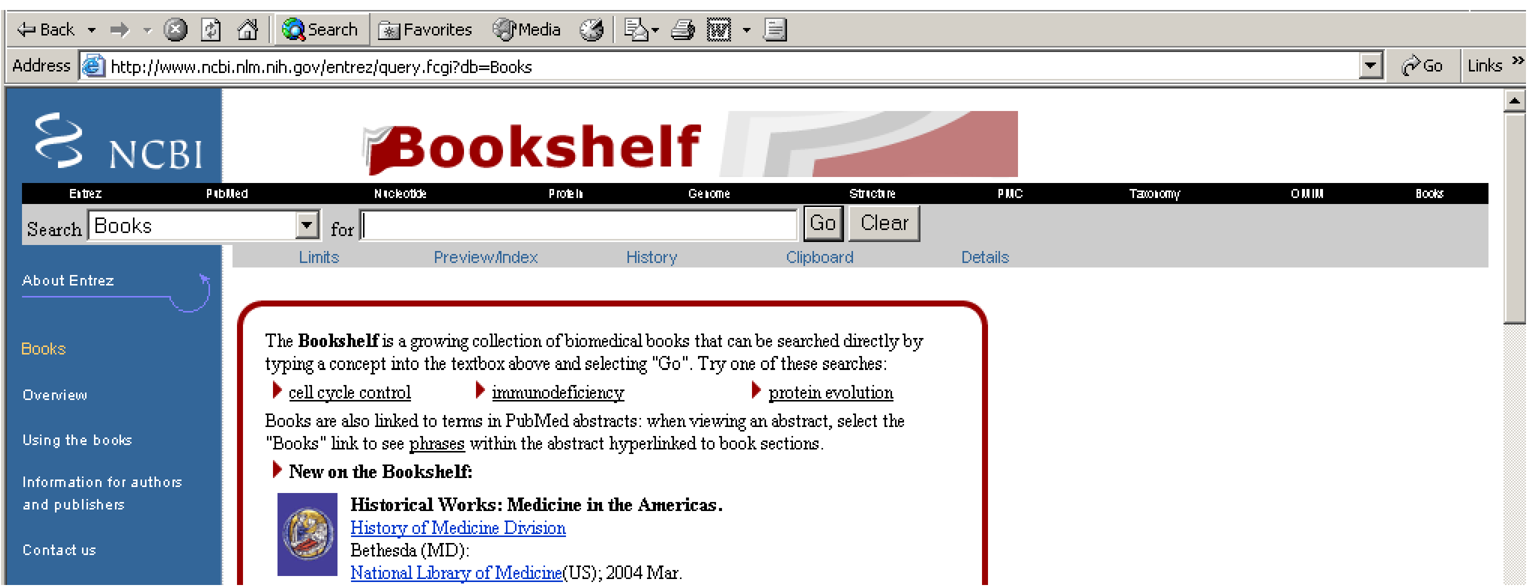
Neurobiology

Mostly Neurophysiology, Really

Acutally Mostly Electrophysiology

Study Material

- Textbooks..
  - Library, collegue, buy
  - neurobiology textbook pdf ...
- Online Texts
  - http://neuroscience.uth.tmc.edu/s1/introduction.html
- Papers......



Overall Aims

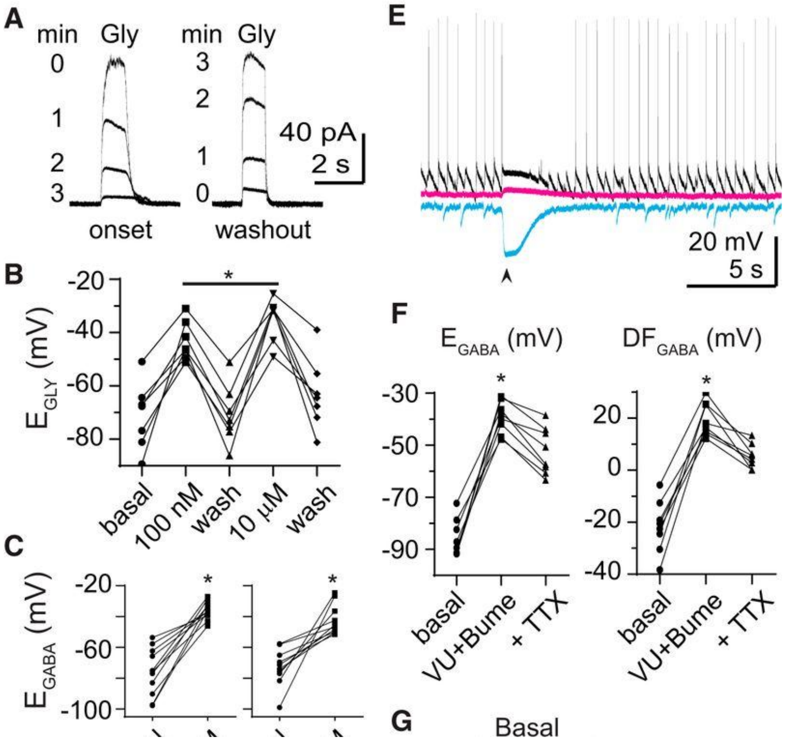
- Understand the basic concepts behind the electrical signals generated at the cell membrane.
- Know the values of the key parameters involved.
- Remember the typical techniques used to investigate these phenomena

Overall Aims

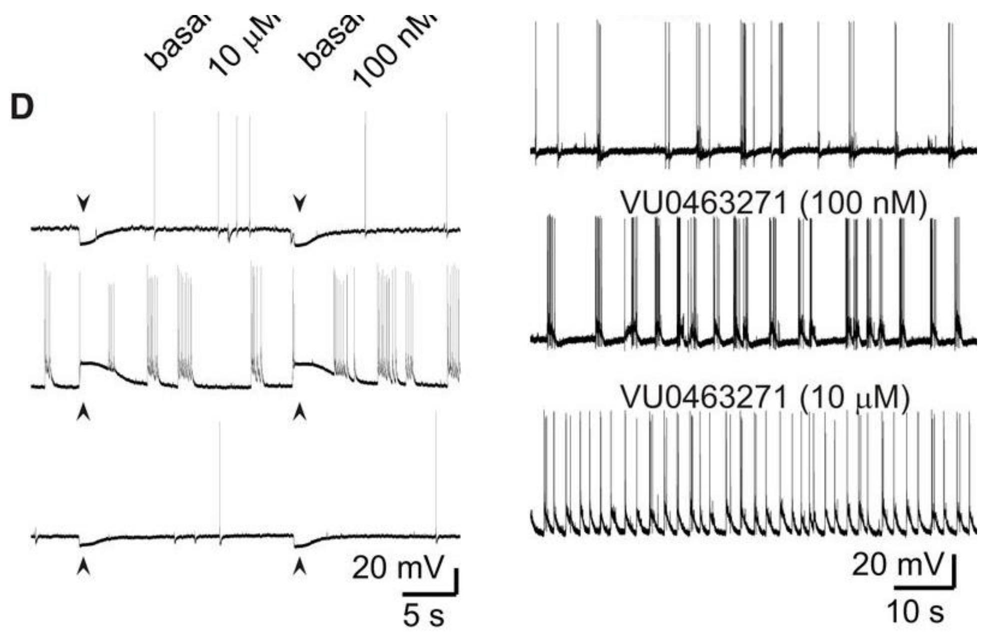
...recordings were performed at 34°C, and HEK cells were recorded at room temperature in the bath saline. For perforated patch experiments, pipettes contained saline (in mm): 140 KCl and 10 HEPES, pH 7.4 KOH. For whole-cell experiments, pipettes contained saline (in mm): 130 K-gluconate, 10 KCl, 0.1 $CaCl_2$, 2 Mg-ATP, 1.1 EGTA, and 10 HEPES, pH 7.4 KOH. Bath saline contained the following (in mm): 140 NaCl, 2.5 KCl, 2.5 $CaCl_2$, 1.2 $MgCl_2$, 10 HEPES, and 11 glucose, pH 7.4 NaOH. We performed an equimolar substitution of NaCl by KCl for the 10 mm $[K^+]_o$ experiments..... We used 20 mV voltage-ramp protocols over 1 s periods to determine the reversal potentials of the leak-subtracted muscimol-activated or glycine-activated currents. The voltages from whole-cell experiments were corrected offline using a calculated liquid junction potential value (16.3 mV) in Clampex (Molecular Devices). Transverse hippocampal slices (400 µm) were immersed in ice-cold cutting solution containing the following (in mm): 87 NaCl, 2.5 KCl, 0.5 $CaCl_2$, 25 $NaHCO_3$, 1.25 NaH_2PO_4, 7 $MgCl_2$, 50 sucrose, and 25 glucose (equilibrated with 95% O_2 and 5% CO_2). Slices recovered for 1 h in ACSF containing the following: (in mm) 126 NaCl, 26 $NaHCO_3$, 1.5 NaH_2PO_4, 2.5 KCl, 2 $CaCl_2$, 2 $MgCl_2$, and 10 glucose at 34°C. Electrodes filled with normal ACSF (1–5 MΩ resistance) and positioned in layer III of the medial entorhinal cortex were used to record epileptiform activity in normal ACSF with elevated KCl (5 mm) and lacking $MgCl_2$.

Sudhir Sivakumaran et al. J. Neurosci. 2015;35:8291-8296

Overall Aims



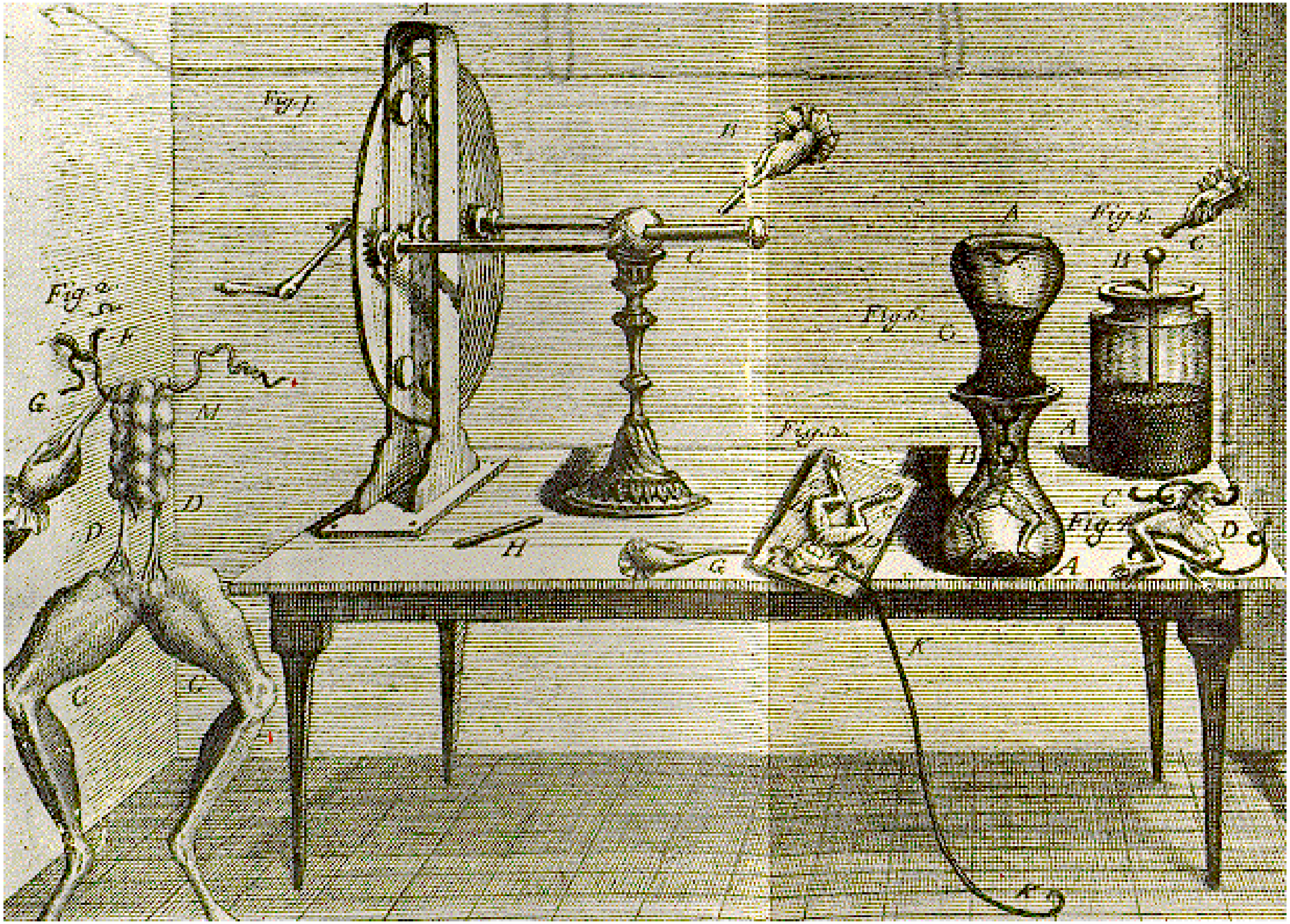
Sudhir Sivakumaran et al. J. Neurosci. 2015;35:8291-8296



Proposed Syllabus

Topics I	Topics II
Introduction & Electrochemical Gradients	Synaptic Transmission
Passive Membrane Properties	Electrophysiology Techniques
Action Potentials	Basic Circuits (Spinal Cord)
Voltage-Gated Ion Channels	Sensory Systems Overview
Ligand-Gated Ion Channels	Synaptic Plasticity

Discovering Electricity

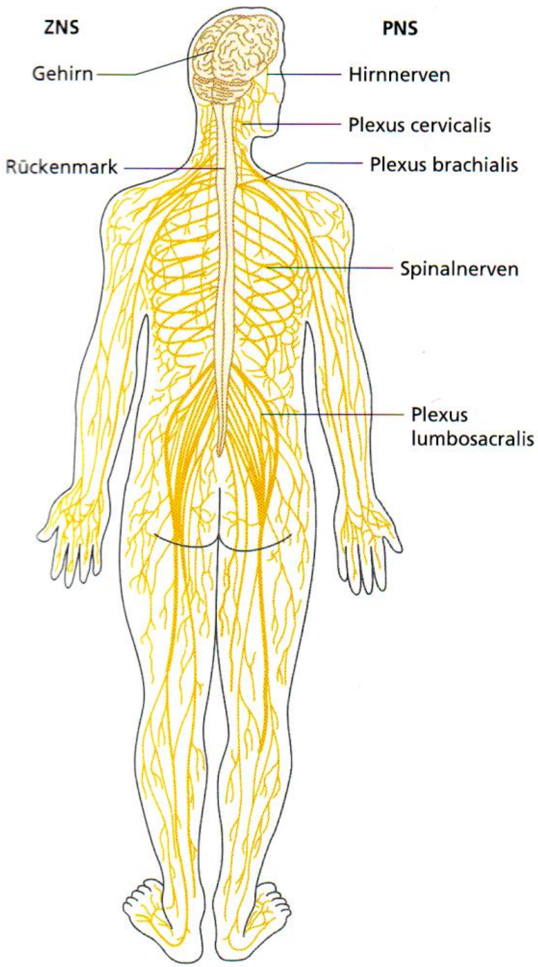


The Role of Electrical Signals

Ion channels and the electrical properties they confer on cells are involved in every human characteristic that distinguishes us from the stones in a field. Every perception, thought, movement, and heartbeat depends on electrical signals generated by the activity of ion channels.........

Science. STKE (Signal transduction knowledge environment)
Vol. 2003, Issue 188, pp. re10, 24 June 2003
„Voltage-Gated K Channels“
Clay M. Armstrong

Why a Nervous System?



Moving Around

Elements of Molecular Neurobiology. C. U. M. Smith
Copyright © 2002 John Wiley & Sons, Ltd.
ISBNs: 0-470-84353-5 (HB); 0-471-56038-3 (PB)

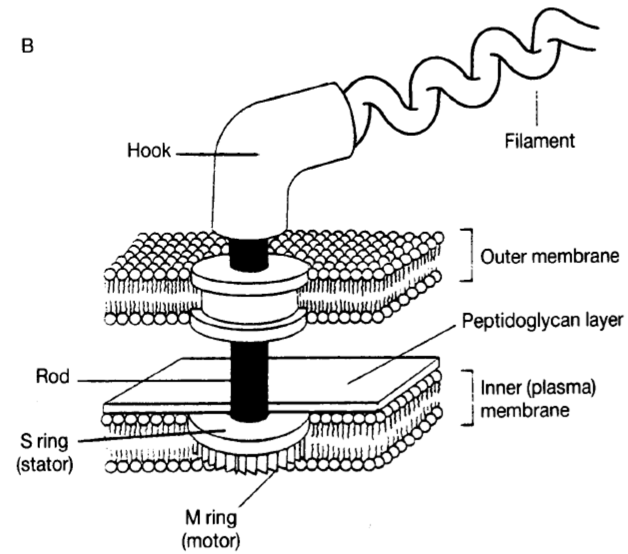


Figure 13.1 Rotary mechanism of a bacterial flagellum. The mechanism penetrates both the outer and inner membranes surrounding the bacterium. Energy derived from a proton gradient causes the 'M ring' (or motor) to rotate relative to the 'S ring' (or stator) at about 100 revolutions per second. The stator is embedded in the peptidoglycan layer. A rod links the M ring to a hook and then to a helical flagellar filament. A 'bearing' in the outer membrane acts as a seal. From Adler (1976), in Goldman, Pollard and Rosenbaum (eds), *Cell Motility*, Cold Spring Harbor, NY: Cold Spring Harbor Laboratory; with permission.

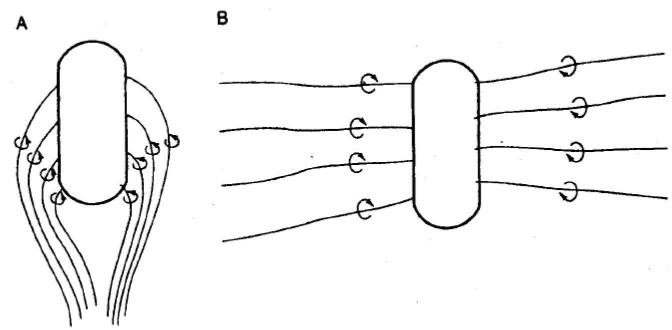
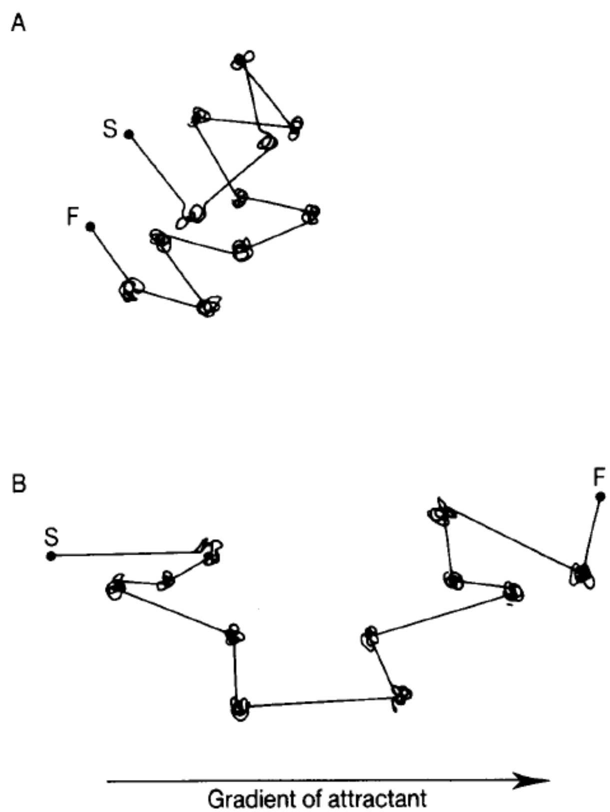
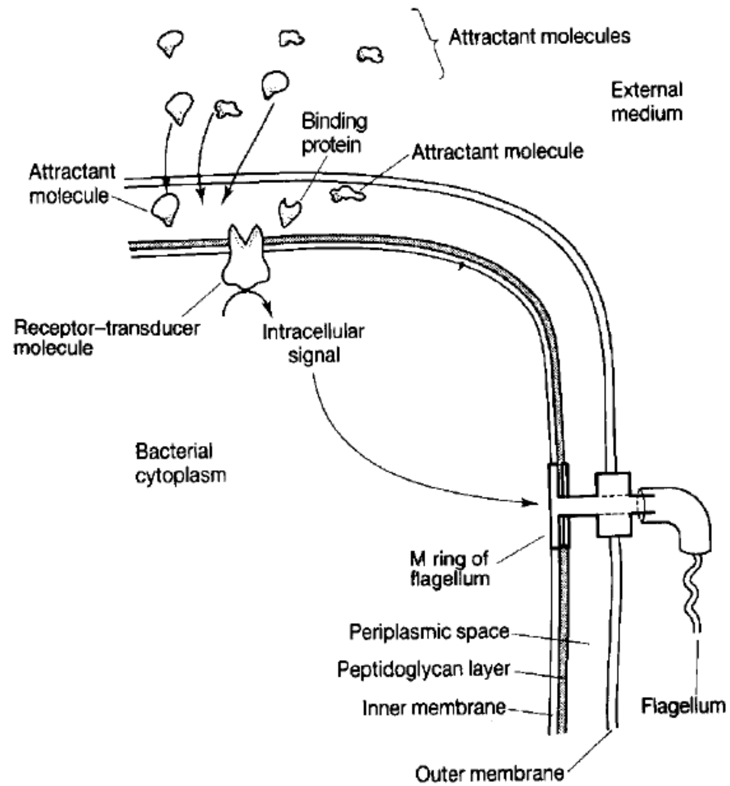


Figure 13.2 Anticlockwise and clockwise rotation of bacterial flagella. (A) Anticlockwise rotation. The flagella stream together as a single bundle which propels the bacterium forwards. (B) Clockwise rotation. The flagella each pull away from the bacterium in the direction of the straight arrows. According to the varying strength of the pull from each flagellum the bacterium veers from side to side and tumbles hither and thither.

Reacting to a Gradient





Elements of Molecular Neurobiology. C. U. M. Smith

ISBNs: 0-470-84353-5 (HB); 0-471-56038-3 (PB)

Diffusion Times

Structure	**Dimension**	**Time**
Cell Membrane	1 nm	100 nano seconds
Mitochondria	1 µm	1 millisecond
Small Cell	10 µm	100 milliseconds
Large Cell	100 µm	10 seconds
Cortical Column	1 mm	16.7 minutes
Cortical Region	2 cm	4.6 days
Body	1 m	31 years

Adapted from page 105 - Cellular Biophysics V1 - T.F. Weiss, MIT Press 1996

Review

- Nervous systems can quickly transmit information.
- Central nervous systems can integrate large amounts of sensory data, compute appropriate reactions and control effector organs (muscles and glands).
- This is achieved by an interplay of electrical and chemical signals.
- Within neurons the signals are generally electrical, between neurons they are mostly chemical.

Electrochemical Gradients

Ion Gradients

Cell Membranes

Ion Channels

Topics I	Topics II
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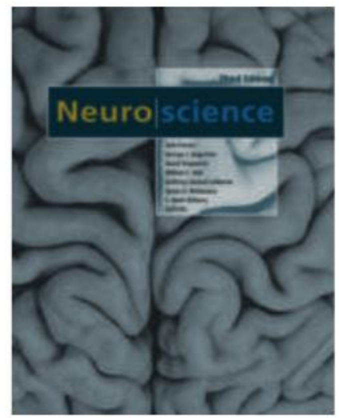
Passive Membrane Properties	Electrophysiology Techniques
Action Potentials	Basic Circuits (Spinal Cord)
Voltage-Gated Ion Channels	Sensory Systems Overview
Ligand-Gated Ion Channels	Synaptic Plasticity

Study Material

- NEUROSCIENCE Third Edition
  - Dale Purves
- Chapter 2 pages 34-43



THE COVER
Dorsal view of the human brain.
(Courtesy of S. Mark Williams.)

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Aims for this Lecture

- Know the values of the main ion concentration gradients.
- Understand the interaction between diffusion gradients and electrical fields.
- Know and understand the Nernst equation.
- Understand the effect of multiple permeabilities (Goldman equation).
- Know the typical resting membrane potential.

Recapitulation L1

- Diffusion is fast over short distances (organelles to small cells), but very slow over long distances (many cells to organs).
- Multicellular organisms need to communicate and need to do so quickly and efficiently.
- Electrical signals across cell membranes are the main signals in nervous systems.

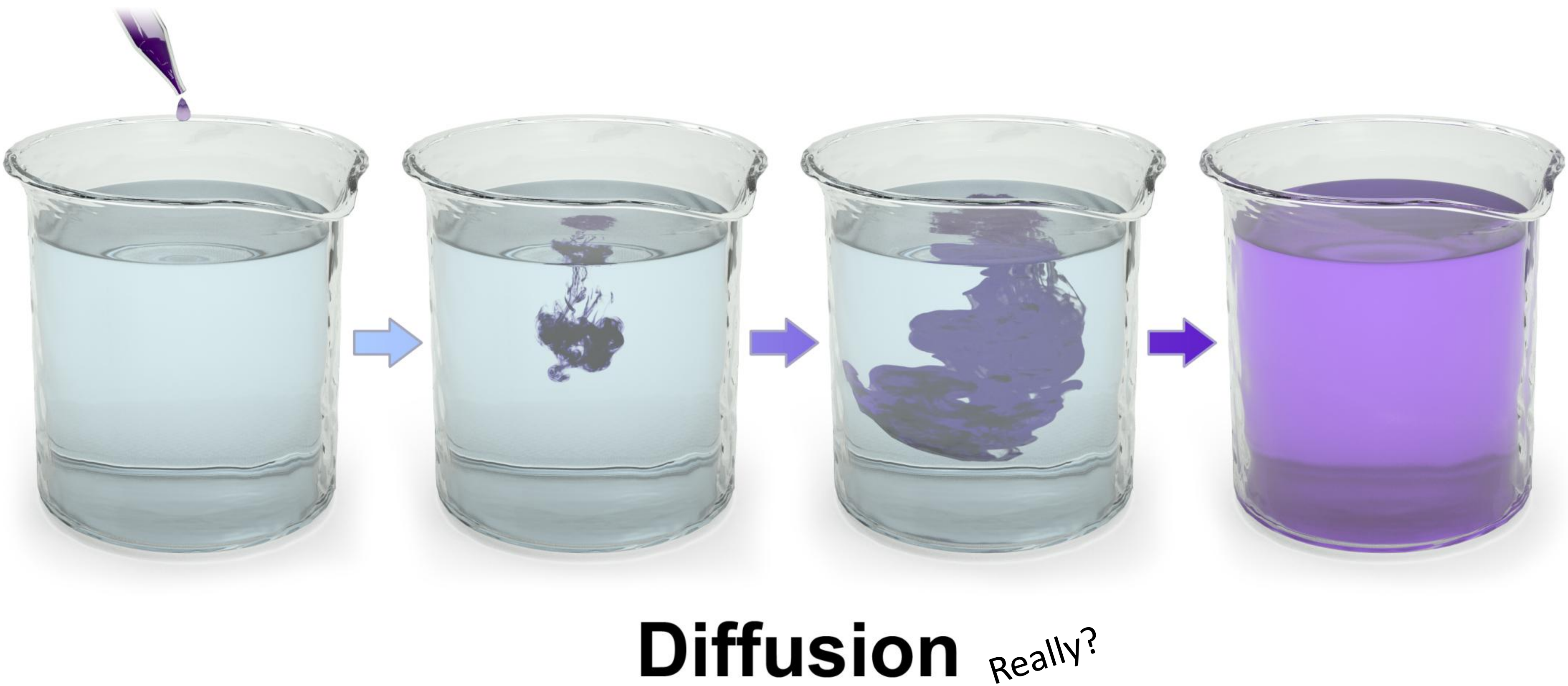
Biophysical Basis

All the phenomena that we will be talking about in the next two hours are manifestations of ion flux across membranes.

Which brings us to the central question…

… why did the ion cross the membrane?

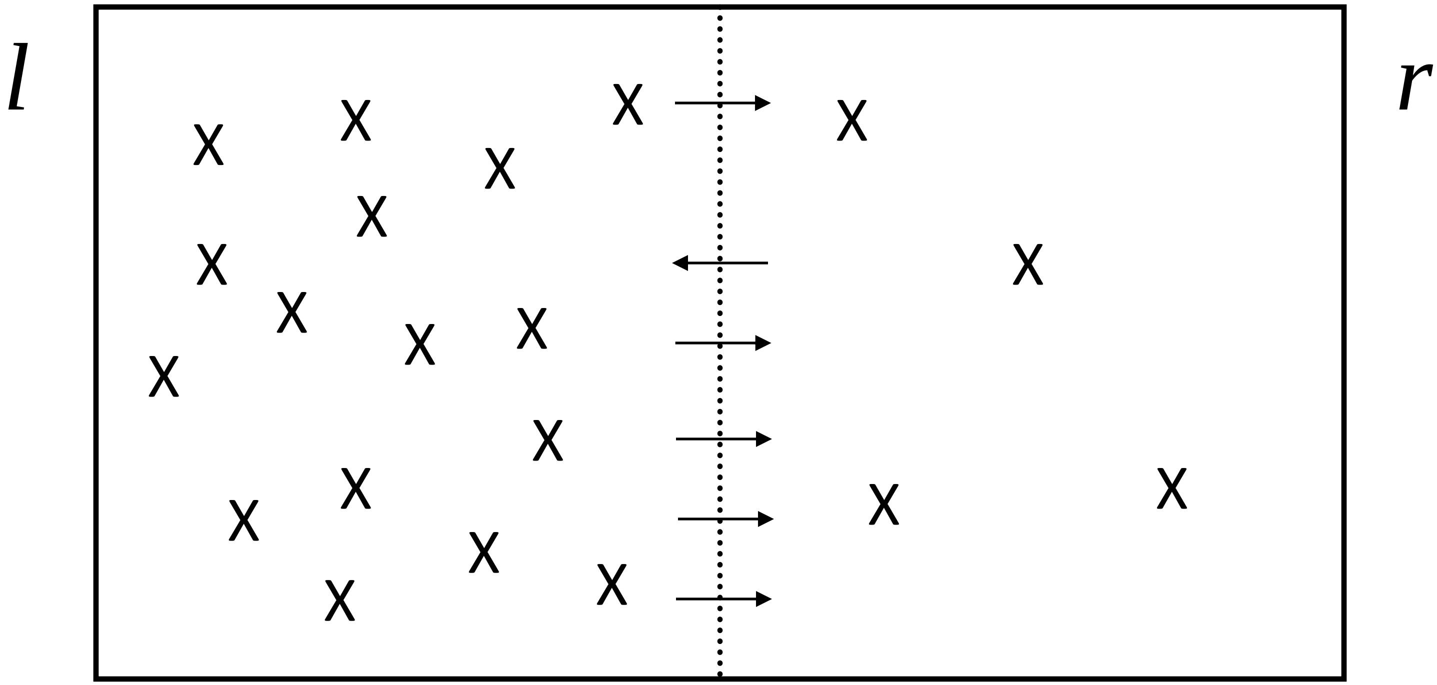
Diffusion and Movement



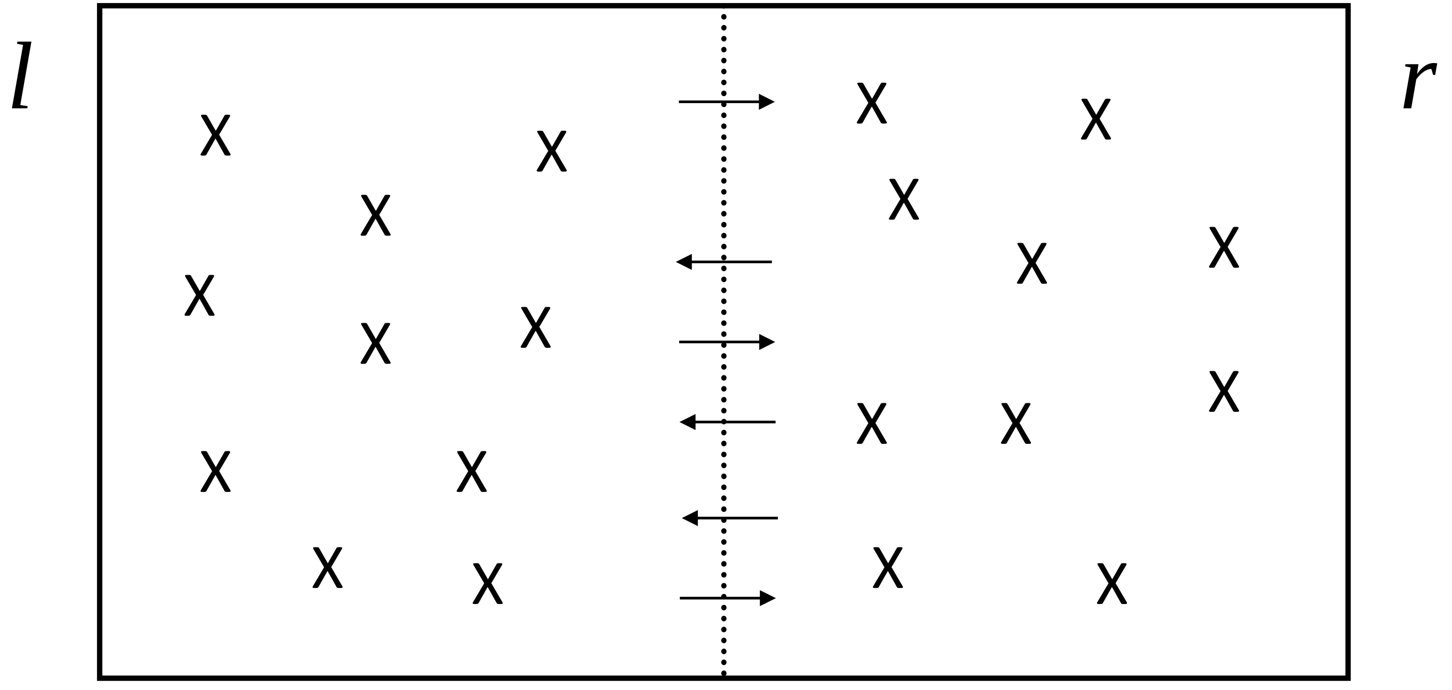
Really?

Wikimedia: Diffusion

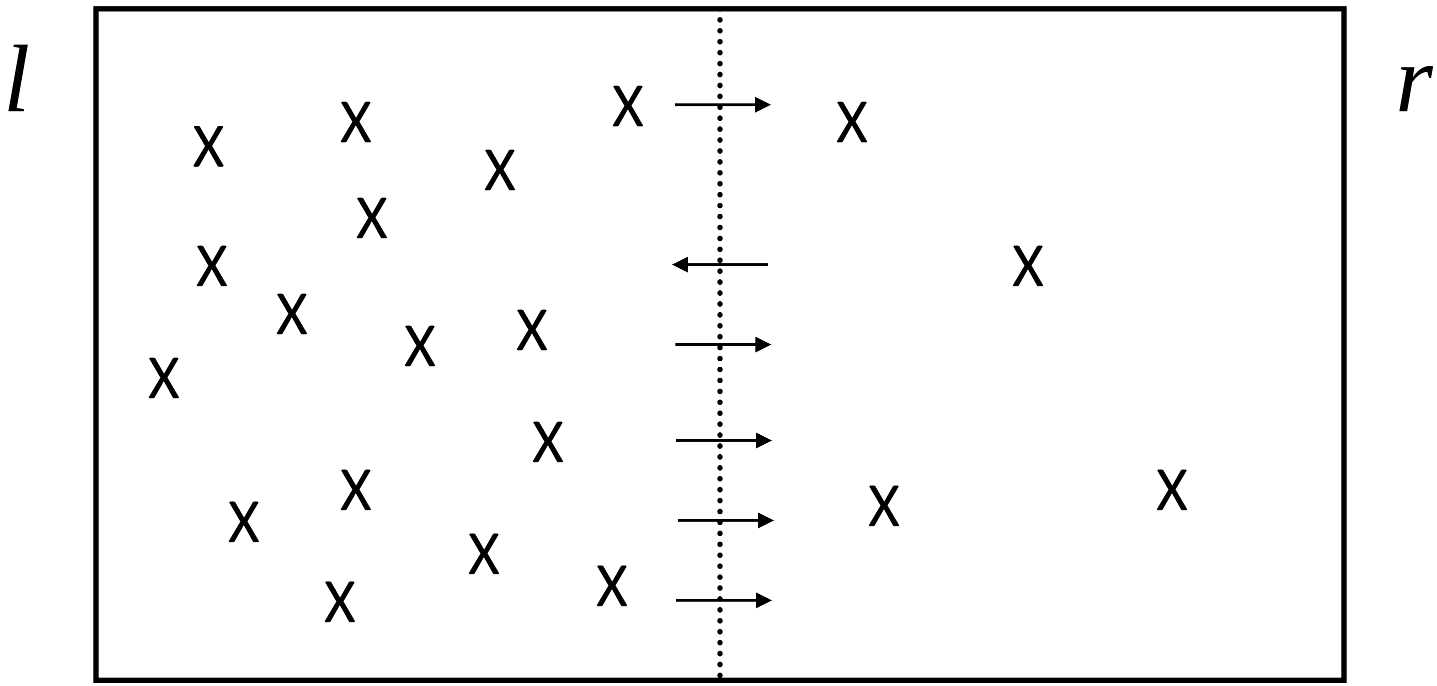
Diffusion



Diffusion



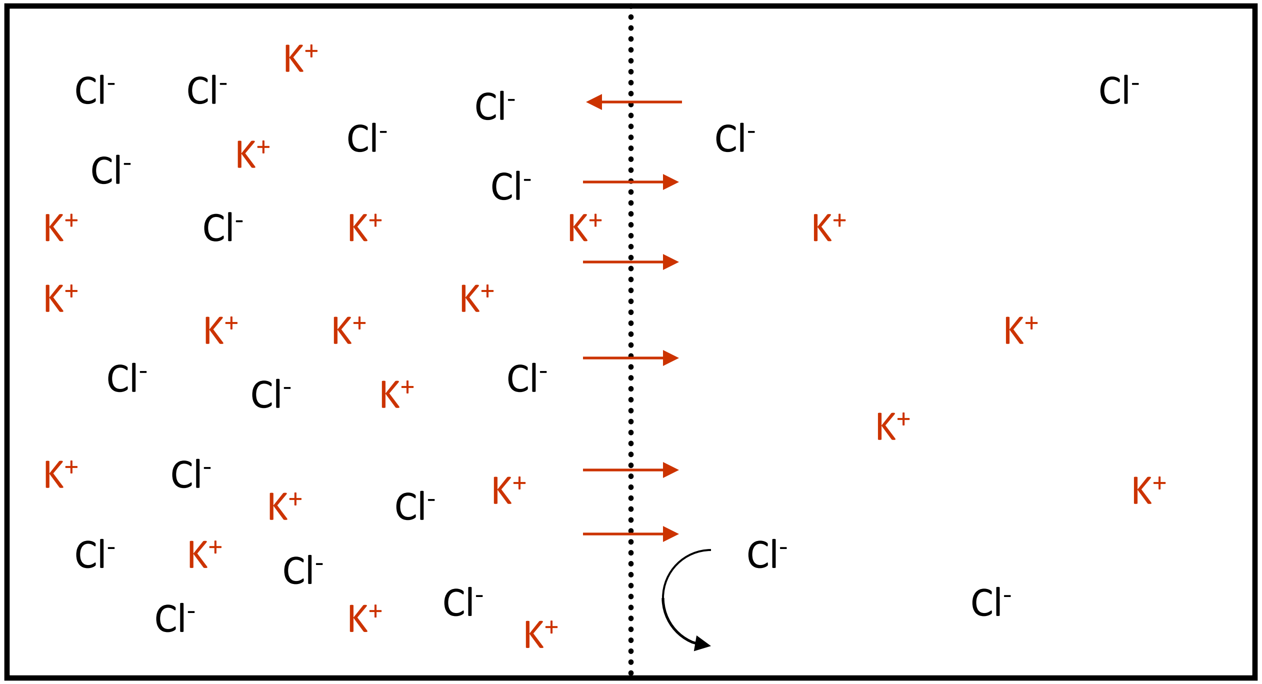
Chemical Potential



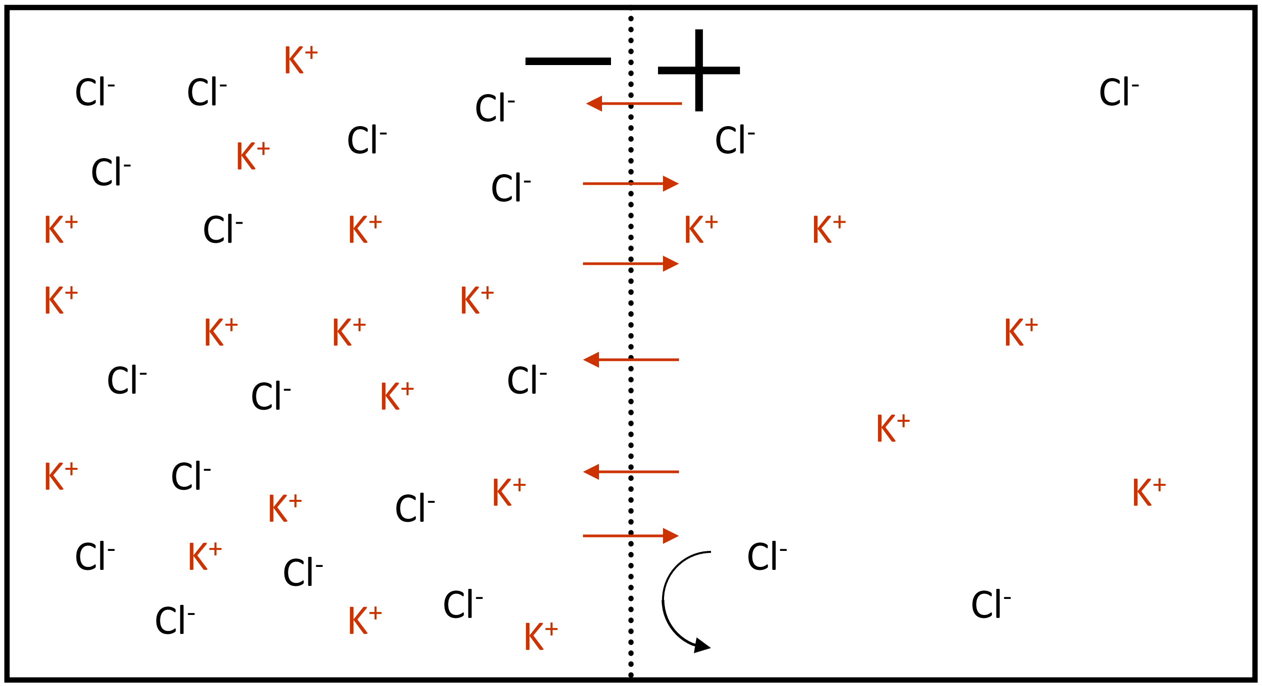
$$\Delta\mu = RT \ln \frac{[X]_r}{[X]_l}$$

Constant pressure and temperature

Electrical Potential



Electrical Potential



Equilibrium

Electrical Field

$$nzF(E_i - E_o)$$

Chemical Energy

$$nRT \ln \frac{[K^+]_o}{[K^+]_i}$$

n	Amount (Mol)
z	Valence (+1 for K)
F	Faraday‘s constant (charge of one Mole of Ions)
E	Potential
R	Gas constant
T	Temperature (absolute K)
[ ]	Concentration in the respective compartment

Nernst Equation

$$zF(E_i - E_o) = RT \ln \frac{[K^+]_o}{[K^+]_i}$$

$$E_i = \frac{RT}{zF} \ln \frac{[K^+]_o}{[K^+]_i}$$

Nernst Equation
Describes equilibrium
No **net** flux

Specific Equilibrium Potentials

$$E_i = \frac{RT}{zF} \ln \frac{[K^+]_o}{[K^+]_i}$$

$$E_i = 61(mV) \log \frac{[K^+]_o}{[K^+]_i}$$

	Intracellular	Extracellular	E
Potassium Ions	155 mM	4 mM	-98 mV
Sodium Ions	12 mM	145 mM	+67mV

Mixed Potentials

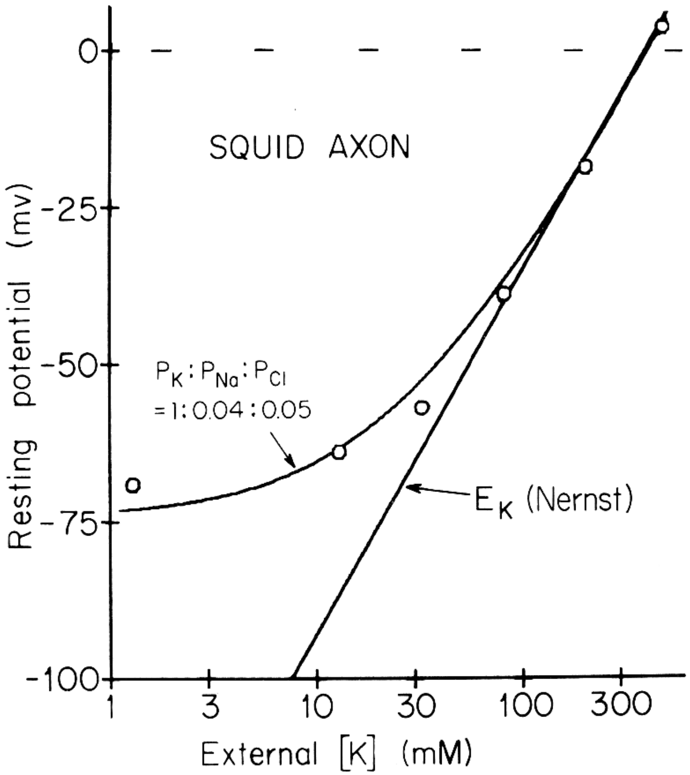


Figure 1–8 Dependence of resting membrane potential on the external K^+ ion concentration in a squid giant axon. The sum of $[Na^+]_o$ and $[K^+]_o$ were kept constant as $[K^+]_o$ was varied. The line labeled E_K shows the expected Nernst potential for K^+ ions, and the curved line is a solution of the Goldman-Hodgkin-Katz voltage equation (Equation 10), assuming that P_K:P_{Na}:P_{Cl} of the axon membrane is 1.0:0.04:0.05. (Data after Curtis and Cole. *J. Cell Comp. Physiol.* 19:135–144, 1942.)

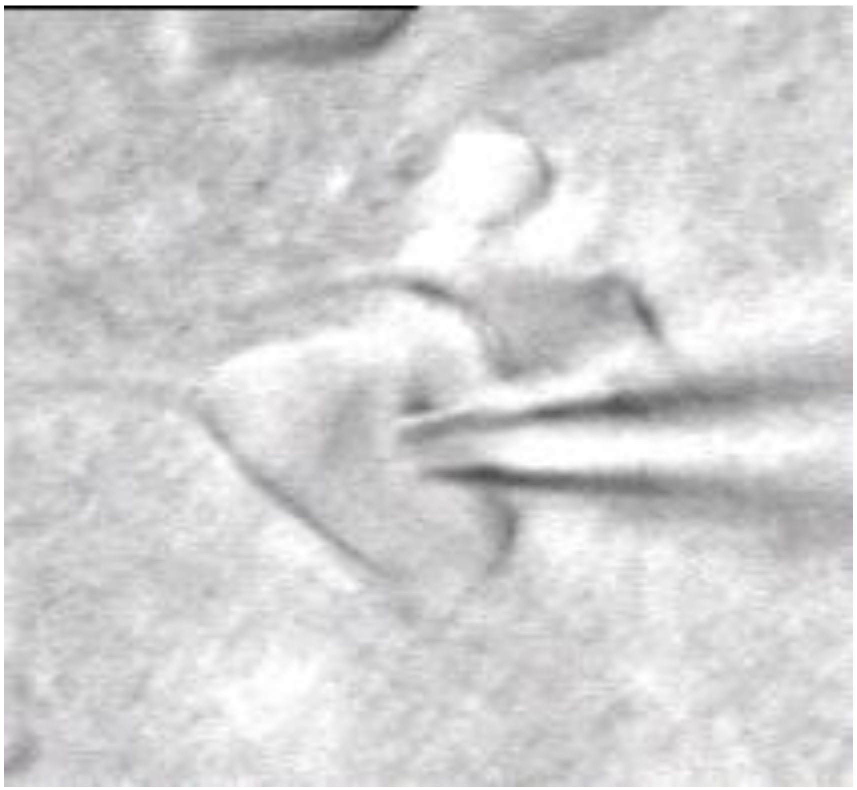
Goldmann, Hodkin, Katz

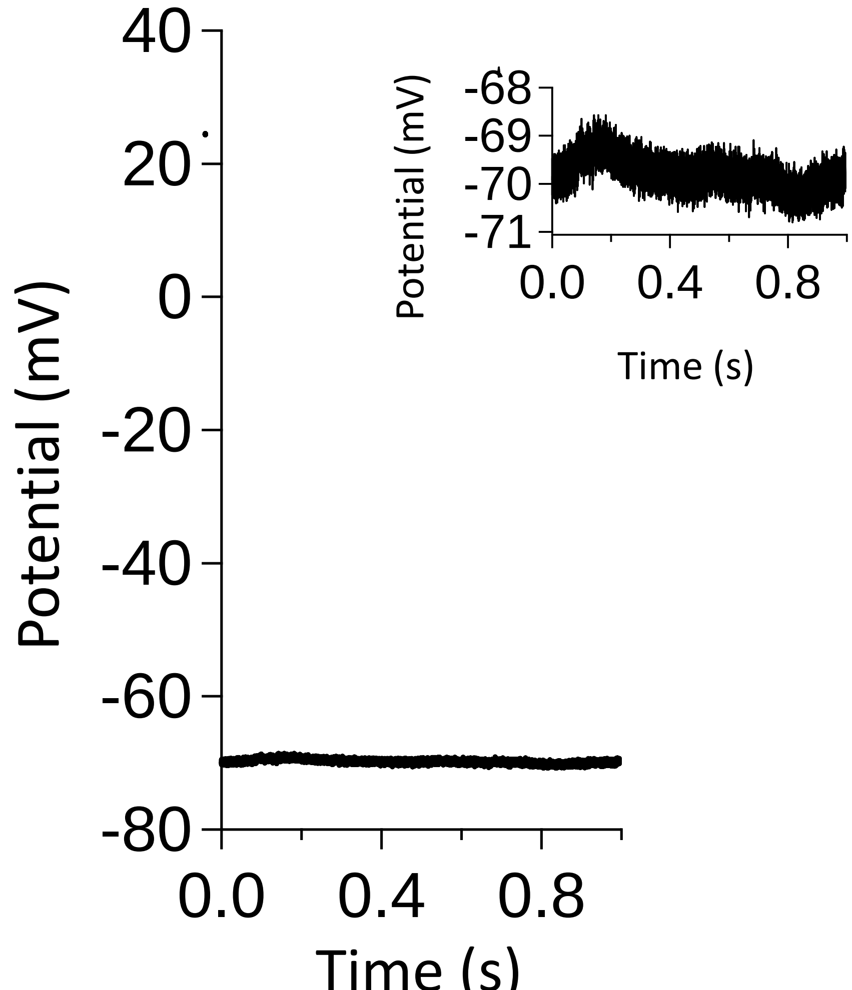
$$E_i = \frac{RT}{F}\ln\left(\frac{P_K[K^+]_o + P_{Na}[Na^+]_o}{P_K[K^+]_i + P_{Na}[Na^+]_i}\right)$$

Table 1–4 Free Ionic Concentrations and Equilibrium Potentials for Mammalian Skeletal Muscle

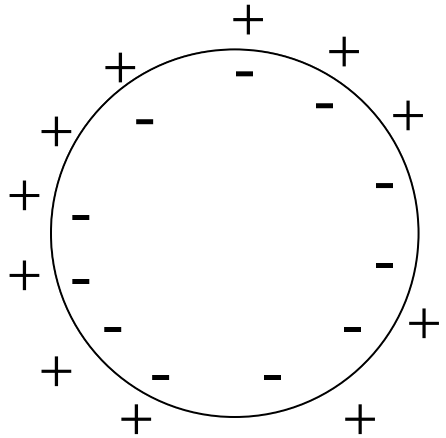
Ion	Extracellular Concentration (mM)	Intracellular Concentration (mM)	$\frac{[Ion]_o}{[Ion]_i}$	Equilibrium Potential[a] (mV)
Na^+	145	12	12	+67
K^+	4	155	0.026	−98
Ca^{2+}	1.5	$<10^{-7}$ M	>15,000	>+128
Cl^-	123	4.2[b]	30[b]	−90[b]

Resting Membrane Potential





A simple calculation..



Spherical cell 25 µm radius

$$A = 4\pi r^2 = 7.85 \times 10^{-5} cm^2$$
$$V = \frac{4}{3}\pi r^3 = 6.5 \times 10^{-8} cm^3$$

Avogadro's number 6.0221 10^23
Elementary charge 1.6022 10^-19 C

Specific capacitance **1 µF/cm²**

Number of ions, needed to charge this capacitor to 100 mV

$$= 4.9 \times 10^7$$

Total number of ions in a saline solution of 300 mM

$$= 1.2 \times 10^{13}$$

Fraction of ions needing to cross the membrane

$$\frac{4.9 \times 10^7}{1.2 \times 10^{13}} = 3.9 \times 10^{-6} = 0.00039\%$$

Electrostatic Force

- 2 individuals (weighing 72 kg – made up of water for simplicity's sake).
- At a distance of 10 m from each other
- Of all their charged particles (protons electrons..) they decide to exchange 0.5% such that person 1 is positively and person 2 negatively charged.
- What force do these two persons exert on each other.

Result

Coulomb's law

$$F = \frac{1}{4\pi\varepsilon_0}\frac{Q_1Q_2}{r^2}$$

$$\varepsilon_0 = 8.85 \cdot 10^{-11} \frac{A^2 s^2}{Nm^2}$$

$$r = 10m$$

$$Q_1 = Q_2 = 7.7 \cdot 10^8 C$$

Force = $5.35*10^{25}$ N

As a comparison:
The gravitational attraction between moon and earth is around $1.98*10^{20}$ N

If the two individuals were as far apart from each other as earth and moon, the force would amount to about $3.6*10^{10}$ N

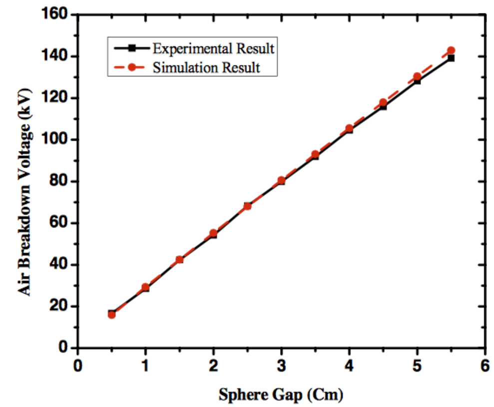
Voltage?

Subrata Karmakar | J. Electrical Systems 8-2 (2012): 209-217

Regular paper

An Experimental Study of Air Breakdown Voltage and its Effects on Solid Insulation

Journal of Electrical Systems



How does 70 mV over 8 nm compare to the breakdown voltage in air?

Equivalent to 87.5 kV per cm!

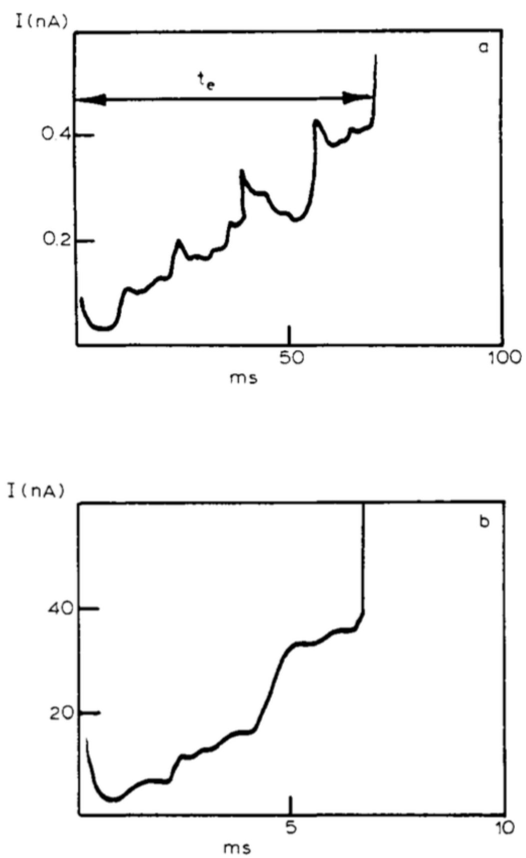


Fig. 1. The breakdown current at the stepwise voltage application. (a) The erythrocyte membrane, $\boldsymbol{U} = 300$ mV. (b) The planar bilayer of the oxidized cholesterol membrane, $\boldsymbol{U} = 200$ mV. The drastic current jump in both the cases corresponds to the irreversible membrane rupture. t_1 is the membrane lifetime.

360 Biochimica et Biophysica Acta 902 (1987) 360–373 Elsevier

BBA 73656

The electrical breakdown of cell and lipid membranes: the similarity of phenomenologies

L.V. Chernomordik, S.I. Sukharev, S.V. Popov, V.F. Pastushenko, A.V. Sokirko, I.G. Abidor and Y.A. Chizmadzhev

Institute of Electrochemistry, USSR Academy of Sciences. Leninsky prospect 31, 117071 Moscow (U.S.S.R.)

Breakdown Voltages

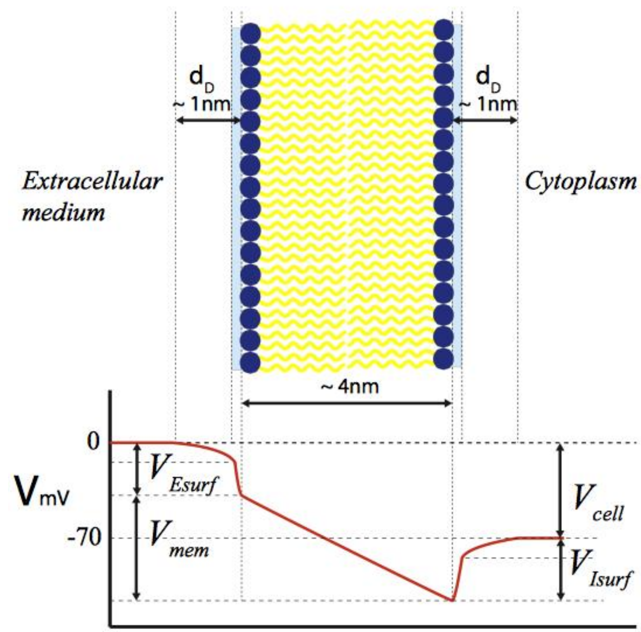
Substance	Dielectric Strength (MV/m)
Helium (relative to nitrogen)[3]	0.15
Air [4]	3.0
Alumina[3]	13.4
Window glass[3]	9.8 - 13.8
Silicone oil, mineral oil[3][5]	10 - 15
Benzene[3]	163
Polystyrene[3]	19.7
Polyethylene[6]	19 - 160
Neoprene rubber[3]	15.7 - 26.7
Distilled water[3]	65 - 70
High vacuum (field emission limited)[7]	20 - 40 (depends on electrode shape)
Fused silica[8]	25–40 at 20 °C
Waxed paper[9]	40 - 60
PTFE (Teflon, extruded)[3]	19.7
PTFE (Teflon, insulating film)[3][10]	60 - 173
Mica[3]	118
Diamond[11]	2000
PZT	10–25[12][13]
Vacuum	10^{12}

Wikipedia

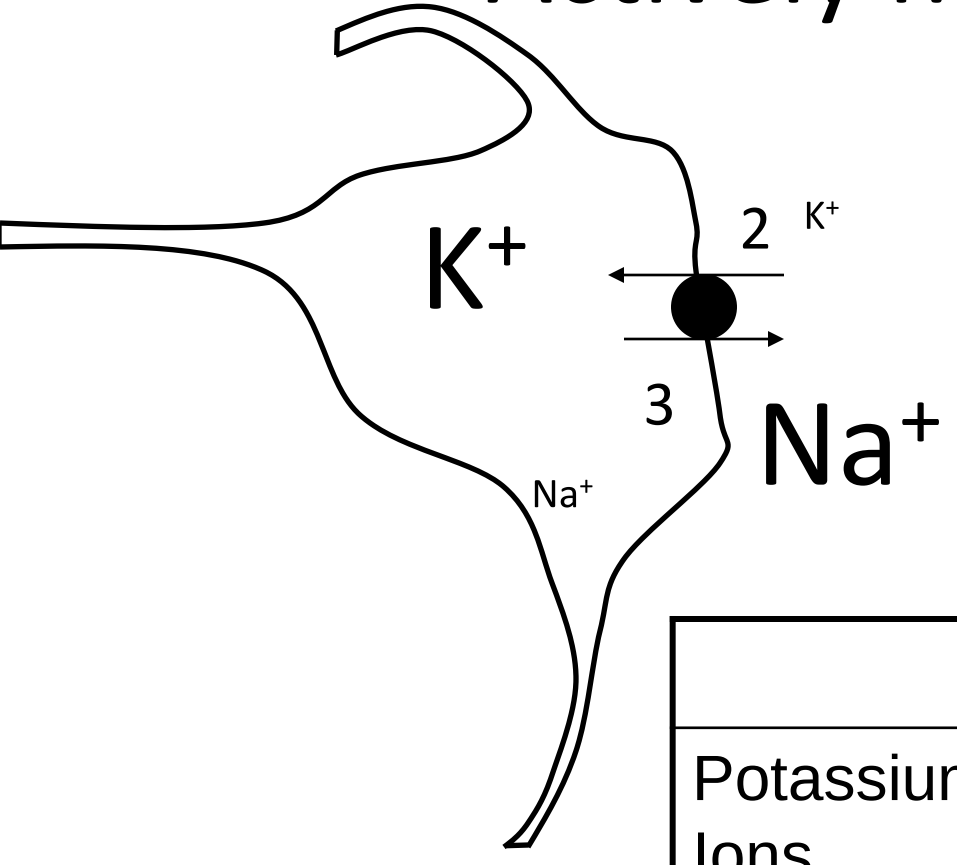
Imaging Voltage in Neurons

Darcy S. Peterka,[1,*] Hiroto Takahashi,[1] and Rafael Yuste[1]
[1]HHMI, Department of Biological Sciences, Columbia University, New York, New York 10027, USA
*Correspondence: dp2403@columbia.edu
DOI 10.1016/j.neuron.2010.12.010

membrane, even briefly reversing its polarity. In fact, the membrane potential changes are sizable (100 mV), and given that they occur across a very narrow section of dielectric material, the plasma membrane (only a few nanometers wide), these changes are associated with an enormous electric field (10^7–10^8 V/m), which can be modulated at kHz frequencies by neurons.



Actively Maintained



	Intracellular	Extracellular
Potassium Ions	155 mM	4 mM
Sodium Ions	12 mM	145 mM

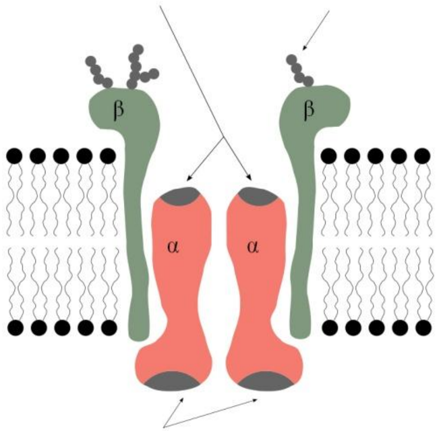
Na/K ATPase

Review

Mechanistic studies of sodium pump

Larry D. Faller

Archives of Biochemistry and Biophysics 476 (2008) 12–21



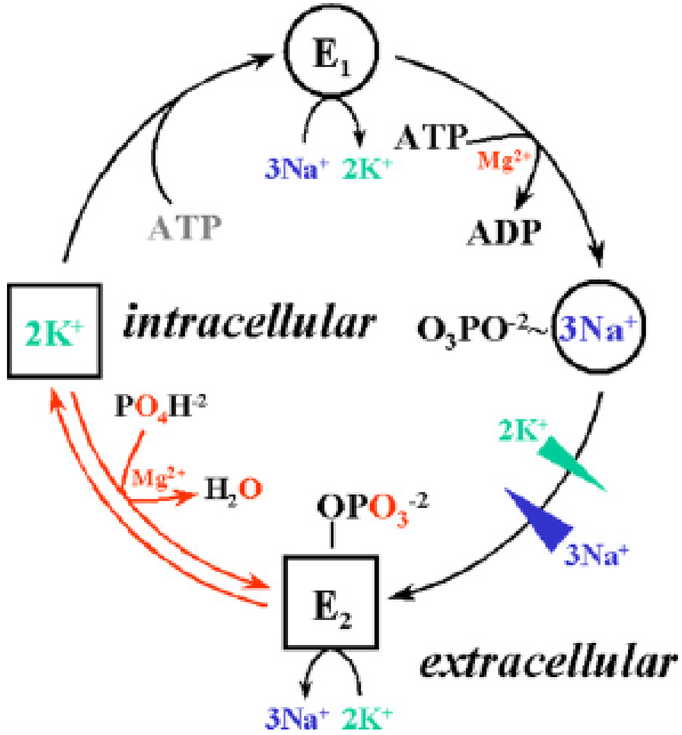


Fig. 1. Reaction cycle. The minimal steps in the catalysis-transport cycle of sodium pump are shown schematically. Black arrows indicate the normal, clockwise direction of the reaction sequence. Oxygen isotope (red) exchange may occur in the reversible step colored red. The cycle represents the sidedness, as well as repetition, of the reactions. For example, the 3 Na^+ (blue) that displace 2 K^+ (green) intracellularly are subsequently displaced by 2 K^+ extracellularly, generating the inward Na^+ (larger) and outward K^+ (smaller) gradients indicated by wedge shapes. A circle and a square symbolize the limiting protein conformations (E_1 and E_2, respectively) with enclosed ions indicating occlusion. Different colors denote ATP acting as an effector (gray) or substrate (black).

First description

The influence of some cations on an adenosine triphosphatase from peripheral nerves.
SKOU JC.
Biochim Biophys Acta. 1957 Feb;23(2):394-401.

Diversity of Na/K ATPases

Isozyme	Na^+ Activation $K_{0.5}$, mM	K^+ Activation $K_{0.5}$, mM	ATP Activation K_m, mM	Ouabain Inhibition K_i, M	Calcium Inhibition K_i, M
Native α1 β1	17.5 ± 0.4	2.1 ± 0.7	0.32 ± 0.04	$9.8 \pm 0.9 \times 10^{-5}$	
α1 β1	16.4 ± 0.7	1.9 ± 0.2	0.46 ± 0.10	$4.3 \pm 1.9 \times 10^{-5}$	$1.0 \pm 0.2 \times 10^{-4}$
α2 β1	12.4 ± 0.5	3.6 ± 0.3	0.11 ± 0.01	$1.7 \pm 0.1 \times 10^{-7}$	
α2 β2	8.8 ± 1.0	4.8 ± 0.4	0.11 ± 0.02	$1.5 \pm 0.2 \times 10^{-7}$	$7.3 \pm 4.6 \times 10^{-6}$
α3 β1	27.9 ± 1.3	5.3 ± 0.3	0.09 ± 0.01	$3.1 \pm 0.3 \times 10^{-8}$	
α3 β2	17.1 ± 1.0	6.2 ± 0.4	0.07 ± 0.02	$4.7 \pm 0.4 \times 10^{-8}$	$1.9 \pm 1.0 \times 10^{-5}$