中国科学院
CHINESE ACADEMY OF SCIENCES

INSTITUTE OF NEUROSCIENCE
中国科学院神经科学研究所

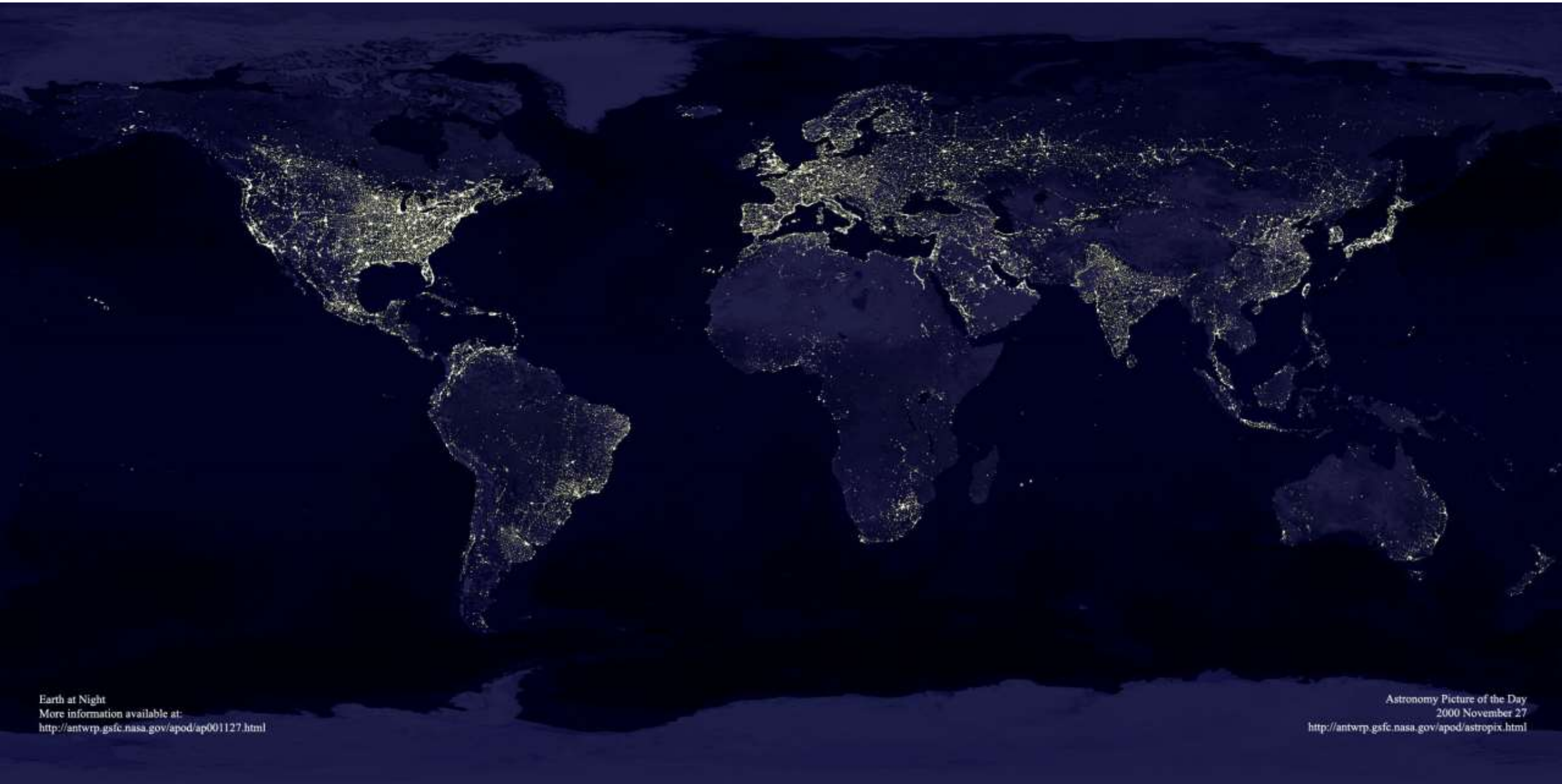
电生理技术

徐春
中科院神经科学所

Menu

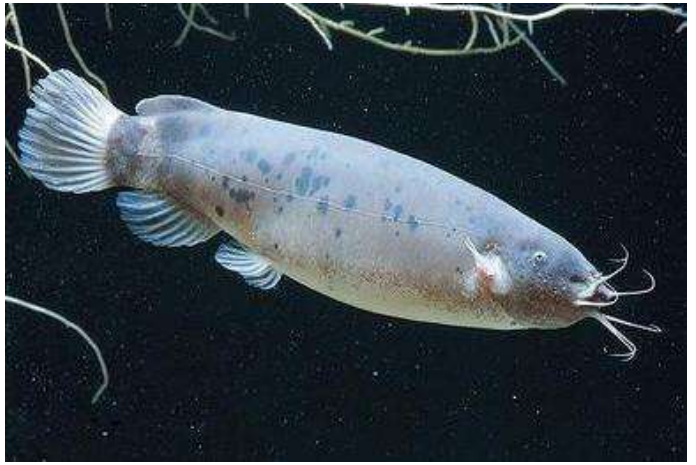
- Why electrophysiology?
- The history and basics of electrophysiology
- Methods in electrophysiology
- Future of electrophysiology

Electricity and human civilization



带电鱼：电鳗、电鳐、电鲶

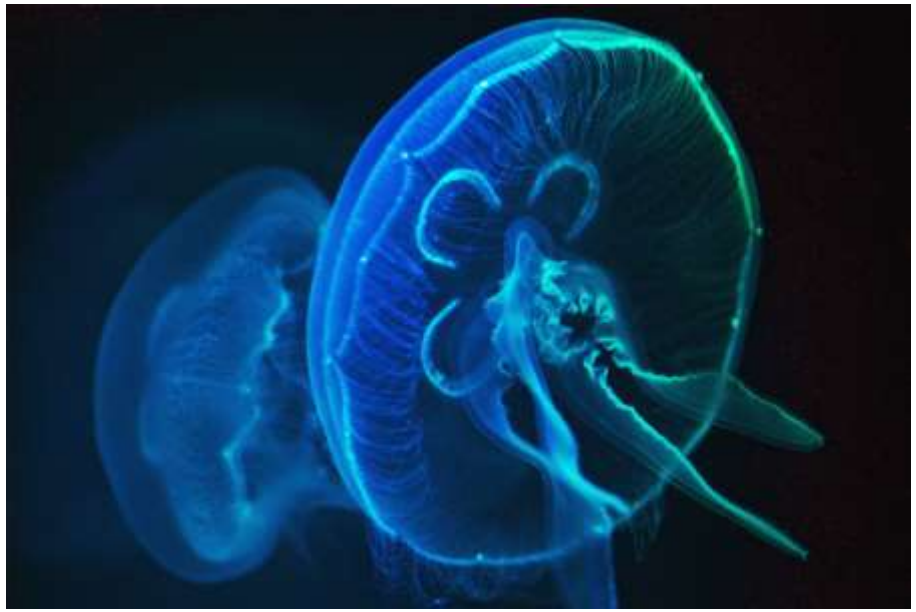
- 电鳗放电电压可达700 ~ 800伏，电鳐放电电压可达300 ~ 500伏。



Electrical signals for sensation

Electrical signals for locomotion

Electrical signals for all kinds of behavior



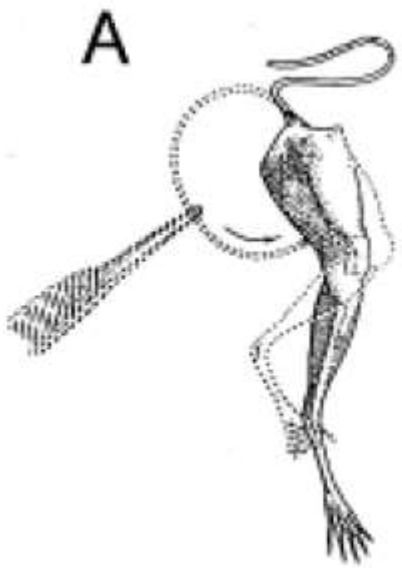
Why electrical signals

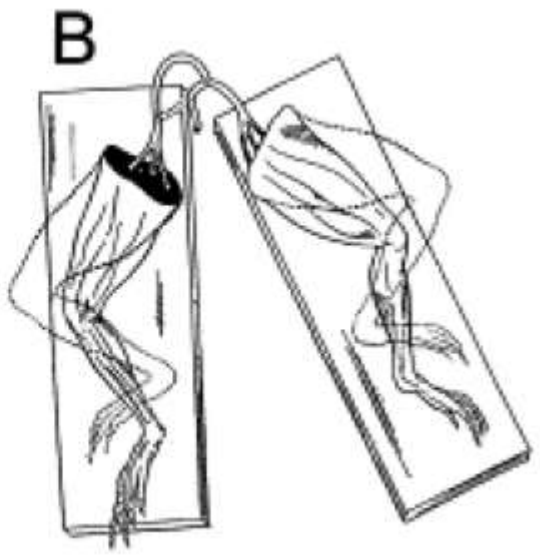
- …

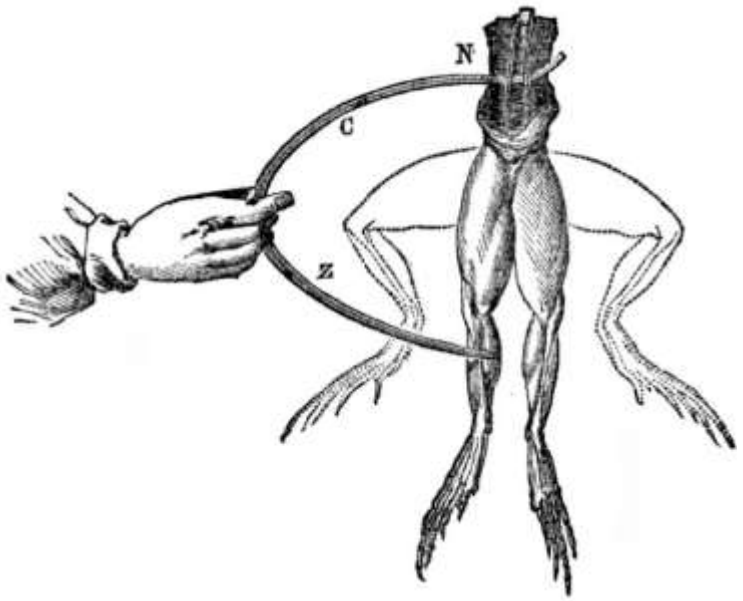
- Why electrophysiology?
- The history and basics of electrophysiology
- Methods in electrophysiology?
- Future of electrophysiology

The muscle contraction is evoked by electrical signals!

Luigi Galvani
1786





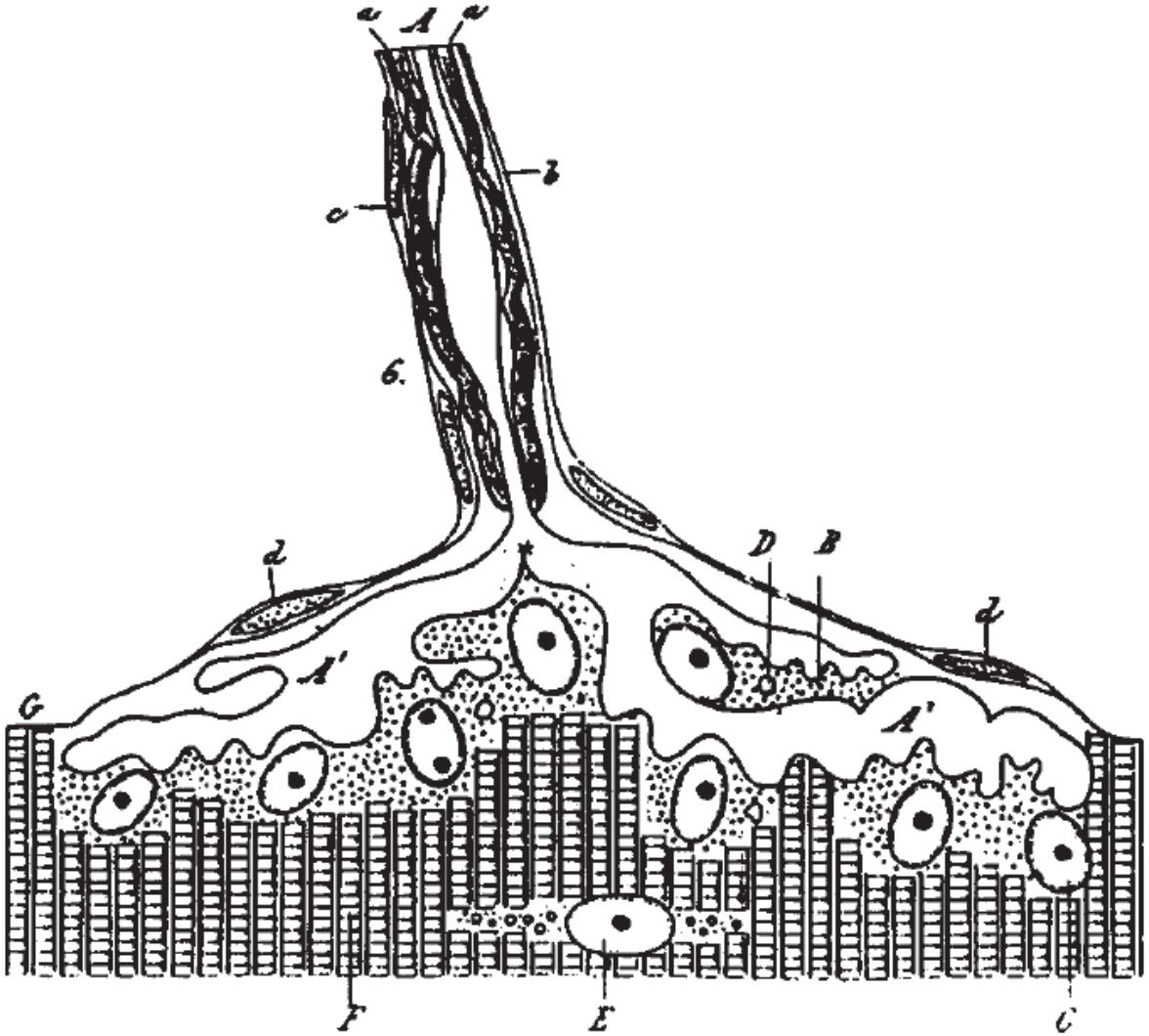


Galvani's experiment demonstrating muscle contraction without using dissimilar substances (metal and tissue). (A) When the surface of section of the nerve touches the muscle, the leg contracts. (B) When the surface of section of the right sciatic nerve touches the intact surface of the left sciatic nerve,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

The muscle contraction is evoked by electrical signals!

Squid Giant Axon and neural muscle junction (NMJ)

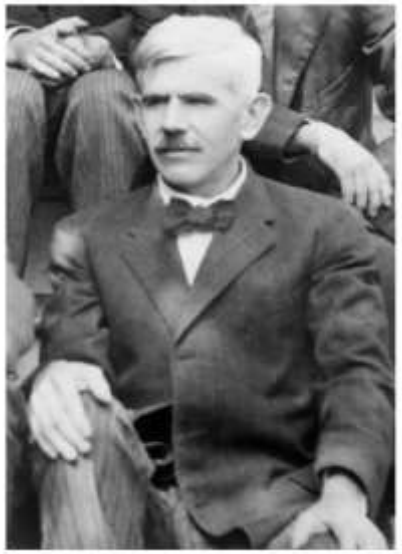
神经肌肉接头利用生物电完成运动



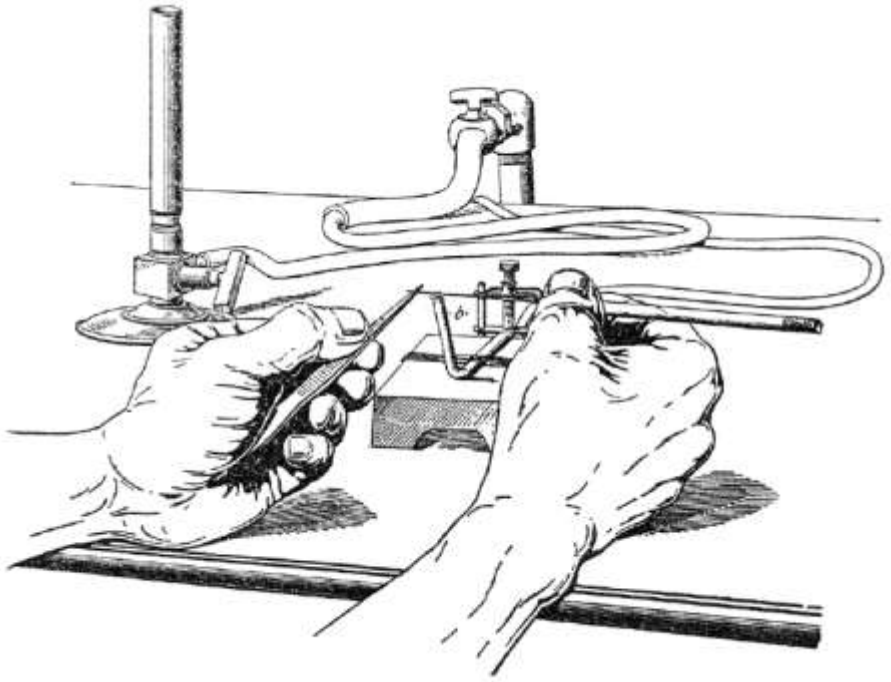
- Still, how is the electrical signals generated and propagated?

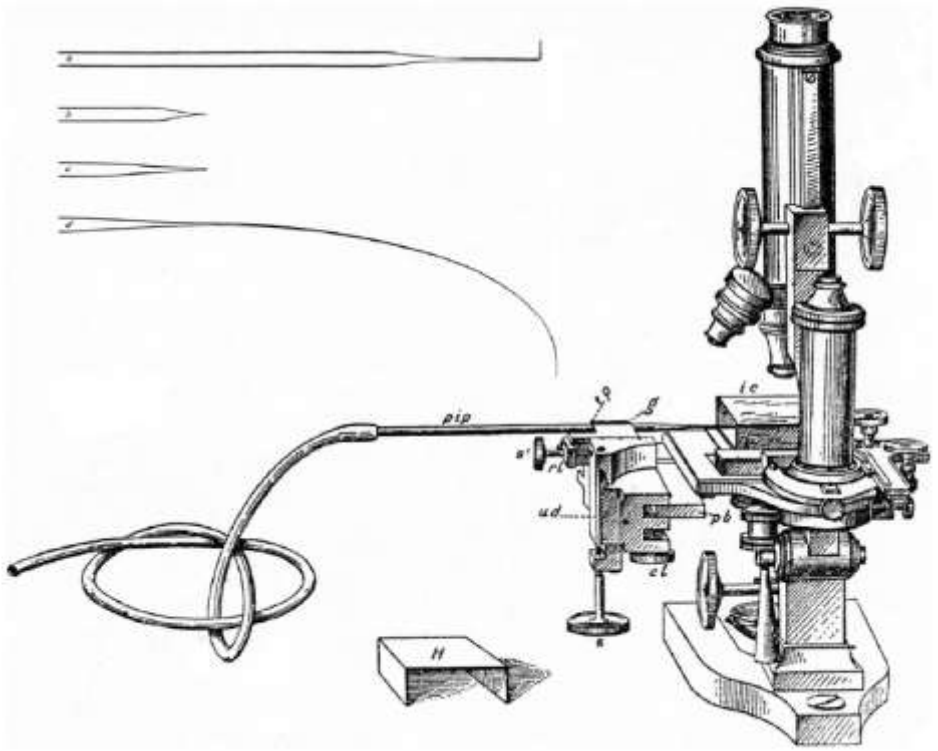
- How to record the electrical signals intracellularly?

Invention of the glass micropipette electrode



Marshall Albert Barber
(circa 1911).





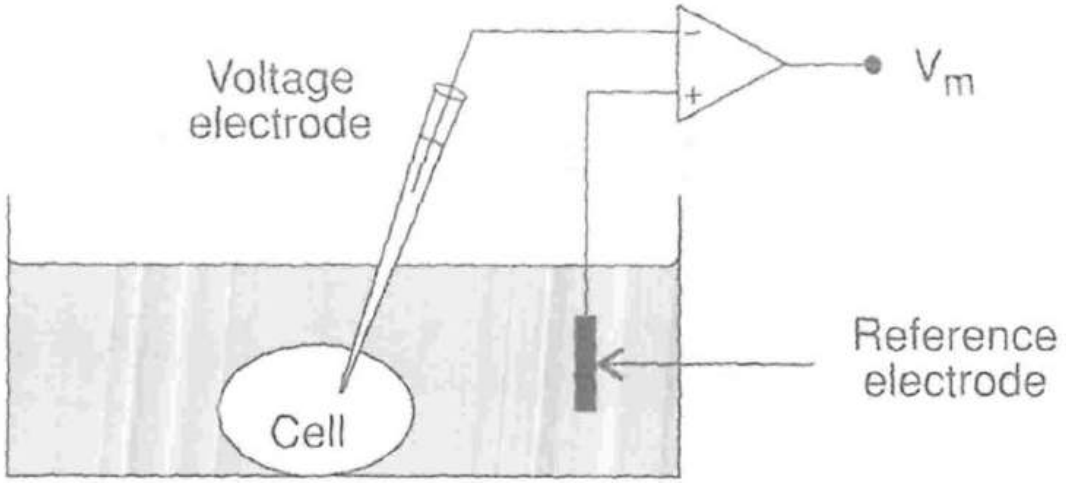
The glass micropipette electrode for intracellular recording

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.

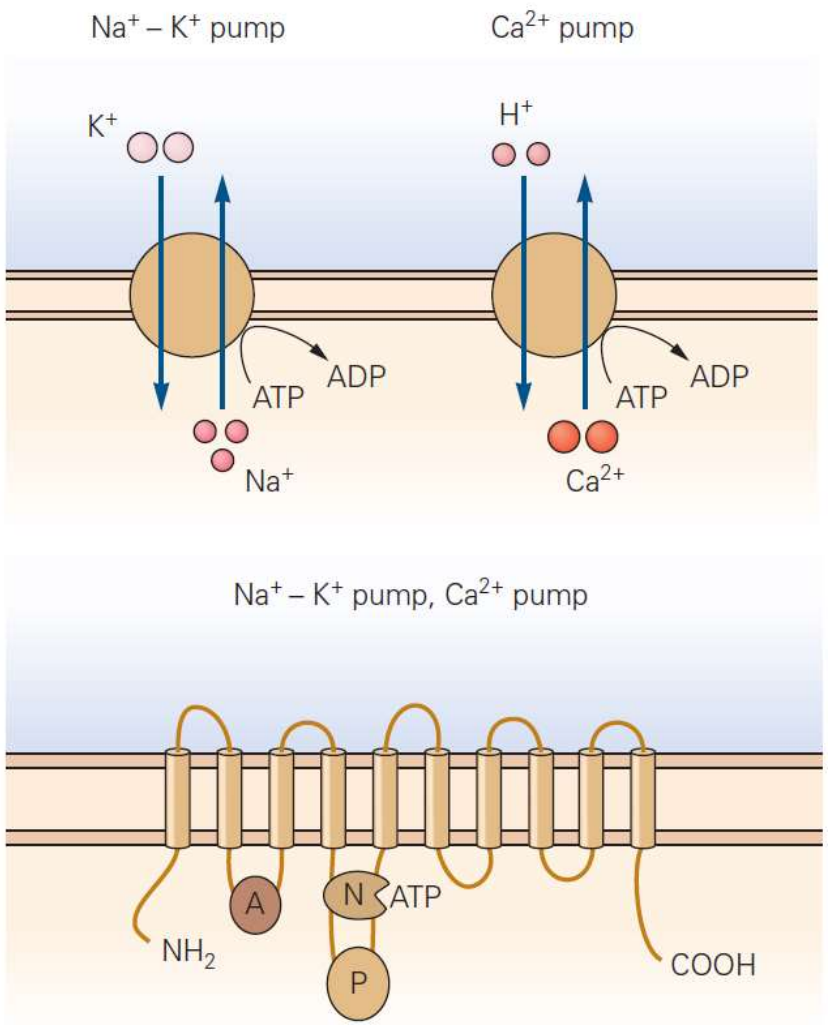
Ketty, Seymour S. (1982).

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.

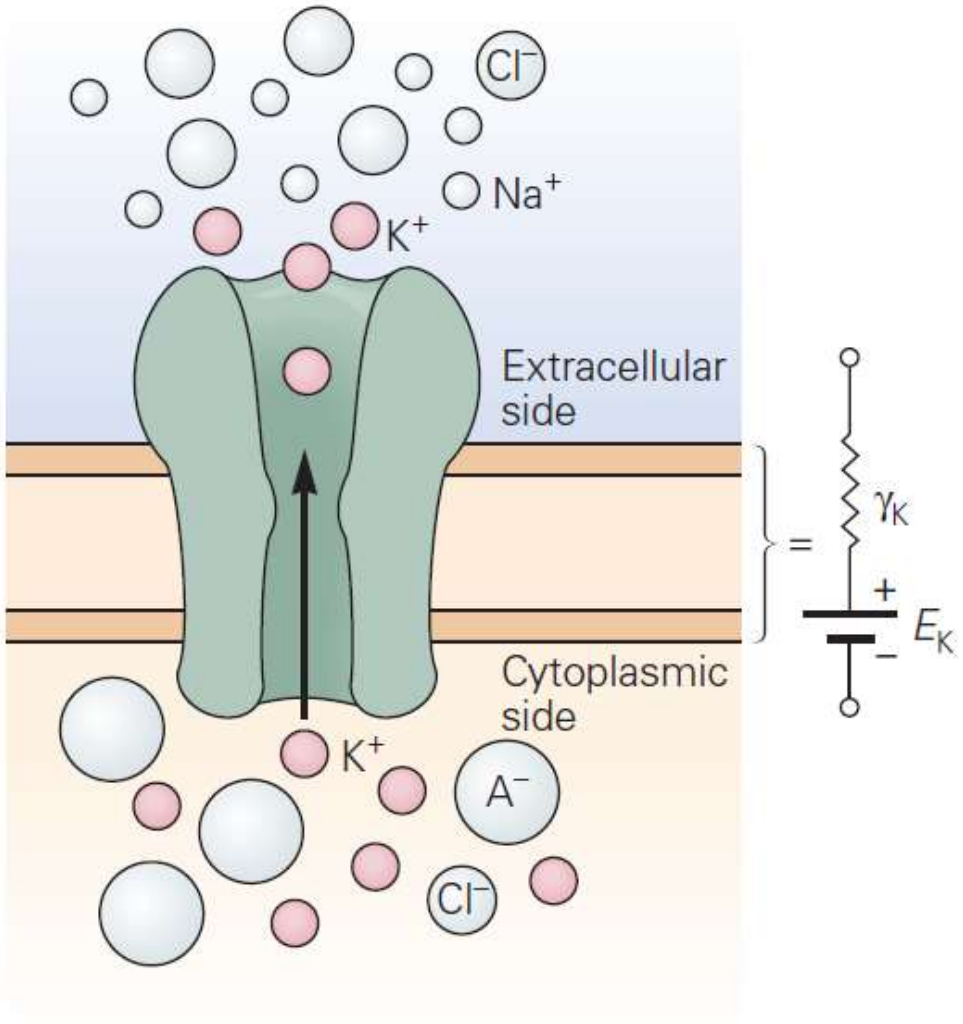
生物电的基础：细胞内外的电势差

生物电的基础：细胞内外的离子流动

Ion pumps



Voltage-gated ion channels



生物电的基础：细胞内外的电势差

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

Here, Vm = Em at resting membrane potential

- E_m = the membrane potential (in volts, equivalent to joules per coulomb)
- P_{ion} = the selectivity for that ion (in meters per second)
- $[ion]_{out}$ = the extracellular concentration of that ion (in moles per cubic meter, to match the other SI units)
- $[ion]_{in}$ = the intracellular concentration of that ion (in moles per cubic meter)
- R = the ideal gas constant (joules per kelvin per mole)
- T = the temperature in kelvins
- F = Faraday's constant (coulombs per mole)

Goldman Equation

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

Here, Vm = Em at the reversal potential

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

Driving force

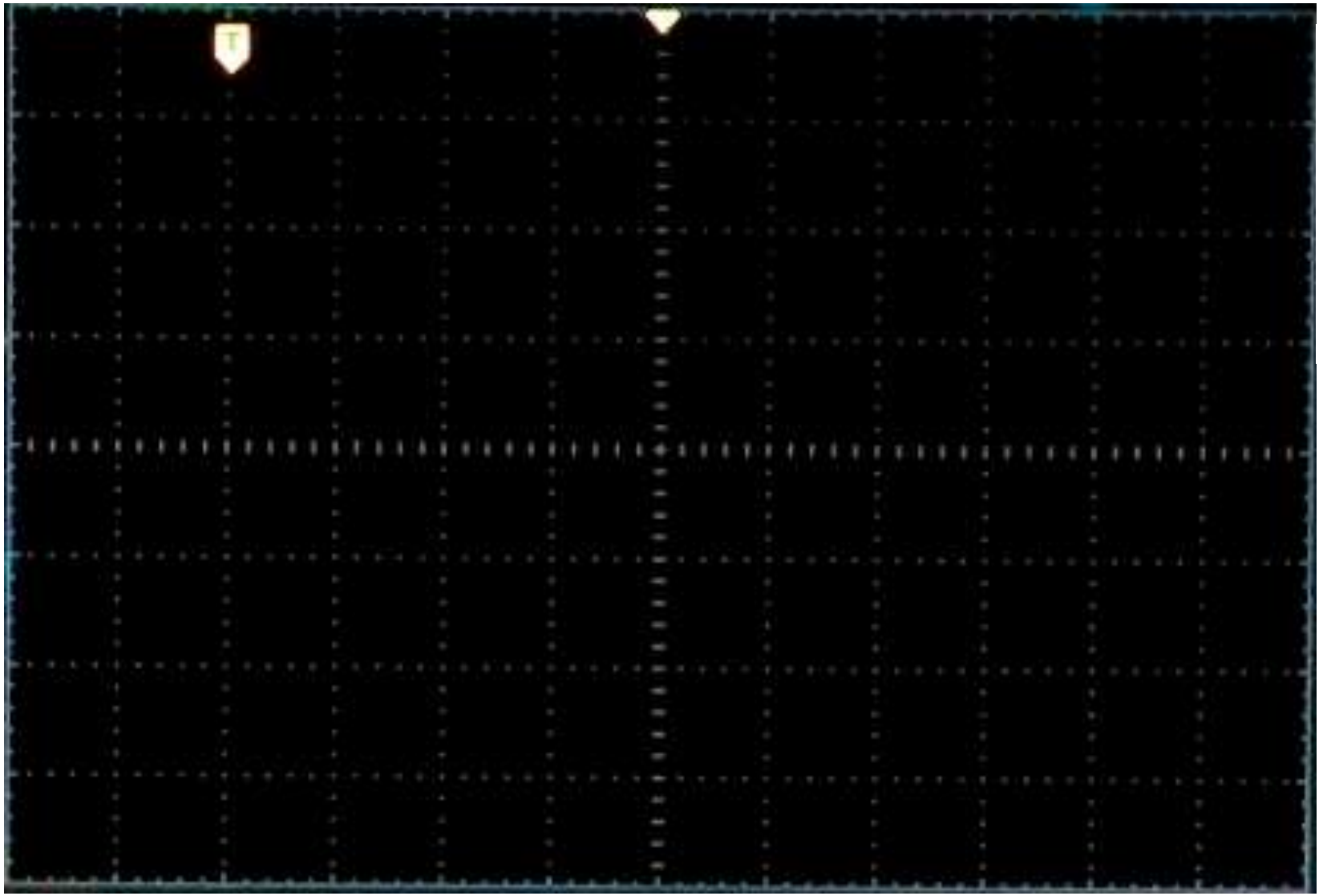
$$i_K = (\gamma_K \times V_m) - (\gamma_K \times E_K) = \gamma_K \times (V_m - E_K).$$

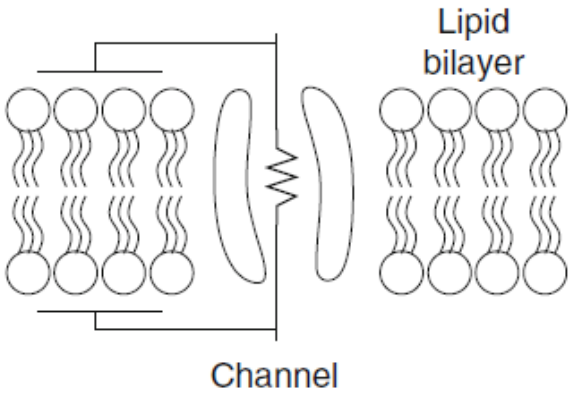
Driving force = Conductance multiplied by Reversal potential

Cell & Ion

- Muscle contraction by electrical signals
- Ion channels (voltage-gated, ligand-gated)
- **Goldman Equation** for membrane potential
- Resting membrane potential
- Reversal potential ($\mathbf{E_{REV}}$)
- Driving force for channels/ion

细胞膜的电学特性

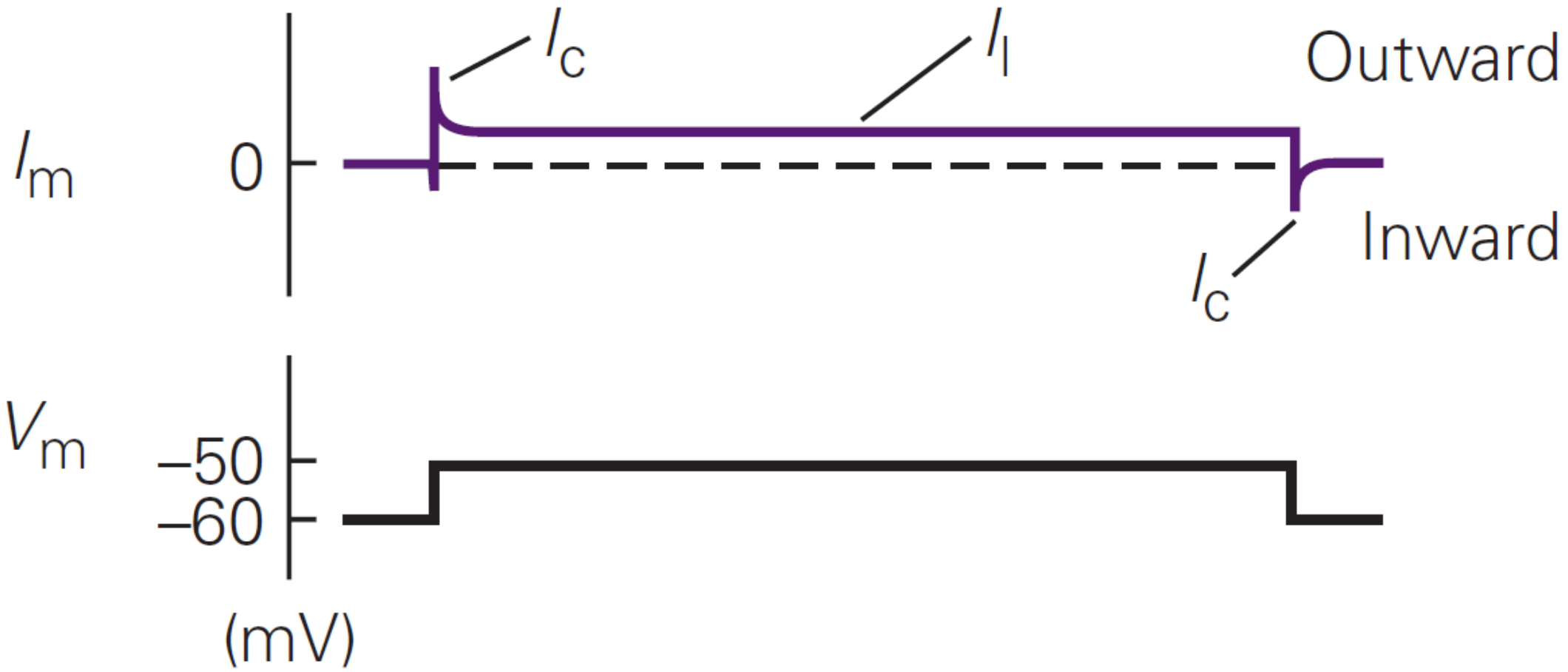




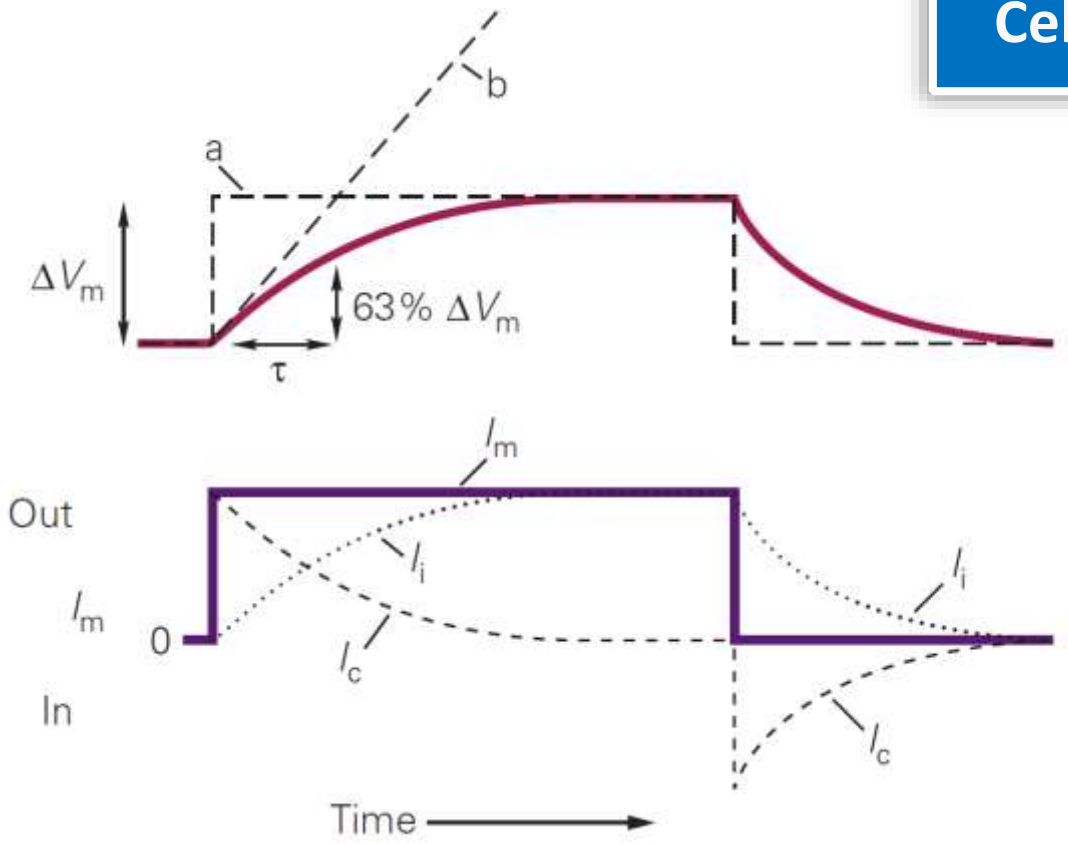
Membrane
Conductance

Ion channels
Receptors

细胞膜的电学特性

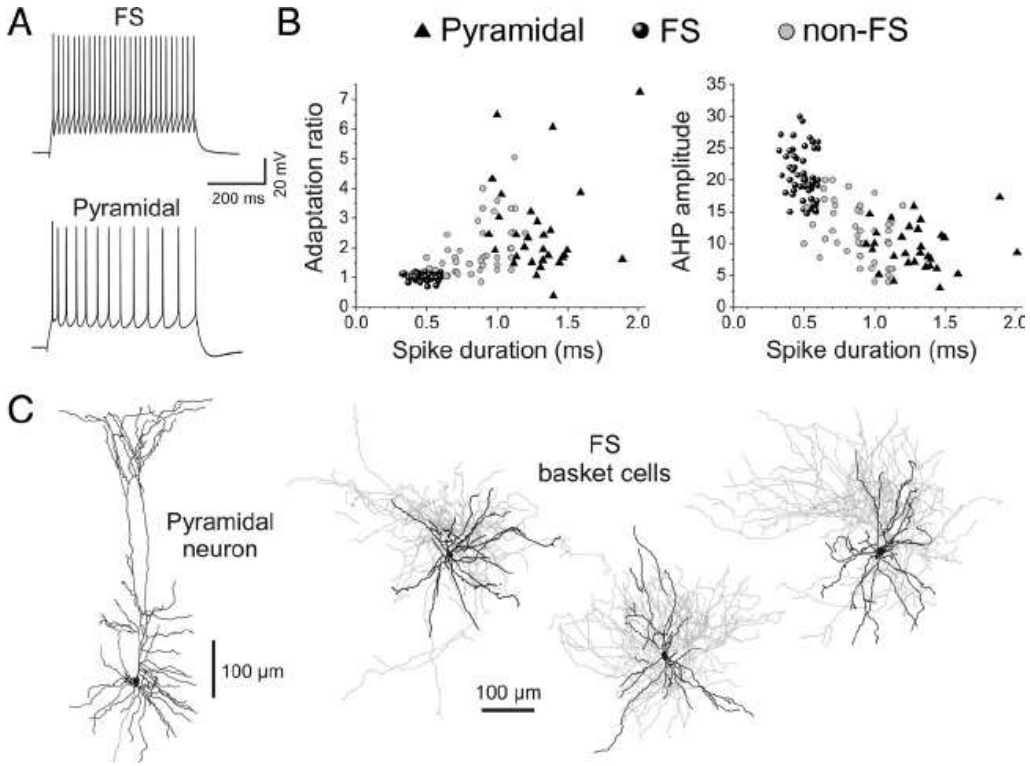


细胞膜的电学特性

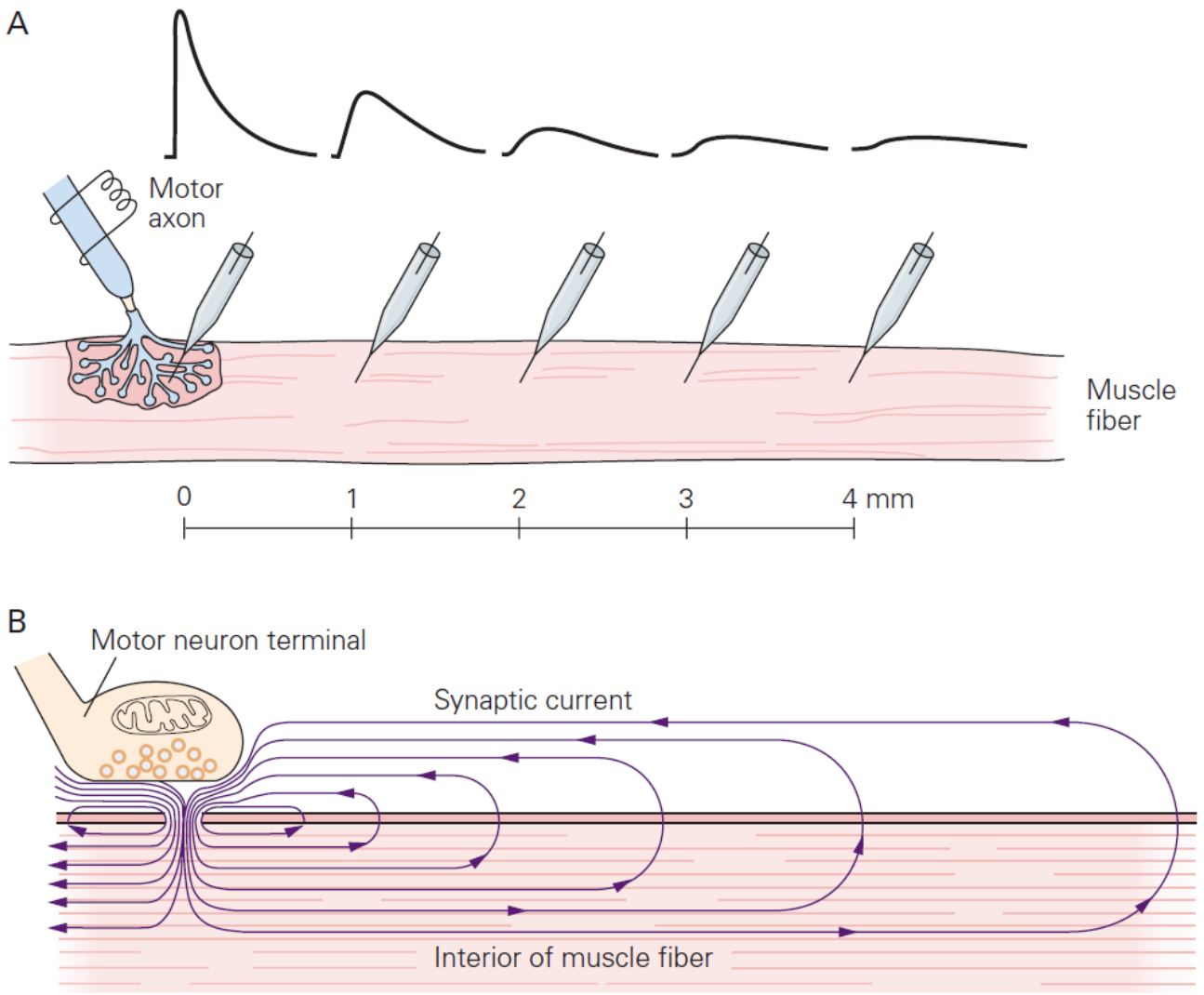


Cell type

- Membrane conductance (intrinsic properties)
- Ion channel types
- Ion channel distribution
- Cell morphology



The end-plate potential (EP) passively propagates



Still, how is the electrical signals generated and propagated?

Squid Giant Axon Recording

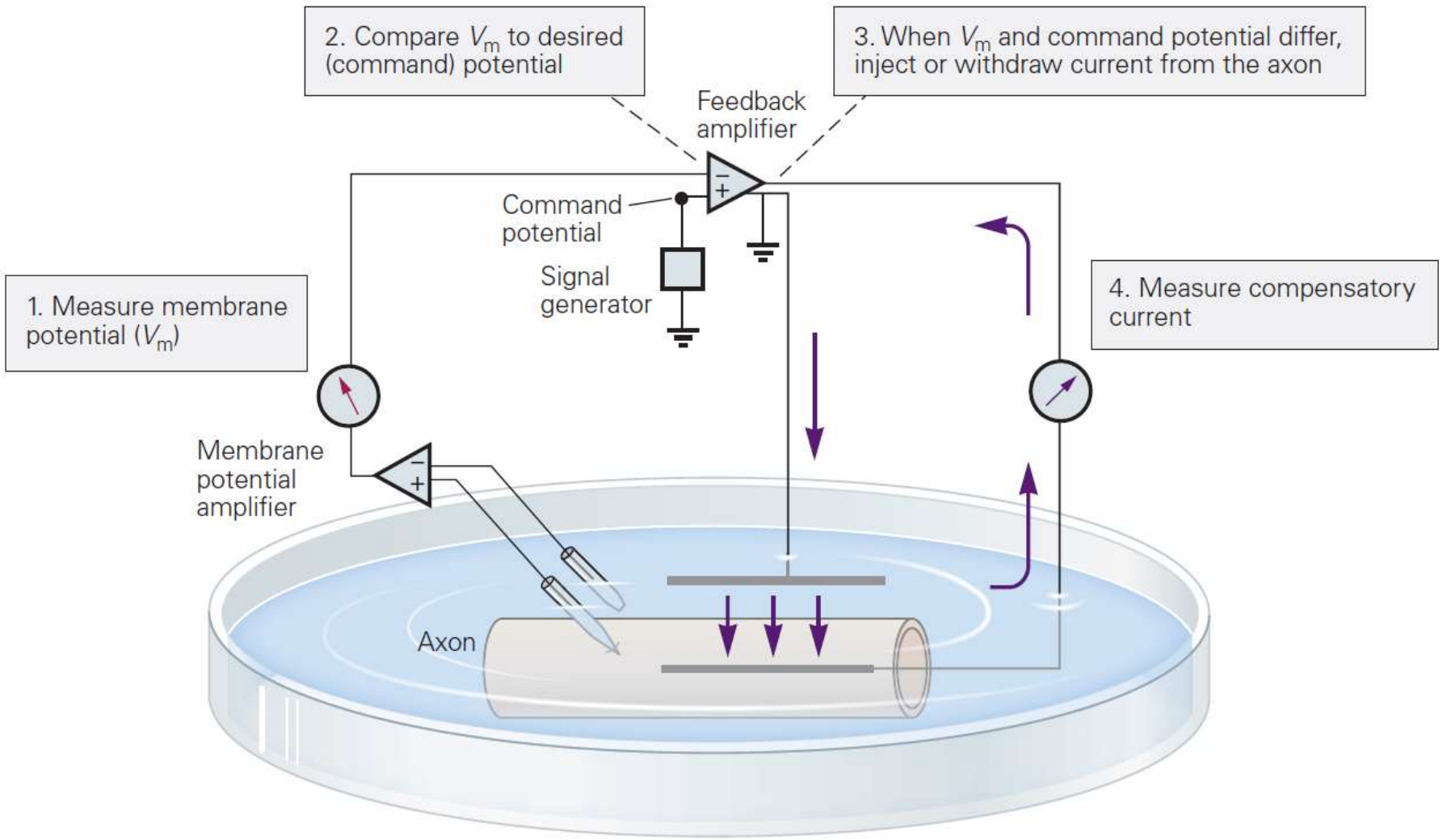
All or none.
Threshold.

Largest axon
Fasted travel

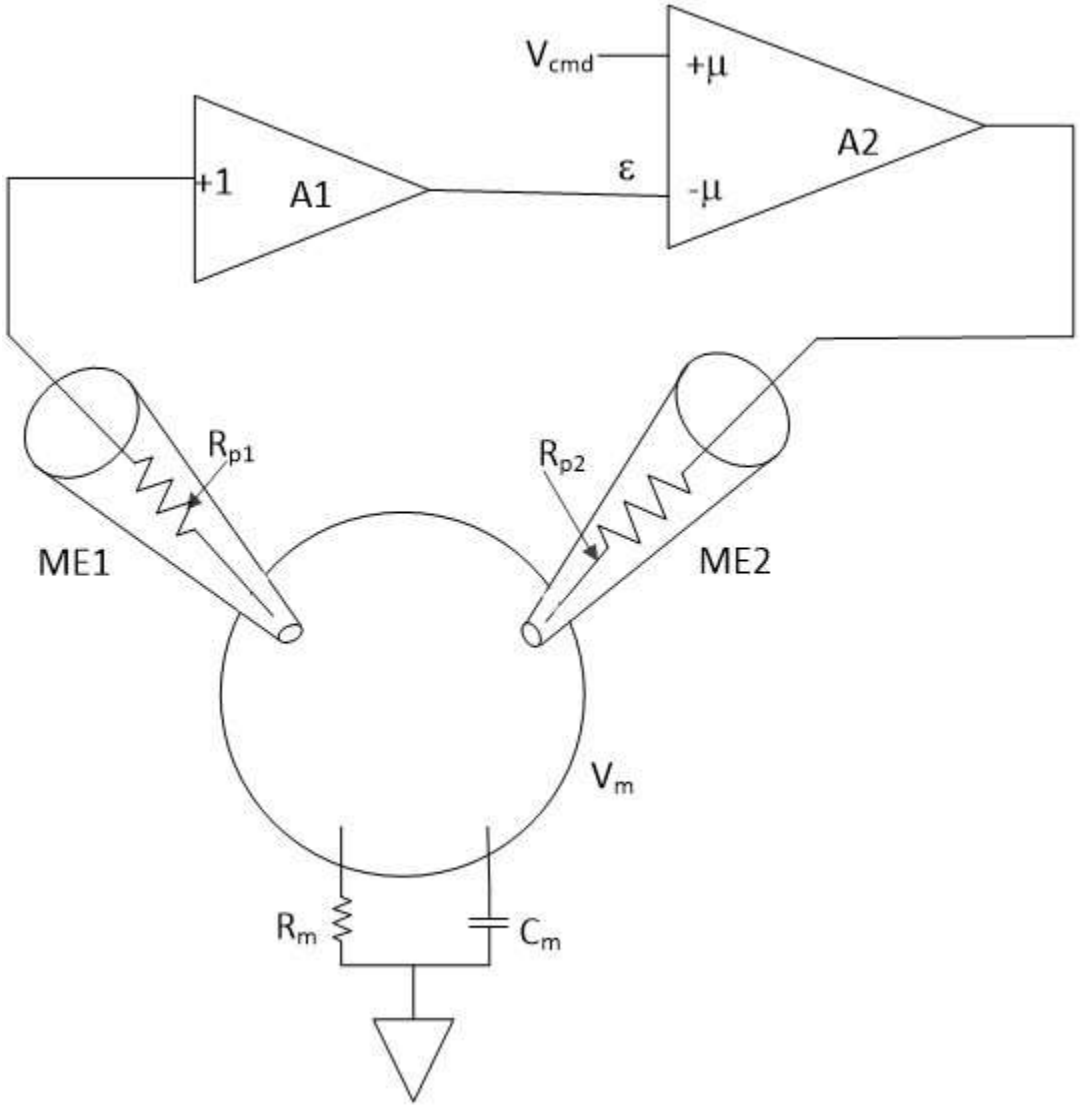
Simultaneously

Since the axon is controlling the muscle contraction, shall we record from them?

Voltage clamp recording

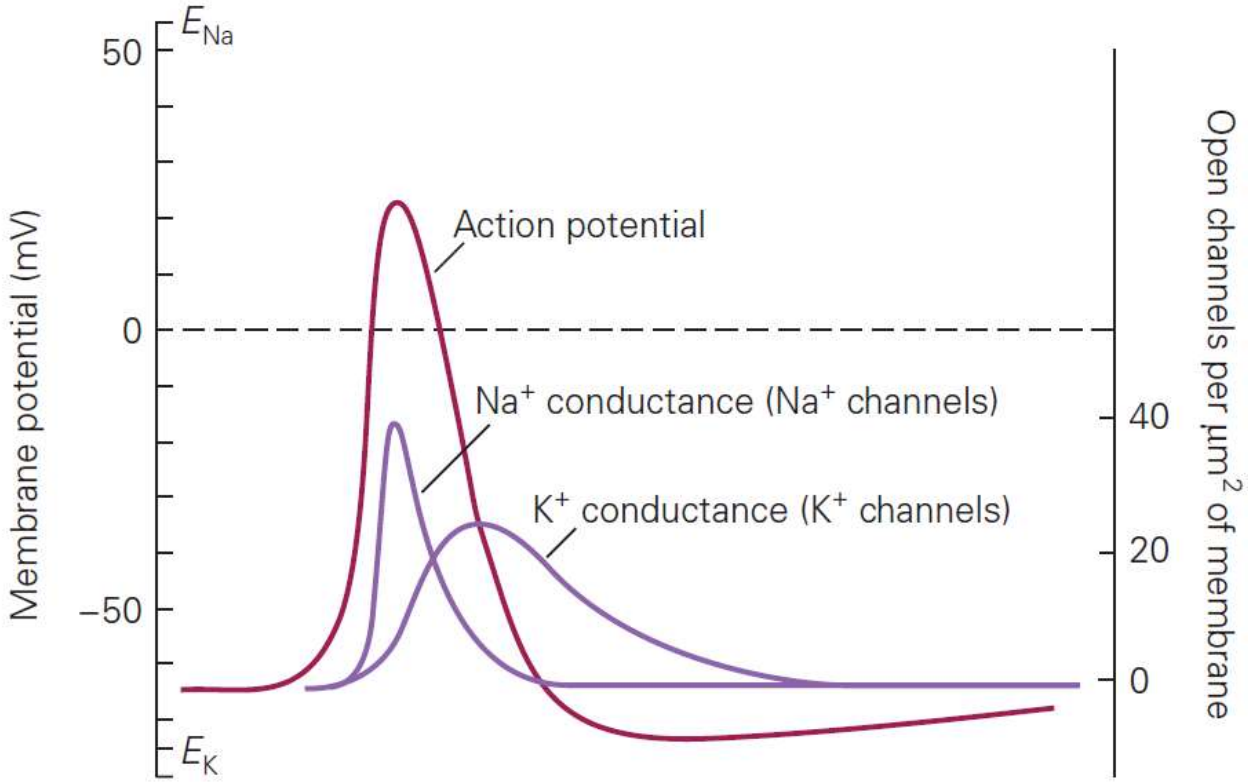


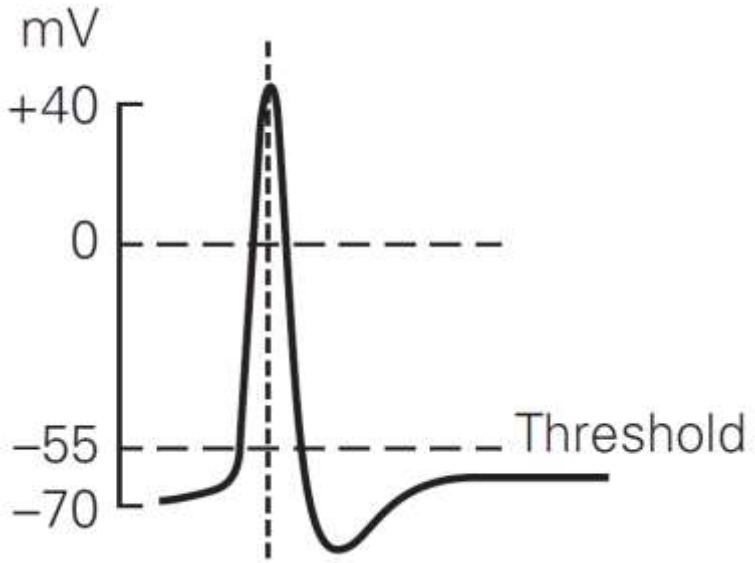
Voltage clamp recording



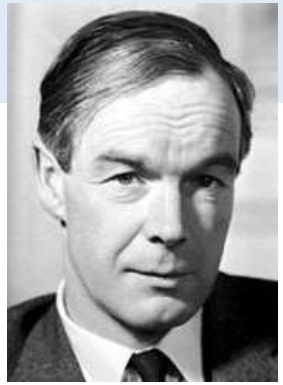
Voltage clamp recording

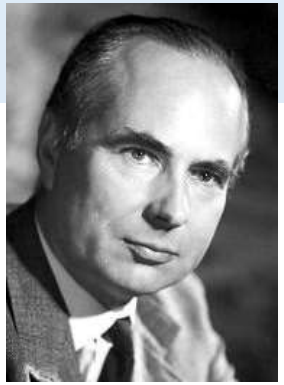
Waveform for action potential





Squid Giant Axon in electrophysiology

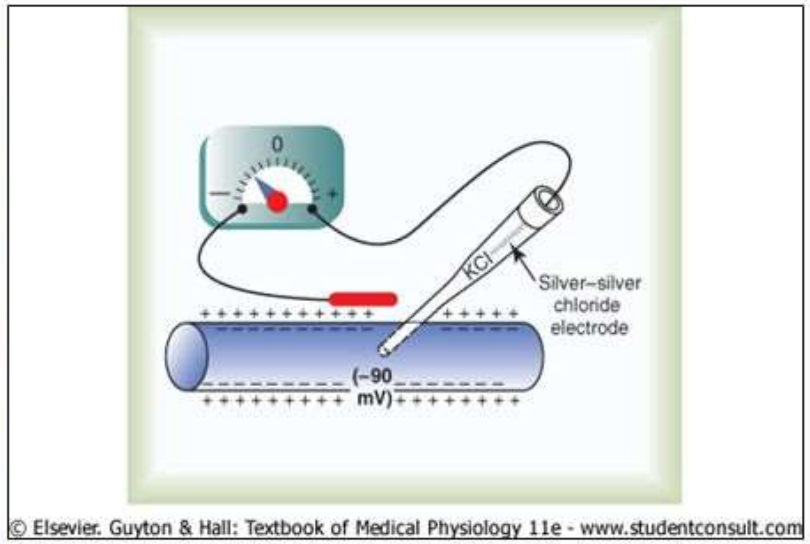


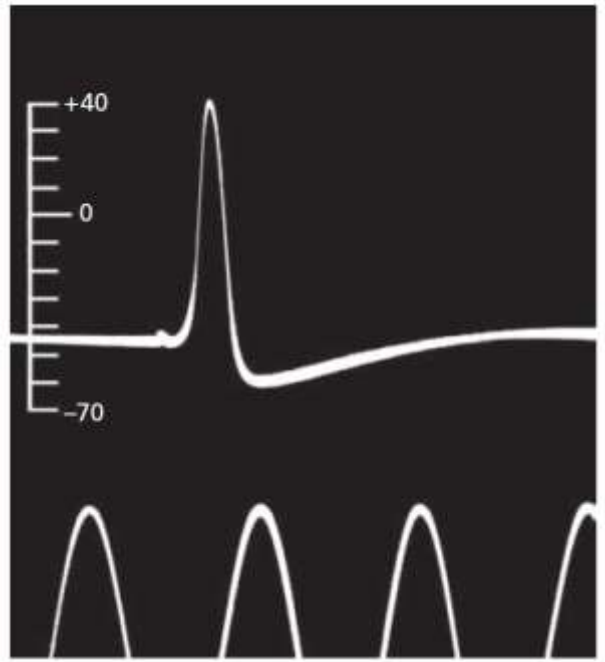


Alan Hodgkin Andrew Huxley

Hodgkin-Huxley Expts, 1952

Squid Giant Axon





1940s

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

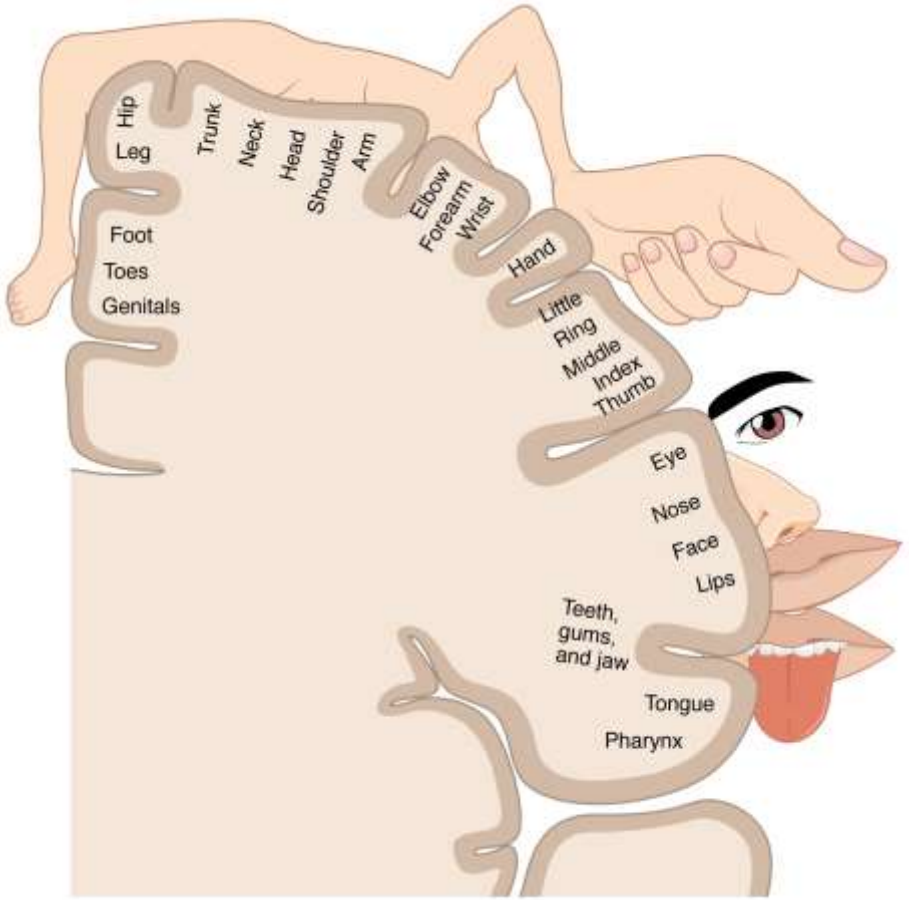
Refractory period of action potentials

Touch is transmitted by electrical signals!

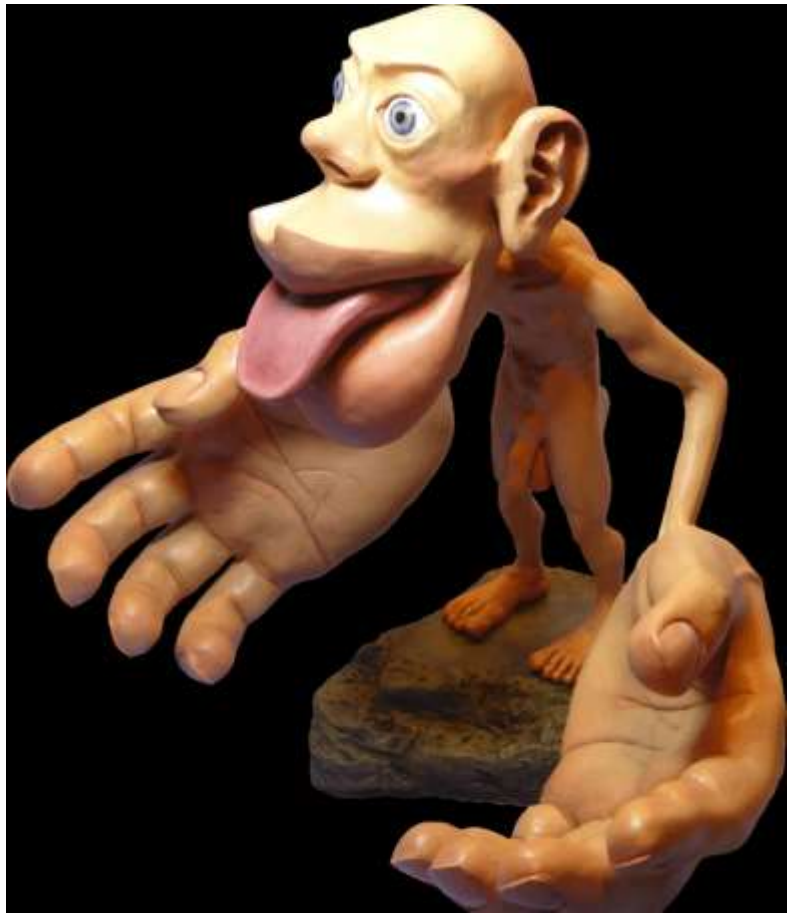
Cortical homunculus ("cortex man“)

Wilder Penfield

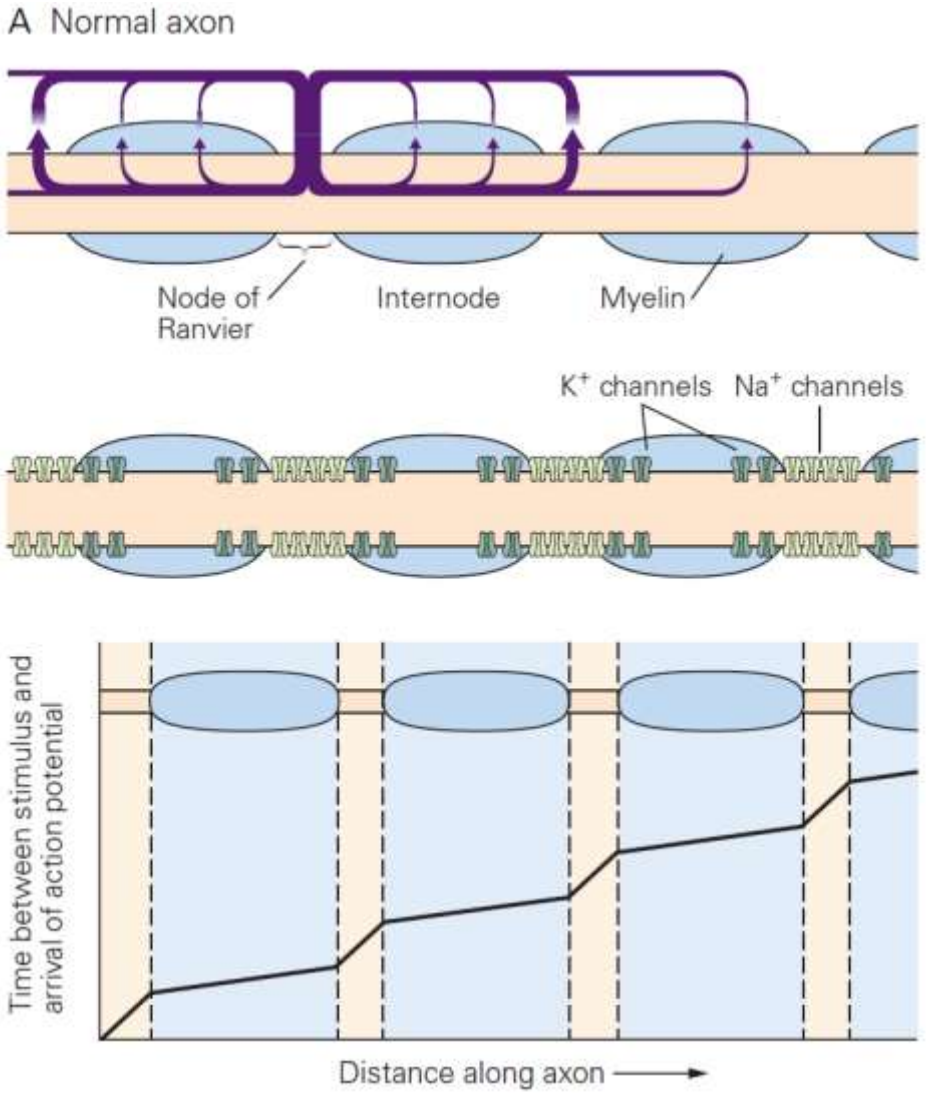
Touch feeling is evoked by electrical signals!



Penfield, 1930s



Action potentials are regenerated at the nodes of Ranvier



Action Potential

- End-plate potential
- Voltage clamp
- Action potential
- All-or-none
- Threshold
- Na+ conductance
- Kinetics
- Waveform
- Refractory period
- Nodes of Ranvier

Keywords

Cell & Ion

- Muscle contraction by electrical signals
- Ion channels (voltage-gated, ligand-gated)
- **Goldman Equation** for membrane potential
- Resting membrane potential
- Reversal potential (E_{REV})
- Driving force for channels/ion

Action Potential

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Cell type

- Membrane conductance (intrinsic properties)
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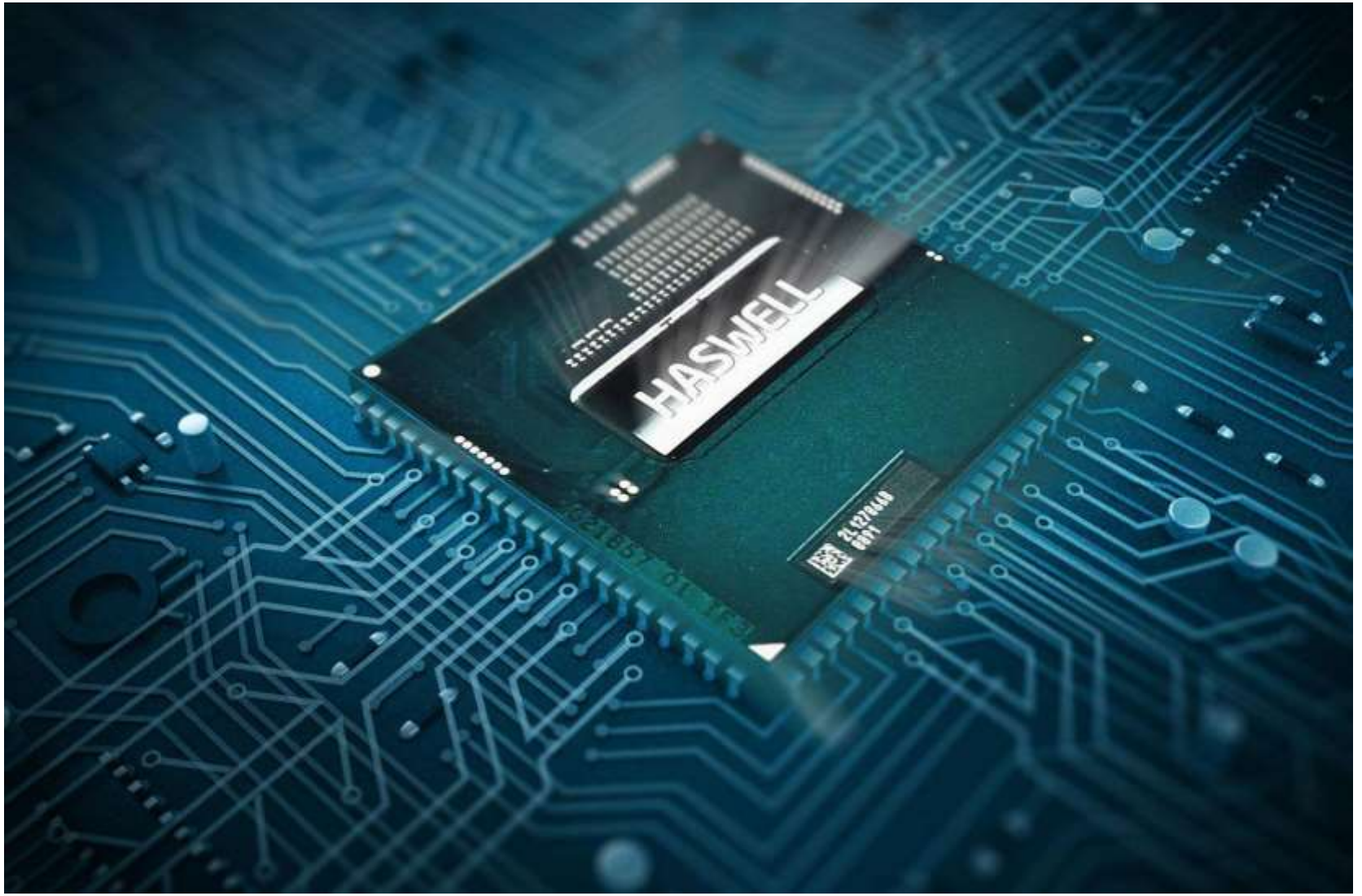
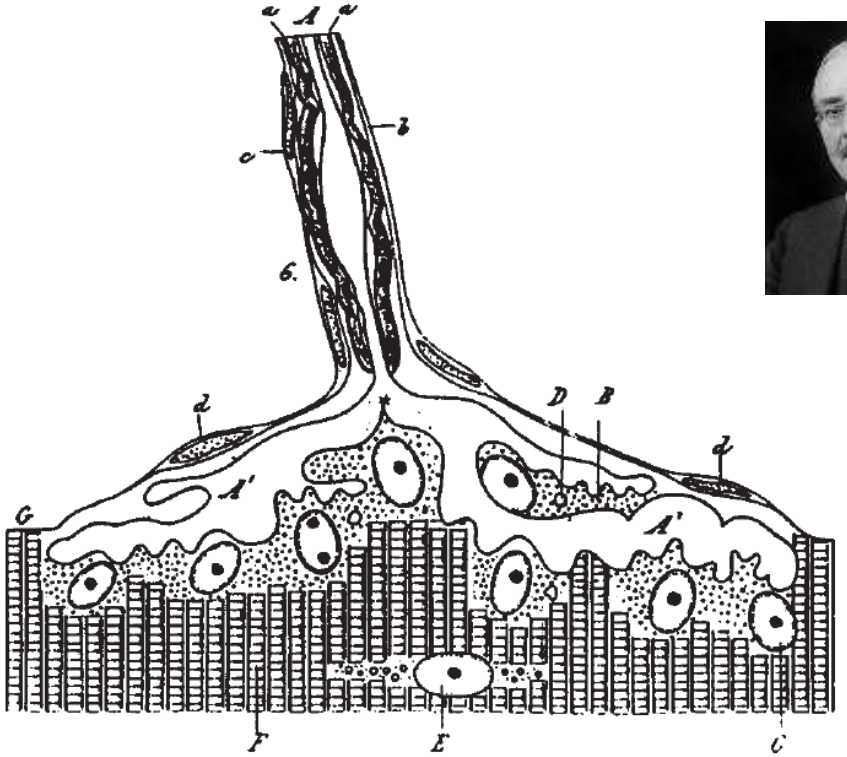
- Ion channel distribution
- Cell morphology

基因 – 分子 – 神经元 – 神经环路 – 行为



The term Synapse by Sherrington in 1897



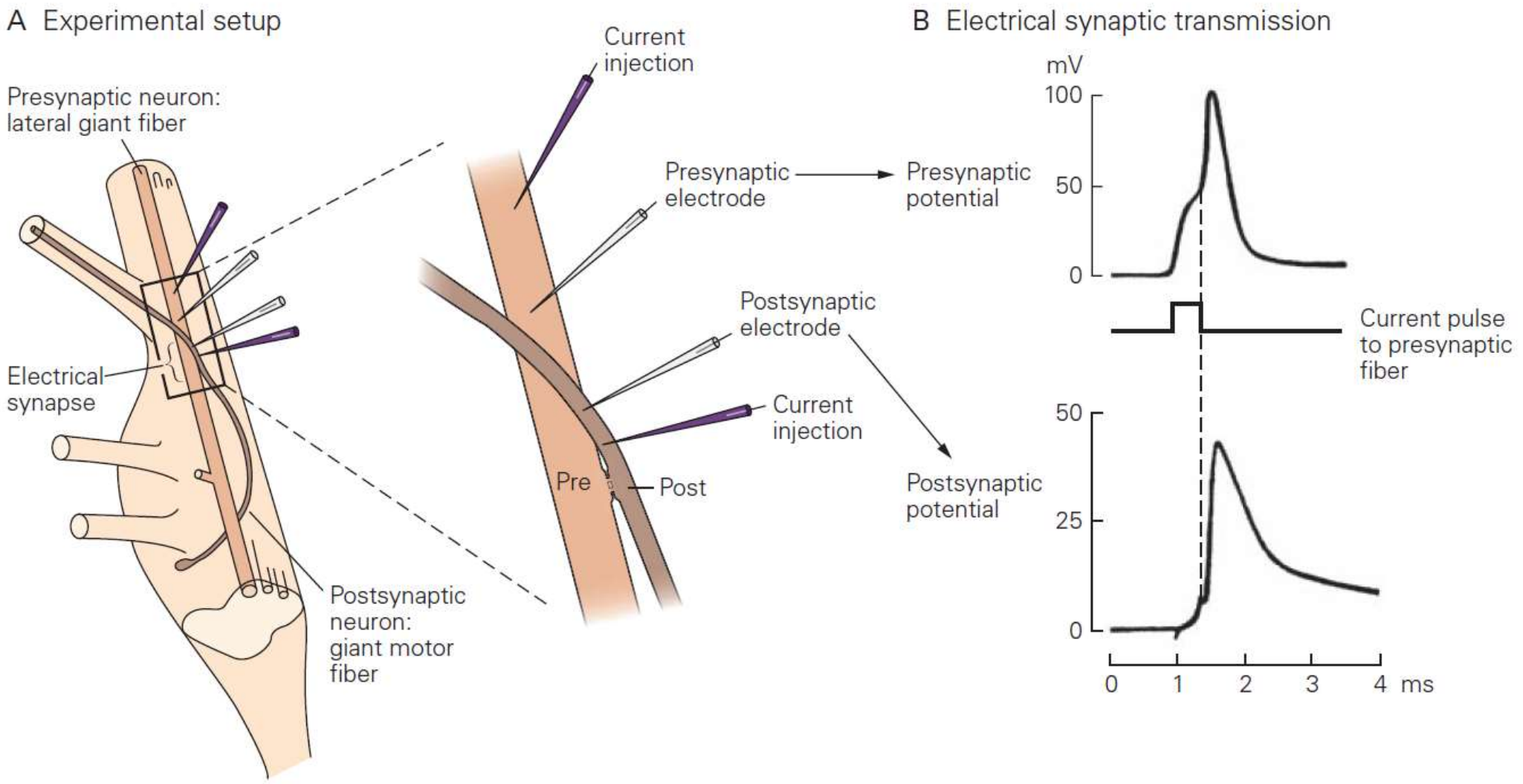
Schematic summary view of the mammalian neuromuscular junction.

'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

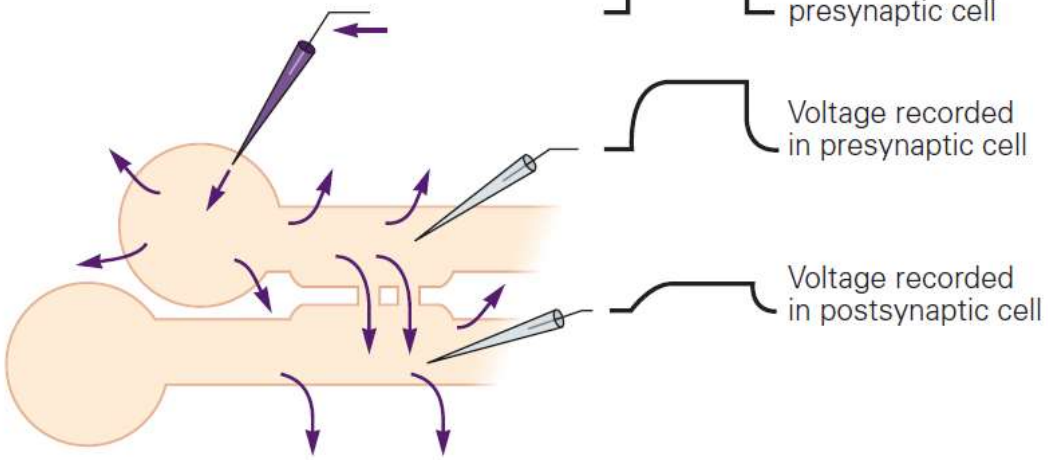
While Ramóny Cajal was laying the anatomical basis for modern neuroscience, Sherrington's work was laying the basis for the physiological principles

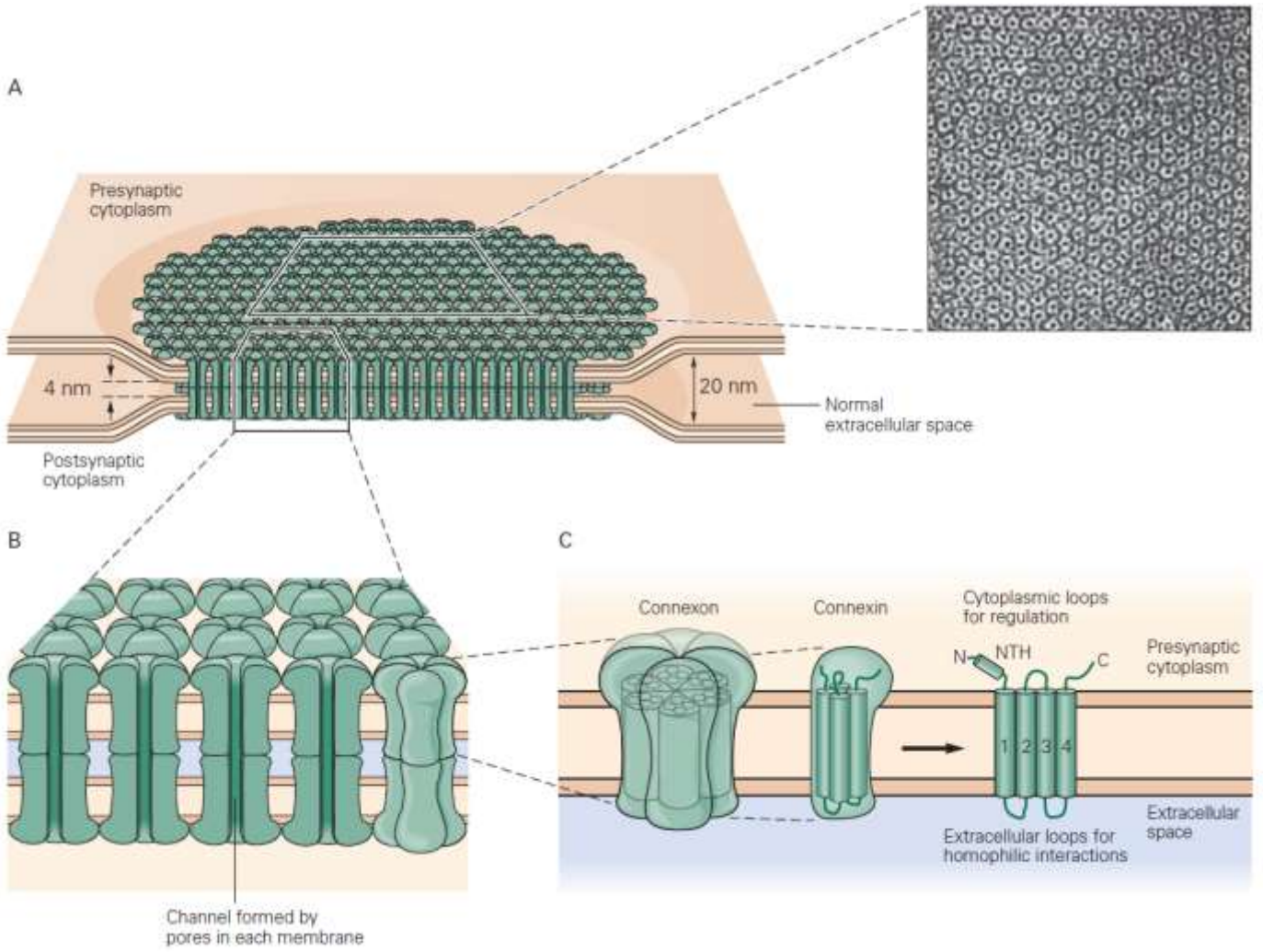
Electrical synaptic transmission



Furshpan and Potter 1957 and 1959

Electrical synaptic transmission

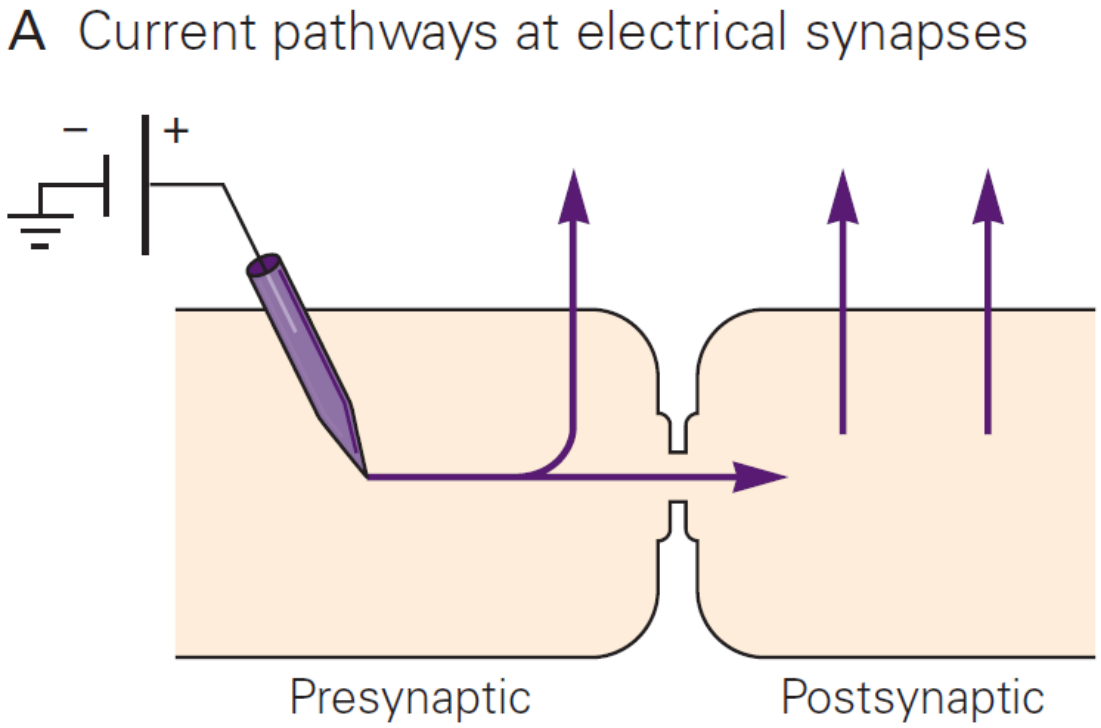




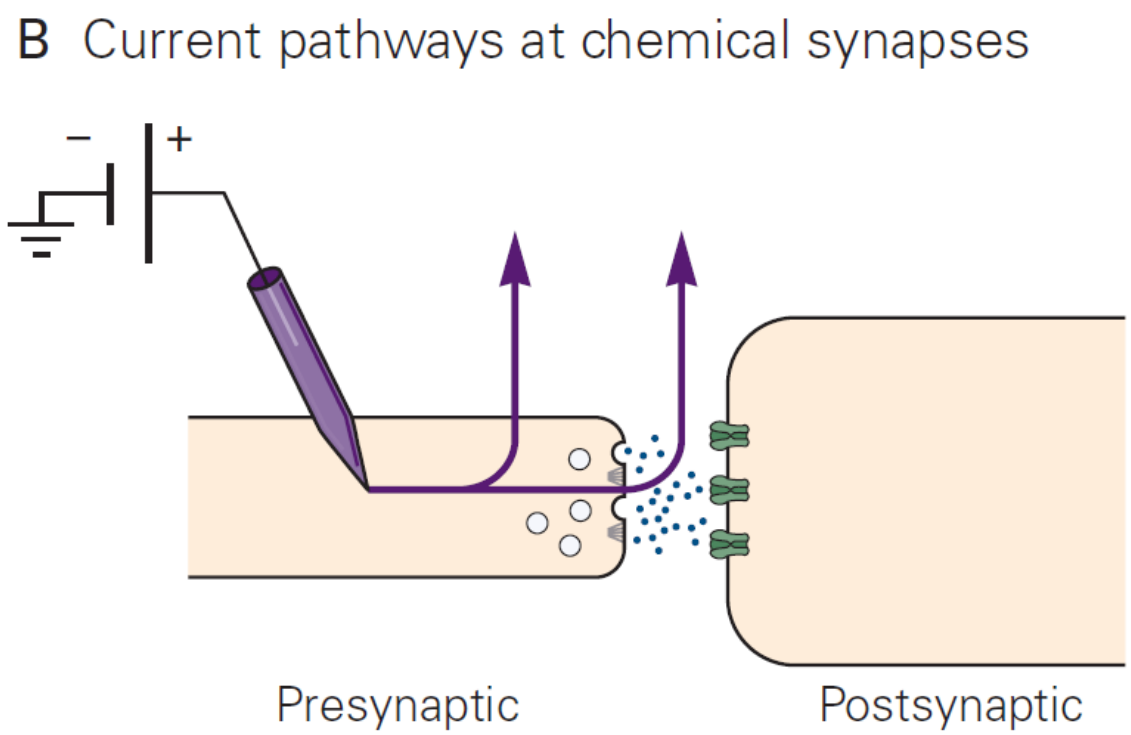
Makowski et al. 1977

Unwin and Zampighi 1980

Neurons communicate through Synapses

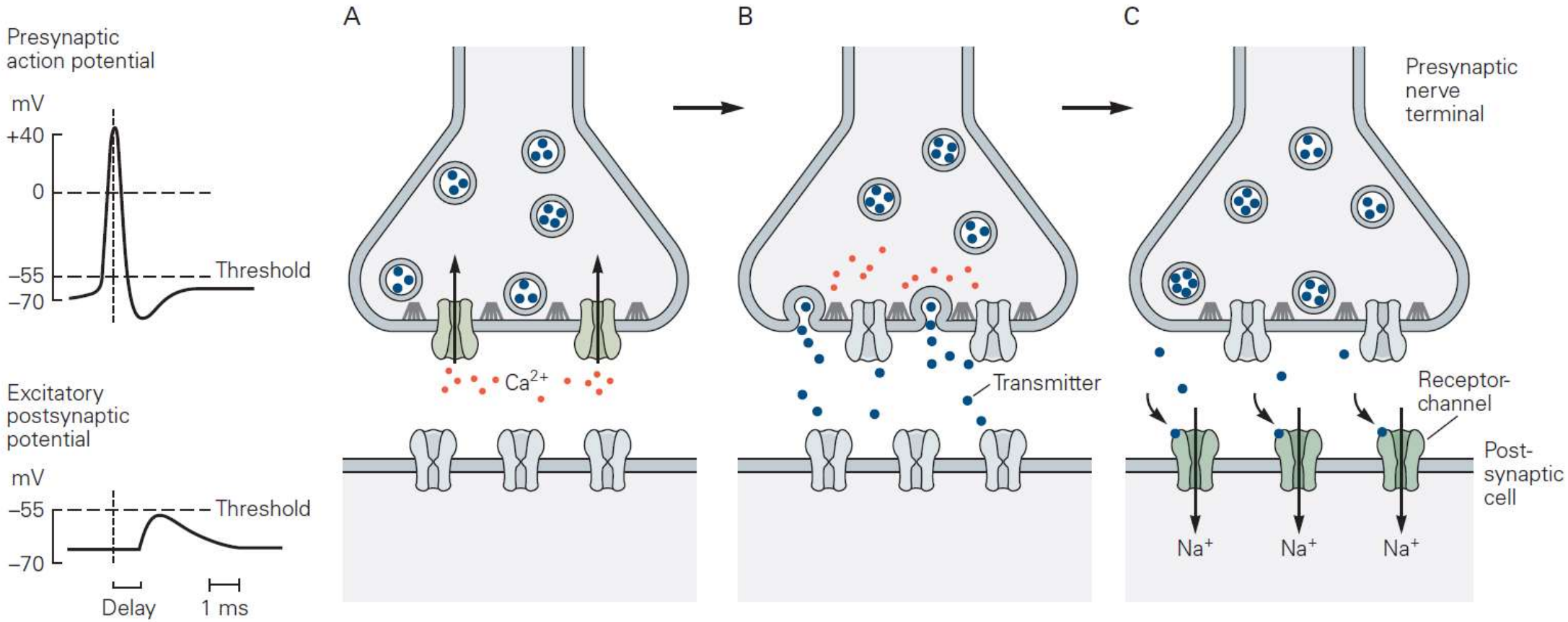


John Eccles

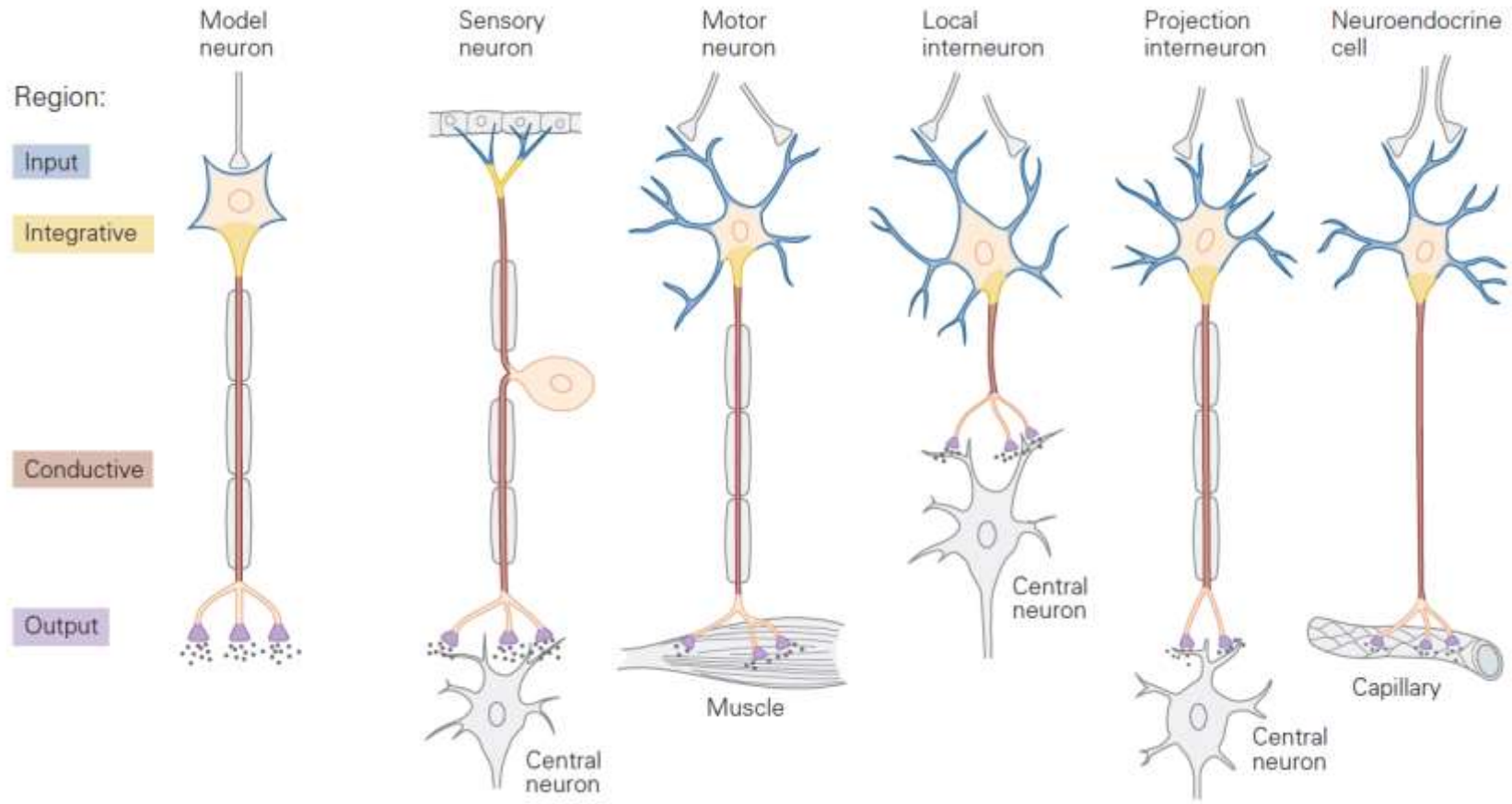


Dale and others

Synaptic transmission at chemical synapses



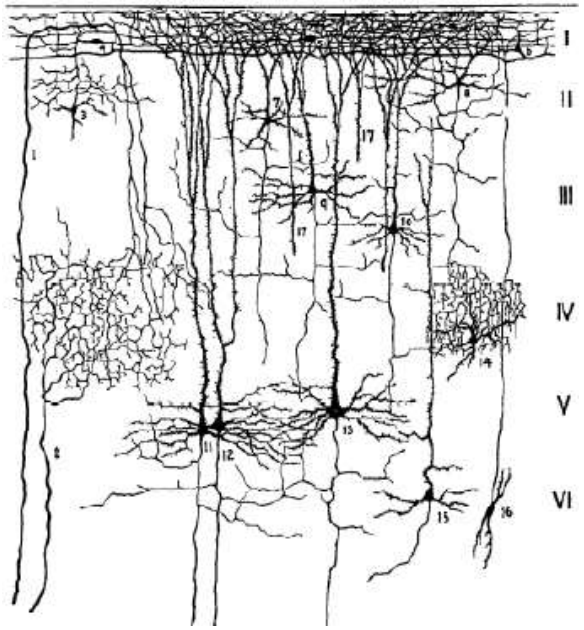
Four regions of a model neuron

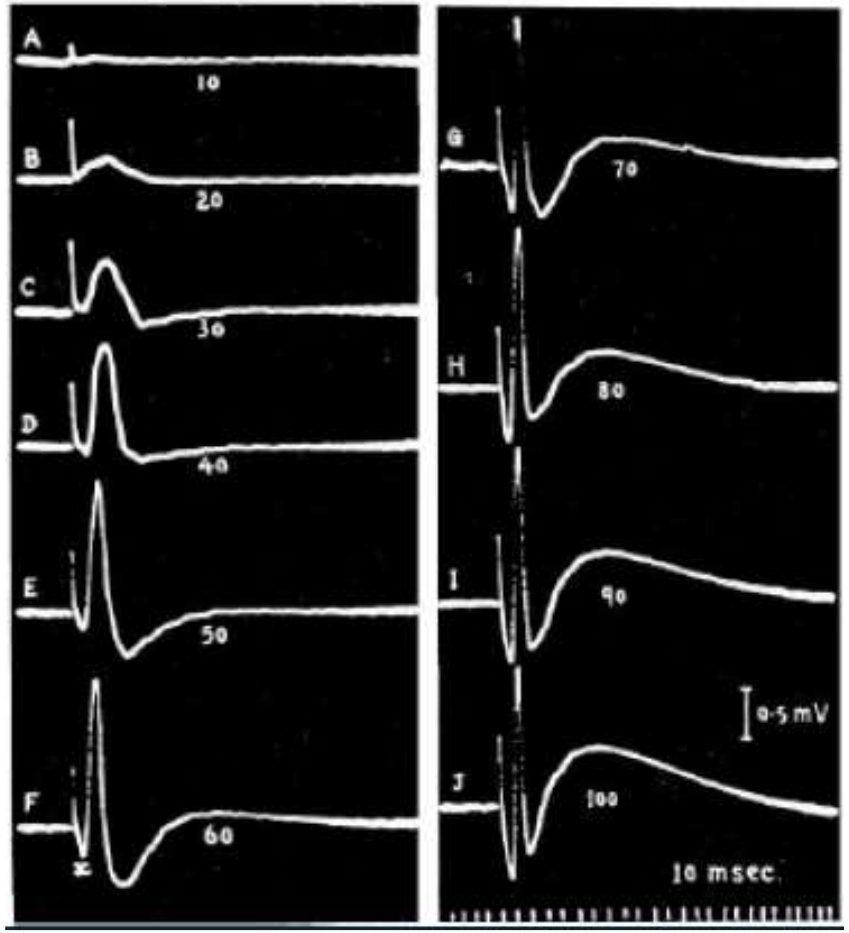


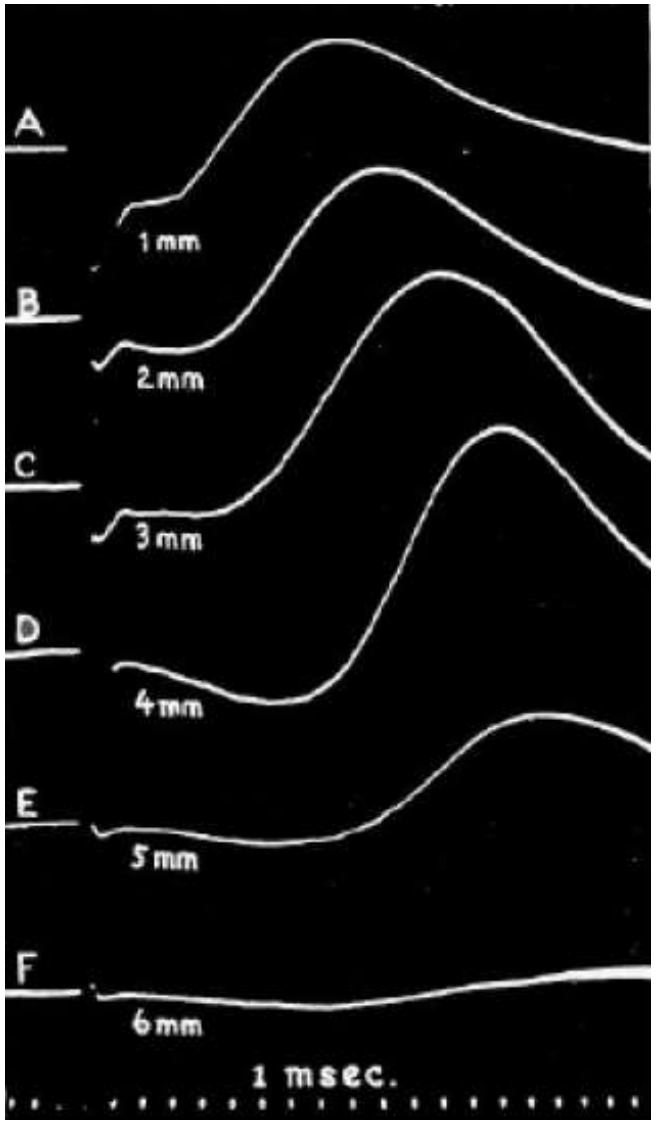
Question: how much sense could electrical signals make regarding information coding and integration?

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

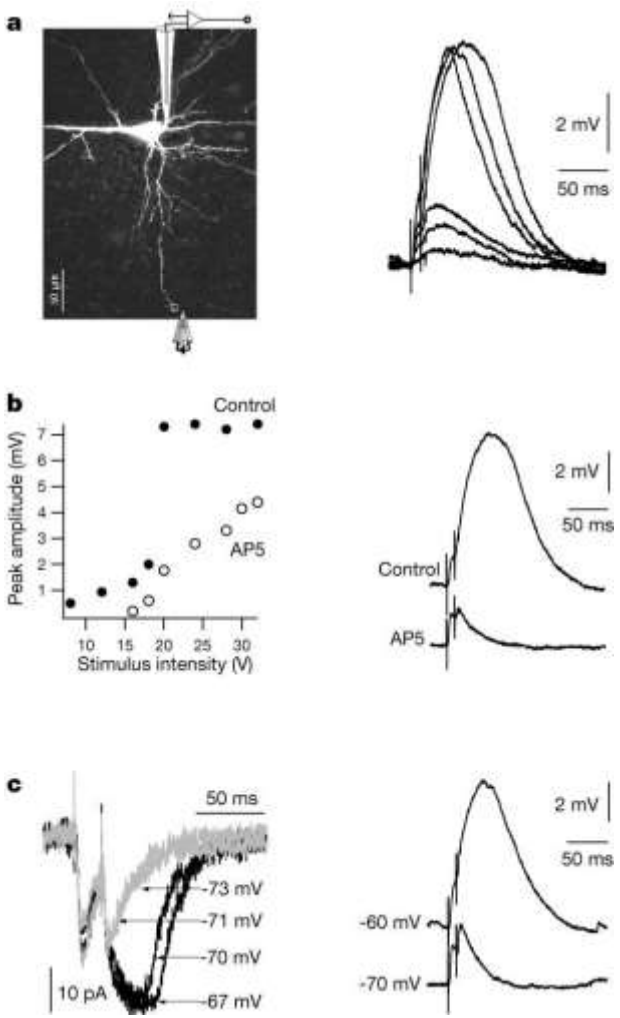
Dear Chang,

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

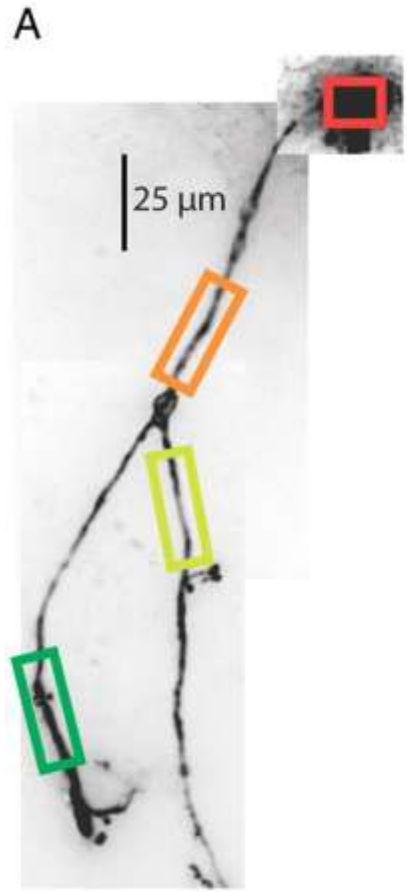
Active conductance at dendrites

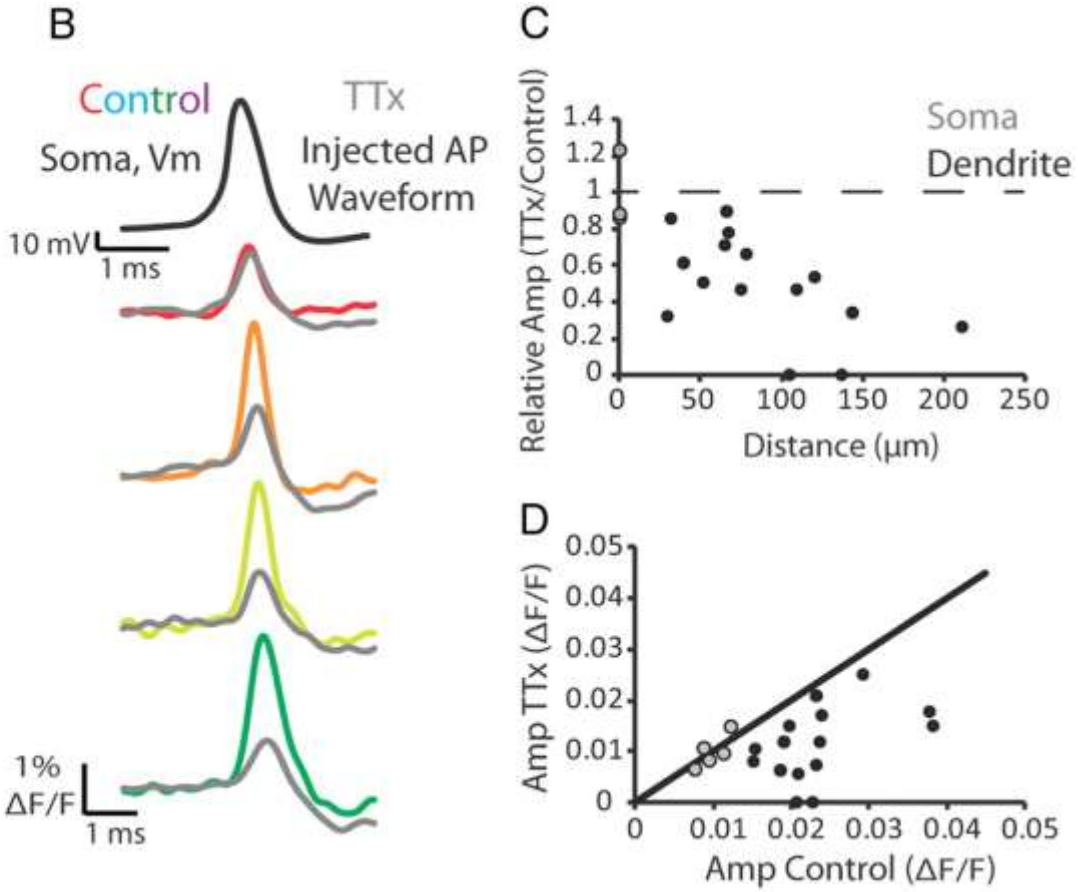
NMDA spikes



Nature volume 404, pages 285–289(2000)

Dendritic action potential





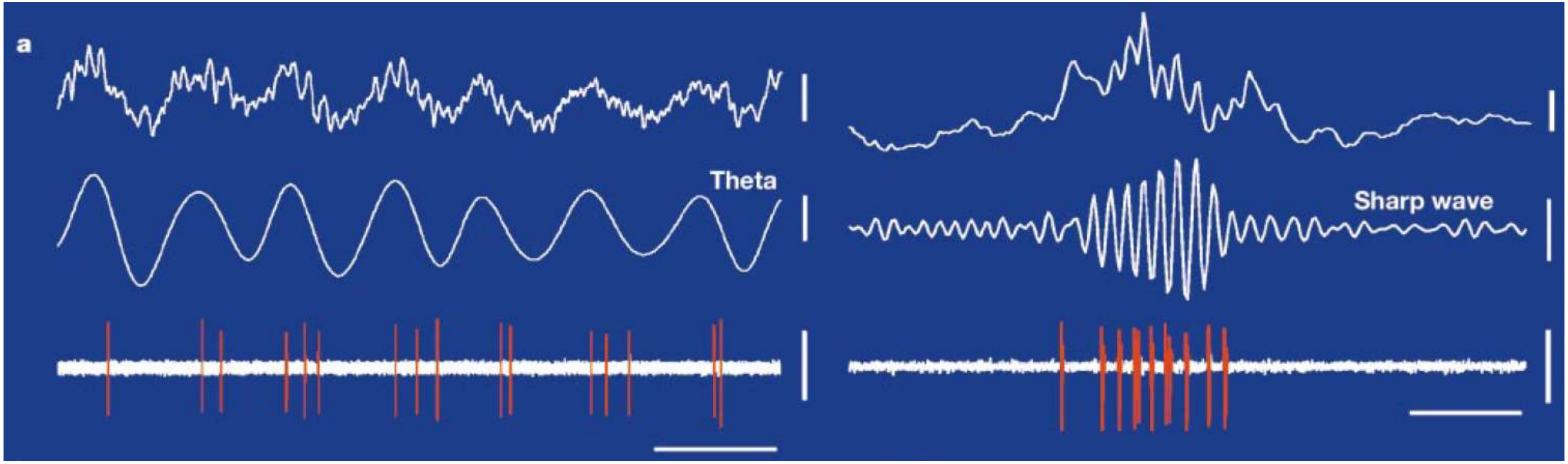
Journal of Neuroscience 14 December 2011, 31 (50) 18289-18302

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

01 Extracellular recording

- Metal electrode
  - Local field potential
  - Single units: single/multiple channles
- Glass capillaries
  - Juxtacellular recording
  - Extracellular recording
  - Cell-attached recording
  - Loose-patch recording

Juxtacellular recording



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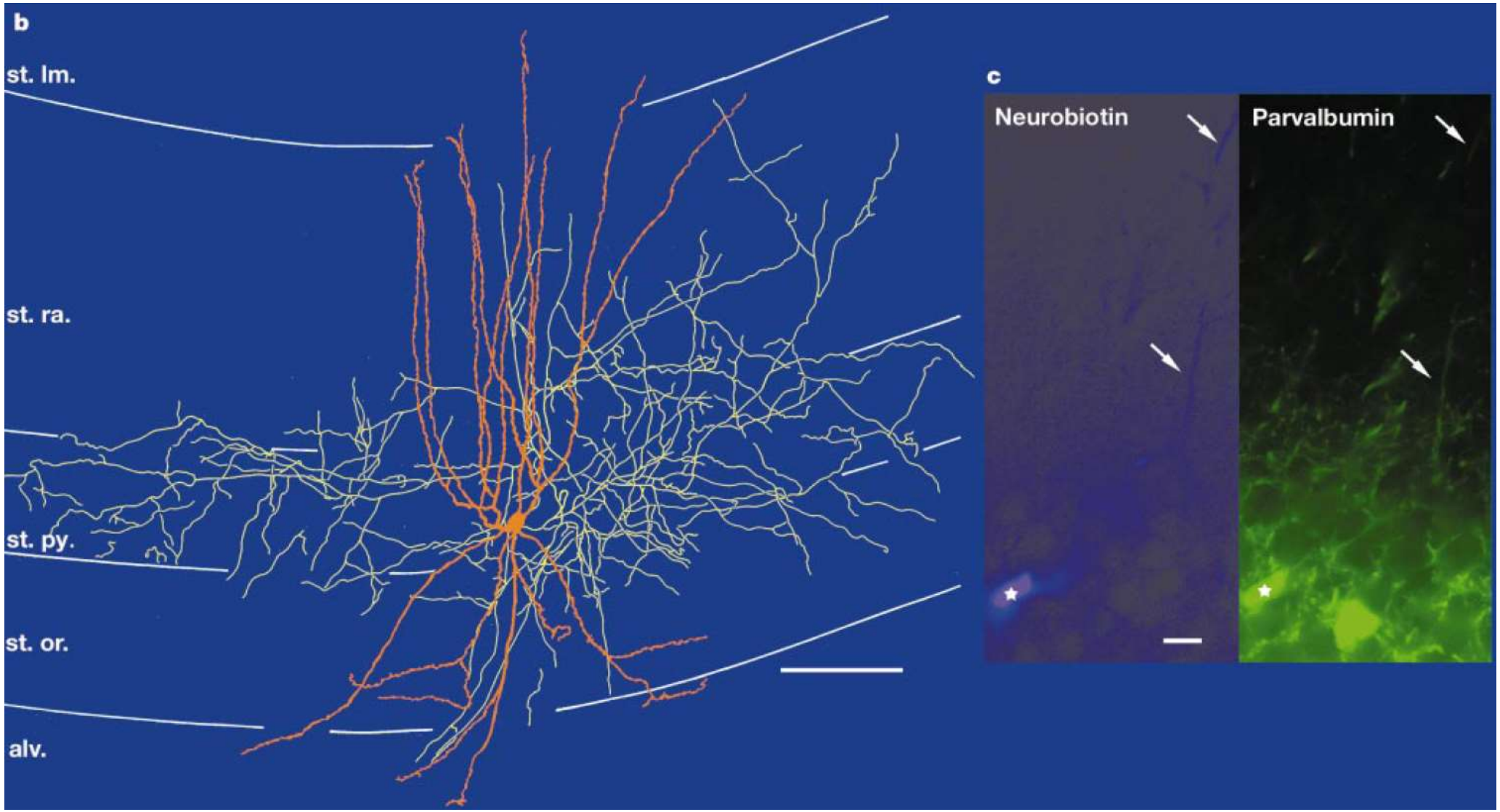
Juxtacellular recording



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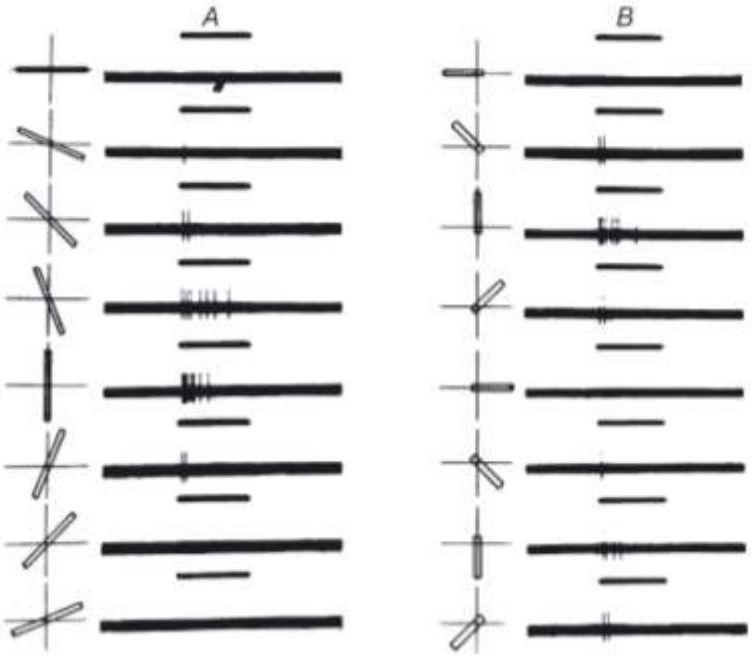
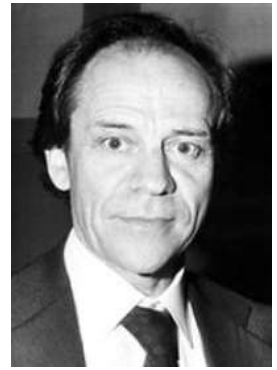
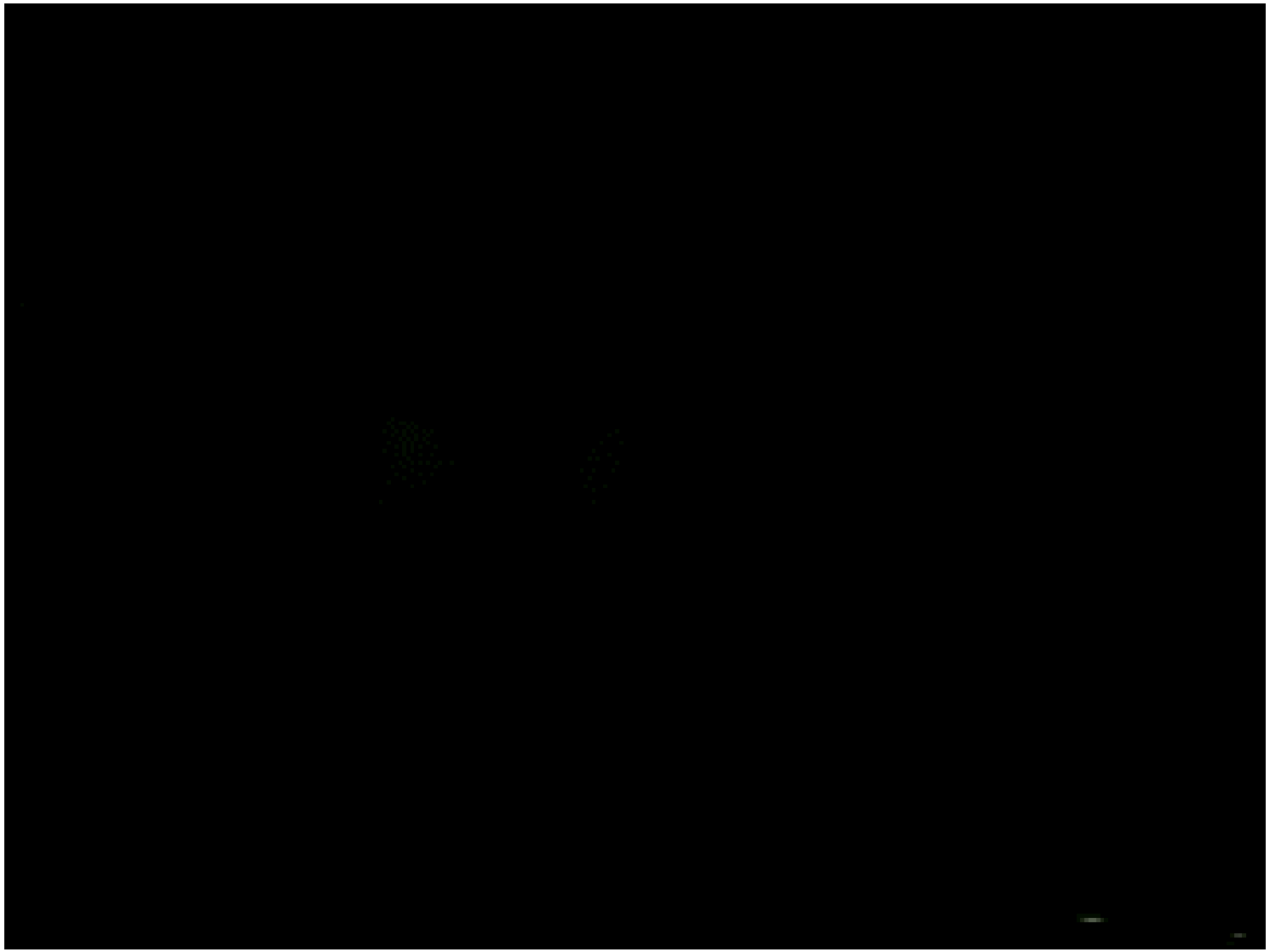
Feature Selectivity & Activity dependency of Visual Pathway





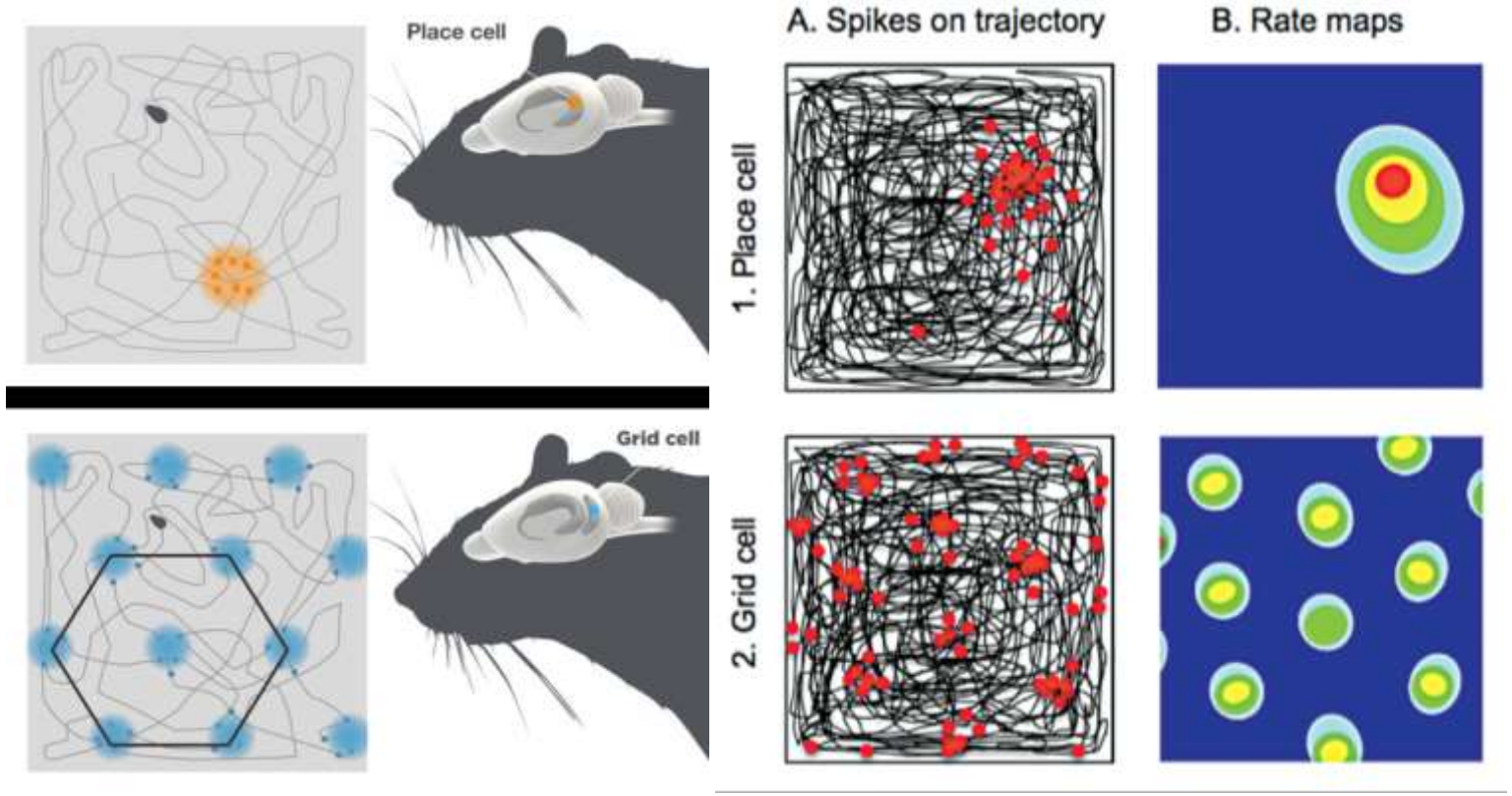
1. D. H. Hubel, T. N. Wiesel, Receptive fields of single neurones in the cat's striate cortex. The Journal of physiology 148, 574 (Oct, 1959).
2. D. H. Hubel, T. N. Wiesel, Receptive fields, binocular interaction and functional architecture in the cat's visual cortex. The Journal of physiology 160, 106 (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



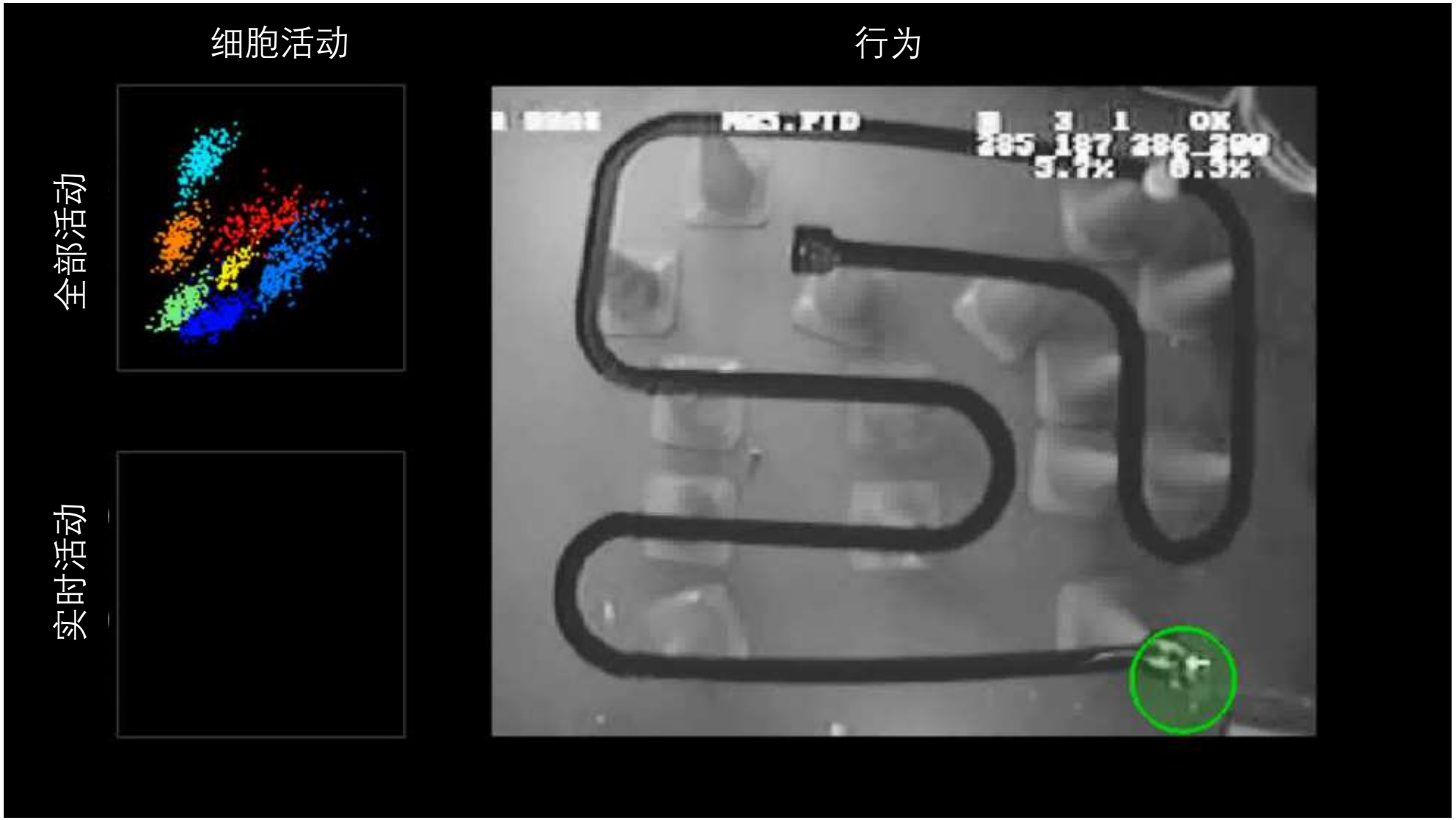
Place Cell and Grid Cell

Nobel Prize 2014



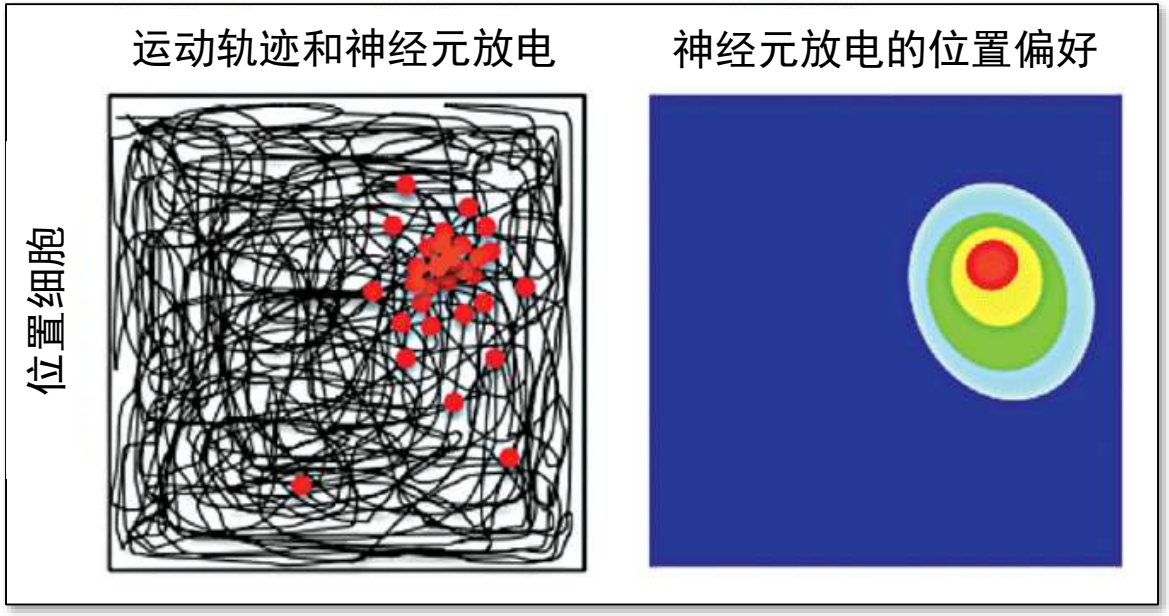
- O'Keefe j, D. J. (1971). "The hippocampus as a spatial map. Preliminary evidence from unit activity in the freely-moving rat". *Brain Research* **34** (1): 171–175.
- Hafting, T.; Fyhn, M.; Molden, S.; Moser, M. -B.; Moser, E. I. (2005). "Microstructure of a spatial map in the entorhinal cortex". *Nature* **436** (7052): 801–806.
- 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*

位置细胞

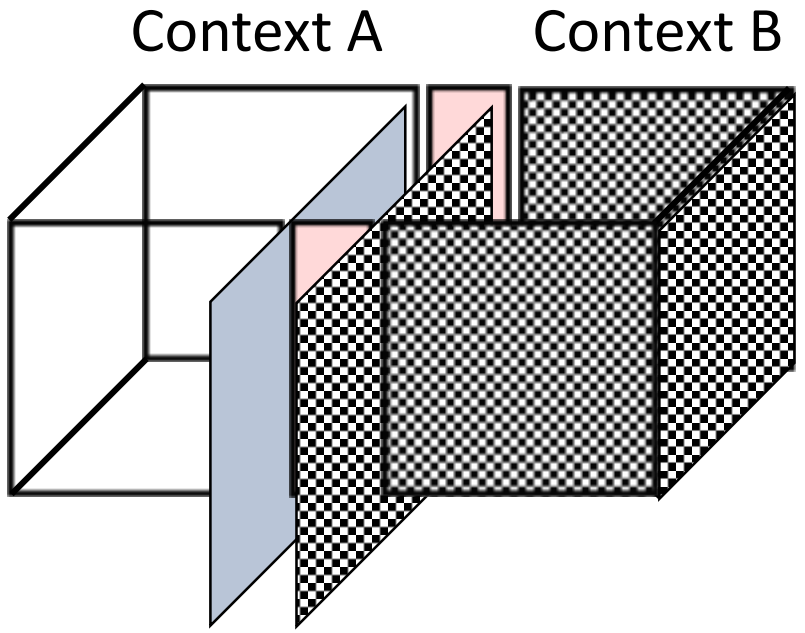


Matthew Wilson @ MIT

Context recognition (Where am I)



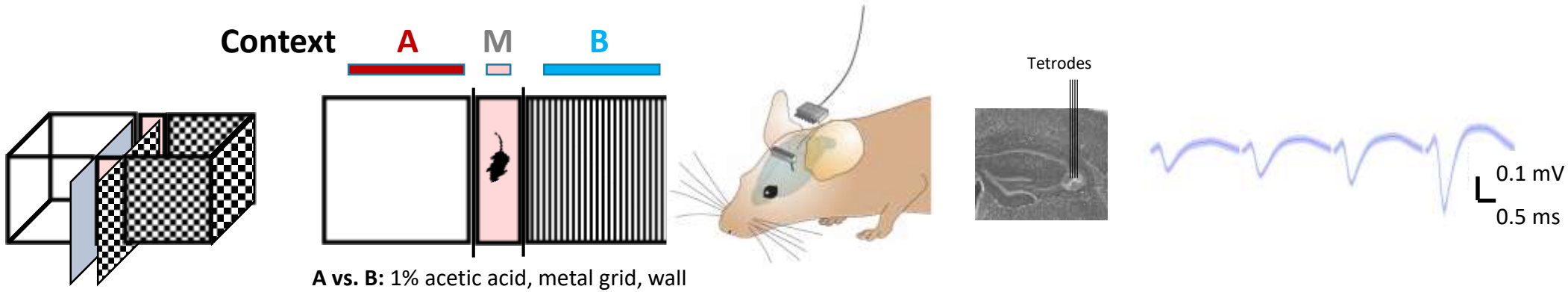
John O'Keefe

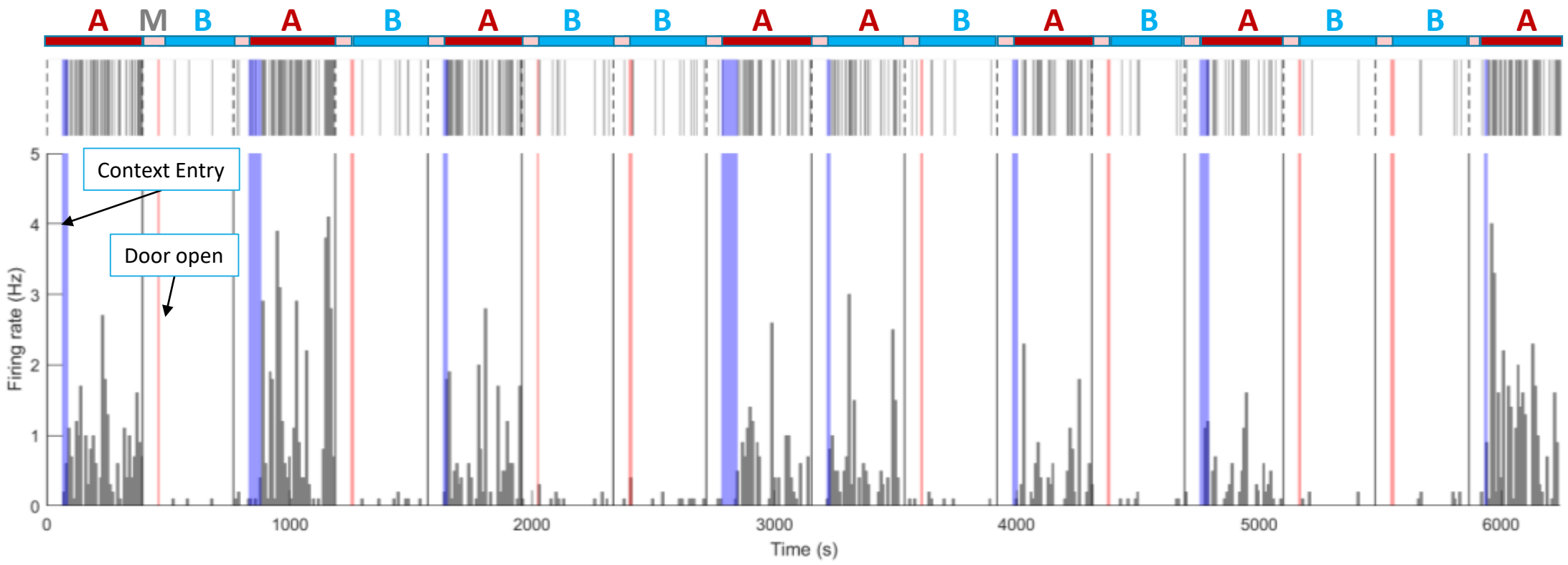


Project 1: The neural correlates of context and place fields in hippocampus

Single-unit recording in dorsal CA3 *in vivo* in distinct contexts

Qiu Shou

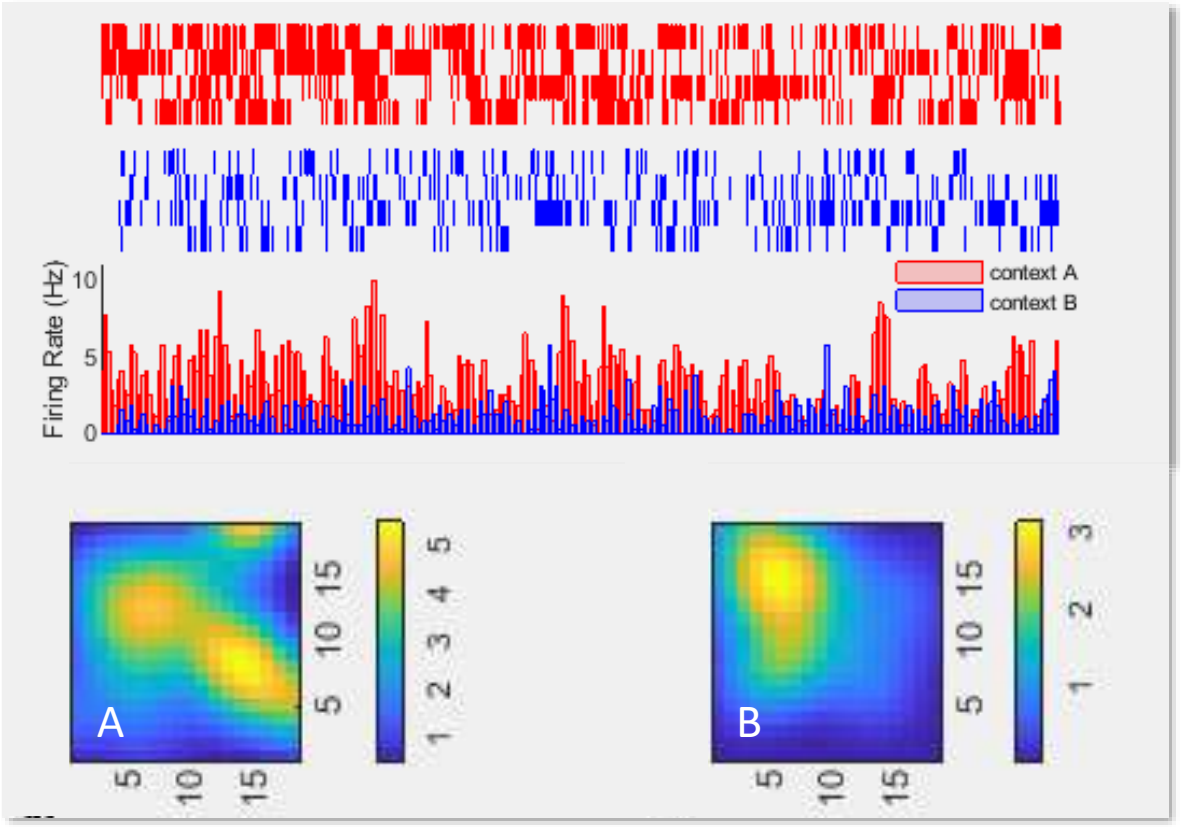




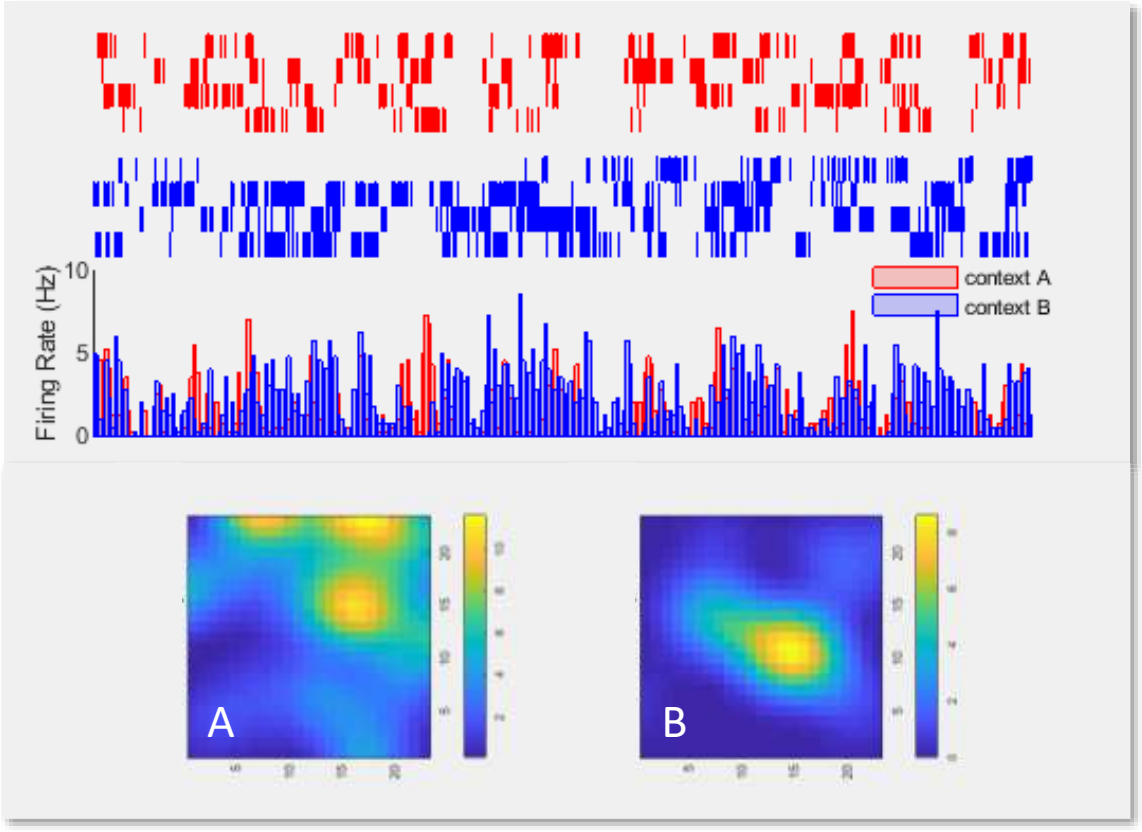
Context-modulated neurons show place fields

Qixin Yang

Context A – preferring unit

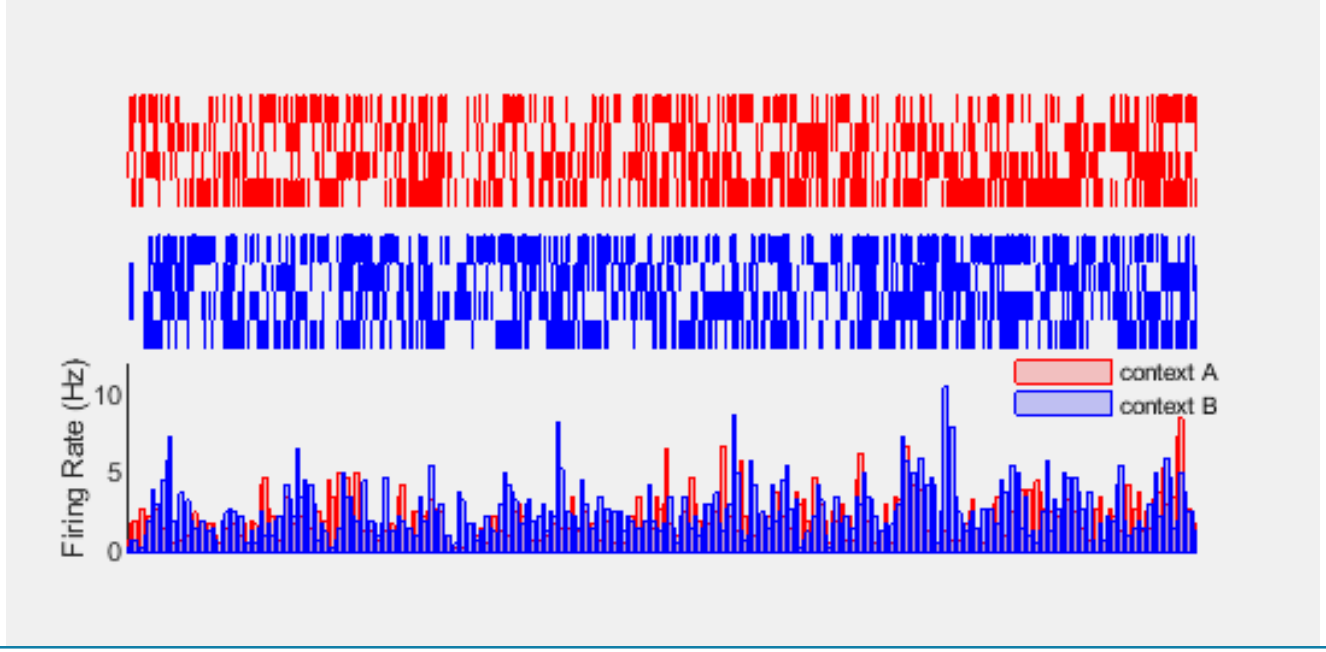


Context B – preferring unit



Context-unmodulated neurons also show place fields

Qixin Yang



- Context cell and place cell are dissociable.
- How about head-direction cells and other types of cells?
- How are different functions represented in the CA3?
- We are using improved paradigm to address these questions.

High-through output recording on the chip (MEA)

MaxOne

High-resolution live cell imaging platform

- Label-free detection of sub-cellular and network activity
- Selective electrical stimulation
- Comprehensive data analysis tools

maxwell BIOSYSTEMS

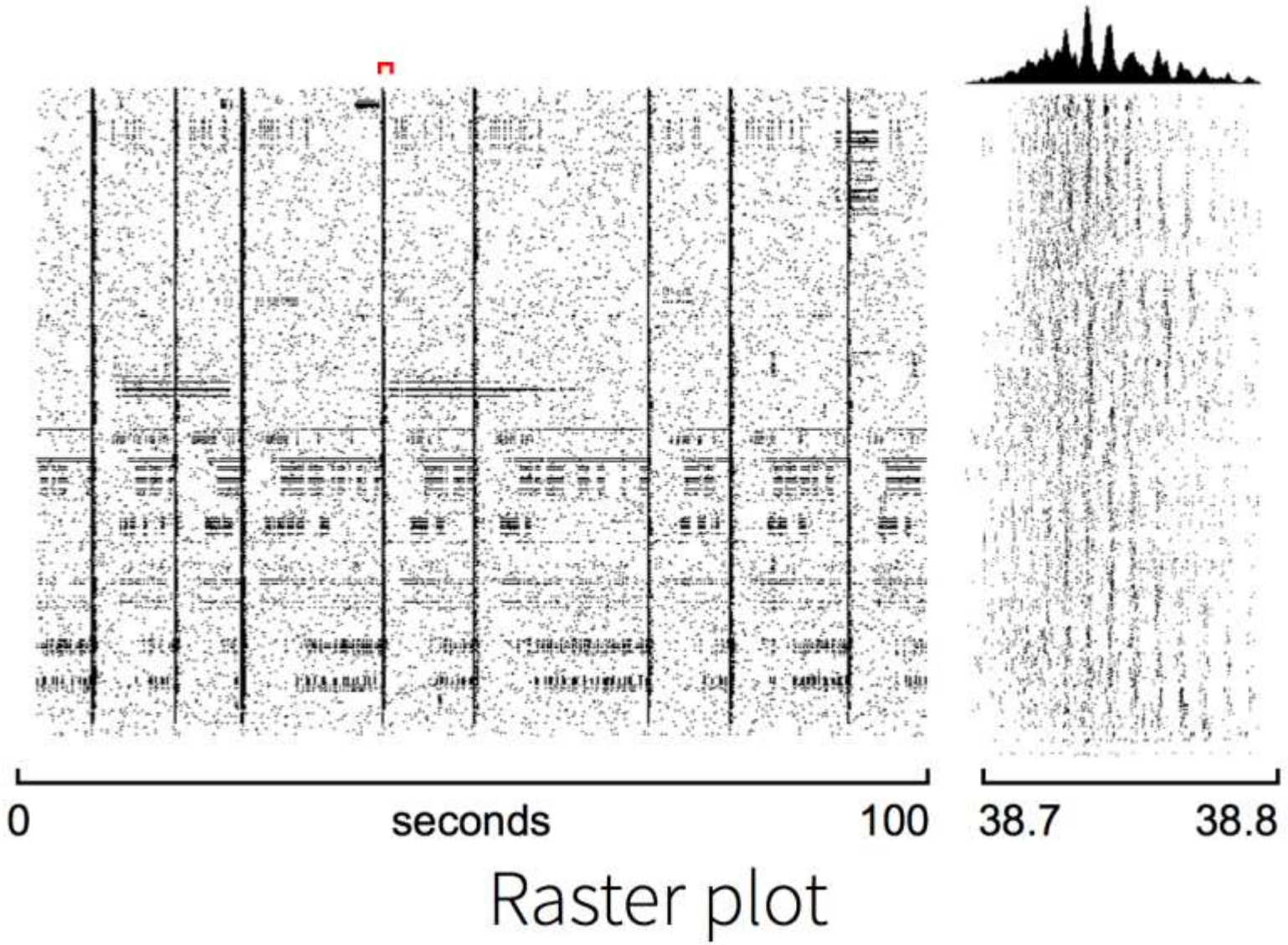
High-through output recording on the chip (MEA)

Key Features per Well

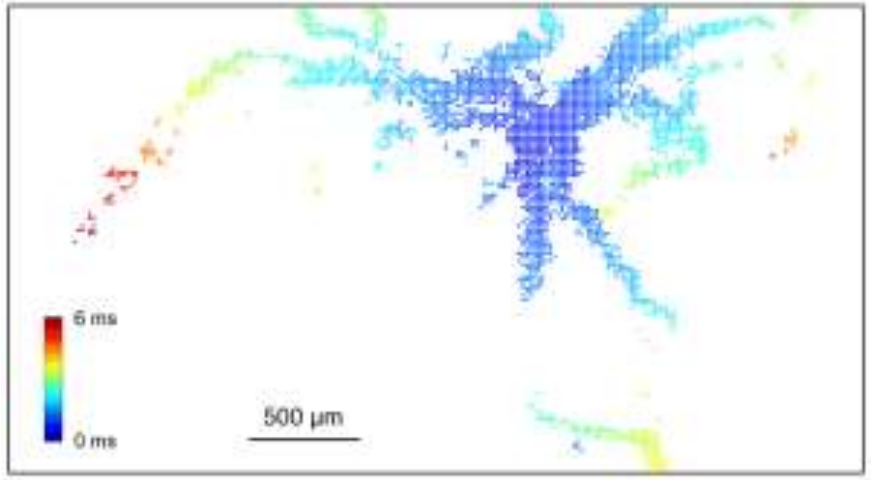
- 26,400 electrodes (9.3×5.45 sq-µm, 17.5 µm pitch)
- 1024 low-noise readout channels
- 32 stimulation channels
- Large sensor area (3.85×2.10 sq-mm)
- Switch-matrix technology for flexible array reconfiguration
- Non-invasive, label-free
- High-resolution activity map
- Axonal action potential propagation tracking

maxwell BIOSYSTEMS

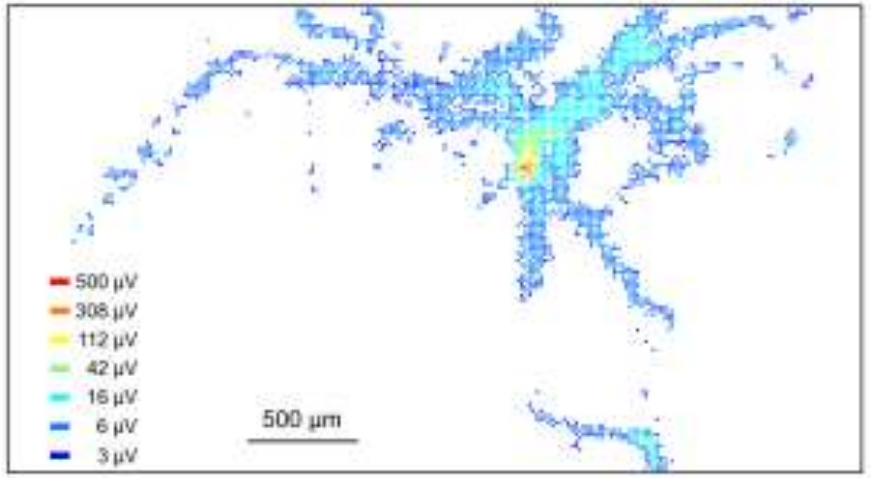
Cells growing on the chip (MEA)



Raster plot



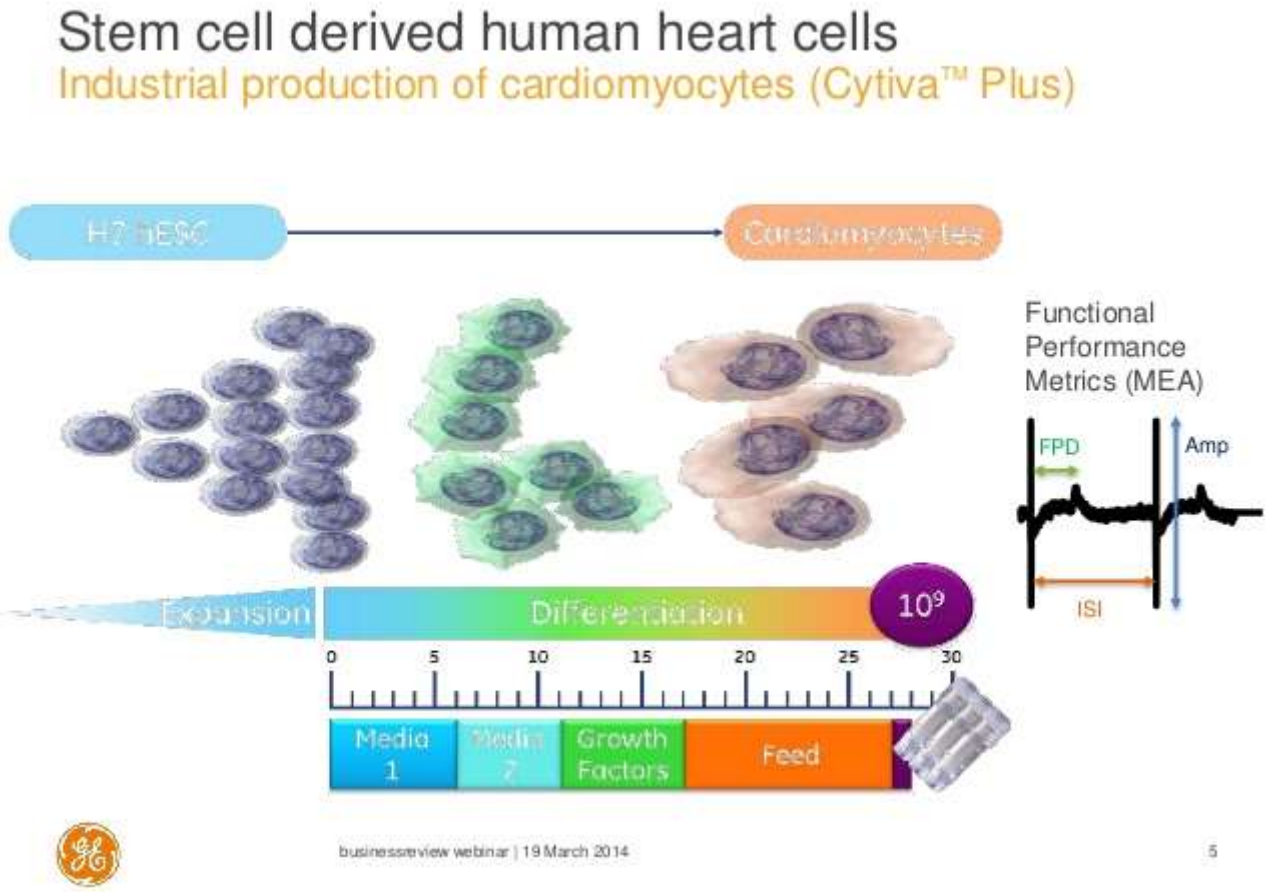
Delay map



Amplitude map

Good for slice recording, retina recording

MEA application in non-neuron cells

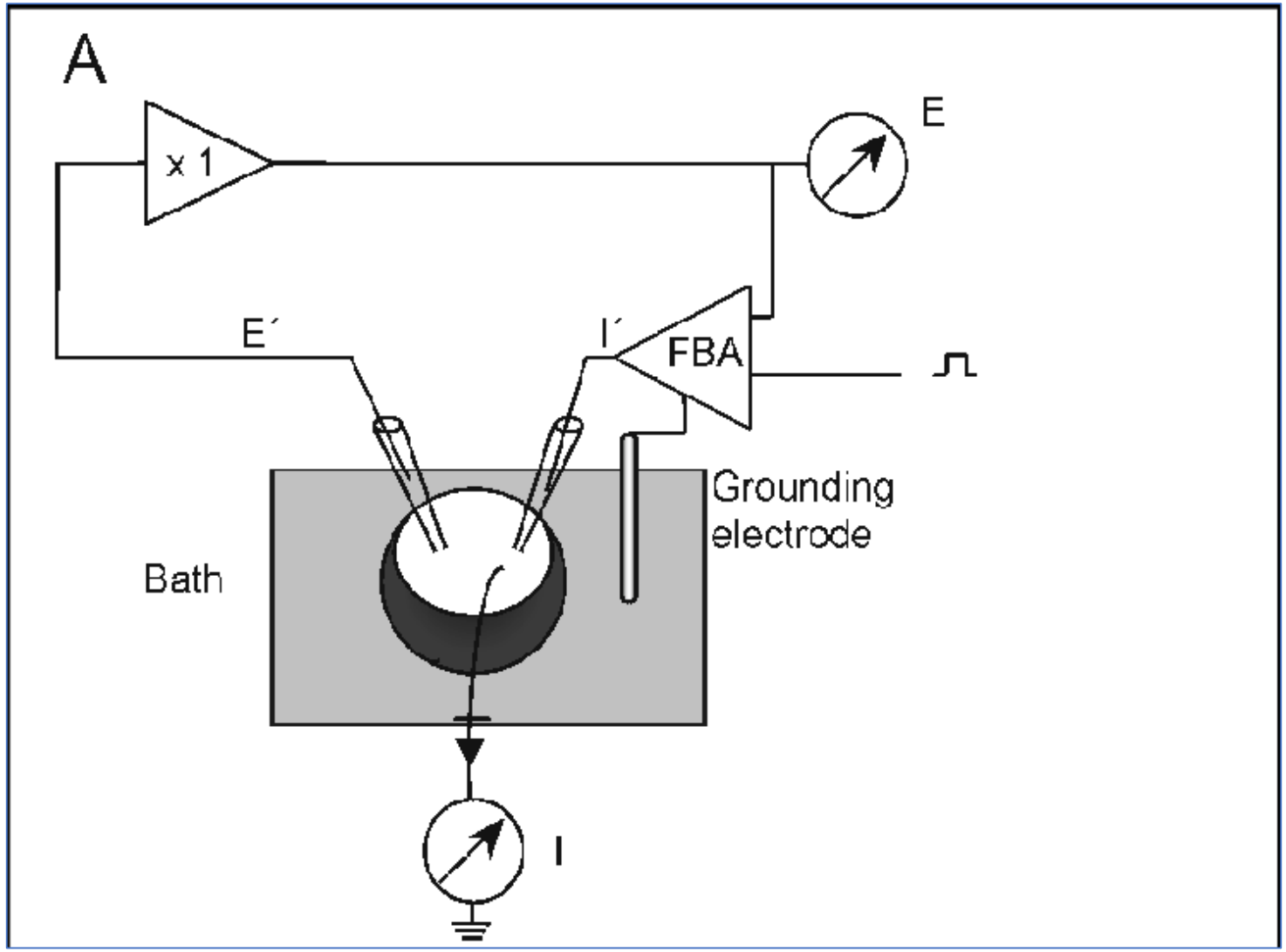


02

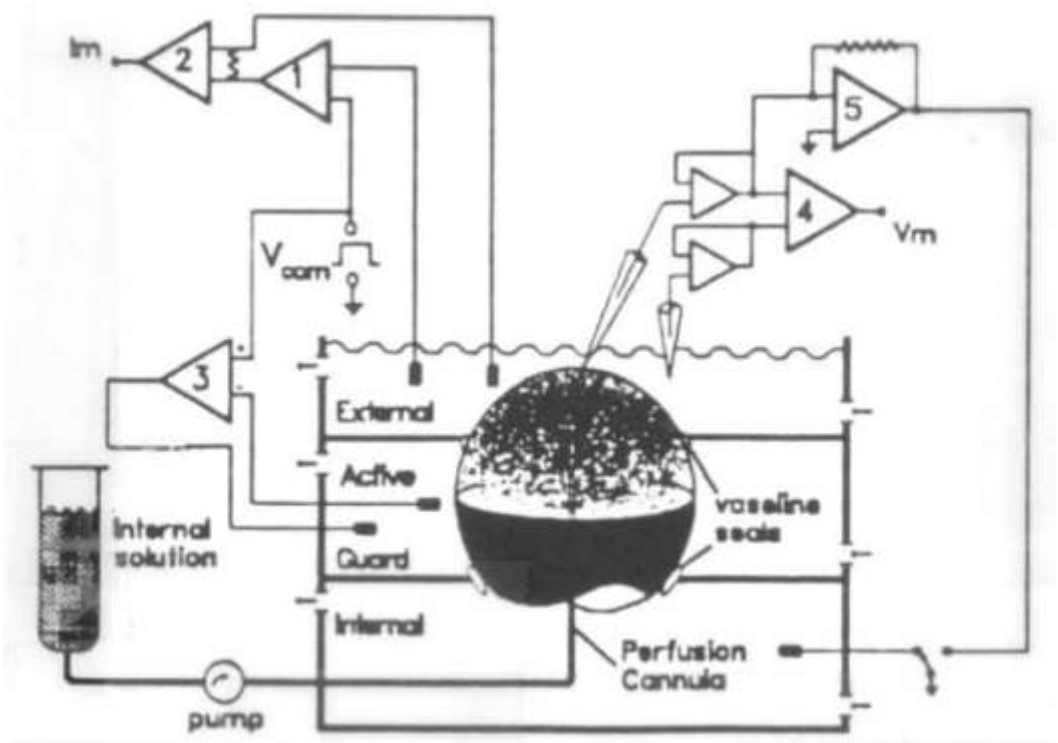
Intracellular recording

- Whole-cell recording
- Single-channel recording
- Sharpe electrodes

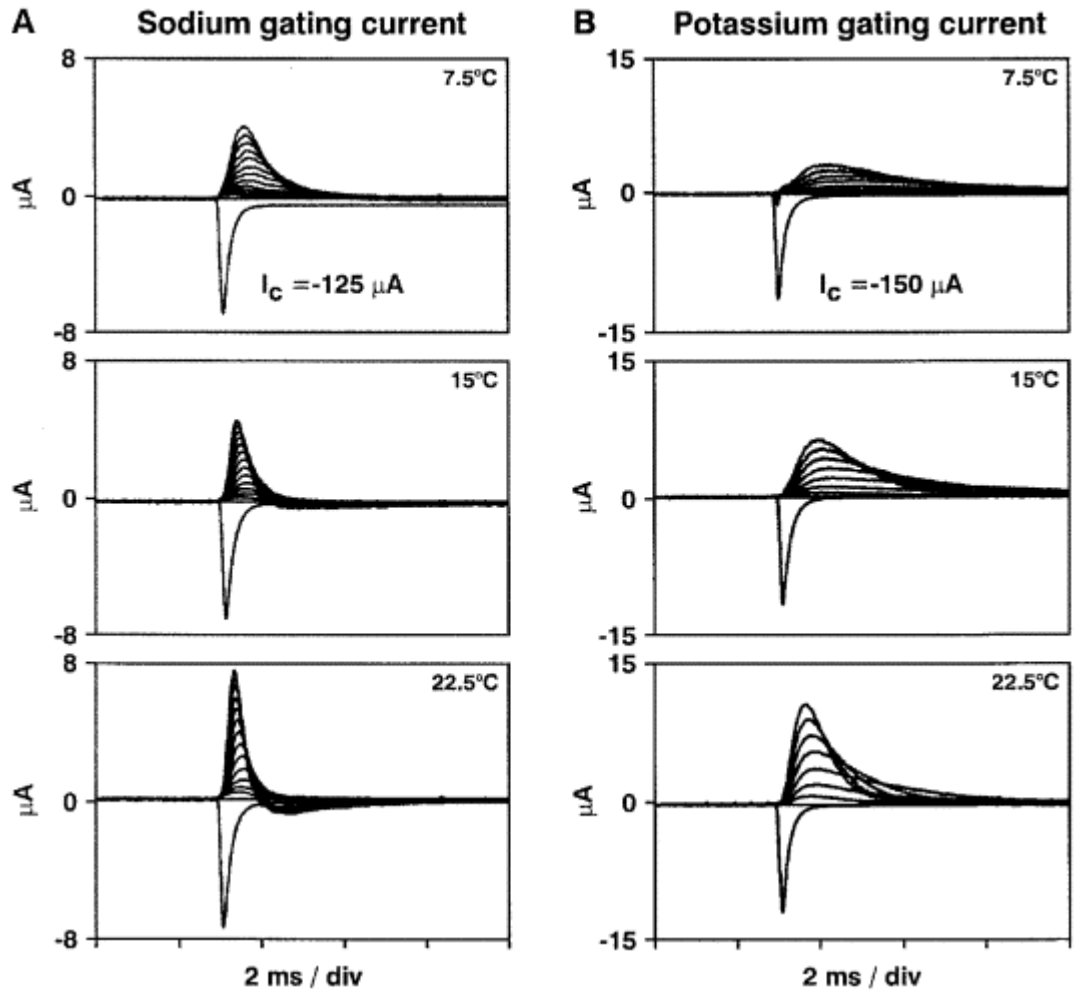
传统研究方式双极电极记录 oocyte recording



Cut-open oocyte recording

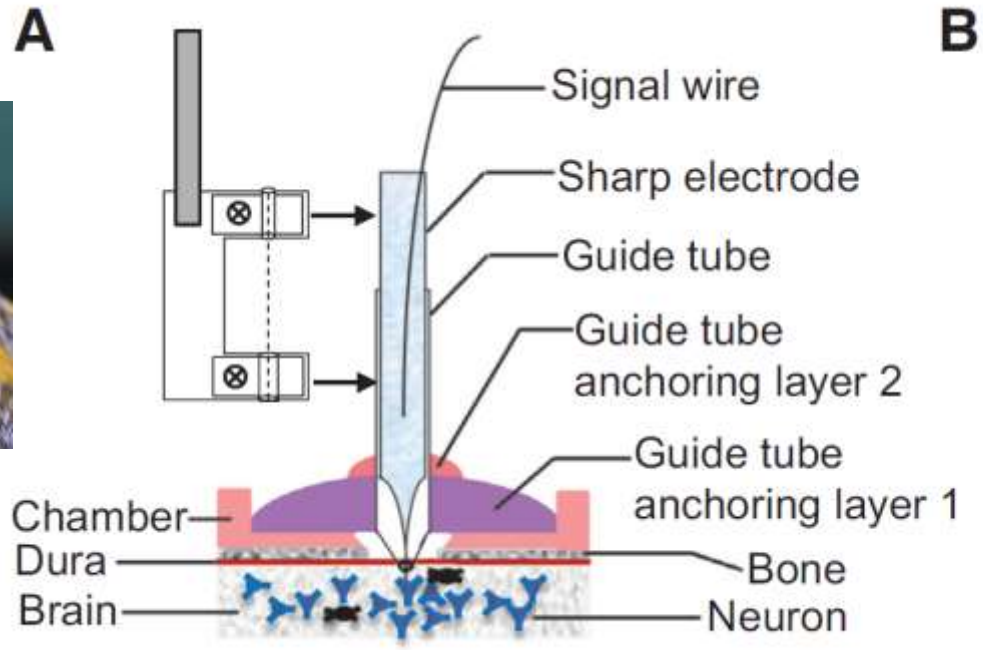


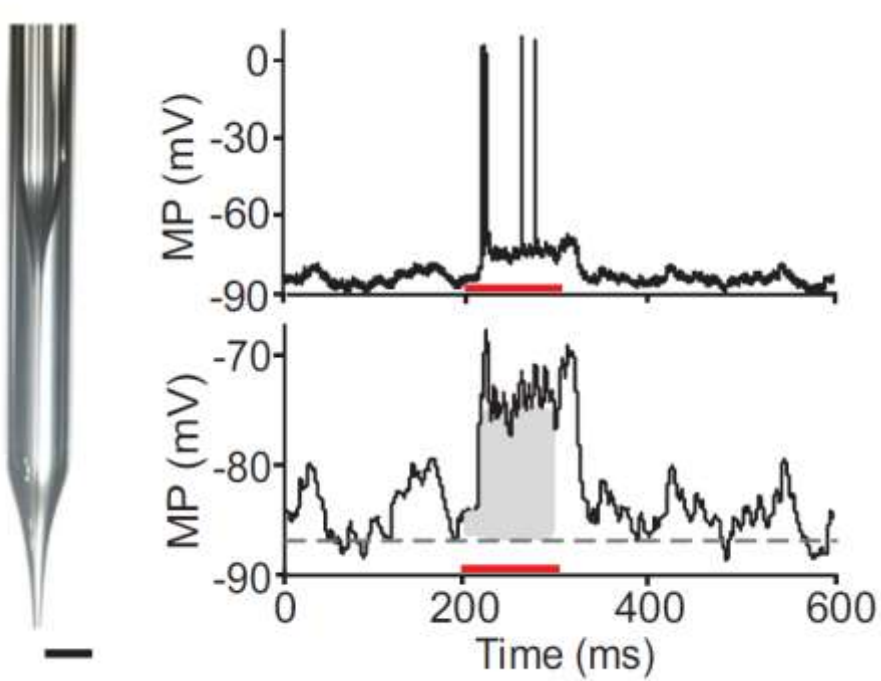
Gating currents by channel opening



Intracellular recording in primates

marmoset





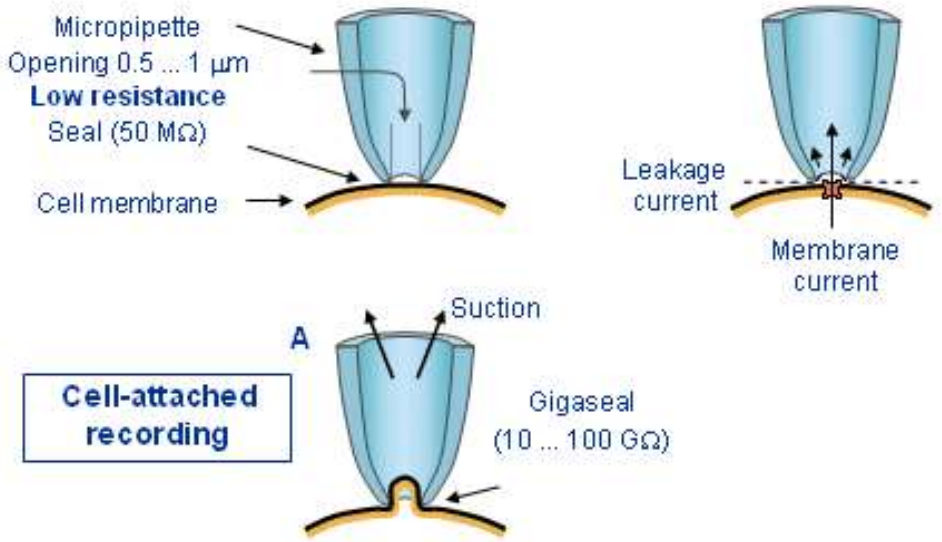
Gao et al., 2016, Neuron 91, 905–919

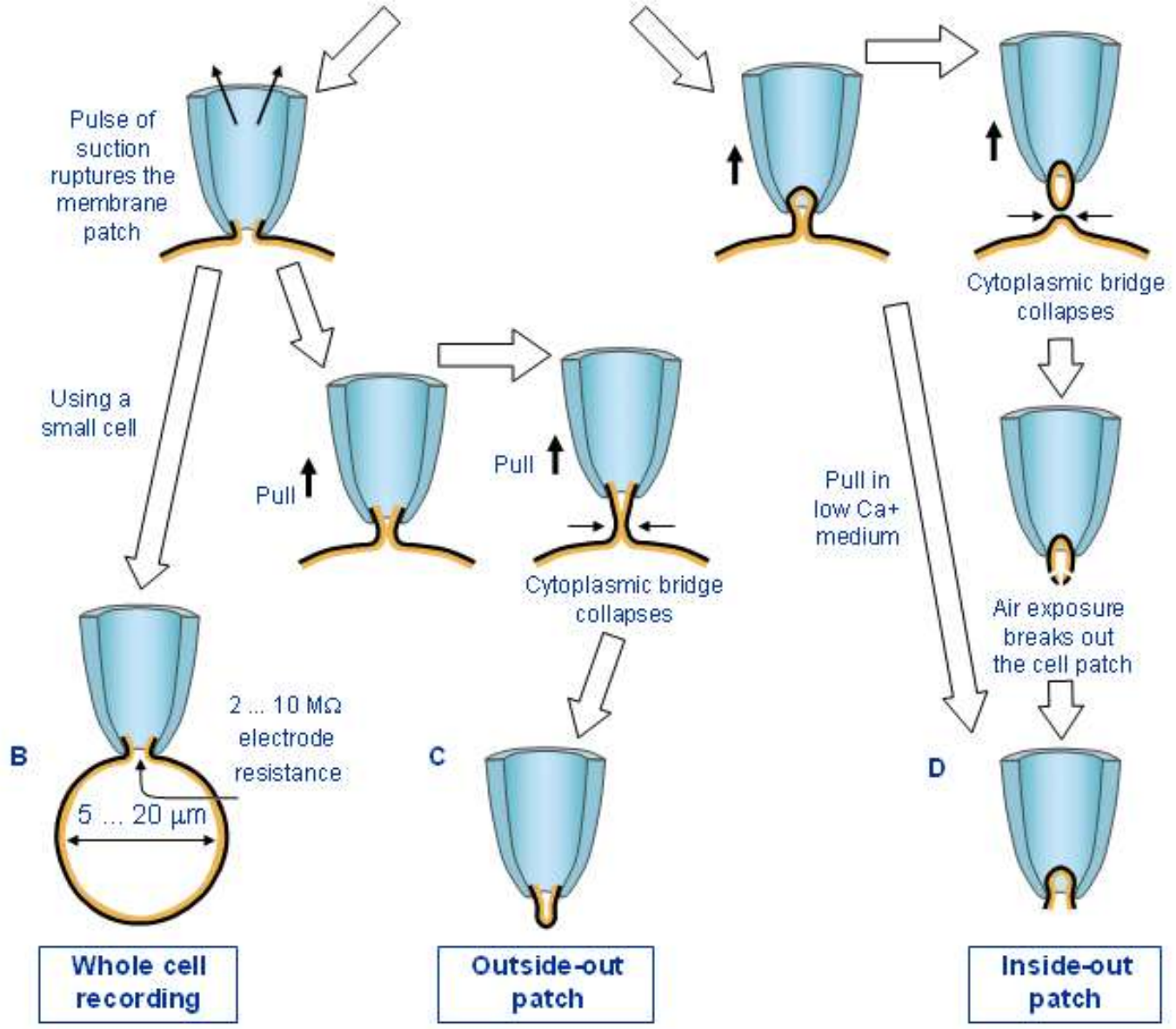
The revolutionary breakthrough – patch clamp technique

Bert Sakmann

Erwin Neher

1991 诺贝尔奖





The revolutionary breakthrough – patch clamp technique

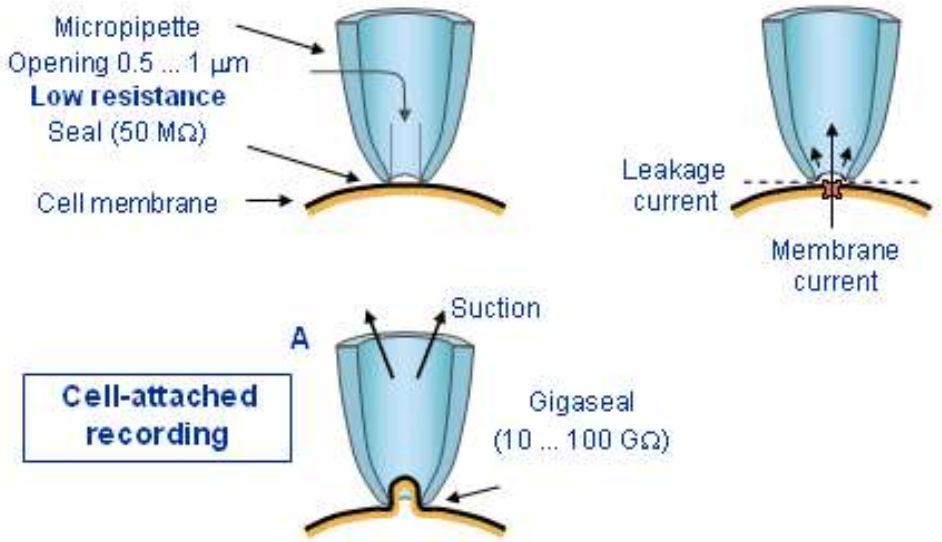
The Patch Clamp Method

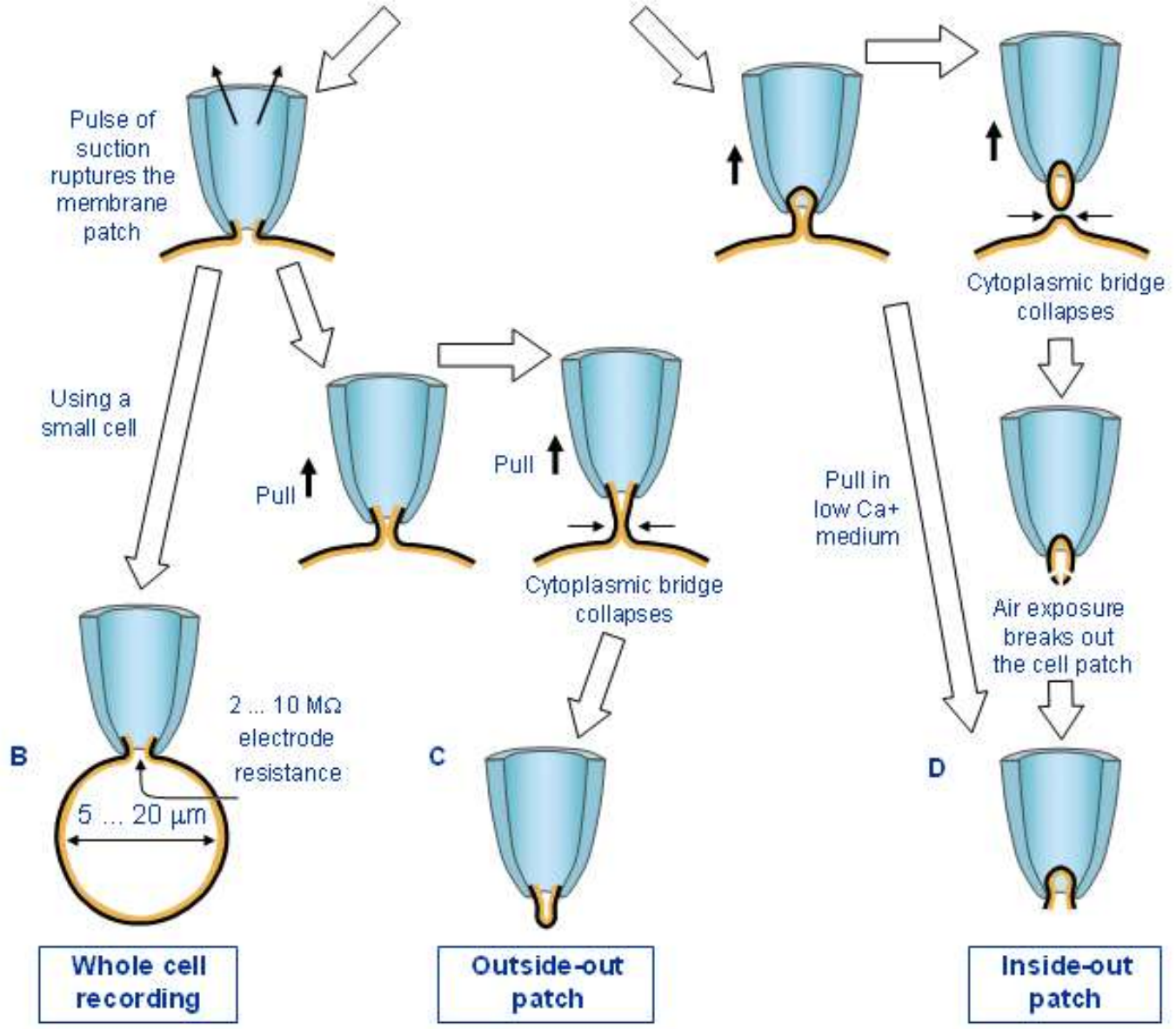
The revolutionary breakthrough – patch clamp technique

Bert Sakmann

Erwin Neher

1991 诺贝尔奖

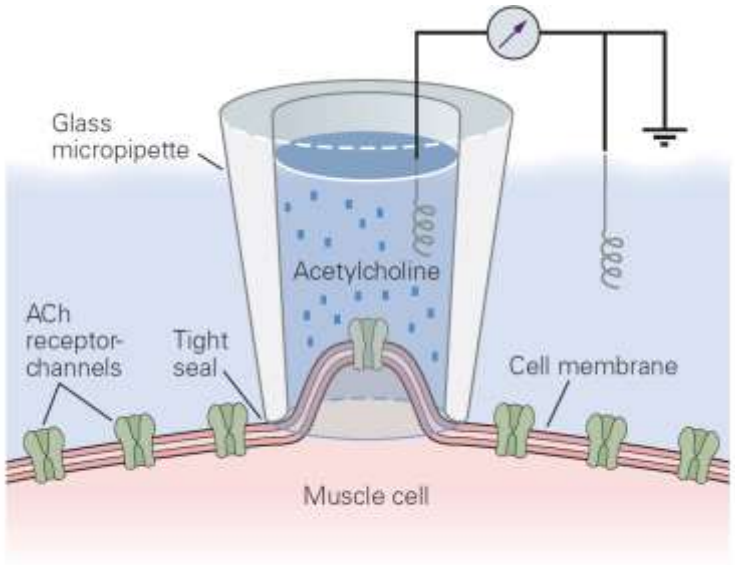


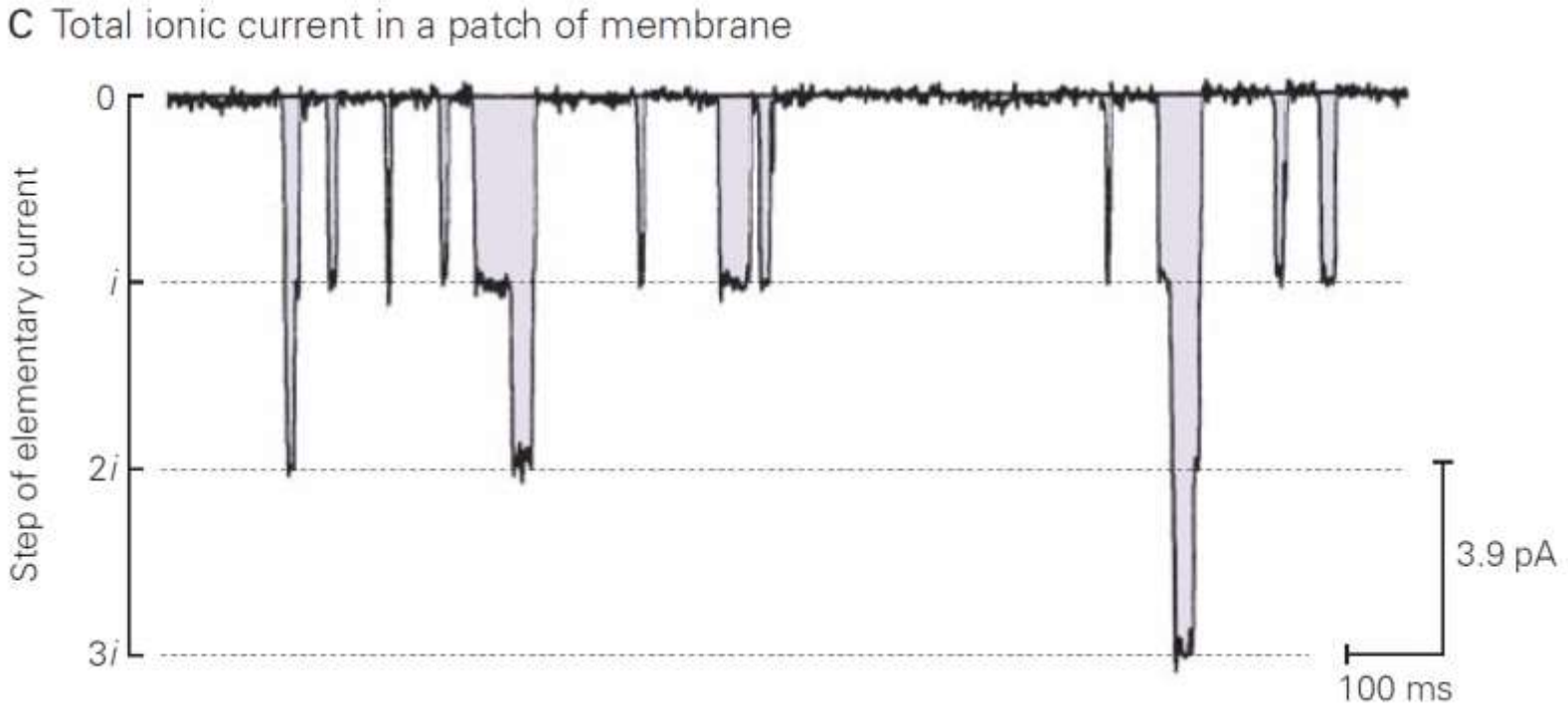


Electrophysiology Development

- 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

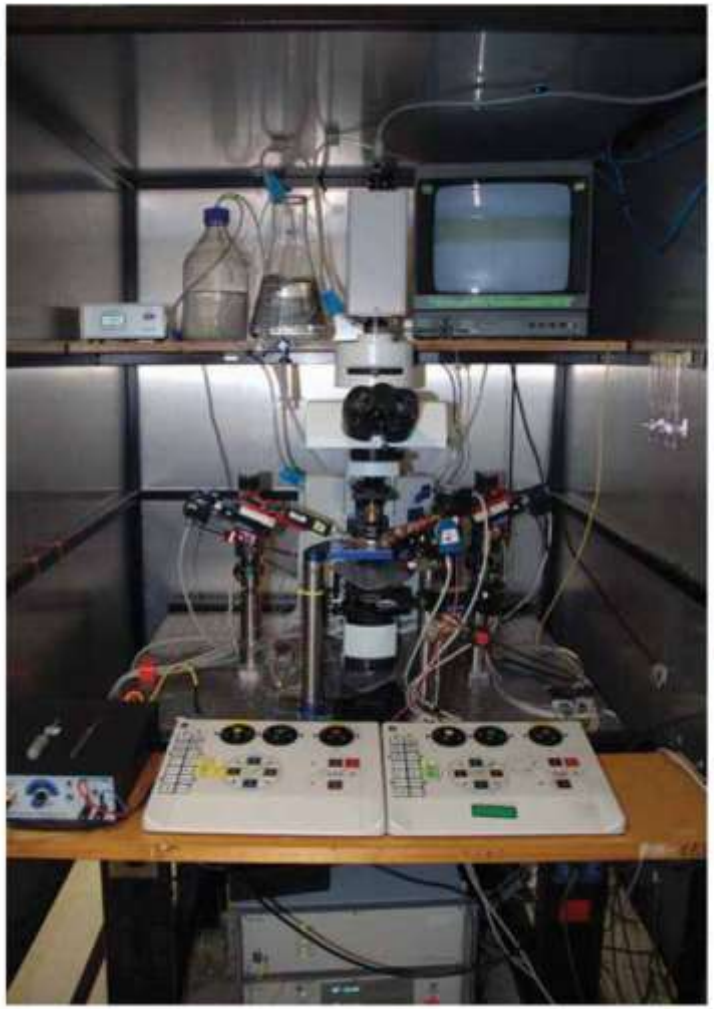
Single-channel recording

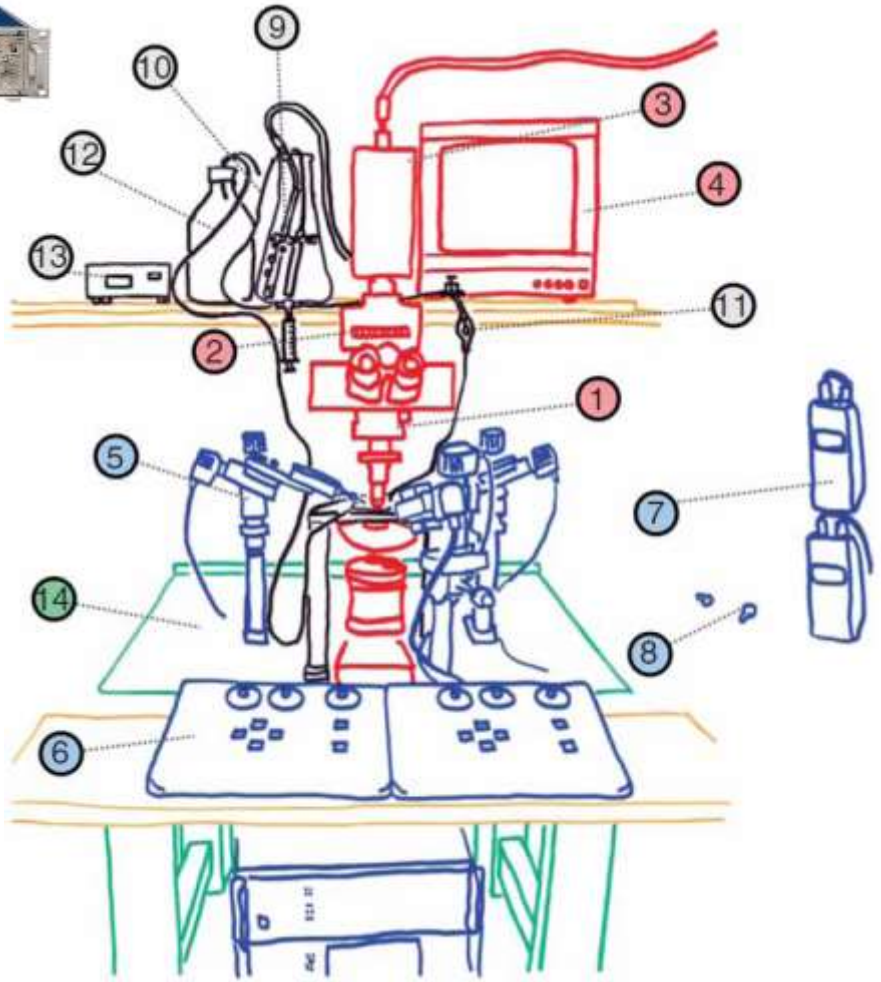




Neher, E. and B. Sakmann (1976). "Single-channel currents recorded from membrane of denervated frog muscle fibres." Nature **260(5554): 799-802.**

A patch-clamp rig





Patch-clamp in acute brain slice

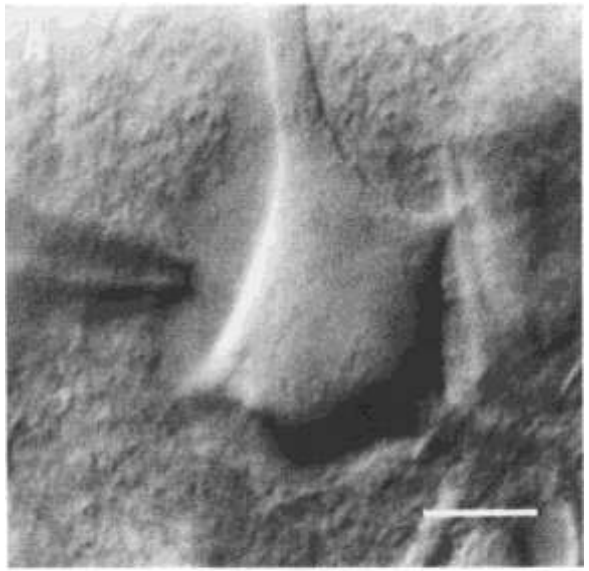
ÉCOLE POLYTECHNIQUE
FÉDÉRALE DE LAUSANNE

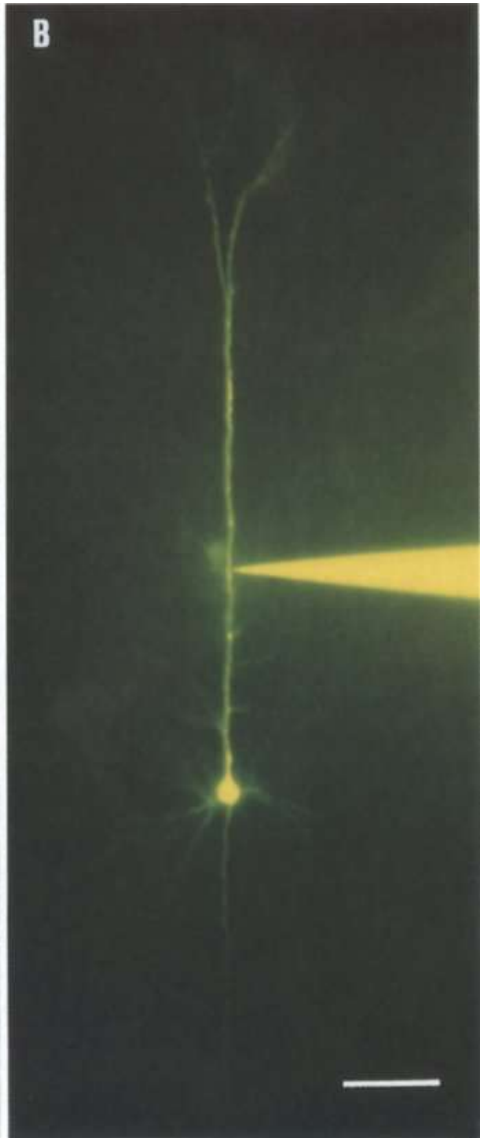
2.5 Whole-cell recordings

Cellular Mechanisms of Brain Function

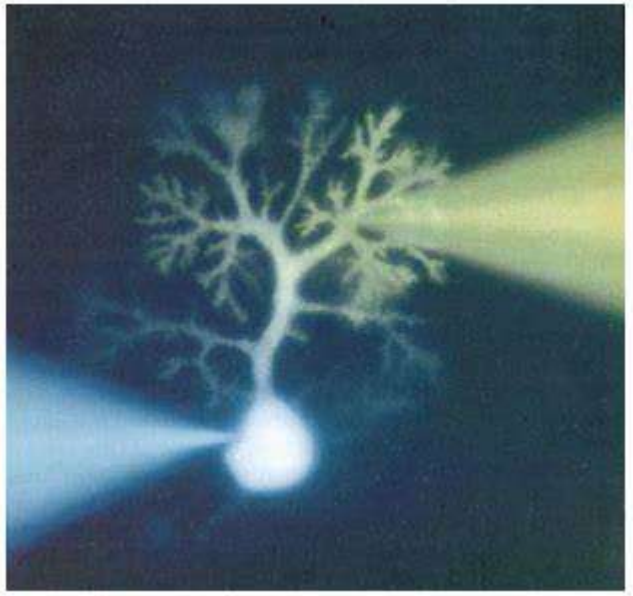
Prof. Carl Petersen

Dendritic recording



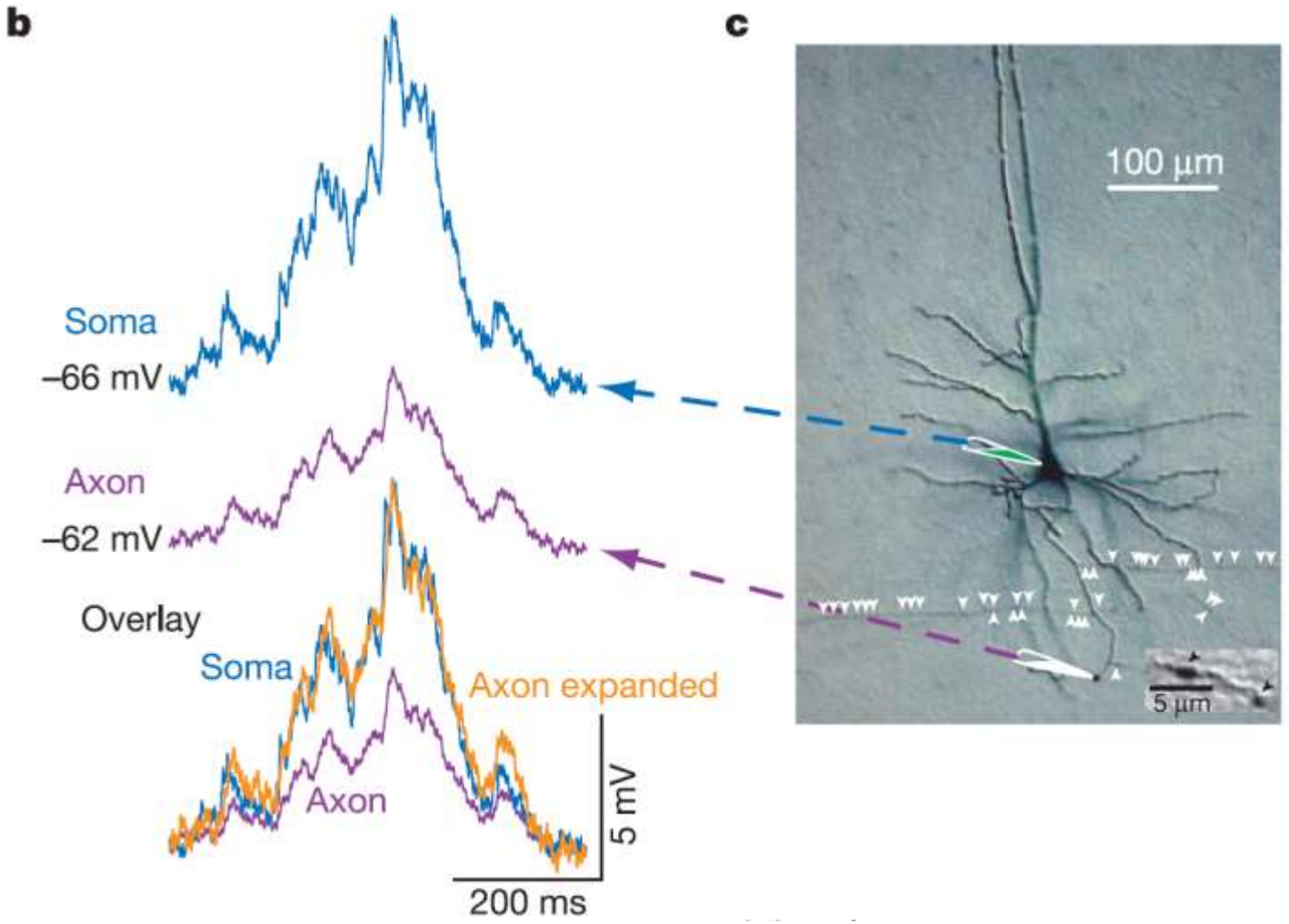


Greg J Stuart



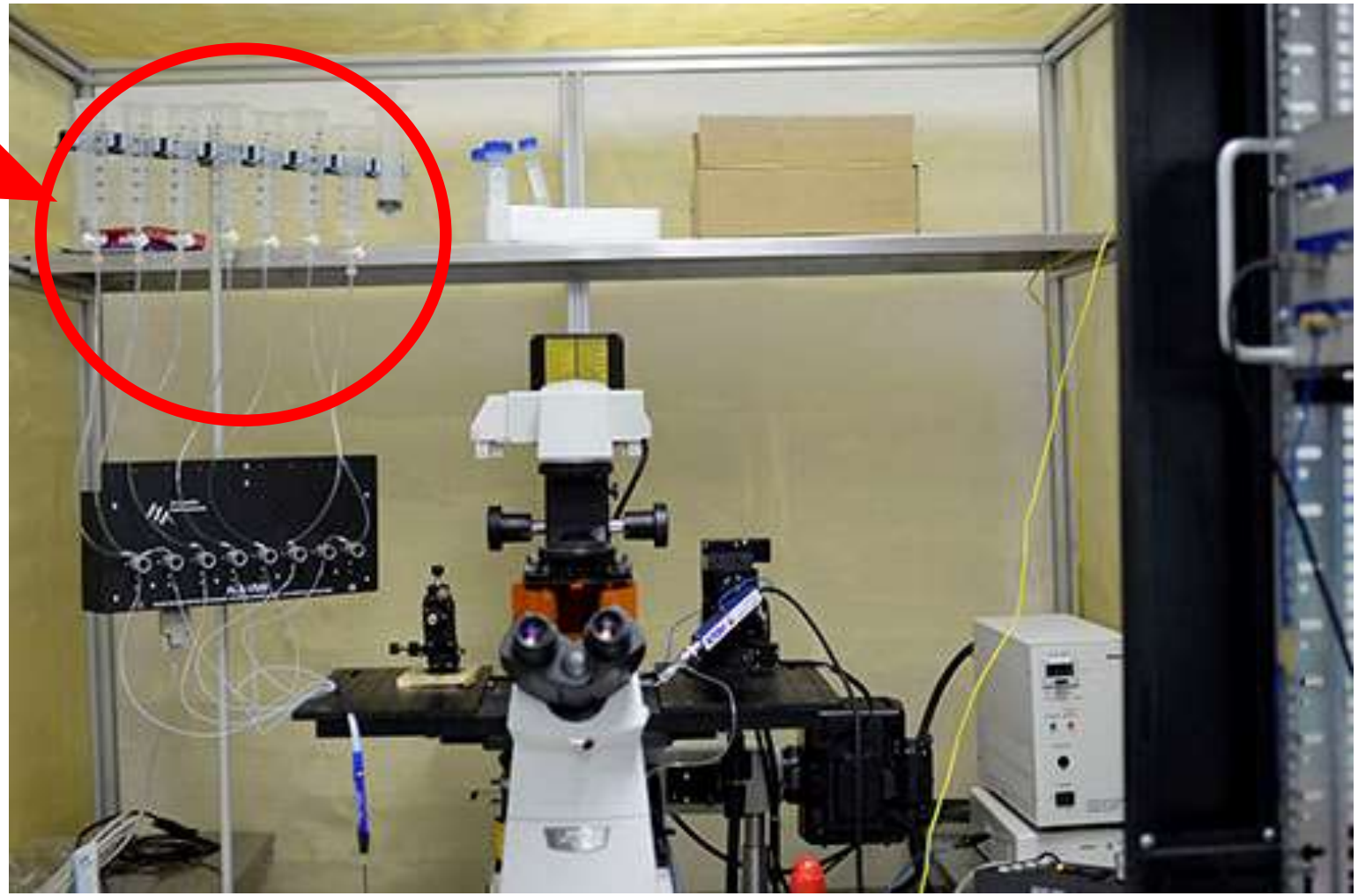
July 1993 Pflügers Archiv - European Journal of Physiology 423(5-6):511-8

Axon recording

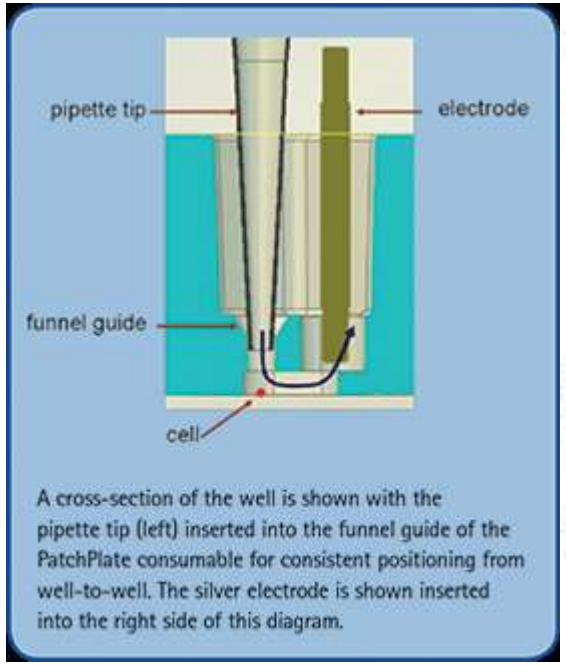


PATCH CLAMP在药物筛选中应用

快速给药系统



High-through output automatic patch clamp in industry



A cross-section of the well is shown with the pipette tip (left) inserted into the funnel guide of the PatchPlate consumable for consistent positioning from well-to-well. The silver electrode is shown inserted into the right side of this diagram.

- Flip-Tip翻转技术

将一定密度的细胞悬液灌注在玻璃电极中，下降到电极尖端的单个细胞通过在电极外施加负压与玻璃电极尖端形成稳定的高阻封接，系统自动判断封接形成是否良好并自动打破露在玻璃微电极尖端外的细胞膜形成全细胞模式。它的显著特点是仍然采用玻璃毛坯作为电极。

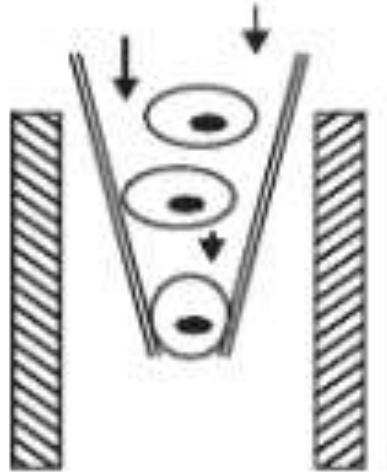


Figure 2. Flow-through Design of the IonWorks Barracuda Consumable Well

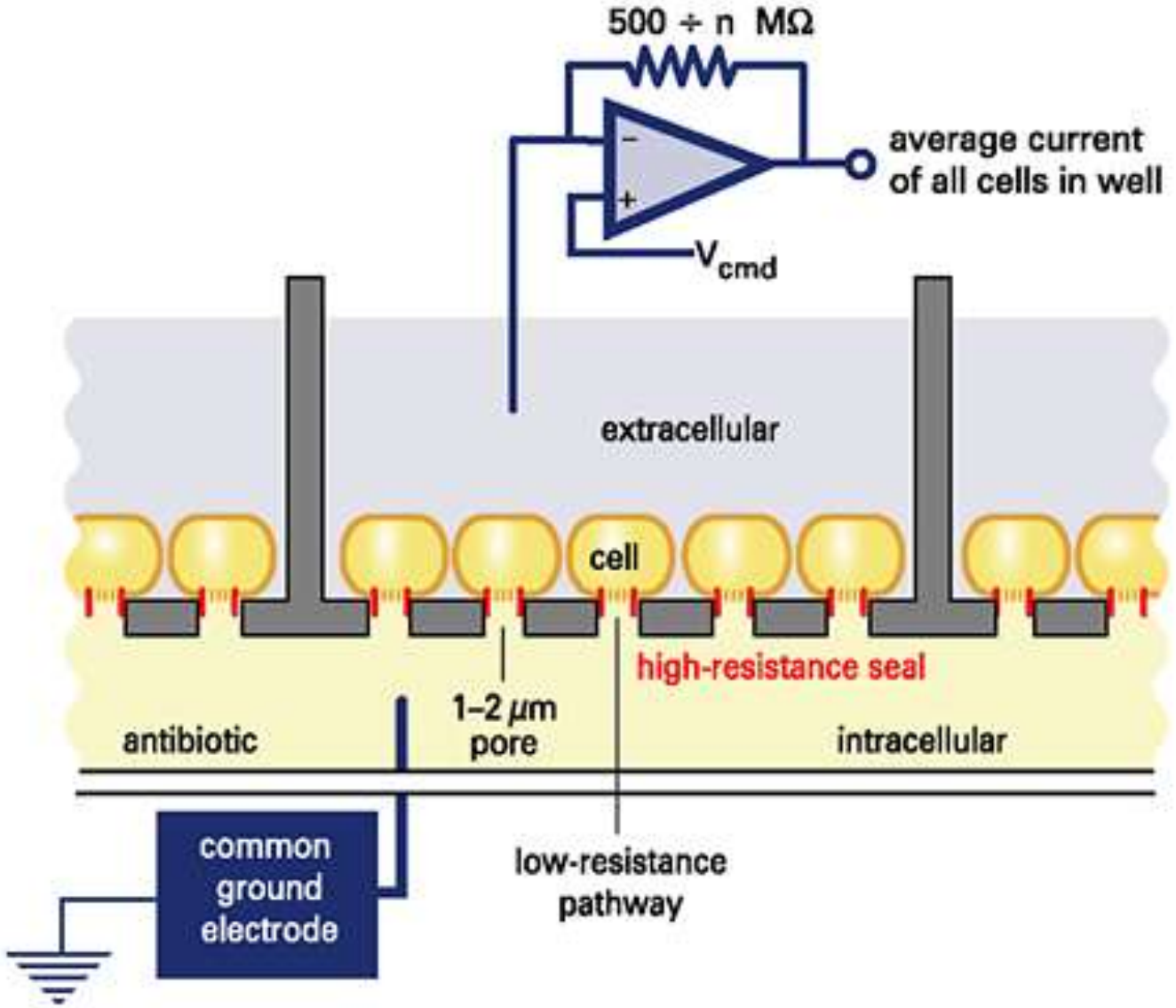
High-through output automatic patch clamp in industry

芯片技术

采用平面电极。平面电极的设计原理来自于传统膜片钳系统的玻璃微电极。在传统的膜片钳实验中，实验人员使用尖端直径 1—2μ M 的玻璃微电极接触细胞表面，与细胞形成紧密封接，再打破细胞膜，形成全细胞记录模式。如果将电极尖端旋转（如下图），使其与细胞接触的部位移至细胞底部，并将电极后端无限拉伸，就形成了平面电极。 平面电极技术摒弃了玻璃微电极，以电极芯片上直径 1—2μ M的小孔来代替玻璃微电极的尖端，从而使同时平行记录多个细胞得以实现。实验中，一定密度的细胞悬液灌注在芯片上面，随机下降到芯片上约 1 - μ2 m 的孔上并在自动负压的吸引下形成高阻封接，打破孔下面的细胞膜形成全细胞记录模式。美国 Molecular Devices（Axon）公司的 Xp 700 系统，采用 Seal Chip 平面电极芯片，是这一技术的代表，其通量最高为 16，即一次可同时记录 16 个细胞，成为高通量全自动膜片钳技术的典范，离子通道药物研发的革命性工具。

- 传统膜片钳微电极
- 旋转
- 拉伸
- 平面电极

High-through output automatic patch clamp in industry



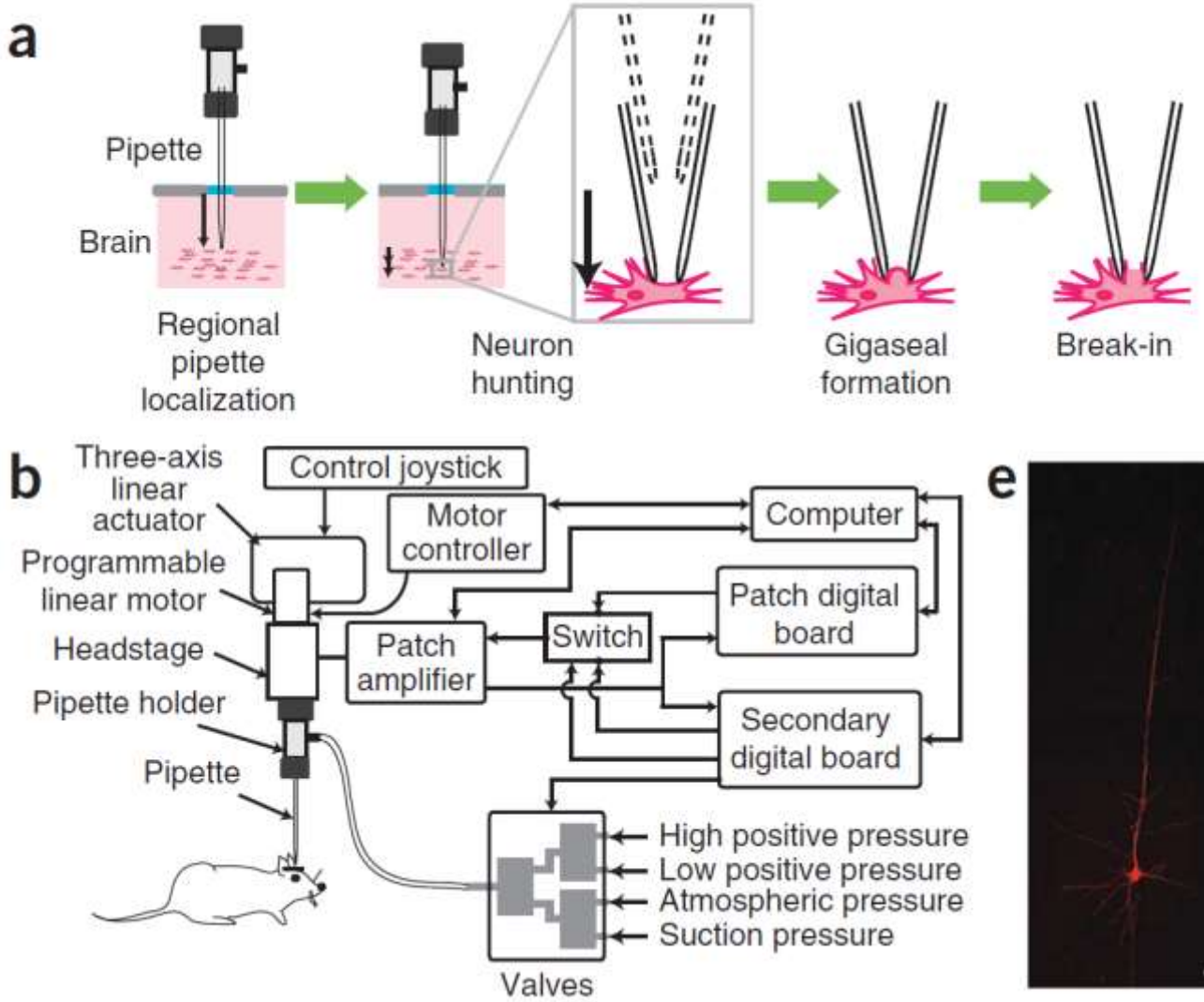
High-through output automatic patch clamp in industry

https://www.moleculardevices.com/en/assets/app-note/dd/cns/patchxpress-7000a-system

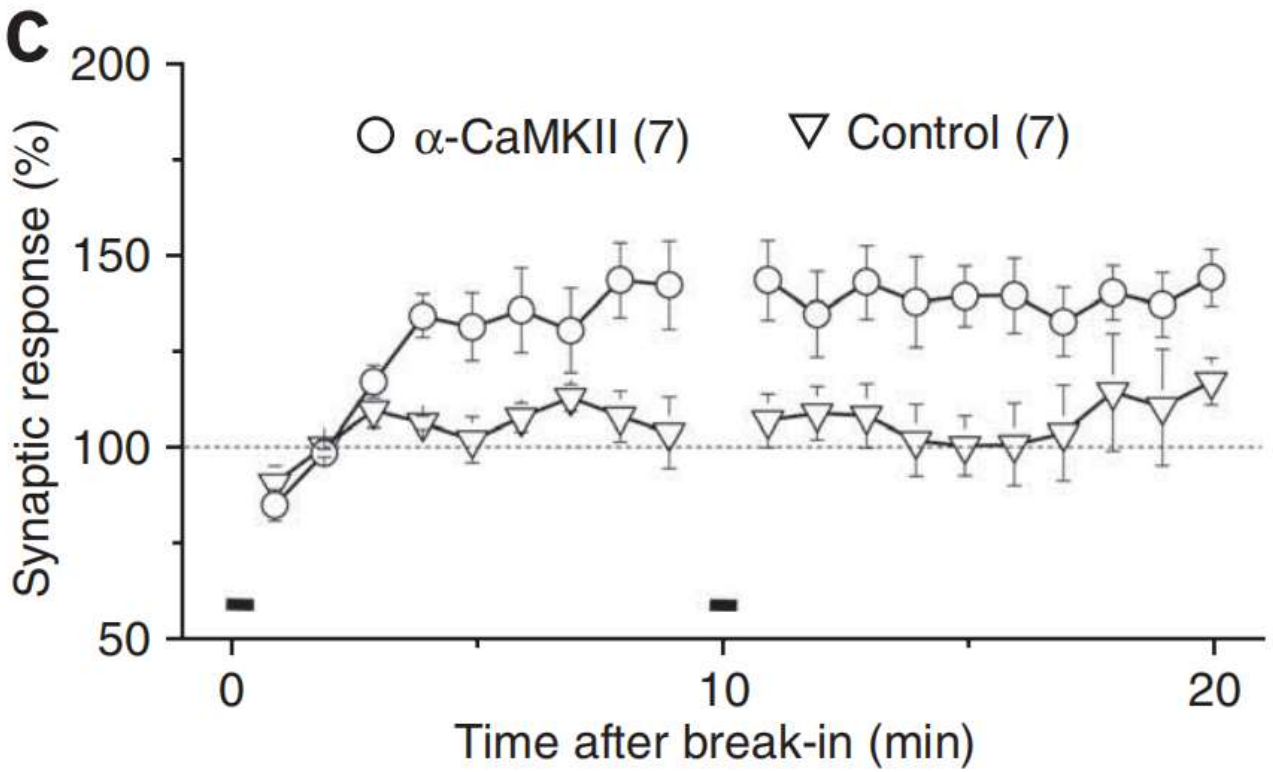
In vivo patch-clamp recording

Automated whole-cell patch-clamp electrophysiology of neurons *in vivo*

Suhasa B Kodandaramaiah[1,2], Giovanni Talei Franzesi[1], Brian Y Chow[1], Edward S Boyden[1,3] & Craig R Forest[2]



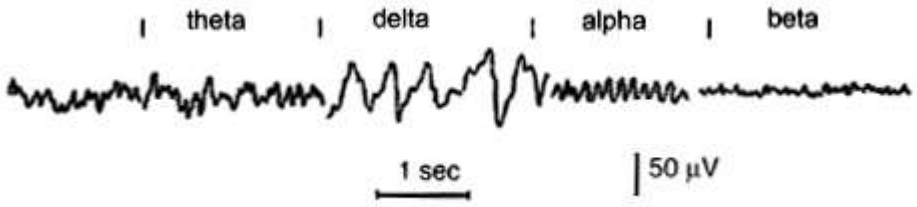
Whole-cell applications



- Whole-cell load molecule
- Whole-cell imaging
- Whole-cell rabies tracing

EEG

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



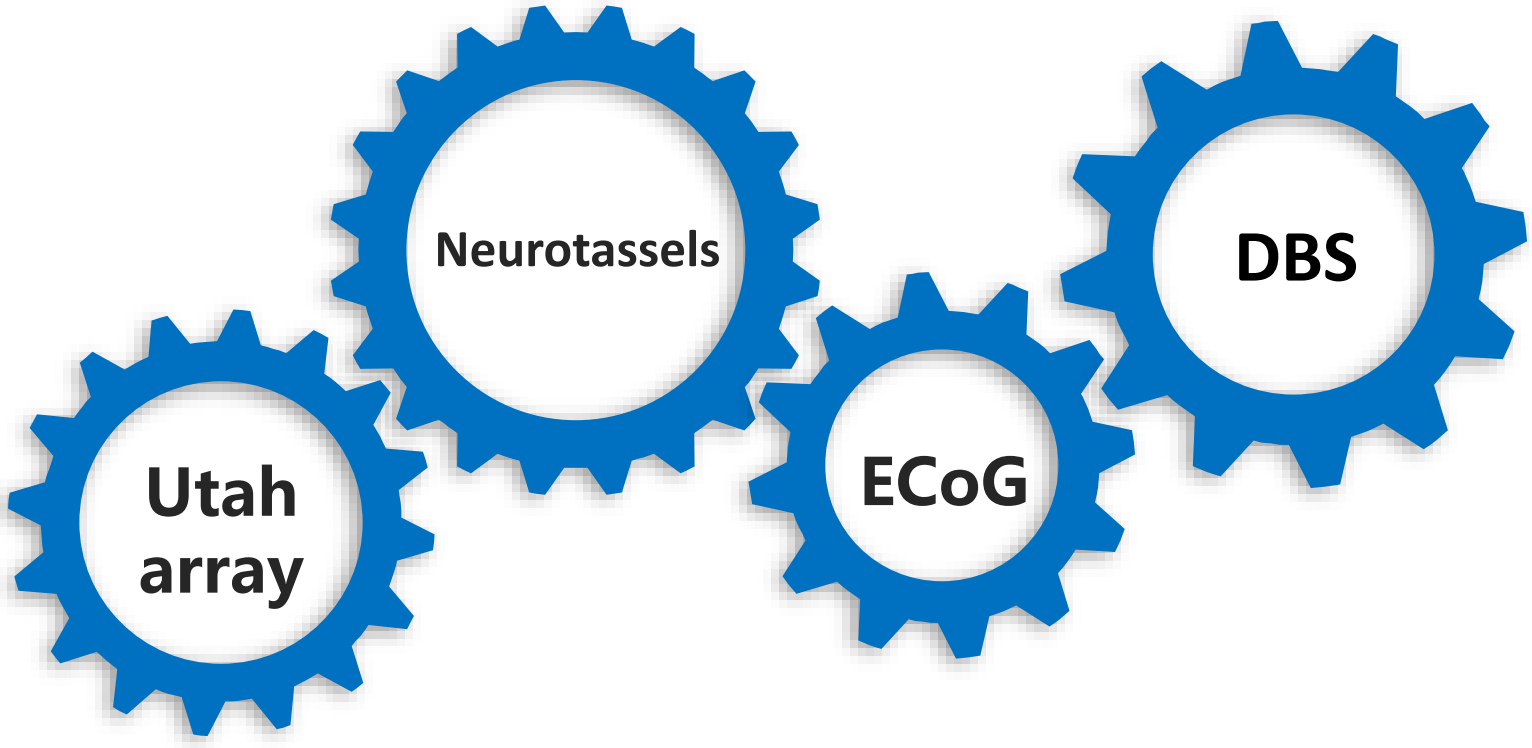
Brain-machine interface

Electrophysiology Development

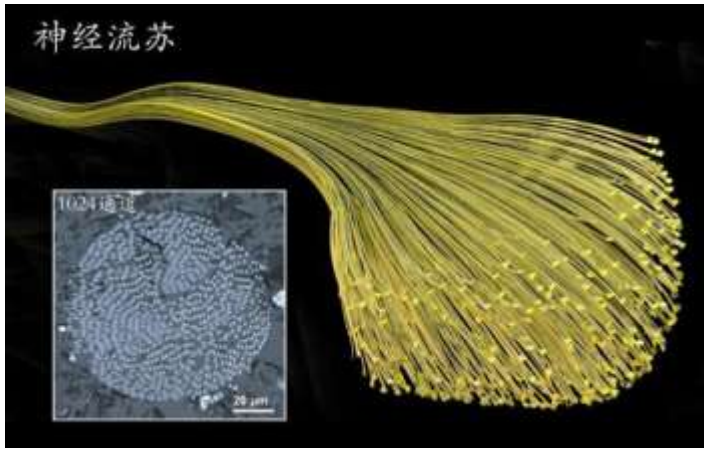
- Extracellular recording
  - Metal electrode
  - Glass micropipette
  - MEA recording
- Intracellular recording
  - Patch-clamp recording
    - Voltage clamp: excitatory post-synaptic current (EPSC)
    - Current clamp: excitatory post-synaptic potential (EPSP)
    - Inside-out
    - Outside-out
  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

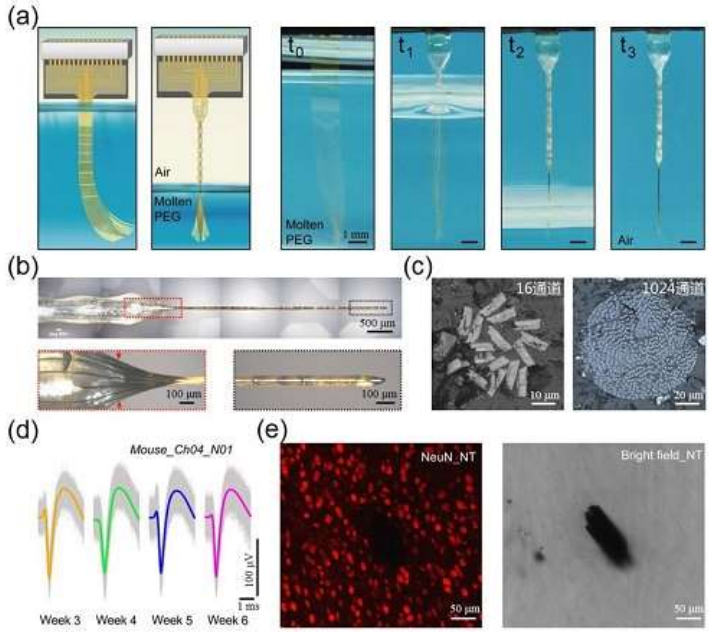
More application in the clinics and system neuroscience

Topics to add in future



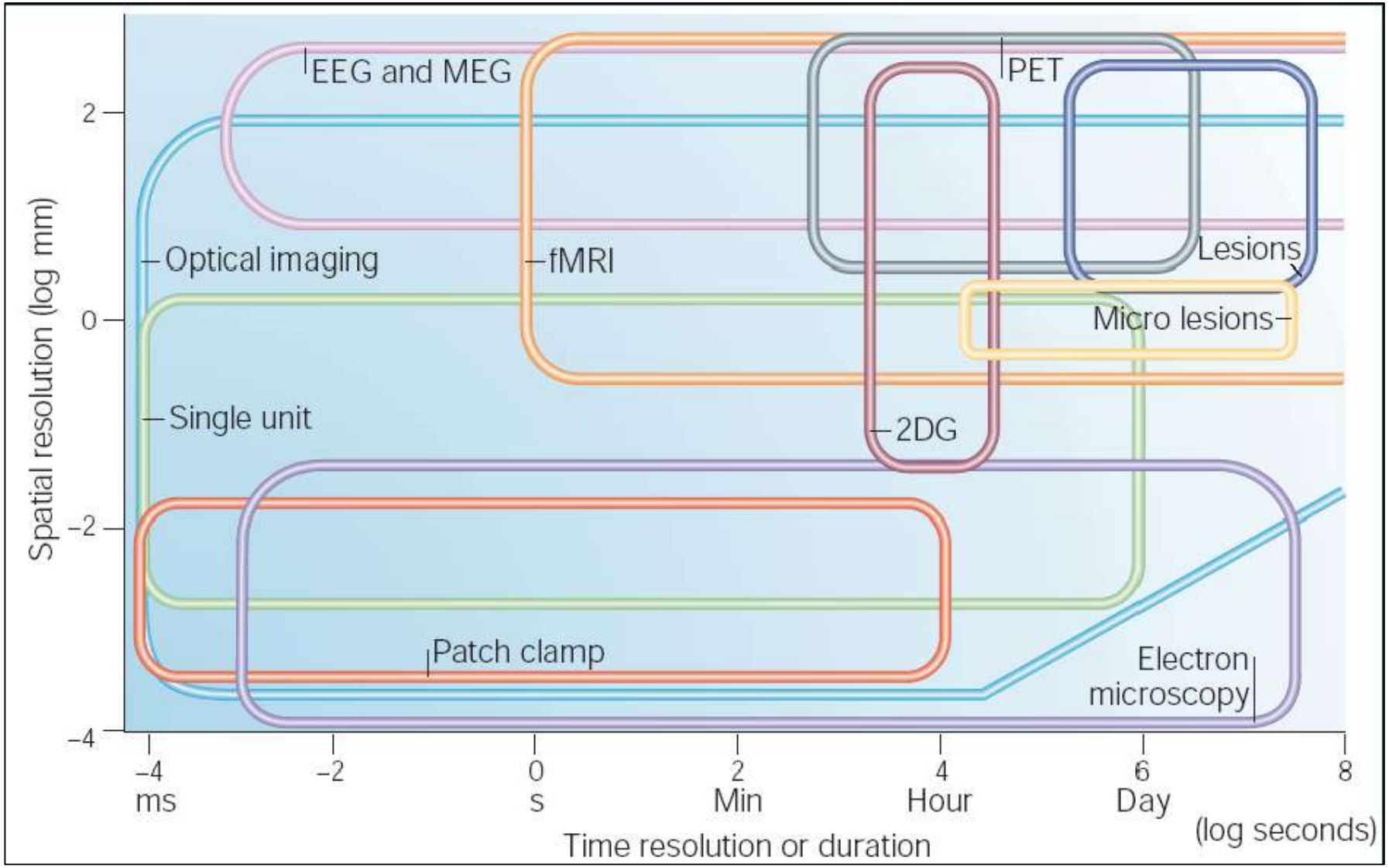
神经流苏



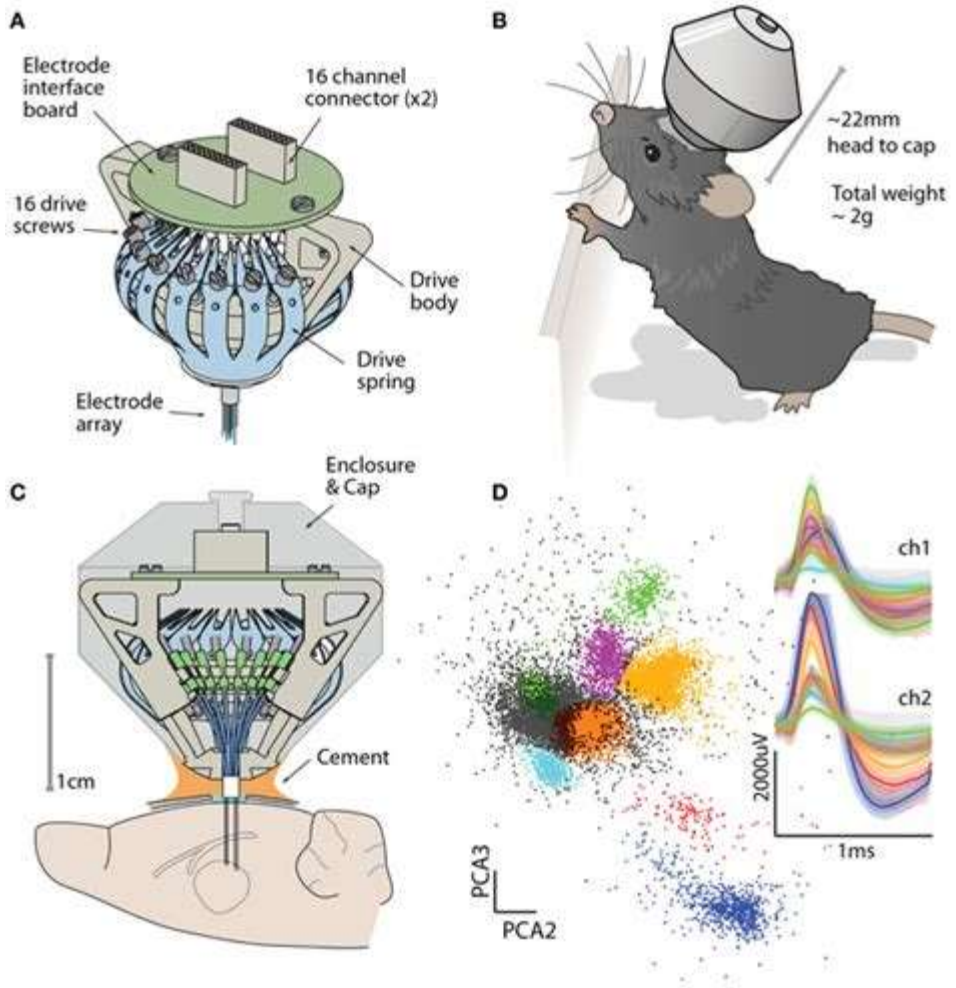


- Why electrophysiology?
- The history and basics of electrophysiology
- Methods in electrophysiology
- Future of electrophysiology

Tools to record biological signals



Increase Channel count



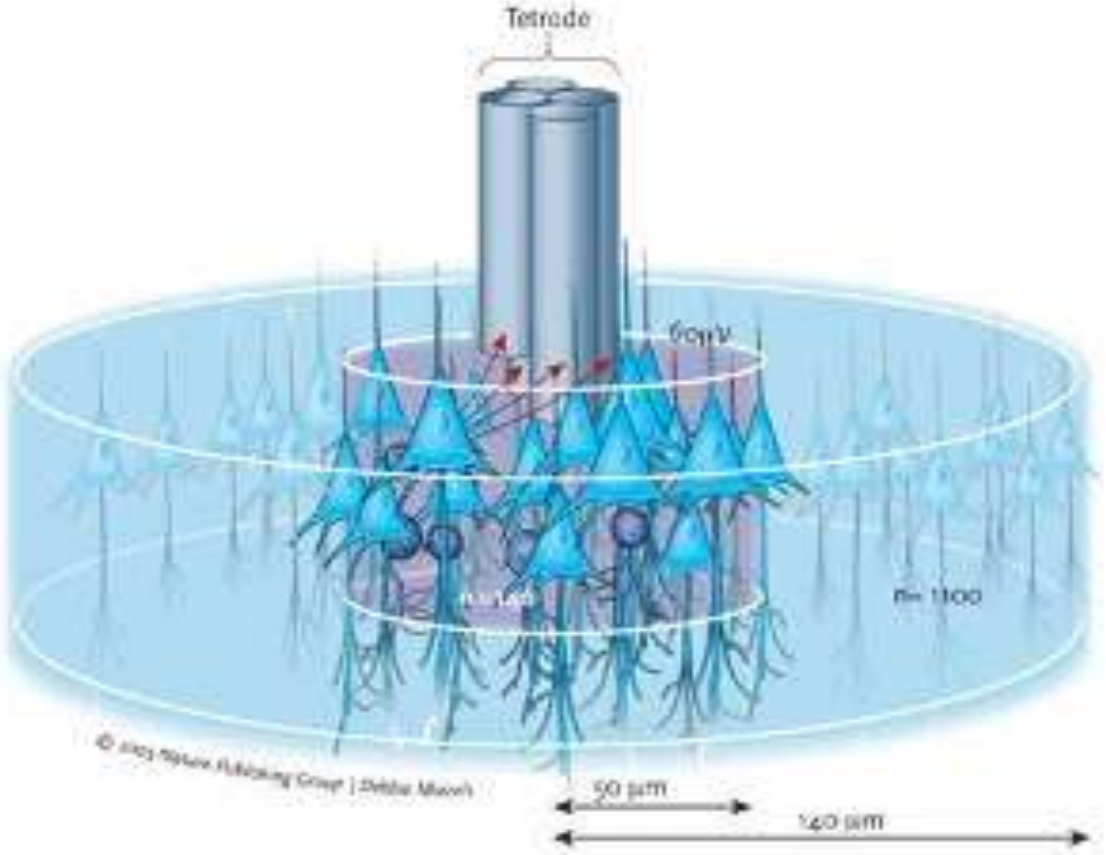
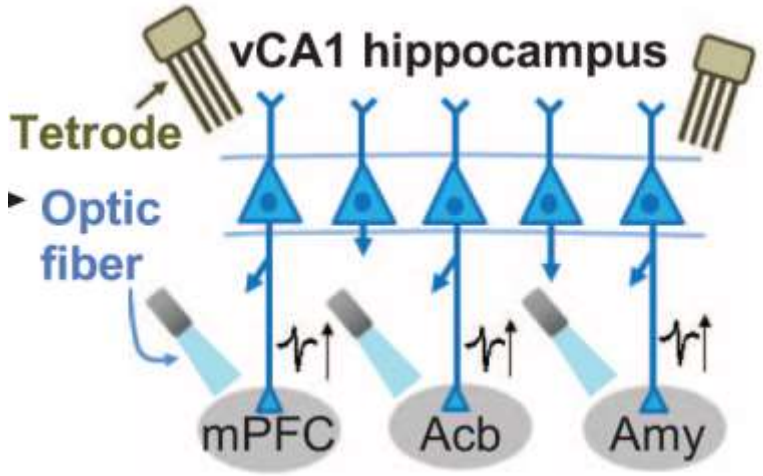
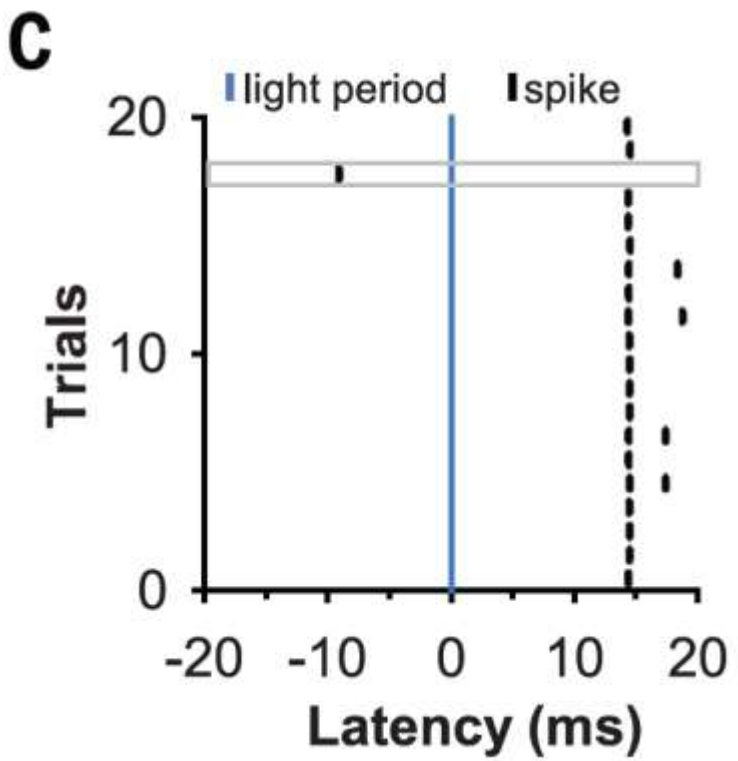
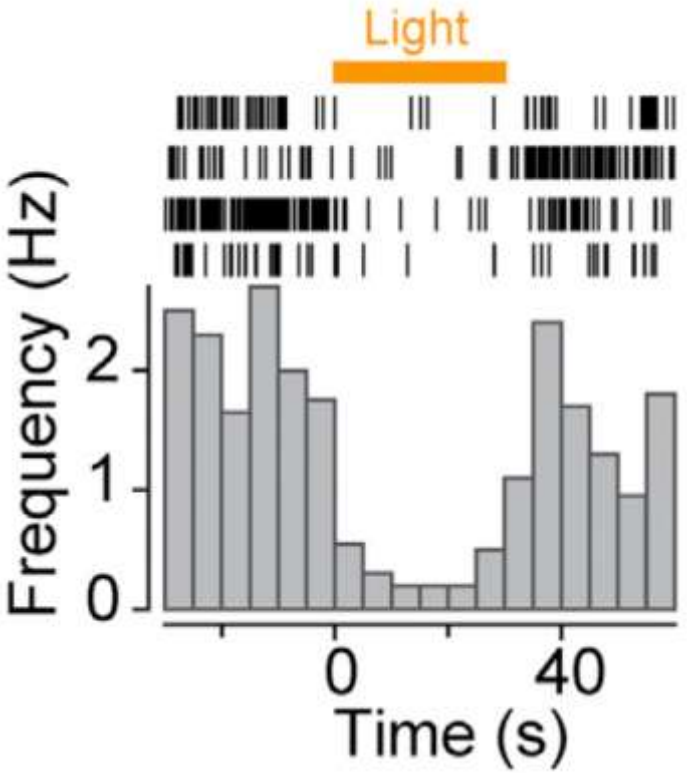


Photo-tagging recording





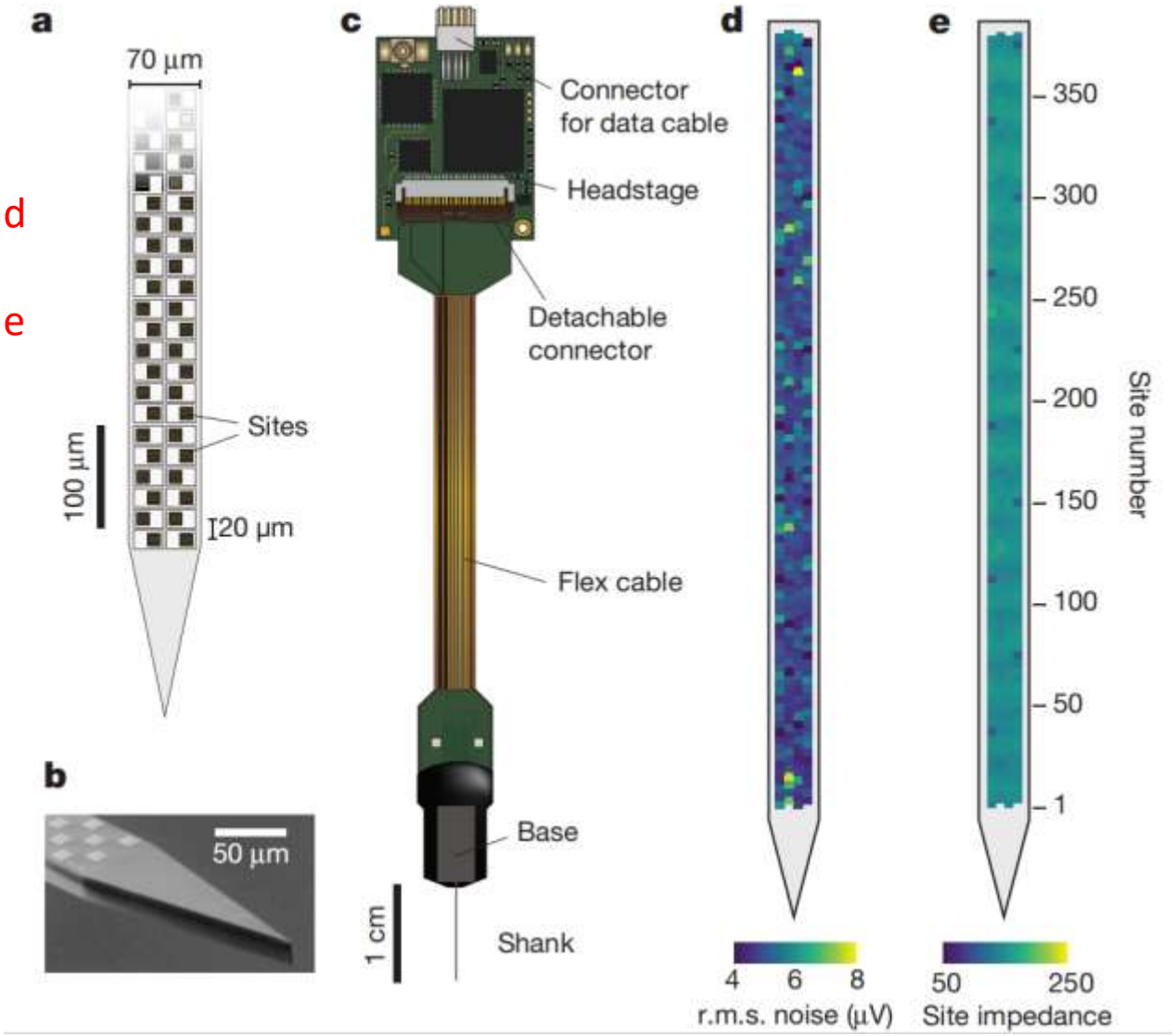
Coicchi et al., 2015

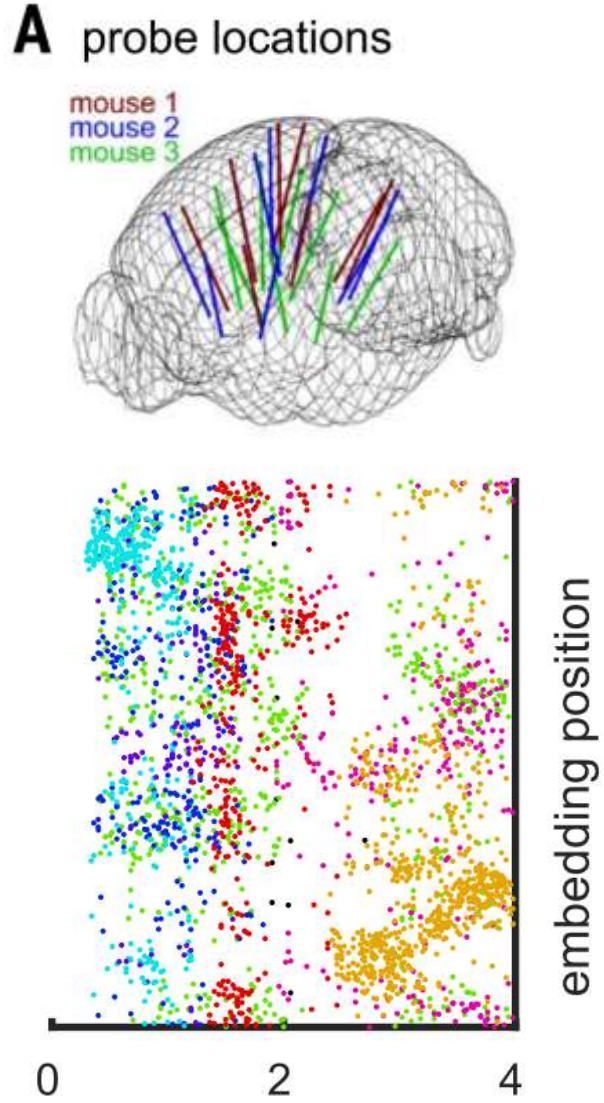


Xu et al., 2016

大范围活动记录单细胞放电活动 Neuropixel

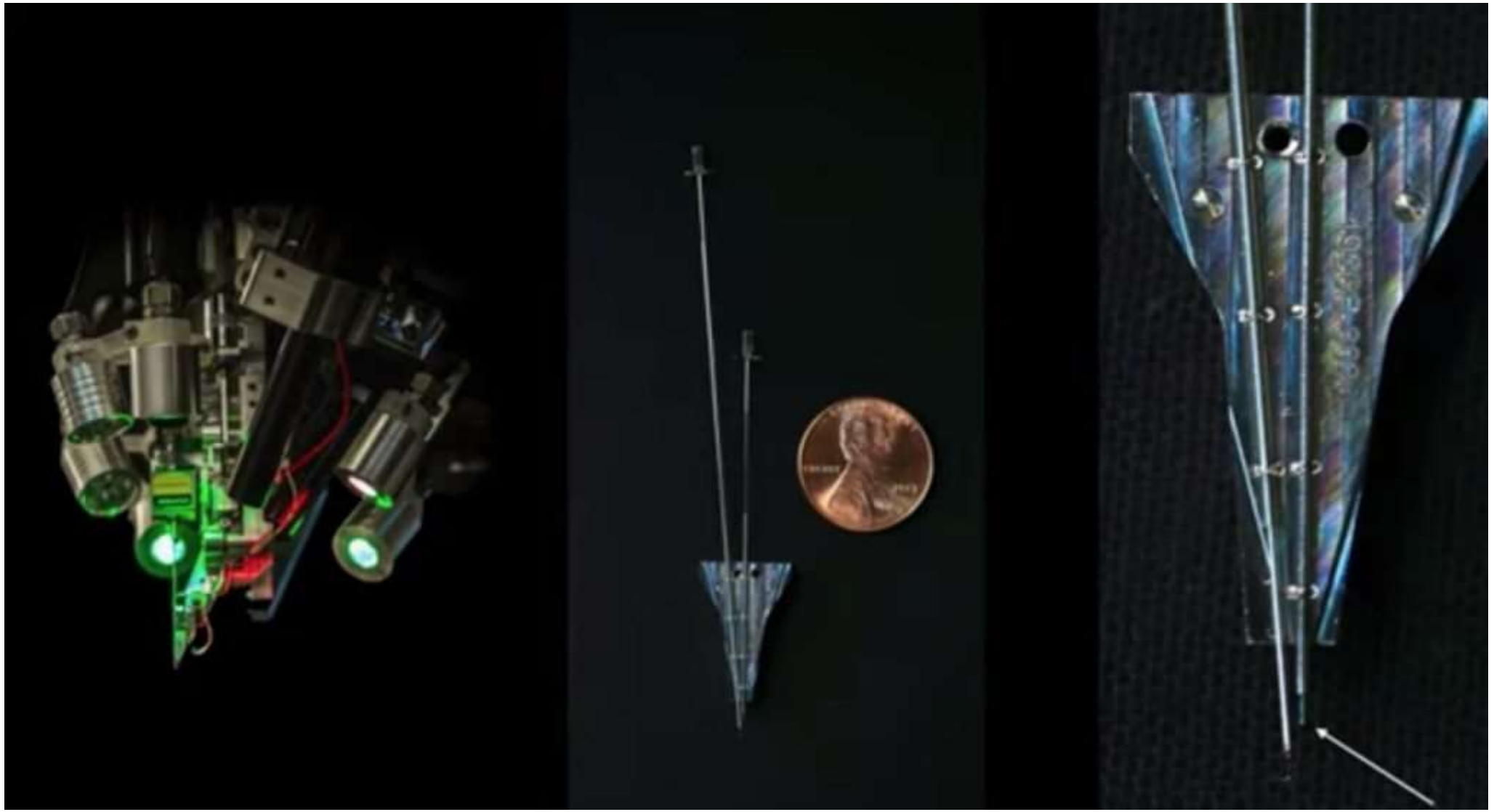
384 dual-band
low-noise
960 selectable
10-mm long





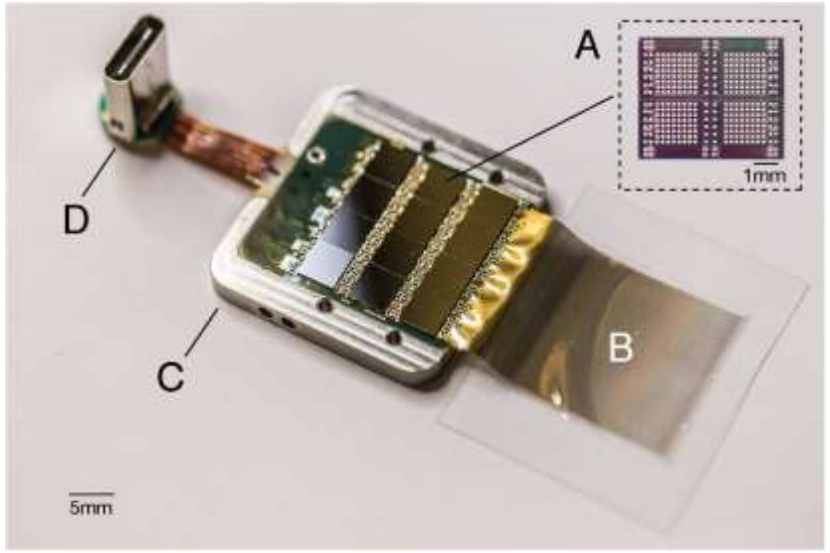
Jun et al., Nature, 2017; Stringer et al., Science, 2019

Neuralink by Elon Musk

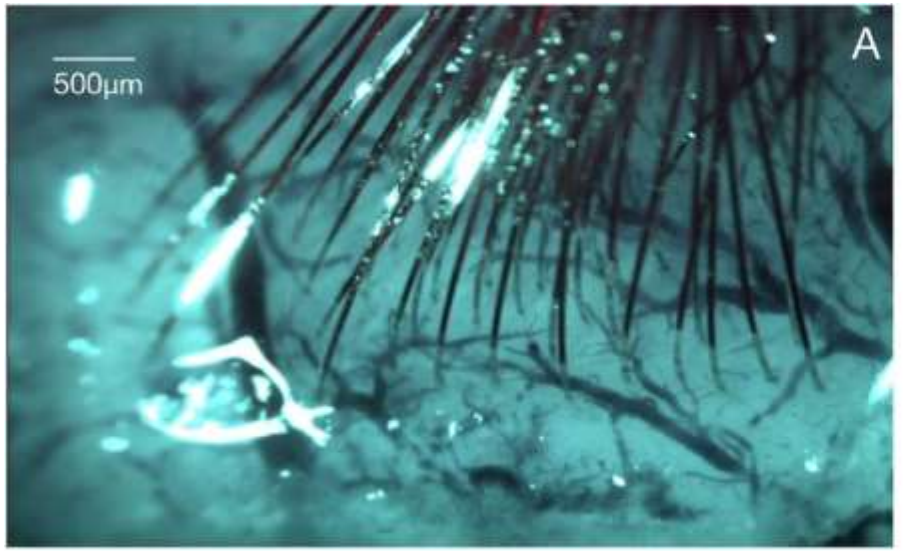


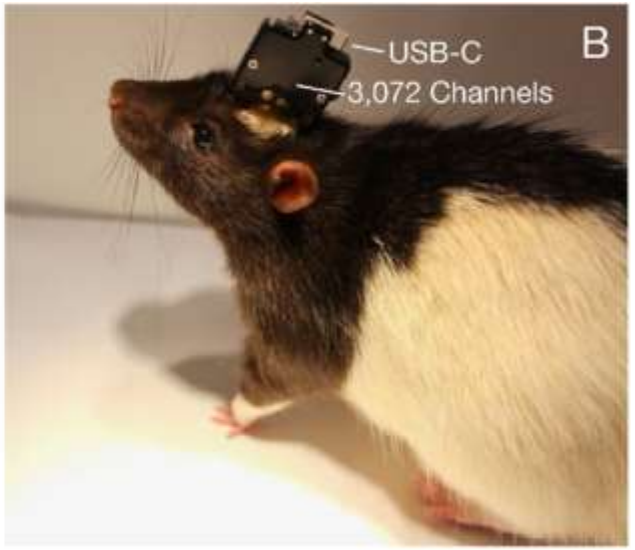
Neuralink by Elon Musk

A packaged sensor device



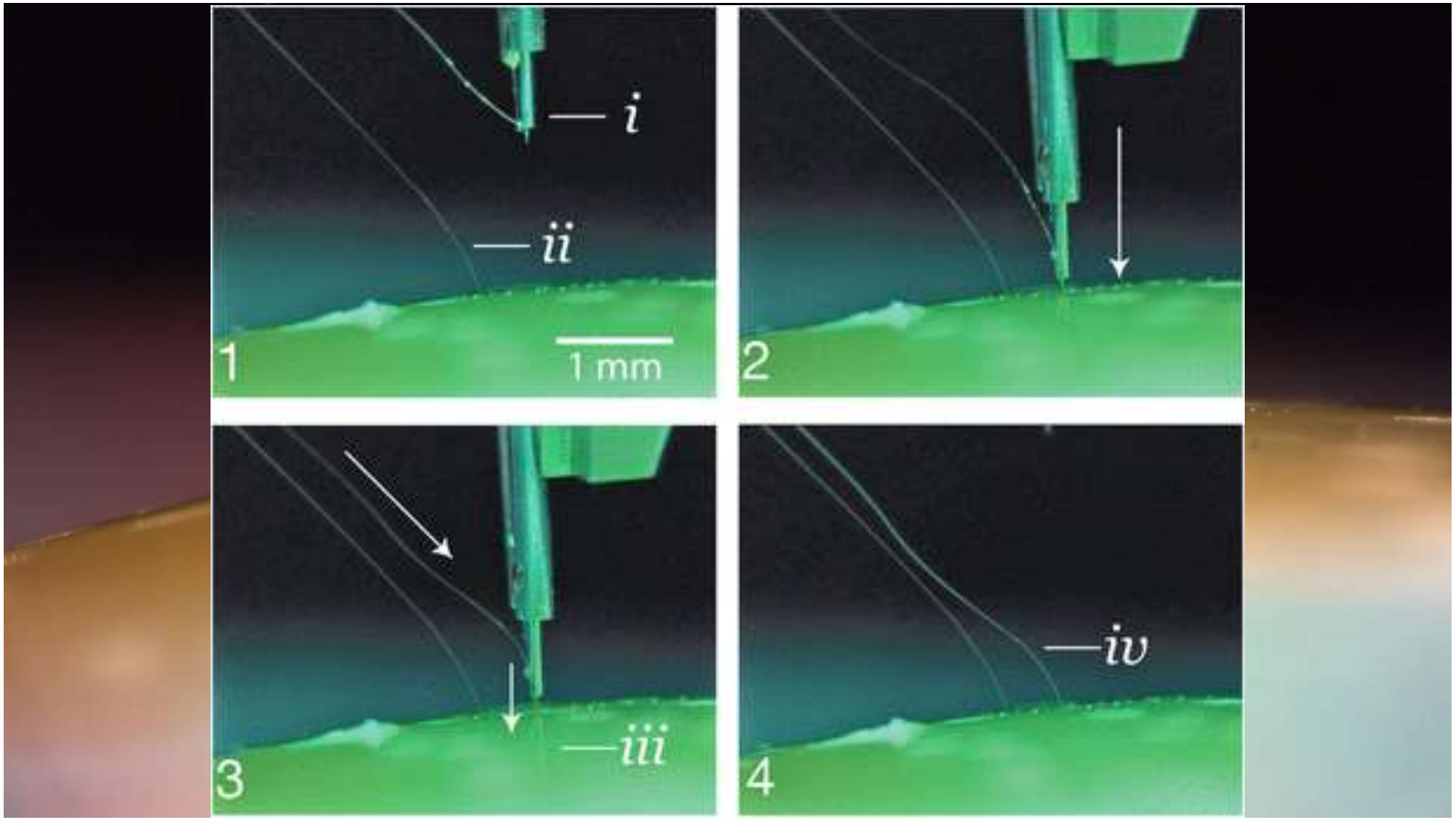
Thread implantation and packaging.





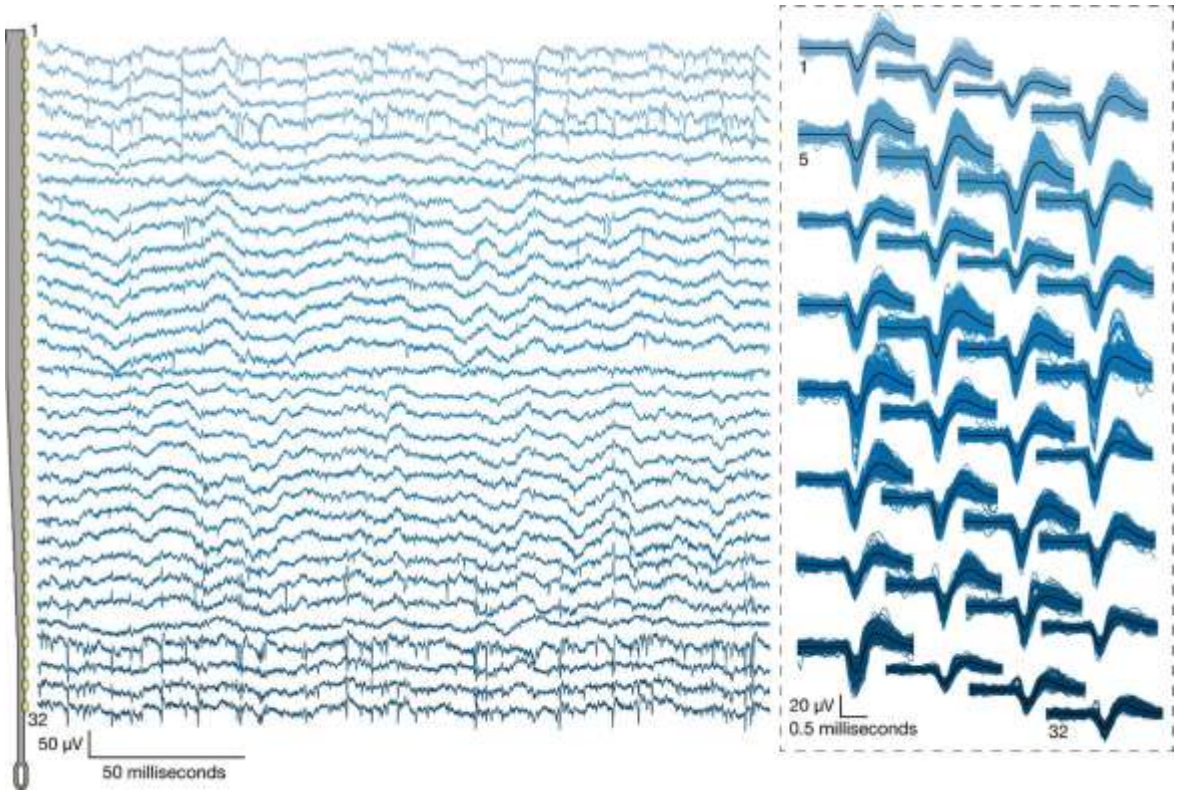
doi: https://doi.org/10.1101/703801

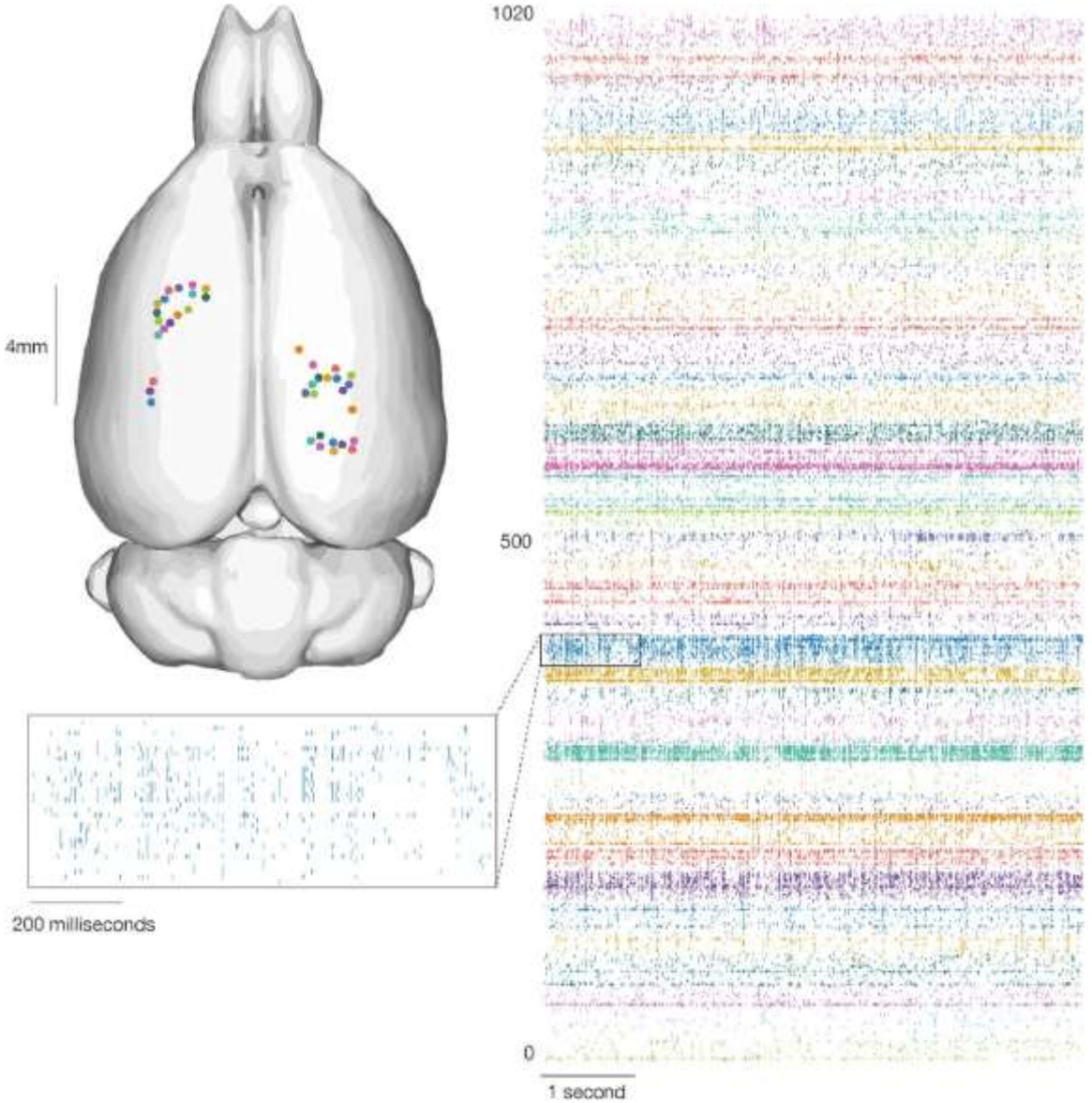
Neuralink by Elon Musk



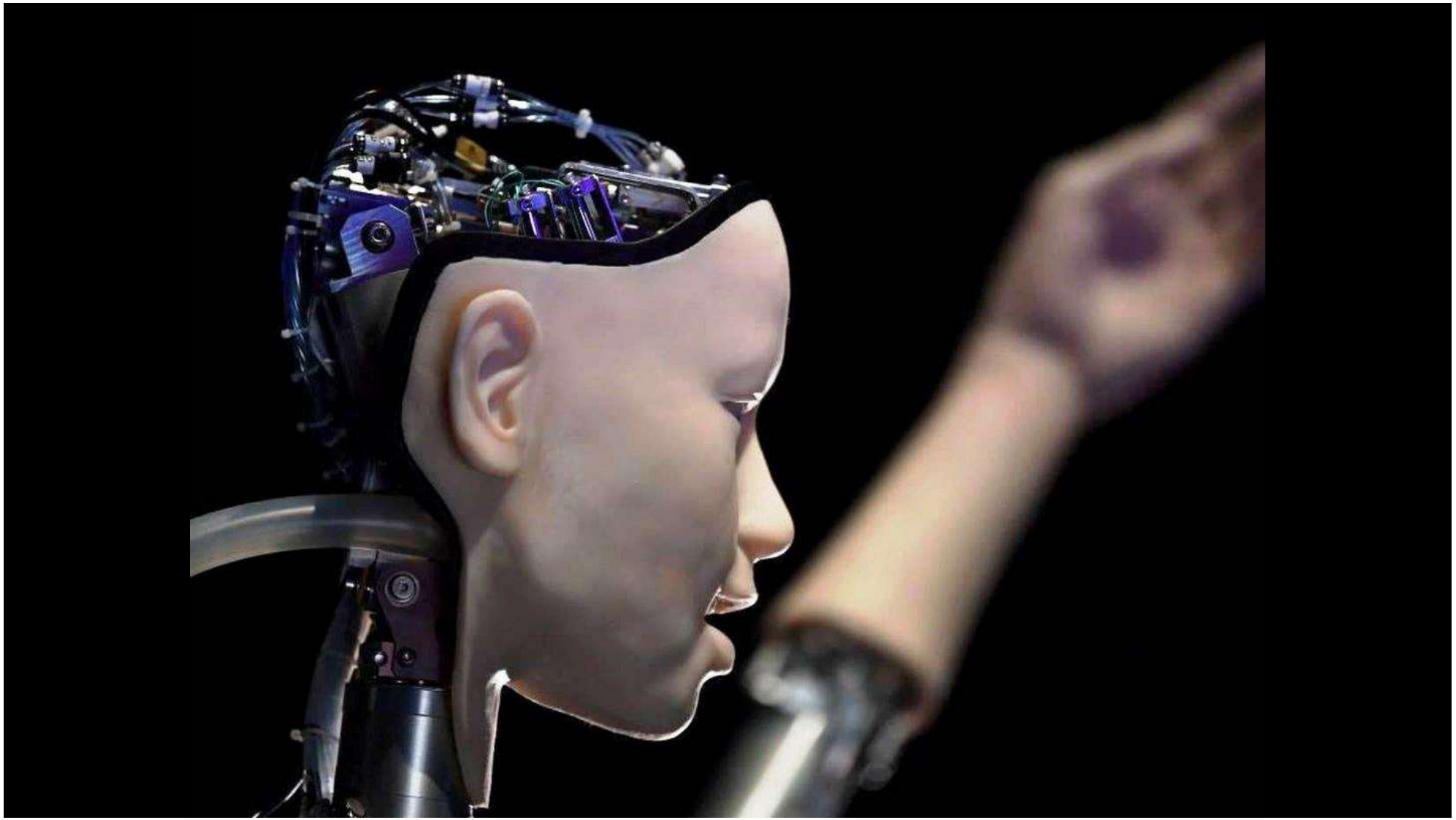
Neuralink by Elon Musk

Neuralink by Elon Musk





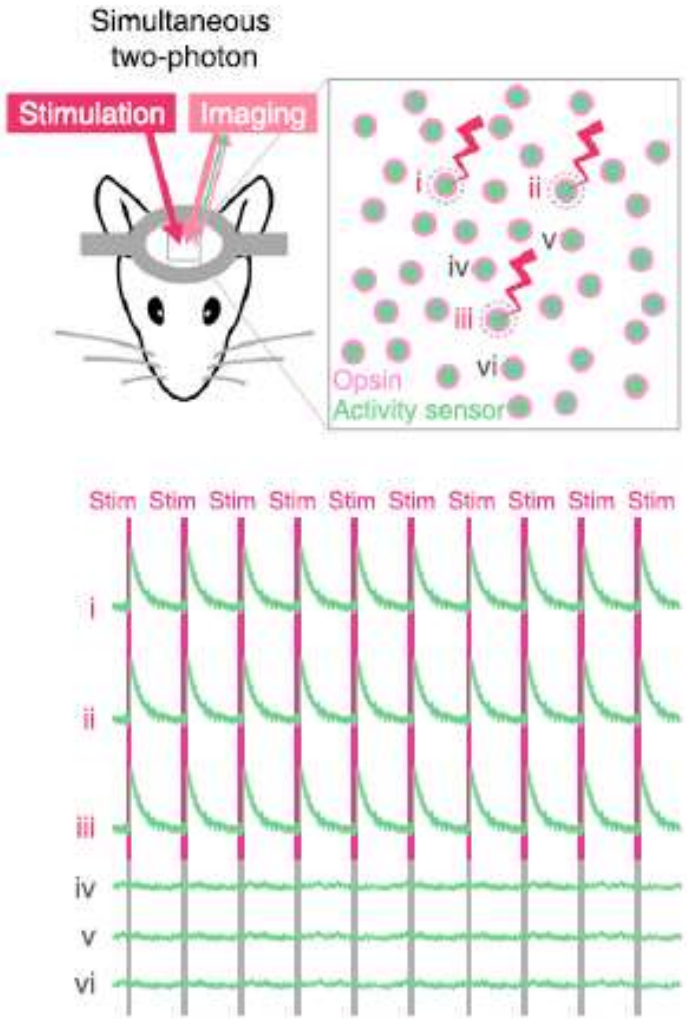
Introducing Neuralink

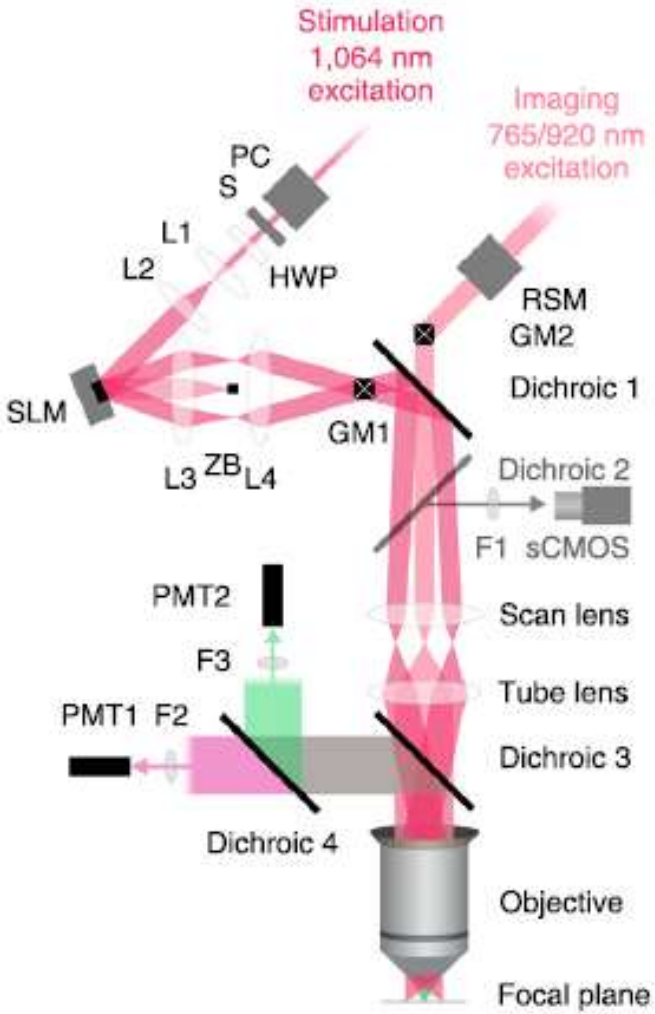


03 Beyond electrophysiology

Combined with optical imaging
in the future.

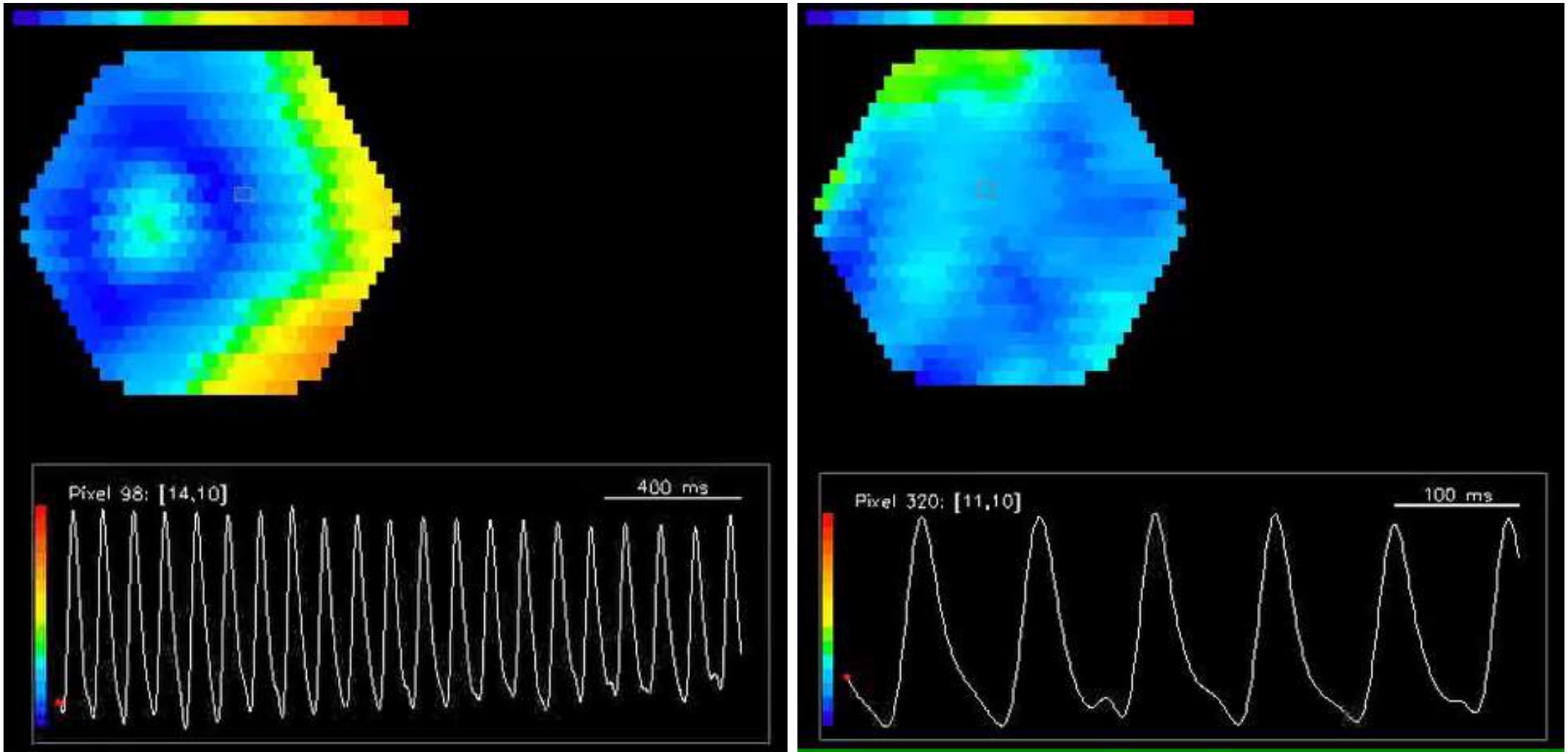
All-optical: calcium imaging + precise optogenetic





Packer et al; 2012

Voltage-sensitive dye imaging



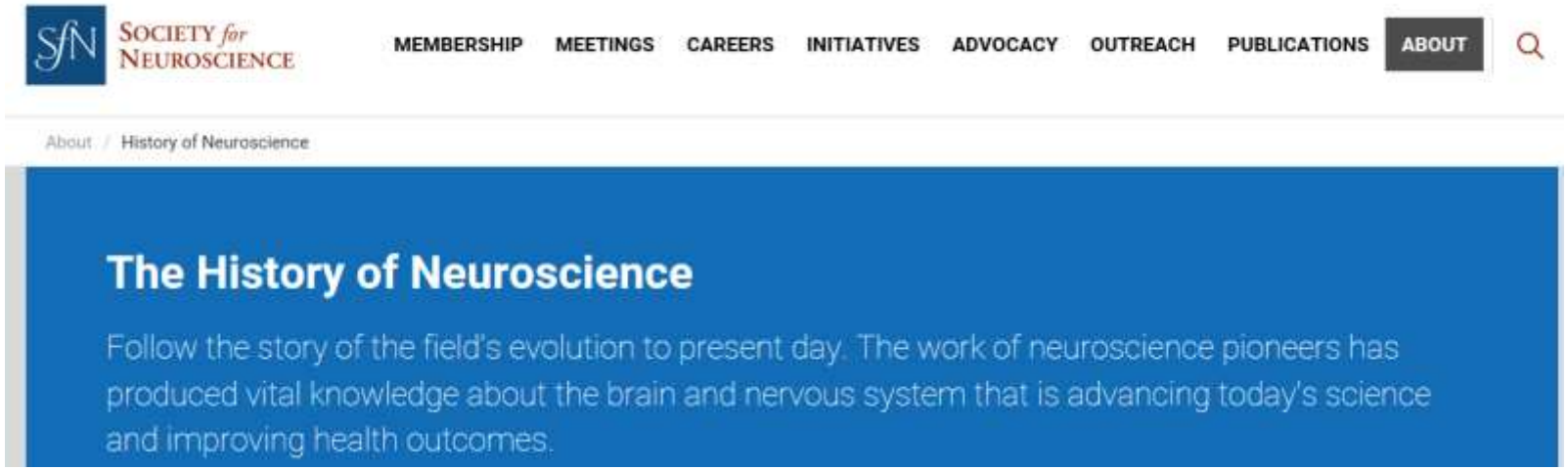
Huang et al., 2004

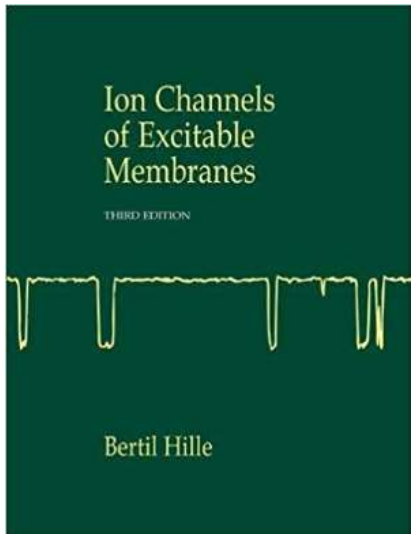
Future of electrophysiology

- Increase Channel count
- Electrode: More stable recording and tissue friendly
- Wireless
- Optical imaging
- All-optical: calcium imaging + precise optogenetic
- Voltage-sensitive dye and indicator
- Miniscope

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

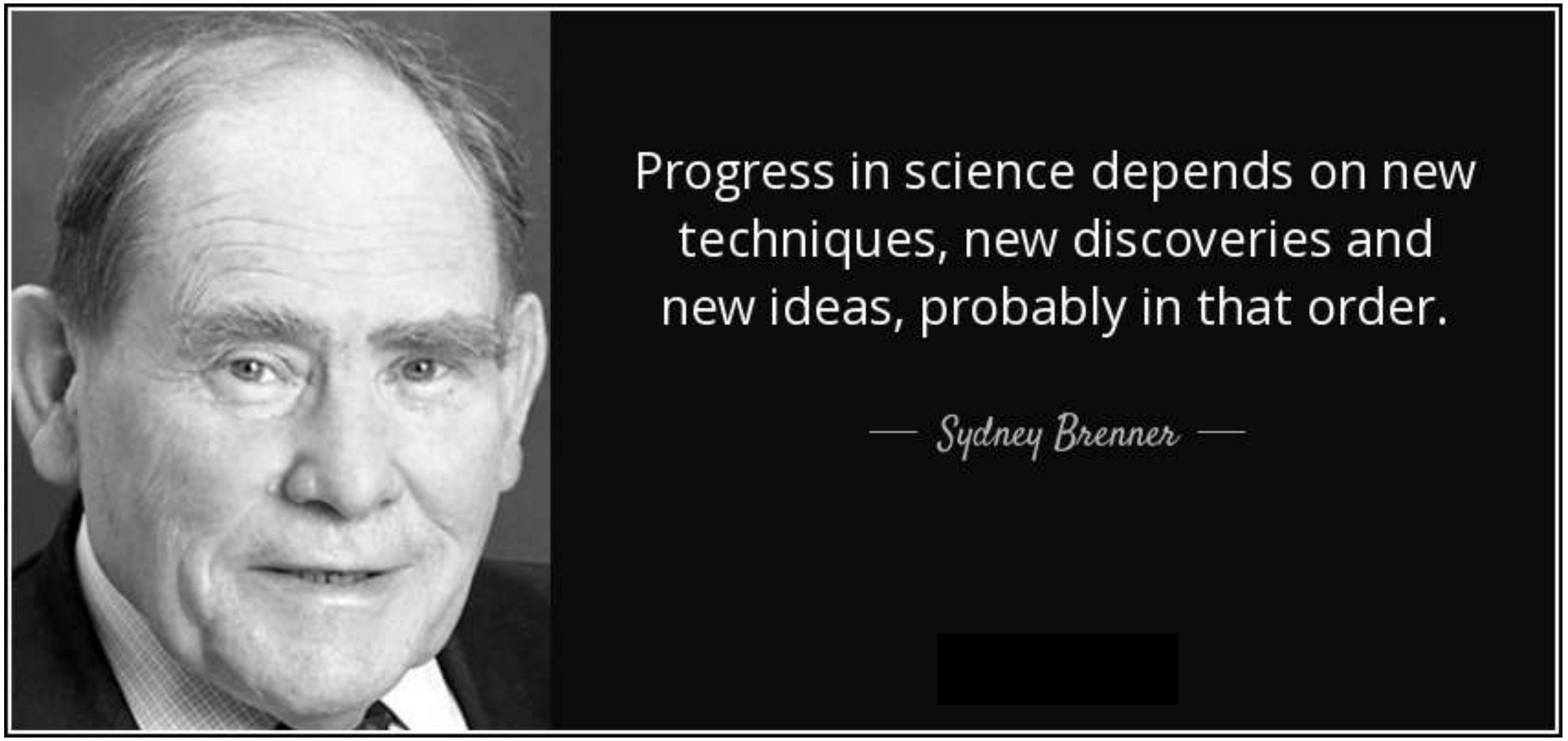
Reference:

Peter Somogyi
GYORGY BUZSAKI
Kenneth Harris
Axon
HEKA

Reference for patch-clamp recording

- 1. E. Neher, B. Sakmann, Single-channel currents recorded from membrane of denervated frog muscle fibres. ***Nature 260, 799 (Apr 29, 1976).***
- 2. H. R. Brenner, B. Sakmann, Gating properties of acetycholine receptor in newly formed neuromuscular synapses. ***Nature 271, 366 (Jan 26, 1978).***
- 3. B. Sakmann, H. R. Brenner, Change in synaptic channel gating during neuromuscular development. ***Nature 276, 401 (Nov 23, 1978).***
- 4. B. Sakmann, G. Boheim, Alamethicin-induced single channel conductance fluctuations in biological membranes. ***Nature 282, 336 (Nov 15, 1979).***
- 5. F. Conti, E. Neher, Single channel recordings of K+ currents in squid axons. ***Nature 285, 140 (May 15, 1980).***
- 6. B. Sakmann, J. Patlak, E. Neher, Single acetylcholine-activated channels show burst-kinetics in presence of desensitizing concentrations of agonist. ***Nature 286, 71 (Jul 3, 1980).***
- 7. F. J. Sigworth, E. Neher, Single Na+ channel currents observed in cultured rat muscle cells. ***Nature 287, 447 (Oct 2, 1980).***
- 8. D. Colquhoun, E. Neher, H. Reuter, C. F. Stevens, Inward current channels activated by intracellular Ca in cultured cardiac cells. ***Nature 294, 752 (Dec 24, 1981).***
- 9. D. Colquhoun, B. Sakmann, Fluctuations in the microsecond time range of the current through single acetylcholine receptor ion channels. ***Nature 294, 464 (Dec 3, 1981).***
- 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).***

- END -



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