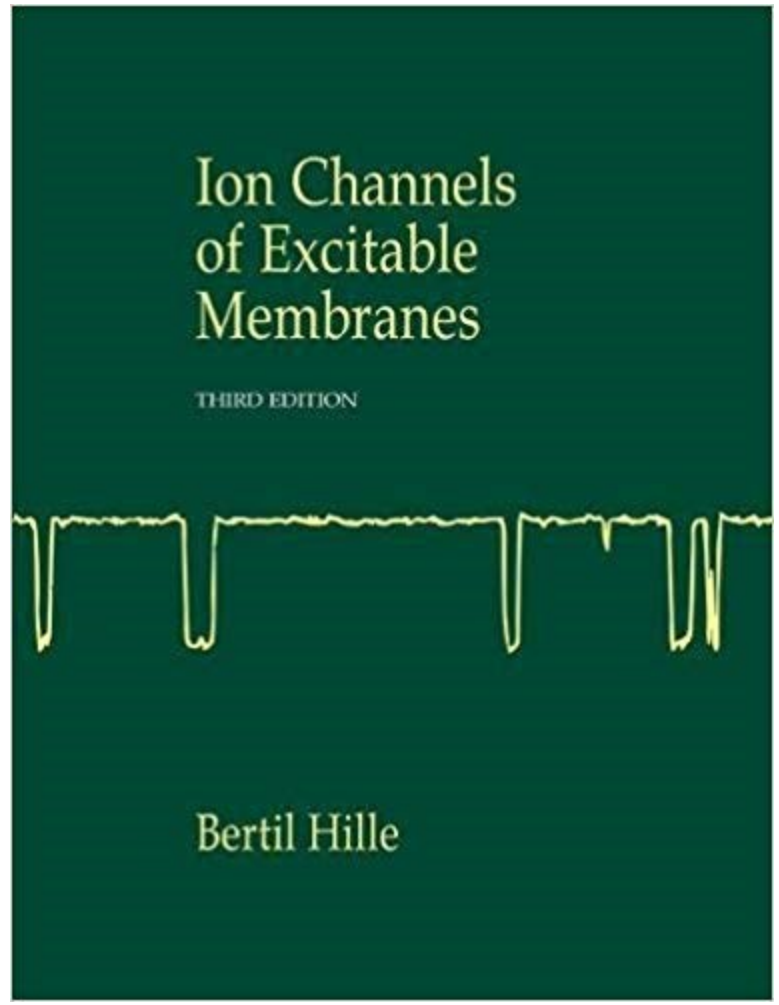
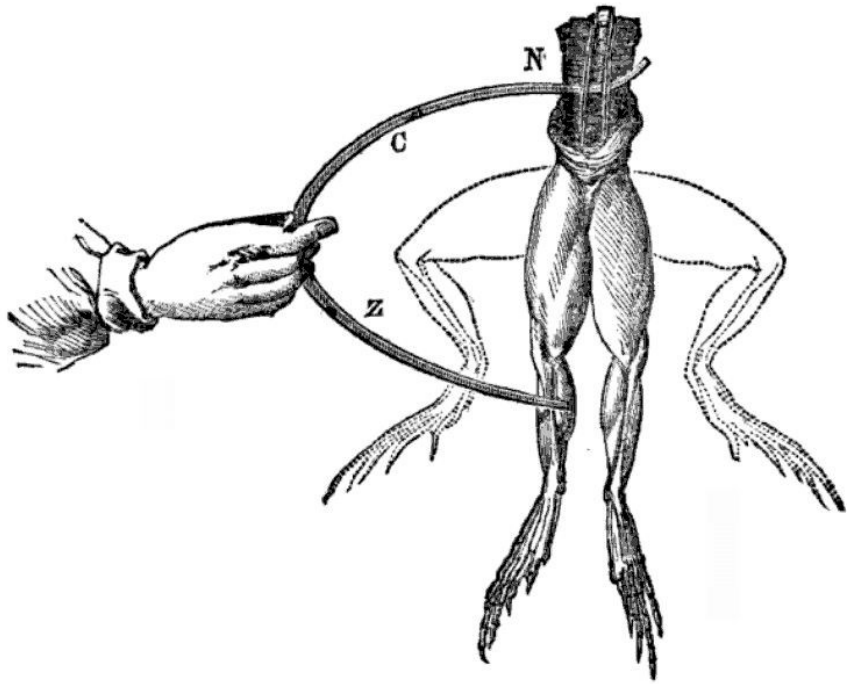
Ion Channels


Ion Channels
of Excitable
Membranes
THIRD EDITION
Bertil Hille

Question: what is the nature of ‘animal spirits’ ?

Luigi Galvani



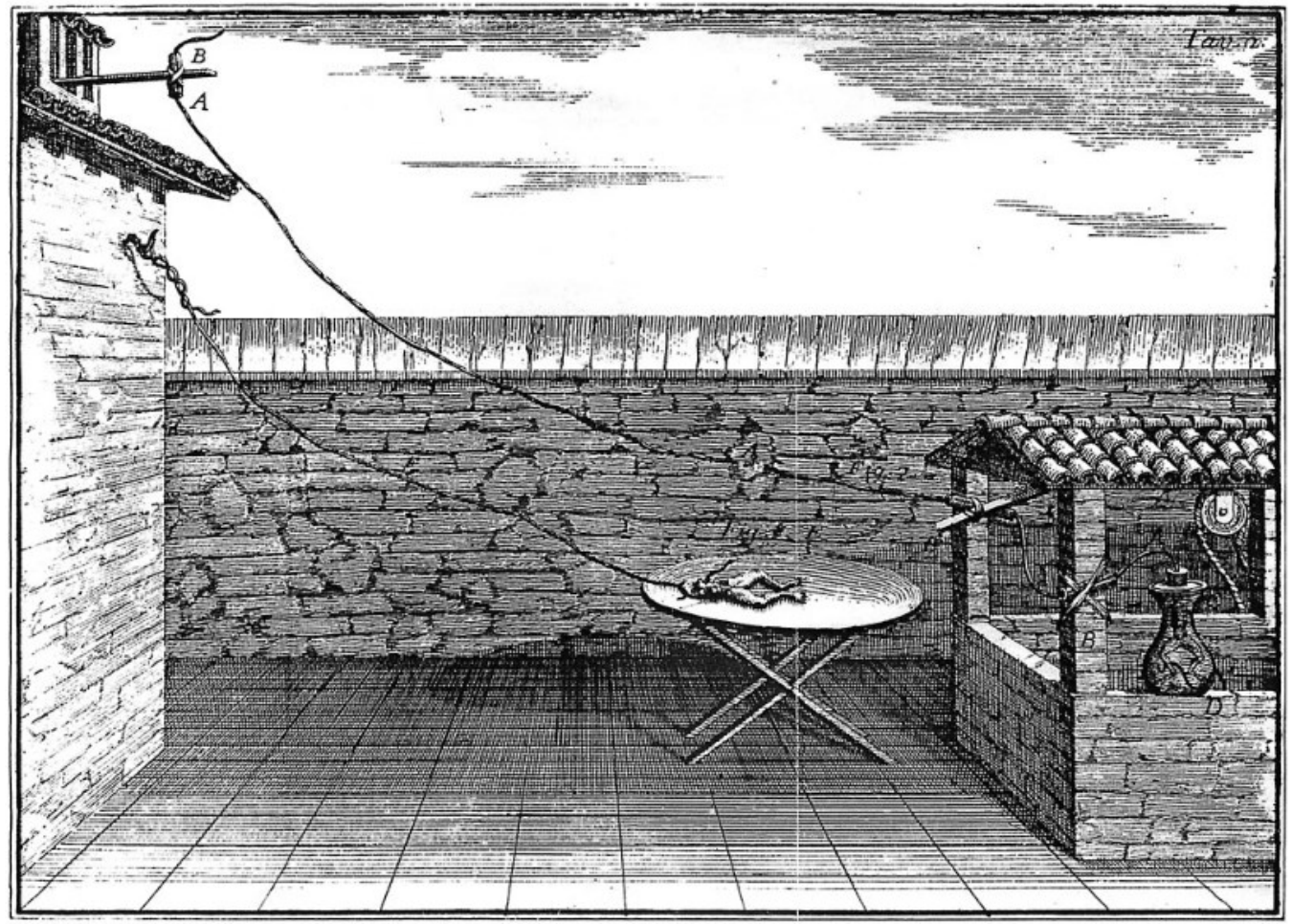


FIG. 3. Plate II of the *Commentarius* (1791 edition): The experiment with the stormy atmospheric electricity.

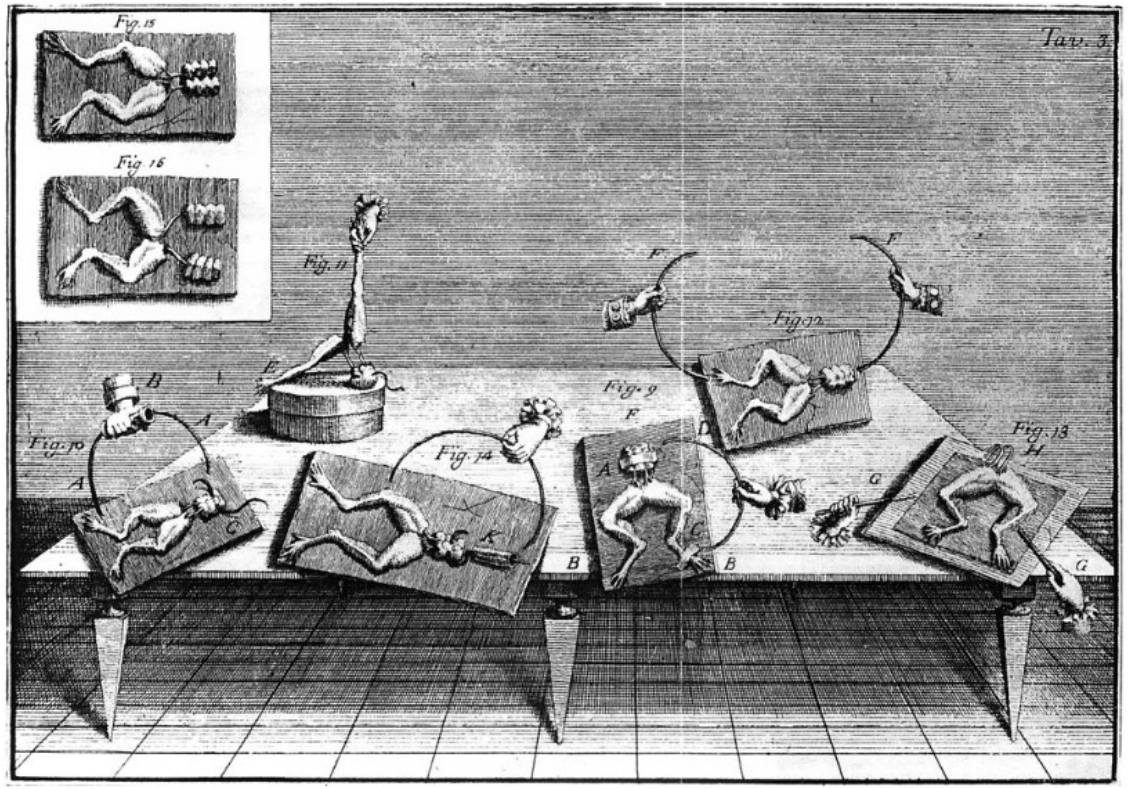


FIG. 4. Plate III of the *Commentarius* (1791 edition): the experiments with metallic arcs.

Conclusion: there is bio-electricity, electrical forces that are intrinsic to the animal (animal electricity)

Question: what is the electrical potential signal of the “excitatory process” in muscle and nerves?

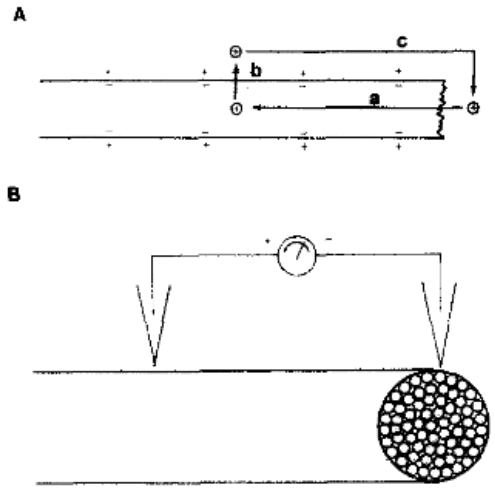
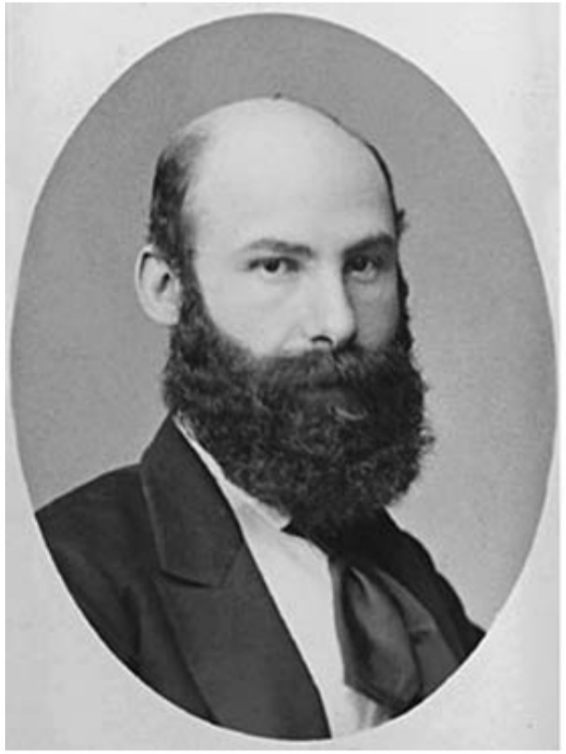


Fig. 1. *Origin of the injury current.* **(A)** *When an axon or muscle fiber is cut, the damaged end forms a leaky, non-specific seal. Because of the inside negative membrane potential, there is a net movement of positive charge through the damaged region into the cell (a). This partially depolarizes the cell, generating an outward current at distal points (b). The return path is along the outside of the fiber (c).* **(B)** *When a peripheral nerve or whole muscle is cut, the injury currents of the individual fibers sum. The net muscle or nerve current is large enough to be detected with a galvanometer.*

Injury current

(Carlo Matteucci)



Julius Bernstein

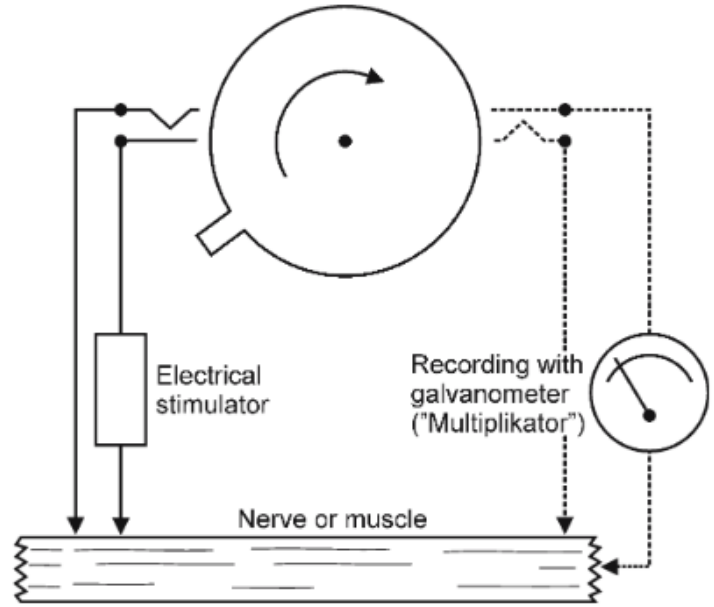


Fig. 2 Differential rheotome ("time slicer"), principle of operation. The cam on a turntable briefly closes a circuit for electrical stimulation and subsequently a second circuit, in which a galvanometer records the nerve or muscle activity ("negative variation"). The delay time between stimulus and recording interval is set by adjusting the angle between the two switches. The speed of the spinning wheel and the width of the cam are set so that the galvanometer is connected to the recording electrodes for only a fraction of a millisecond (adapted from Schuetze 1983)

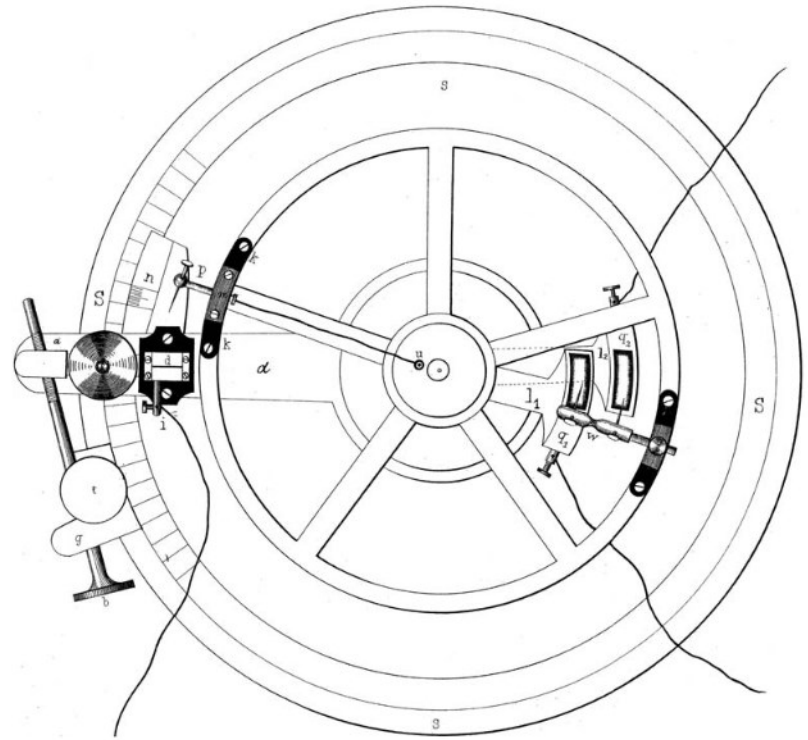
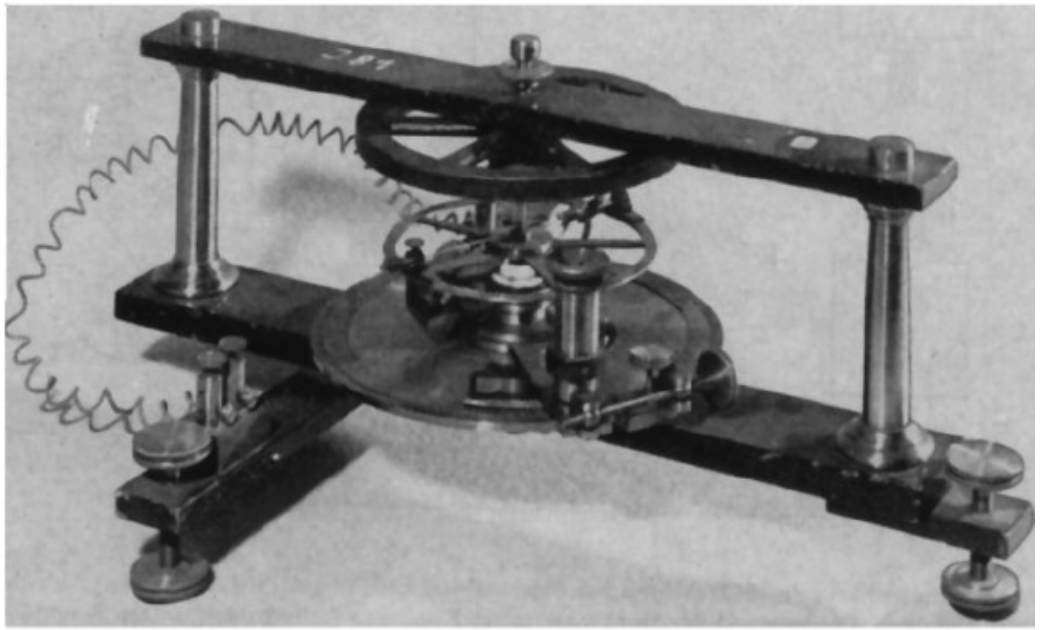
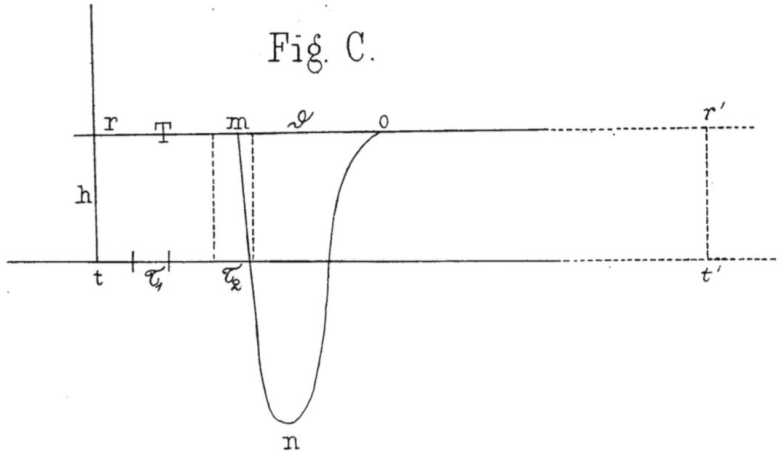
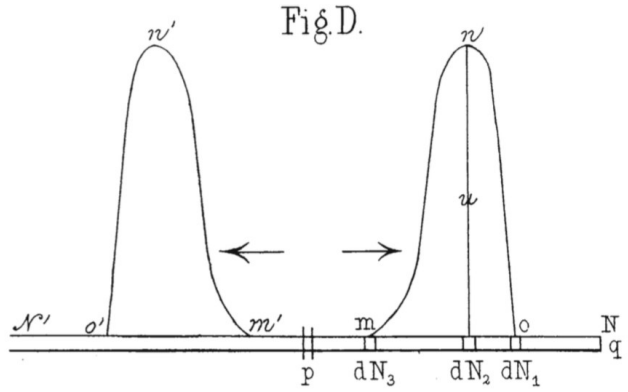


Fig. 3 Bernstein's differential rheotome; top view of instrument (reproduced from Bernstein 1868). A fine pin (p) on the rotating wheel briefly touches a thin copper wire (d) to close the stimulus circuit (*left*). The recording circuit (*right*) is closed when a pair of pins lightly brushes across mercury contacts contained in q_1 and q_2. The closing time (= sampling interval) is set by adjusting the relative positions of the mercury contacts. The delay between stimulus and recording is fine-adjusted by screw b. The whole device is less than 20 cm in diameter







Conclusion: a fast voltage change that is larger then a “resting” potential

Question: what is the physico-chemical nature of bioelectric events?

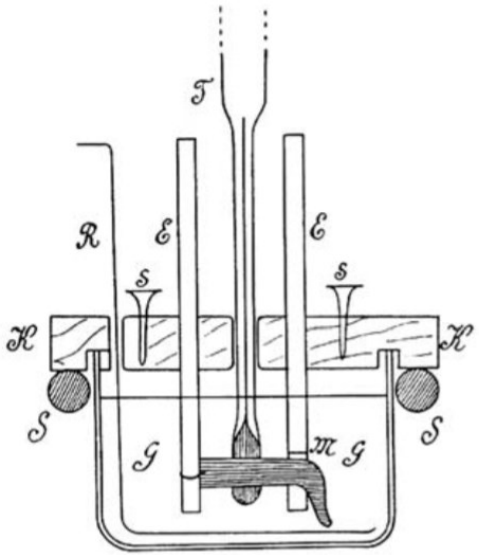


Fig. 1.

Nernst equation:

$$E = \frac{RT}{F} \ln \frac{[K^+]_{out}}{[K^+]_{in}}$$

E: equilibrium potential of potassium

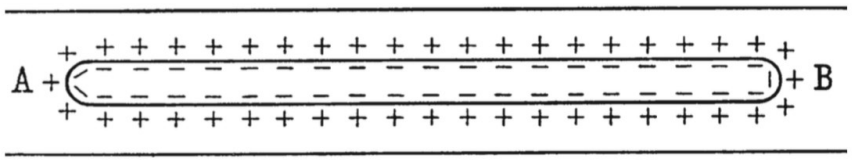
T: absolute temperature

R: gas constant

F: Faraday (electr. charge/mole)

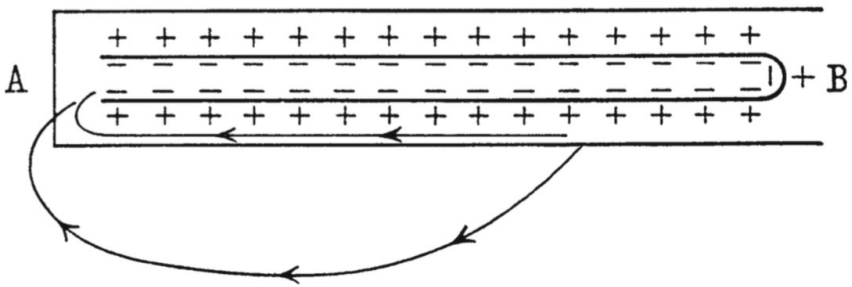
$[K^+]$: potassium concentration (activity)

Fig. 32 a.



Unverletzte Faser mit elektrischer Doppelschicht, stromlos.

Fig. 32 b.

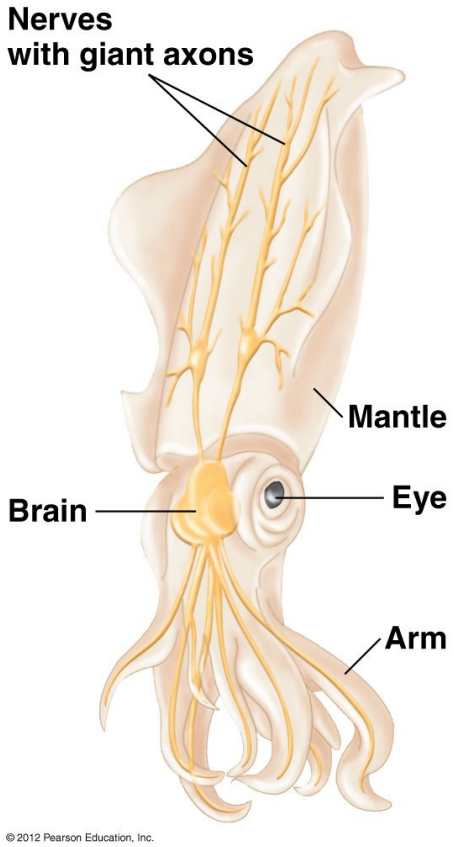


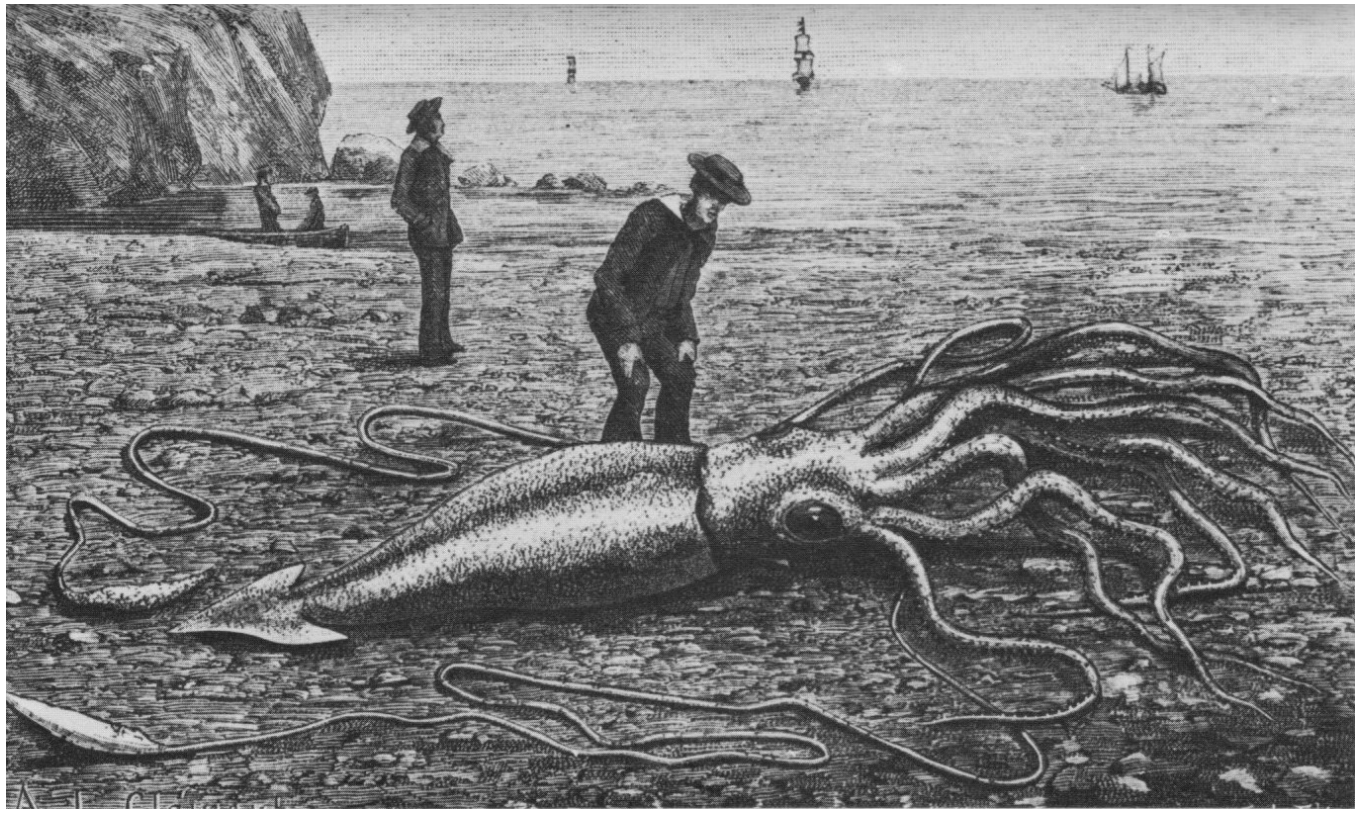
Verletzte Faser mit elektrischer Doppelschicht, Längsquerschnittstrom.

"*Let us imagine that these electrolytes diffuse unhindered from the axial cross section of the fibrils into the surrounding fluid, while they are prevented from diffusing through the longitudinal section by an intact plasmalemma which is impermeable to one kind of ion such as the anion (PO_4^{-} etc.) to a greater or lesser degree. Then an electrical double layer would emerge at the surface of the fibril, with negative charges towards the inside and positive charges towards the outside. Indeed, this electrical double layer must also exist in the undamaged fiber, but would become apparent only in response to lesion or stimulation (negative variation). This assumption would imply a theory of pre-existence. As the semipermeable membrane plays an essential role in this theory, I will succinctly call it '**Membrane Theory**'.*"

Conclusion: ions separated by a membrane with selective permeability, which breaks down during impulses

Question: is the membrane breakdown hypothesis correct?





Kacy Cole and Howard Curtis, 1939

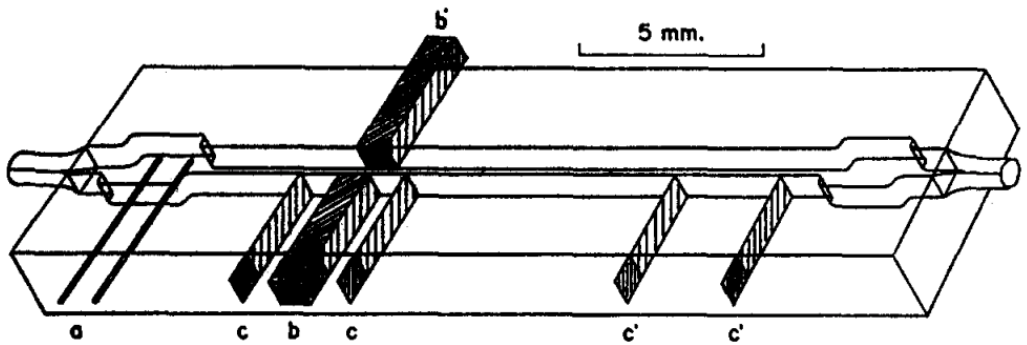


FIG. 1. Measuring cell for squid giant axon. The central trough is for the axon and the connections for circulating sea water are at each end. The axon is stimulated with electrodes, a, the transverse impedance measured between electrodes, b, b', and the action potentials between various combinations of b, c, and c'.

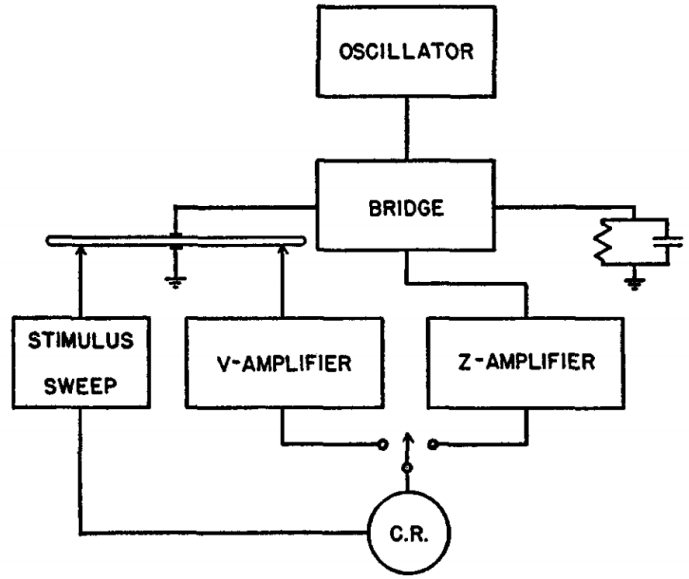


FIG. 2. Schematic diagram of the electrical equipment. The axon is at the left, and the balancing resistance and capacity at the right, of the bridge. The action potential and bridge amplifiers are represented by V-amplifier and Z-amplifier respectively and the cathode ray oscillograph by C. R.

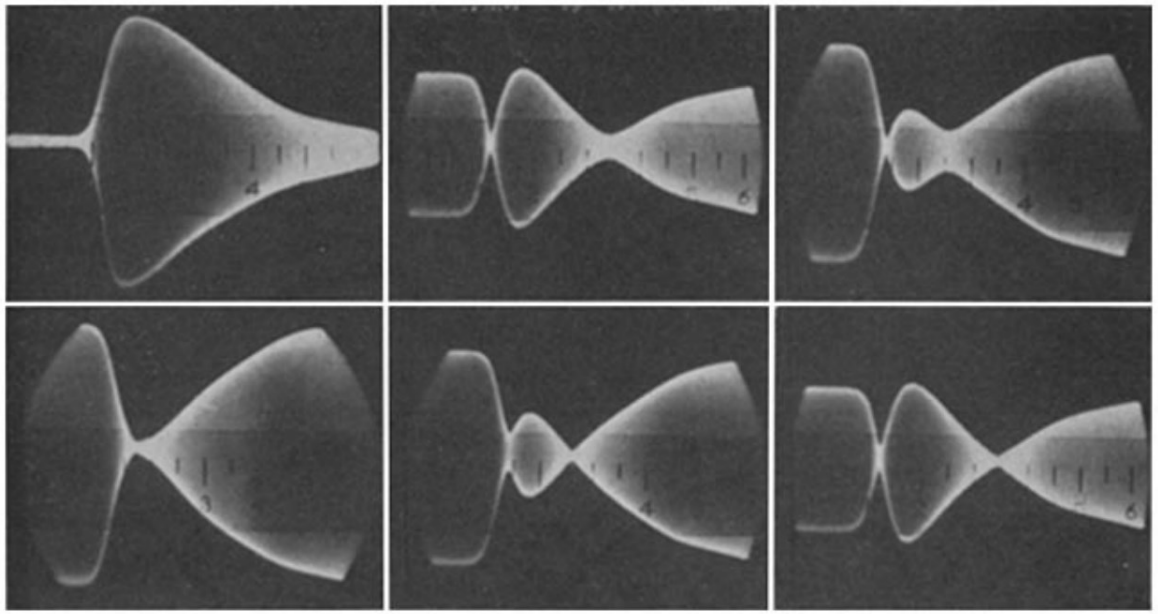


FIG. 3. Bridge output during the passage of an impulse with the bridge balanced for the impedance of the axon first at rest and then at various times during the action. Frequency 50 kc.; maximum change, 7 per cent.

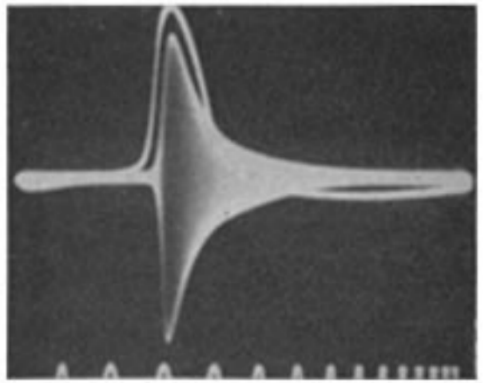


FIG. 4. Double exposure of the 2 per cent maximum bridge unbalance at 20 kc. and the monophasic action potential at one of the impedance electrodes. The time marks at the bottom are 1 millisecond apart.

Conclusion: Membrane breakdown hypothesis holds

Question: is the membrane breakdown hypothesis correct?

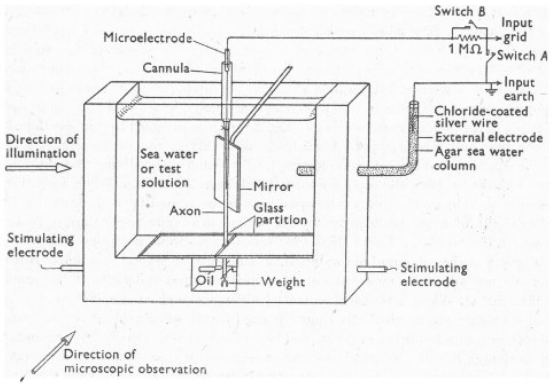


Fig. 1. Simplified diagram of recording cell.

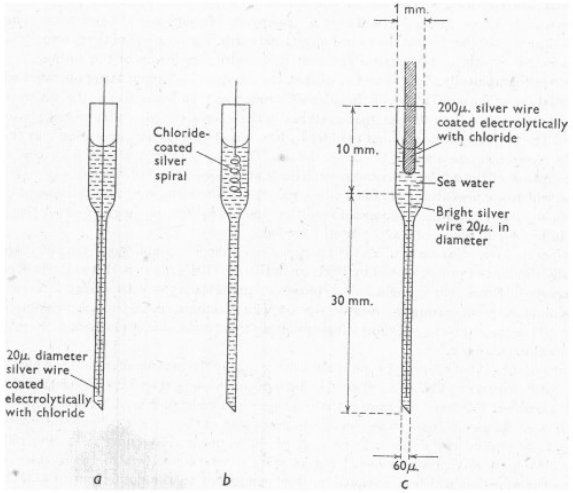


Fig. 2. Diagrams of microelectrodes: *a* and *b*, types formerly employed; *c*, type used in present work.

Alan Hodgkin and Andrew Huxley, 1939

Action Potentials Recorded from Inside a Nerve Fibre

NERVOUS messages are invariably associated with an electrical change known as the action potential. This potential is generally believed to arise at a membrane which is situated between the axoplasm and the external medium. If this theory is correct, it should be possible to record the action potential between an electrode inside a nerve fibre and the conducting fluid outside it. Most nerve fibres are too small for this to be tested directly, but we have recently succeeded in inserting micro-electrodes into the giant axons of squids (*Loligo forbesi*)[1].

The following method was used. A 500 μ axon was partially dissected from the first stellar nerve and cut half through with sharp scissors. A fine cannula was pushed through the cut and tied into the axon with a thread of silk. The cannula was mounted with the axon hanging from it in sea water. The upper part of the axon was illuminated from behind and could be observed from the front and side by means of a system of mirrors and a microscope; the lower part was insulated by oil and could be stimulated electrically. Action potentials were recorded by connecting one amplifier lead to the sea water outside the axon and the other to a micro-electrode which was lowered through the cannula into the intact nerve beneath it. The micro-electrode consisted of a glass tube about 100 μ in diameter and 10–20 mm. in length; the end of the tube was filled with sea water, and electrical contact with this was made by a 20 μ silver wire which was coated with silver chloride at the tip. Fig. 1 is a photograph of an electrode

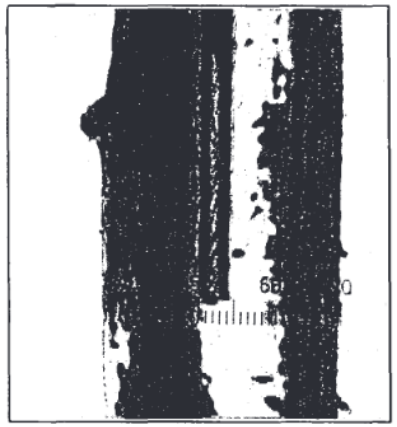


Fig. 1.

PHOTOMICROGRAPH OF ELECTRODE INSIDE GIANT AXON. 1 SCALE DIVISION = 33 μ.

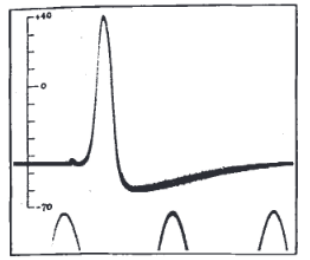


Fig. 2.

ACTION POTENTIAL RECORDED BETWEEN INSIDE AND OUTSIDE OF AXON. TIME MARKER, 500 CYCLES/SEC. THE VERTICAL SCALE INDICATES THE POTENTIAL OF THE INTERNAL ELECTRODE IN MILLIVOLTS, THE SEA WATER OUTSIDE BEING TAKEN AT ZERO POTENTIAL.

inside the living axon. The giant axon shows as a clear space and is surrounded by the small fibres and connective tissue which make up the rest of the nerve trunk. The silver wire can be seen inside the electrode and about 1 mm. from its tip. A small action potential was recorded from the upper end of the axon and this gradually increased as the electrode was lowered, until it reached a constant amplitude of 80–95 mv. at a distance of about 10 mm. from the cannula. In this region the axon appeared to be in a completely normal condition, for it survived and transmitted impulses for several hours. Experiments with external electrodes showed that the action potential was conducted for at least a centimetre past the tip of the micro-electrode.

These results are important for two reasons. In the first place they prove that the action potential arises at the surface, and in the second, they give the absolute magnitude of the action potential as about 90 mv. at 20° C. Previous measurements have always been made with external electrodes and give values which are reduced by the short-circuiting effect of the fluid outside the nerve fibre.

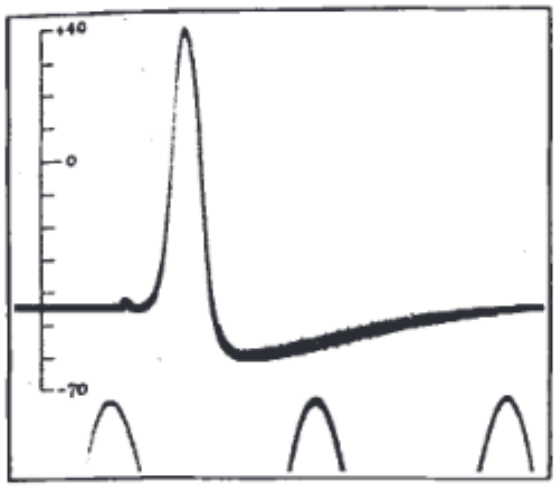
The potential difference recorded between the interior and exterior of the resting fibre is about 50 mv. The potential difference across the membrane may be greater than this, because there may be a junction potential between the axoplasm and the sea water in the tip of the electrode. This potential cannot be estimated, because the anions inside the nerve fibre have not been identified.

We wish to express our indebtedness to Mr. J. Z. Young, whose discovery of the giant axon in *Loligo* made this work possible.

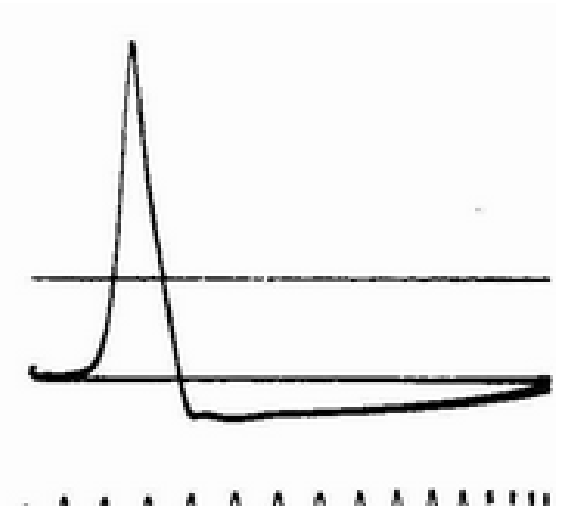
A. L. HODGKIN.
A. F. HUXLEY.

Laboratory of the
Marine Biological Association,
Plymouth.
August 26.

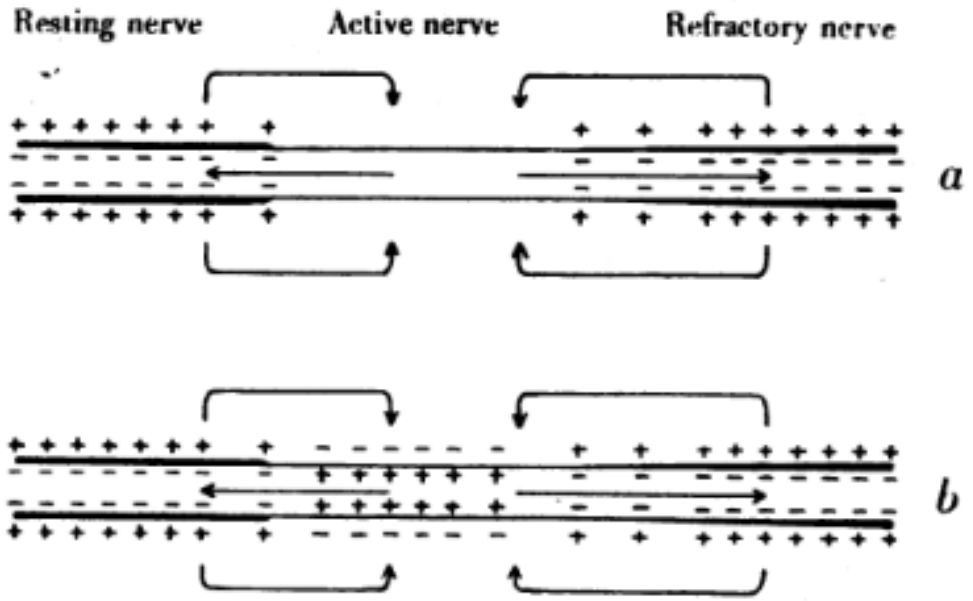
[1] Young, J. Z., *Proc. Roy. Soc.*, B, 121, 319 (1936).



Alan Hodgkin and Andrew Huxley, 1939



Kacy Cole and Howard Curtis, 1939



Text-fig. 6. Diagram illustrating (*a*) classical and (*b*) revised concepts of nervous activity.

Conclusion: Membrane breakdown hypothesis needs revising

Question: is sodium responsible for the overshoot of the action potential?

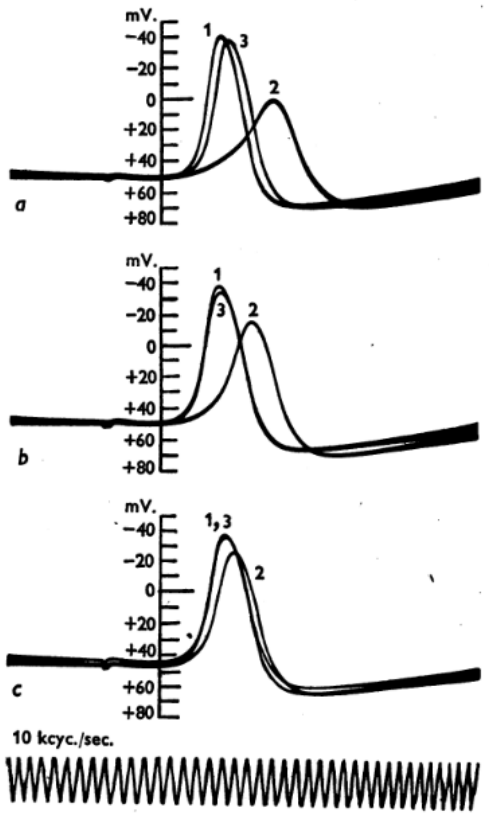
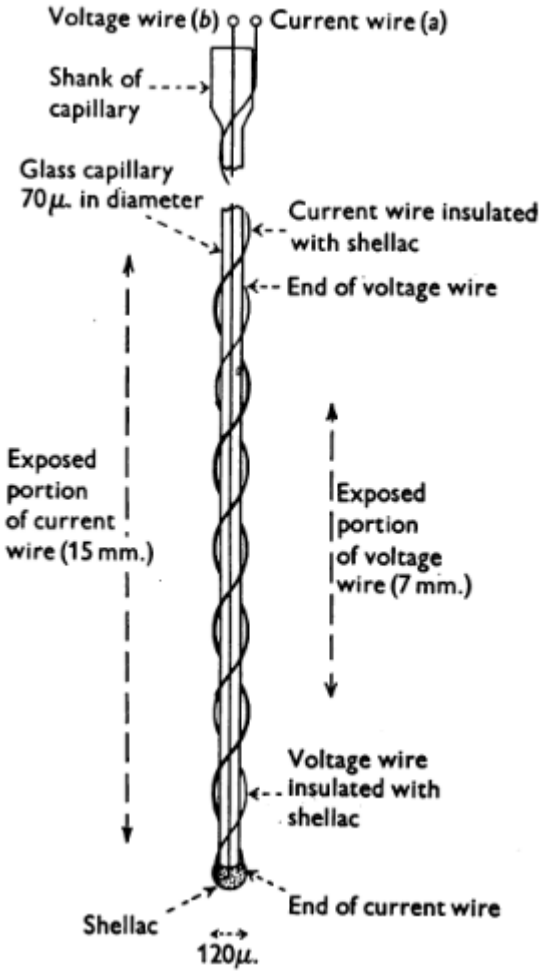


Fig. 4. Action of sodium-deficient solutions on the resting and action potential. *a* 1, response in sea water; *a* 2, after 16 min. in 33% sea water, 67% isotonic dextrose; *a* 3, 13 min. after reapplication of sea water. *b* 1, response in sea water, *b* 2, after 15 min. in 50% sea water, 50% isotonic dextrose; *b* 3, 6 min. after reapplication of sea water. *c* 1, response in sea water; *c* 2, after 16 min. in 71% sea water, 29% isotonic dextrose; *c* 3, 7 min. after reapplication of sea water. The scale gives the potential difference across the nerve membrane (outside – inside) with no allowance for the junction potential between the axoplasm and the sea water in the microelectrode.

Conclusion: Yes

Question: what are the ionic currents that generate the action potential?



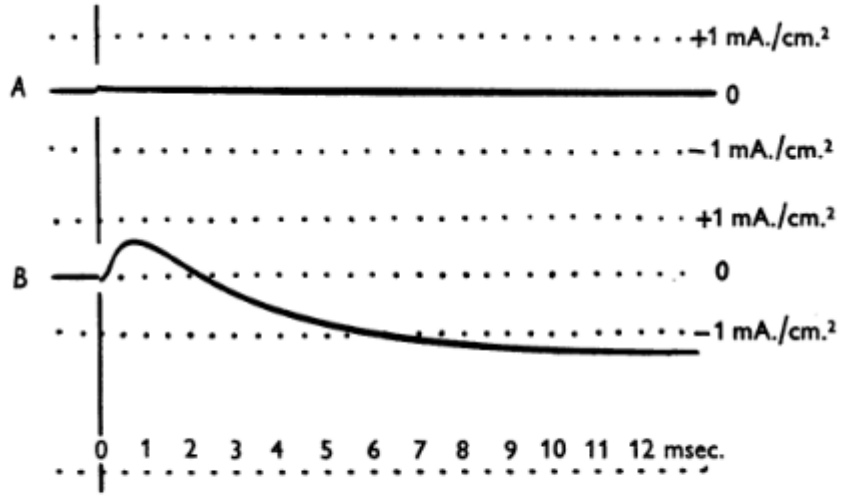


Fig. 11. Records of membrane current under a voltage clamp. At zero time the membrane potential was increased by 65 mV. (record *A*) or decreased by 65 mV. (record *B*); this level was then maintained constant throughout the record. Inward current is shown as an upward deflexion. Axon 41; diameter 585 μ. Temperature 3·8° C. Compensated feed-back.

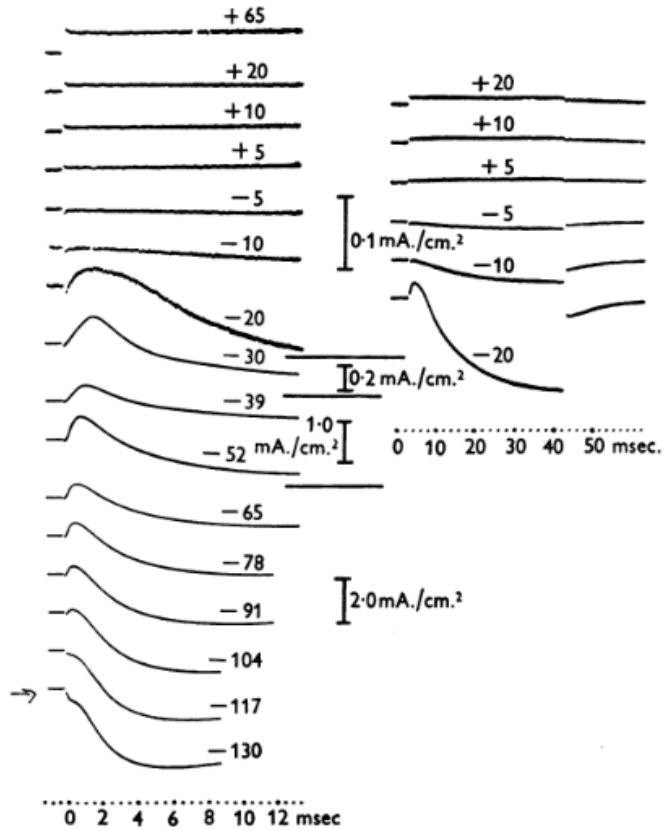


Fig. 12. Records of membrane current under a voltage clamp. The displacement of membrane potential (V) is given in millivolts by the number attached to each record. Inward current is shown as an upward deflexion. Six records at a lower time base speed are given in the right-hand column. Experimental details as in Fig. 11.

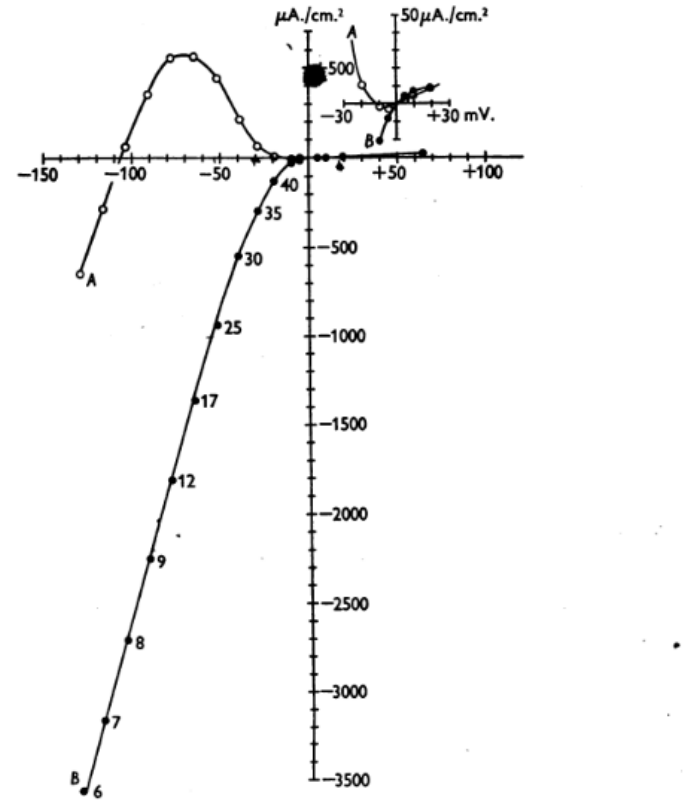


Fig. 13. Relation between membrane current density and membrane potential. Abscissa: displacement of membrane potential from its resting value in mV. Ordinate: membrane current density at 0·63 msec. after beginning of voltage step (curve A) and in 'steady state' (curve B). The numbers attached to curve B indicate the times in msec. at which the measurements were made. Inset: curves in region of origin drawn with a tenfold increase in the vertical scale. Inward current density is taken as positive and the membrane potential is given in the sense external potential minus the internal potential. Measurements were made from the records reproduced in Fig. 12 (3·8° C.).

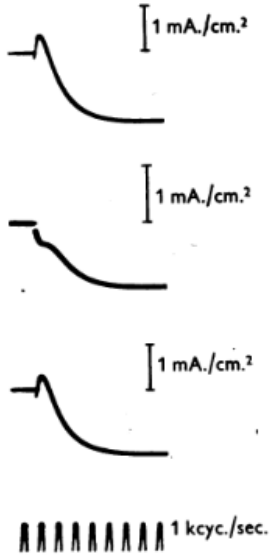


Fig. 1

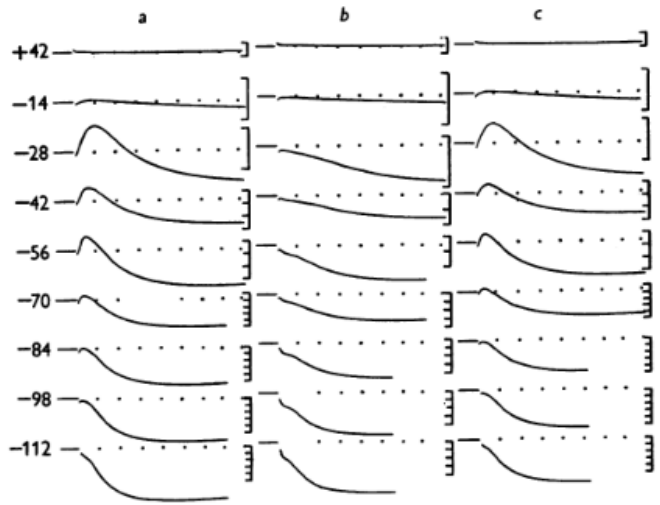


Fig. 2

Fig. 1. Records of membrane current during 'voltage clamps' in which membrane potential was lowered by 65 mV. Top record: axon in sea water. Centre record: axon in choline sea water. Bottom record: after replacing sea water. Axon no. 15; temperature 11° C. Inward current is shown upwards in this and all other figures.

Fig. 2. Records of membrane current during 'voltage clamps'. *a*, axon in sea water; *b*, axon in choline sea water; *c*, after replacing sea water. Displacement of membrane potential indicated in mV. Axon no. 21; temperature 8·5° C. Vertical scale: 1 division is 0·5 mA./cm.². Horizontal scale: interval between dots is 1 msec.

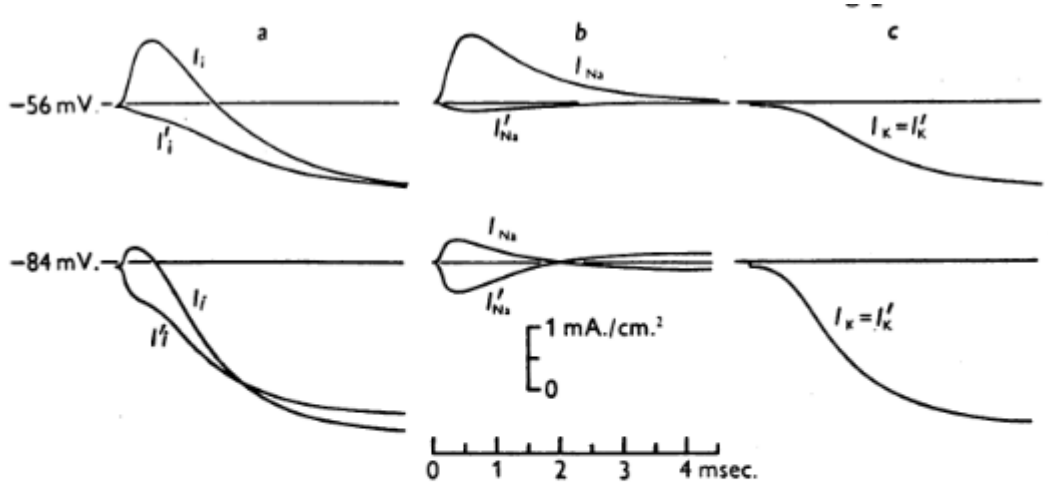


Fig. 5. Curves illustrating separation of ionic current into I_{Na} and I_K. Upper part of figure. *a*, ionic currents: I_i, axon in sea water, membrane potential lowered by 56 mV.; I'_i, axon in 10% sodium sea water, membrane potential lowered by 60 mV. (average of curves taken before and after I_i). *b*, sodium currents: I_{Na},-sodium current in sea water; I'_{Na}, sodium current in 10% sodium sea water. *c*, potassium current, same in both solutions. Lower part of figure. Same, but membrane potential lowered by 84 mV. in sea water and 88 mV. in 10% sodium sea water. Current and time scales same for all curves. Axon no. 21; temperature 8·5° C.

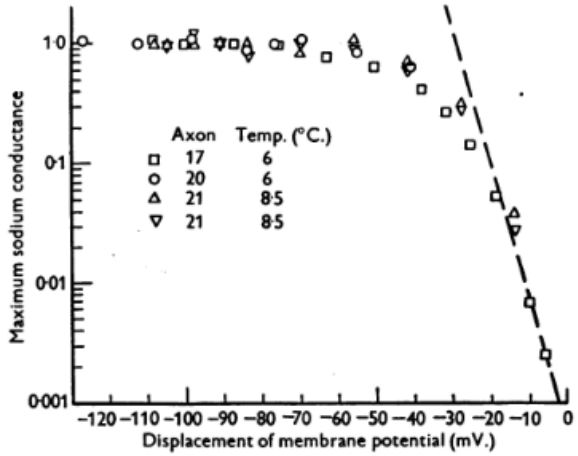


Fig. 9. Maximum sodium conductance reached during a voltage clamp. Ordinate: peak conductance relative to value reached with depolarization of 100 mV., logarithmic scale. Abscissa: displacement of membrane potential from resting value (depolarization negative).

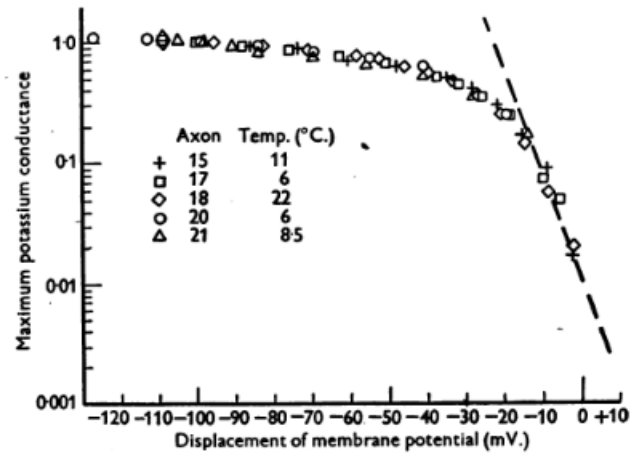


Fig. 10. Maximum potassium conductance reached during a voltage clamp. Ordinate: maximum conductance relative to value reached with depolarization of 100 mV., logarithmic scale. Abscissa: displacement of membrane potential from resting value (depolarization negative).

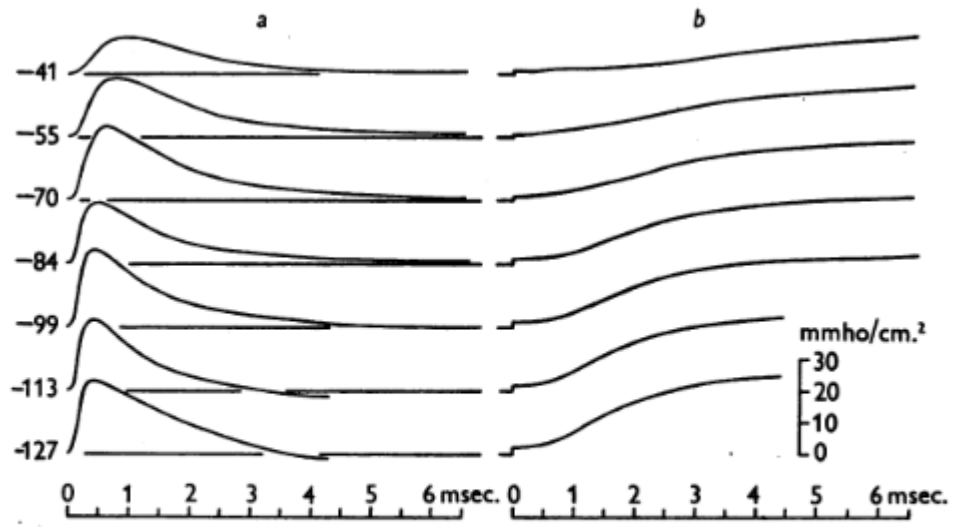
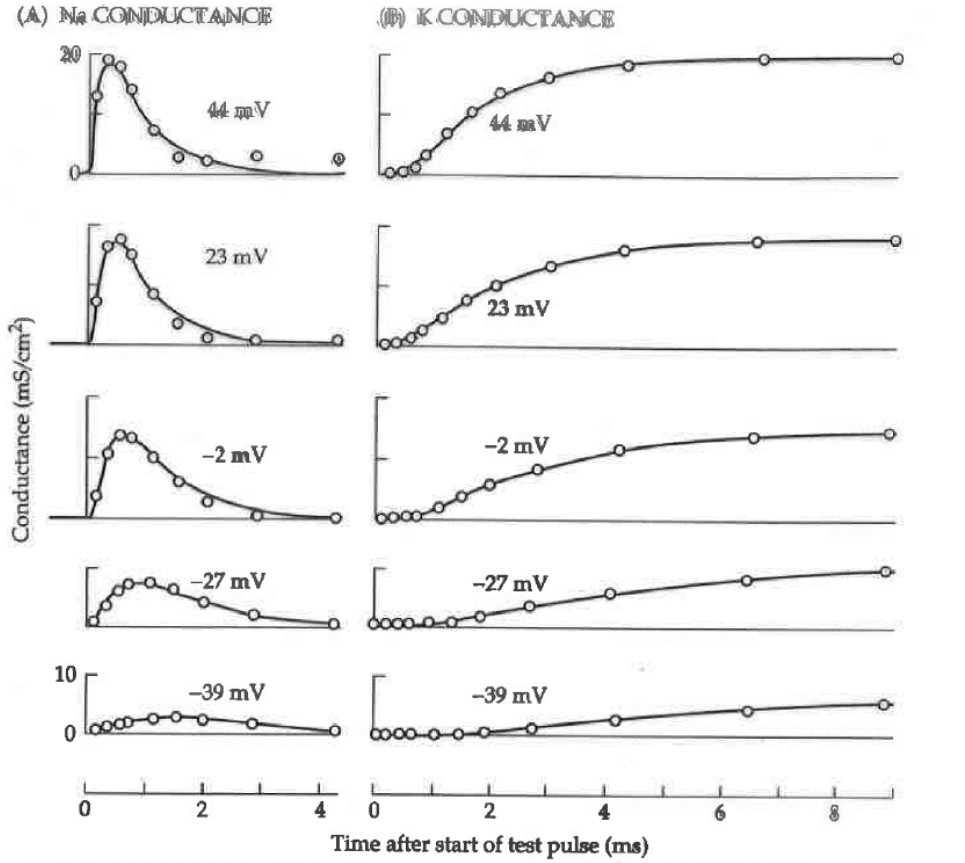
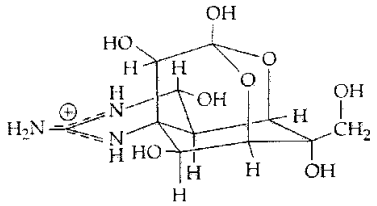


Fig. 8. Curves of sodium conductance (*a*) and potassium conductance (*b*). Displacement of membrane potential (millivolts) when axon was in sea water is indicated on each curve. Curves of I_t and I_K in same experiment are shown in Figs. 3 and 6*a* respectively. Axon no. 20; temperature 6·3° C.

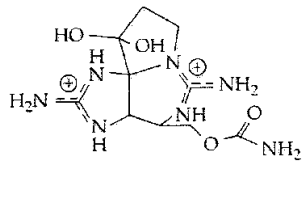


Conclusion: Fast activating/inactivating sodium current and slow activating/non-inactivating potassium current

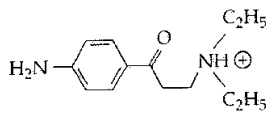
Question: how do ions cross membranes?



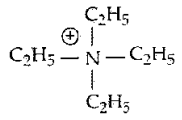
TTX



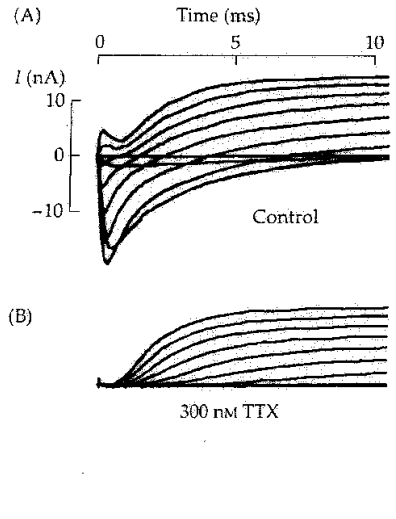
STX

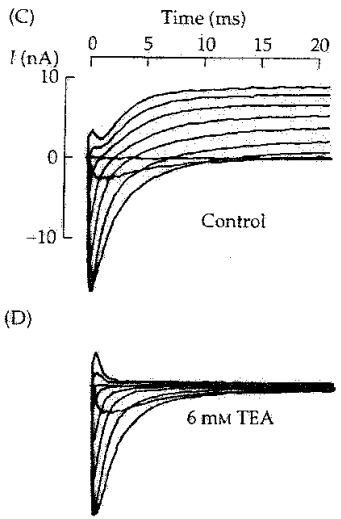


Procaine



TEA





(Narahashi 1964)

(Armstrong & Binstock 1965)

Erwin Neher

Bert Sakmann

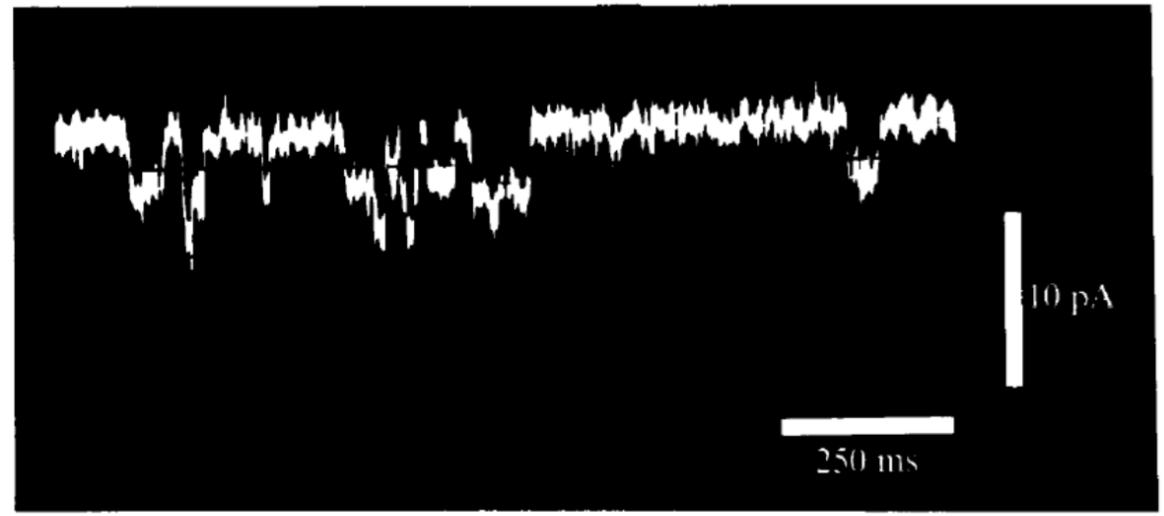
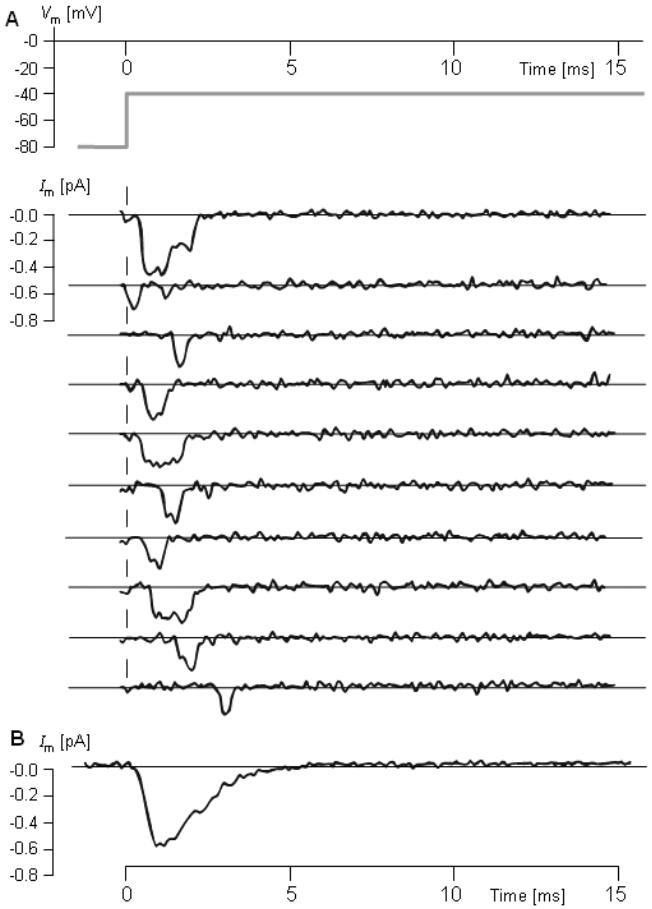
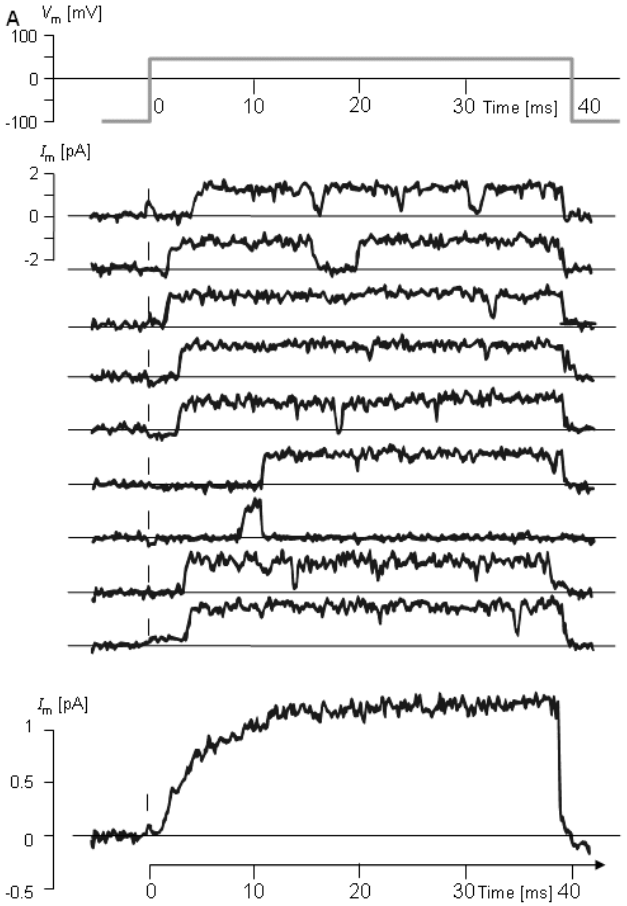


Figure 2. **Early single-channel currents from denervated frog** *(Ram pipiens)* **cutaneous pectoris muscle. The pipette contained 0.2 μM suberyldicholine, an analogue of acetylcholine which induces very long-lived channel openings. Membrane potential – 120 mV; temperature 8°C. Reproduced from Neher & Sakmann 1976.**





Conclusion: It is most likely a pore

Question: How can a pore be selective?

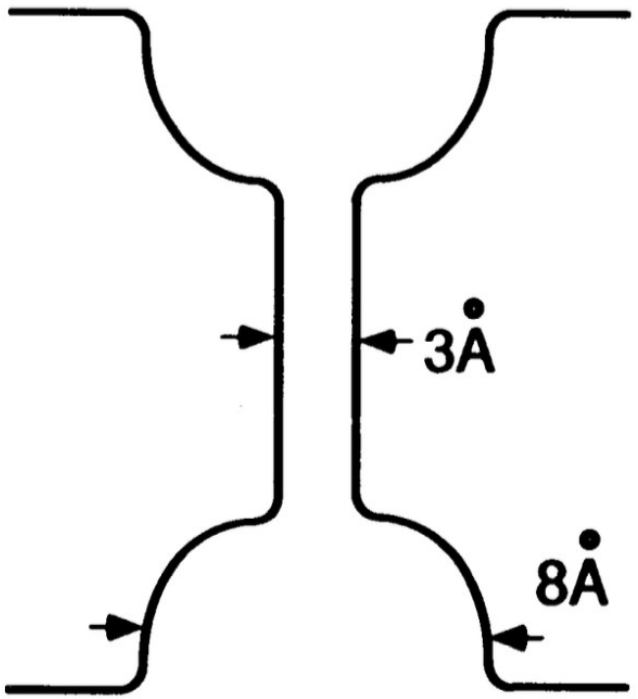
TABLE II

PERMEABILITY RATIOS FOR ALL CATIONS IN THE POTASSIUM CHANNEL

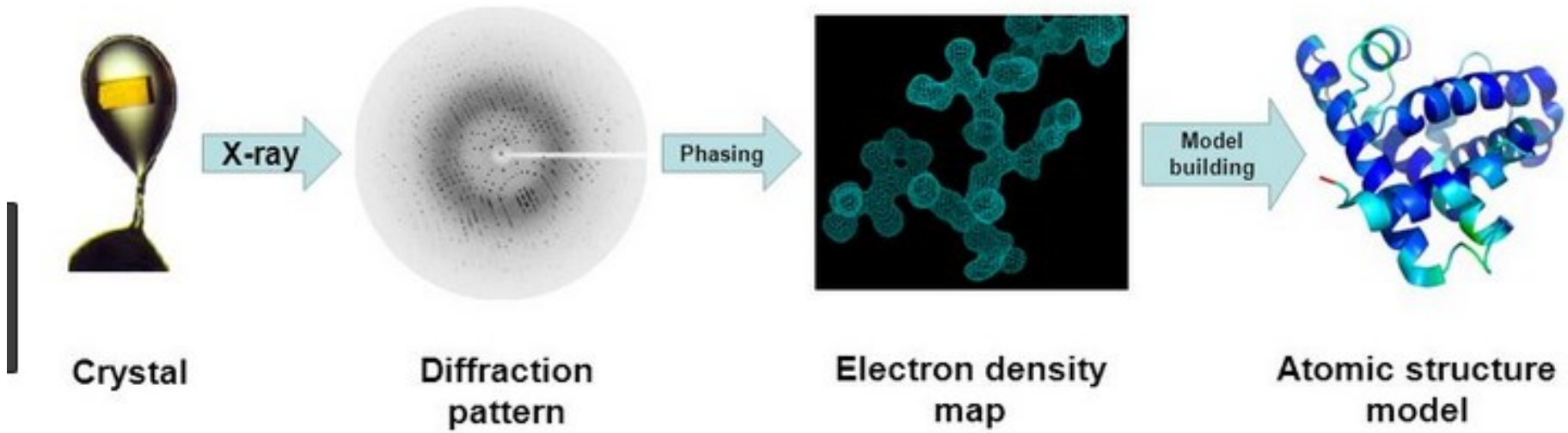
P_X/P_K	X	Minimum pore diameter*
		Å
<0.018‡	Lithium	1.20
<0.010‡	Sodium	1.90
1.00	Potassium	2.66
2.3	Thallium	2.80
0.91	Rubidium	2.96
0.13	Ammonium	3.0
<0.029‡	Hydrazine	3.3
<0.025‡	Hydroxylamine	3.3
<0.077‡	Cesium	3.38
<0.021‡	Methylamine	3.6
<0.020‡	Formamidine	3.6
<0.013‡	Guanidine	4.8

* Measured from atomic models with a pentagonal pore described in Discussion section.

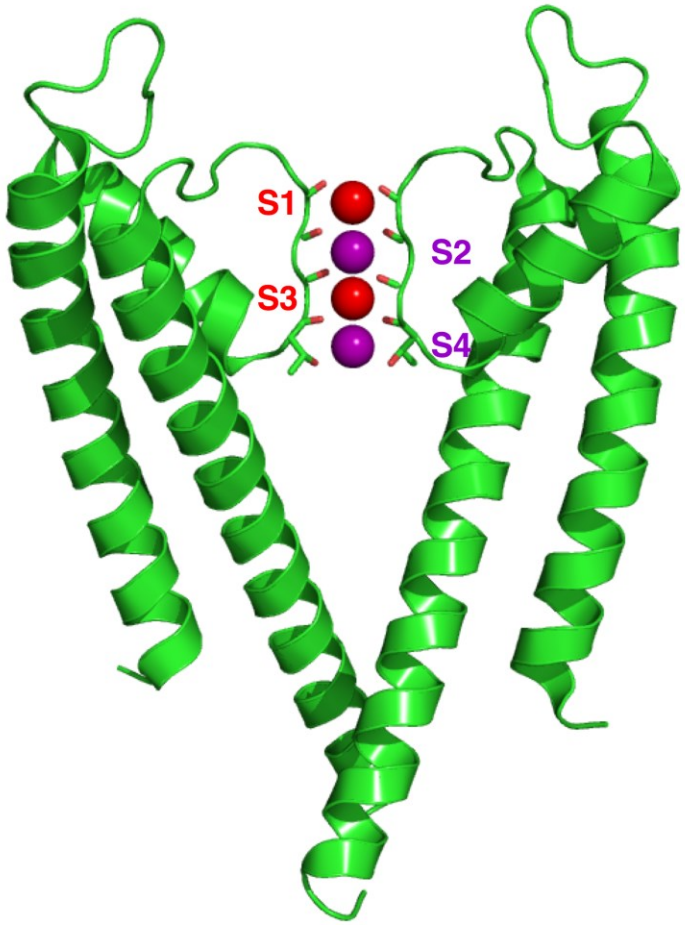
‡ Permeabilities measured using NH_4 Ringer as a reference and calculated as $P_X/P_K = 0.13 \times P_X/P_{NH_4}$.

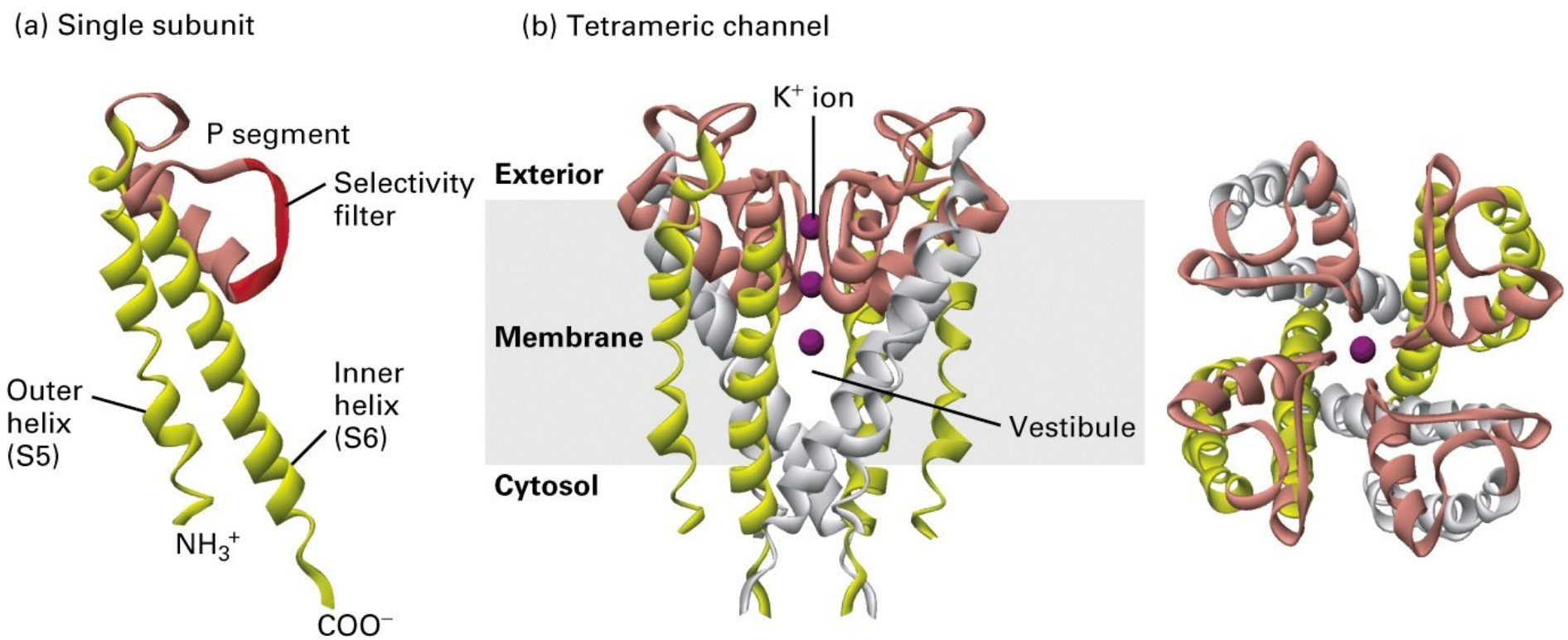


Macromolecular crystallography

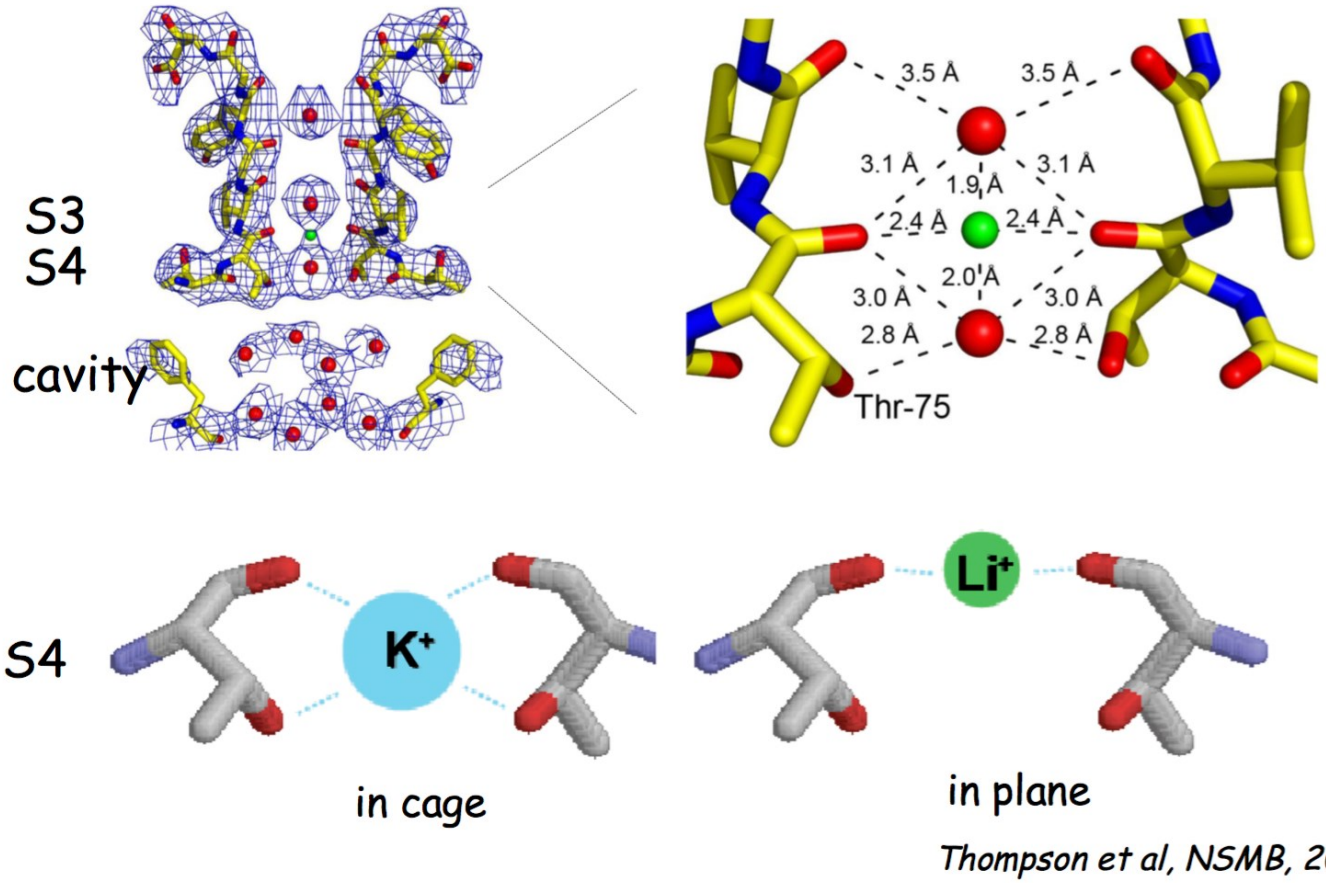


Kcsa channel




(a) Single subunit
P segment
Selectivity filter
Outer helix (S5)
Inner helix (S6)
NH_3^+
COO^-
(b) Tetrameric channel
K^+ ion
Exterior
Membrane
Cytosol
Vestibule

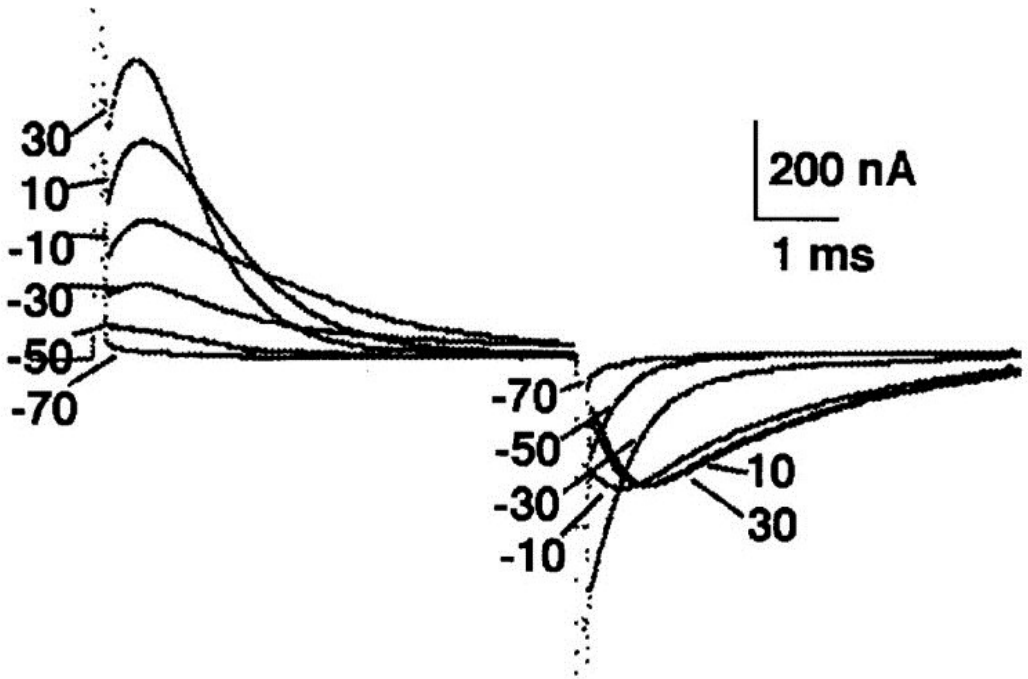
Li^+/Na^+ bind "in-plane" in the selectivity filter



Thompson et al, NSMB, 2009

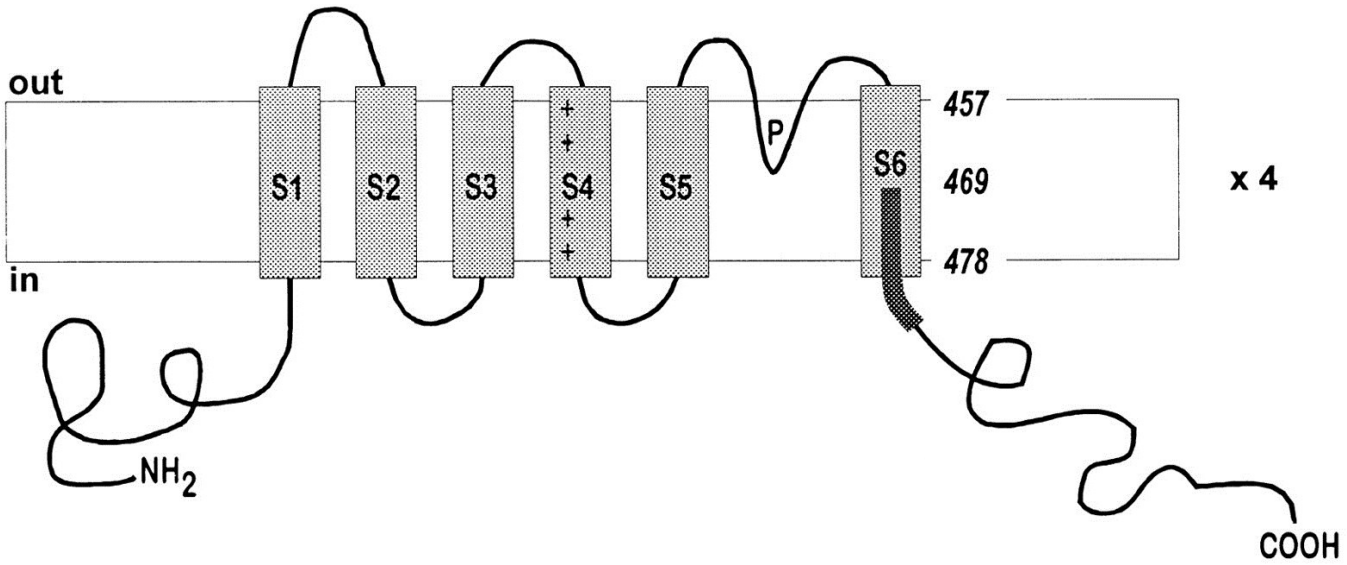
Conclusion: It's a pore, and the energy landscape determines selectivity

Question: How is a channel gated by voltage?



(Keynes & Riojas 1974)

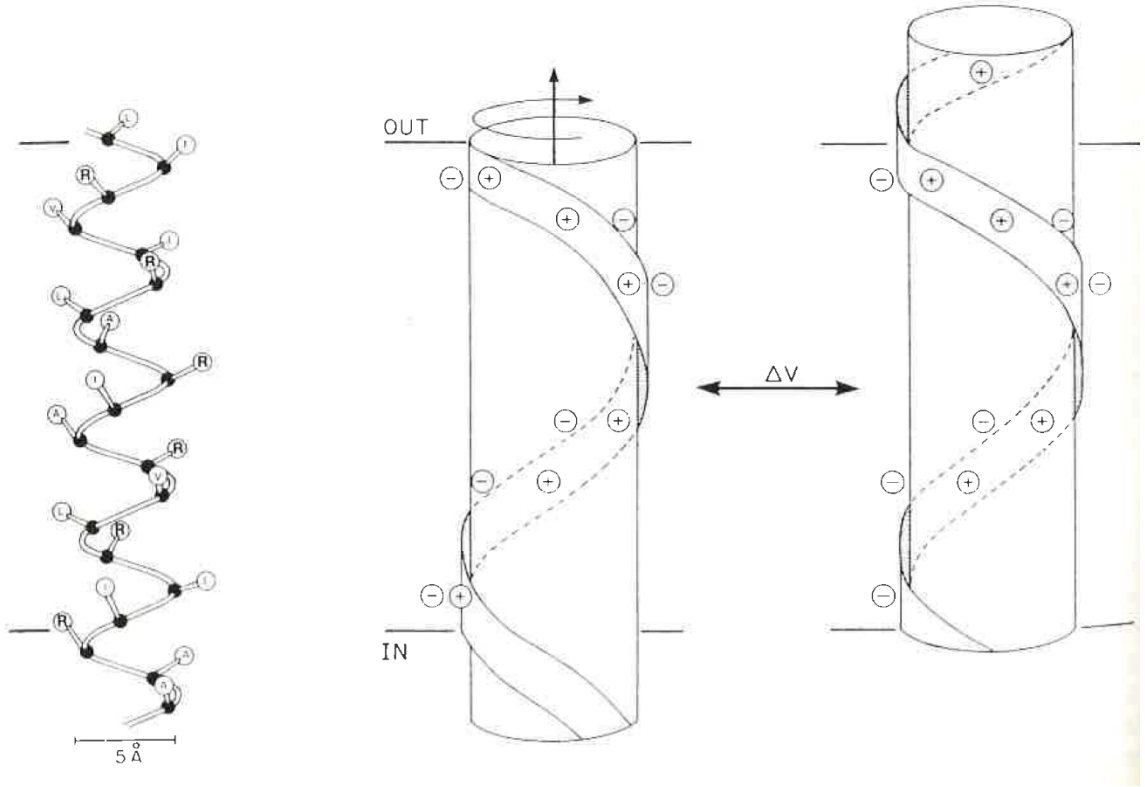
(Armstrong & Bezanilla 1973)



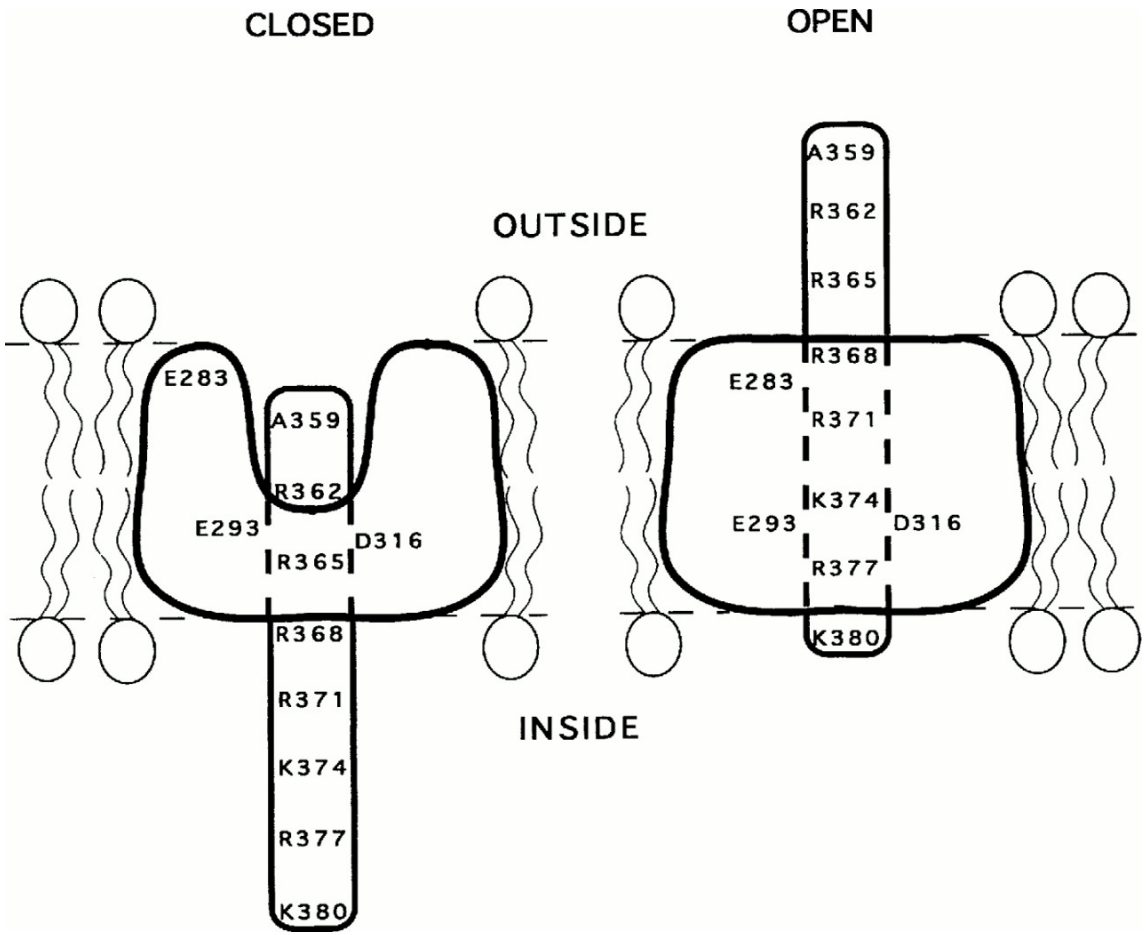
S4

	259
Shaker	/AMSLAILRVIRLVRVFRIFKLSRHSK
Kv1.2	/AMSLAILRVIRLVRVFRIFKLSRHSK
Kv2.1	FQNVRRVVQIFRIMRILRILKLARHST
Kv3.1	KDVLGFLRVVRFVRILRIFKLTRHFV
Kv4.1	VSGAFVTLRVFRVFRIFKFSRHSQ

Helical screw model of S4 movement

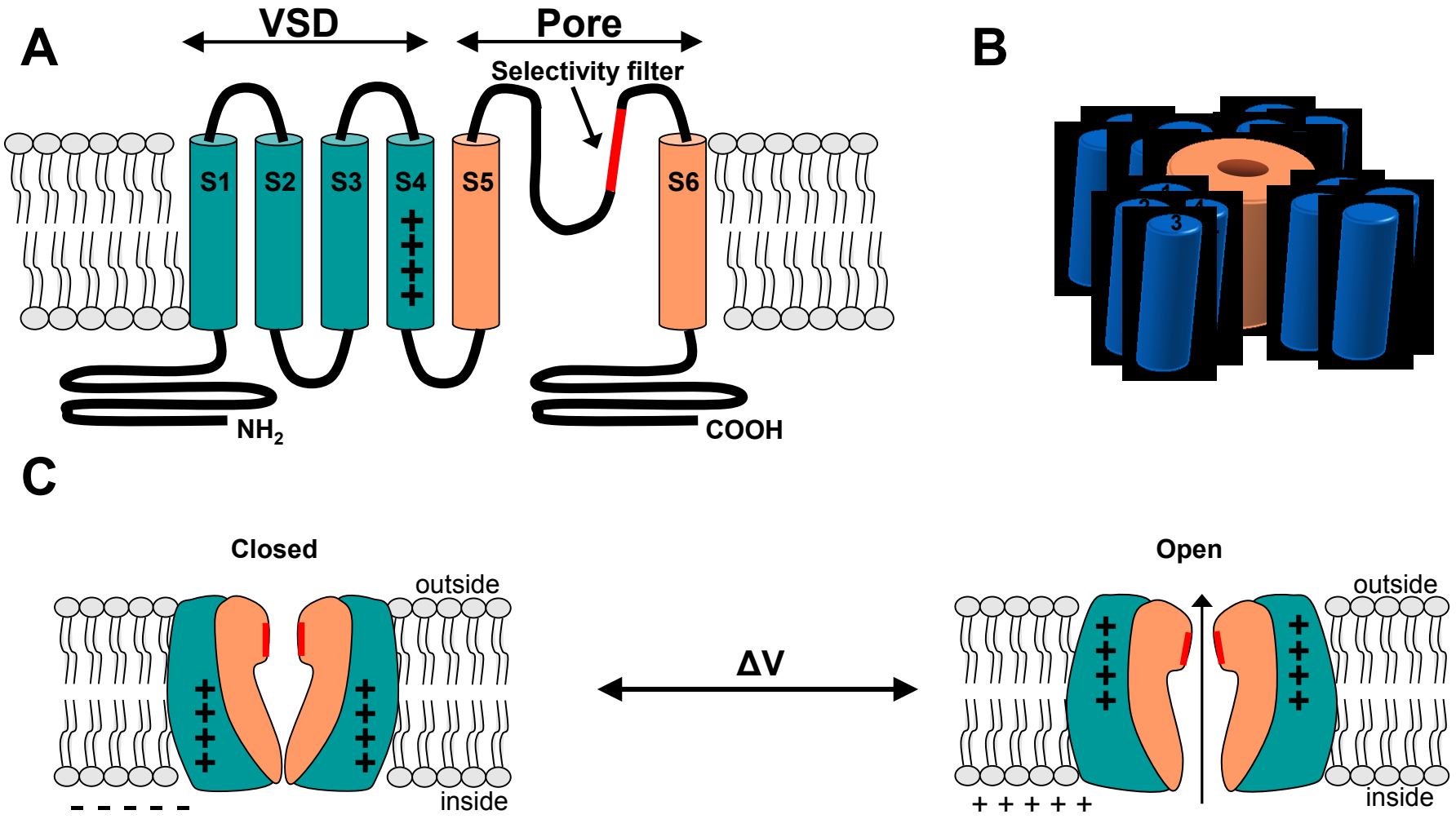


(Catterall 1986, Guy 1986)

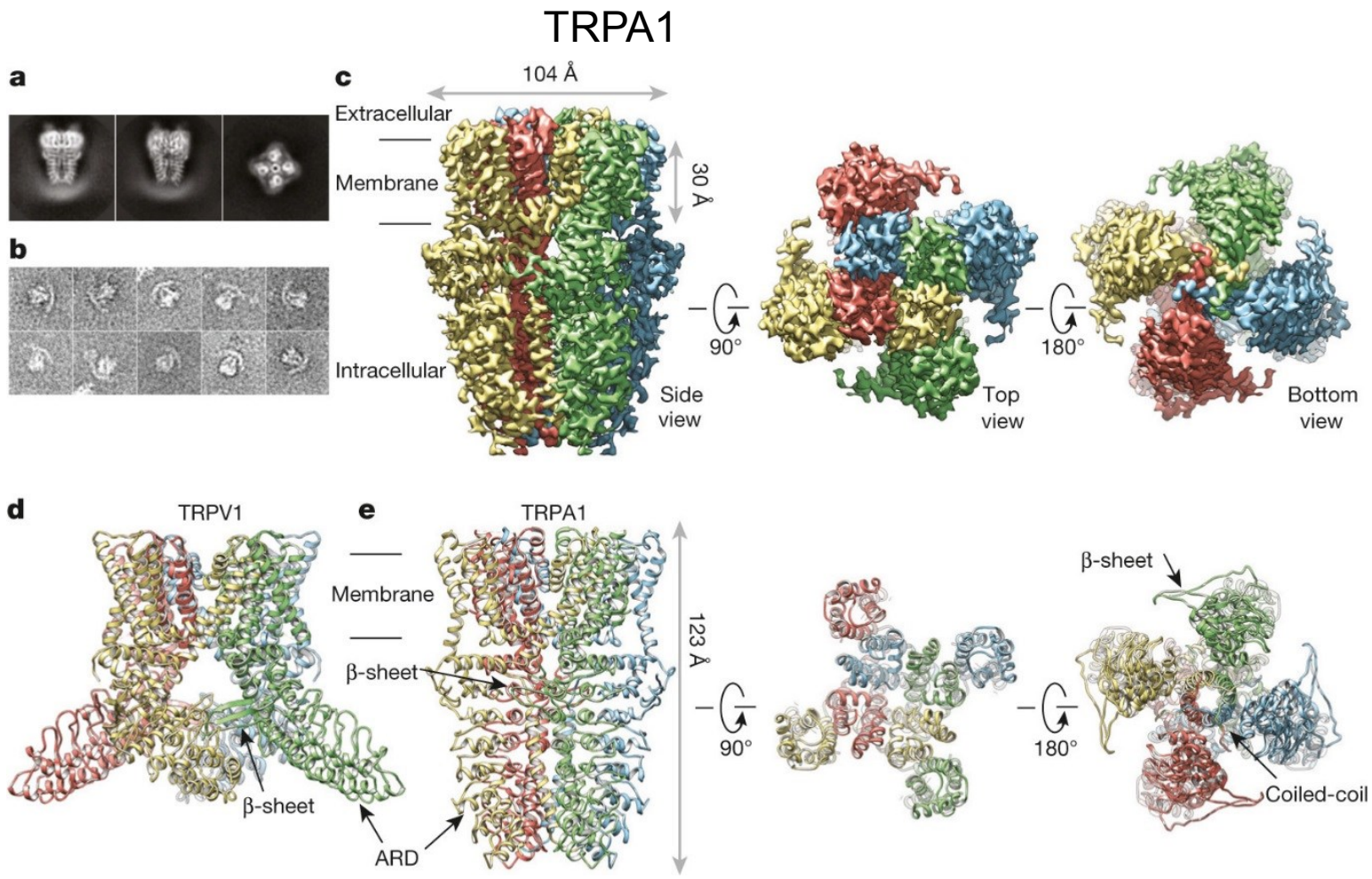


Conclusion: movement of charged residues lining the pore

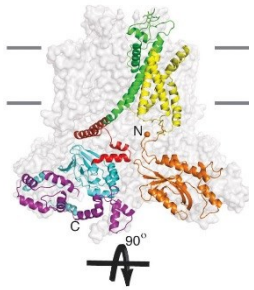
A voltage-gated ion channel

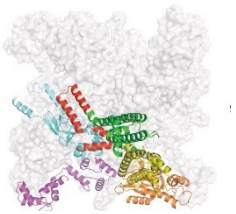


Question: how is functional diversity generated?

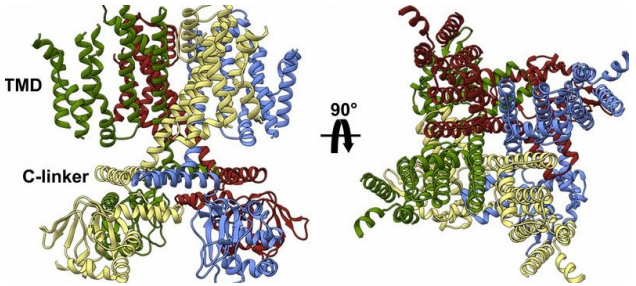


EAG

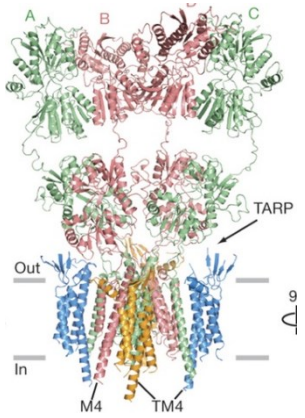




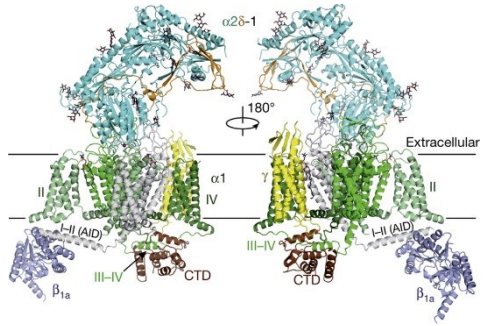
CNG



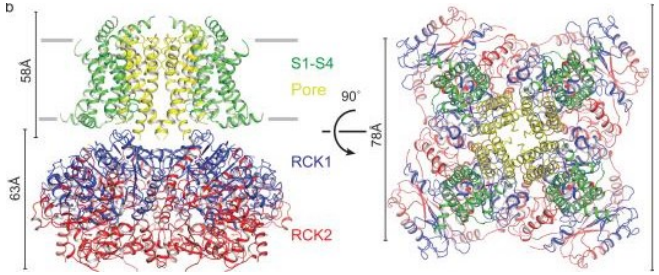
GluA2



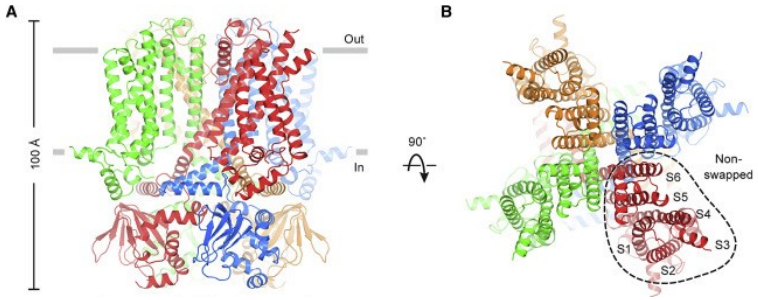
CaV1.1



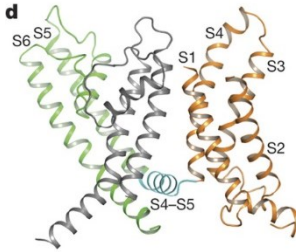
Slo2.2



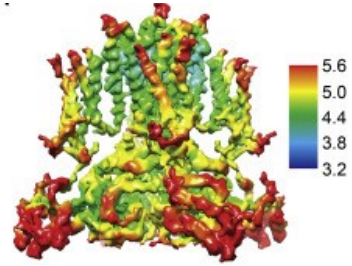
HCN1

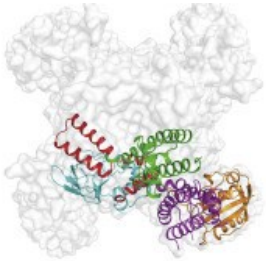


BK



hERG





KCNQ1