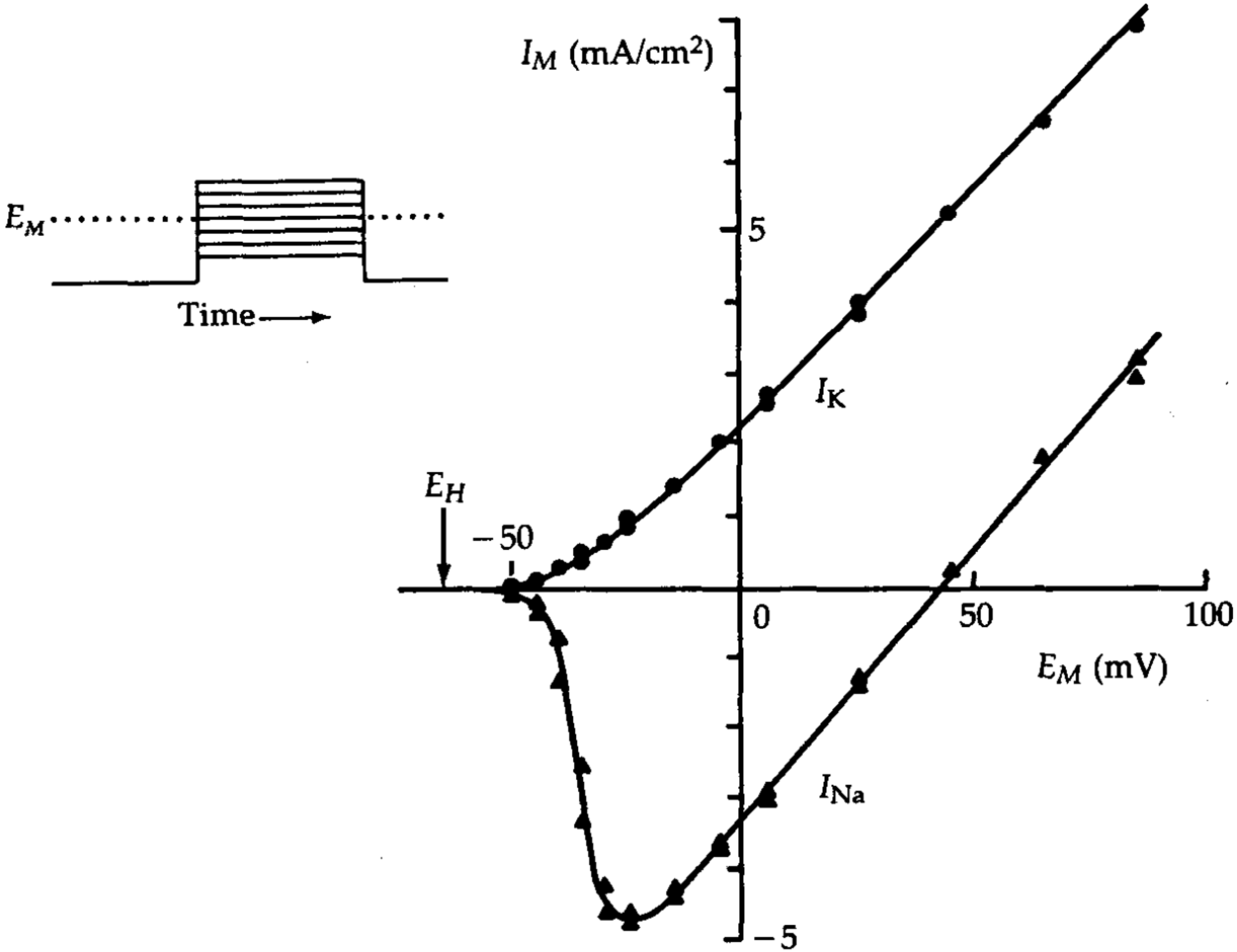
Hodgkin A L. Series B, Biological Sciences, 1958



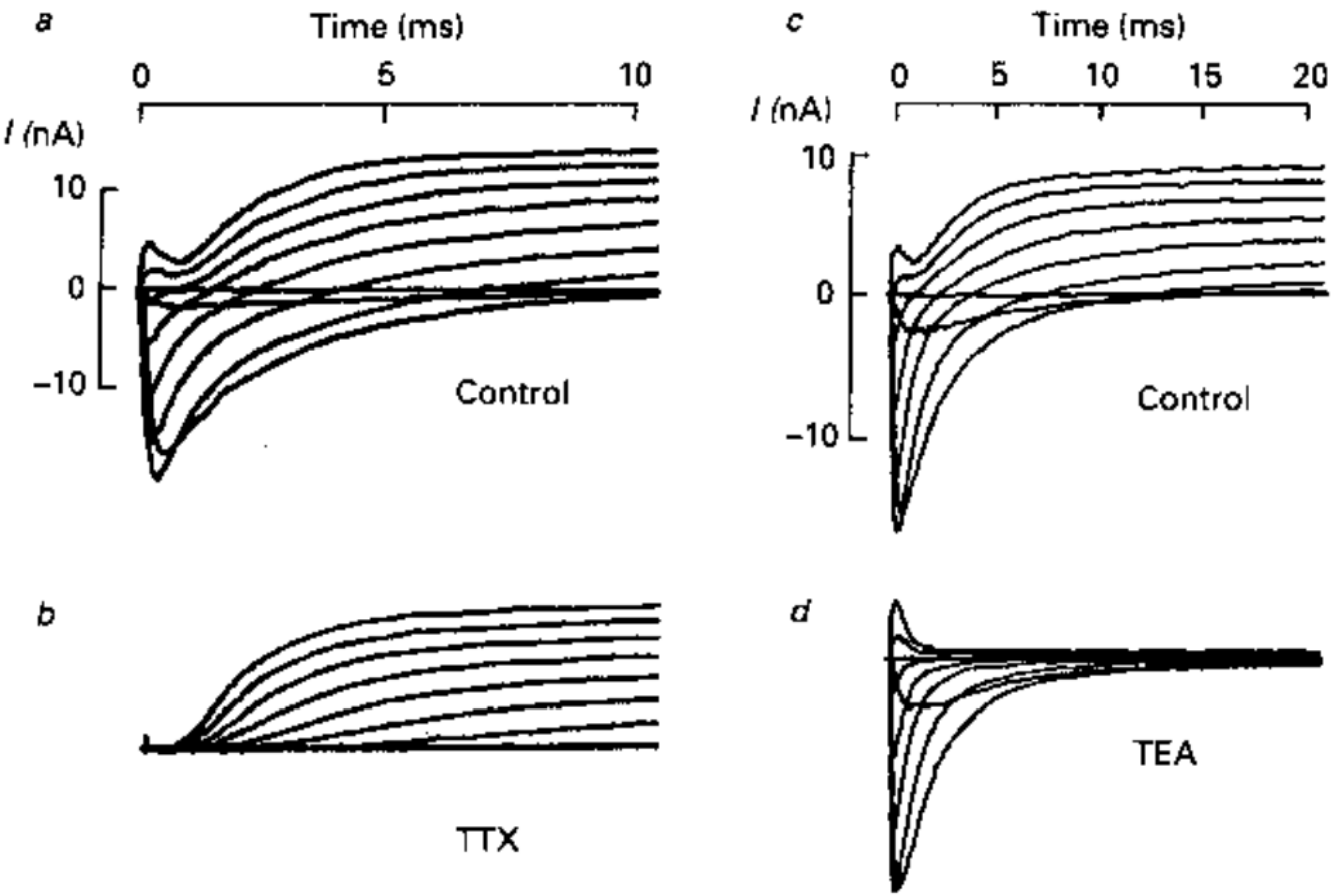
From traces of Na current as a function of time we can obtain g_{Na} by using the equation $I_{Na}=g_{Na}(E-E_{Na})$. E_{Na} is the potential at which the current is nulled.

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

Hodgkin A L. Series B, Biological Sciences, 1958



Hille B. Sunderland, MA: Sinauer, 2001.



Hille B. Sunderland, MA: Sinauer, 2001.

$g_K = \bar{g}_K\ n^4$

n=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

$g_{Na} = \bar{g}_{Na}\ m^3 h$

n=probability of 3 charged particles being In the correct configuration.1-h=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.

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

$$= C_m dV/dt + g_k(E-E_k) + g_{Na}(E-E_{Na}) + g_l(E-E_l)$$

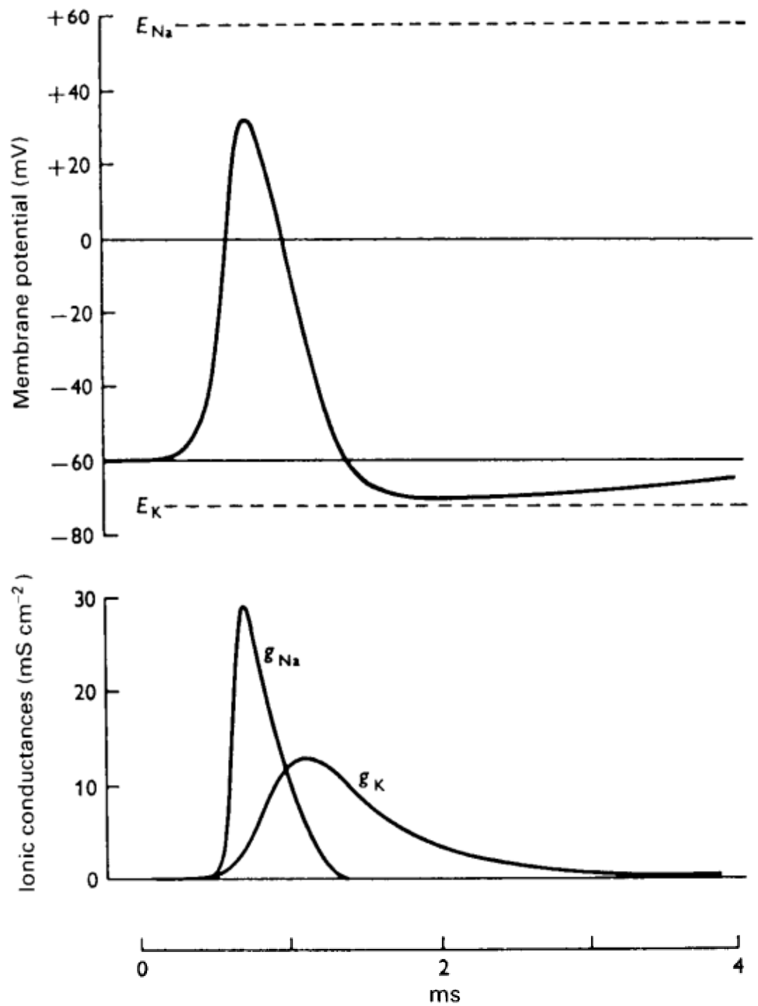
$$= C_m dV/dt + \bar{g}_{Na} n^4(E-E_k) + \bar{g}_{Na} m^3 h\ (E-E_{Na}) + g_l(E-E_l)$$

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, 1952*d*; redrawn.)

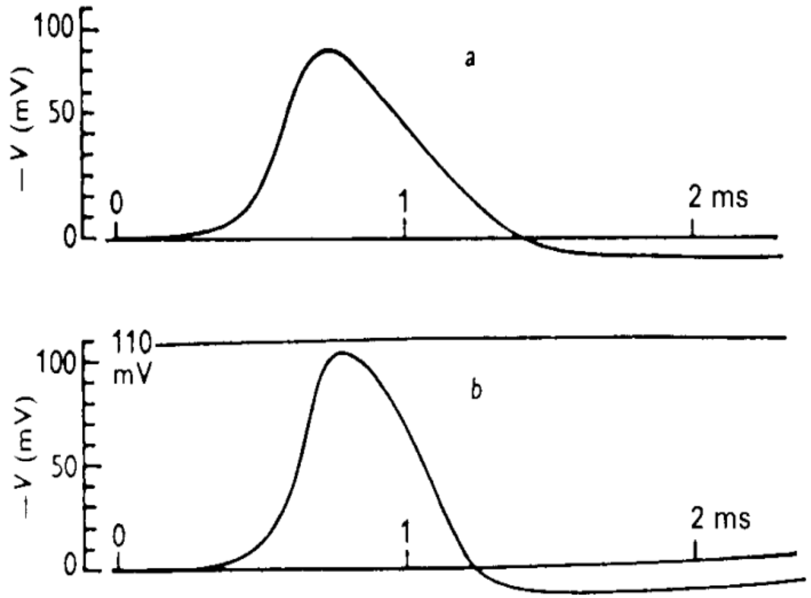


上图：动作电位电压-时间曲线

下图：钠离子、钾离子电导与时间的关系

Hodgkin A L , Huxley A F ,The Jounal of physiology

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

Hodgkin A L, Huxley A F. The Journal of physiology, 1952

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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* Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo 060, Japan
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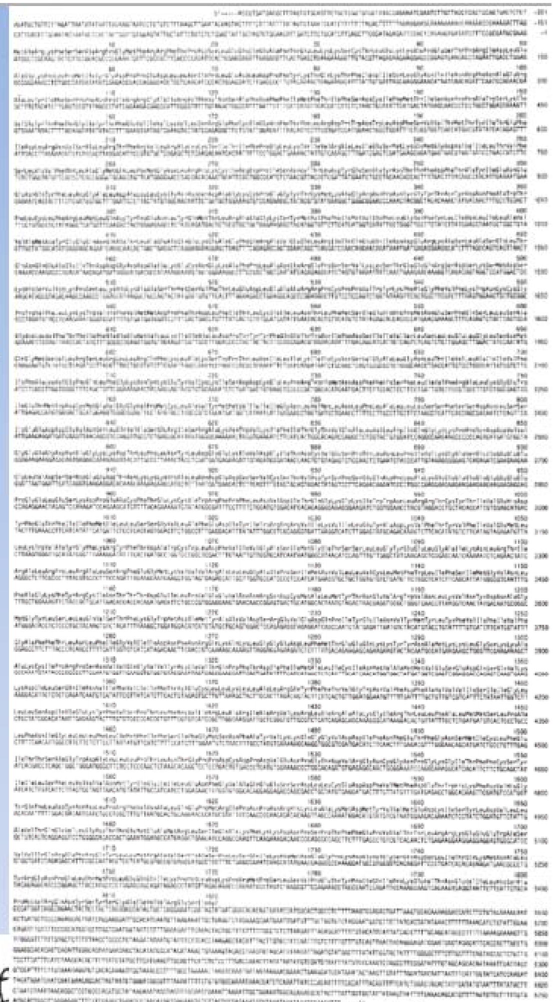
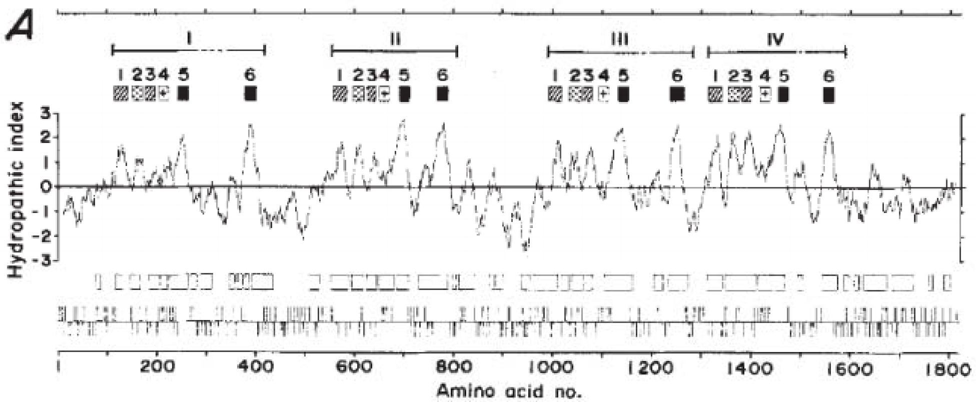
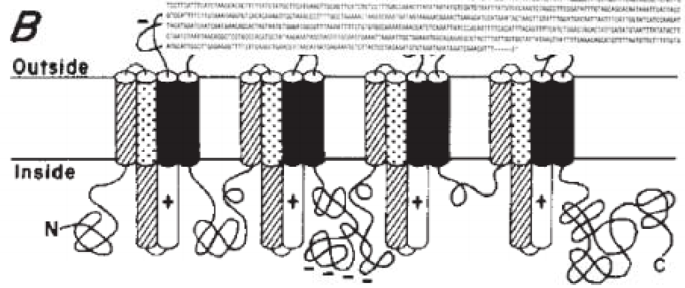
‖ Department of Biology, Kyushu University Faculty of Science, Fukuoka 812, Japan

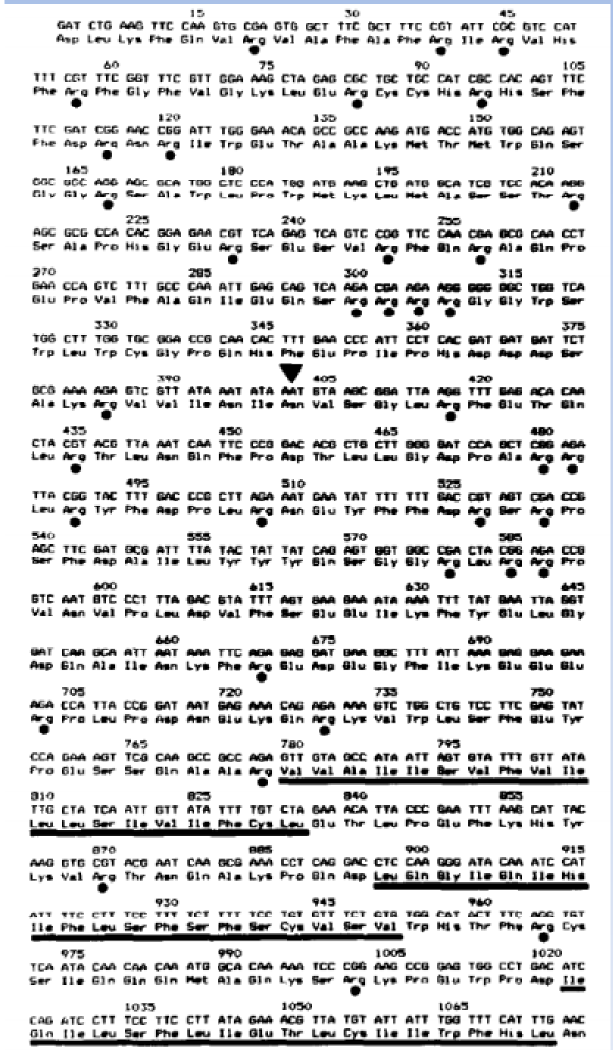
NATURE VOL. 312 8 NOVEMBER 1984





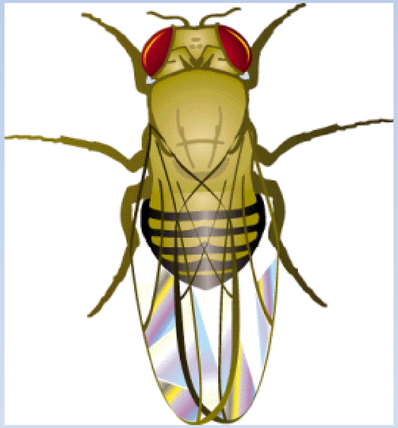


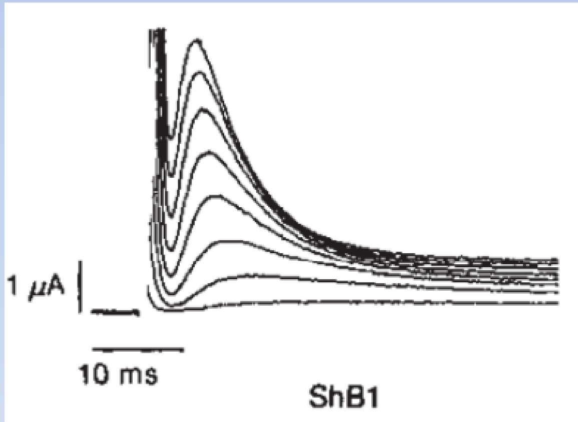
Molecular Characterization of *Shaker*, a Drosophila Gene That Encodes a Potassium Channel



Alexander Kamb, Linda E. Iverson, and Mark A. Tanouye
Division of Biology 216-76
California Institute of Technology
Pasadena, California 91125

***Cell* (1987) 50:405**





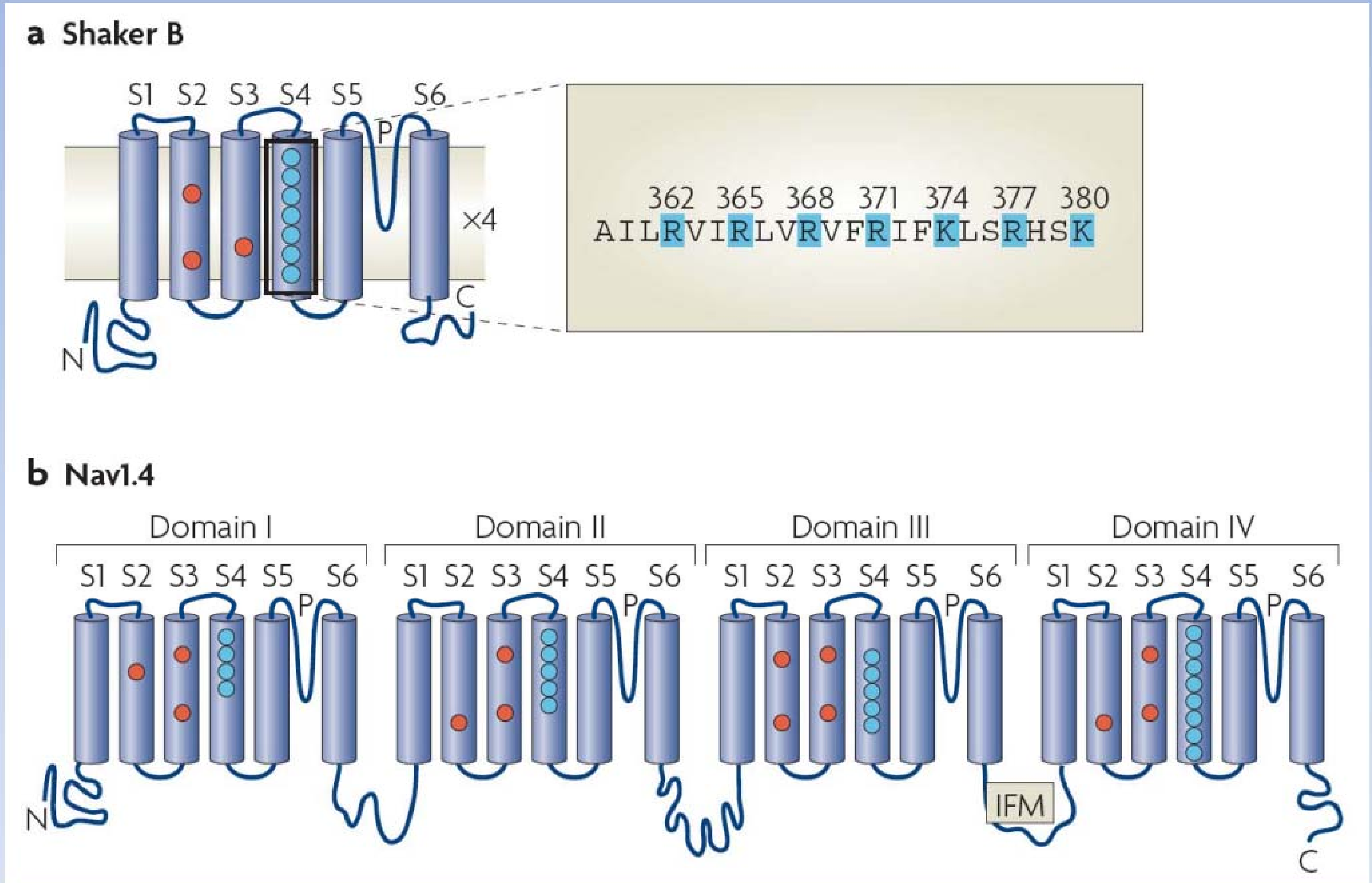
Timpe et al (1988) *Nature* 331:143

Voltage sensing

VSD: the voltage sensor domain

VSD：电压敏感结构域

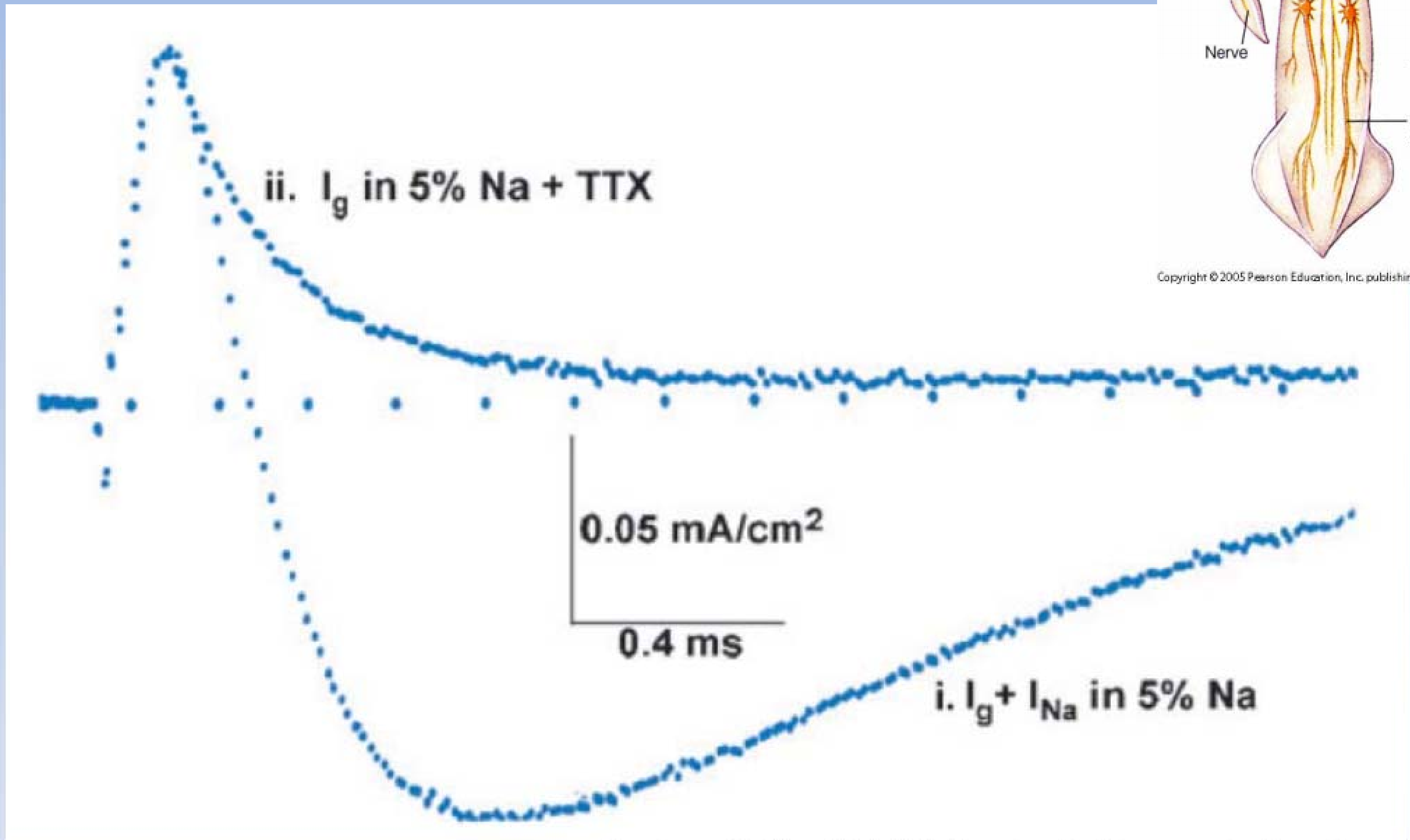
VSD = S2/S3/S4

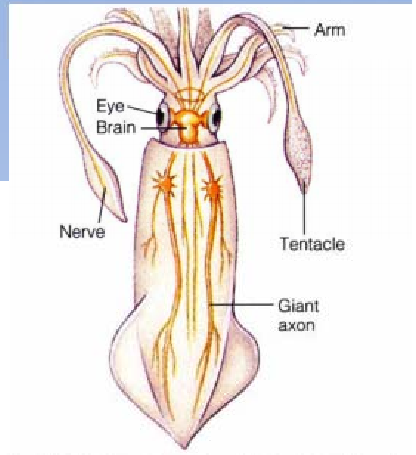


- Salt bridge forms between acidic residues in S2/S3 (red spheres) and basic residues of S4 (blue spheres)
- Consistent with helical screw motion

Bezanilla (2008)
Nature Reviews Mol Cell Biol 9:323

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





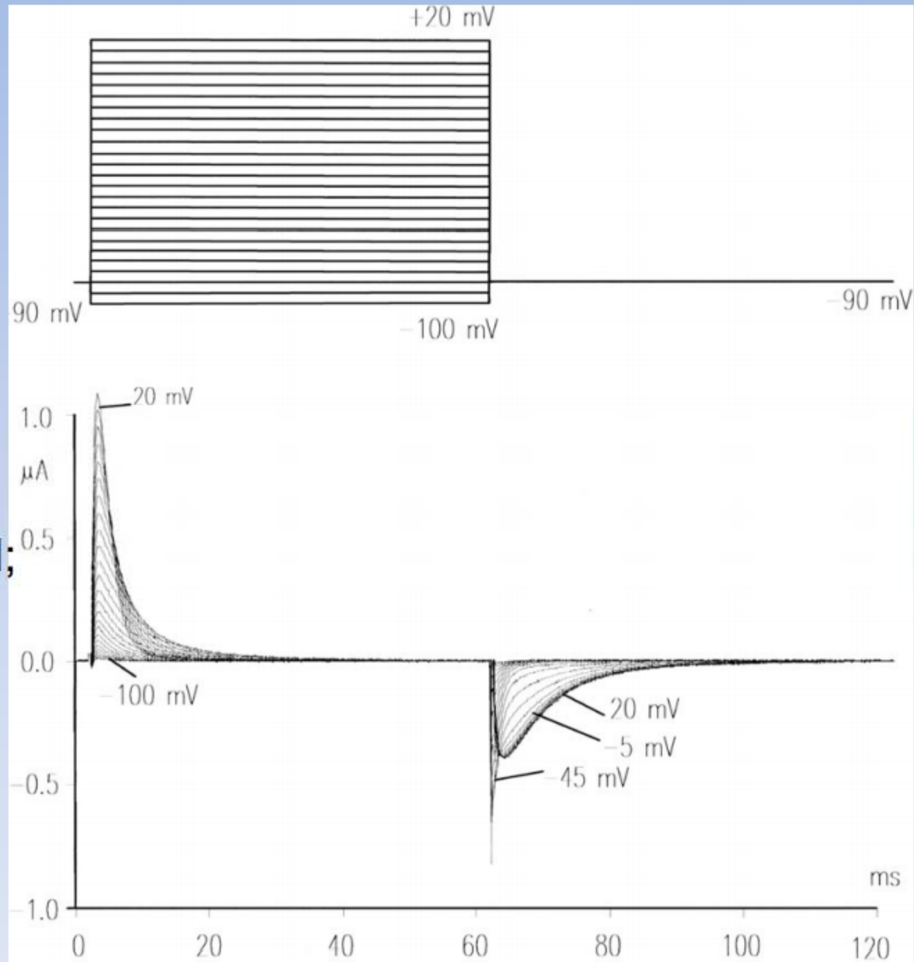
Armstrong CM, Bezanilla F. 1973. Currents related to movement of the gating particles of the sodium channels. *Nature* 242:459–61

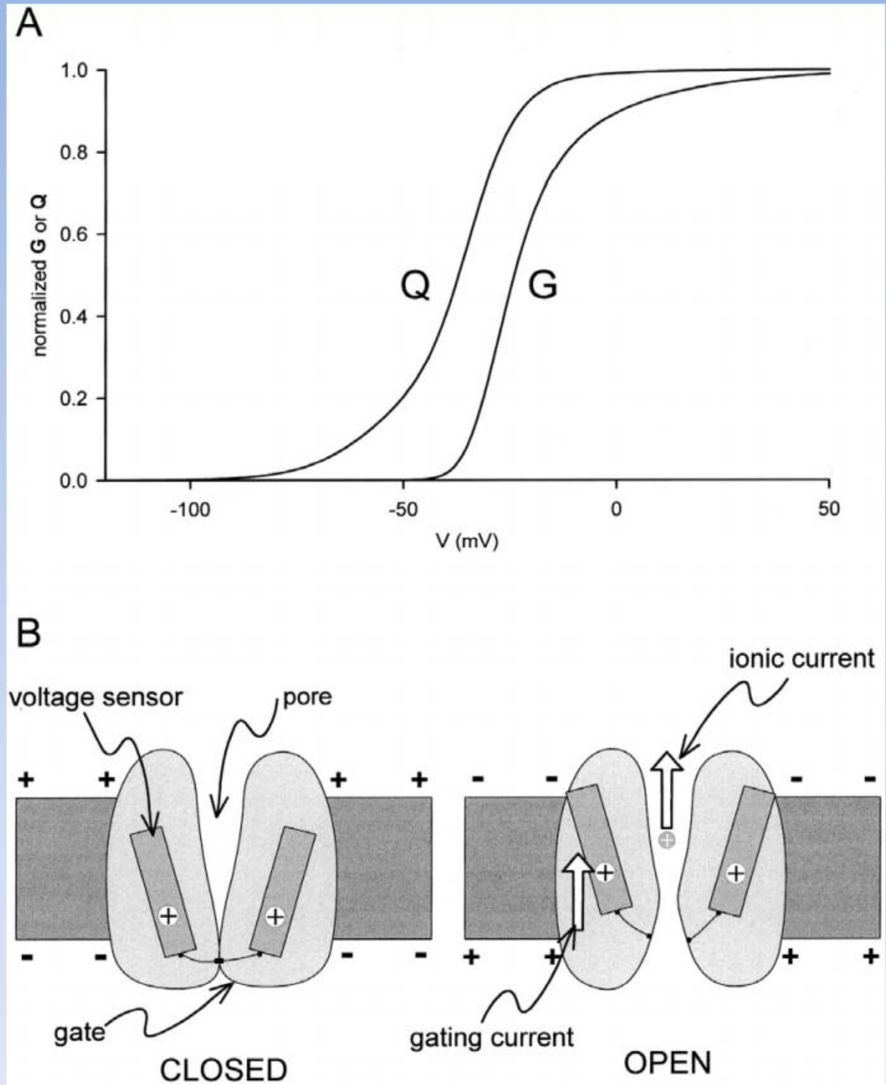
I_K was eliminated by removing all K^+, I_{Na} was reduced by lowering [Na^+].
I_{cap} was removed by subtraction, then eliminated with tetrodotoxin (TTX)

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)

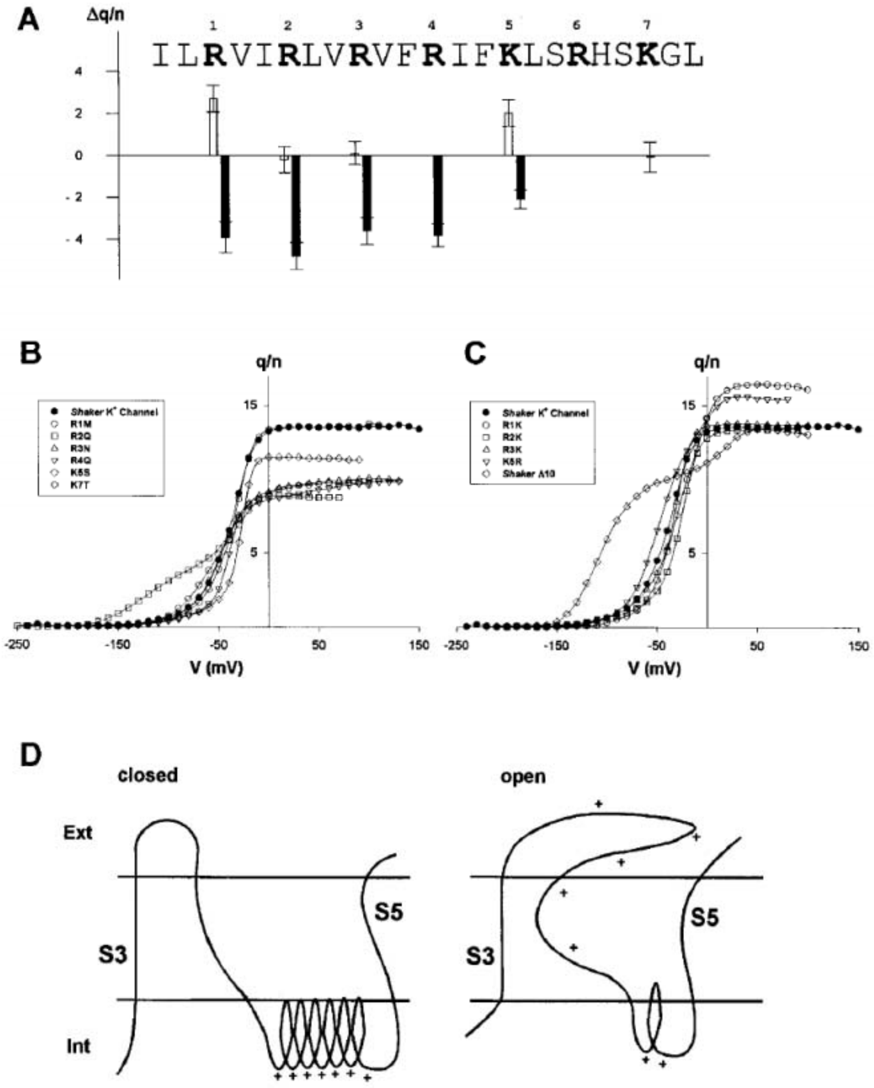
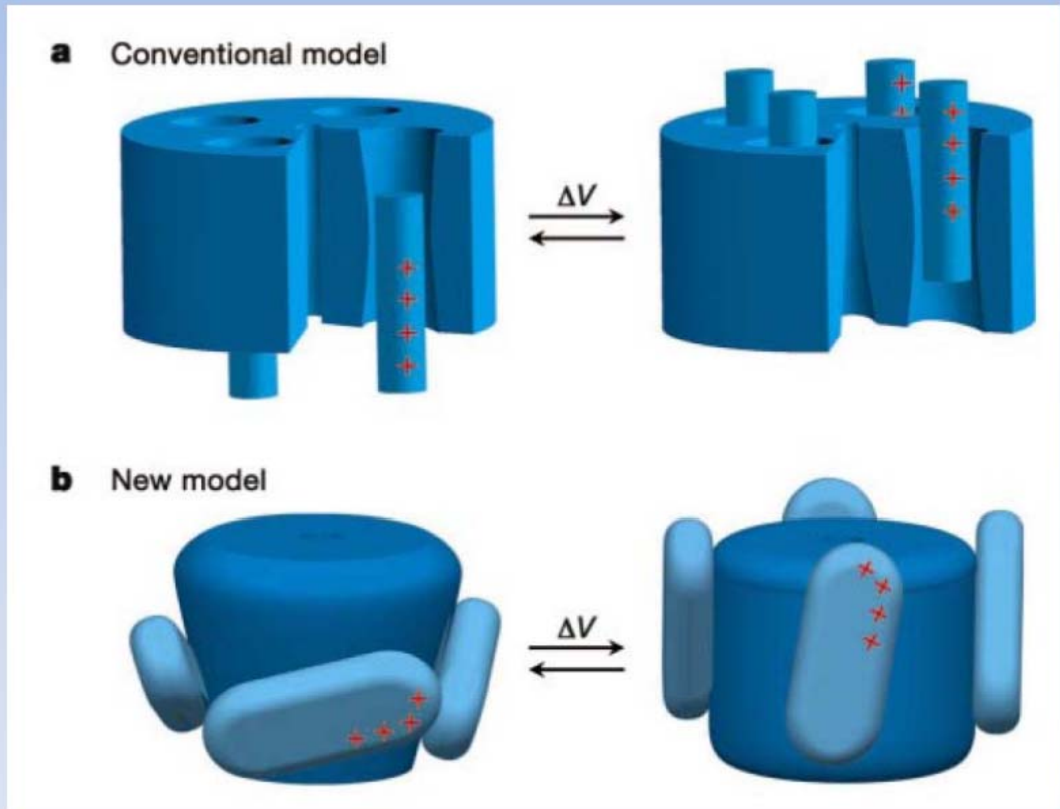


Figure 7. Summary

Aggarwal and MacKinnon (1996) Neuron **16**, 1169

controversy

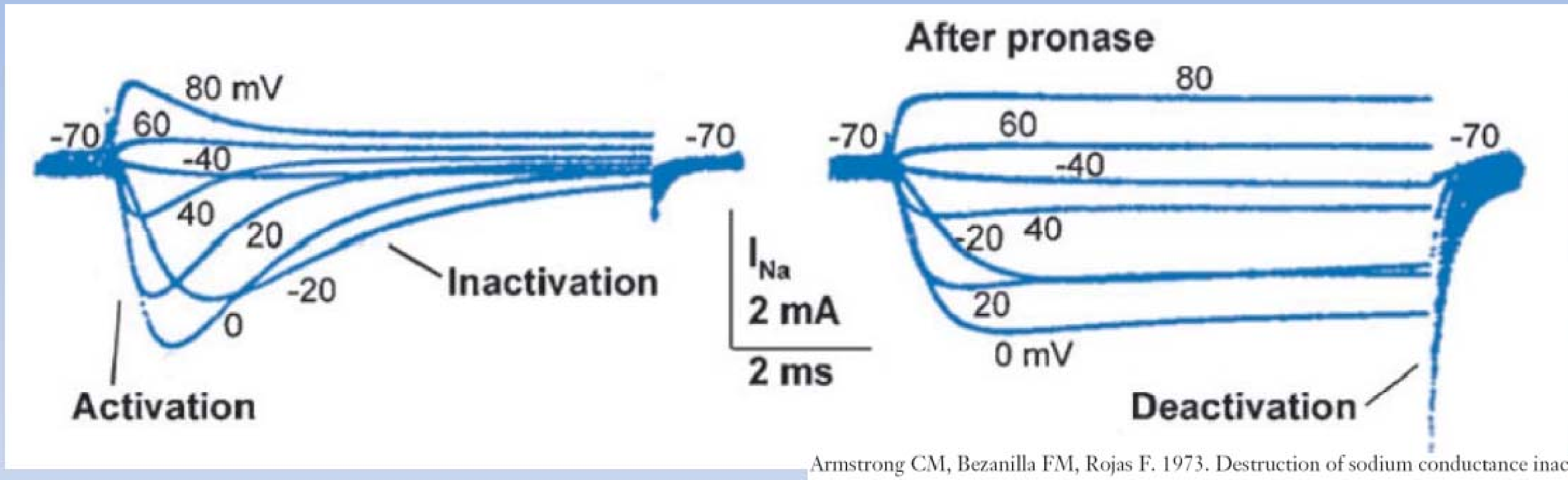


Shaker

KvAP

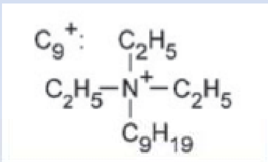
Inactivation gates

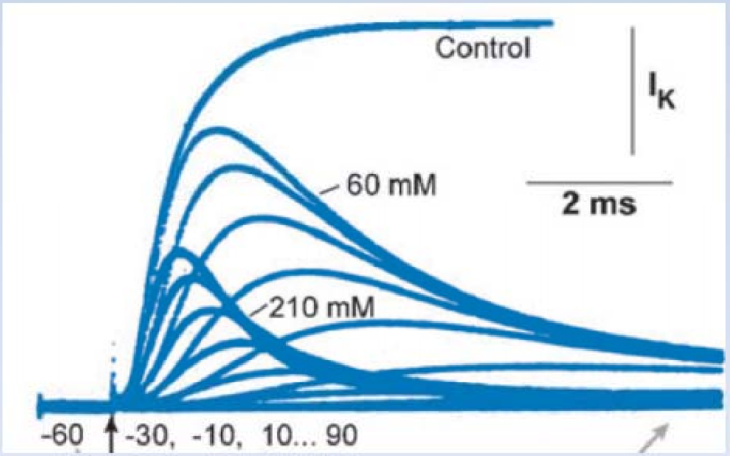
Pronase, a proteolytic enzyme applied internally to squid giant axons eliminates inactivation of Na channels

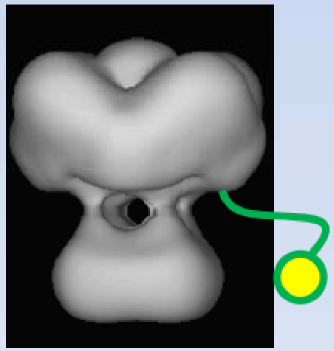


Armstrong CM, Bezanilla FM, Rojas F. 1973. Destruction of sodium conductance inactivation in squid axons perfused with pronase. *J. Gen. Physiol.* 62:375–91

Looks similar to block of K channels by internal C9:



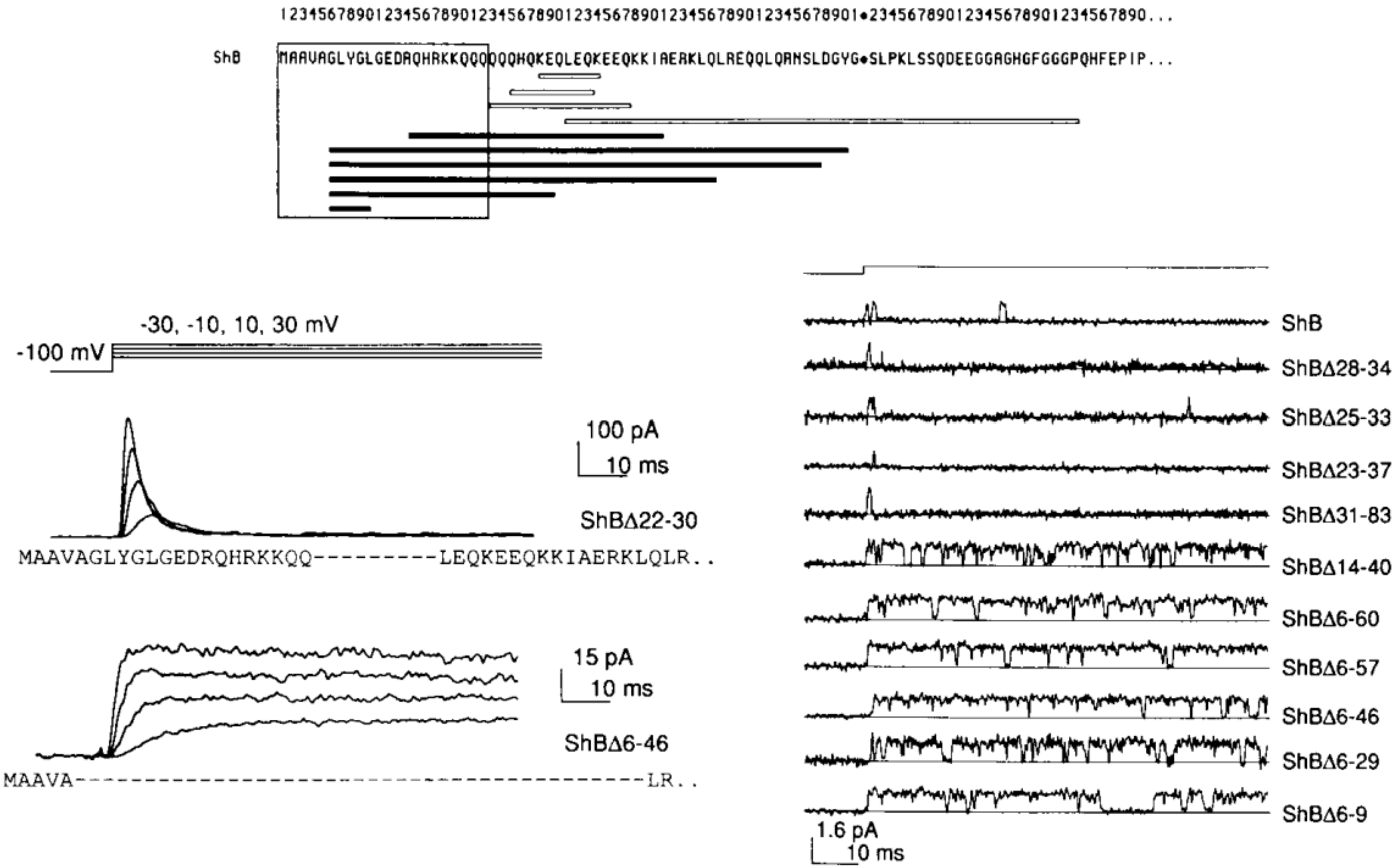




“ball and chain”

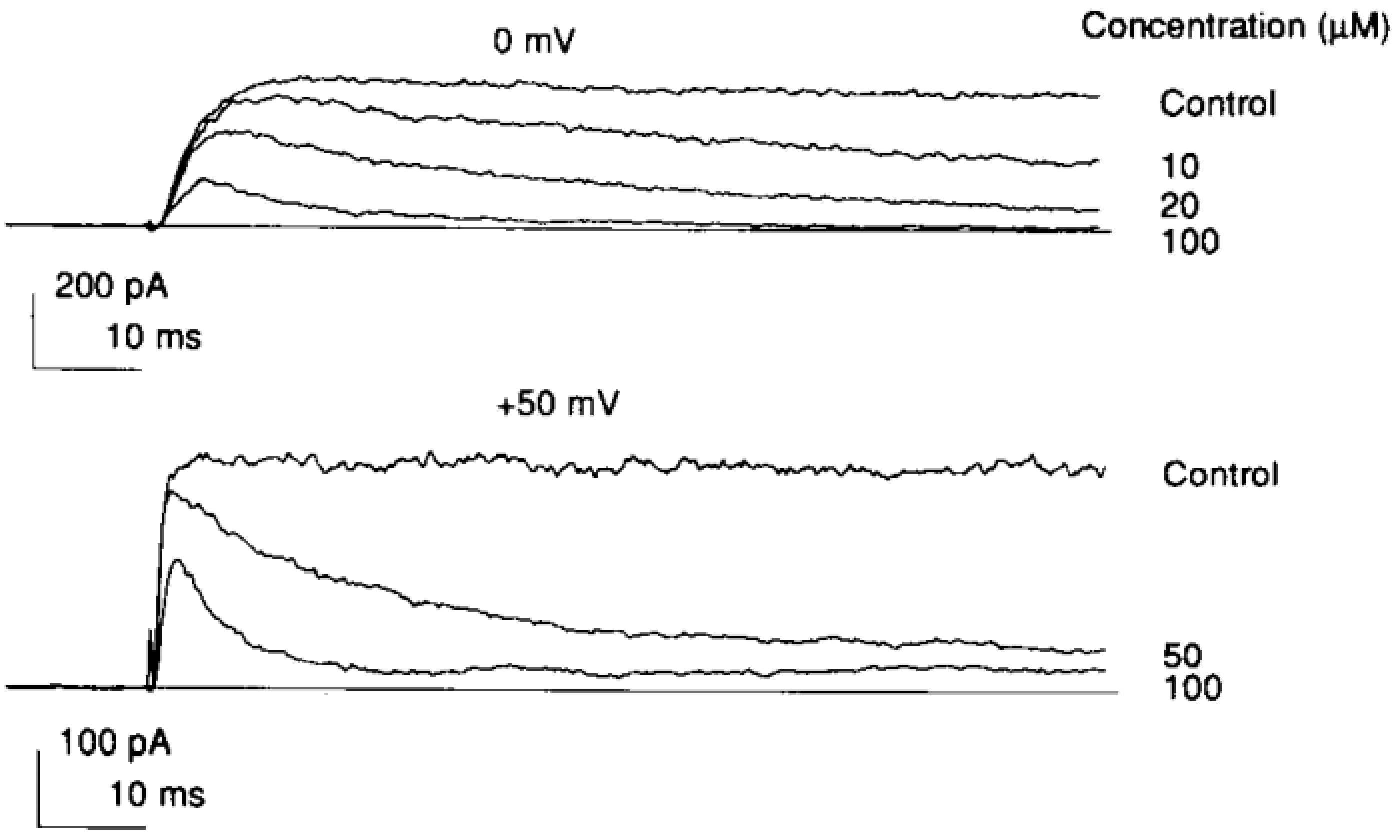
Armstrong (2007) *Ann Rev Physiol* 69:1-18

Deletion assay to map the ball



Biophysical and Molecular Mechanisms of Shaker Potassium Channel Inactivation
Hoshi, Toshinori;Zagotta, William N;Aldrich, Richard W
Science; Oct 26, 1990; 250, 4980

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