SIBS 中国科学院上海生命科学研究院
SHANGHAI INSTITUTES FOR BIOLOGICAL SCIENCES

砺志求真 笃学明德

电生理技术

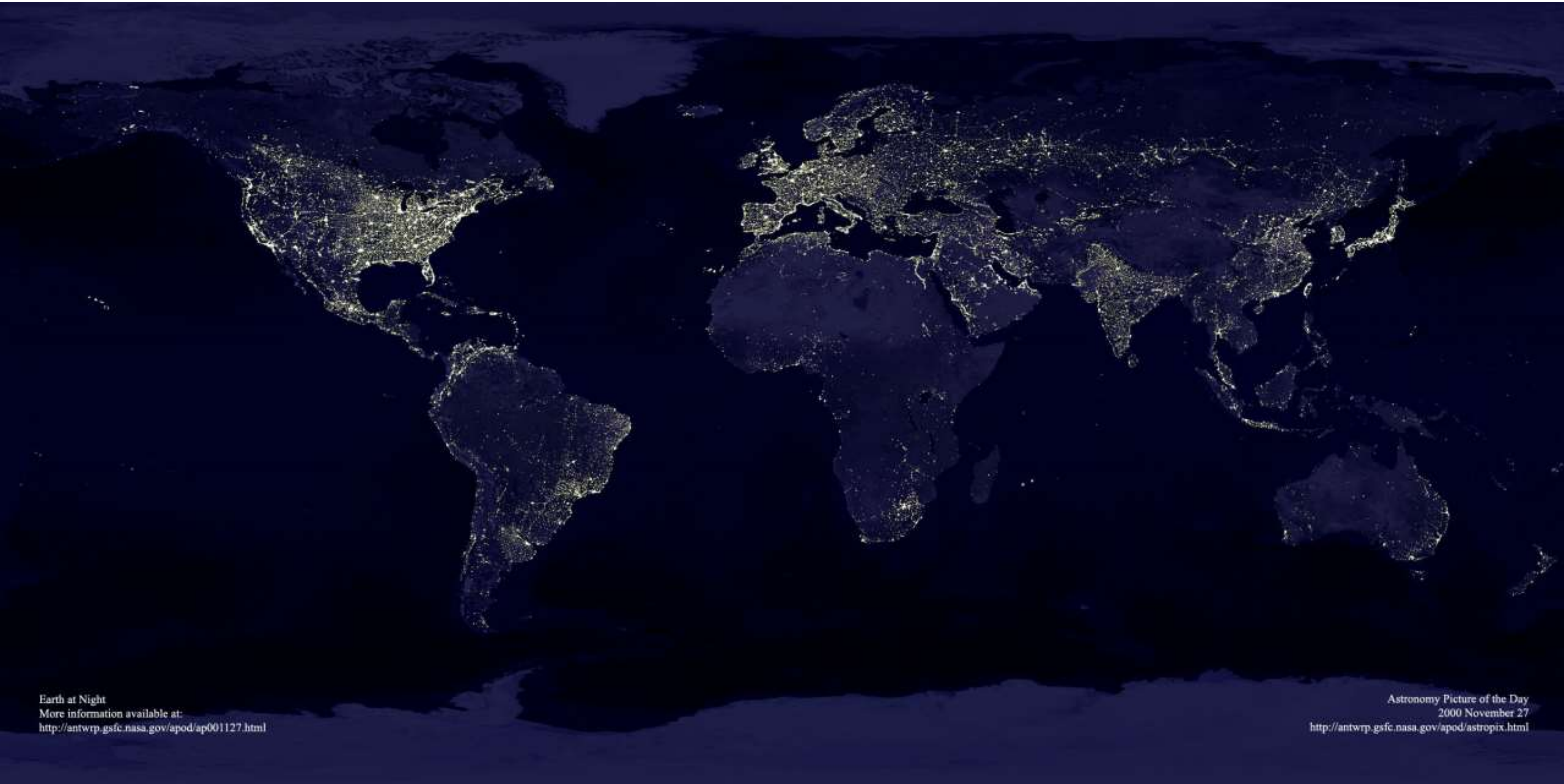
Chun XU （徐春）

Institute of Neuroscience, CAS

Menu

- Why electrophysiology?
- The history and basics of electrophysiology
- Methods in electrophysiology
- Future of electrophysiology

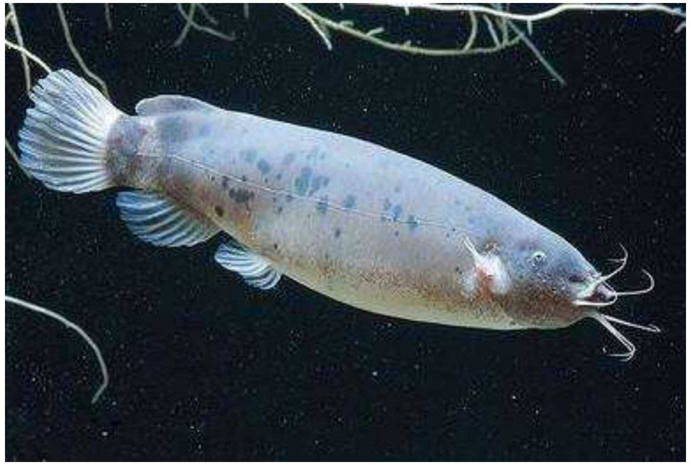
Electricity and human civilization



带电鱼：电鳗、电鳐、电鲶

- 电鳗放电电压可达700～800伏，电鳐放电电压可达300～500伏。





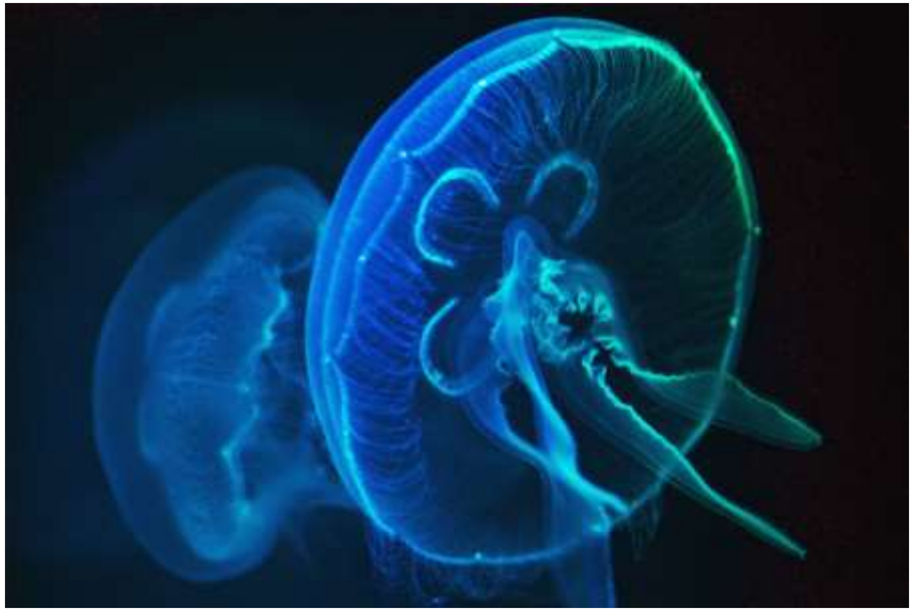
Electrical signals for sensation

Electrical signals for locomotion





Electrical signals for all kinds of behavior



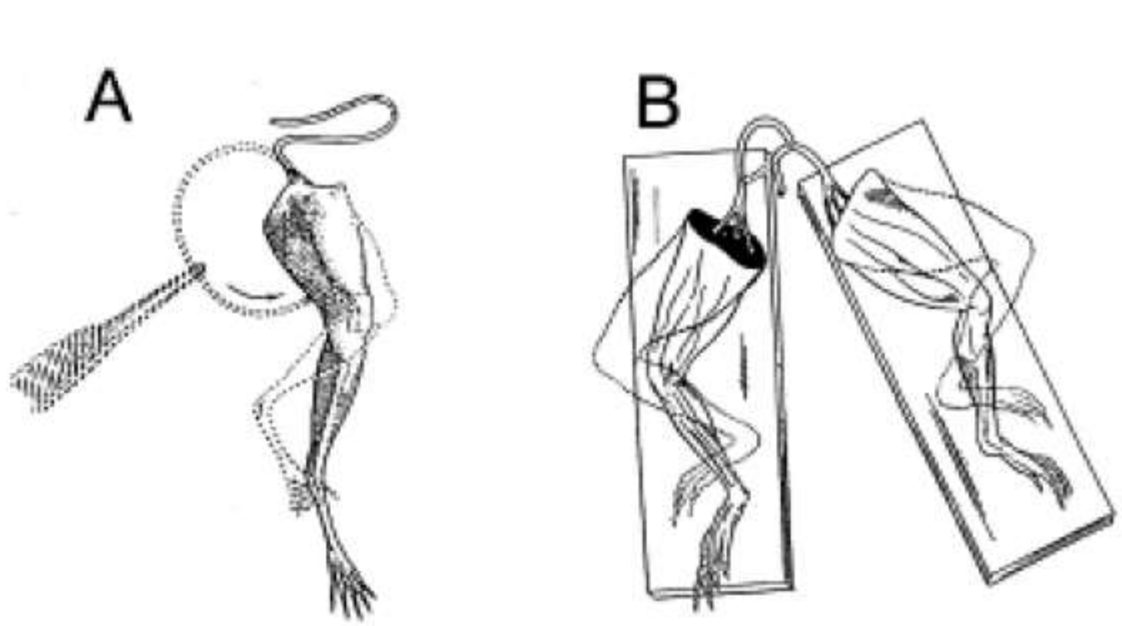
Why electrical signals

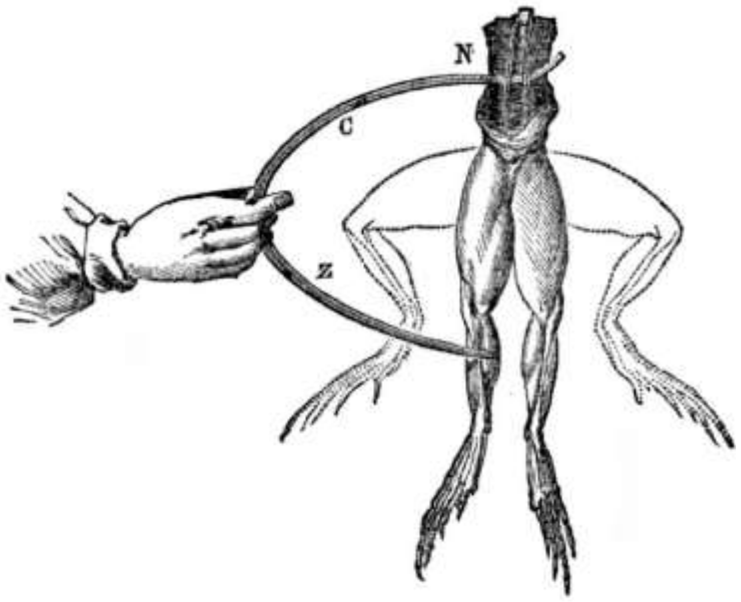
- …

- Why electrophysiology?
- The history and basics of electrophysiology
- Methods in electrophysiology?
- Future of electrophysiology

The muscle contraction is evoked by electrical signals!

Luigi Galvani
1786





Galvani's experiment demonstrating muscle contraction without using dissimilar substances (metal and tissue). (A) When the surface of section of the nerve touches the muscle, the leg contracts. (B) When the surface of section of the right sciatic nerve touches the intact surface of the left sciatic nerve, both legs contract

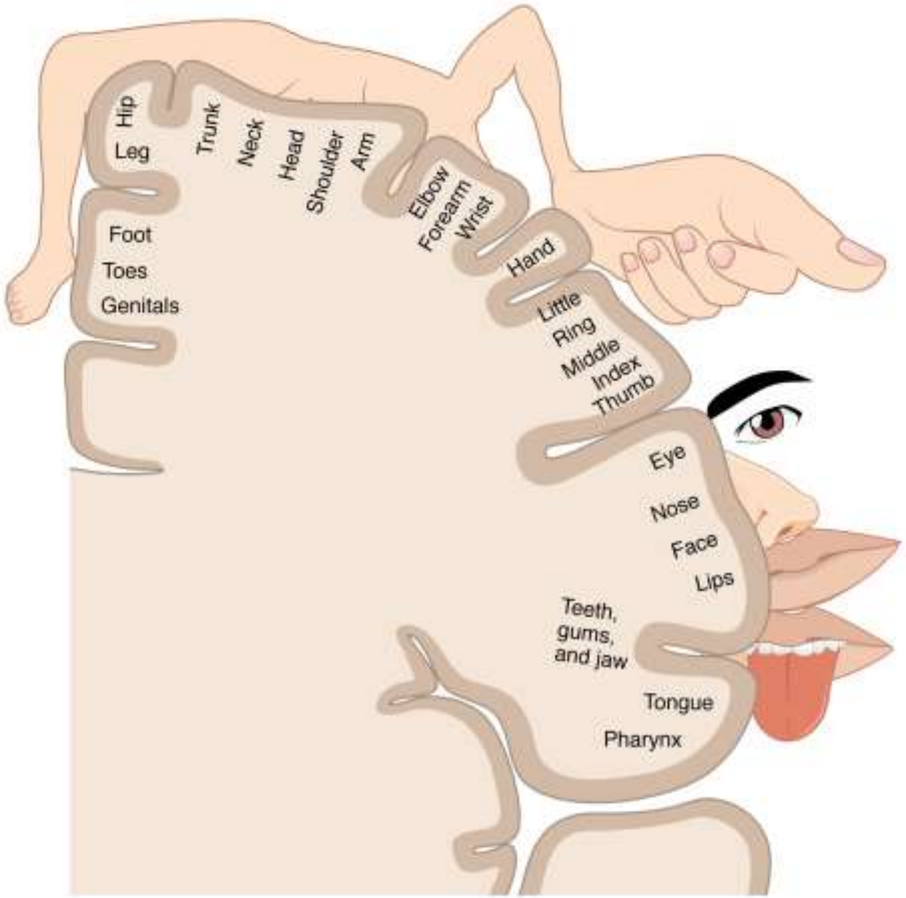
The muscle contraction is evoked by electrical signals!

Squid Giant Axon and neural muscle junction (NMJ)

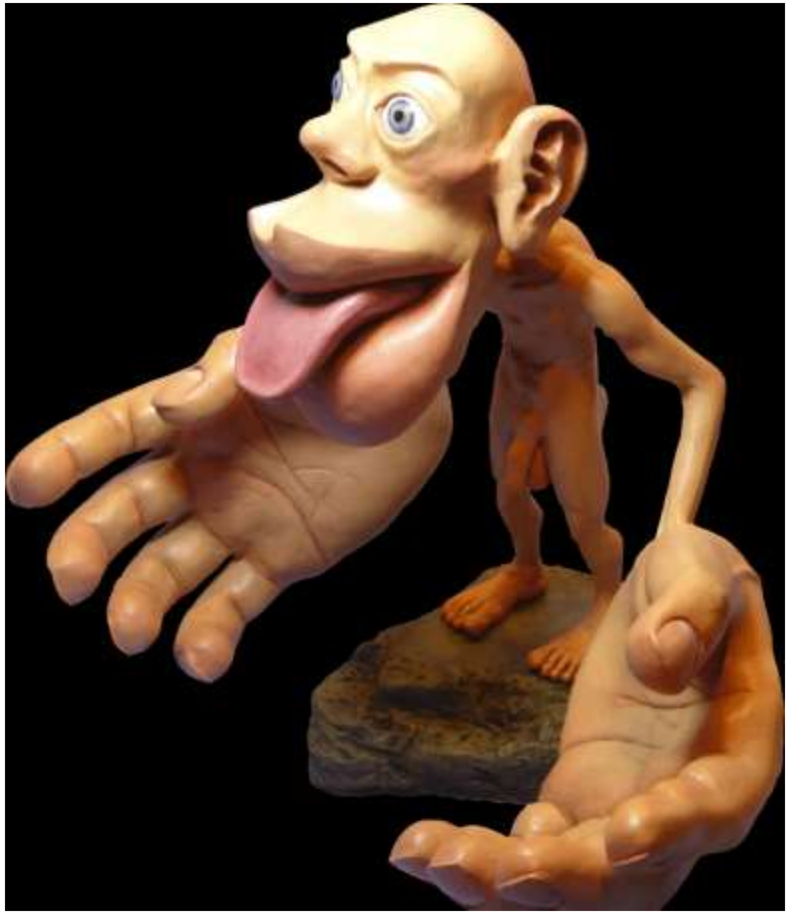
Cortical homunculus ("cortex man“)

Wilder Penfield

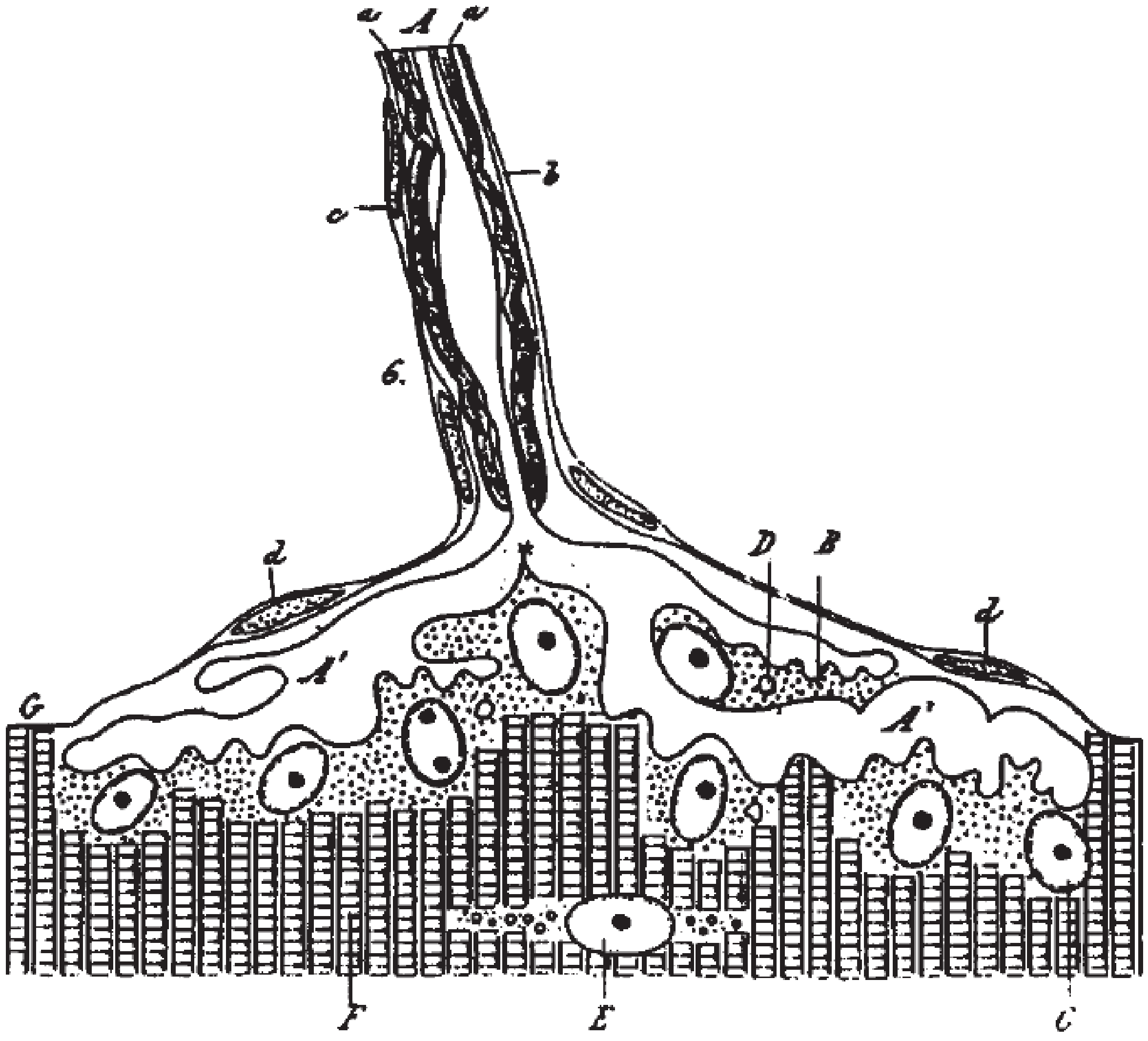
Touch feeling is evoked by electrical signals!



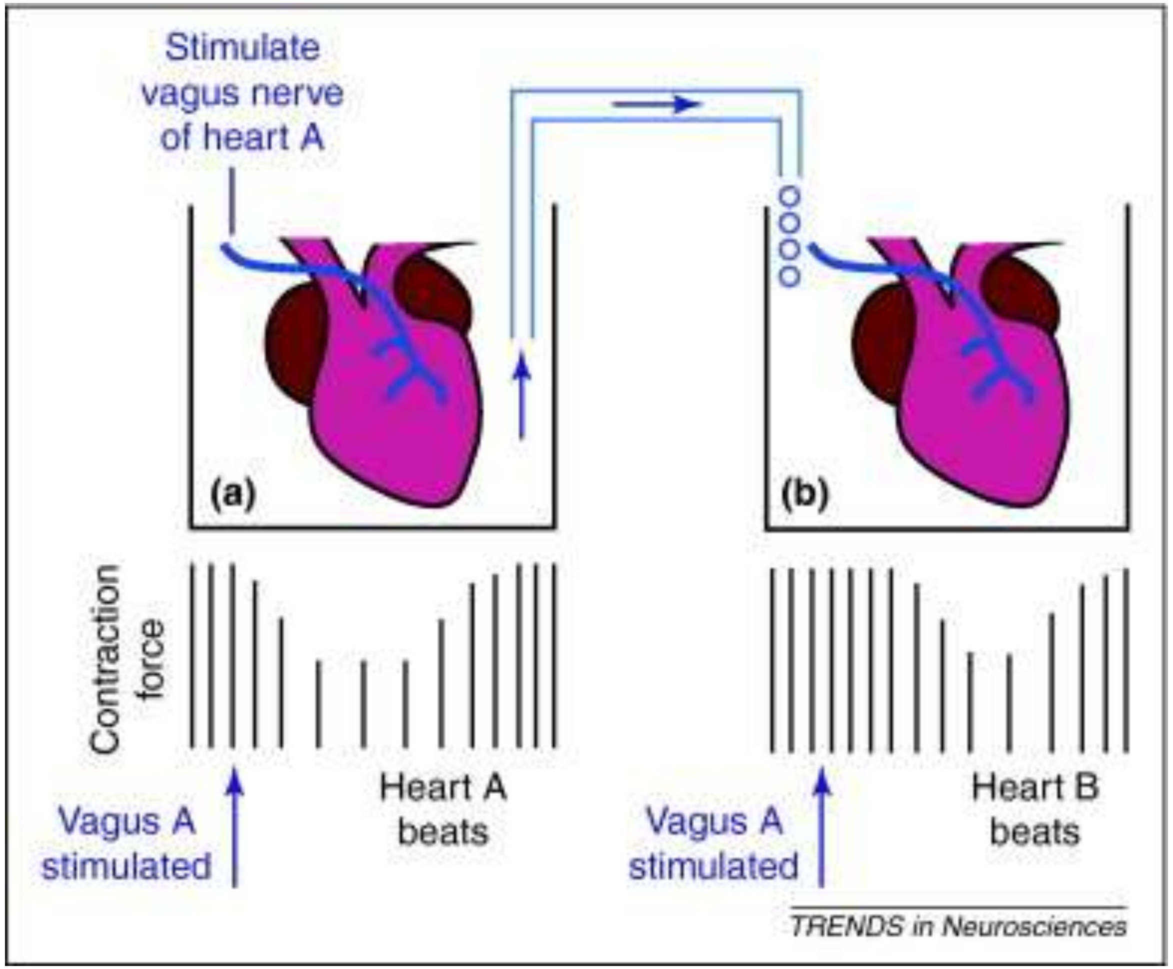
Penfield, 1930s



神经肌肉接头利用生物电完成运动



One of the key evidences for chemical ‘synaptic’ transmission



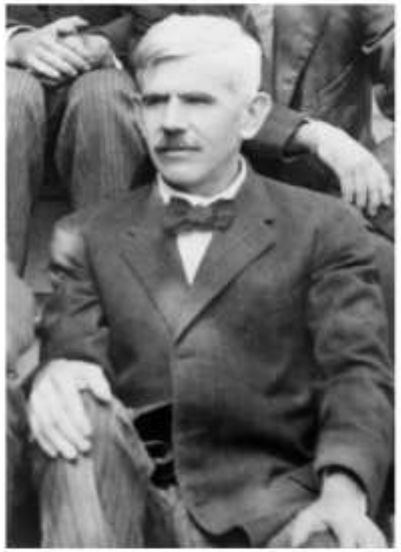
One of the key evidences for chemical 'synaptic' transmission

"In the night of Easter Saturday, 1921, I awoke, turned on the light, and jotted down a few notes on a tiny slip of paper. Then I fell asleep again. It occurred to me at six o'clock in the morning that during the night I had written down something most important, but I was unable to decipher the scrawl. **That Sunday was the most desperate day in my whole scientific life.** During the next night, however, I awoke again, at three o'clock, and I remembered what it was. This time I did not take any risk; **I got up immediately, went to the laboratory, made the experiment on the frog's heart** ··· and at five o' clock the chemical transmission of nervous impulse was conclusively proved." --- quoted from Loewi, O., From the Workshop of Discoveries, Lawrence: University of Kansas Press, 1953.

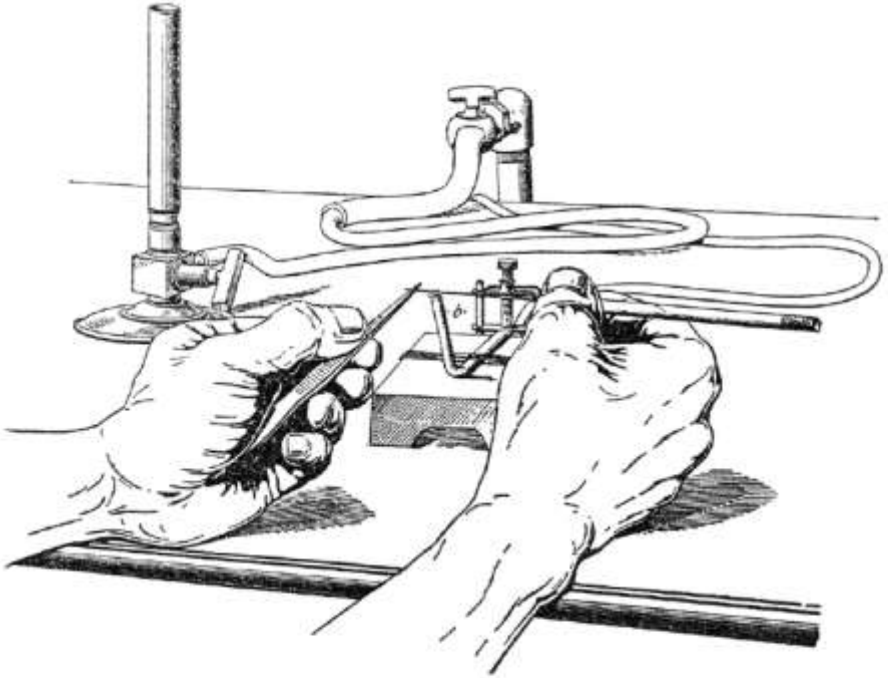
- **Still, how is the electrical signals generated and propagated?**

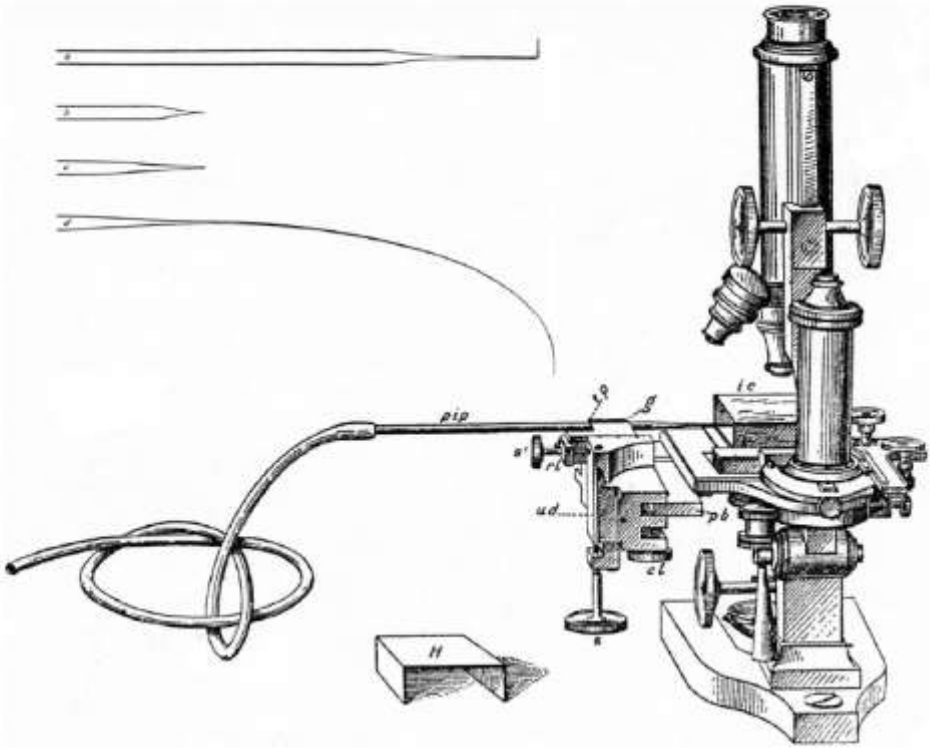
- **How to record the electrical signals intracellularly?**

Invention of the glass micropipette electrode



Marshall Albert Barber
(circa 1911).



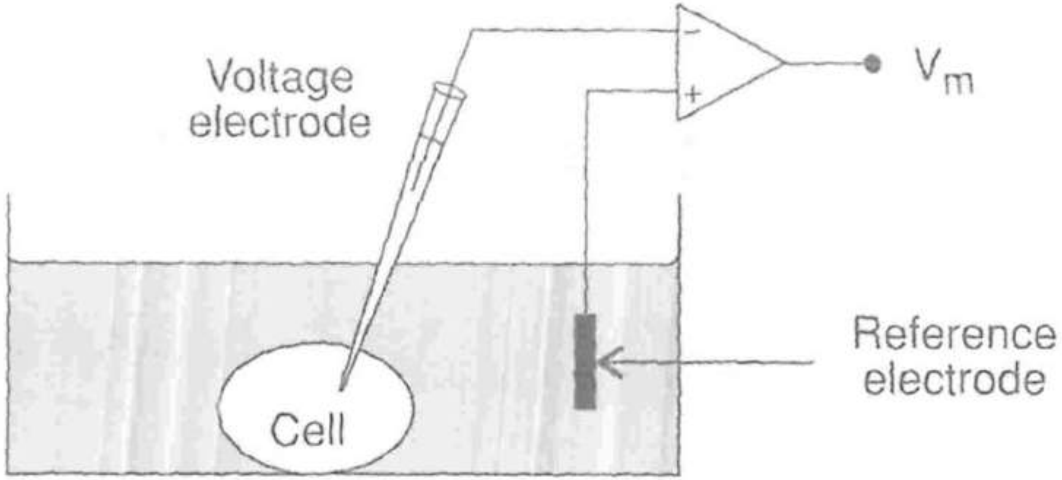


The glass micropipette electrode for intracellular recording

Milestone in Physiology
JGP 100th Anniversary

Gilbert Ning Ling

Ralph Gerard



It would be difficult to exaggerate the important role that the capillary microelectrode has played in Neurophysiology in the thirty years since its development.

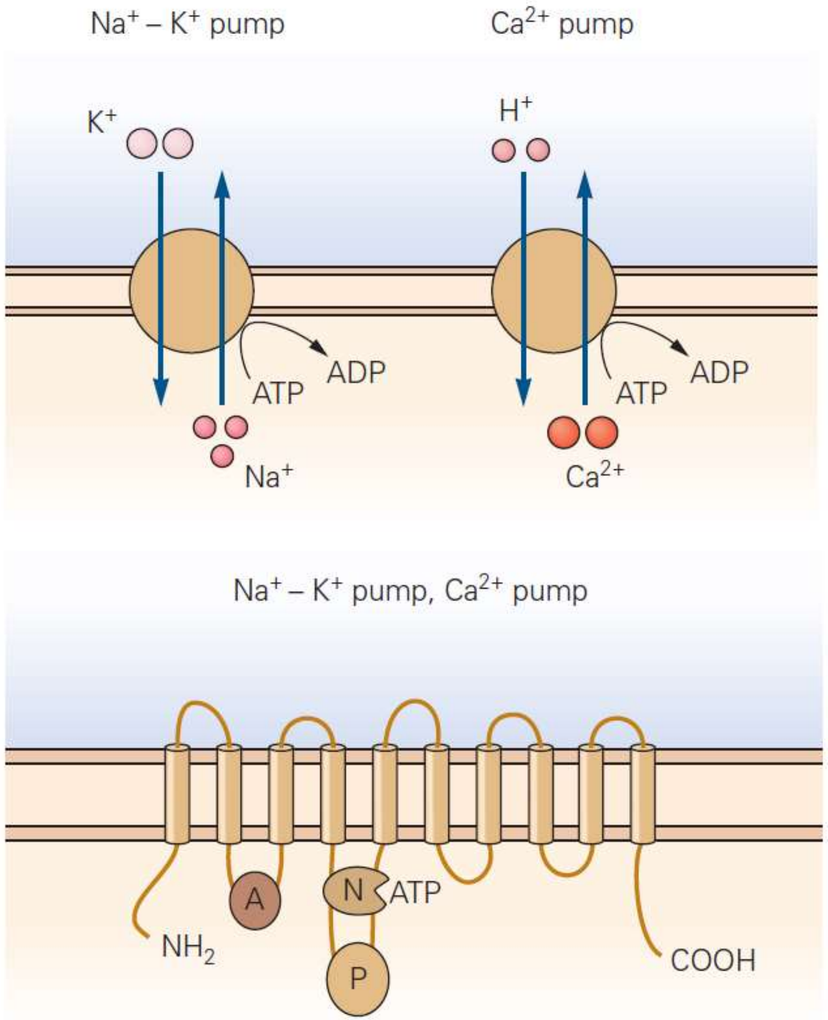
Ketty, Seymour S. (1982).

Ling, Gilbert; Gerard, R. W. (December 1949). Journal of Cellular and Comparative Physiology 34 (3): 383–396.
Ling, G.; Gerard, R. W. (December 1949). Journal of Cellular and Comparative Physiology 34 (3): 397–405.
Ling, G.; Woodbury, J. W. (December 1949). Journal of Cellular and Comparative Physiology 34 (3): 407–412.
Ling, G.; Gerard, R. W. (December 1949). Journal of Cellular and Comparative Physiology 34 (3): 413–438.

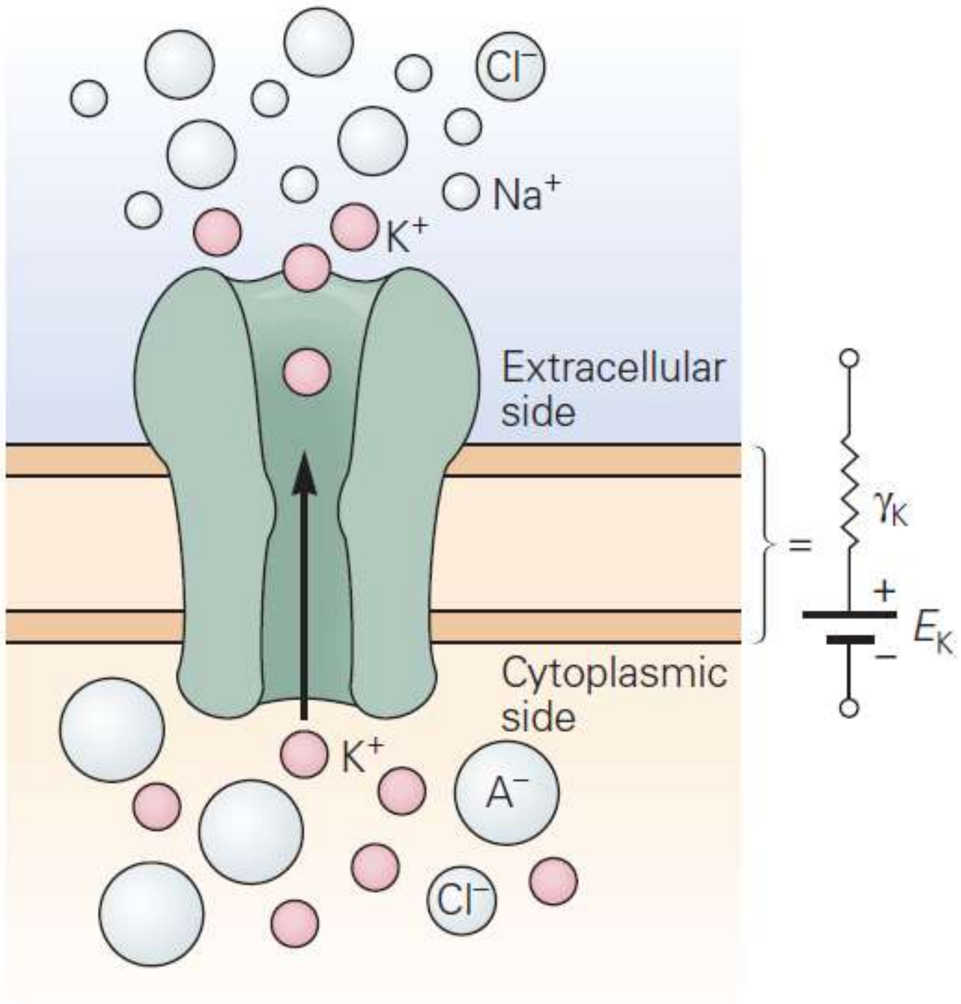
生物电的基础：细胞内外的电势差

生物电的基础：细胞内外的电势差

Ion pumps



Voltage-gated ion channels



生物电的基础：细胞内外的电势差

$$V_m = \frac{RT}{F} \ln \frac{P_K[K^+]_o + P_{Na}[Na^+]_o + P_{Cl}[Cl^-]_i}{P_K[K^+]_i + P_{Na}[Na^+]_i + P_{Cl}[Cl^-]_o}.$$

Goldman Equation

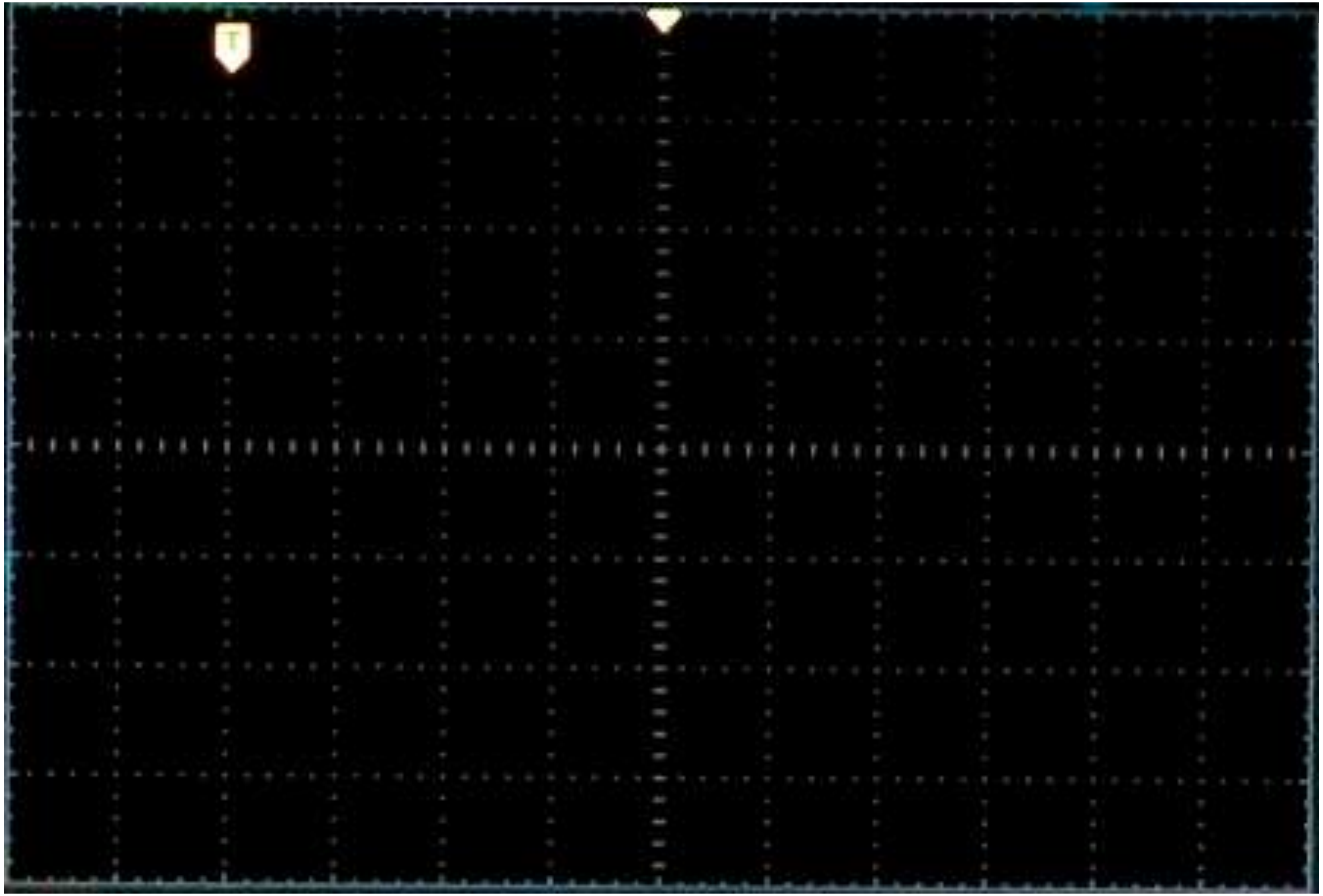
$$V_m \cong \frac{RT}{F} \ln \frac{[K^+]_o}{[K^+]_i}$$

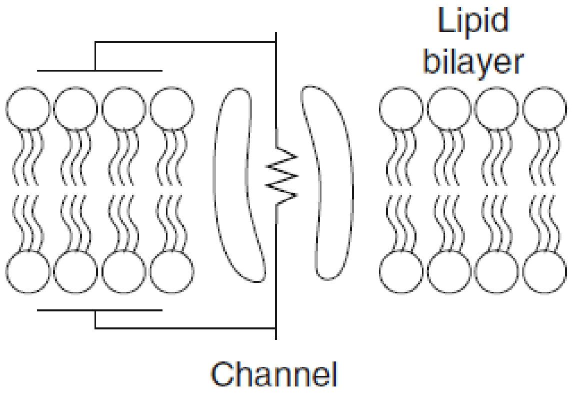
At room temperature (25 °C), RT/F may be treated as a constant and replaced by 25.693 mV for cells.

Driving force

$$i_K = (\gamma_K \times V_m) - (\gamma_K \times E_K) = \gamma_K \times (V_m - E_K).$$

细胞膜的电学特性

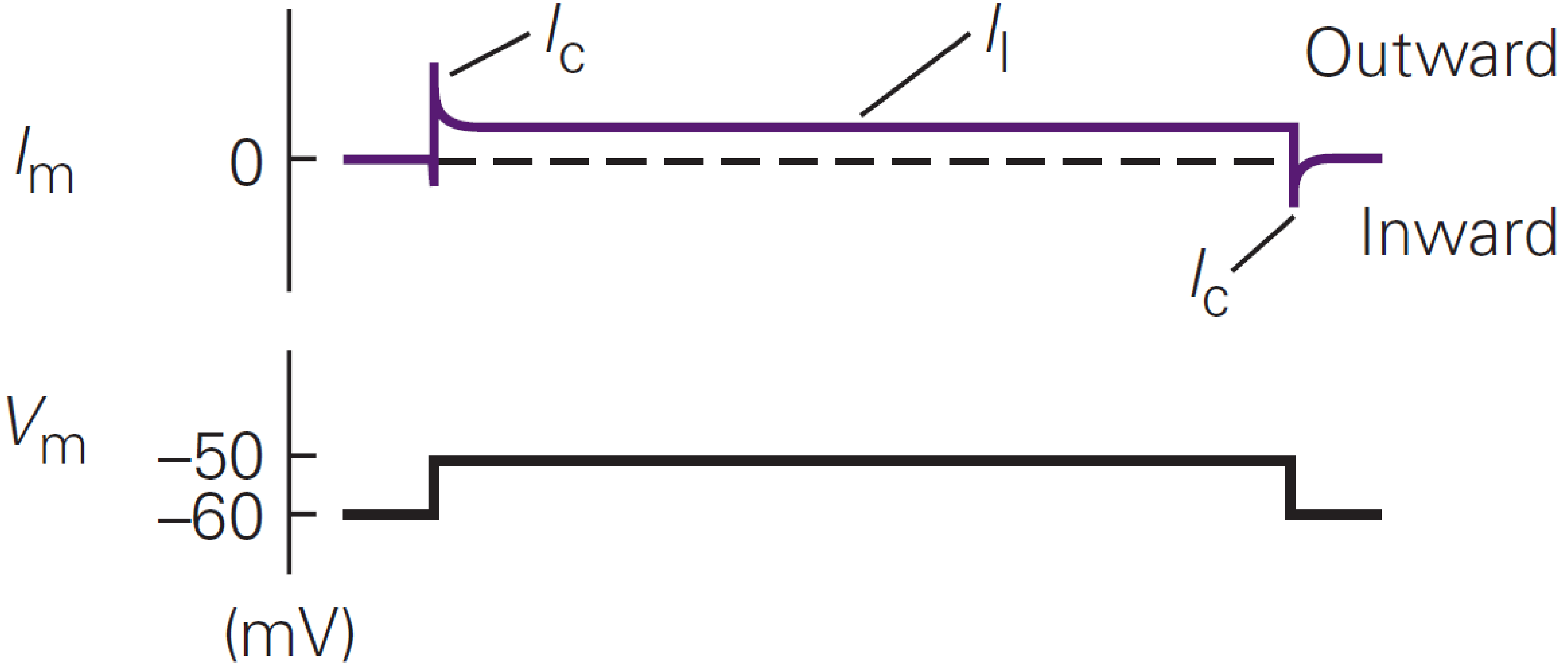




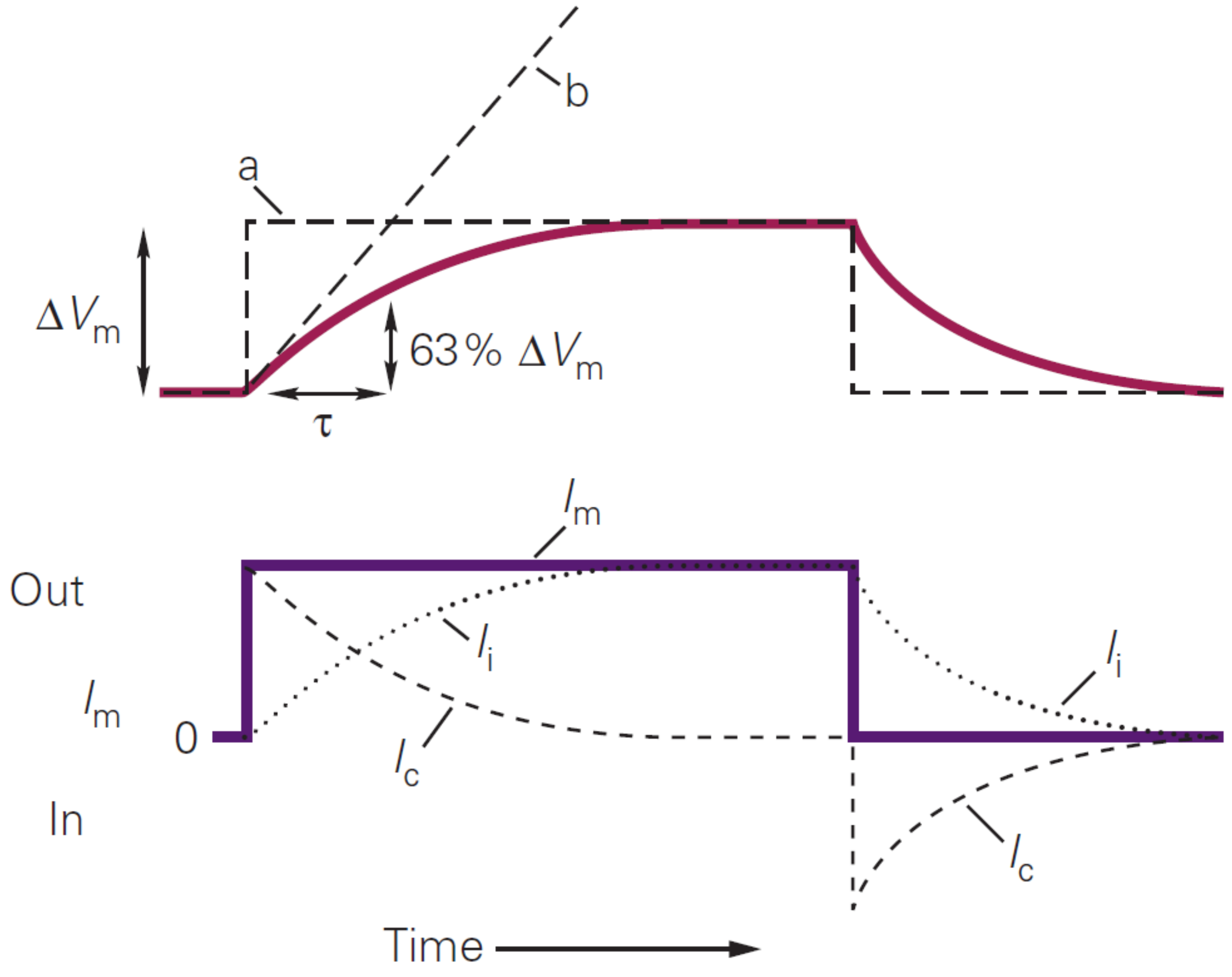
Membrane Conductance

Ion channels
Receptors

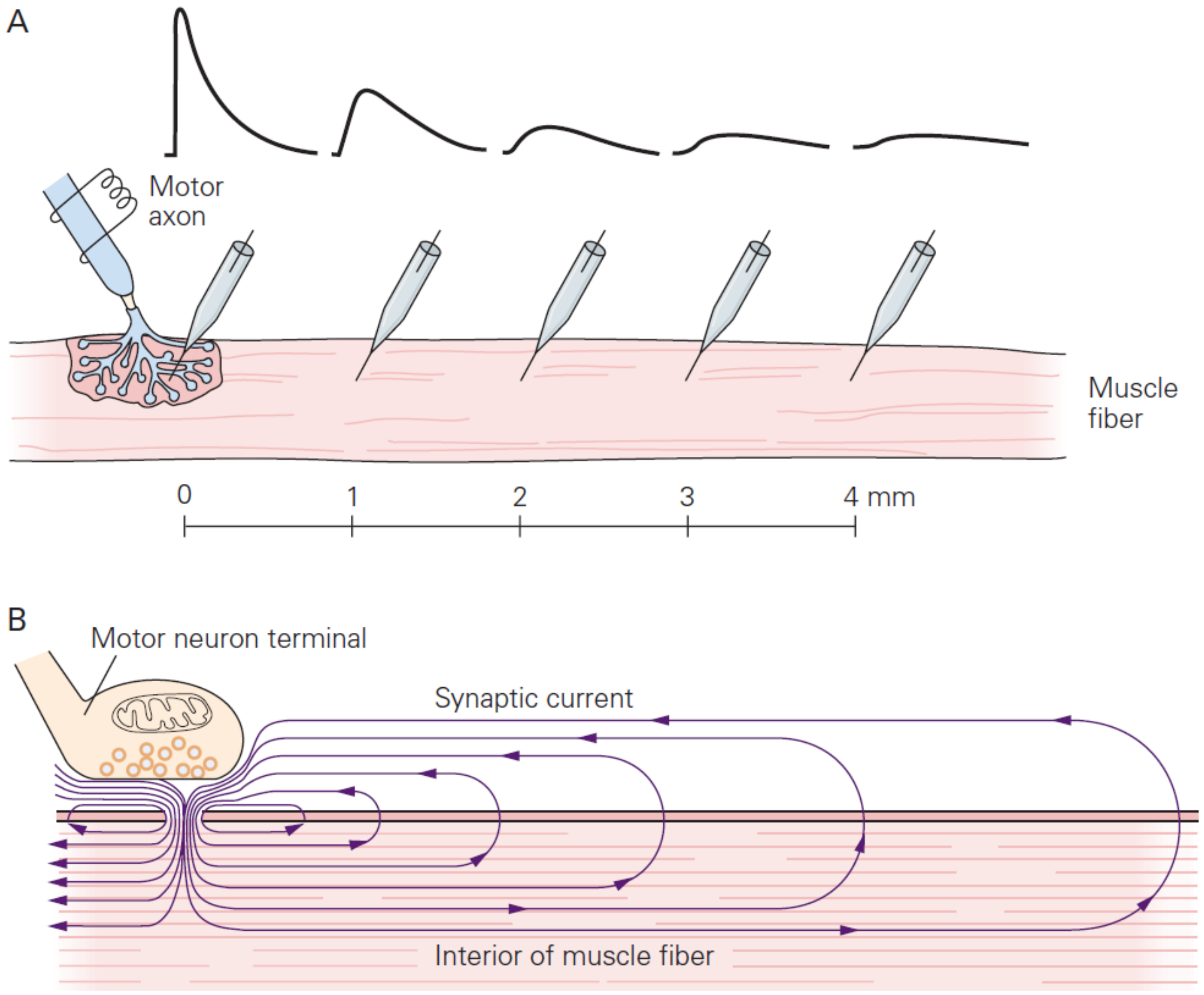
细胞膜的电学特性



细胞膜的电学特性



The end-plate potential (EP) passively propagates



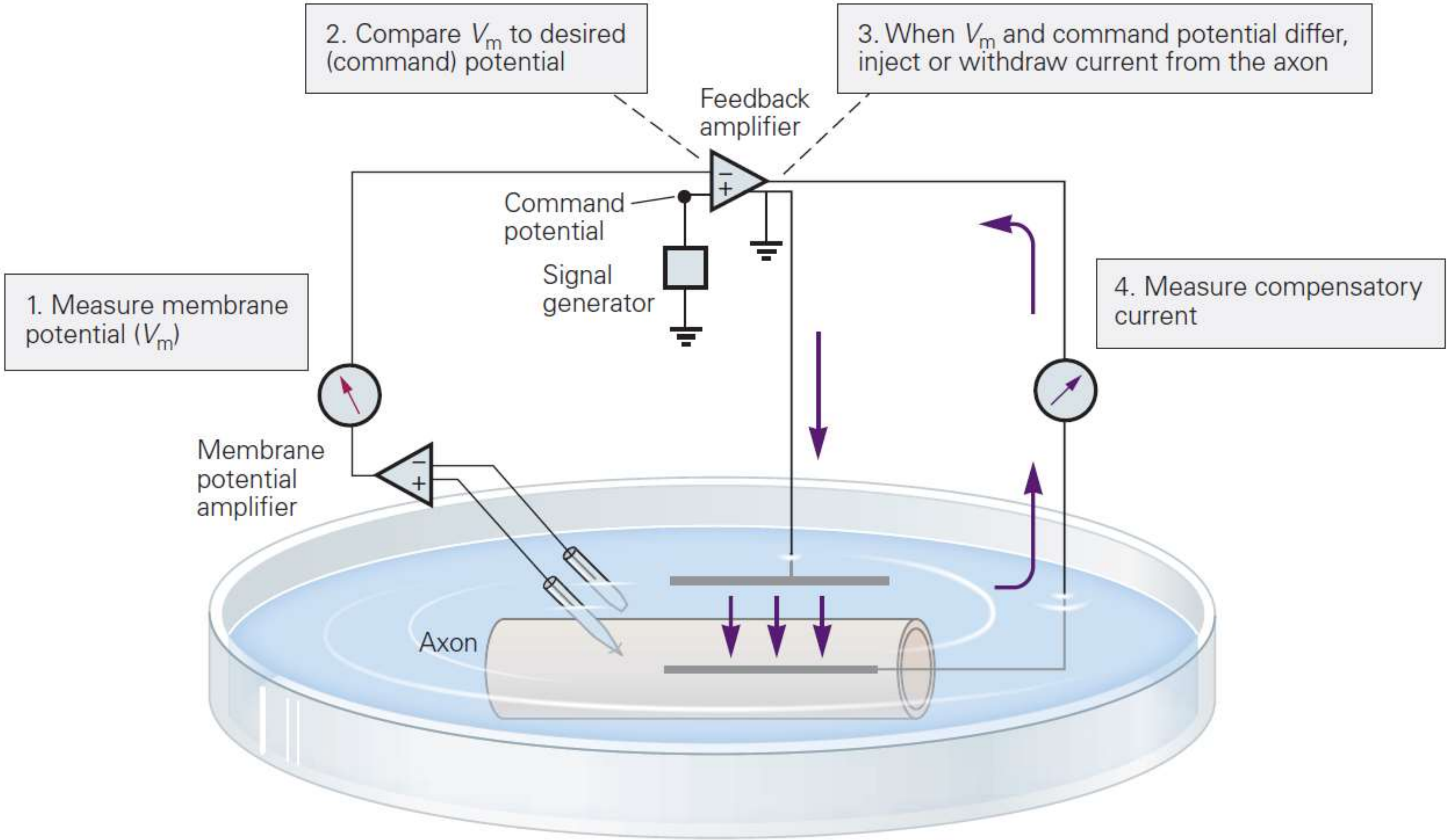
Squid Giant Axon Recording

All or none.
Threshold.

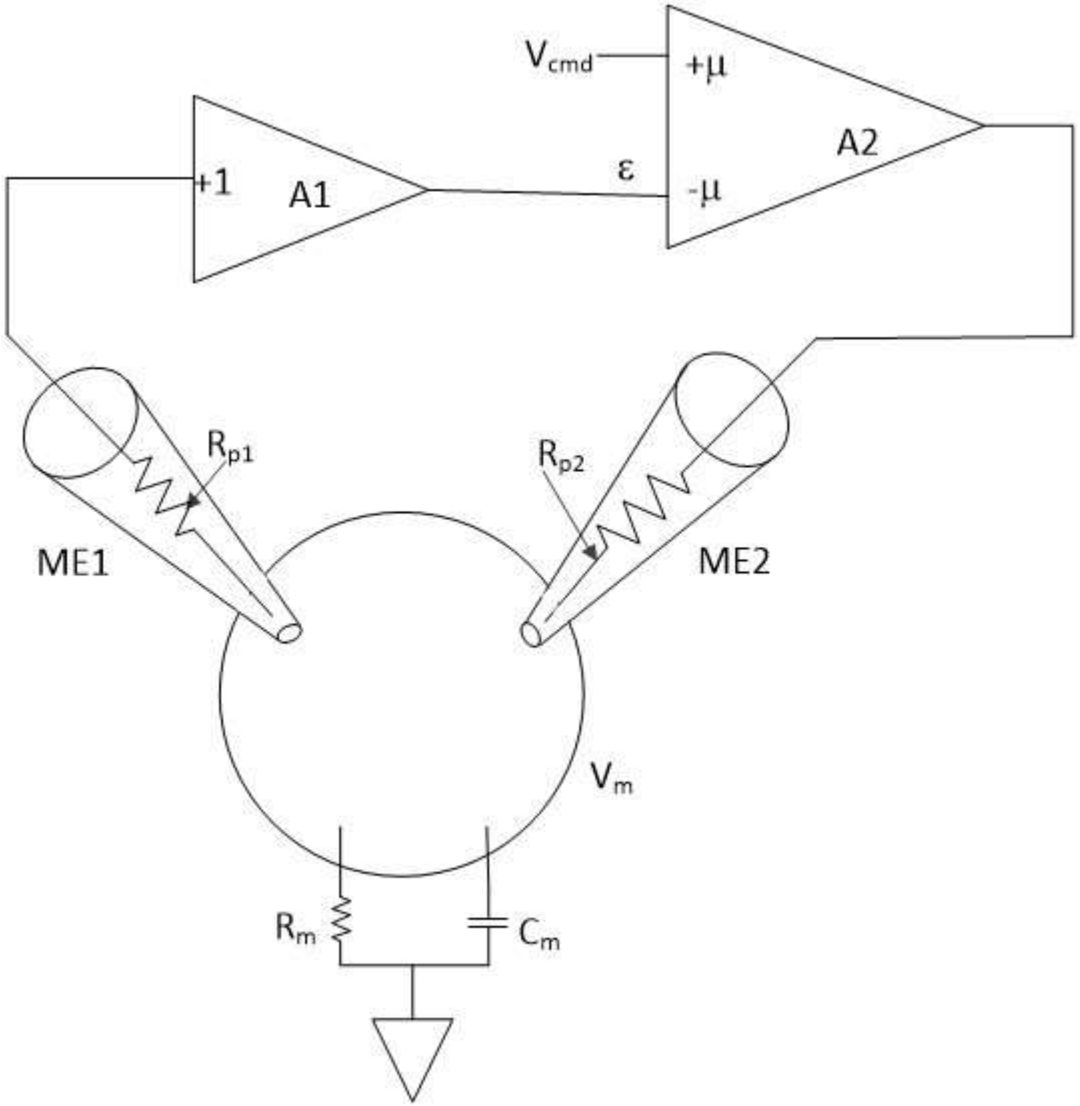
Largest axon
Fasted travel

Simultaneously

Voltage clamp recording

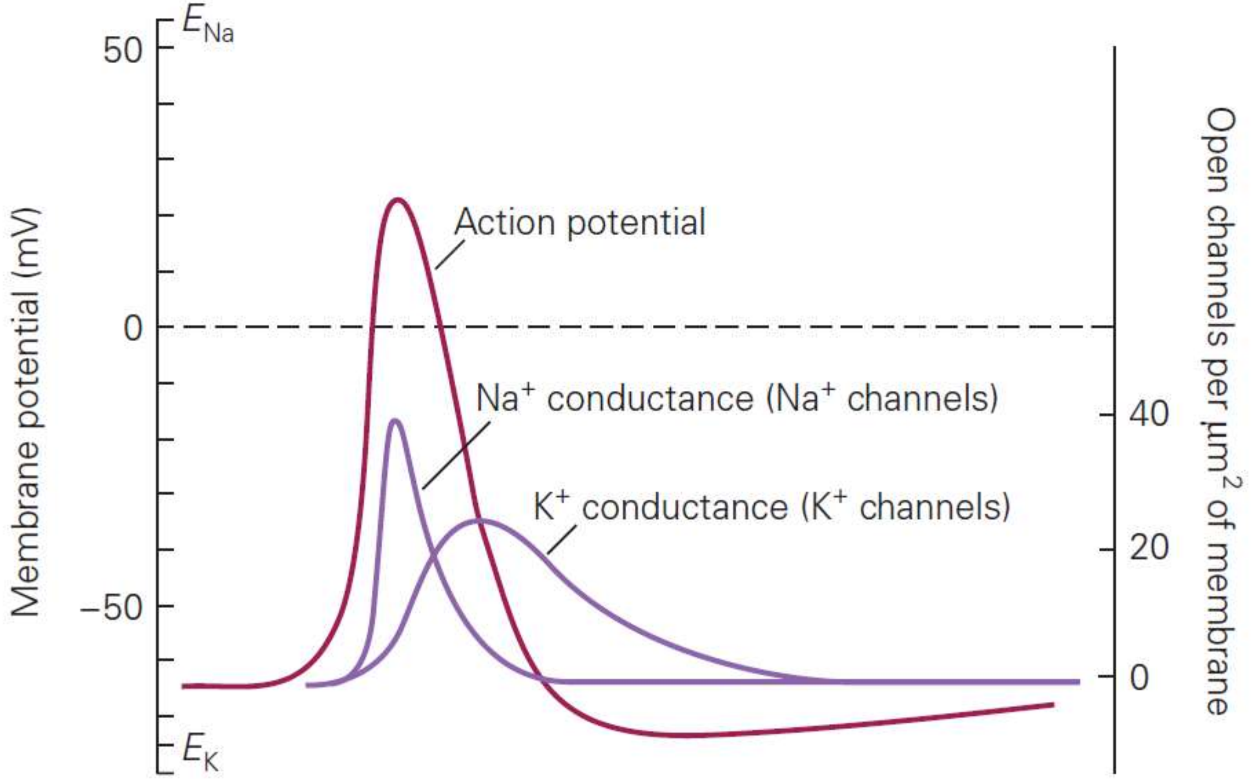


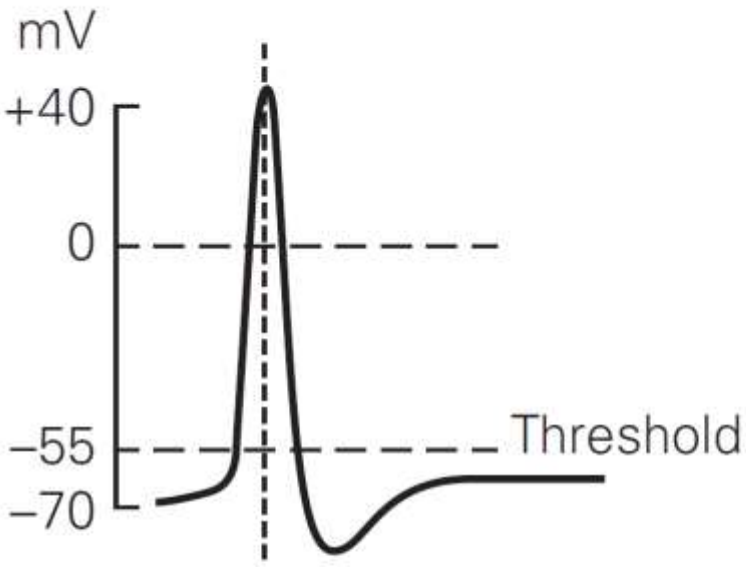
Voltage clamp recording



Voltage clamp recording

Waveform for action potential

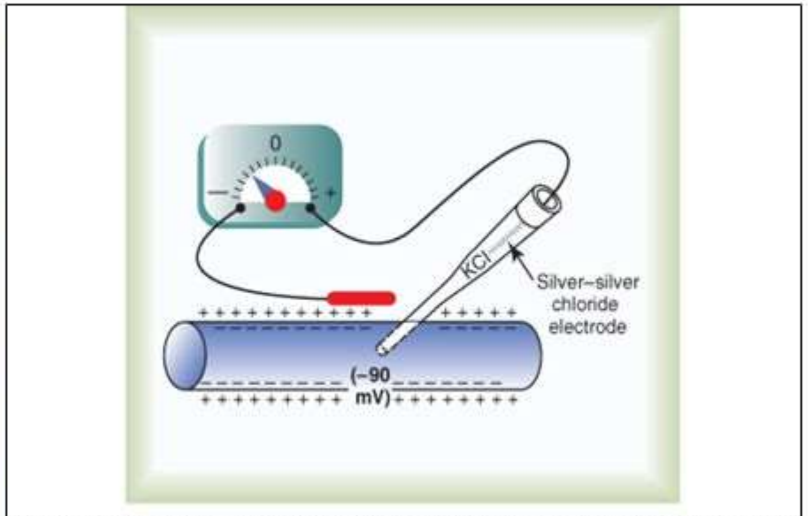




Squid Giant Axon in electrophysiology

Hodgkin-Huxley Expts, 1952

Squid Giant Axon



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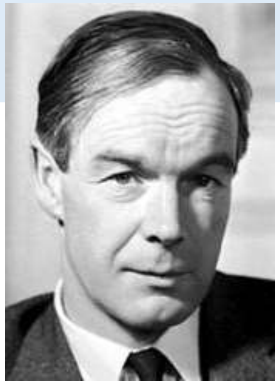
Few neurons, large diameter

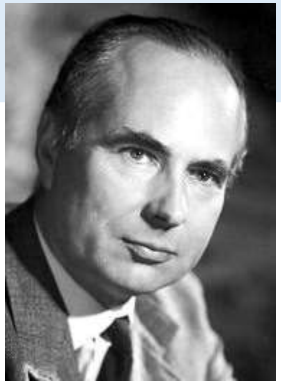
Large enough to insert microelectrodes

Stimulating microelectrodes (inject current) to disturb cell with electrical stimuli

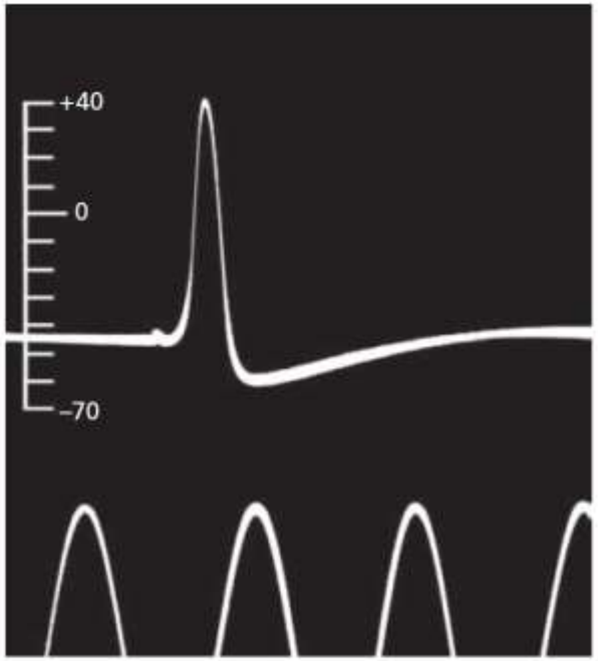
Recording microelectrodes (see current changes in cell and record them)

http://www.science.smith.edu/departments/NeuroSci/courses/bio330/squid.html





Alan Hodgkin Andrew Huxley

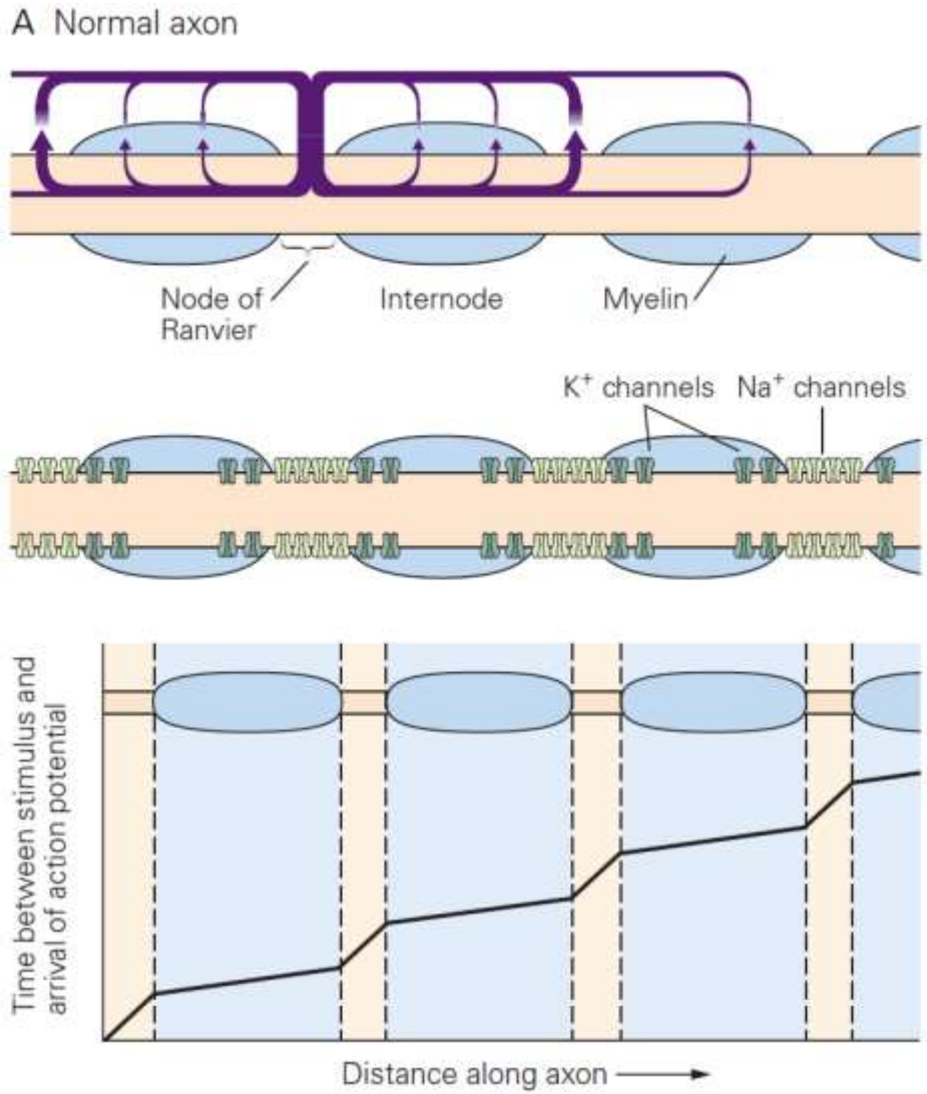


1940s

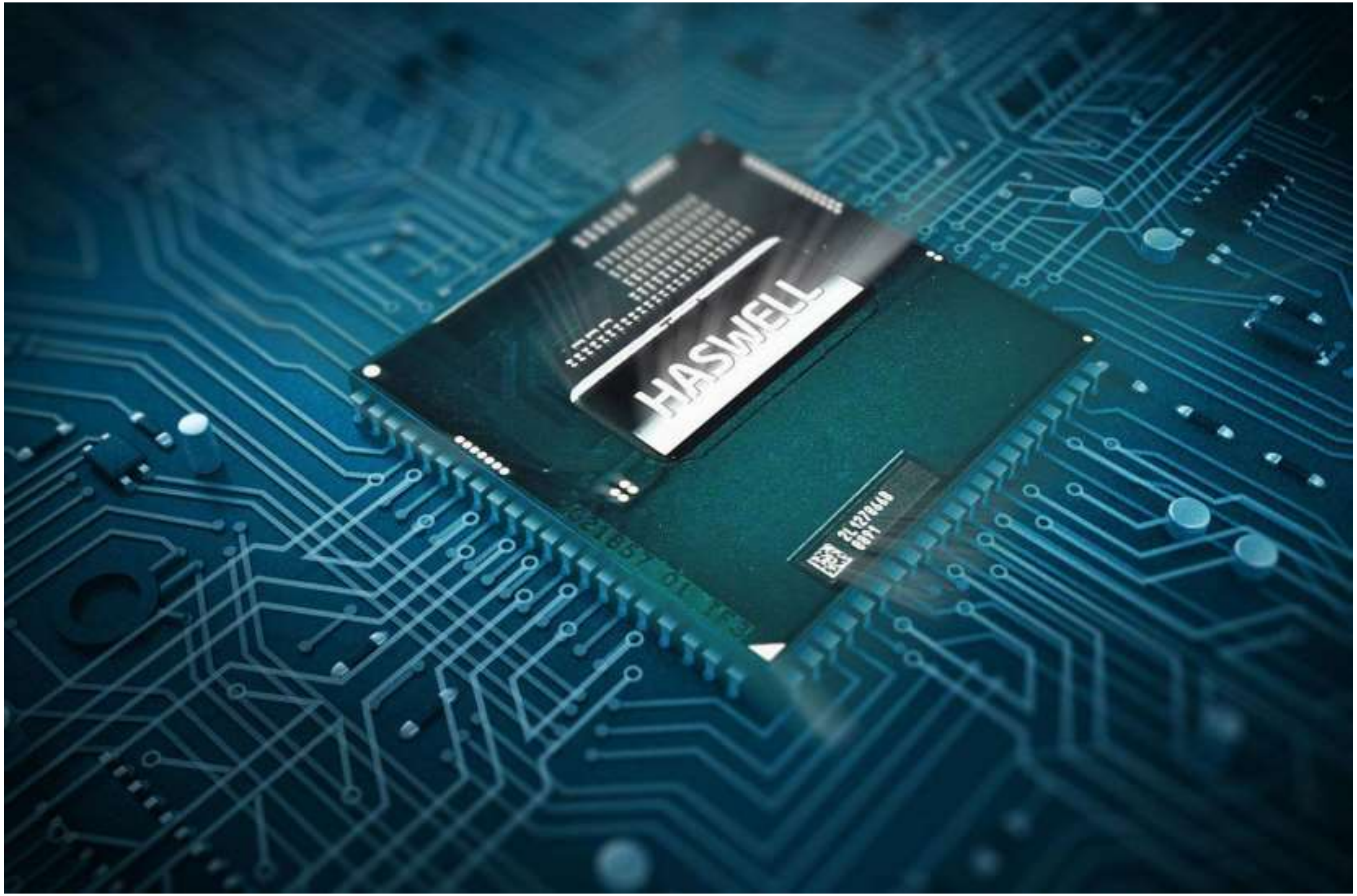
Refractory period of action potentials

Touch is transmitted by electrical signals!

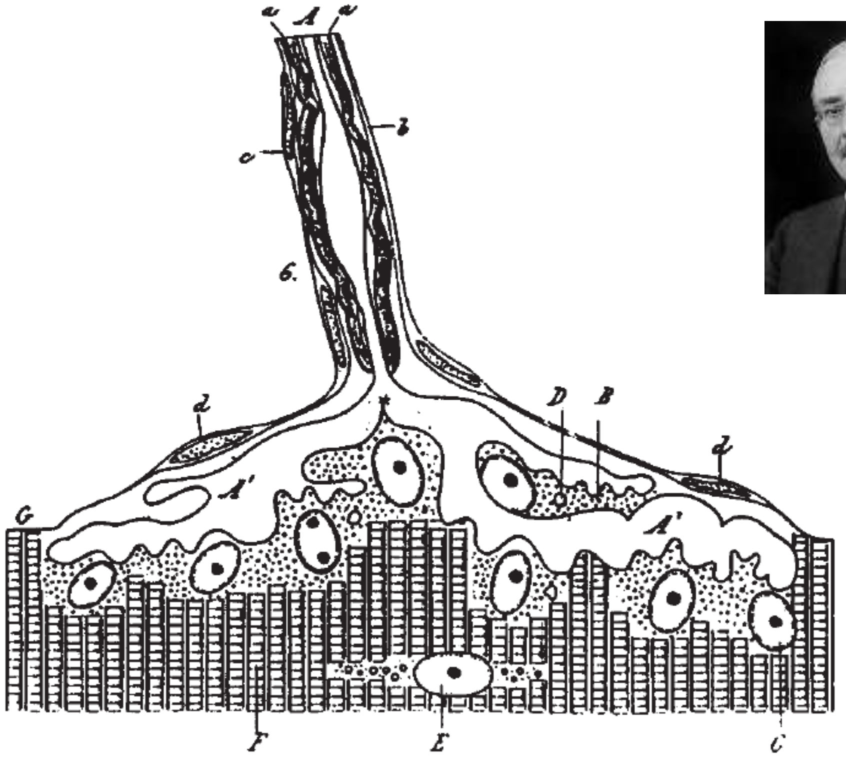
Action potentials are regenerated at the nodes of Ranvier



基因 – 分子 – 神经元 – 神经环路 – 行为



The term Synapse by Sherrington in 1897



Schematic summary view of the mammalian neuromuscular junction.

'So far as our present knowledge goes, we are led to think that the tip of a twig of the arborescence is not continuous with but merely in contact with the substance of the dendrite or cell body on which it impinges. Such a special connection of one nerve cell with another might be called a synapse.'

Sherrington, C.S. (1897) in Textbook of Physiology (Foster, M., ed.), p. 60

While Ramóny Cajal was laying the anatomical basis for modern neuroscience, Sherrington's work was laying the basis for the physiological principles

The debate for synaptic transmission in CNS

One of the most important experiments in neurophysiology in the twentieth century took place in the physiology laboratories at the University of Otago, New Zealand, in August 1951.

In the Nobel lecture Dale speculated on the possibility of neurochemical transmission in the CNS. He cited the known reservoirs of acetylcholine in the basal ganglia and other brain structures. He tentatively proposed "I take the view however that we need a much larger array of well authenticated facts before we can theorise."

Photograph taken at University of Otago, 1952, just before John Eccles left for Oxford to deliver the Waynflete lectures. Front row, Rosalind Eccles, Jack Coombs, Wilfred Rall, John Eccles, Lawrence Brock, Bronwen Broomfield

The scientific history for chemical synaptic transmission

Emil DuBois-Reymond, 1877

Thomas Elliott

1904

Impulses by adrenaline

Walter Dixon

1907

Muscarine-like substance

Henry Dale

1914

Adrenaline and acetylcholine

Otto Loewi

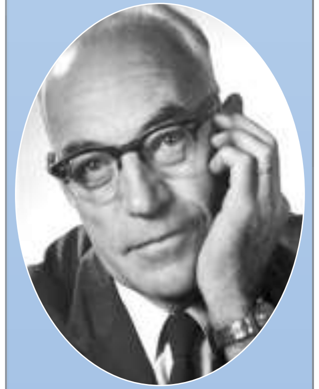
1921

Chemical transmission

Te-Pei Feng

1940

End-plate potential



Bernard Katz

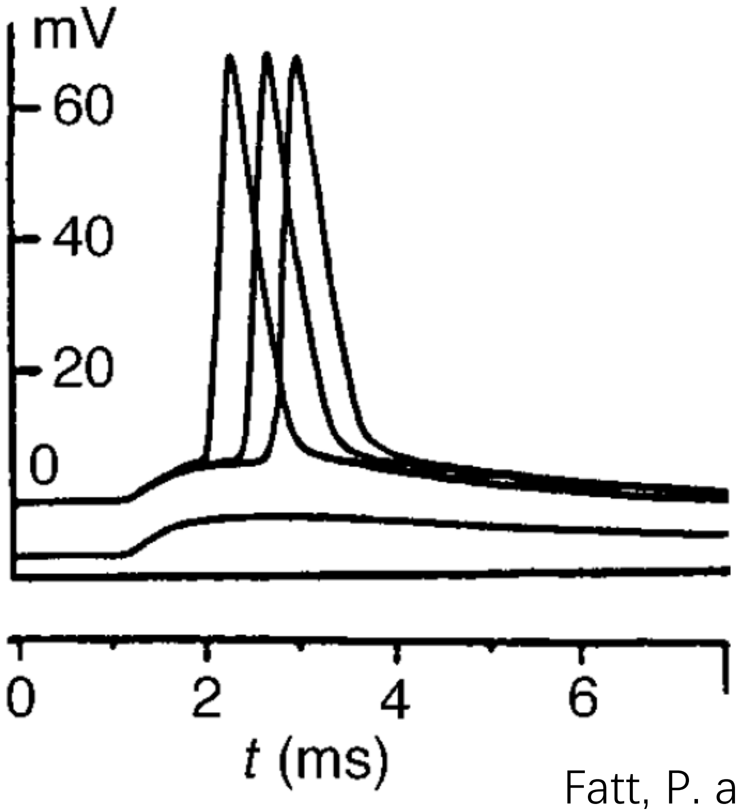
1940s

End-plate potential

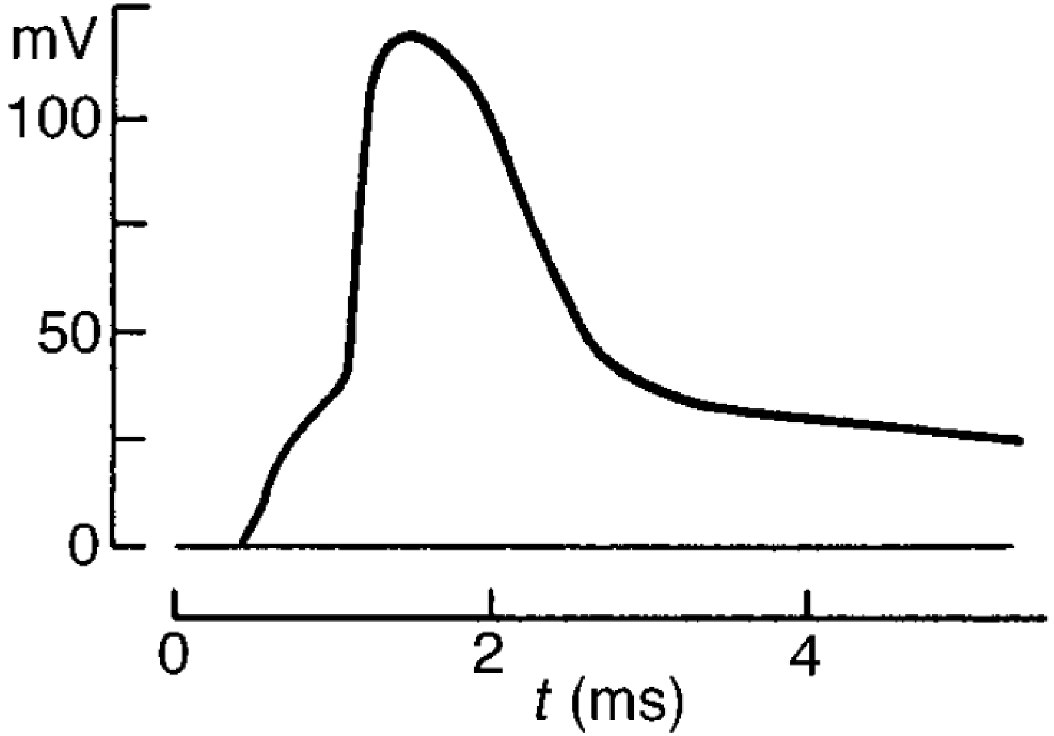
"Either there exists at the boundary of the contractile substance a stimulatory secretion . . . or the phenomenon is electrical in nature." (Reymond, 1877)

One of the key evidences for chemical synaptic transmission

Presyanptic impulse potential



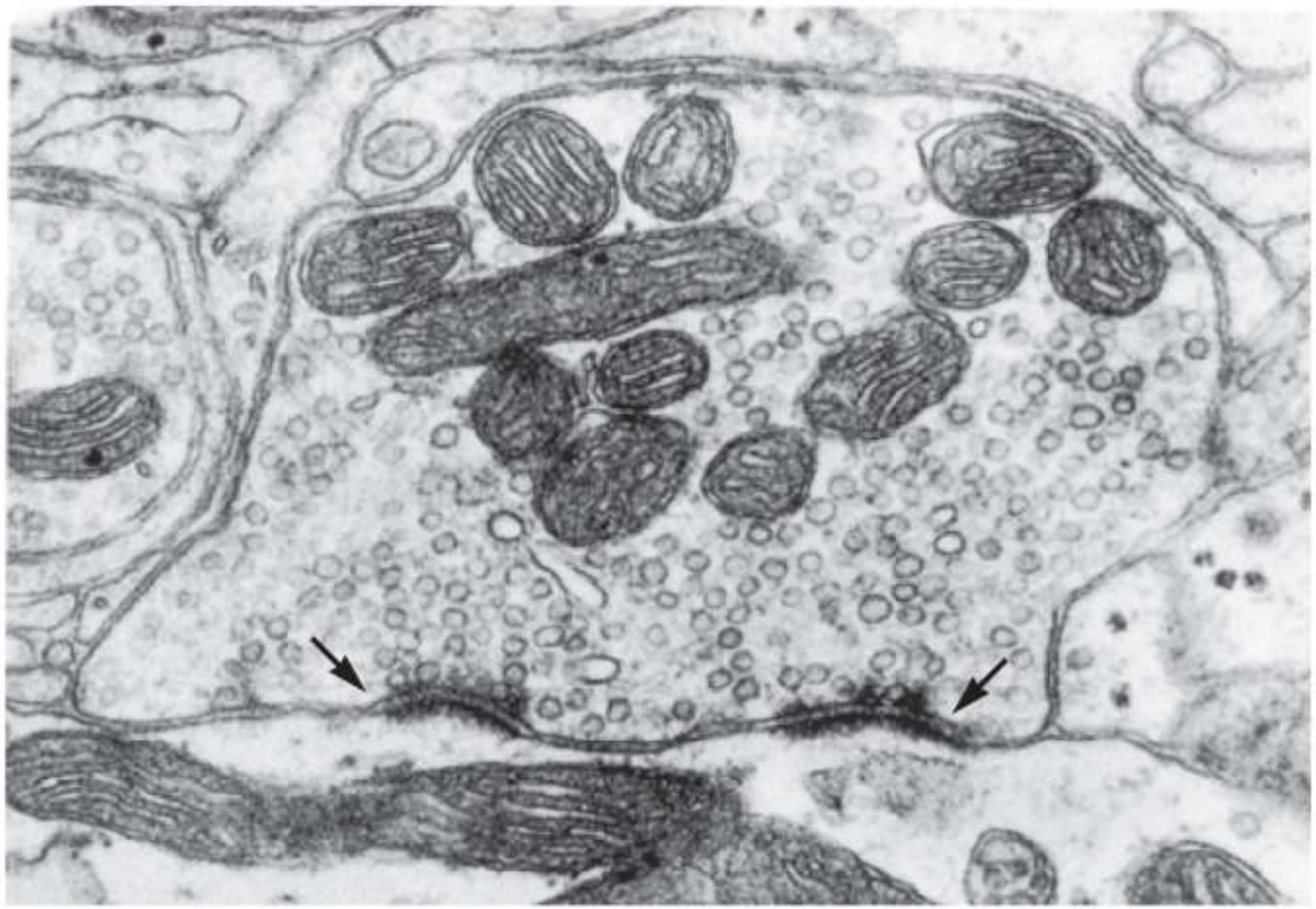
End-plate potential



Fatt, P. and Katz, B. (1950) J. Physiol. 111, 46P–47P

Impulse potential is much smaller than **End-plate potential**

The fine structure of a presynaptic terminal of Chemical Synapse.



early 1950s

Another key evidences for chemical synaptic transmission by EM technique

Quantal release in synaptic transmission

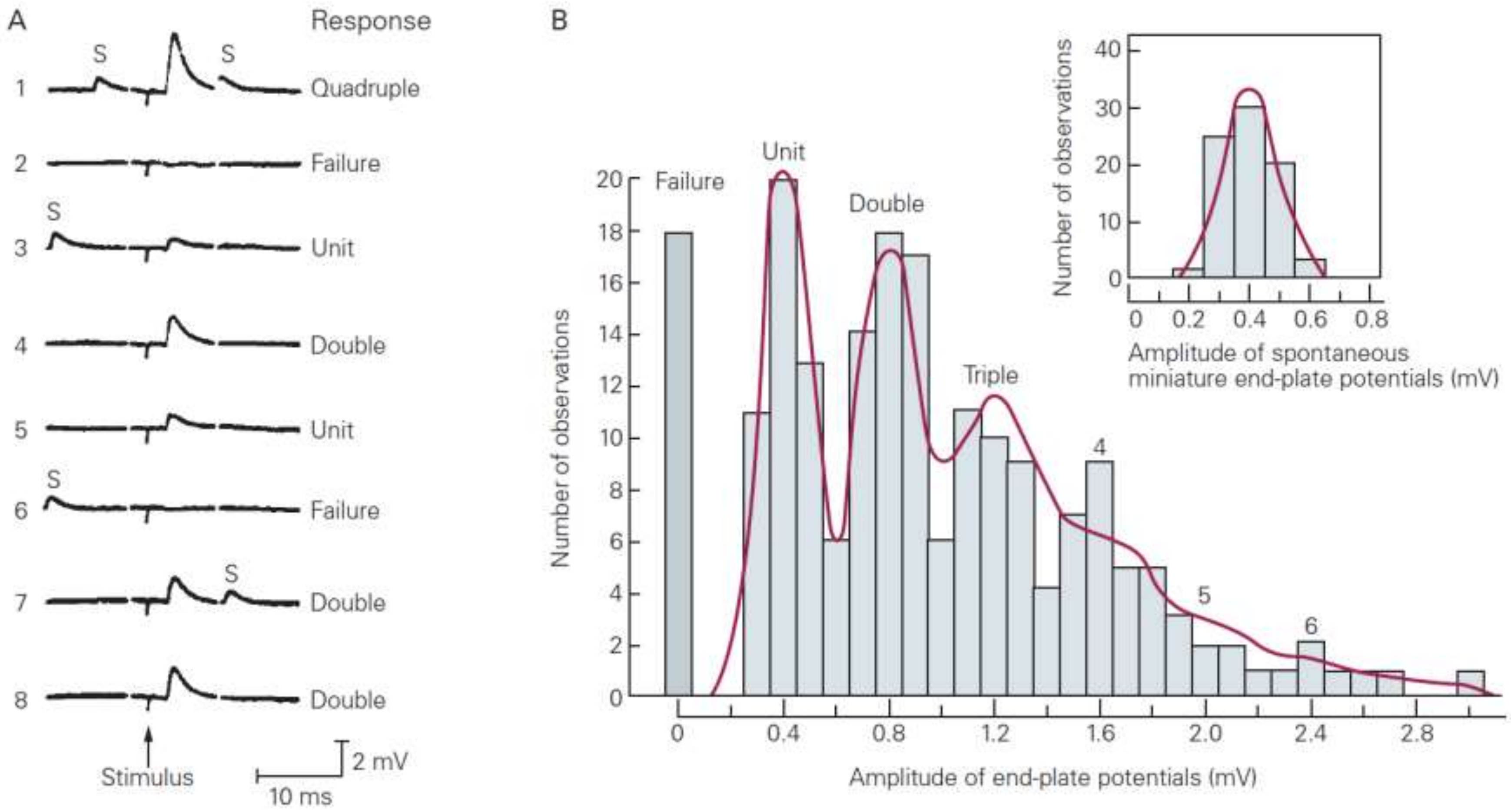
Bernard Katz

For his work in synapse, he shared Nobel prize with Julius Axelrod and Ulf von Euler

Quantal release in synaptic transmission

Bernard Katz

For his work in synapse, he shared Nobel prize with Julius Axelrod and Ulf von Euler

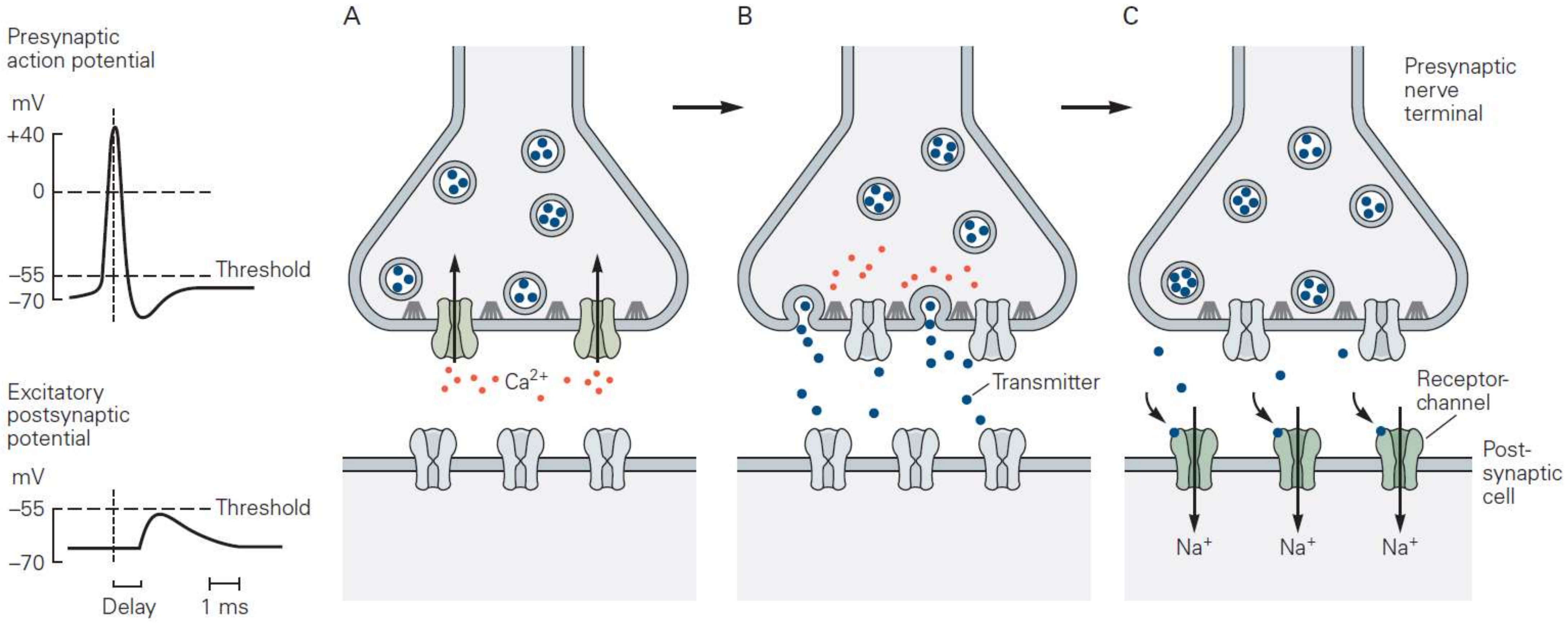


P. Fatt and B. Katz, J. Physiol. 117, 109 (1952).

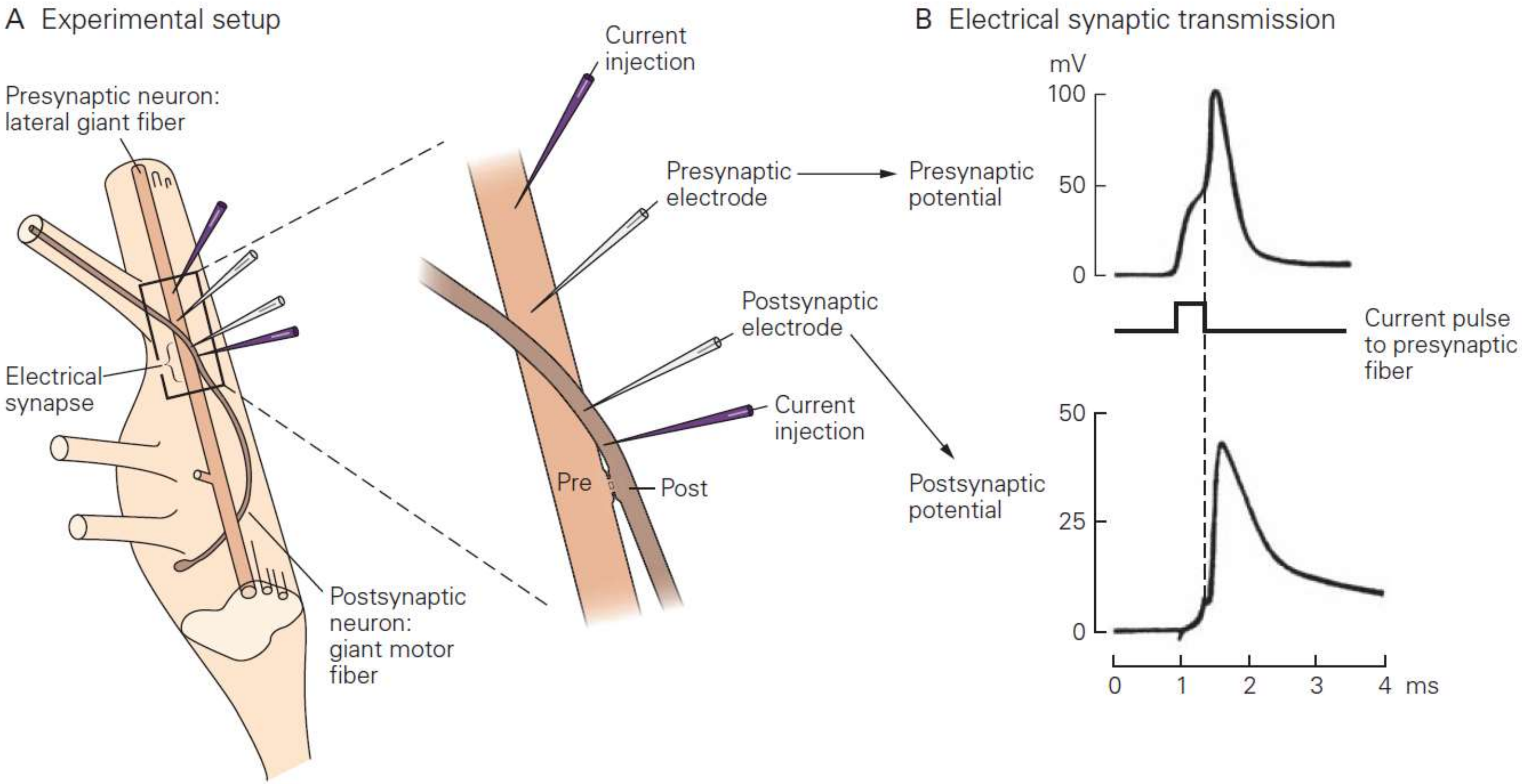
Each vesicle stores one quantum of transmitter

Coming to CNS

Synaptic transmission at chemical synapses

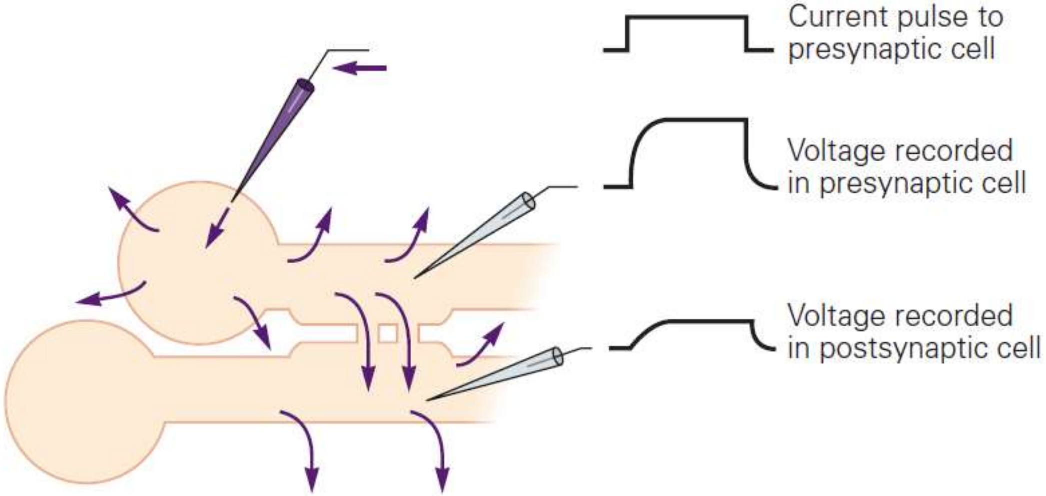


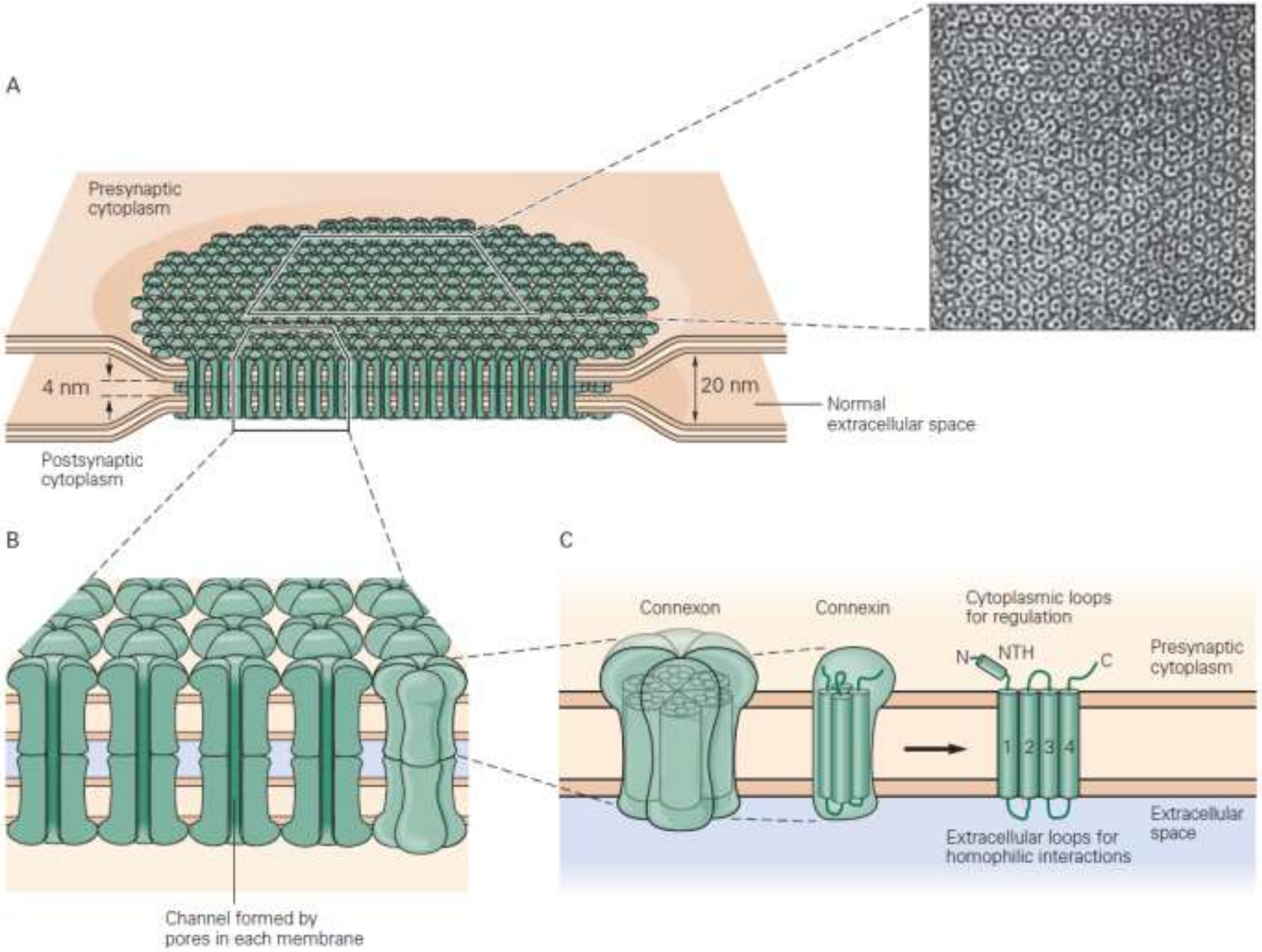
Electrical synaptic transmission



Furshpan and Potter 1957 and 1959

Electrical synaptic transmission

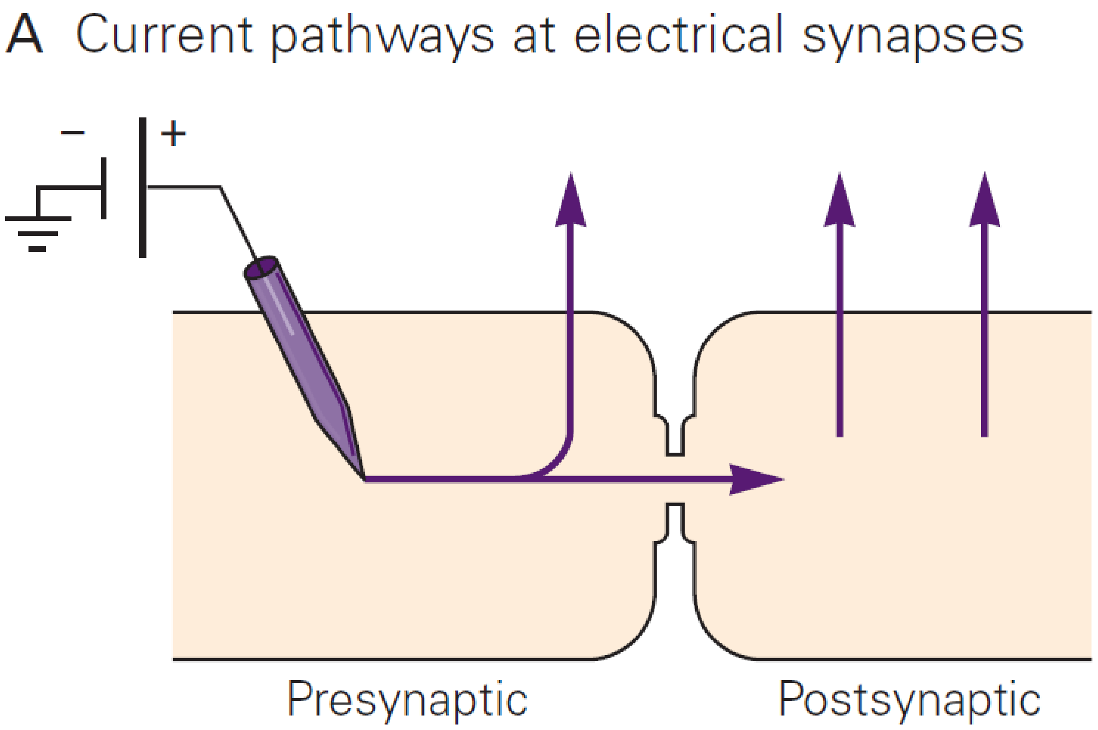




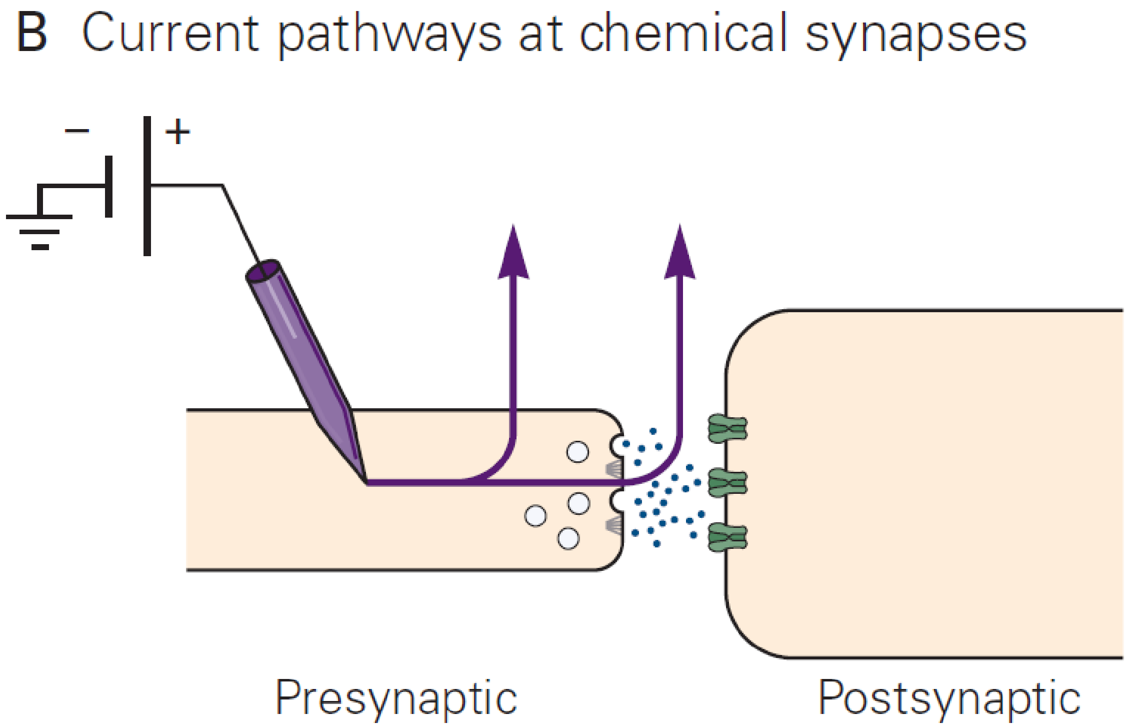
Makowski et al. 1977

Unwin and Zampighi 1980

Neurons communicate through Synapses



John Eccles



Dale and others

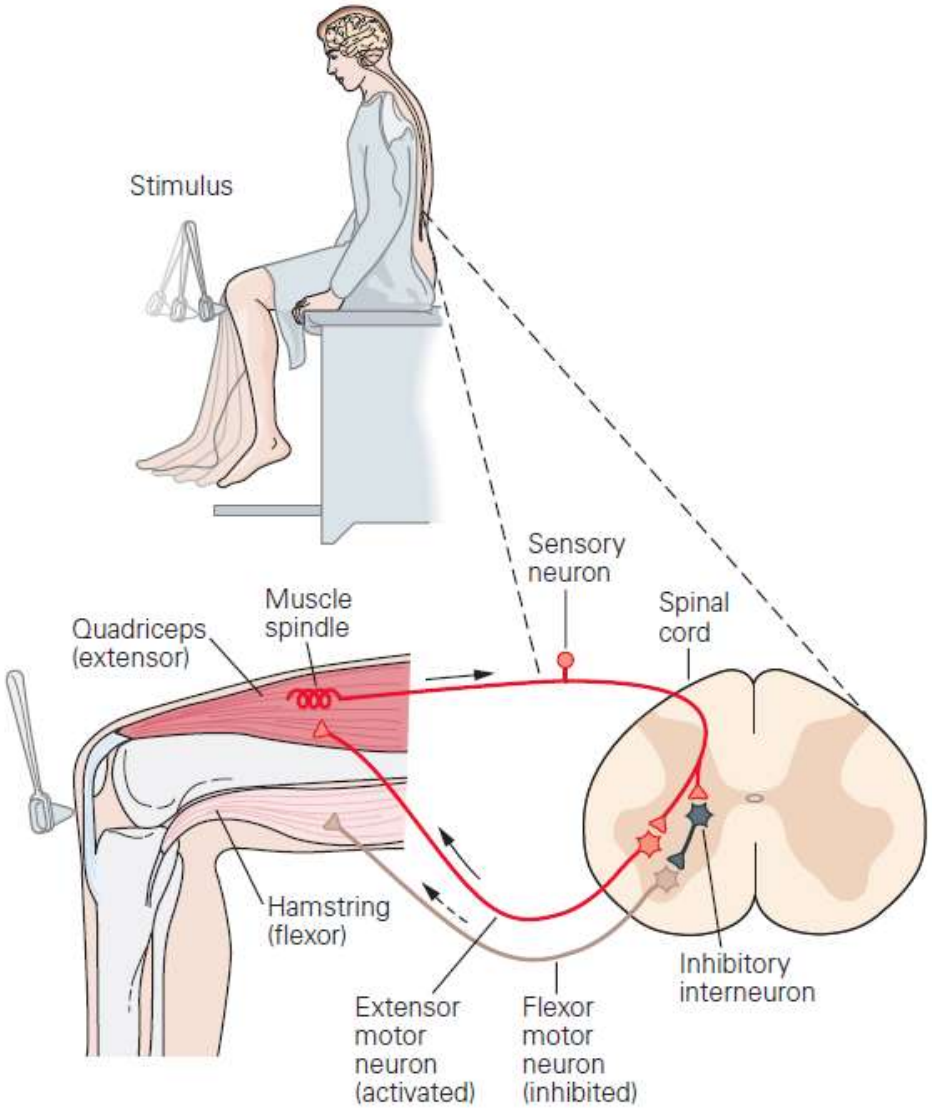
Compare electric and chemical synapse

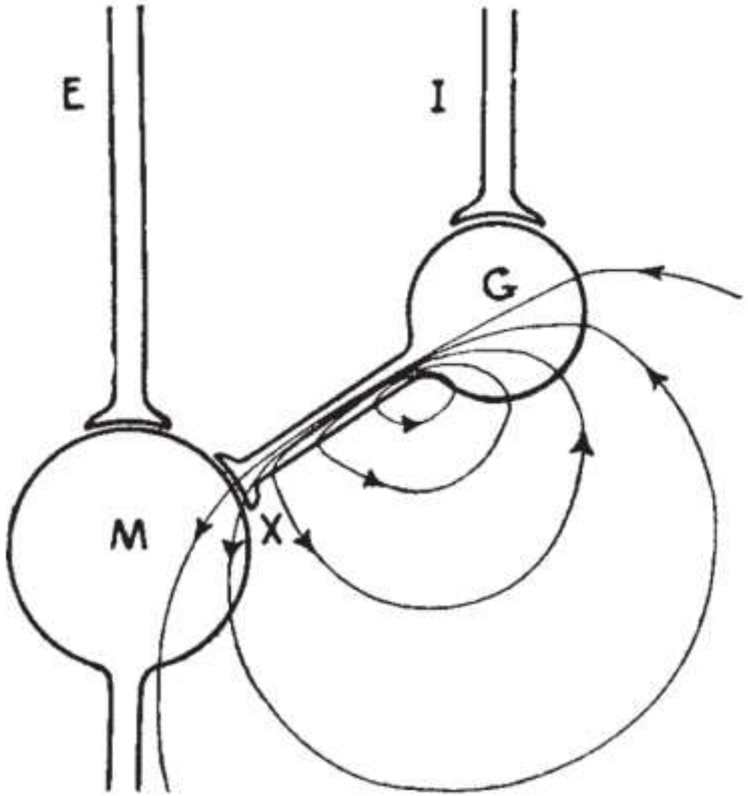
	Electric synapse	Chemical synapse
speed		

A problem for electrical synapse

- How is inhibition achieved by electrical synapses?

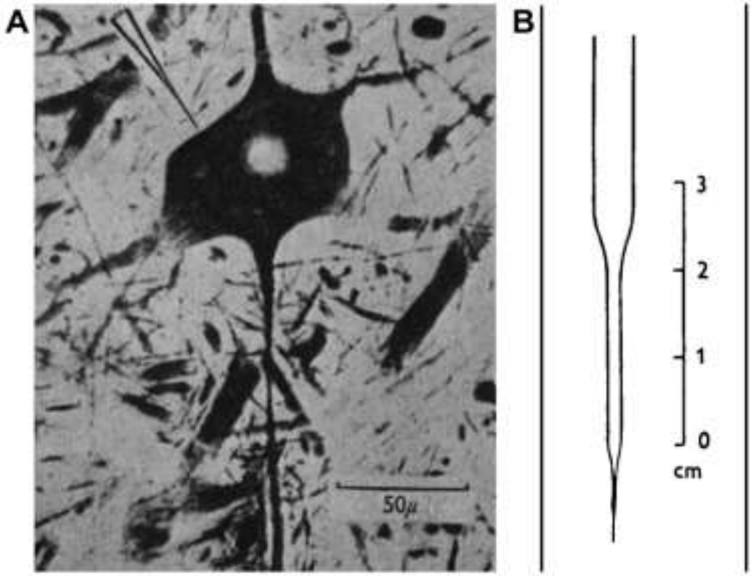
Golgi-cell hypothesis

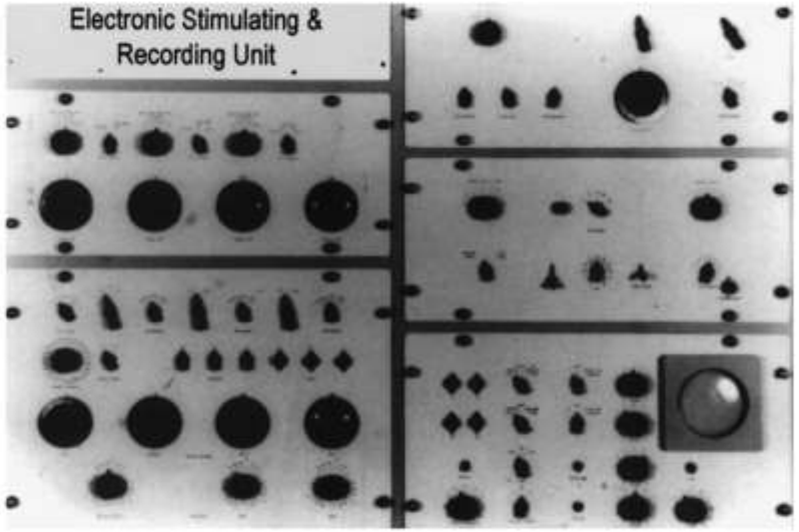


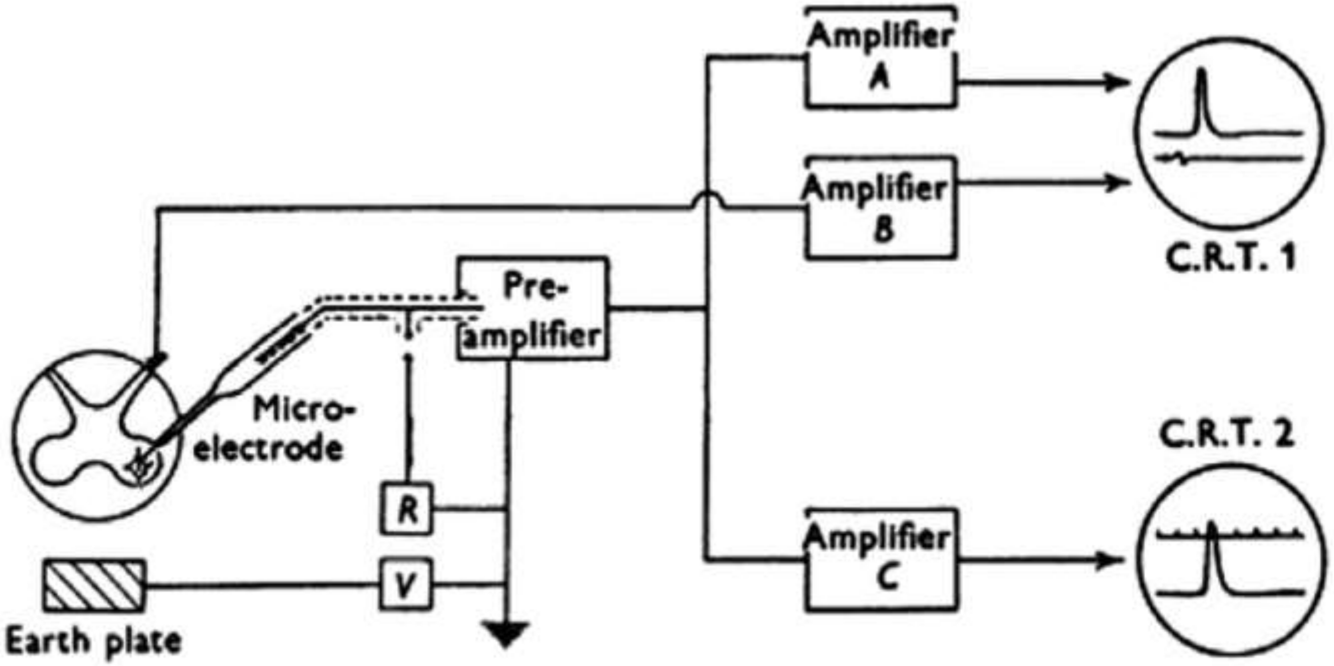


Golgi-cell hypothesis (Brooks & Eccles, 1947)

The crucial experiments by the team of Eccles

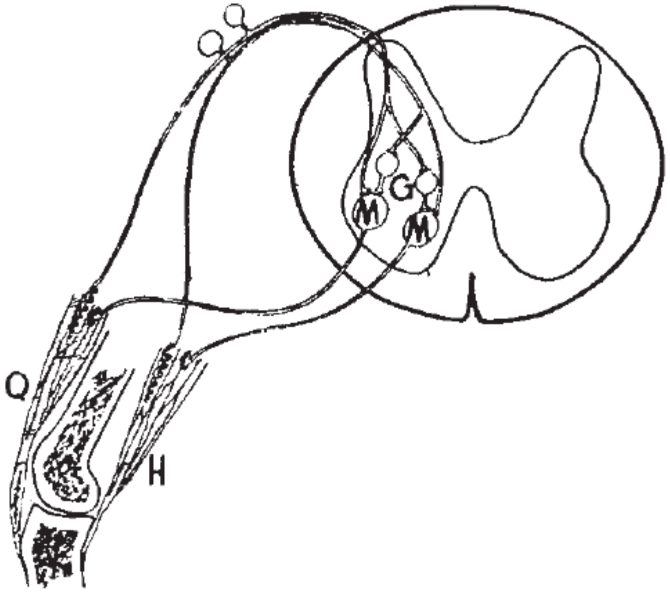


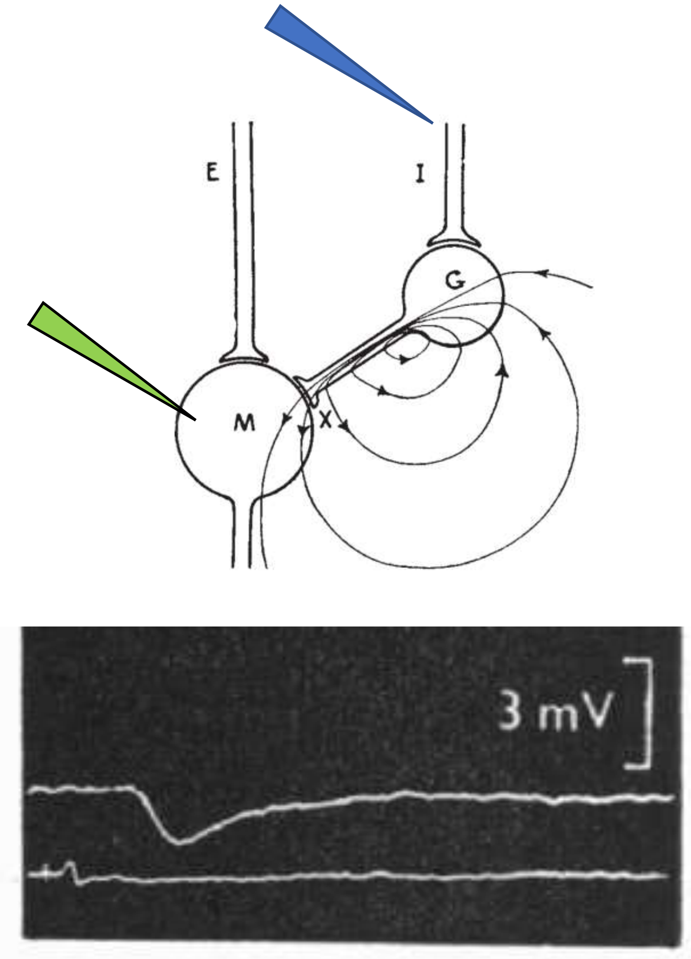


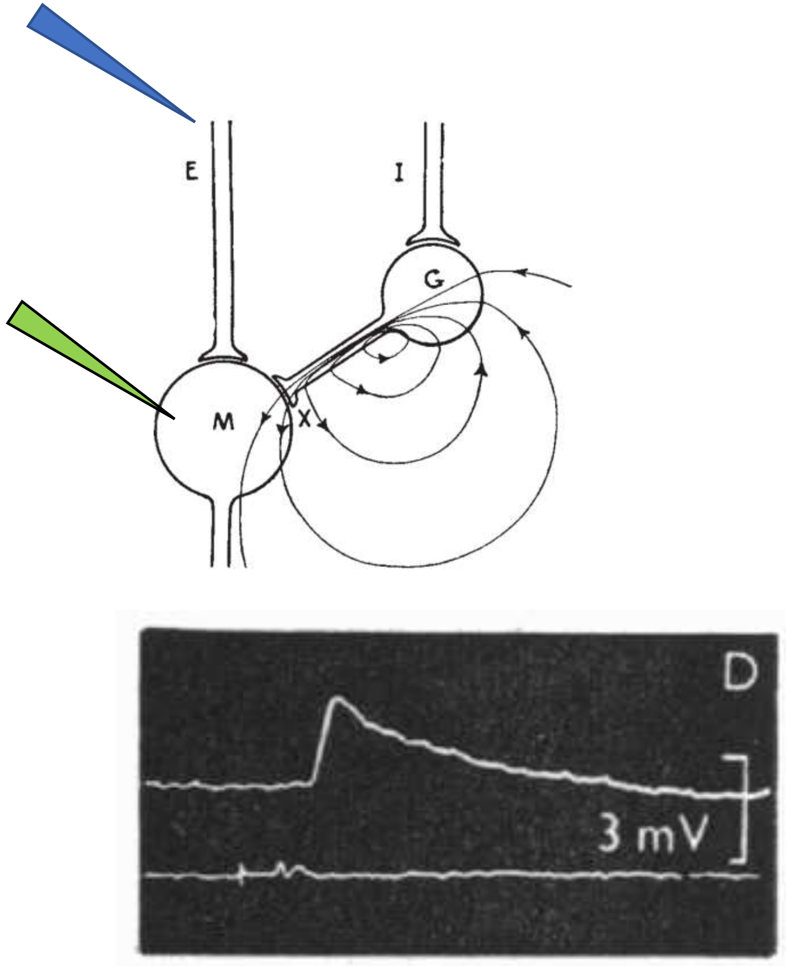


Microelectrode and spinal cord motor neuron. (From Brock LG, Coombs JS, Eccles JC. The recording of potentials from motor-neurones with an intracellular electrode. J Physiol 1952:117:431–60.)

Golgi-cell hypothesis is falsified

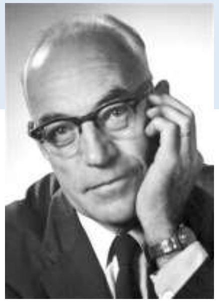






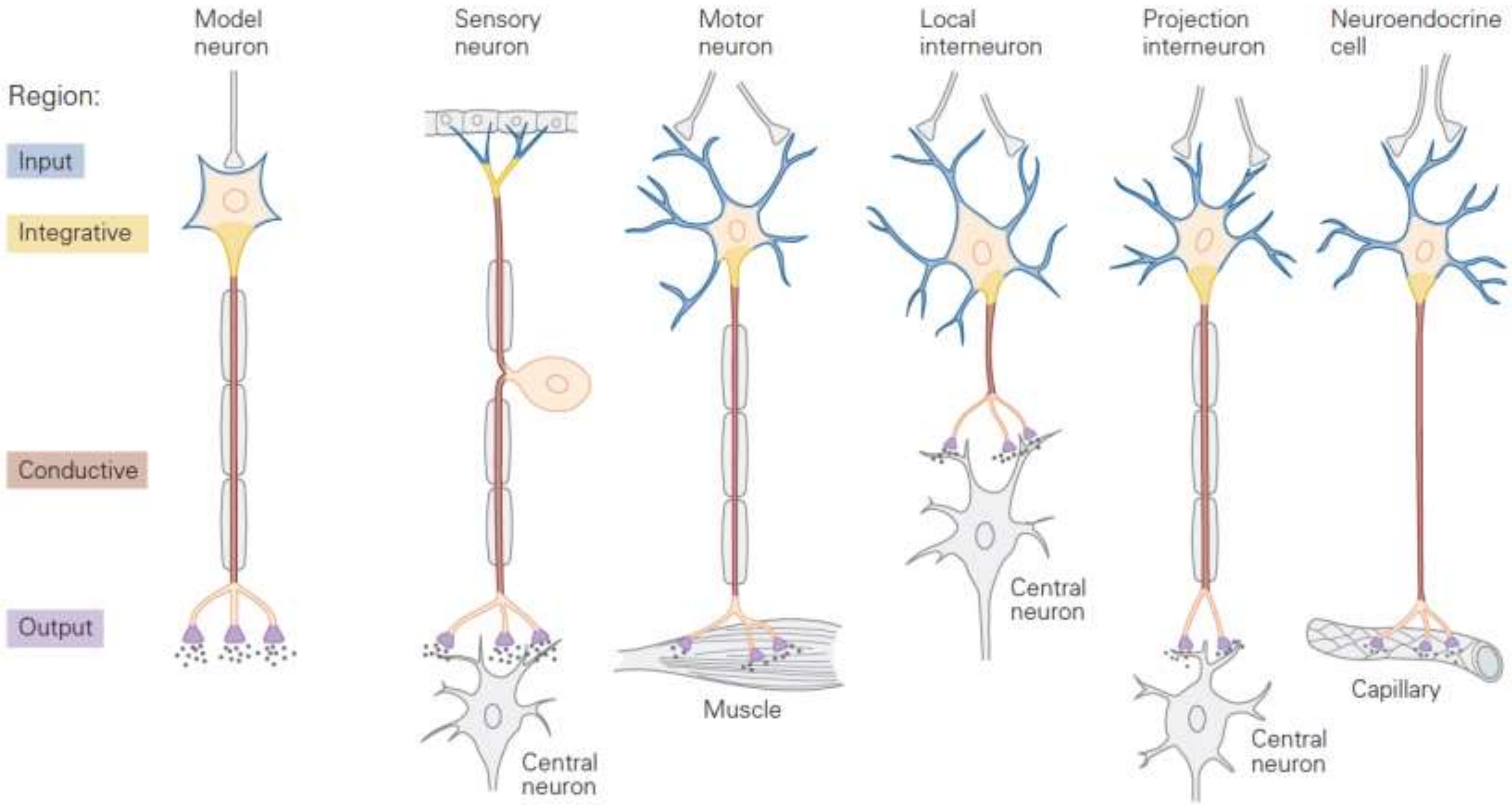
J Physiol 1952:117:431–60

The credit to falsify oneself



- Eccles and Dale had a long association which began as adversaries, sometimes expressed in tense exchanges. The warmth of their personal relationship and mutual respect is clearly evident in their correspondence. In the closing address at the 1975 Sir Henry Dale Centennial Symposium in Cambridge Eccles remarked, ''It was a great privilege to have been so closely associated with him in those great creative years, first as a sparring partner in opposition and then as a convert. Such great men are infinitely precious in our lives and in our culture."
- This remarkable experiment demonstrated the tenacity of John Eccles to pursue scientific research even if the outcome could falsify his previously strongly held views. This important chapter in the history of neuroscience demonstrates the collaboration of scientists in different disciplines, establishing the mechanism by which neurons communicate in the CNS.

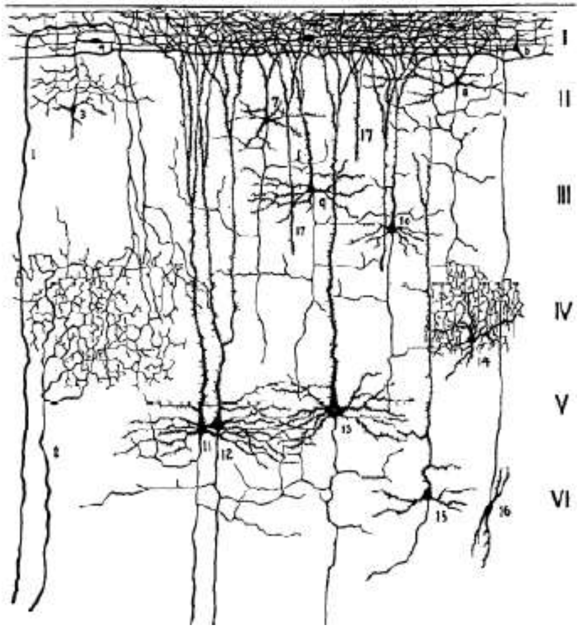
Four regions of a model neuron

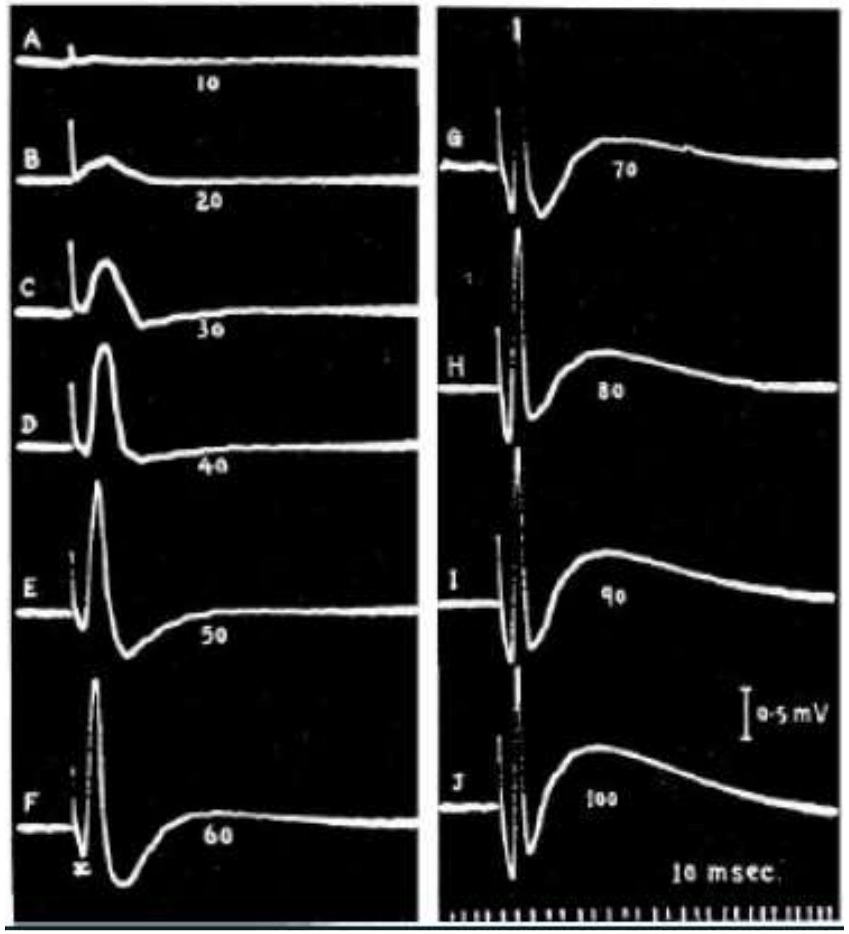


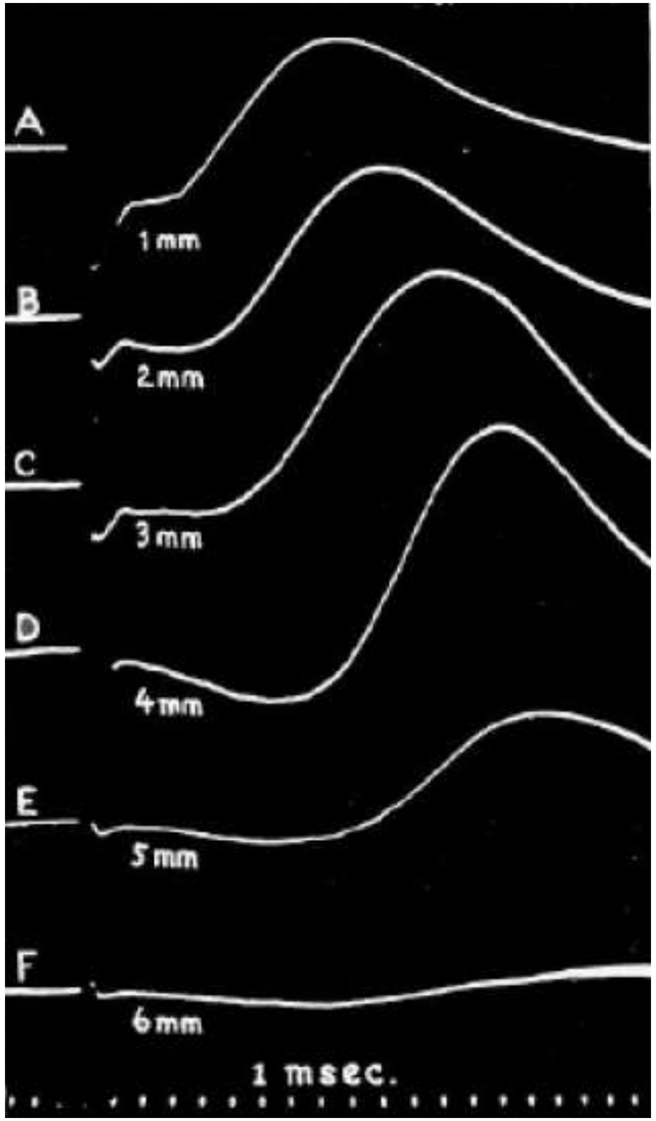
Question: how much sense could electrical signals make regarding information coding and integration?

Synaptic Potential will be Propagated and computed along Dendrites

张香桐树突研究的先驱之一
中国神经科学奠基人之一
岳阳路320号 脑所所长







Cheng, HT. (1951) Dendritic potential of cortical neurons produced by direct electrical stimulation of the cerebral cortex. J Neurophysiol. 1951 Jan;14(1):1-21.

Synaptic Potential will be Propagated and computed along Dendrites

张香桐树突研究的先驱之一
中国神经科学奠基人之一
岳阳路320号 脑所所长

Dear Chang,

I have just finished reading 'The Repetitive Discharges of Reverberating Cortico-Thalamic Circuits.'
I thank you for sending me your work. Without hesitation, I can readily say that your article is a masterpiece. Your deep and systematic analysis of the experimental data and your observations are of a great importance. Moreover, I must congratulate you for the clarity of your presentation and your impartial view of the state of previous works. Your article set a good example to us all.
I thank you for giving me the opportunity of reading it. As your elder, I am happy to say that you are one of the key figure of contemporary physiology. I wish you many other successes.

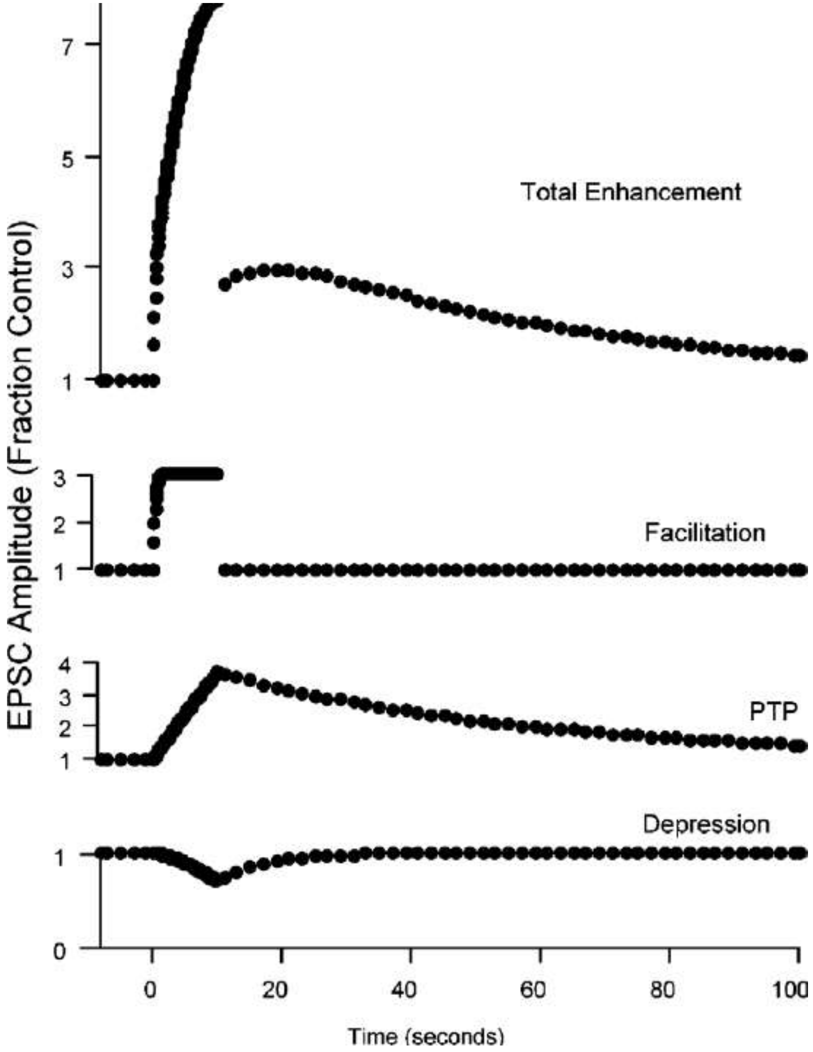
Yours
Lorente de No

The pioneering work before LTP discovery

冯德培

神经可塑性研究的先驱之一
岳阳路320号 生理研究所

美国国家科学院外籍院士、第三世界科学院院士、英国伦敦大学学院院士、印度国家科学院外籍院士，神经肌肉接头研究领域国际公认的先驱者之一



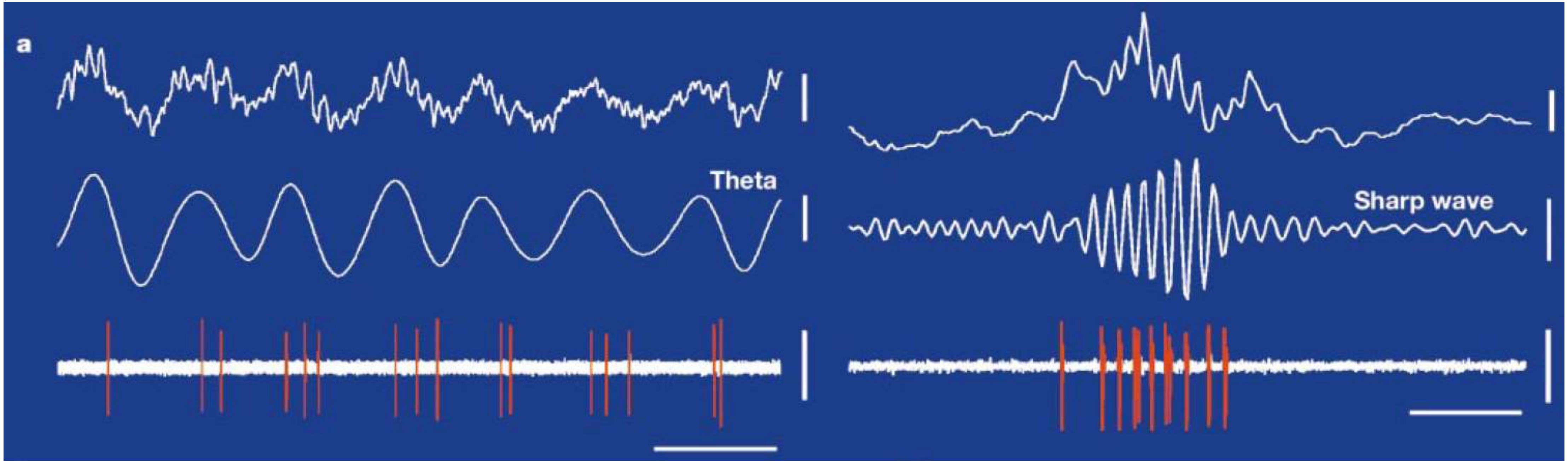
Feng TP. 1941. The changes in the endplate potential during and after prolonged stimulation. Chin. J. Physiol. 13:79–107

- Why electrophysiology?
- The history and basics of electrophysiology
- Methods in electrophysiology
- Future of electrophysiology

Electrophysiology Development

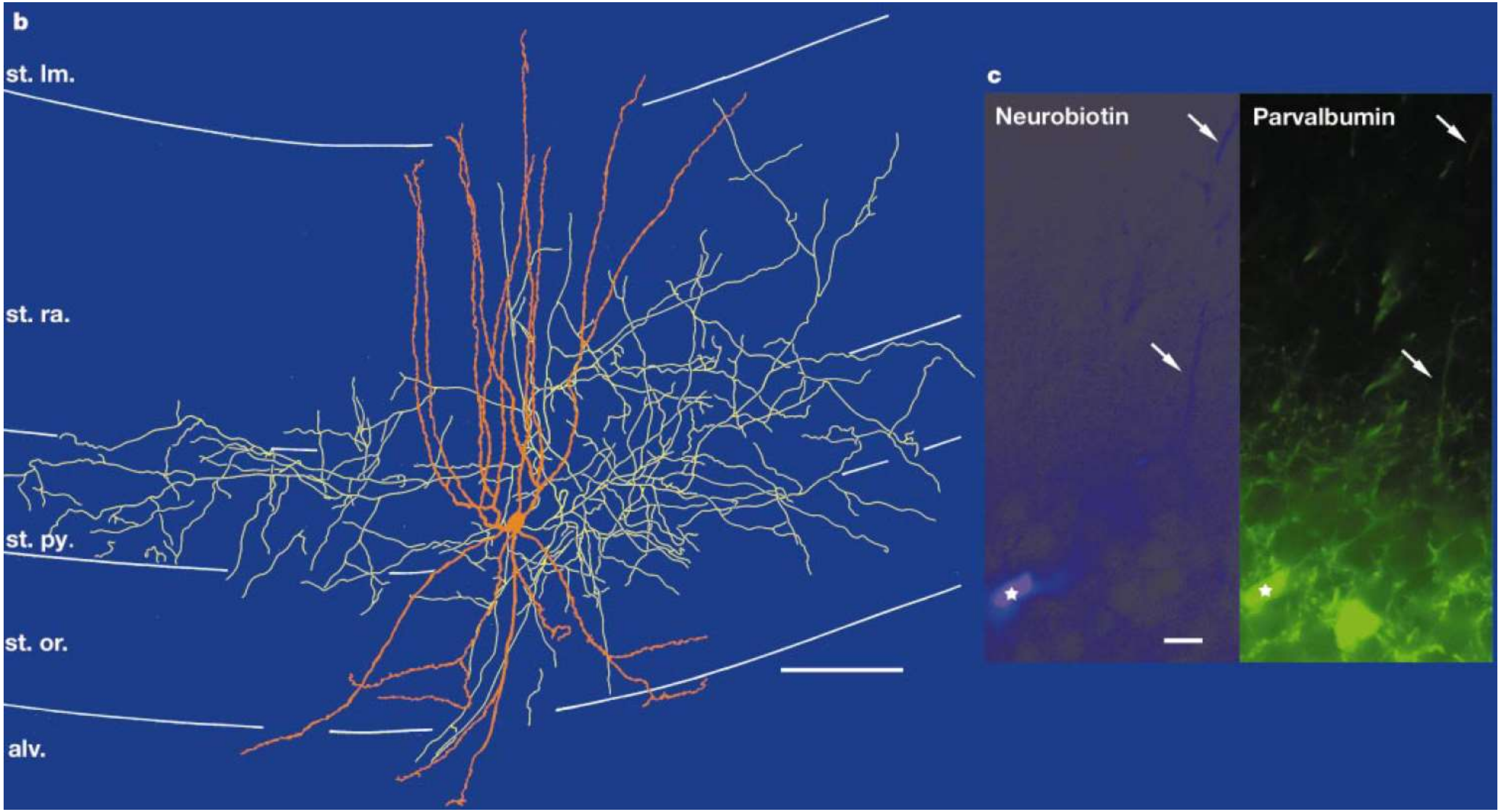
- Extracellular recording
  - Metal electrode
  - LFP / Oscillations
- Intracellular recording

Juxtacellular recording



NATURE | VOL 421 | 20 FEBRUARY 2003

Juxtacellular recording



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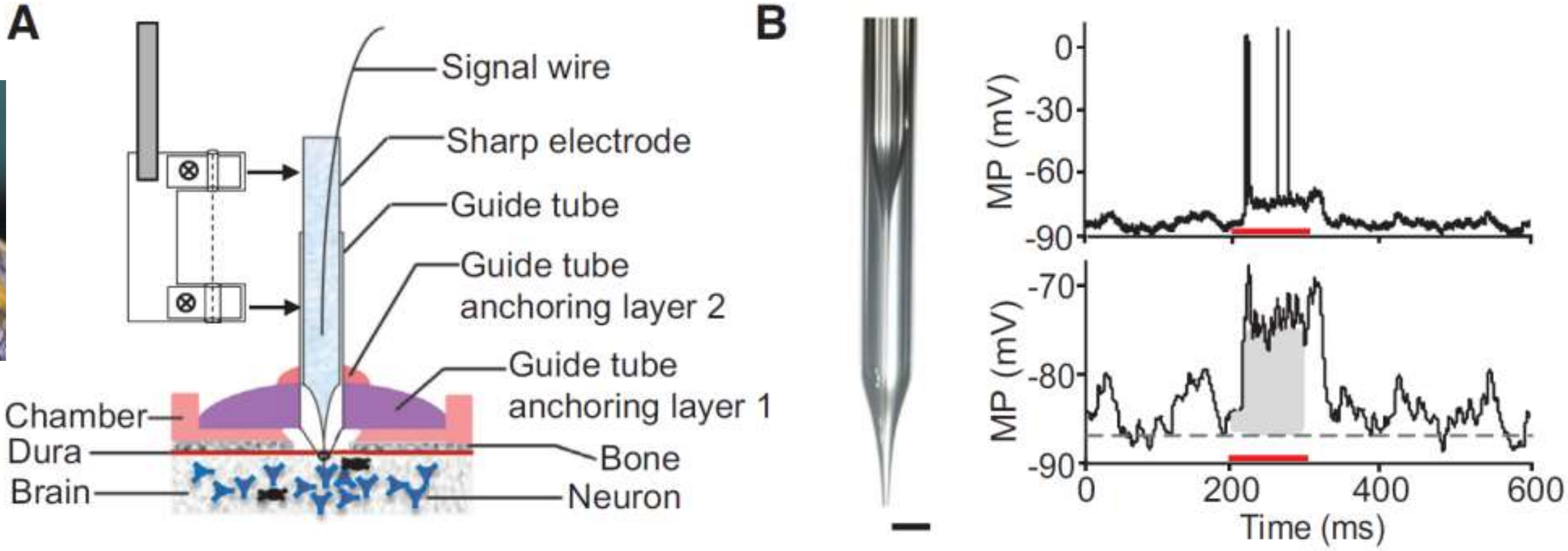
Intracellular recording in primates

marmoset

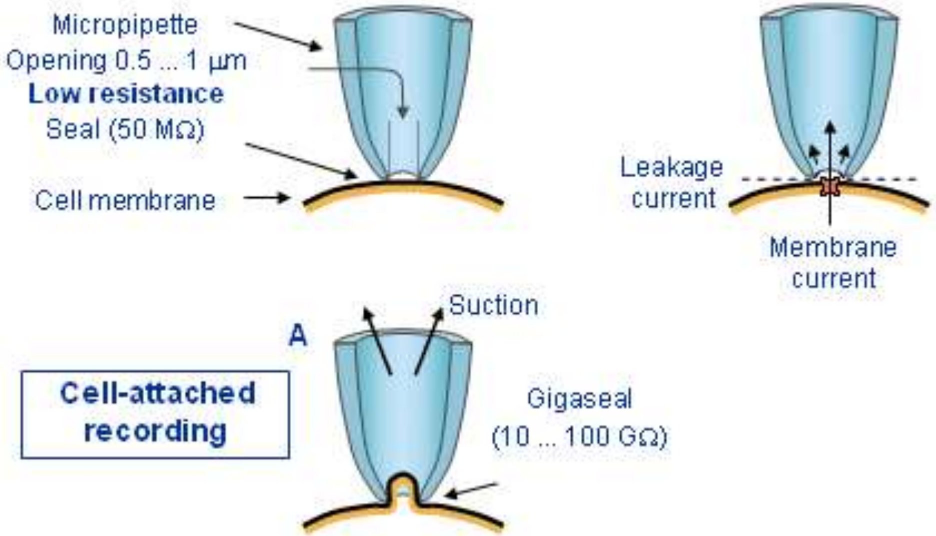


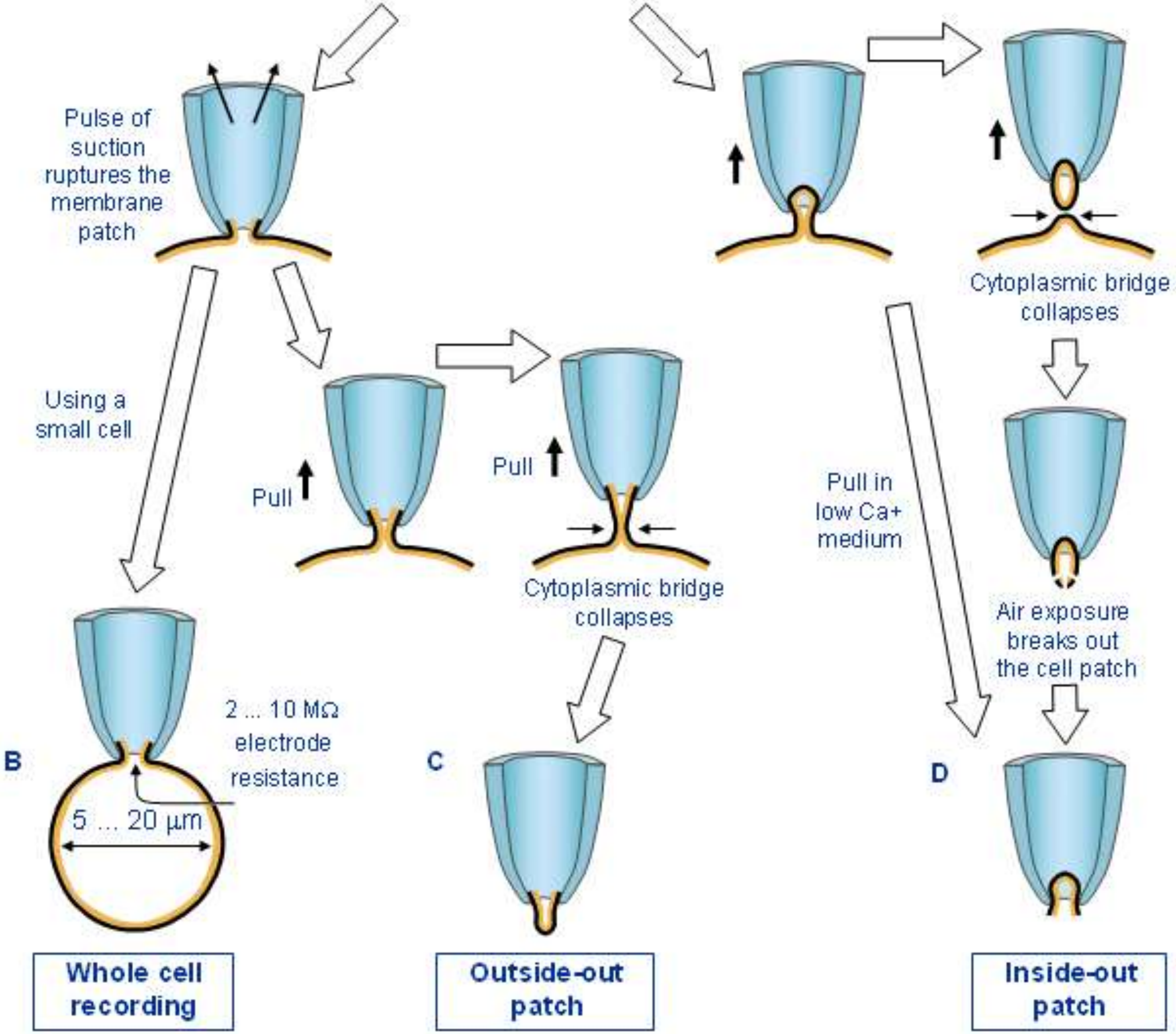
Gao et al., 2016, Neuron 91, 905–919

The revolutionary breakthrough – patch clamp technique

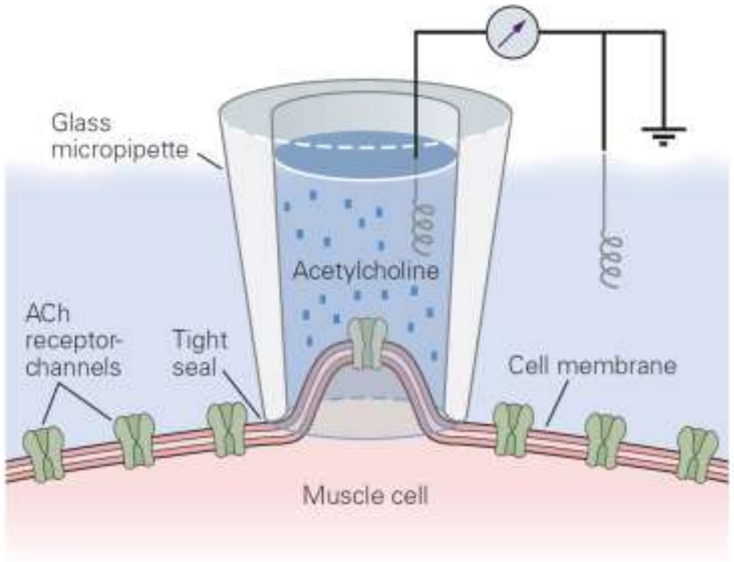
Bert Sakmann

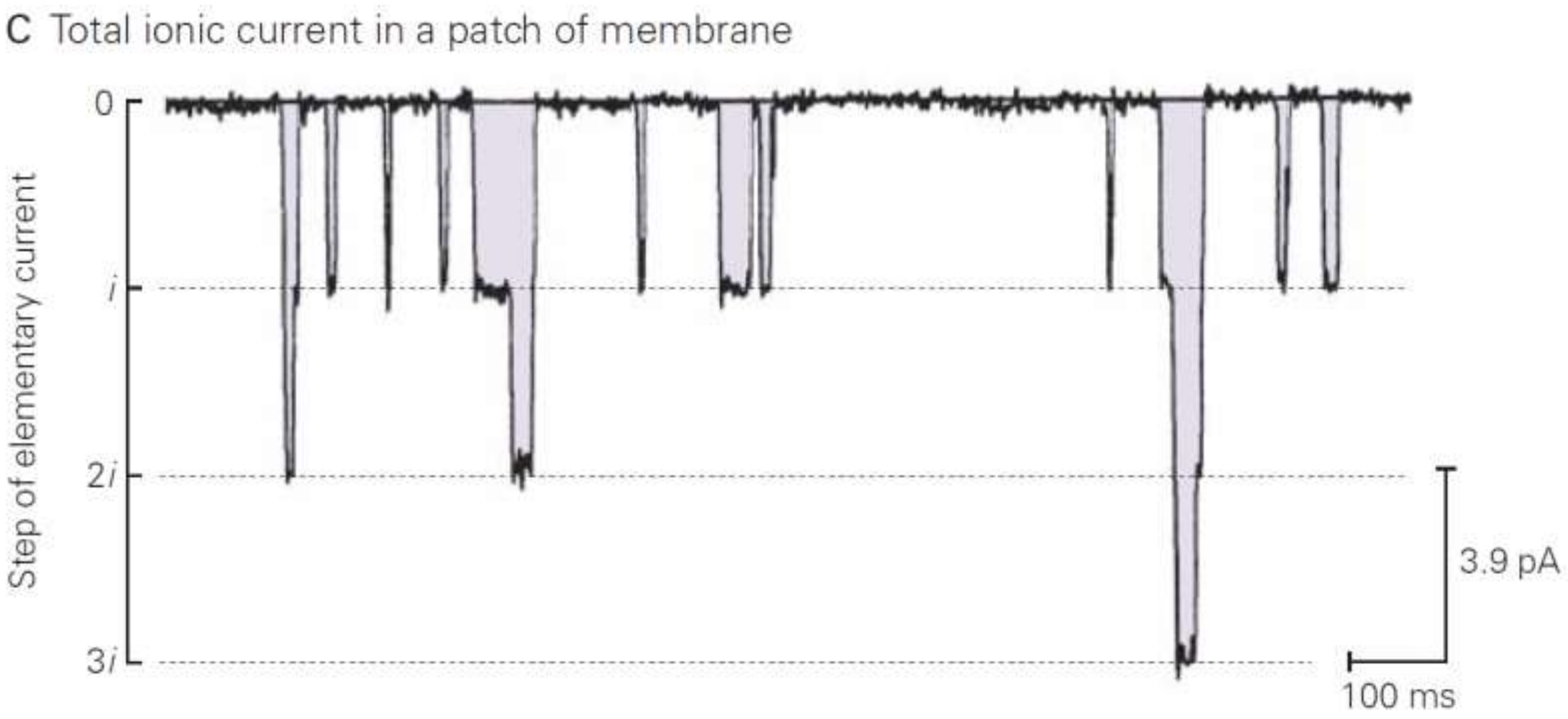
Erwin Neher





Single-channel recording



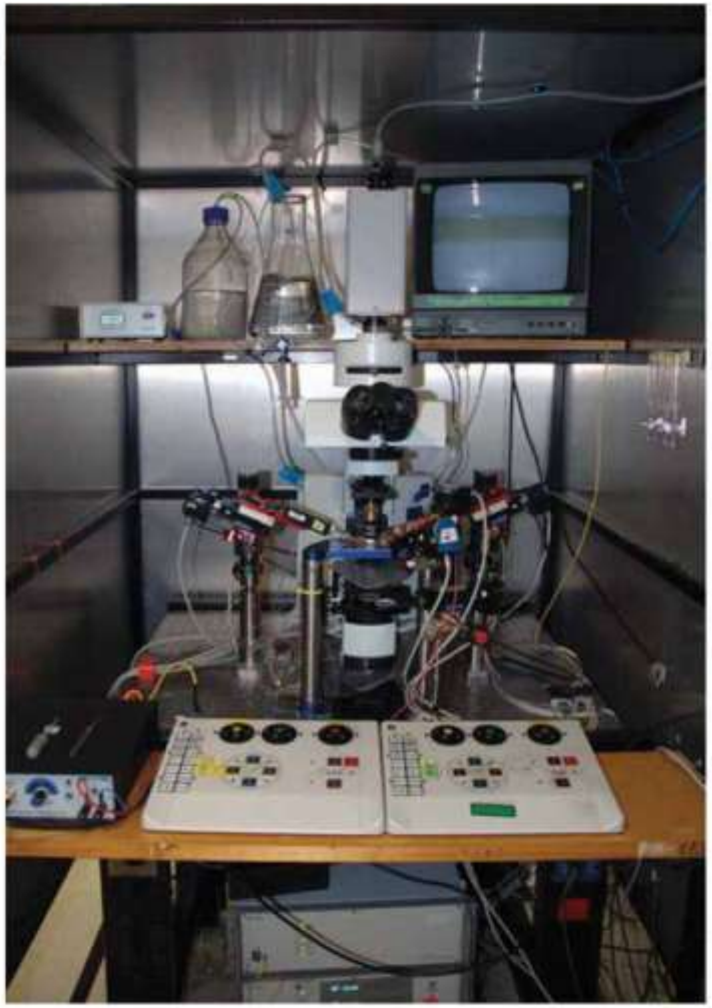


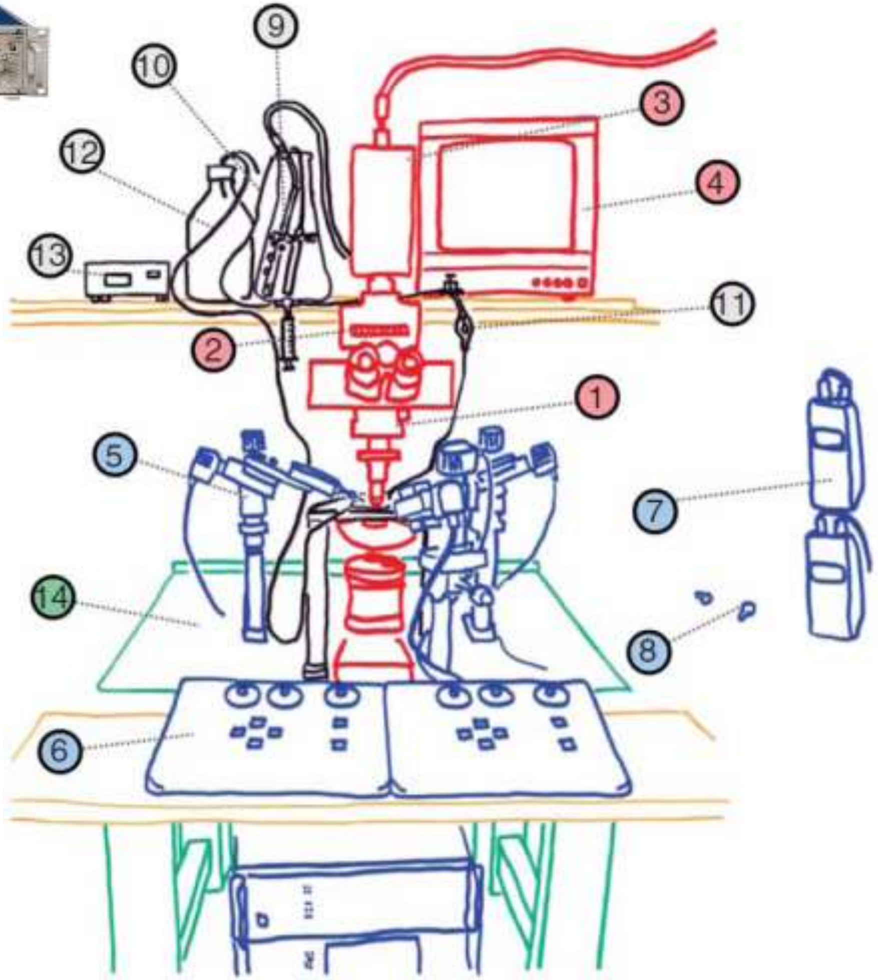
Neher, E. and B. Sakmann (1976). "Single-channel currents recorded from membrane of denervated frog muscle fibres." Nature **260(5554): 799-802.**

Electrophysiology Development

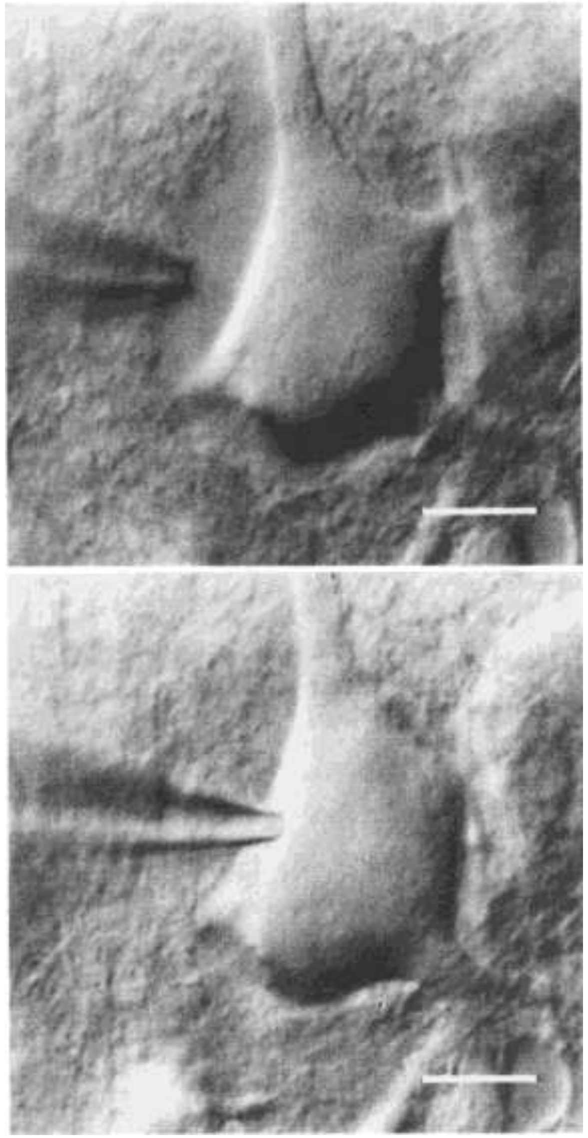
- Extracellular recording
  - Metal electrode
  - LFP / Oscillations
- Intracellular recording
  - Patch-clamp recording
    - Voltage clamp: excitatory post-synaptic current (EPSC)
    - Current clamp: excitatory post-synaptic potential (EPSP)
    - Inside-out
    - Outside-out
    - Whole-cell
    - Dendritic recording
    - Axon recording
    - Capacitance recording

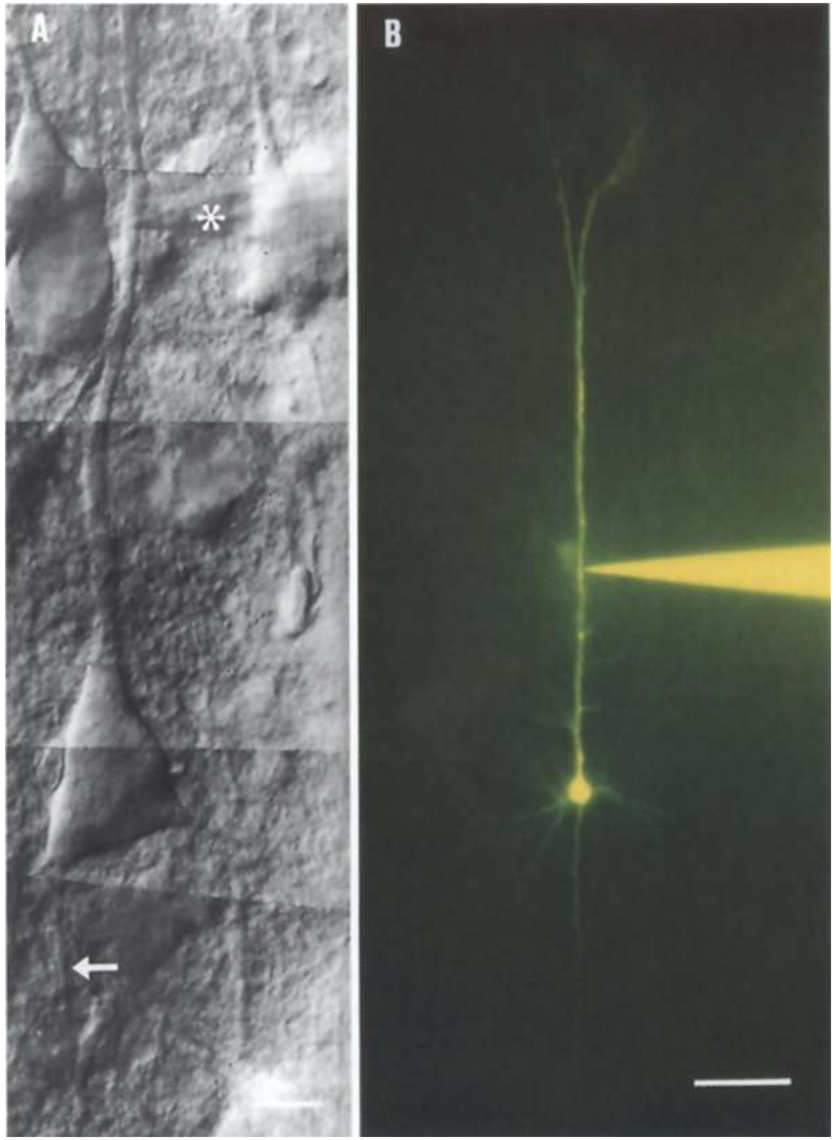
A patch-clamp rig

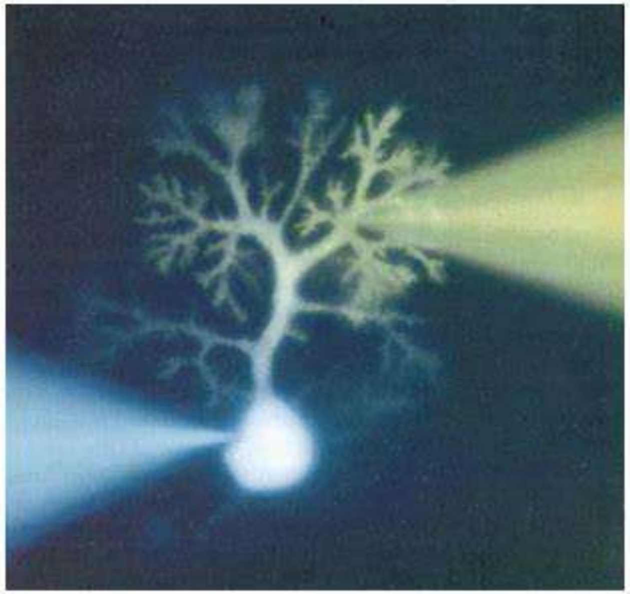




Dendritic recording

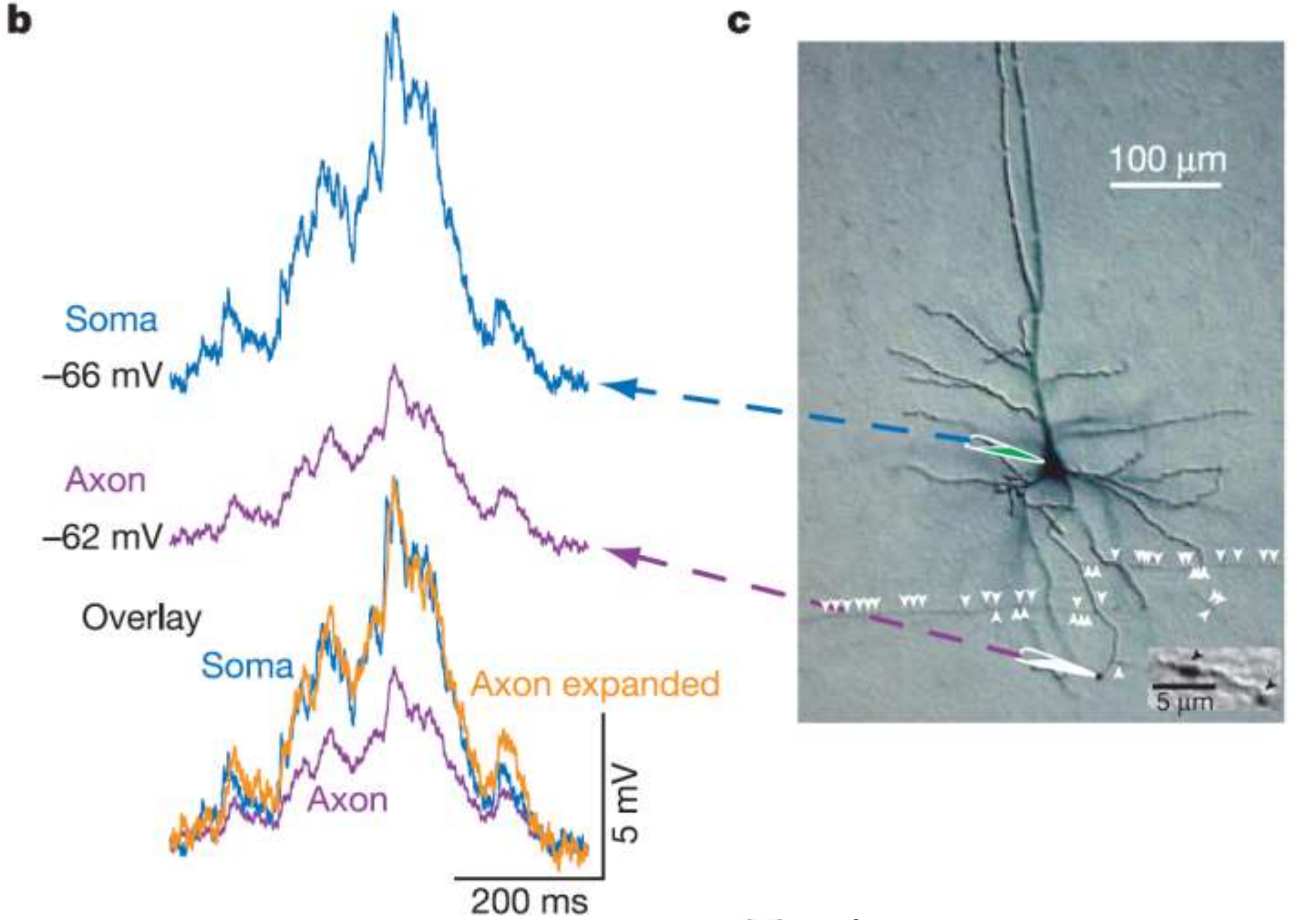




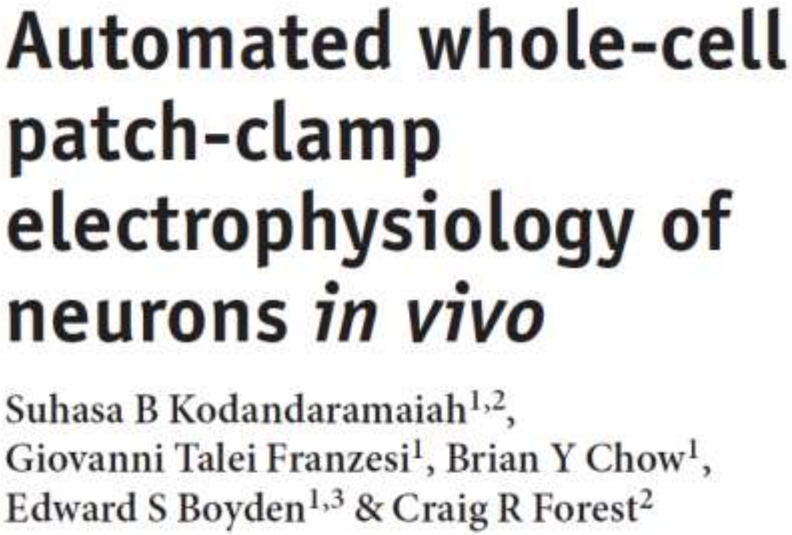


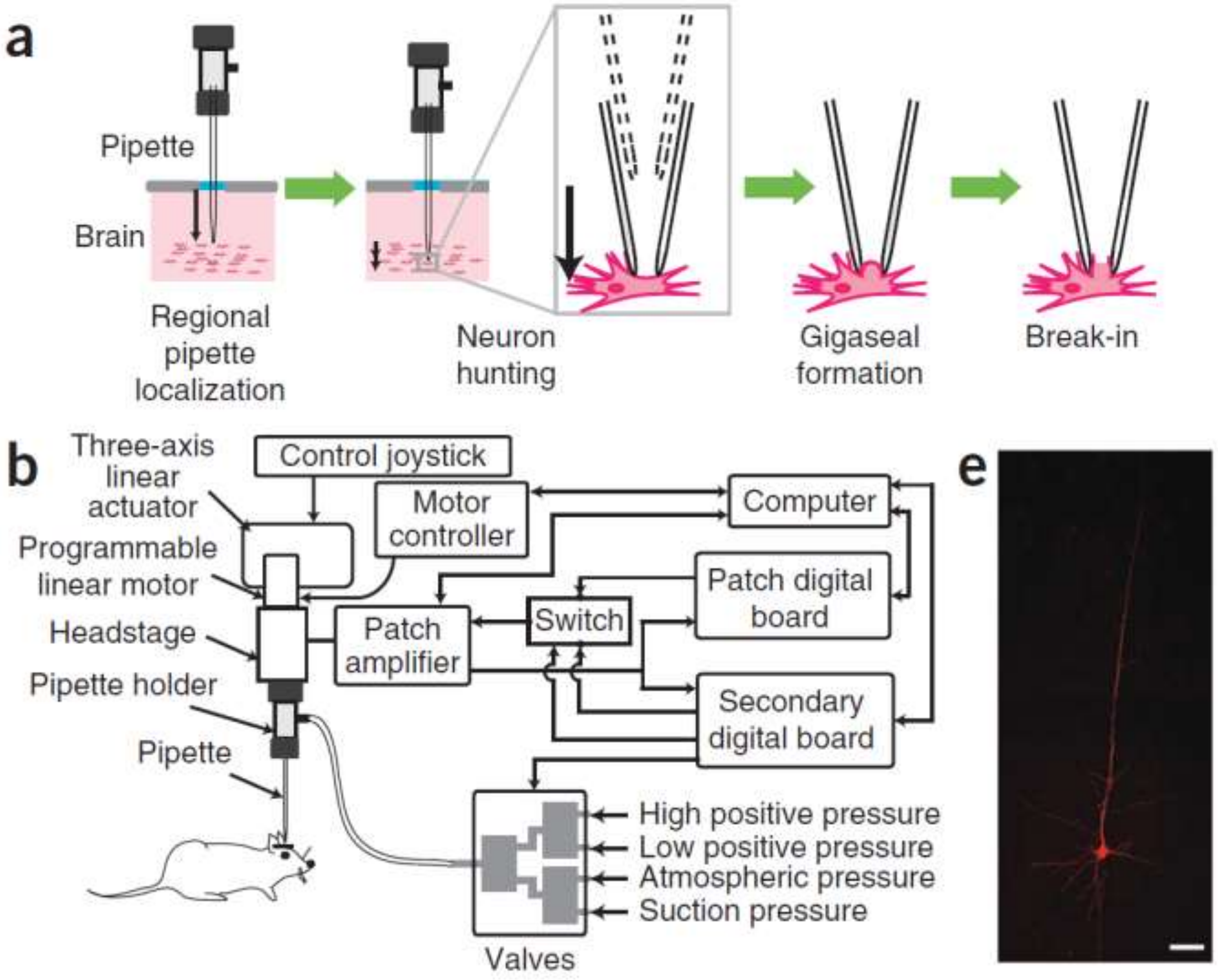
July 1993 Pflügers Archiv - European Journal of Physiology 423(5-6):511-8

Axon recording

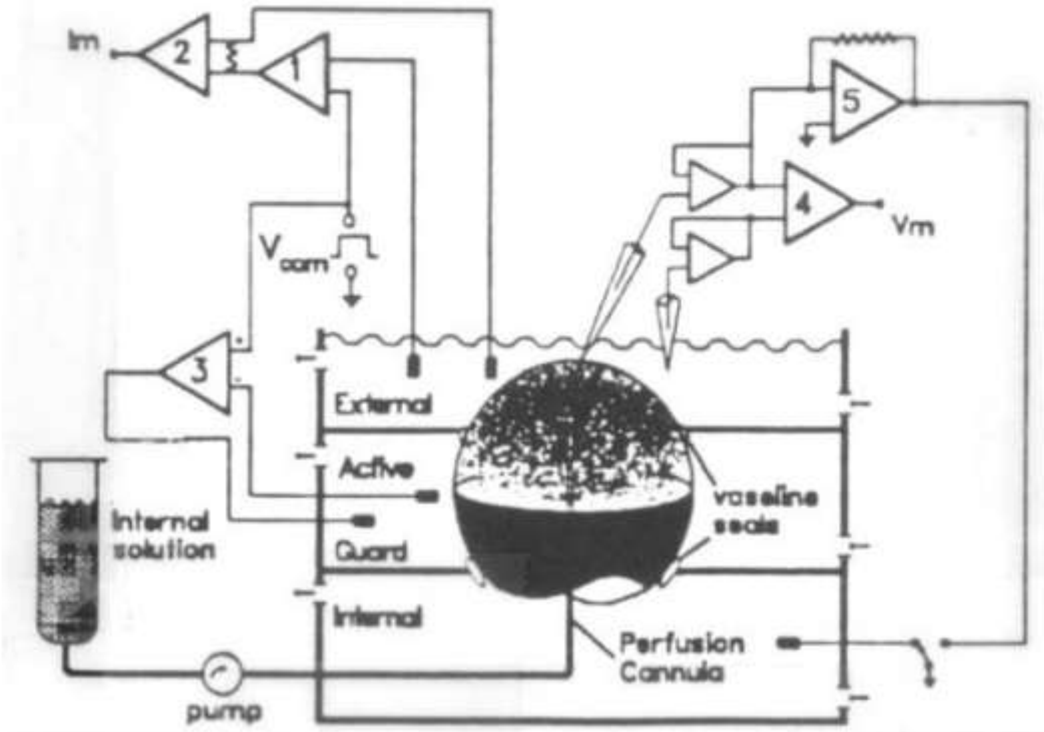


In vivo patch-clamp recording

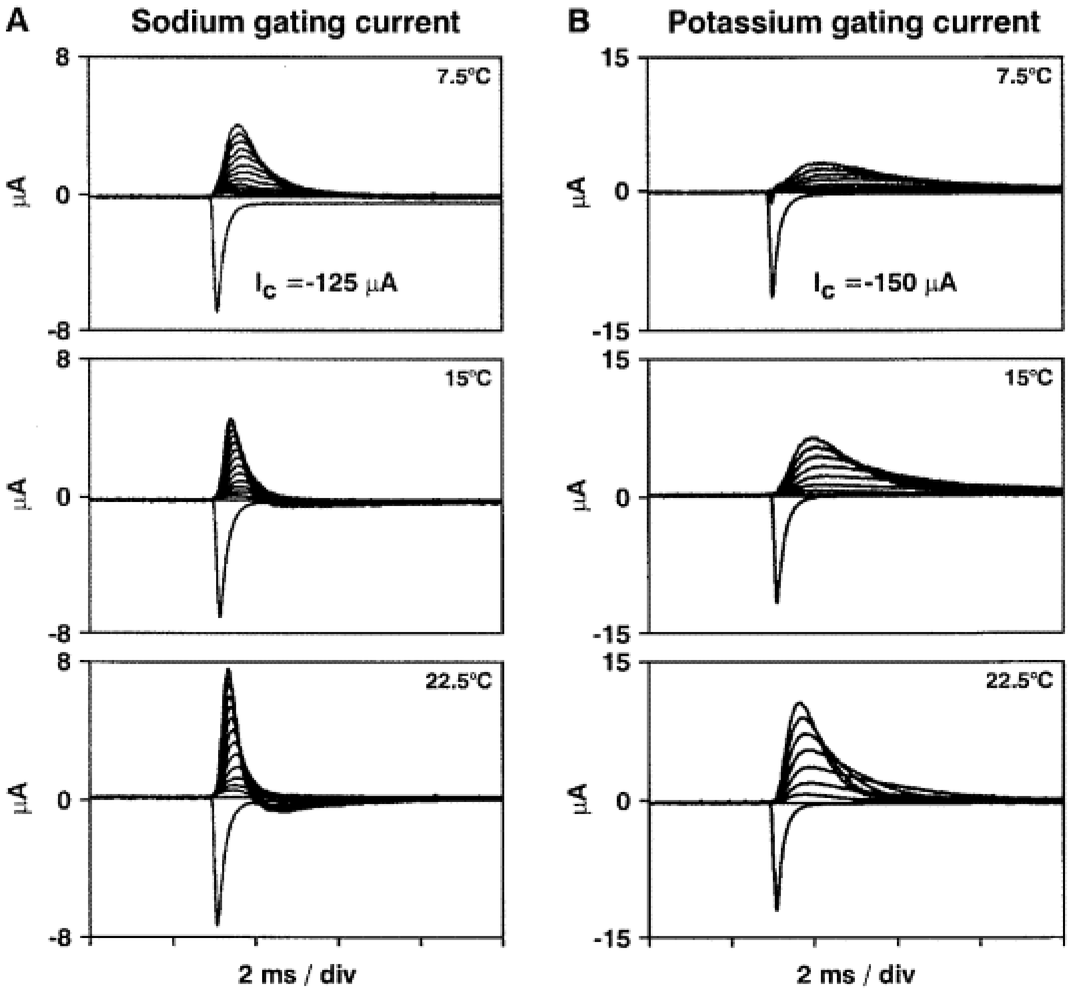




Cut-open oocyte recording



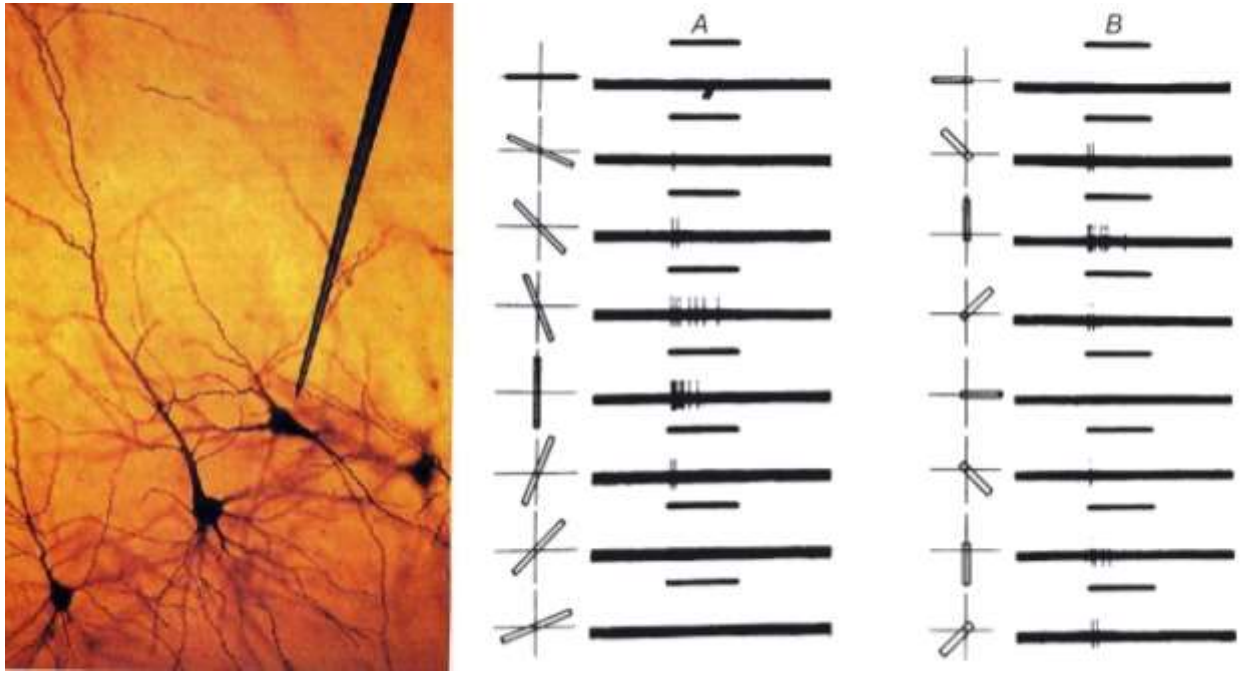
Gating currents by channel opening

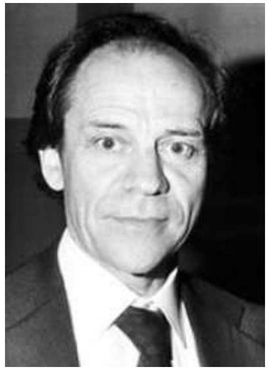


Electrophysiology Development

- Extracellular recording
  - Metal electrode
  - Glass micropipette
- Intracellular recording
  - Patch-clamp recording
    - Voltage clamp: excitatory post-synaptic current (EPSC)
    - Current clamp: excitatory post-synaptic potential (EPSP)
    - Inside-out
    - Outside-out
    - Whole-cell
    - Dendritic recording
    - Axon recording
    - Capacitance recording
  - Cut-open oocyte recording
  - Two-electrode recording

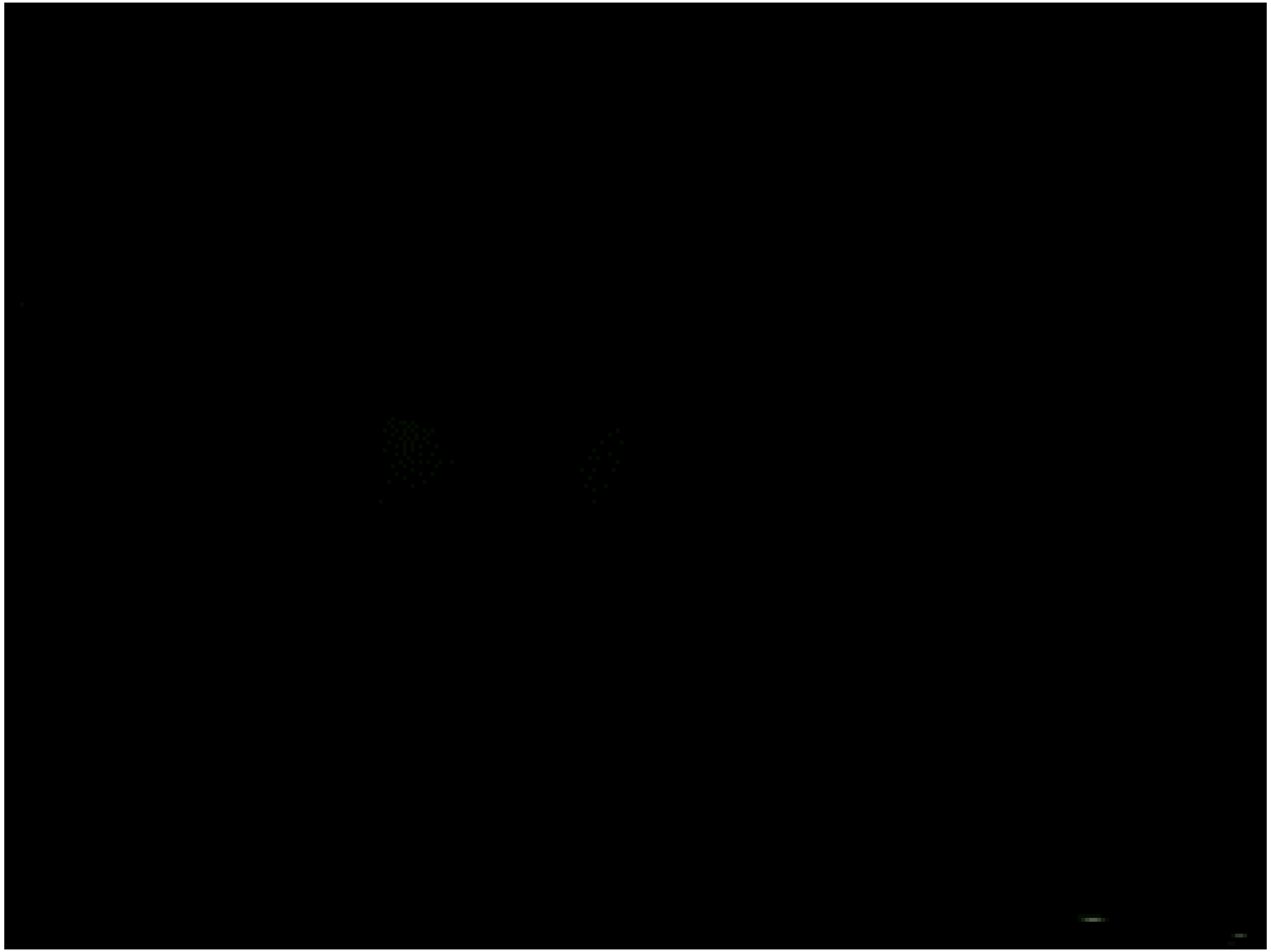
Feature Selectivity & Activity dependency of Visual Pathway





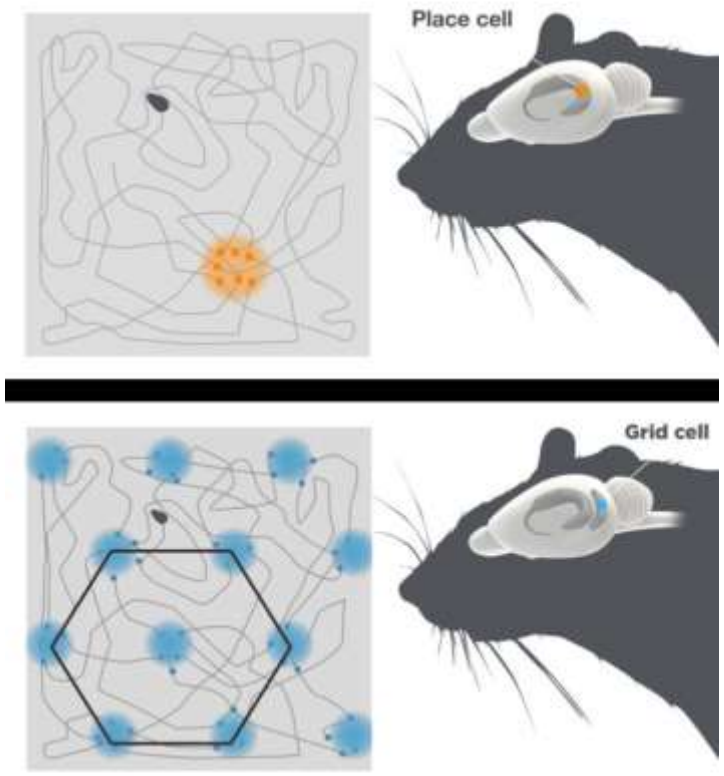
1. D. H. Hubel, T. N. Wiesel, Receptive fields of single neurones in the cat's striate cortex. The Journal of physiology 148, 574 (Oct, 1959).
2. D. H. Hubel, T. N. Wiesel, Receptive fields, binocular interaction and functional architecture in the cat's visual cortex. The Journal of physiology 160, 106 (Jan, 1962).
3. T. N. Wiesel, D. H. Hubel, Effects of Visual Deprivation on Morphology and Physiology of Cells in the Cats Lateral Geniculate Body. Journal of neurophysiology 26, 978 (Nov, 1963).
4. T. N. Wiesel, D. H. Hubel, Single-Cell Responses in Striate Cortex of Kittens Deprived of Vision in One Eye. Journal of neurophysiology 26, 1003 (Nov, 1963).

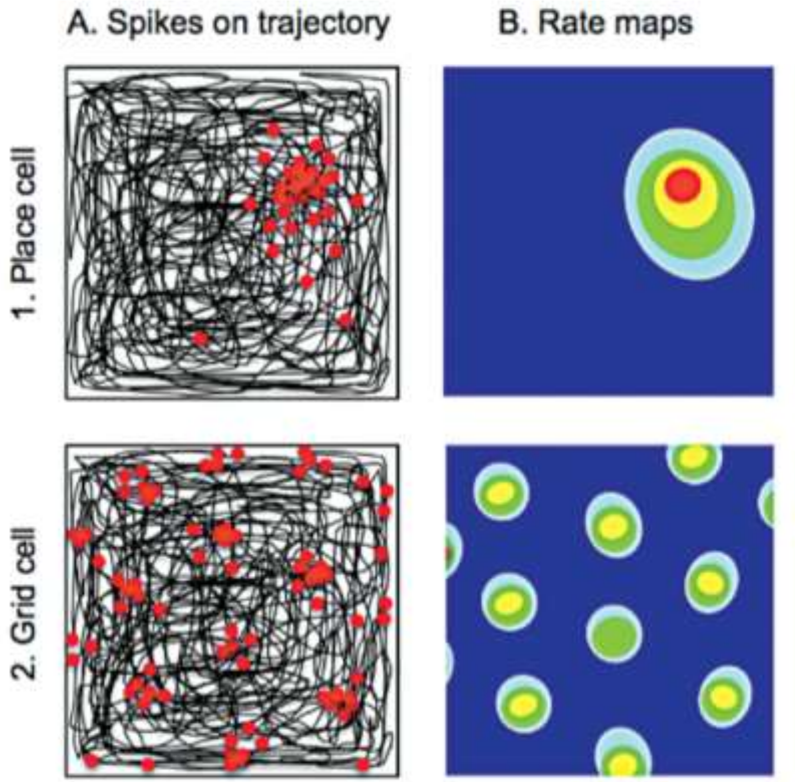
The historical moments for electrophysiology and vision research



Place Cell and Grid Cell

Nobel Prize 2014





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- Jacobs, J.; Weidemann, C. T.; Miller, J. F.; Solway, A.; Burke, J. F.; Wei, X. X.; Suthana, N.; Sperling, M. R.; Sharan, A. D.; Fried, I.; Kahana, M. J. (2013). "Direct recordings of grid-like neuronal activity in human spatial navigation". *Nature Neuroscience*

A context-dependent memory effect for divers

Glen Egstrom

UCLA Dive Physiology Researcher

Egstrom et al. (1972) observed that divers had considerable difficulty in recalling material learnt under water.

http://www.internationallegendsofdiving.com/FeaturedLegends/Glen_Egstrom_bio.htm

EGSTROM, G. H., WELTMAN, G., BADDELEY, A. D., CUCCARO, W. J. & WILLIS, M. A. (1972). Underwater work performance and work tolerance. Report no. 51, Bio-Technology Laboratory, University of California, Los Angeles.

Godden & Baddely,1975

In one condition the divers recalled in the same location where they learnt the words

In the other condition they recalled in the other location

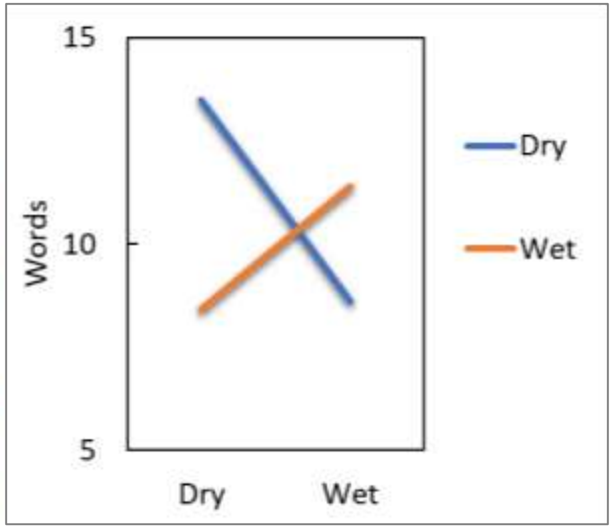


Table 1. *Mean number of words recalled in Expt. I as a function of learning and recall environment*

	Recall environment				
	Dry		Wet		
Learning environment	Mean recall score	S.D.	Mean recall score	S.D.	Total
Dry	13·5	5·8	8·6	(3·0)	22·1
Wet	8·4	3·3	11·4	(5·0)	19·8
Total	21·9	—	20·0	—	—

Br. J. Psychol. (1975), 66, 3, pp. 325-331

Two forms of context-dependent learning and memory

1. Context regulates CS-US association

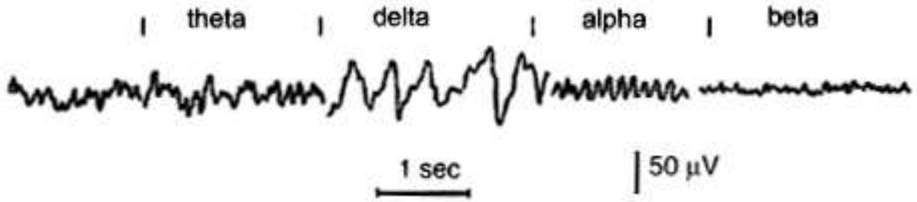
2. Context per se is associated with US

What are the neural circuit mechanism underlying context-dependent learning and memory?

CS: conditional stimulus (neutral stimuli such as tone, light, odor etc.)
US: unconditional stimulus (stimuli with emotional valence such as shock, food, predator odor etc.)

EEG

- The electroencephalogram (EEG) is a recording of the electrical activity of the brain from the scalp.



- Why electrophysiology?
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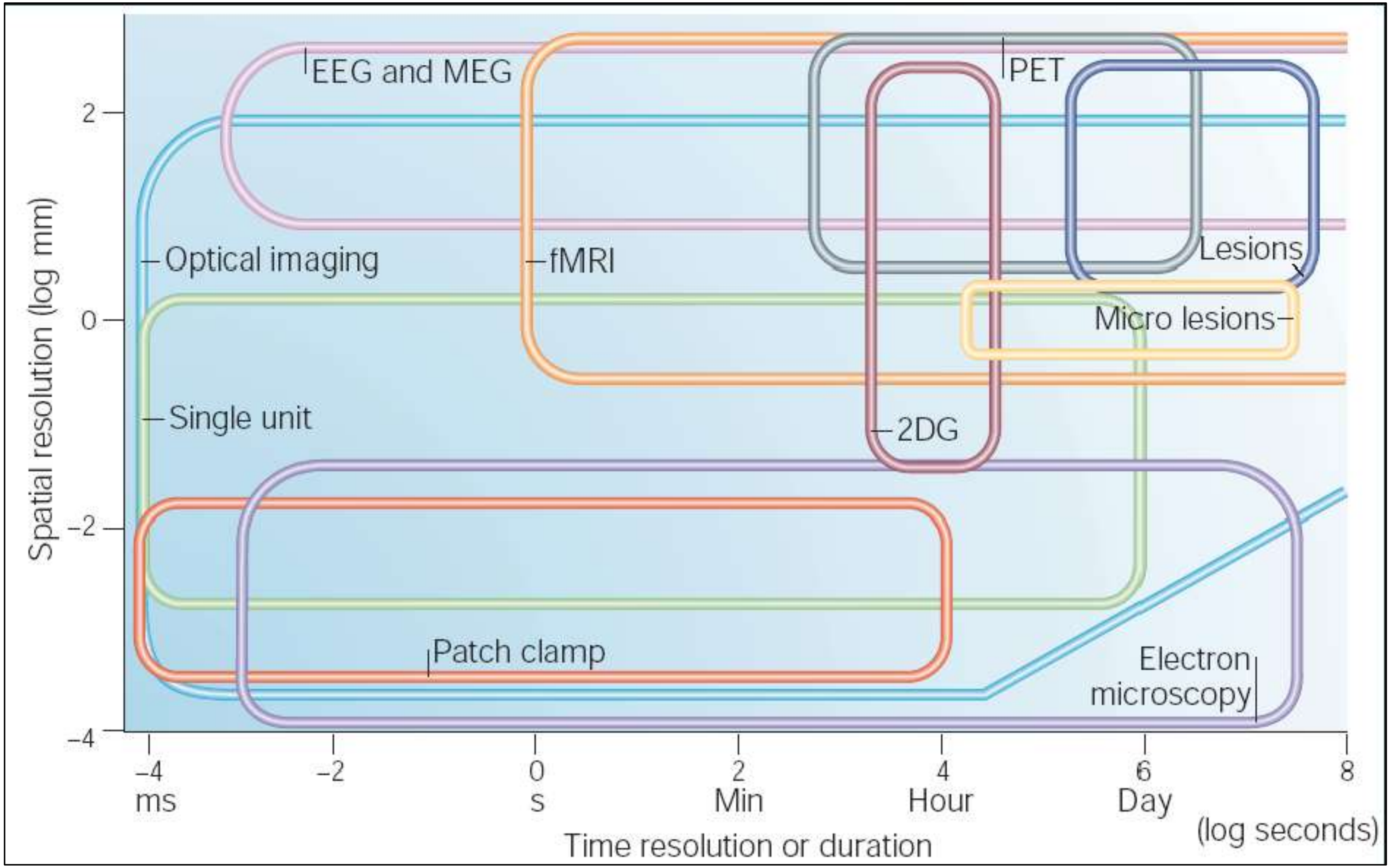
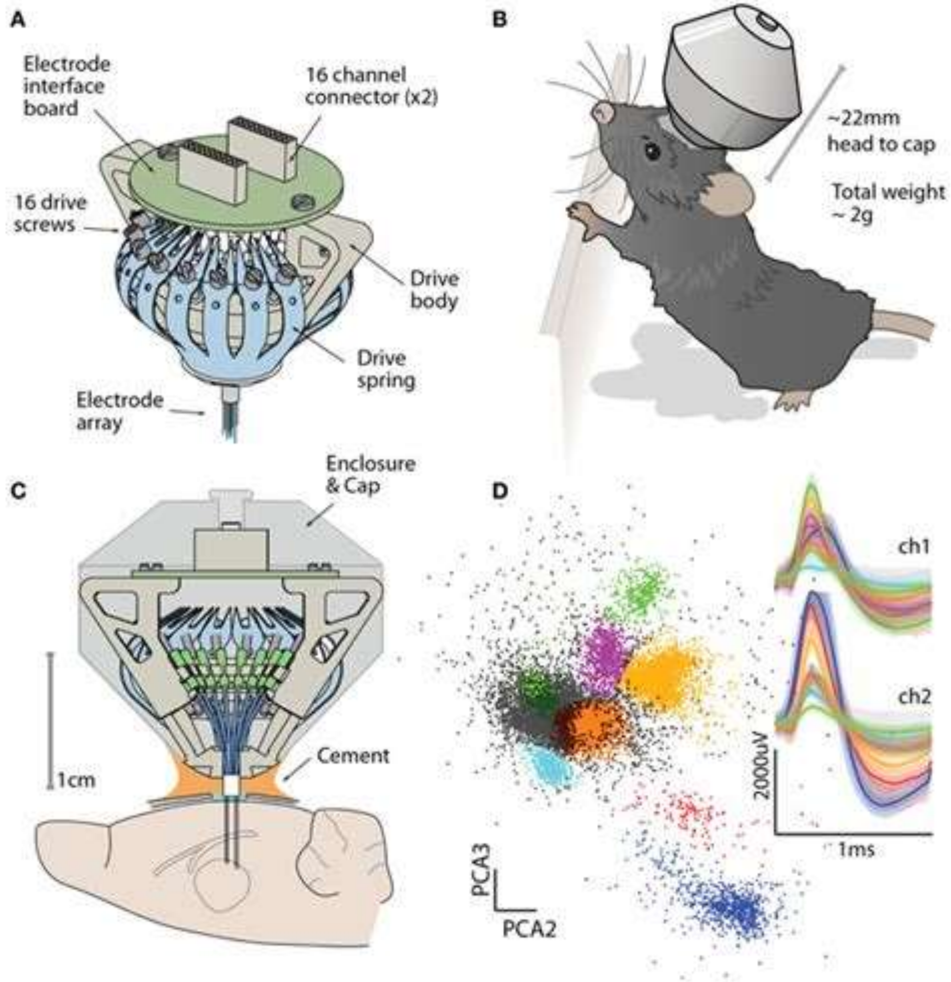
Tools to record biological signals

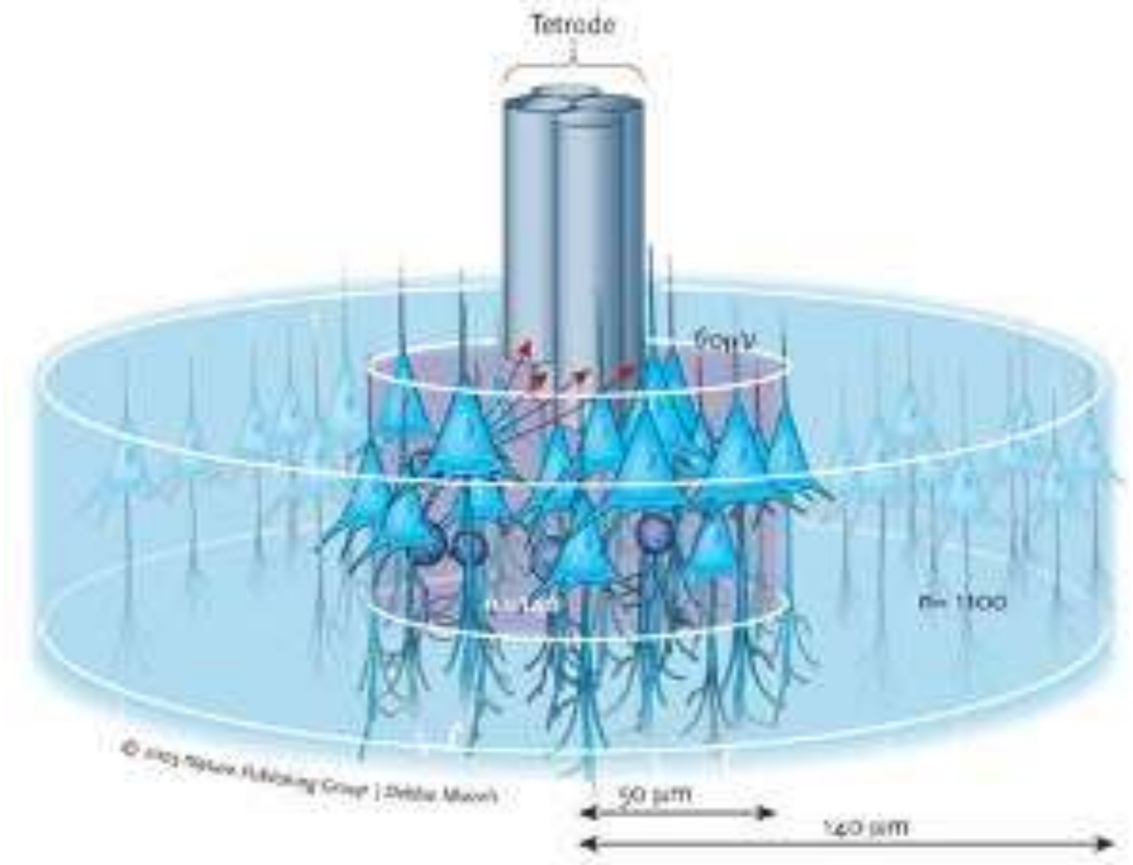


Future of electrophysiology

- Increase Channel count
- Electrode: More stable recording and tissue friendly
- Wireless
- Optical imaging
- All-optical: calcium imaging + precise optogenetic
- Voltage-sensitive dye (mention but not in detail).
- Miniscope

Increase Channel count





Increase the channel account

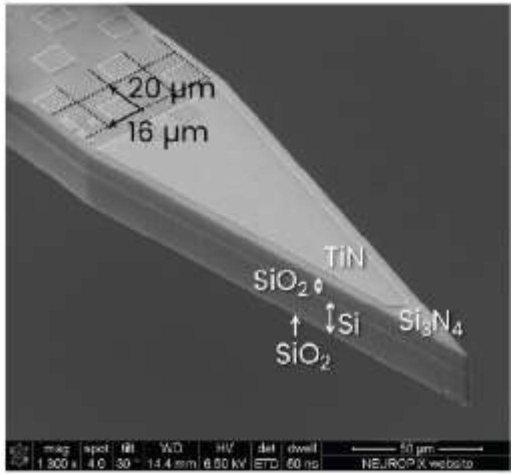
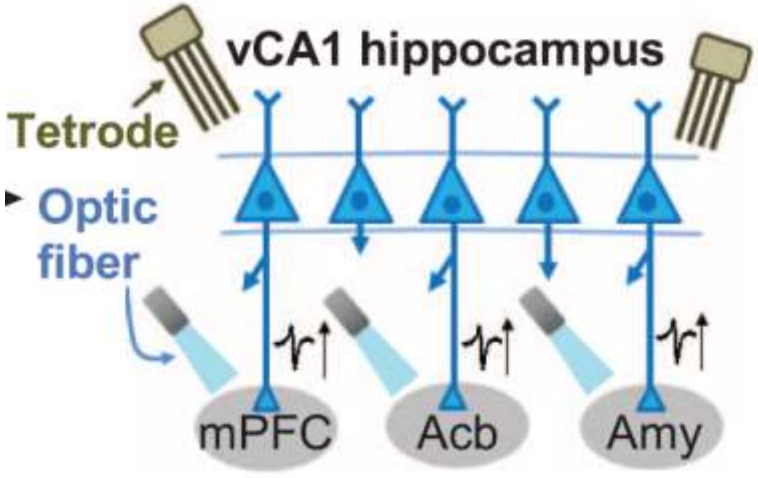
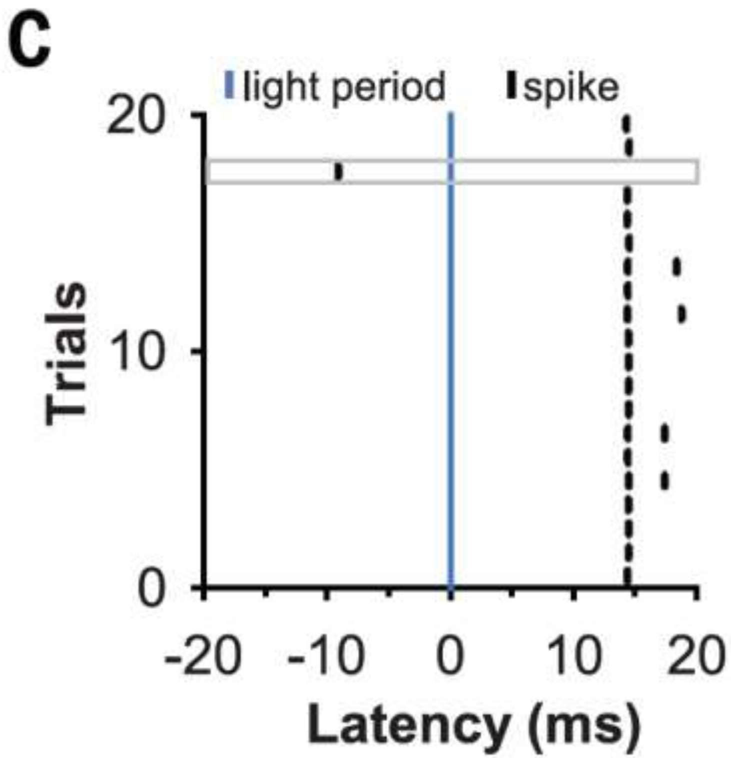
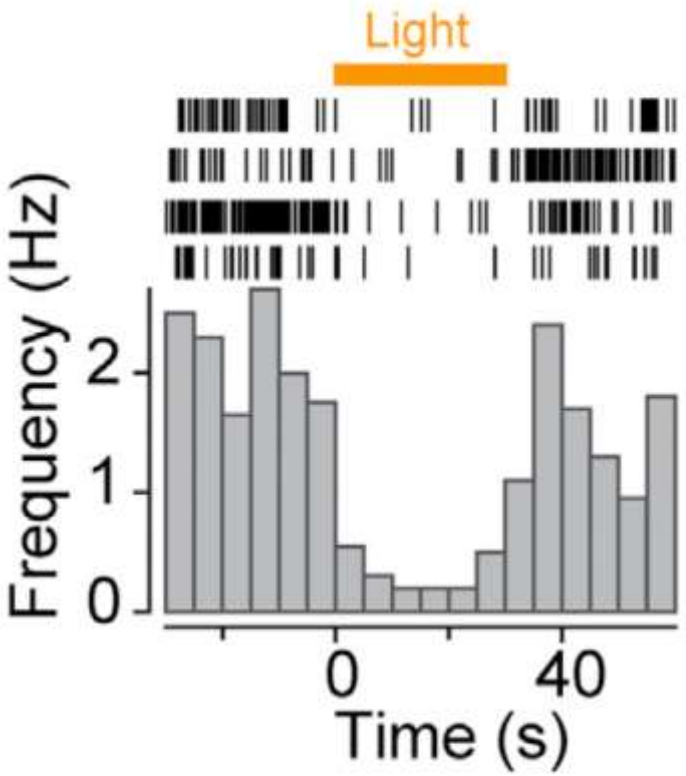


Photo-tagging recording



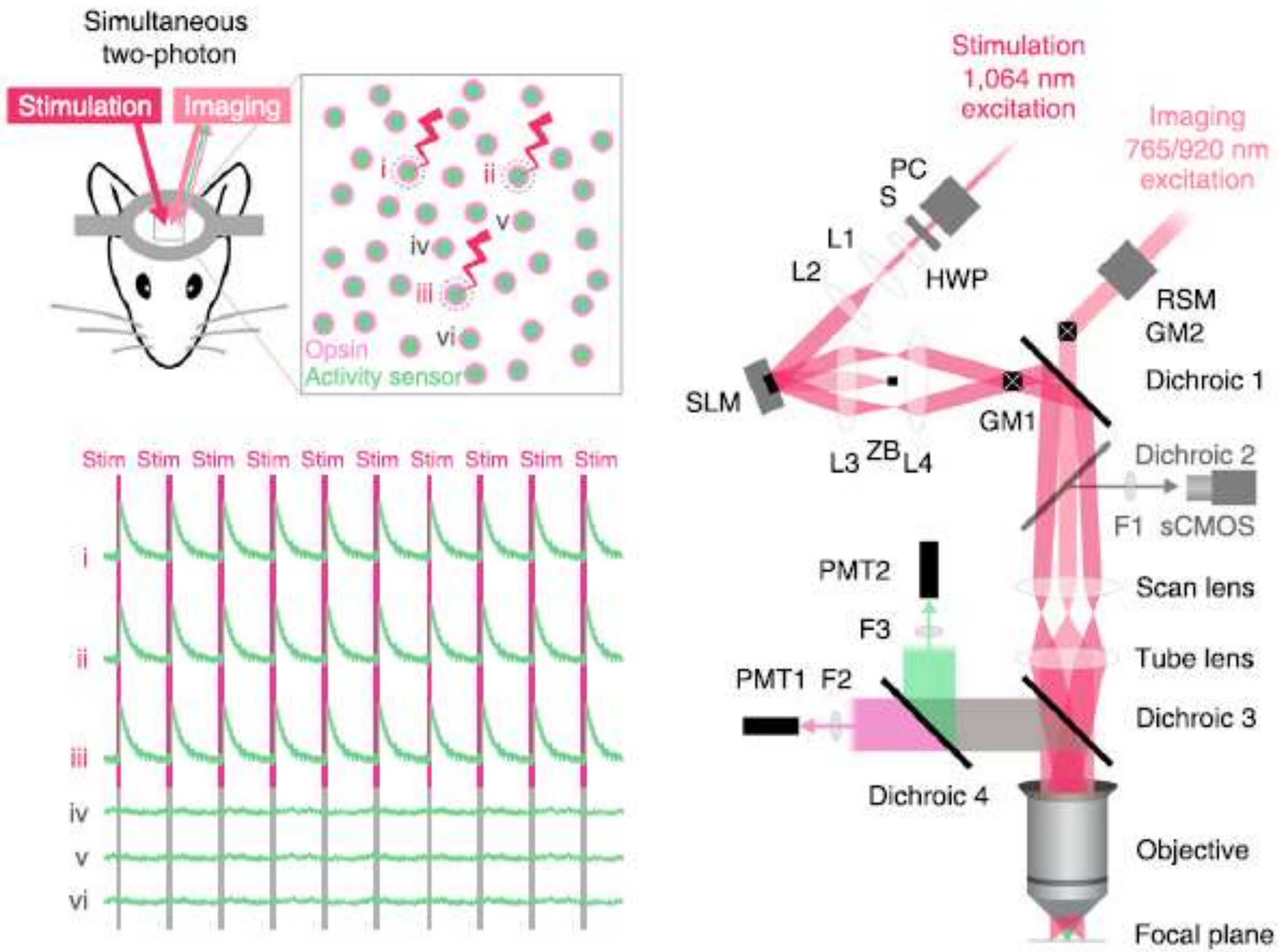


Coicchi et al., 2015



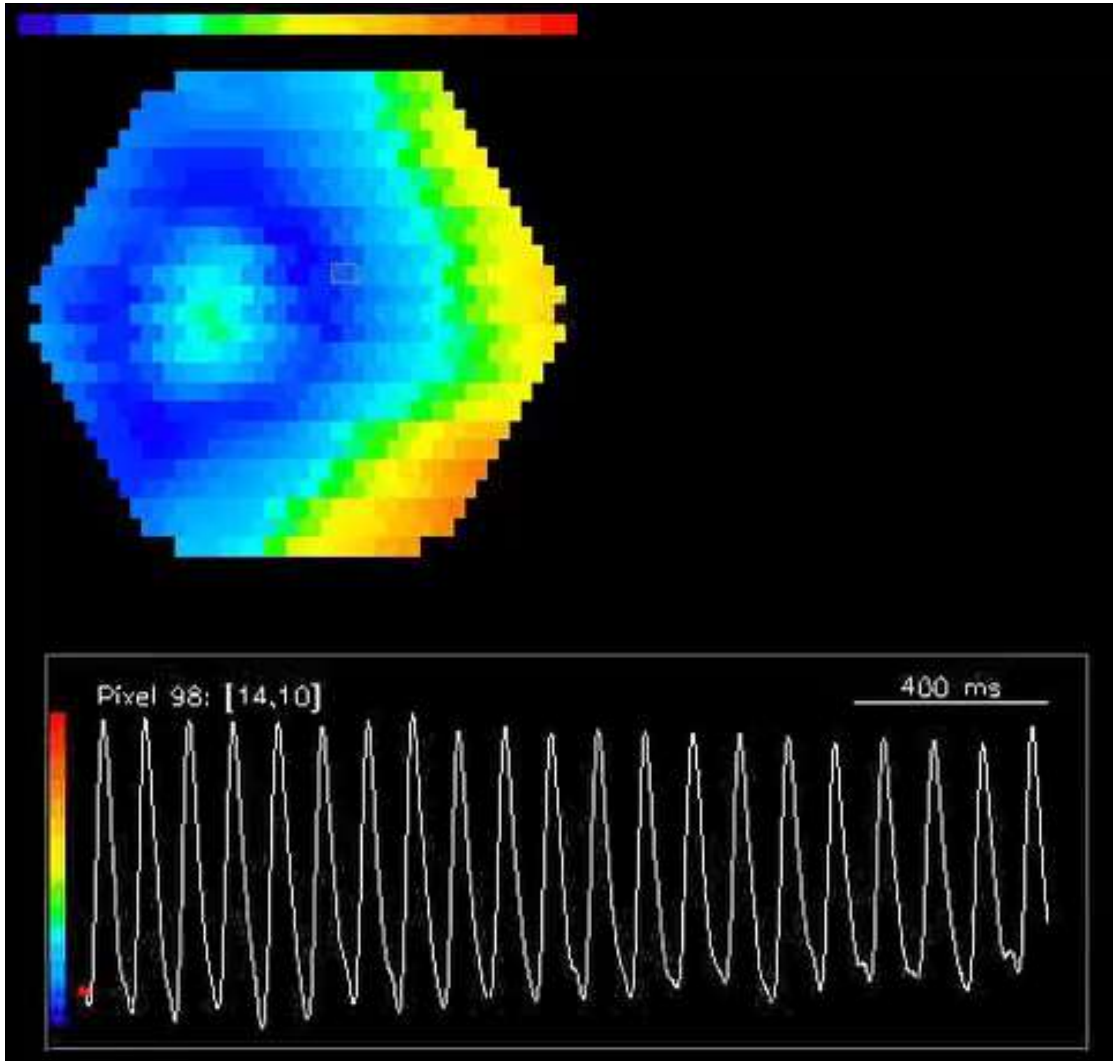
Xu et al., 2016

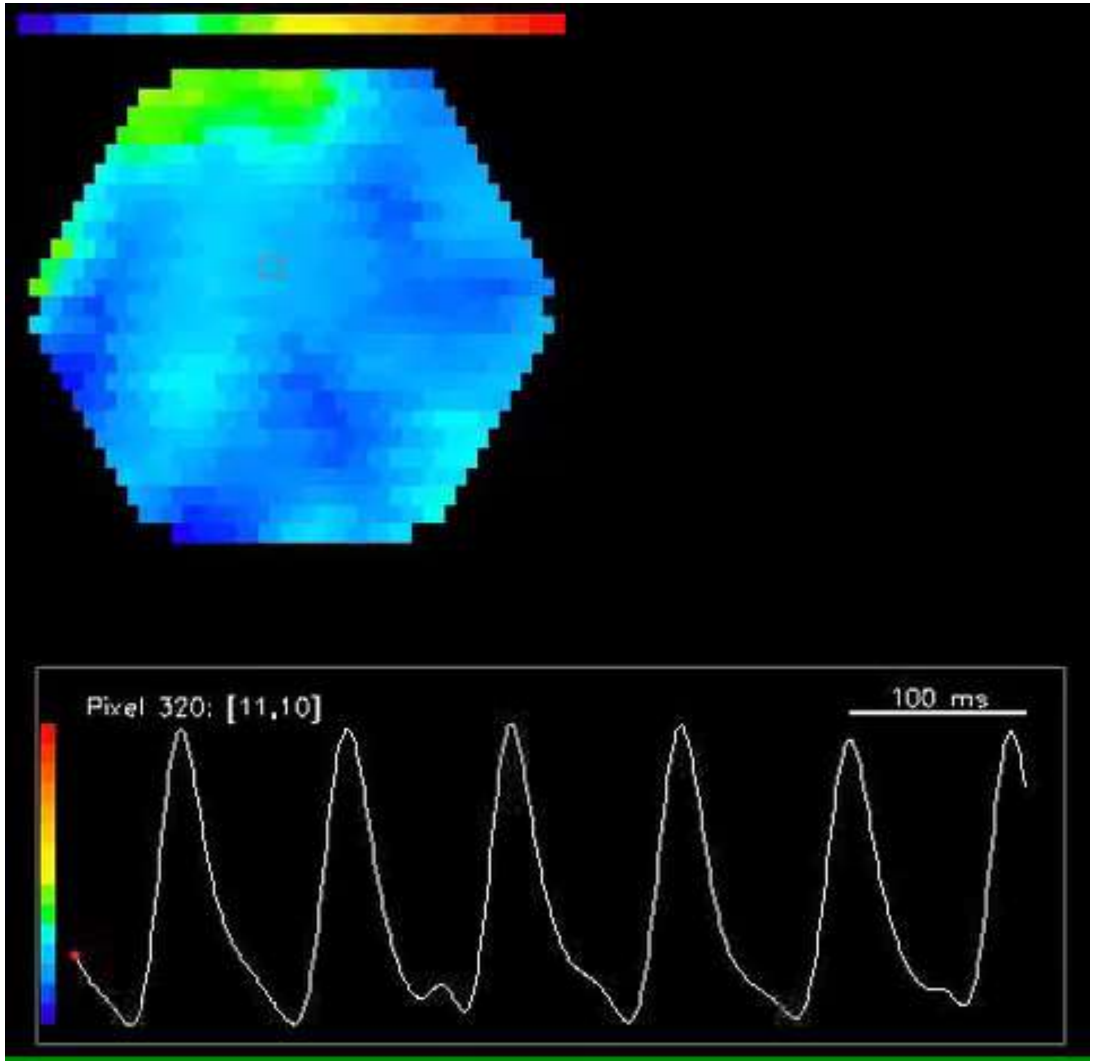
All-optical: calcium imaging + precise optogenetic



Packer et al; 2012

Voltage-sensitive dye imaging

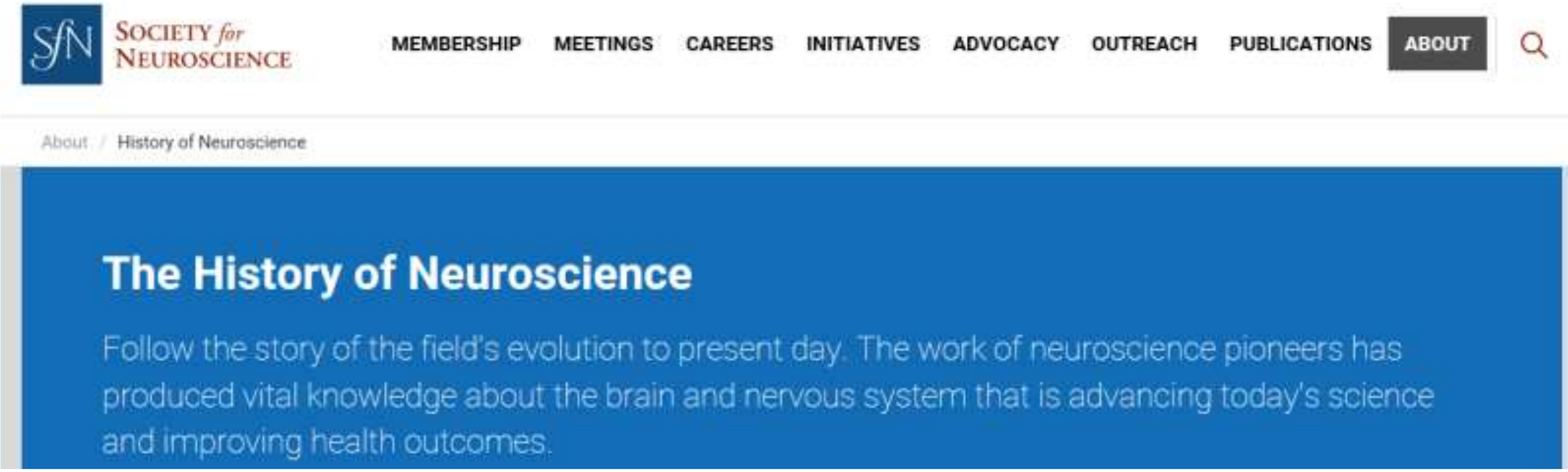


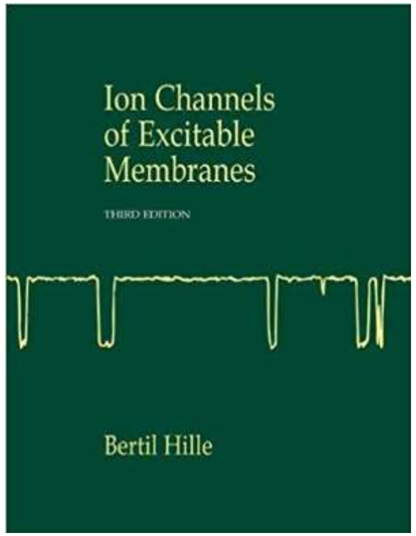


Huang et al., 2004

Recommendation for further reading

- http://www.sfn.org/about/history-of-neuroscience





《膜片钳实验技术》

作　　者：陈军 编 著
出 版 社：科学出版社
出版时间：2001-10
I S B N ：9787030088208

Reference:

Peter Somogyi
GYORGY BUZSAKI
Kenneth Harris
Axon
HEKA

Reference for patch-clamp recording

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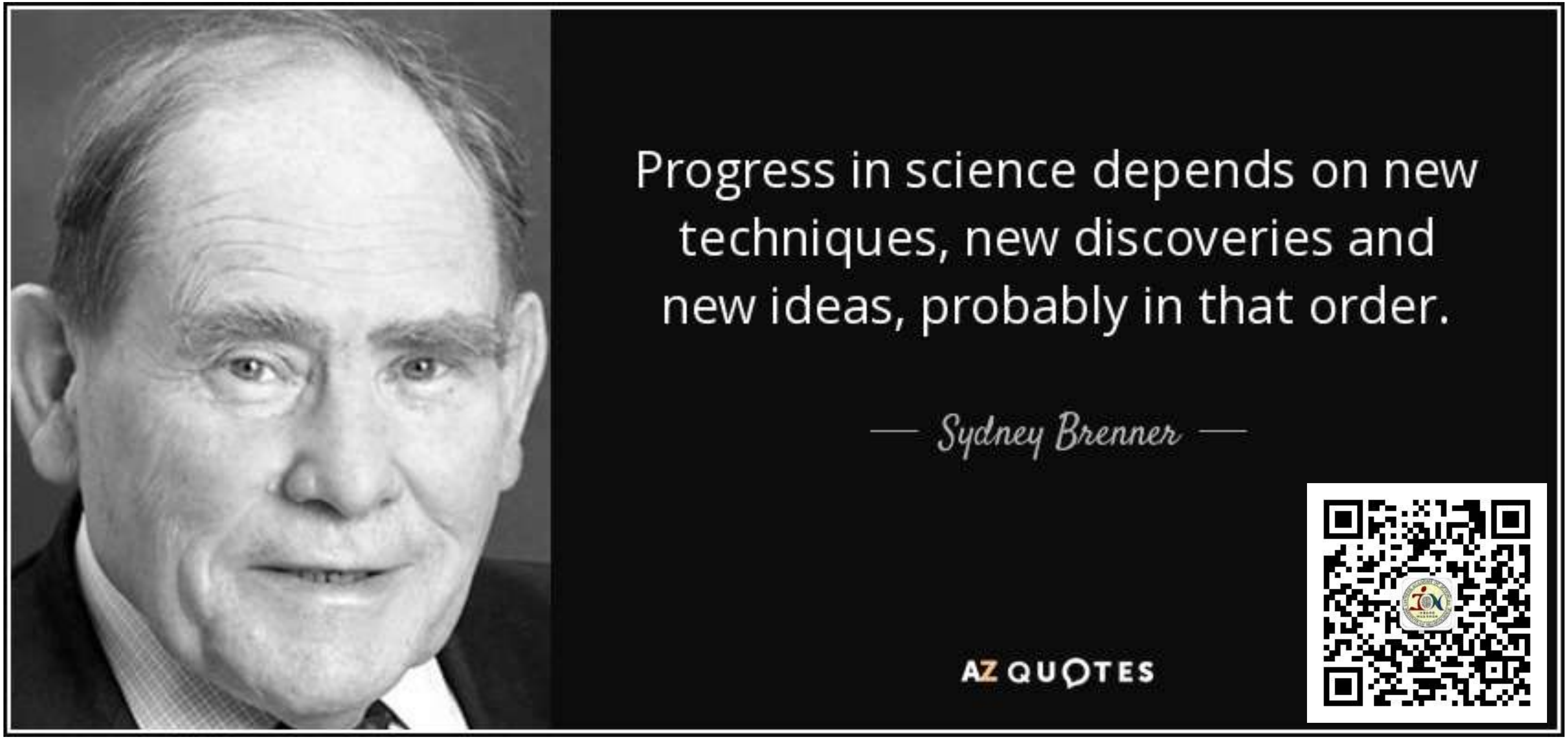
- 10**.** O. P. Hamill, B. Sakmann, Multiple conductance states of single acetylcholine receptor channels in embryonic muscle cells. ***Nature 294, 462 (Dec 3, 1981).***
- 11. O. P. Hamill, A. Marty, E. Neher, B. Sakmann, F. J. Sigworth, Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches**. *Pflugers Archiv : European journal of physiology 391, 85 (Aug, 1981).***


Progress in science depends on new techniques, new discoveries and new ideas, probably in that order.
— Sydney Brenner —
AZ QUOTES

- END -