



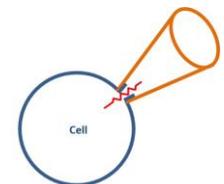
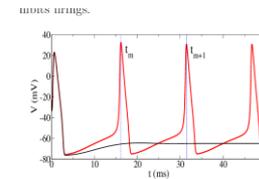
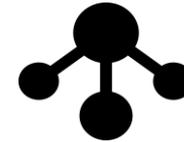
PÓS-GRADUAÇÃO
ENGENHARIA ELÉTRICA
FLORIANÓPOLIS | SANTA CATARINA



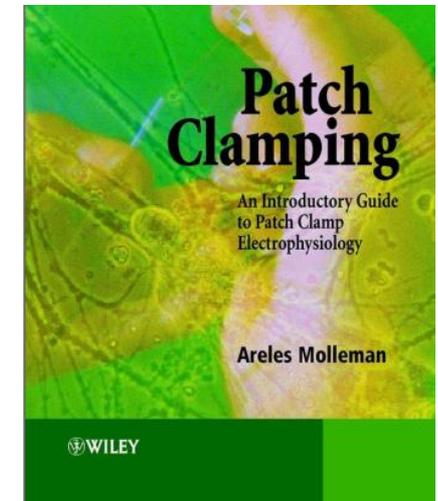
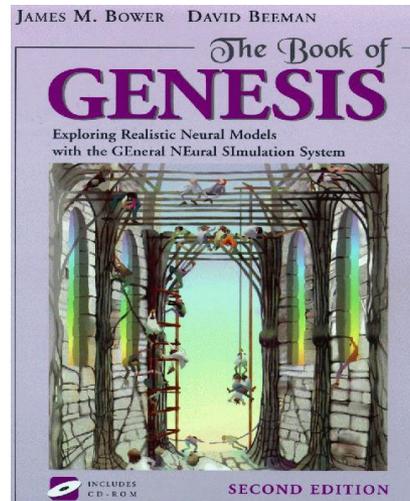
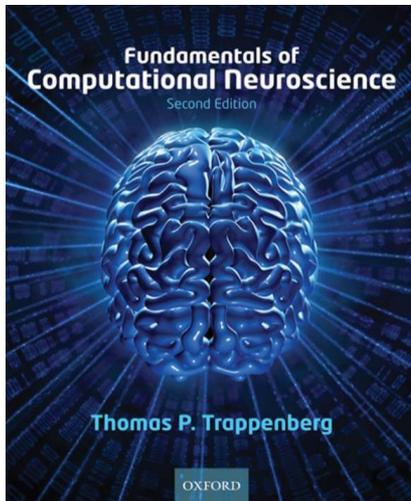
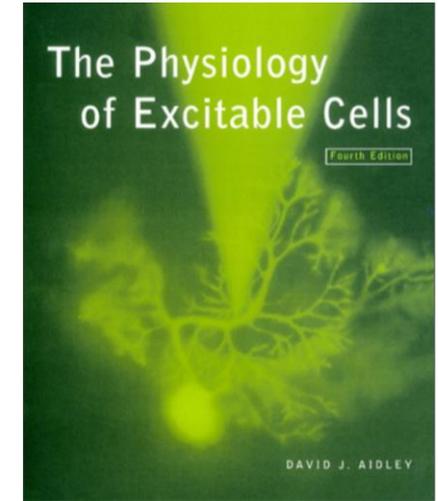
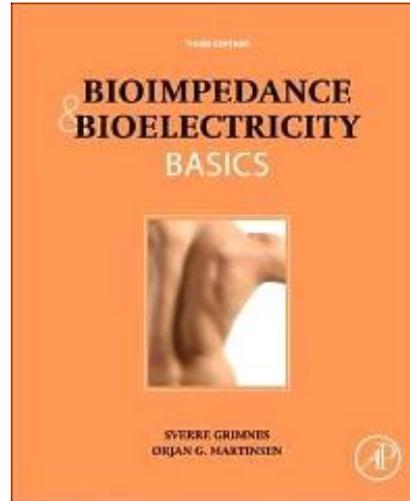
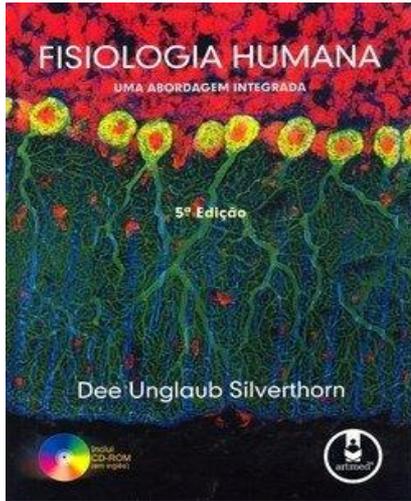
Membranas Celulares: Modelagem e Leitura de Potenciais Elétricos

Profa. Daniela O. H. Suzuki
Guilherme B. Pintarelli

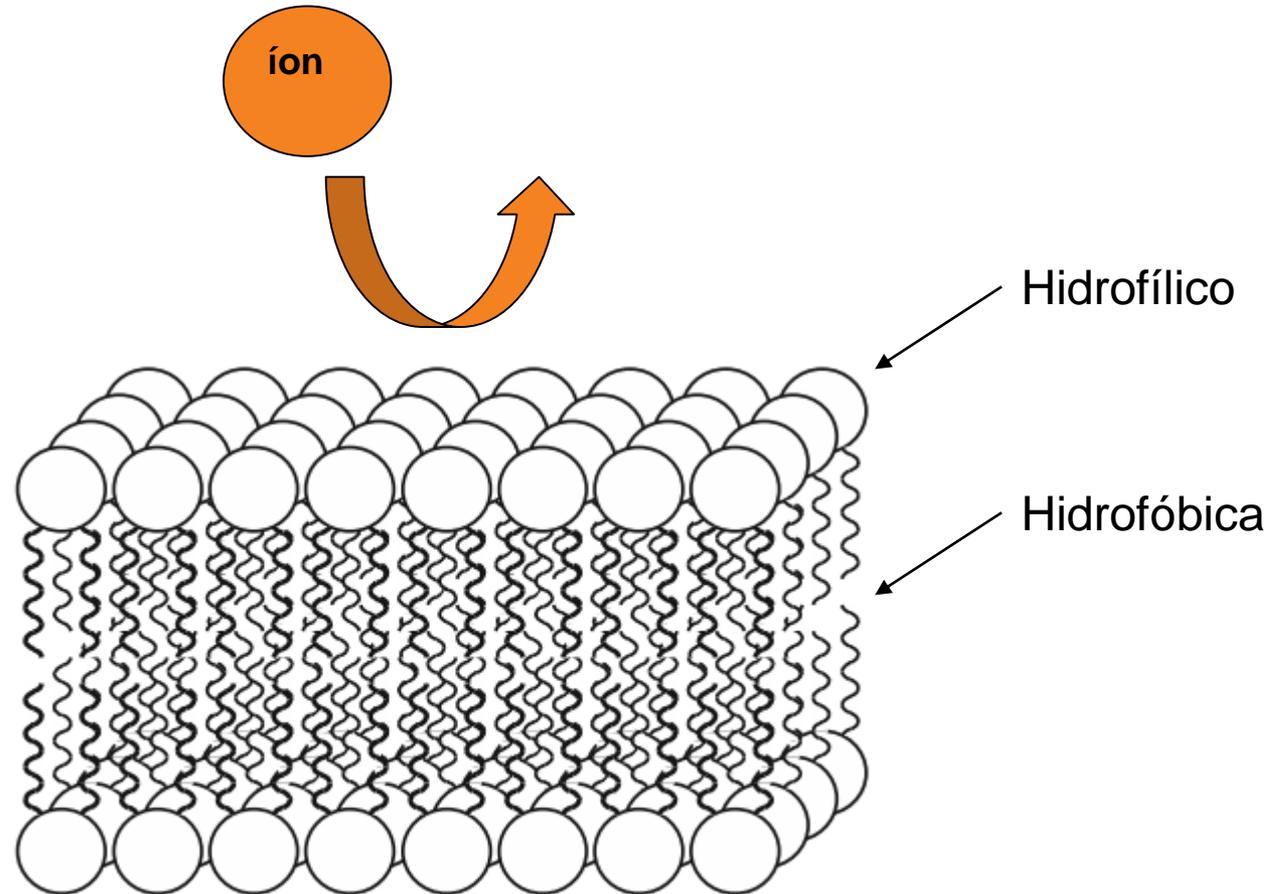
- Estrutura da membrana celular;
- Circuito elétrico equivalente da membrana;
- Modelo de Hodgkin-Huxley;
- Técnicas medições intracelulares e de membrana (*patch clamp*);
- Eletroporação ou Eletropermeabilização;



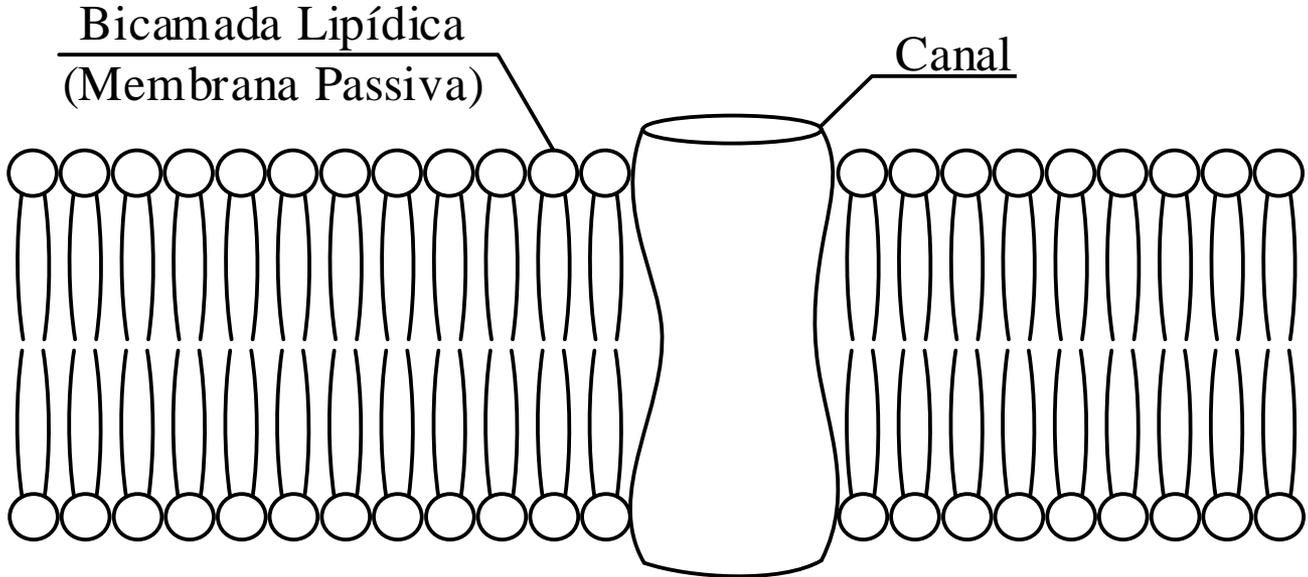
Bioeletricidade // Eletrofisiologia



Membrana Celular



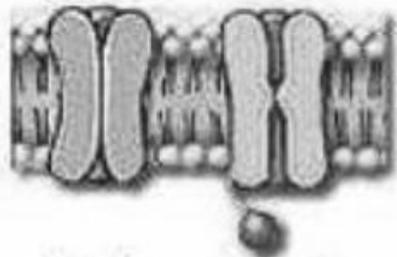
Membrana Celular



Passive channels



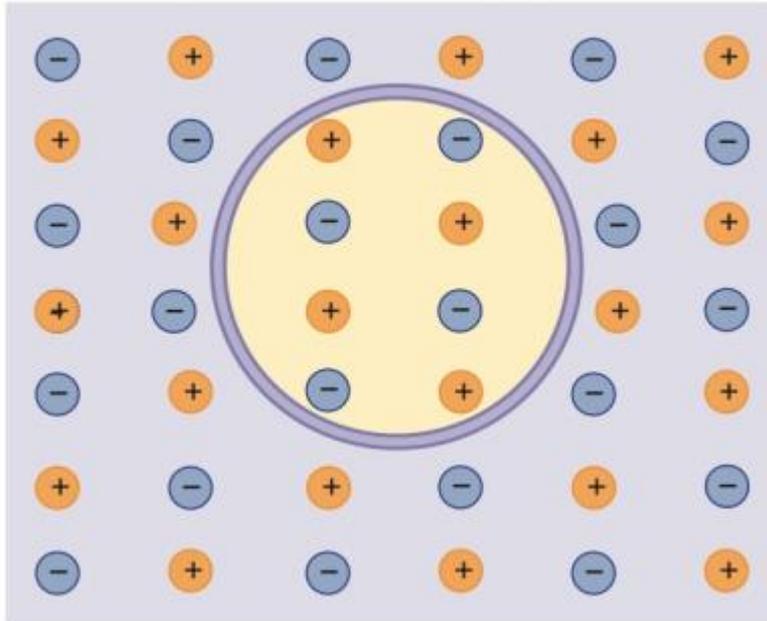
Chemically-gated channels



Voltage-gated channels

Composição Iônica

(a) A célula e a solução estão elétrica e quimicamente em equilíbrio



(b) A célula e a solução estão em desequilíbrio químico e elétrico. É usada energia para bombear um cátion para fora da célula, deixando uma carga resultante de -1 na célula e $+1$ fora dela.

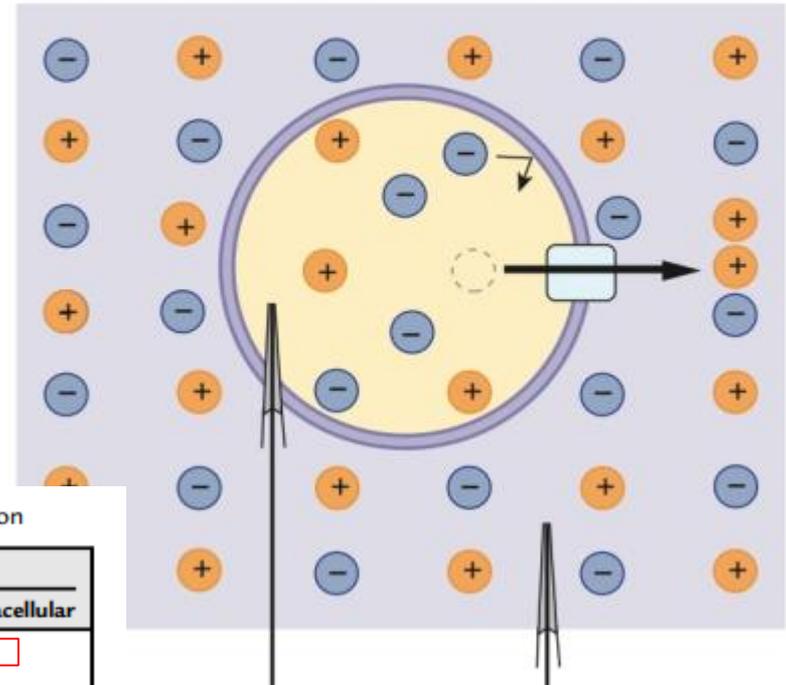


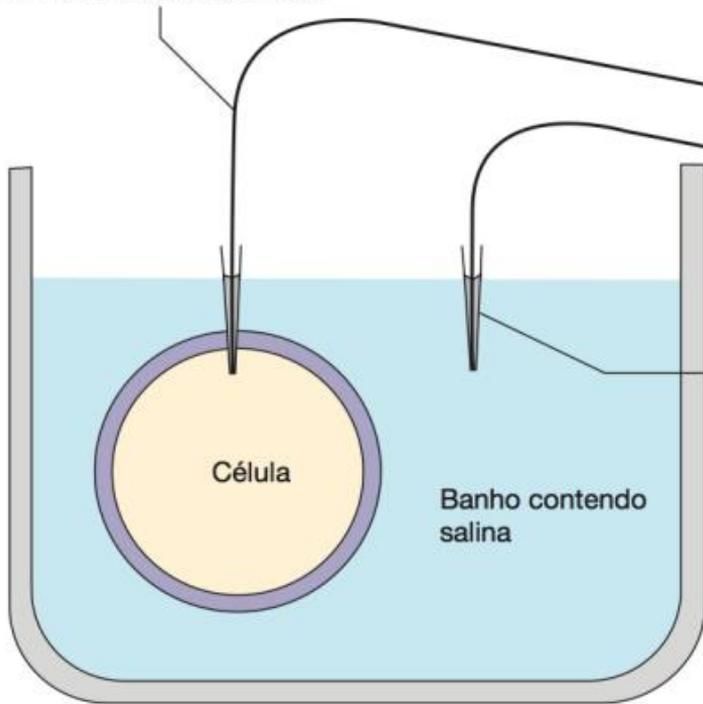
Table 2.6: Concentration of Electrolytes in Body Liquids [meq/L] is Ion Concentration

	Cations, meq/L		Anions, meq/L	
	Plasma	Intracellular	Plasma	Intracellular
Na ⁺	142	10	Cl ⁻	103
K ⁺	4	140	HCO ₃ ⁻	24
Ca ²⁺	5	10 ⁻⁴	Protein ⁻	16
Mg ²⁺	2	30	HPO ₄ ⁻ + SO ₄ ⁻ +	10
H ⁺ (pH = 7.4)	4 × 10 ⁻⁵	4 × 10 ⁻⁵	organic acids	130
Sum	153	180	Sum	153

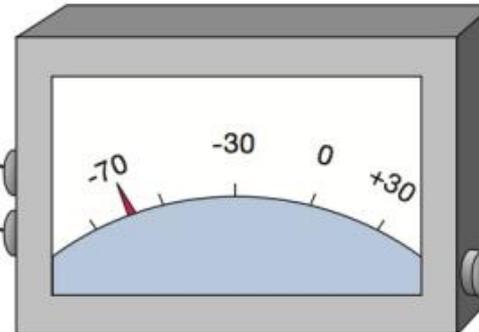
Milliequivalents [mmol × valency z] per liter, and 0.9% NaCl is 154 mmol.

Potencial de Repouso

Um eletrodo de registro é colocado dentro da célula.

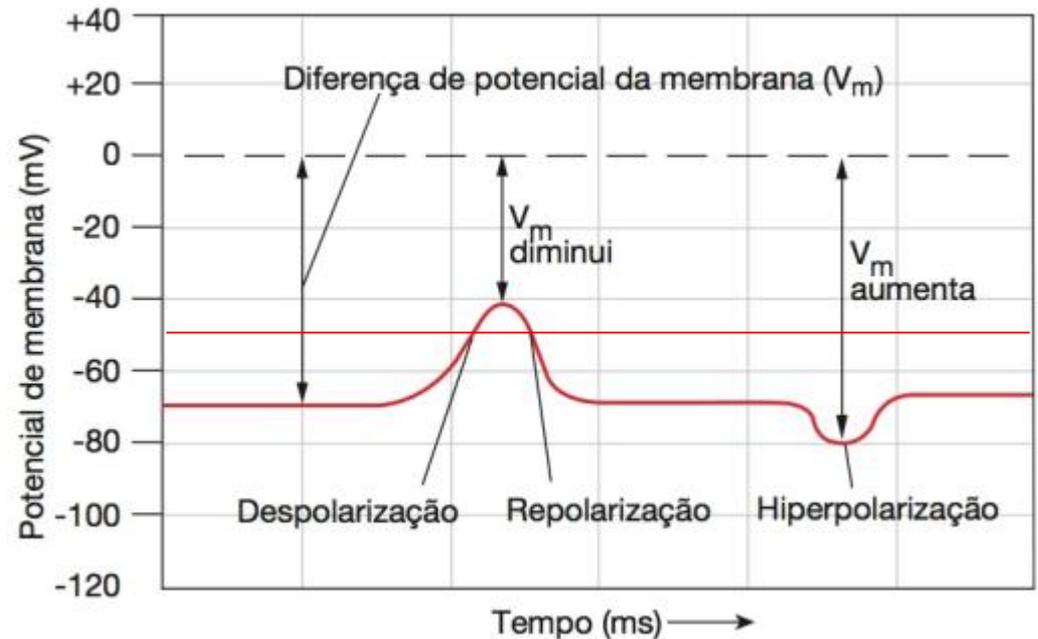


Entrada



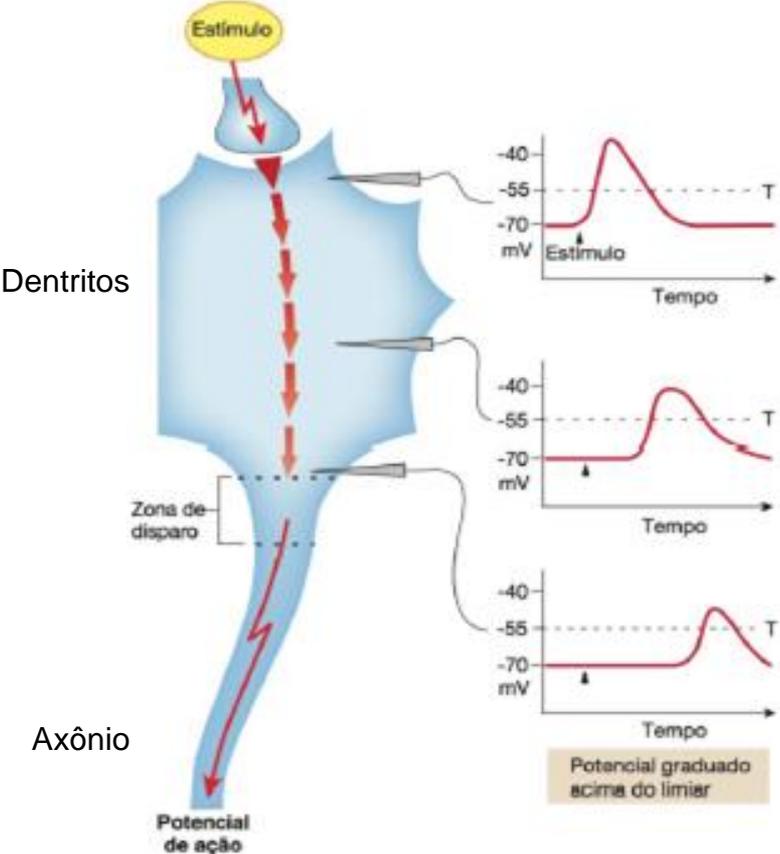
O voltímetro mede a diferença de cargas elétricas entre o interior de uma célula e a solução que a circunda. Este valor é a diferença de **potencial da membrana**, ou V_m .

Saída

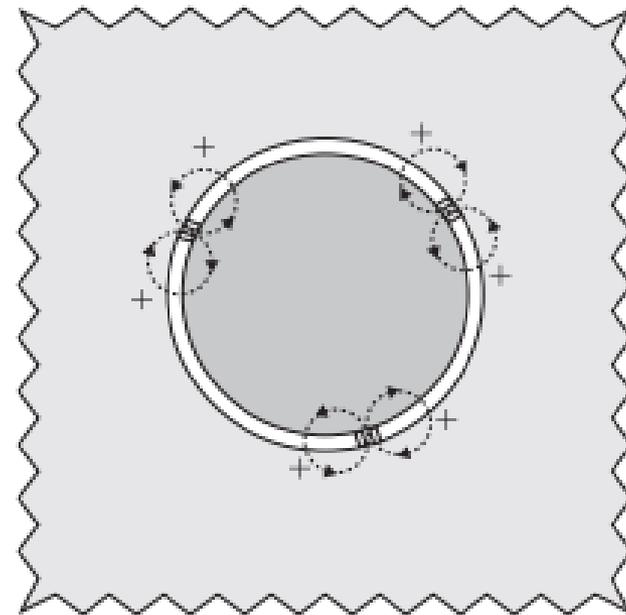
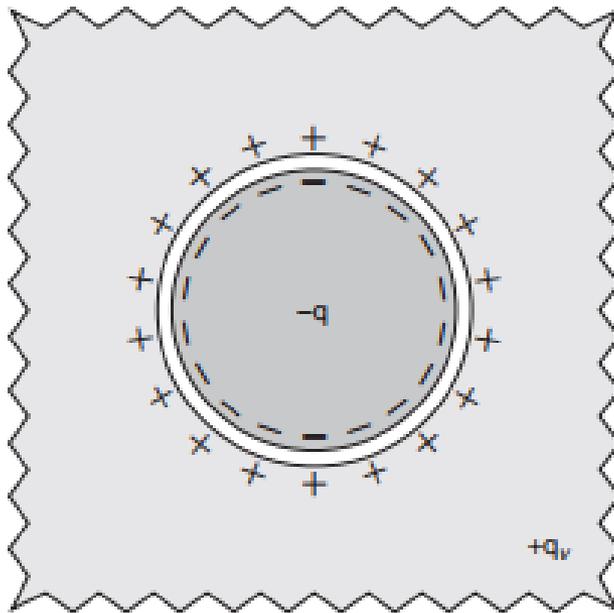


Potencial de Ação

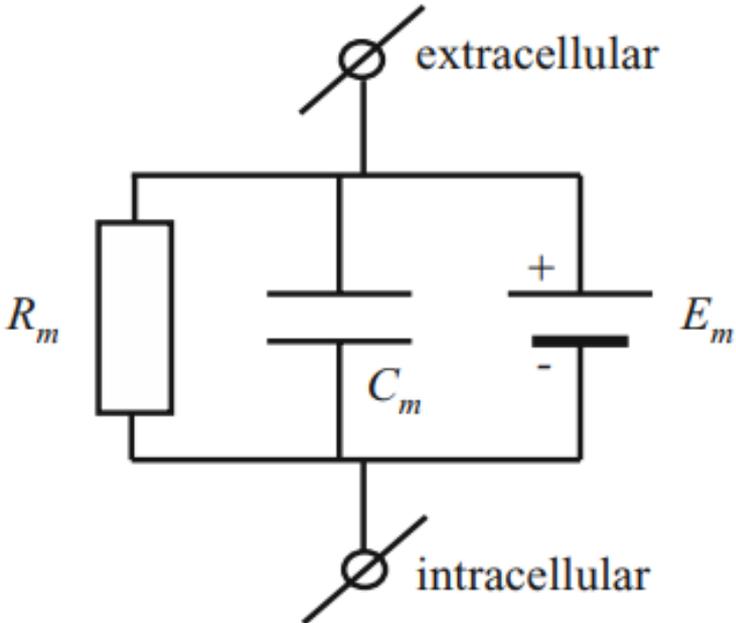
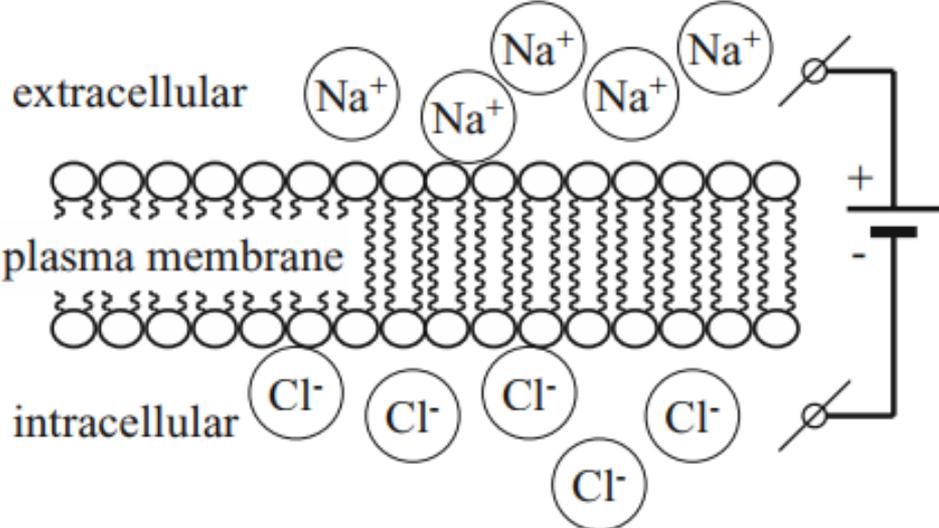
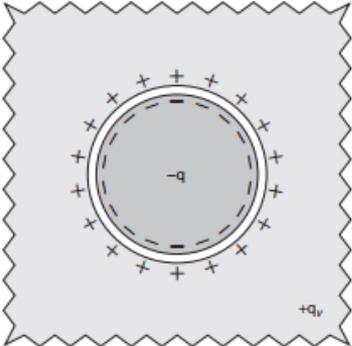
(b) Um estímulo mais forte no mesmo ponto do corpo celular gera um potencial graduado que ainda está acima do limiar no momento em que ele chega na zona de disparo, resultando em um potencial de ação.



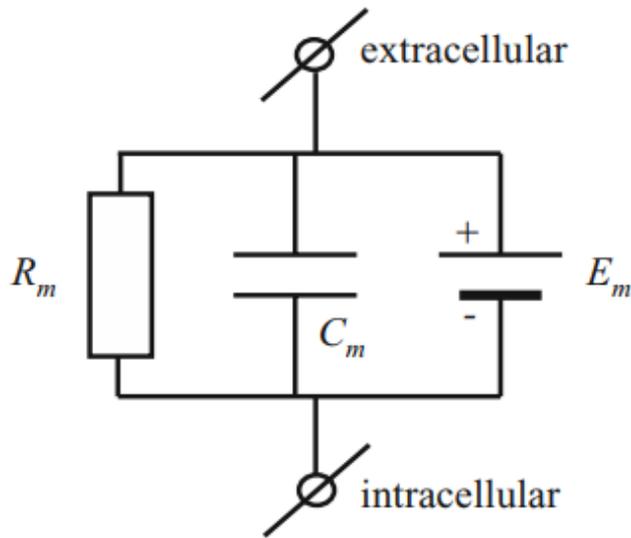
Características Elétricas da Membrana



Características Elétricas da Membrana



Características Elétricas da Membrana



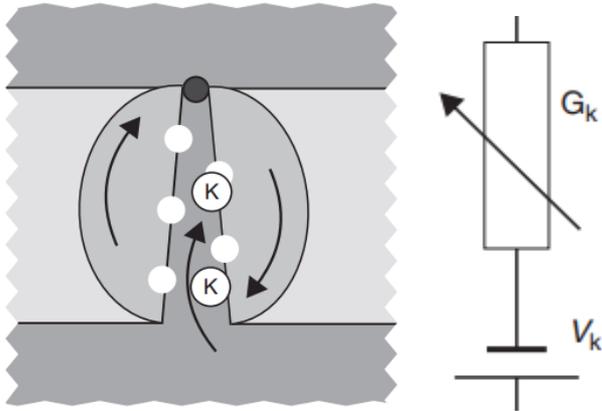
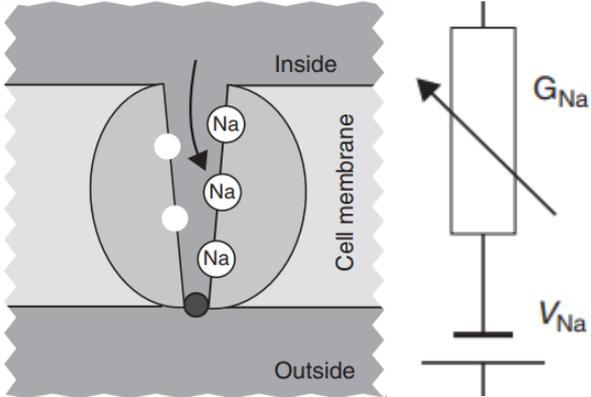
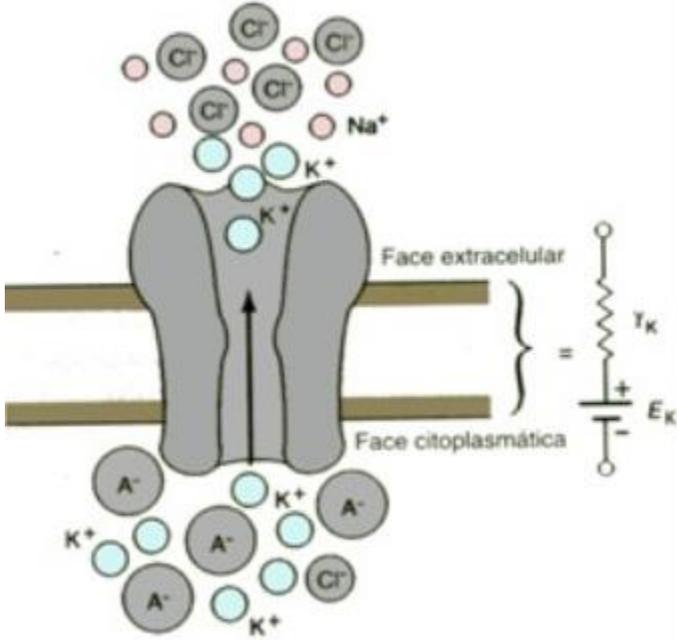
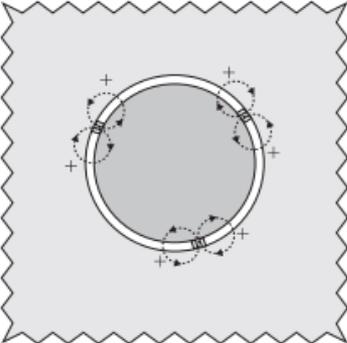
Equação de Goldman-Hodgkin-Katz:

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

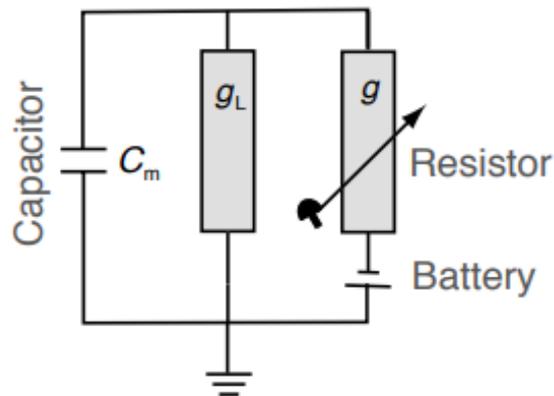
$$C = \frac{A\epsilon_r}{d}$$

Linear e sem informação de canais iônicos

Características Elétricas da Membrana

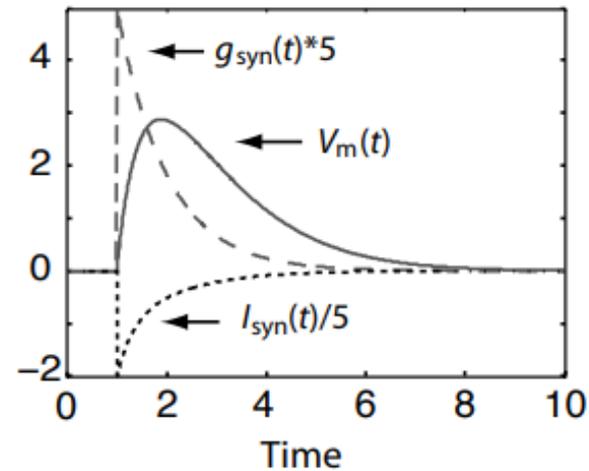


A. Electric circuit of basic synapse



$$I_{\text{ion}} = \bar{g}_{\text{ion}}(V - E_{\text{ion}}),$$

B. Time course of variables



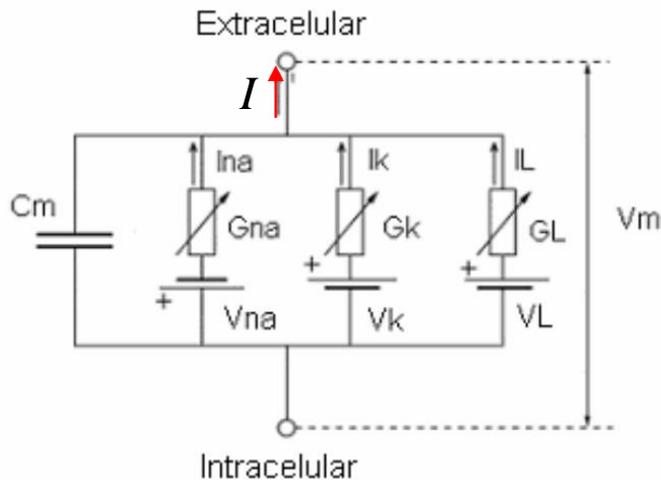


Fig. 2.8 A circuit representation of the Hodgkin–Huxley model. This circuit includes a capacitor on which the membrane potential can be measured and three resistors with their own batteries, modelling the ion channels; two are voltage-dependent and one is static.

$$I = C_m \cdot \frac{dV_m}{dt} + I_{Na} + I_k + I_L$$

$$I_{Na} = \bar{G}_{Na} \cdot m^3 \cdot h \cdot (V_m - V_{Na})$$

$$I_k = \bar{G}_k \cdot n^4 \cdot (V_m - V_k)$$

$$I_L = G_L \cdot (V_m - V_L)$$

Condutância máxima (parâmetro)

Adimensionais $f(v,t)$ ($0 \leq n,m,h \leq 1$):

n: Ativação Canal Potássio [K+]

m: Ativação Canal Sódio [Na+]

h: Inativação Canal Sódio [Na+]

Concentração iônica (parâmetro)

Modelo Hodgkin-Huxley

$$I = C_m \cdot \frac{dV_m}{dt} + I_{Na} + I_k + I_L$$



$$\frac{dV_m}{dt} = \frac{1}{C_m} \cdot (I_{st} - I_{Na} - I_k - I_L)$$

4 equações
diferenciais
ordinárias
(EDO)

$$I_{Na} = \bar{G}_{Na} \cdot m^3 \cdot h \cdot (V_m - V_{Na})$$

$$I_k = \bar{G}_k \cdot n^4 \cdot (V_m - V_k)$$

$$I_L = G_L \cdot (V_m - V_L)$$

Ativação ou probabilidade
do canal estar aberto:
n, m e h
 $0 \leq n, m, h \leq 1$

$$\frac{dm}{dt} = \alpha_m \cdot (1 - m) - \beta_m \cdot m$$

Taxa de
fechamento

$$\frac{dh}{dt} = \alpha_h \cdot (1 - h) - \beta_h \cdot h$$

Taxa de
abertura

$$\frac{dn}{dt} = \alpha_n \cdot (1 - n) - \beta_n \cdot n$$

$$1 - m \begin{matrix} \alpha(v) \\ \rightleftharpoons \\ \beta(v) \end{matrix} m$$

$\alpha_m, \beta_m, \alpha_h, \beta_h, \alpha_n, \beta_n?$

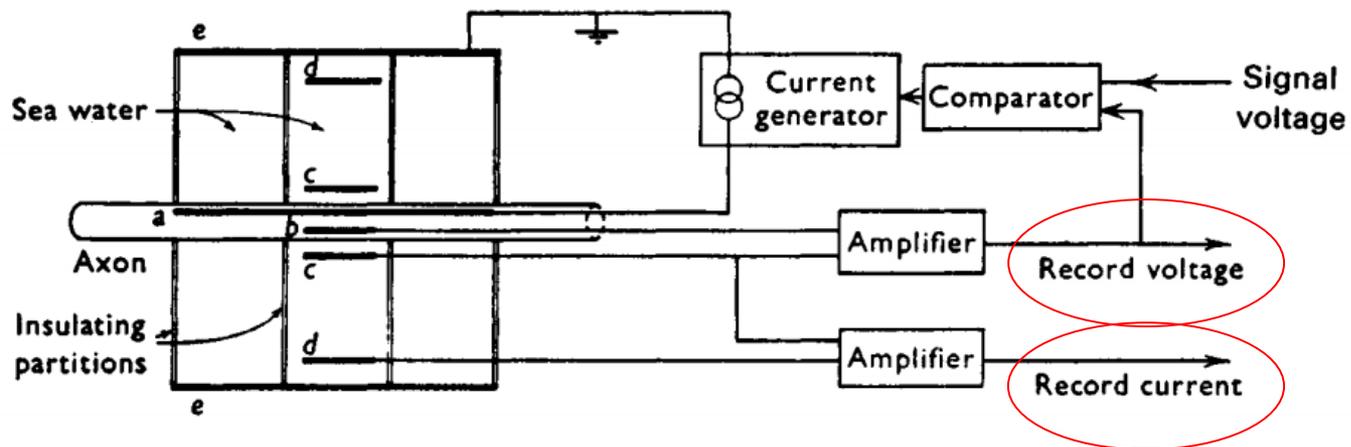
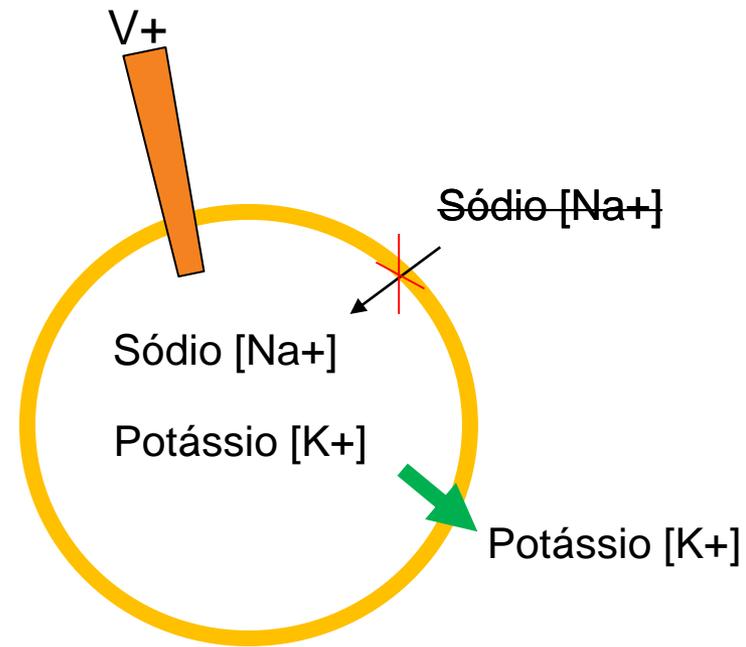
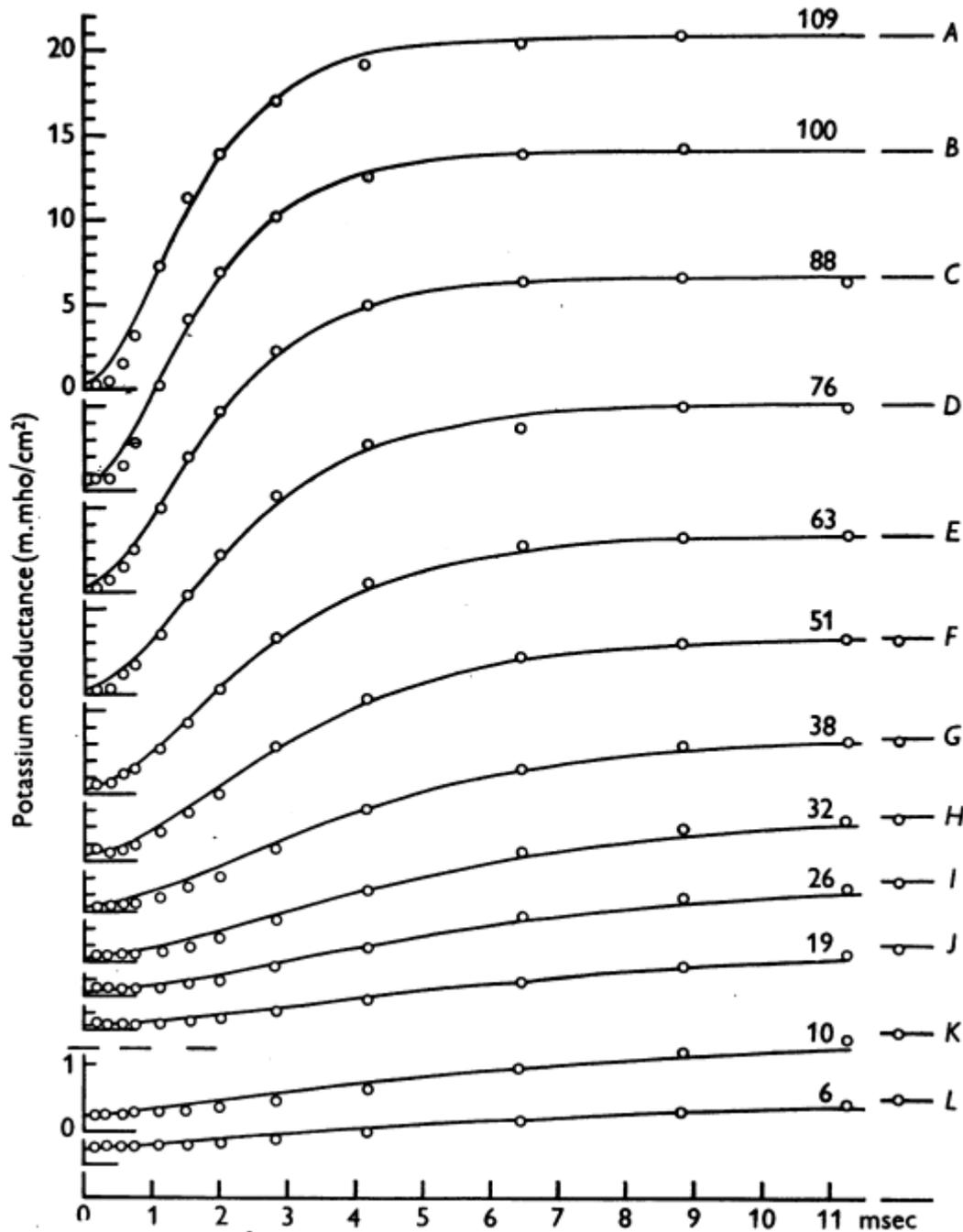


Figure 5.6. Schematic diagram of the method used to determine membrane currents in a squid axon under voltage clamp. (Based on Hodgkin *et al.*, 1952.)

Fixa V -> Força despolarização -> I



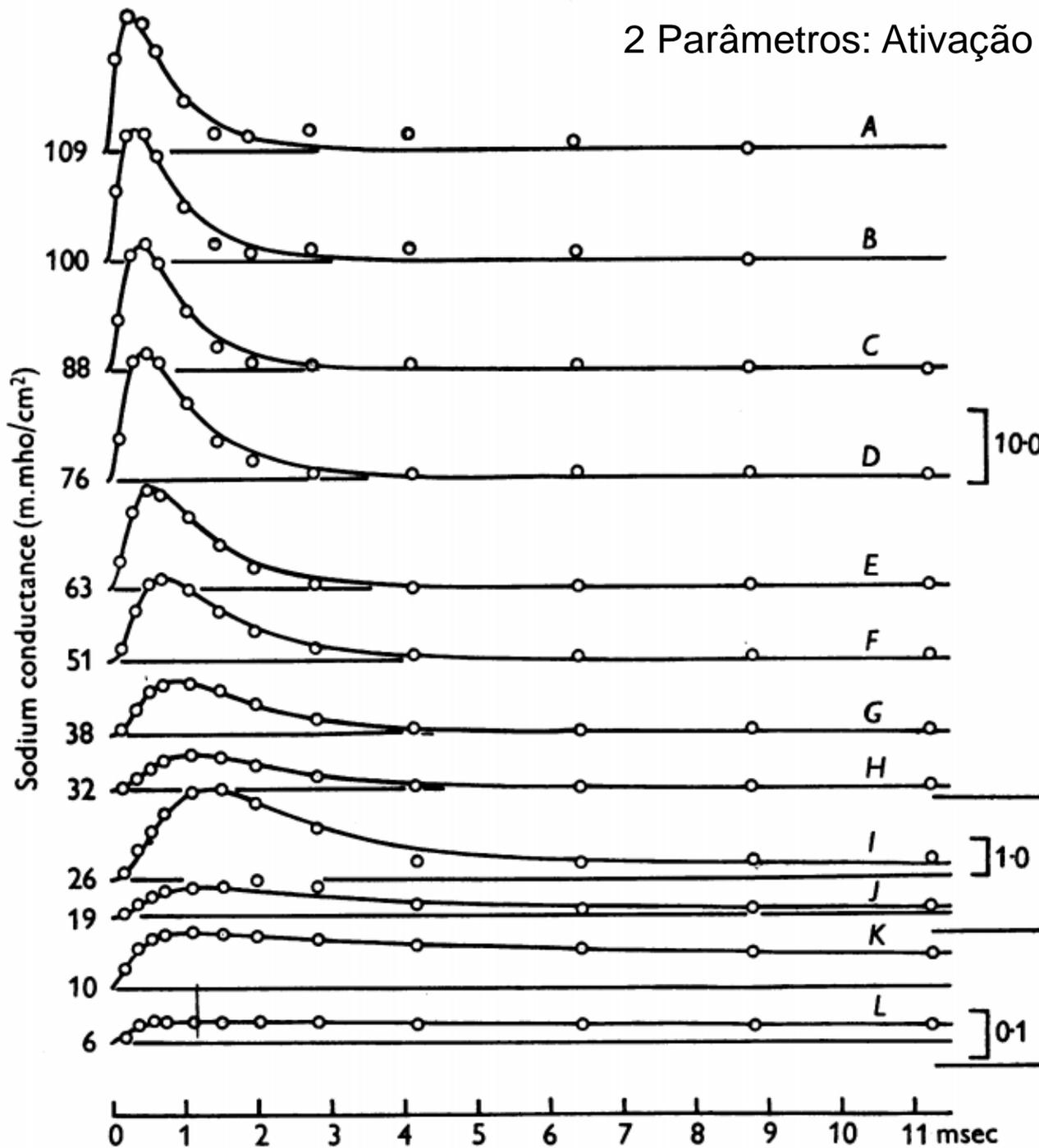
$$g_K = n^4 \bar{g}_K$$

Relação com
condutância era com
ordem 4 de n

$$\alpha_n = 0.01 \frac{10 - v}{\exp\left(\frac{10 - v}{10}\right) - 1}$$

$$\beta_n = 0.125 \exp\left(\frac{-v}{80}\right)$$

2 Parâmetros: Ativação (m) e inativação (h) canal



“Fitting” da curva



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

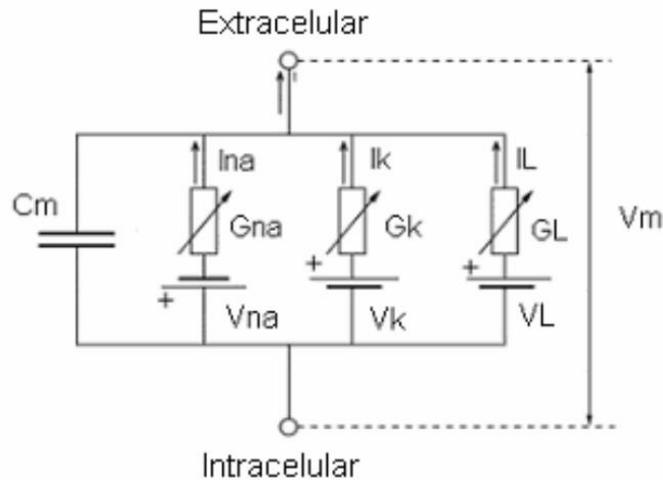
$$\alpha_m = 0.1 \frac{25 - v}{\exp\left(\frac{25 - v}{25}\right) - 1}$$

$$\beta_m = 4 \exp\left(\frac{-v}{18}\right)$$

$$\alpha_h = 0.07 \exp\left(\frac{-v}{20}\right)$$

$$\beta_h = \frac{1}{\exp\left(\frac{30 - v}{10}\right) + 1}$$

Modelo Hodgkin-Huxley



$$\frac{dV_m}{dt} = \frac{1}{C_m} \cdot (I_{st} - I_{Na} - I_k - I_L)$$

$$\frac{dm}{dt} = \alpha_m \cdot (1 - m) - \beta_m \cdot m$$

$$\frac{dh}{dt} = \alpha_h \cdot (1 - h) - \beta_h \cdot h$$

$$\frac{dn}{dt} = \alpha_n \cdot (1 - n) - \beta_n \cdot n$$

$$\alpha_n = 0.01 \frac{10 - v}{\exp\left(\frac{10 - v}{10}\right) - 1}$$

$$\alpha_m = 0.1 \frac{25 - v}{\exp\left(\frac{25 - v}{25}\right) - 1}$$

$$\beta_n = 0.125 \exp\left(\frac{-v}{80}\right)$$

$$\beta_m = 4 \exp\left(\frac{-v}{18}\right)$$

$$\alpha_h = 0.07 \exp\left(\frac{-v}{20}\right)$$

$$\beta_h = \frac{1}{\exp\left(\frac{30 - v}{10}\right) + 1}$$

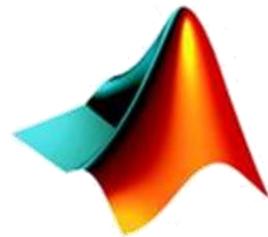
$$I_{Na} = \bar{G}_{Na} \cdot m^3 \cdot h \cdot (V_m - V_{Na})$$

$$I_k = \bar{G}_k \cdot n^4 \cdot (V_m - V_k)$$

$$I_L = G_L \cdot (V_m - V_L)$$

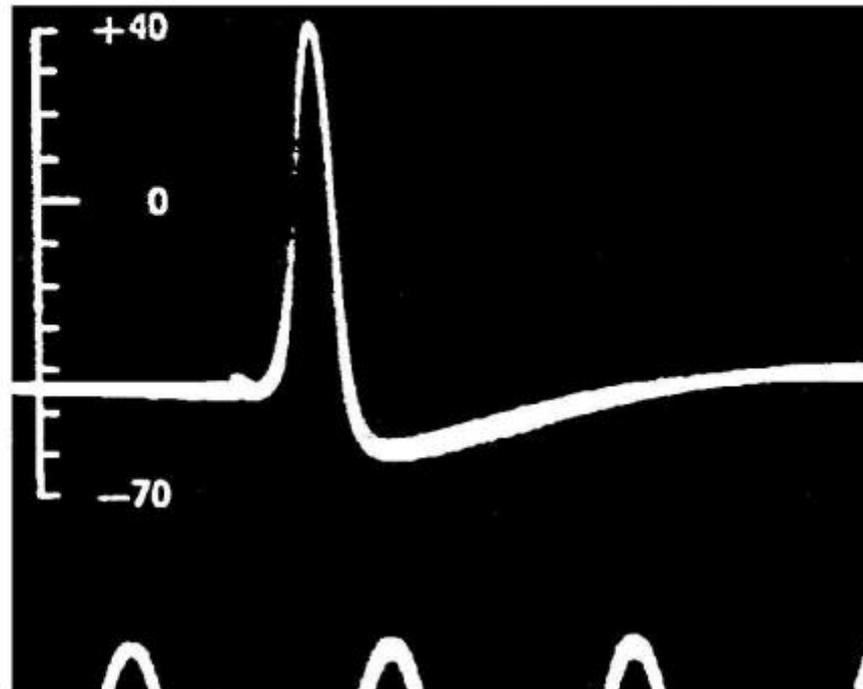
Quantitative description of membrane current and its application to conduction and excitation in nerve (1952)

hh_aula.m

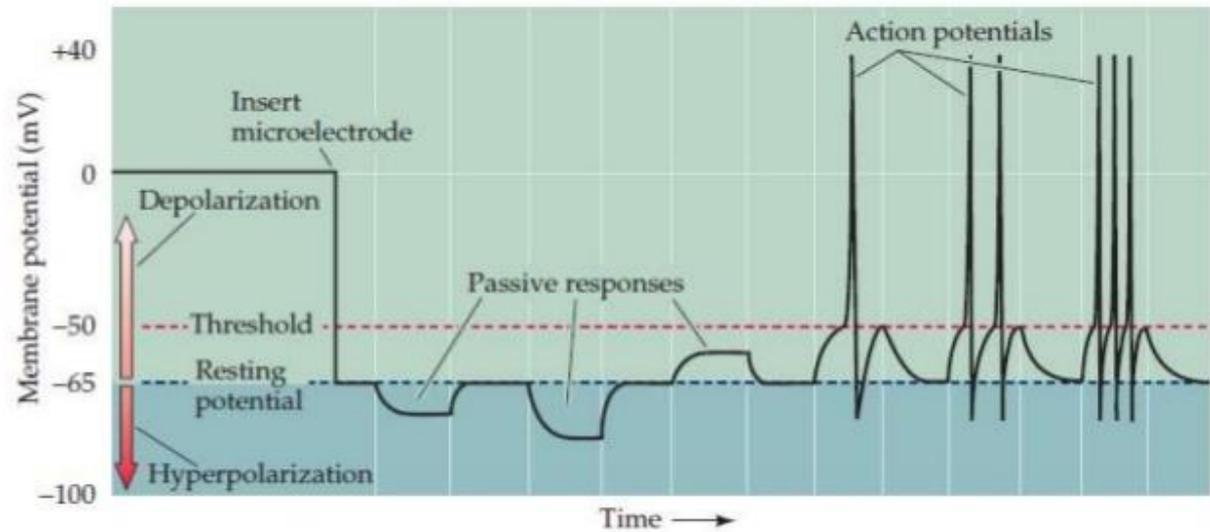
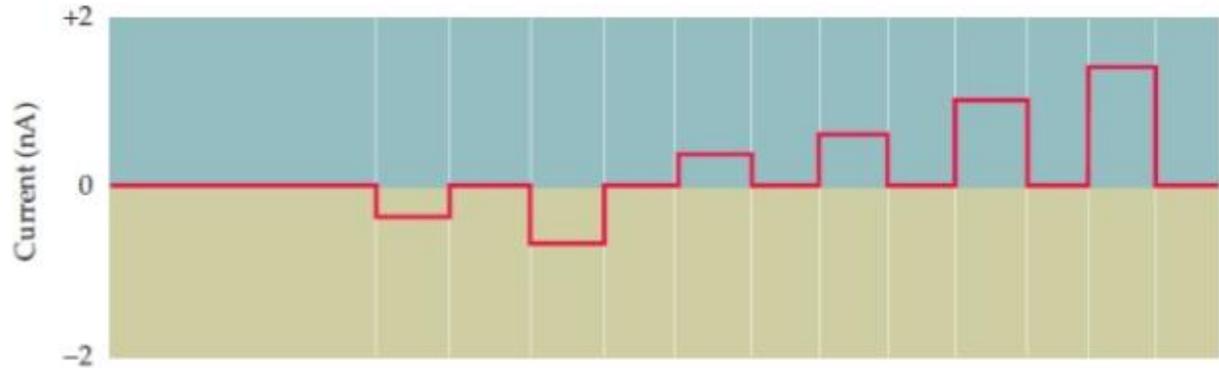
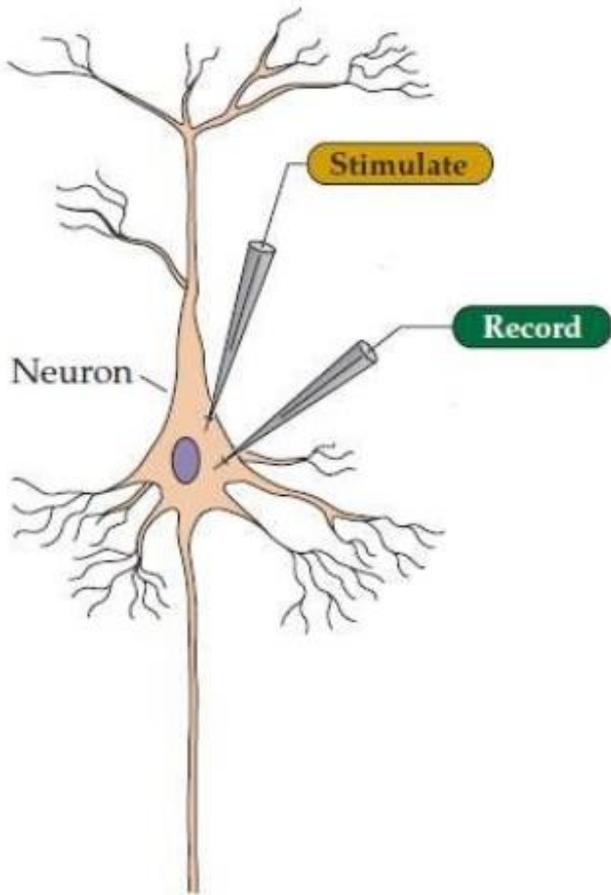


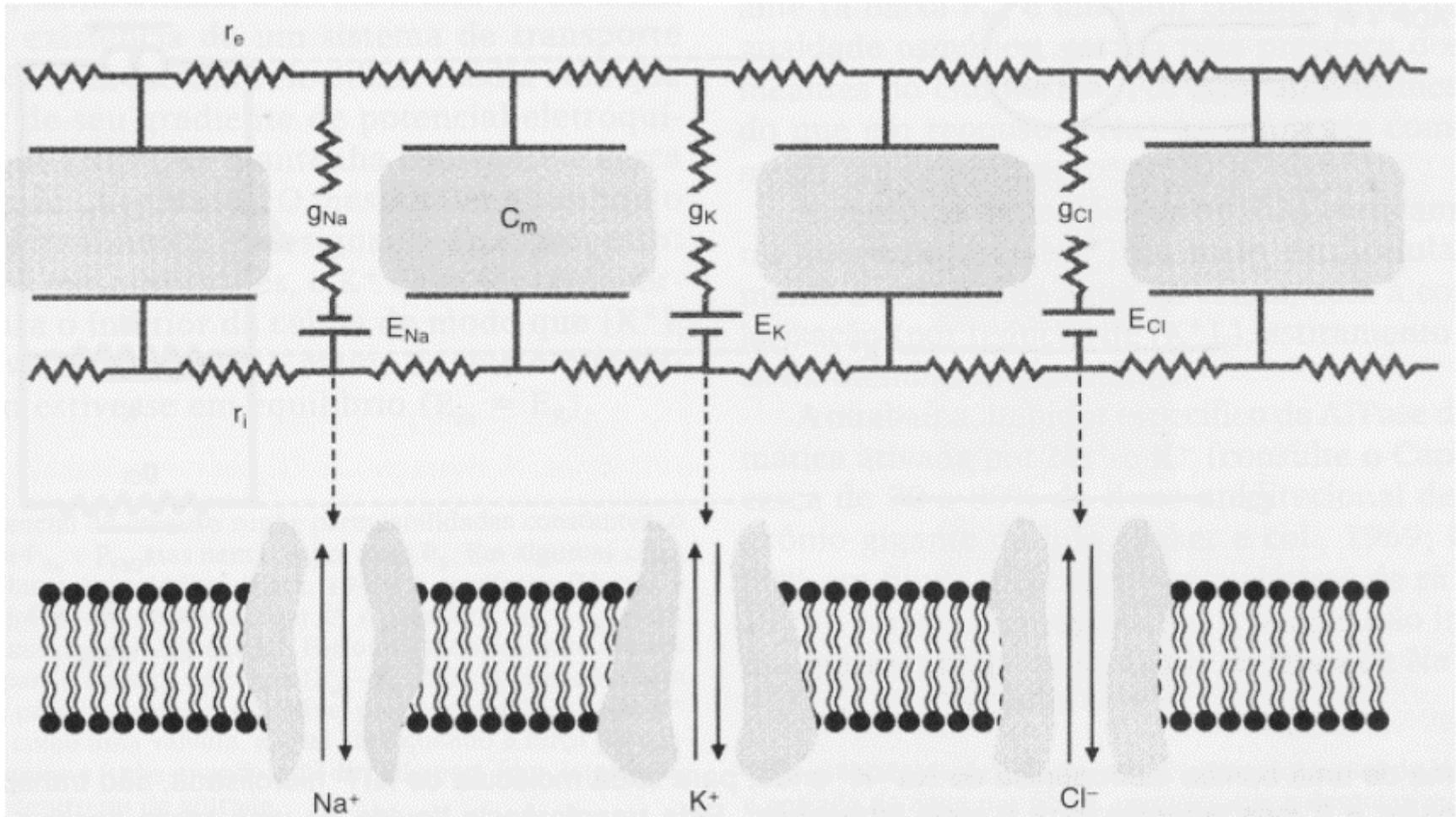
MATLAB[®]

Modelo Hodgkin-Huxley



Quantitative description of membrane current and its application to conduction and excitation in nerve(1952)







Tektronix 502A



Huxley

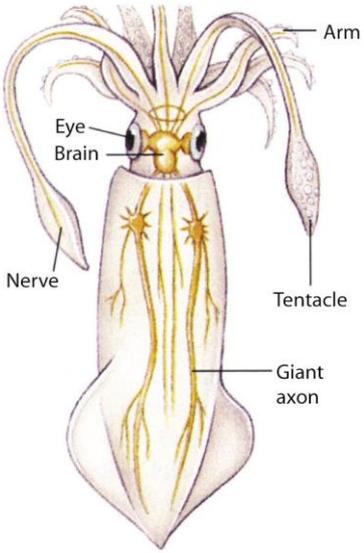
Hodgkin

NERVE-CELL ENIGMA SOLVED

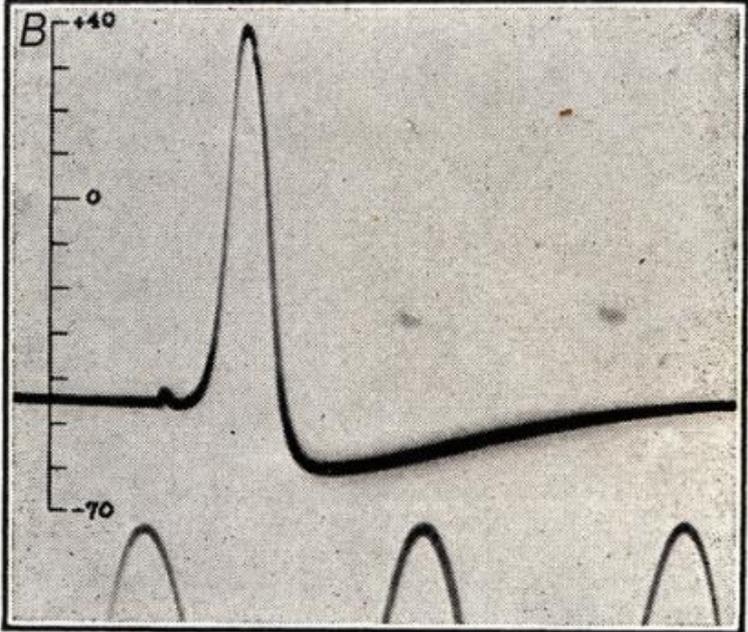
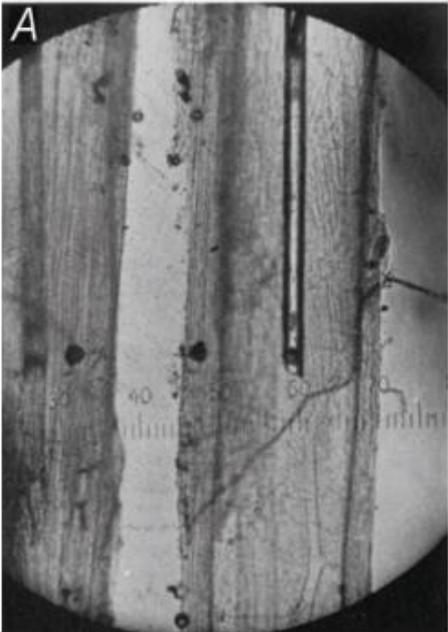
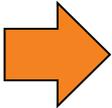
The British scientists, A. L. Hodgkin and A. F. Huxley, experimenting with the nerve fibers of squids and lobsters.

A brief historical perspective: Hodgkin and Huxley

Hodgkin and Huxley 1939



Copyright © 2009 Pearson Education, Inc.



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

Registros Eletrofisiológicos



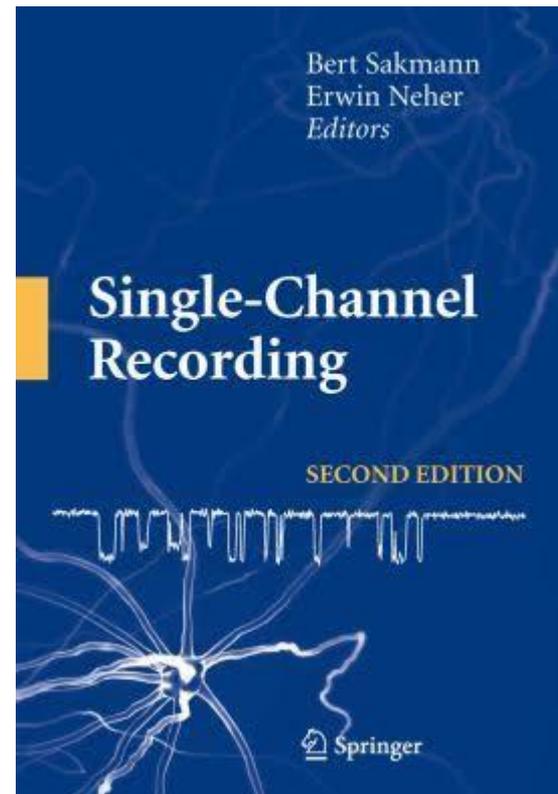
The Nobel Prize in Physiology or Medicine 1991



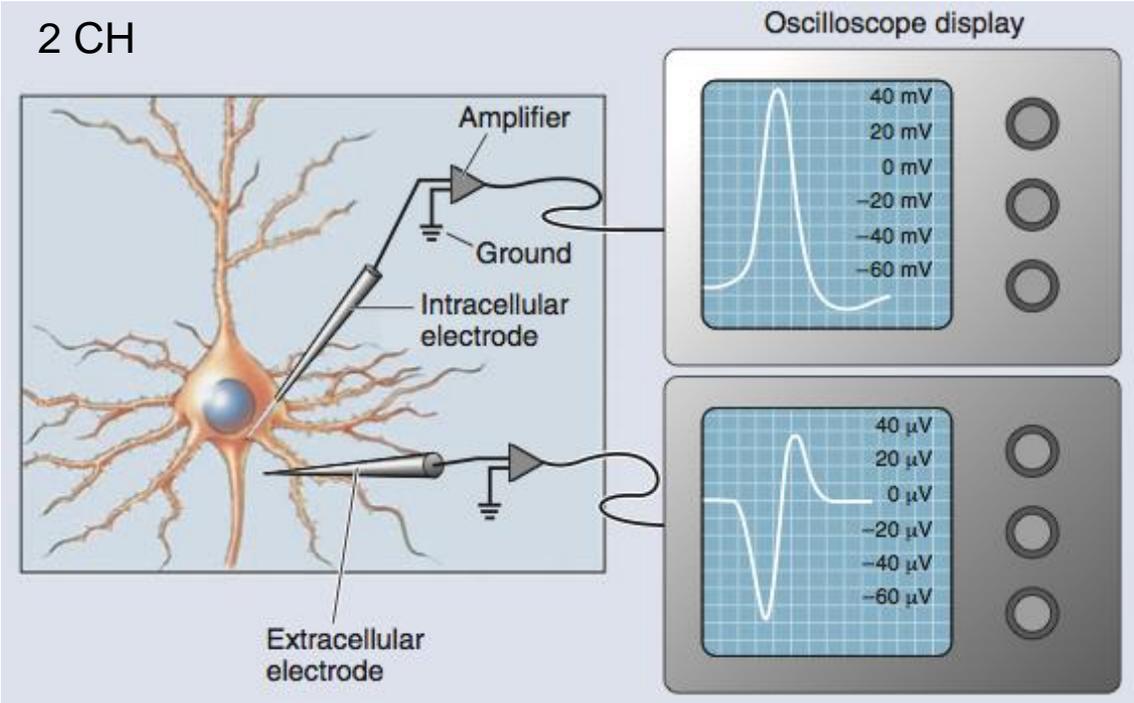
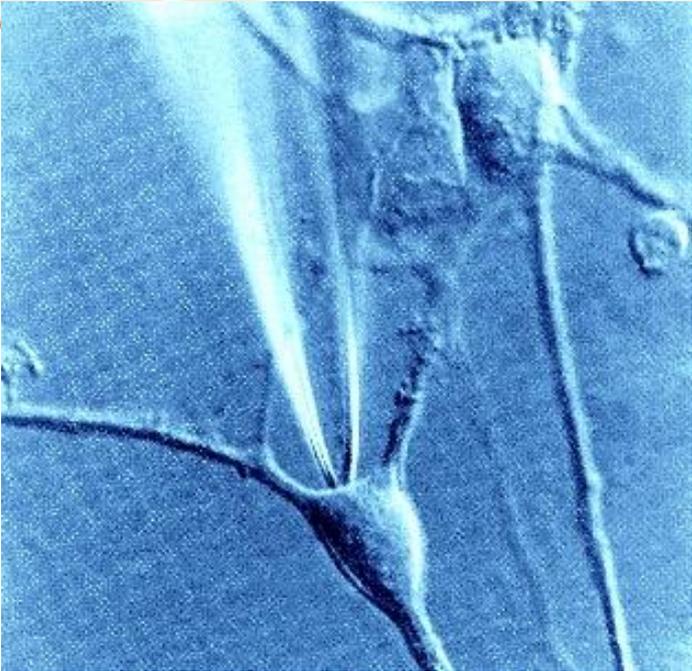
Erwin Neher
Prize share: 1/2

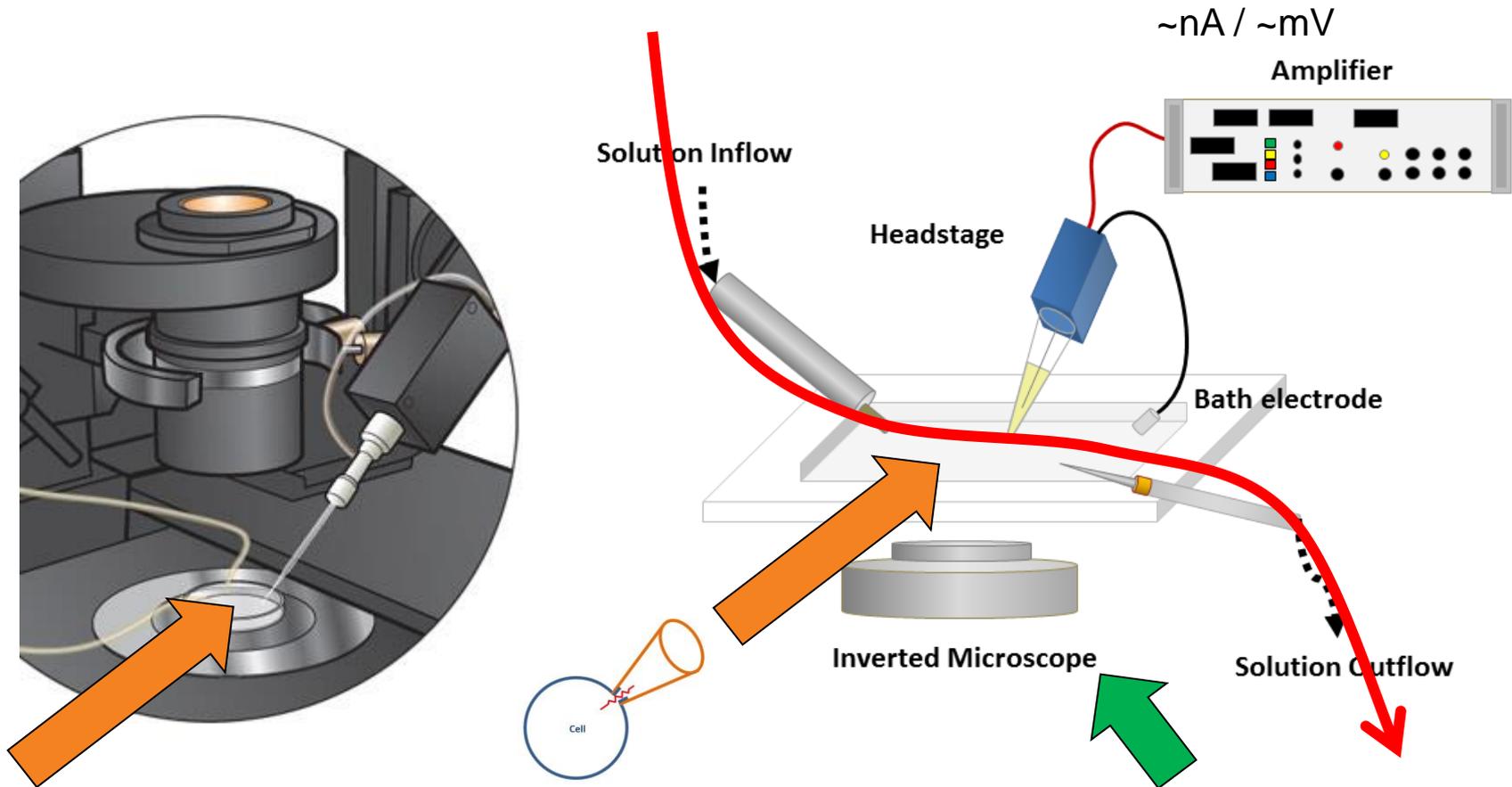


Bert Sakmann
Prize share: 1/2

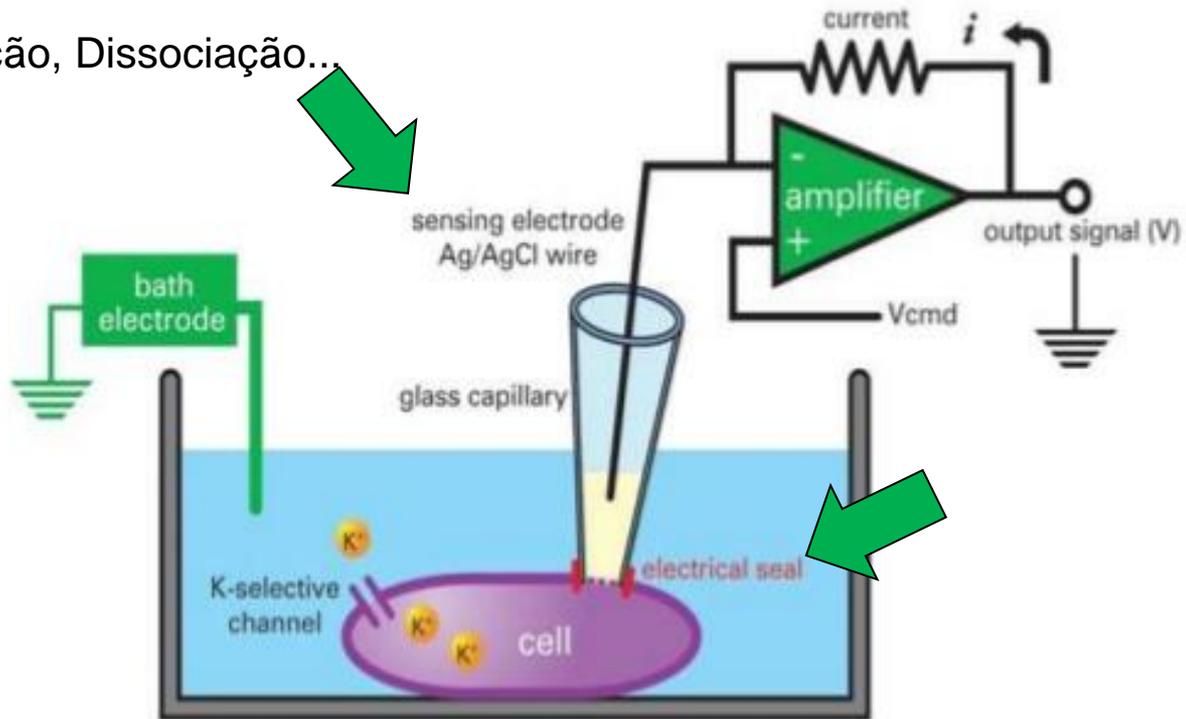


Registros Eletrofisiológicos





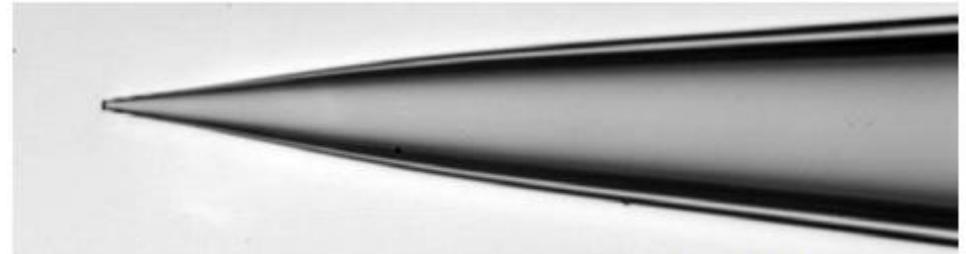
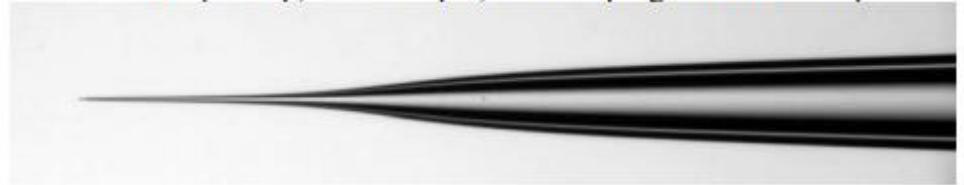
Oxidação, Dissociação...



Micropipeta



Image A (40x mag.) 1.0 x 0.5 thick walled glass (BF100-50-10)
~0.5 μ m Tip, 4-5mm taper, one line program with 3 loops



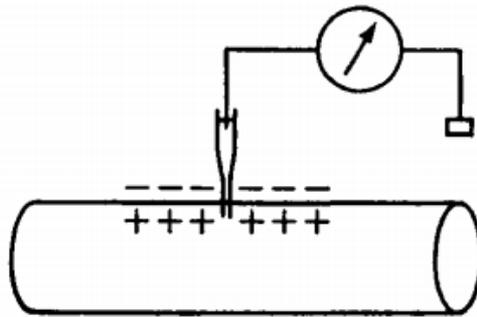
1.5mm x 0.86mm thick walled glass, ~2 μ m Tip, 3-4mm taper (400X mag)

Medium/Large Holding Pipette (400x mag.)

Scored and cut with clean break using a ceramic tile, then fire-polished. 15 μ m ID x 80 μ m OD



Tipos de Registros



1 Intracellular recording

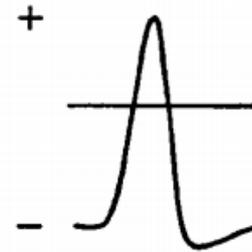
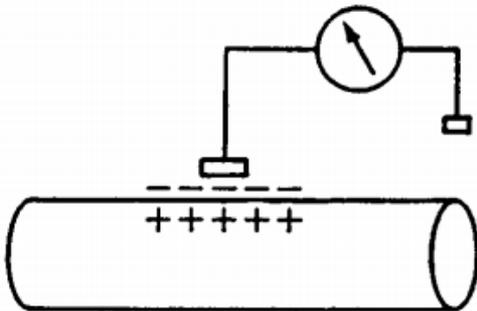
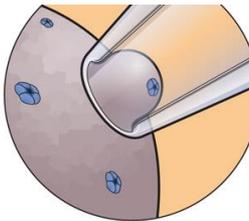


Figure 4.6. Diagram to show the difference in sign of action potentials recorded by intracellular and by extracellular electrodes. Extracellular records are in fact frequently shown with negative potentials upwards.



2 Extracellular recording



3 Patch Clamp

Medição Intracelular

Circuito Equivalente

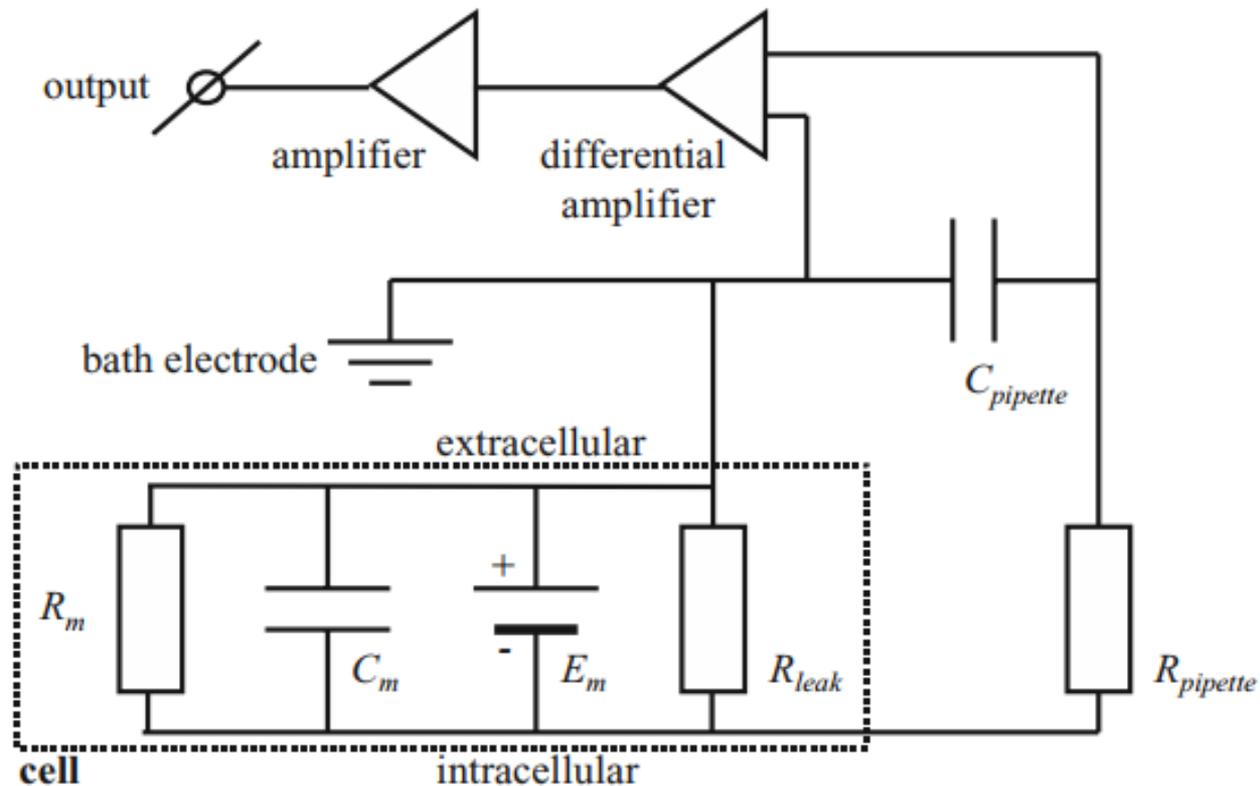
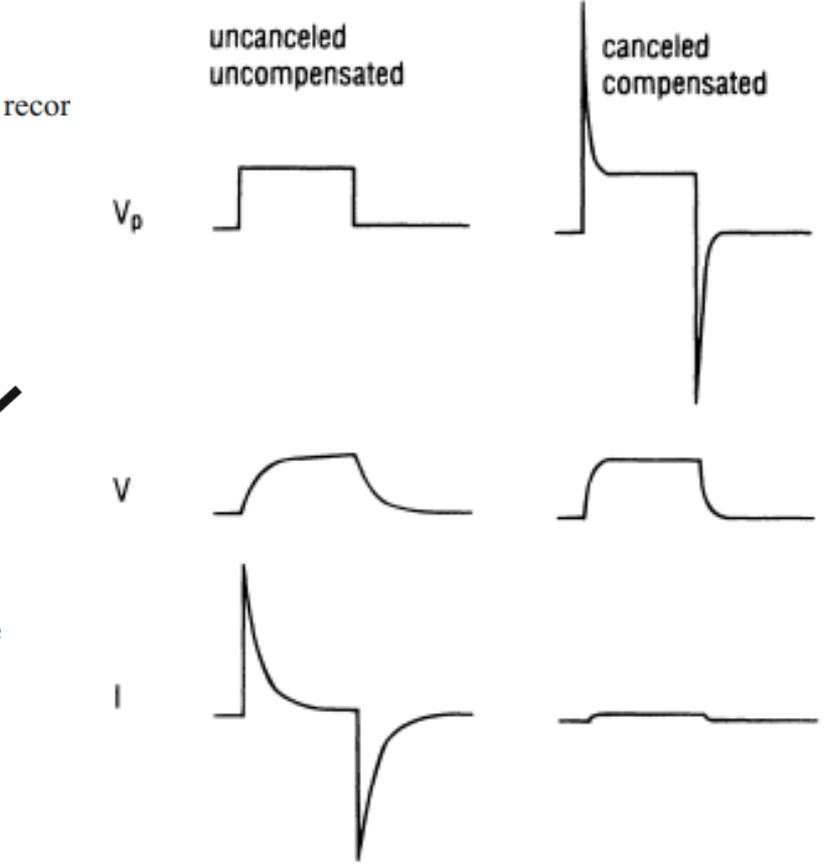
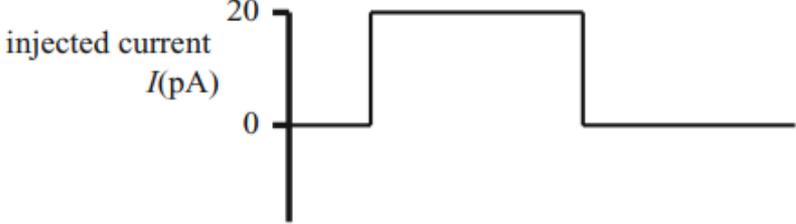
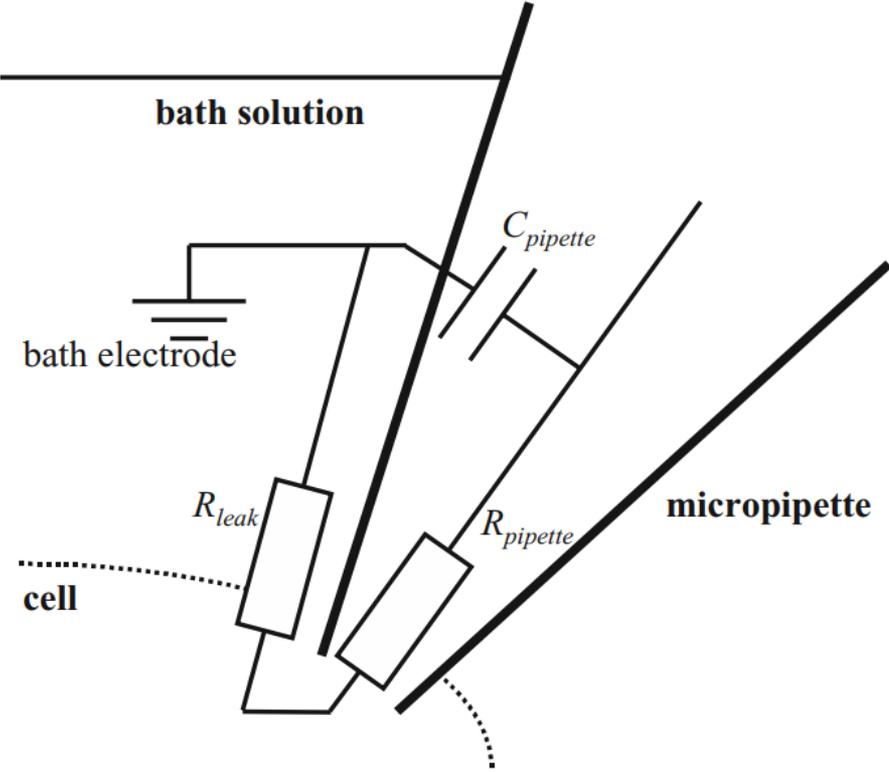


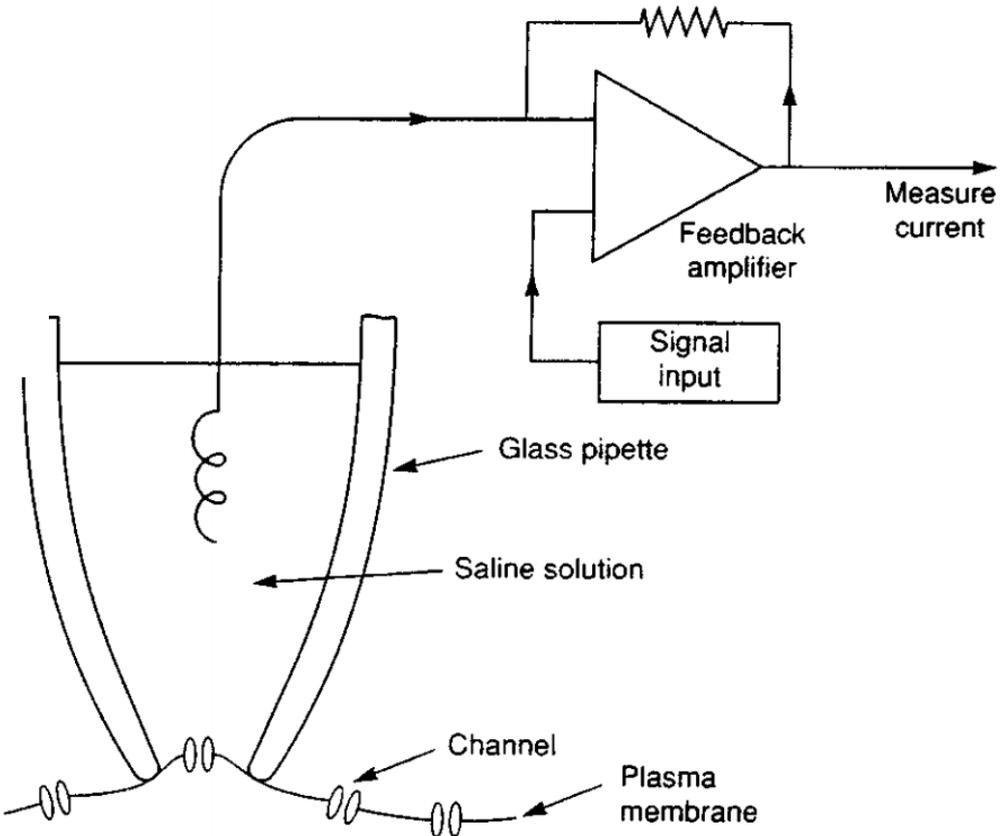
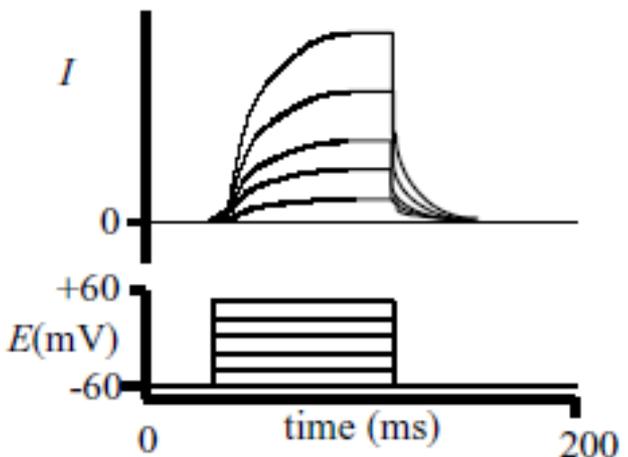
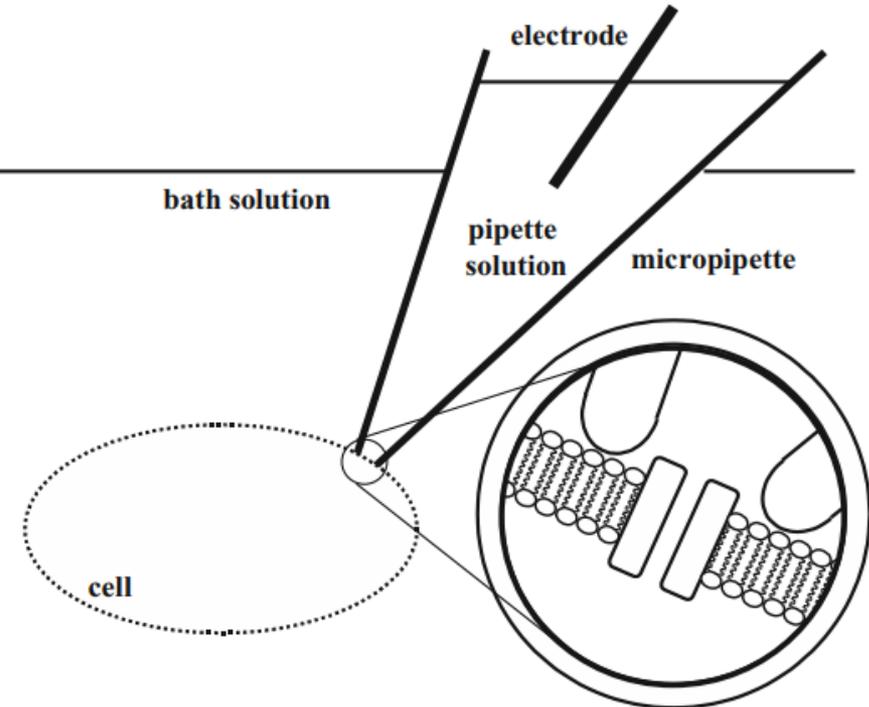
Figure 2.14 Equivalent circuit for an intracellular recording configuration. In addition to

Medição Intracelular

Zoom - Desvios Pipeta



Patch Clamp



Patch Clamping

Patch Clamp Circuito Equivalente

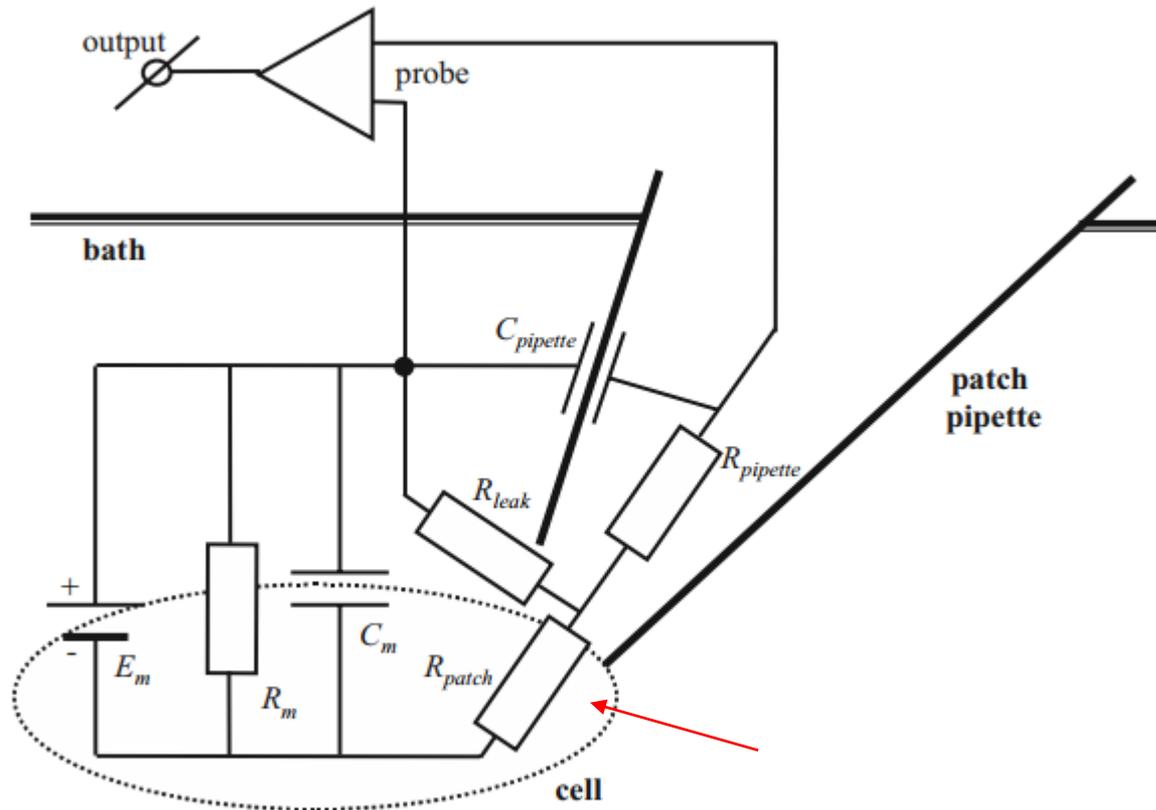
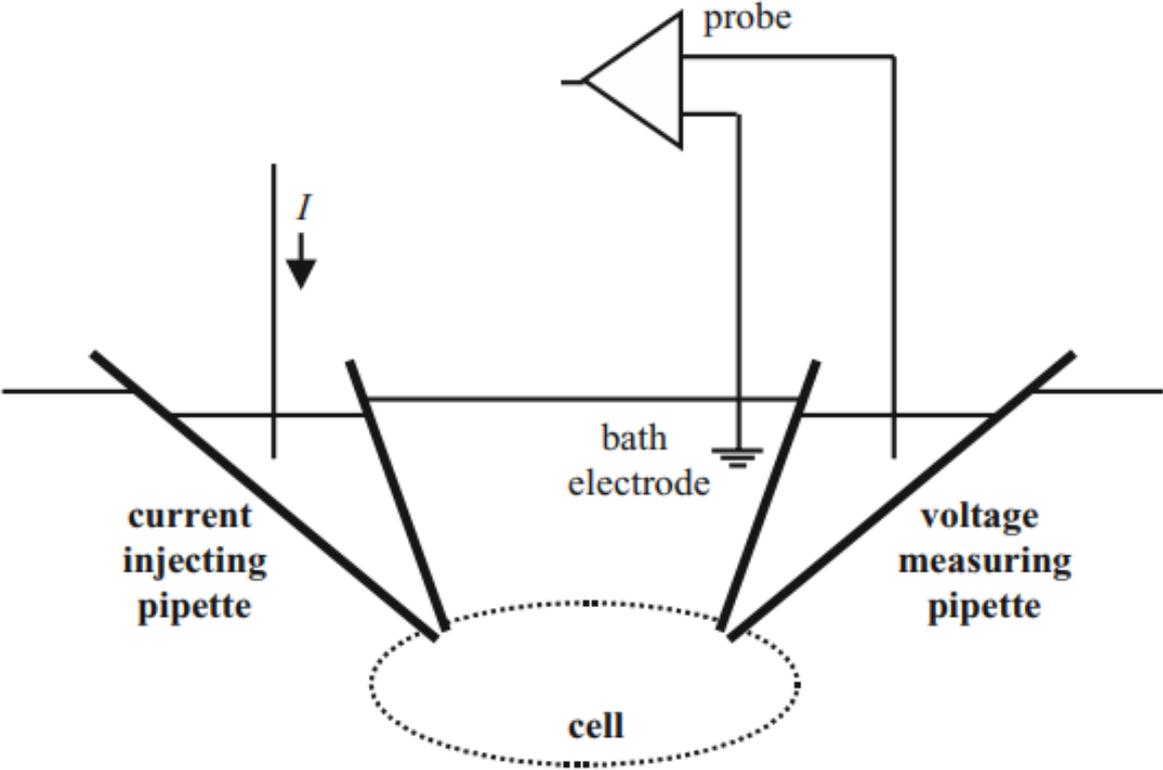
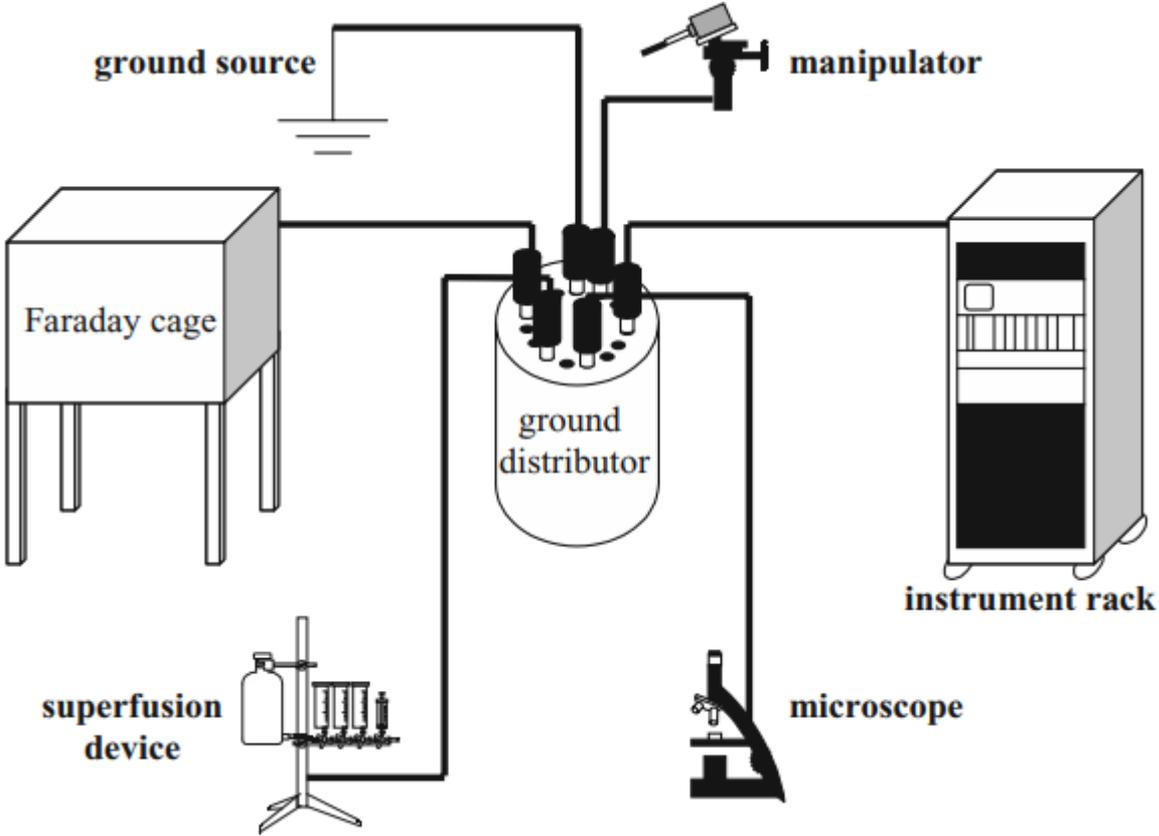


Figure 2.24 Equivalent circuit for the cell-attached patch configuration

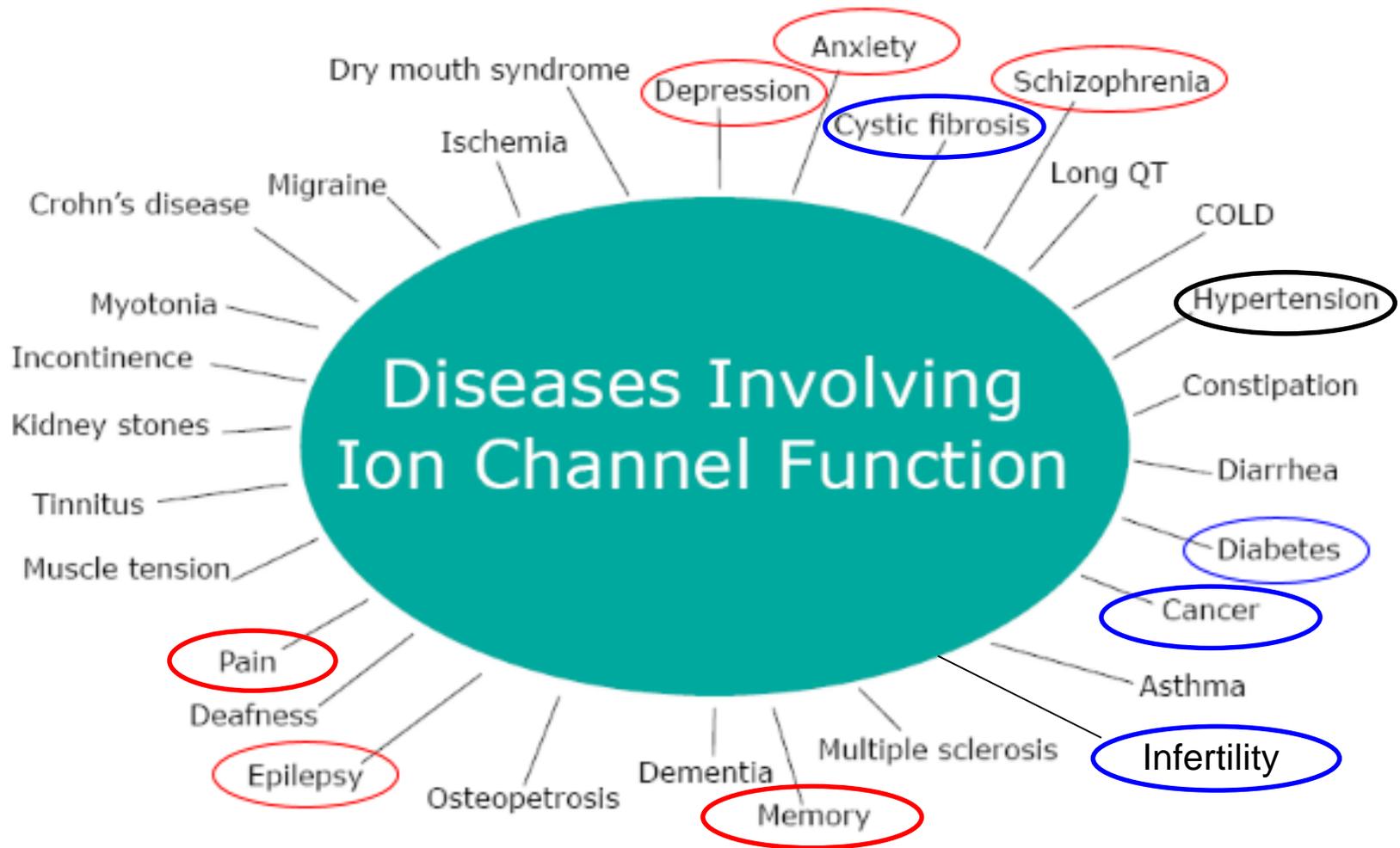
Duplos Eletrodos



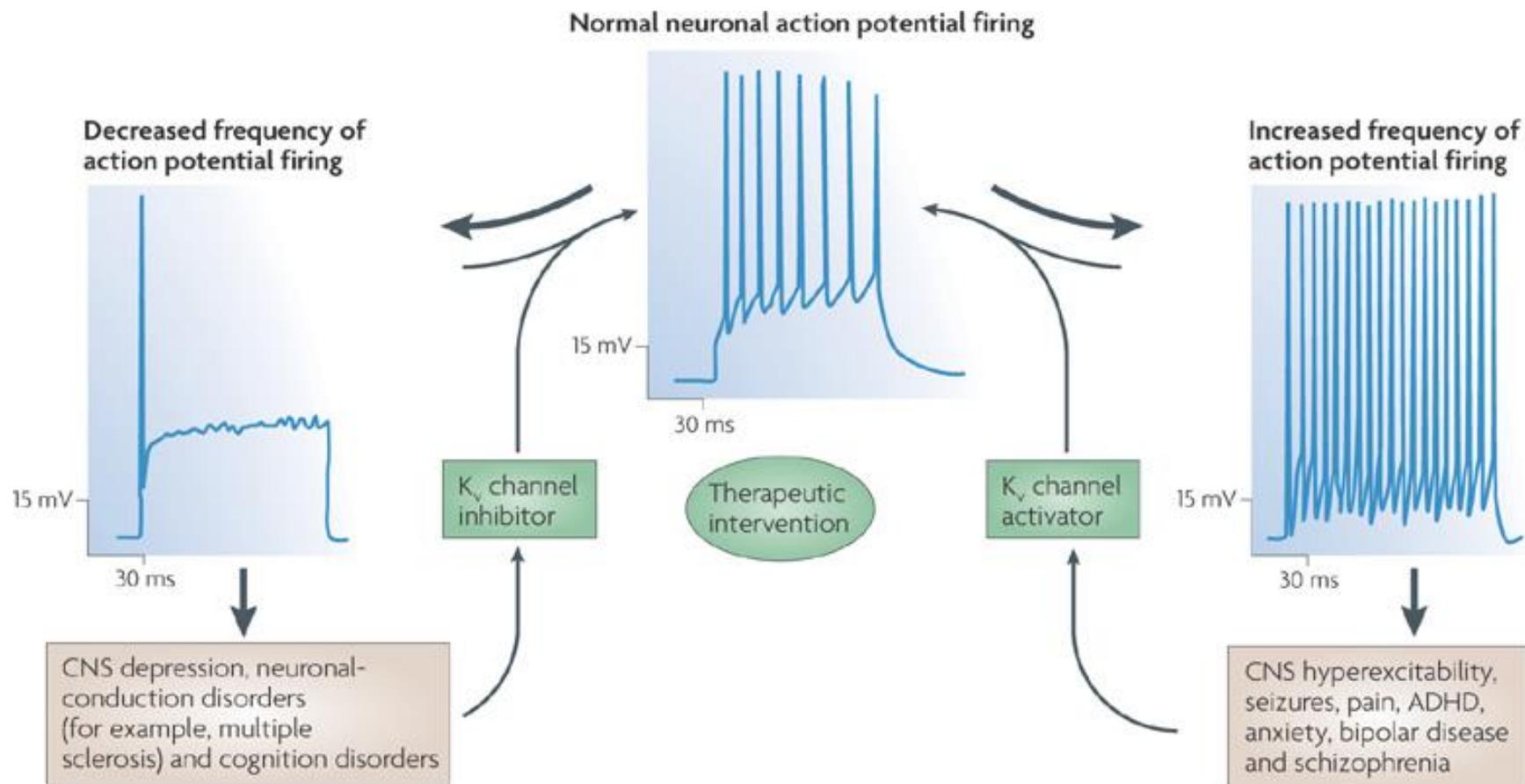
Requisitos - Curiosidade



Canais iônicos como alvos terapêuticos



Neuron Firing in Health and Disease: Therapeutic targets



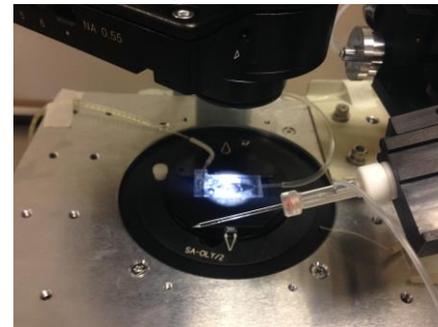
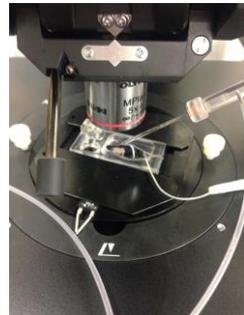
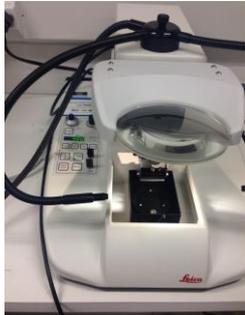
Nubiocel Lab



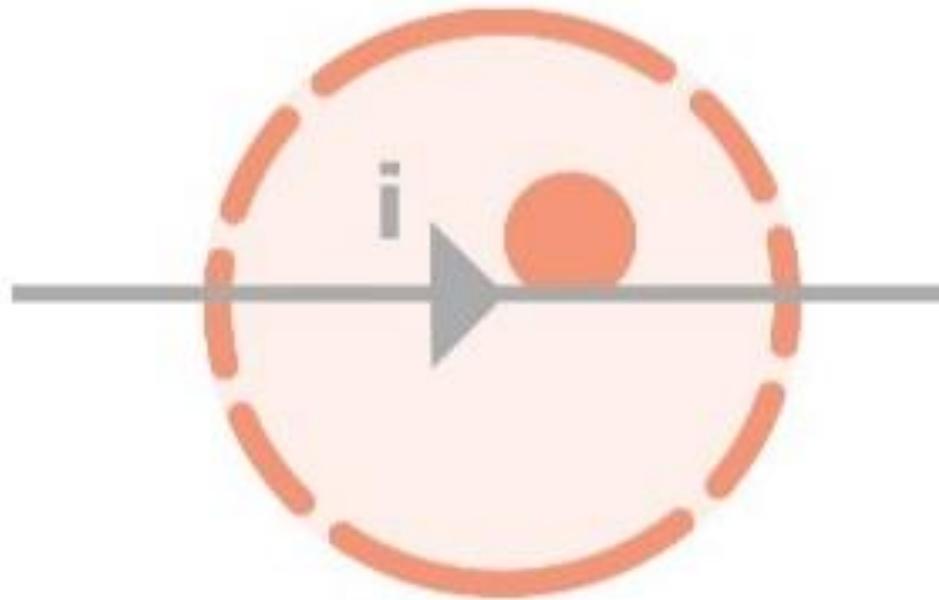
Brain Slices

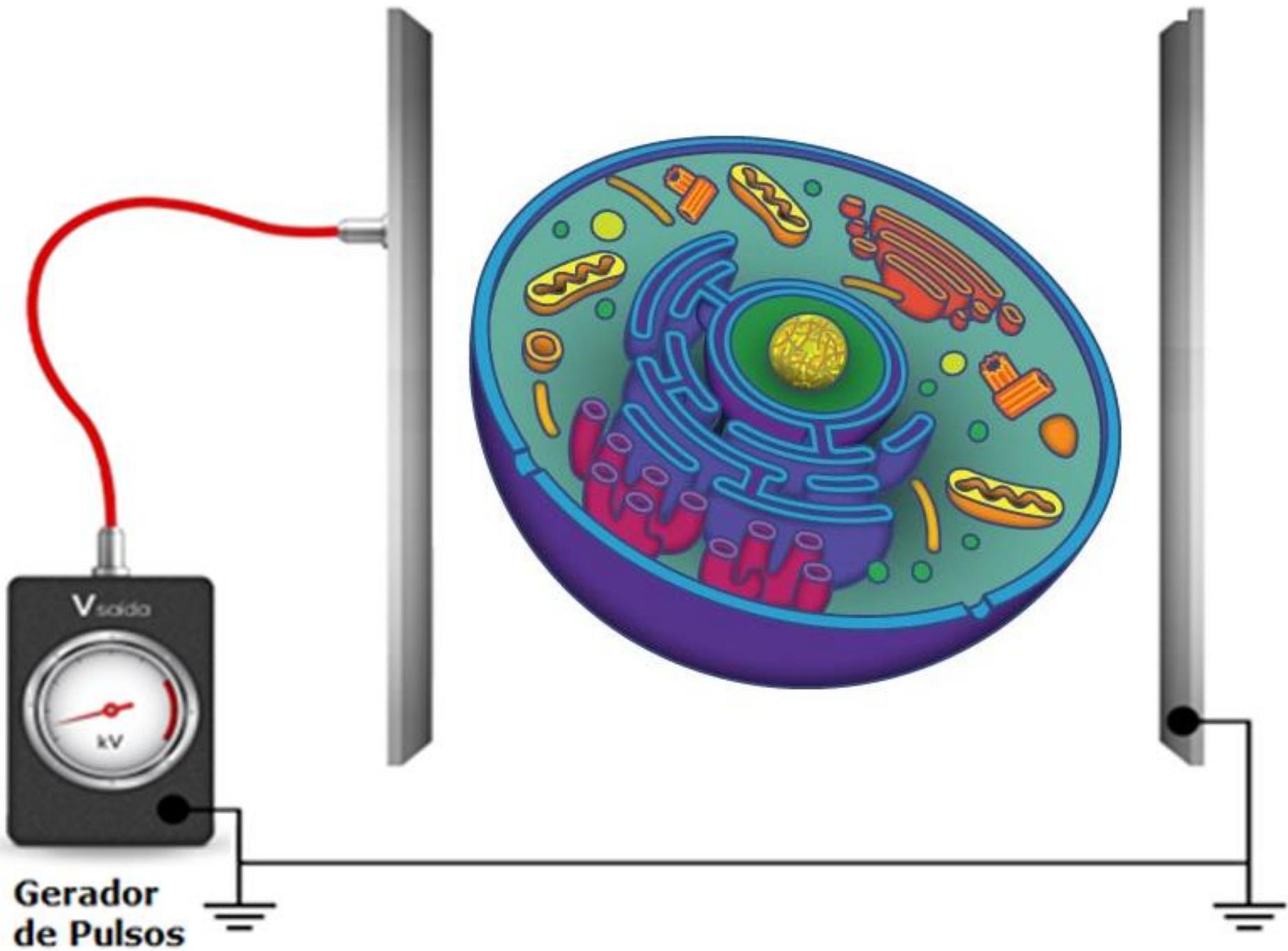


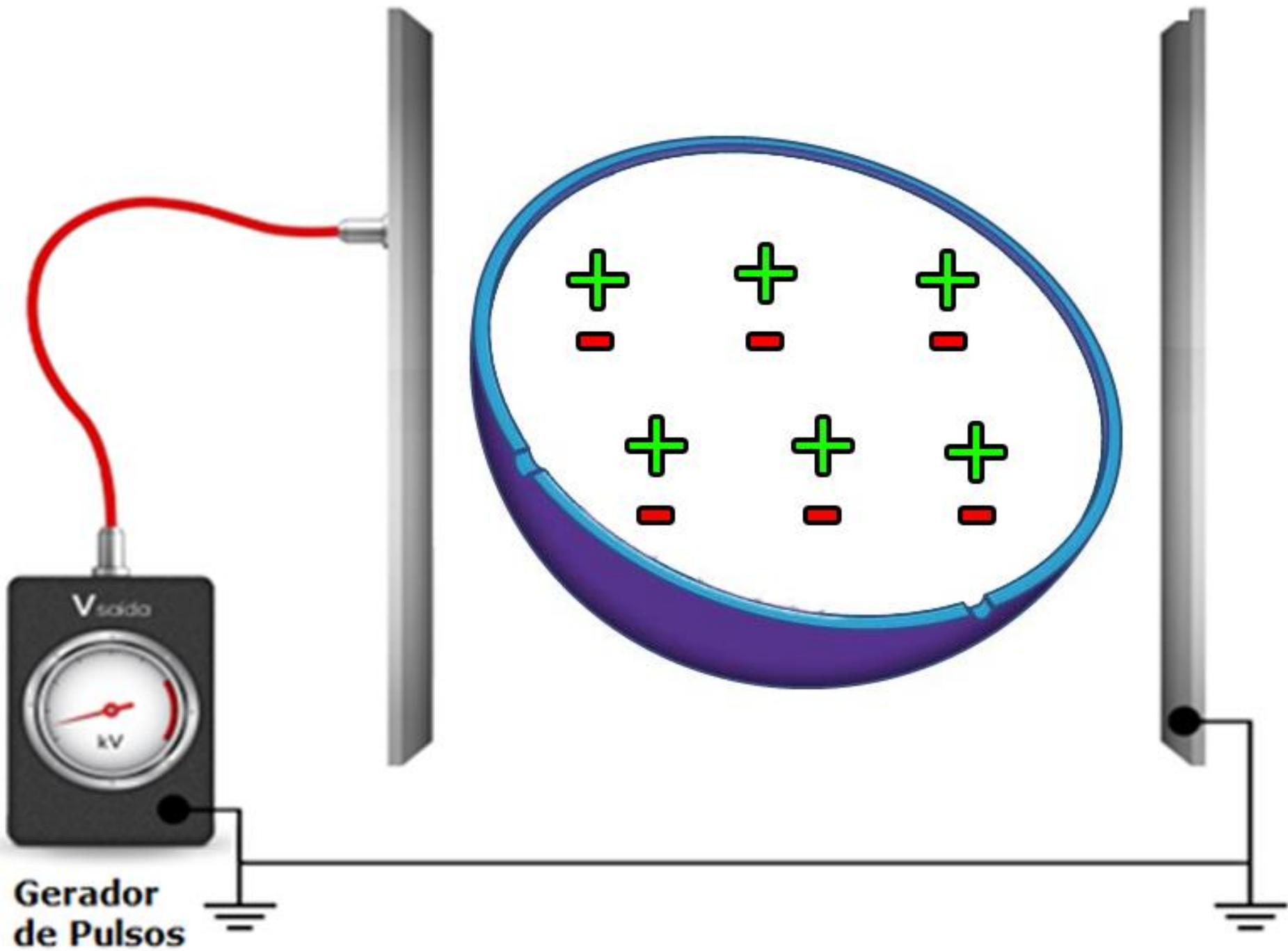
Isolated cells

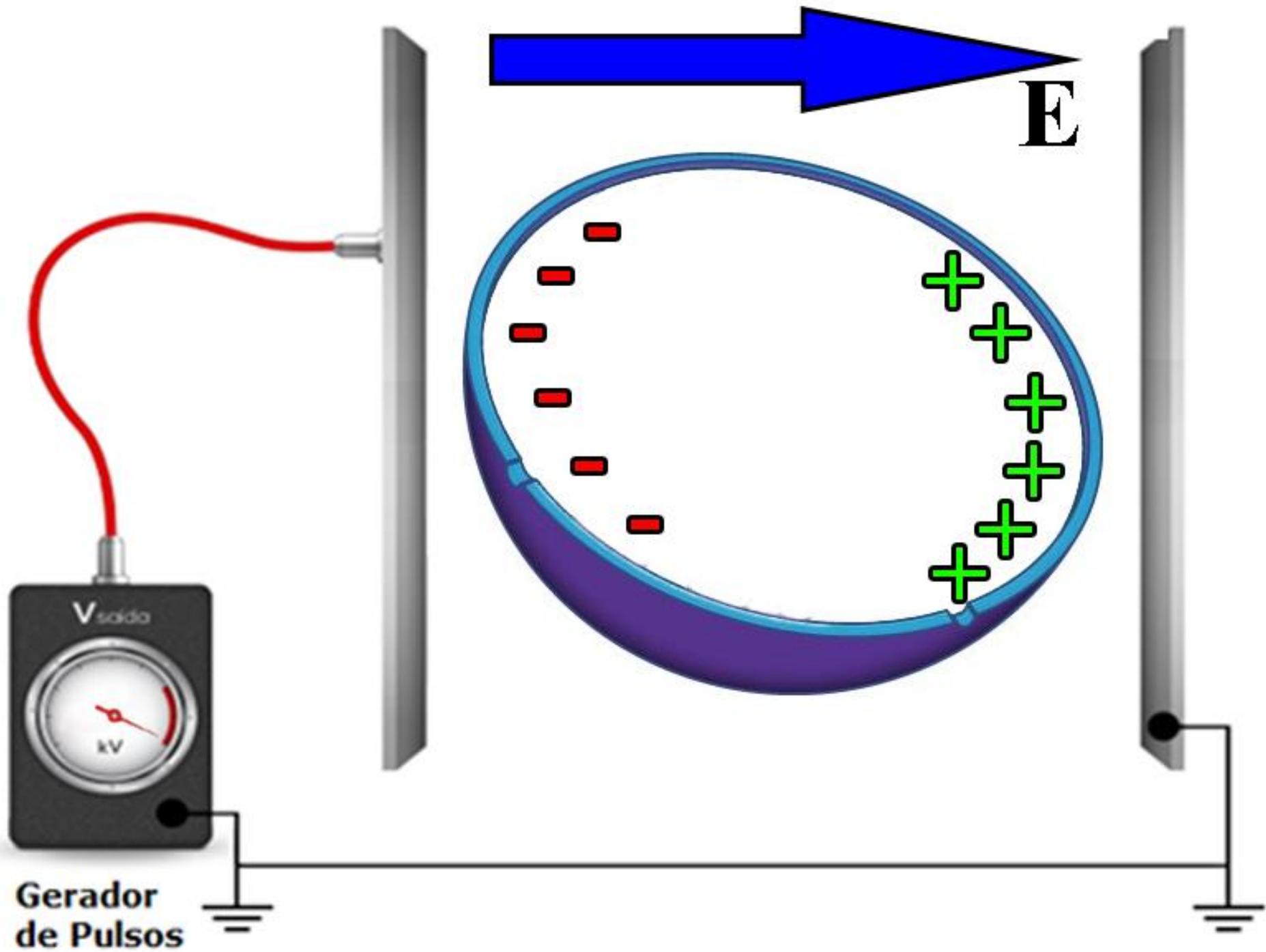


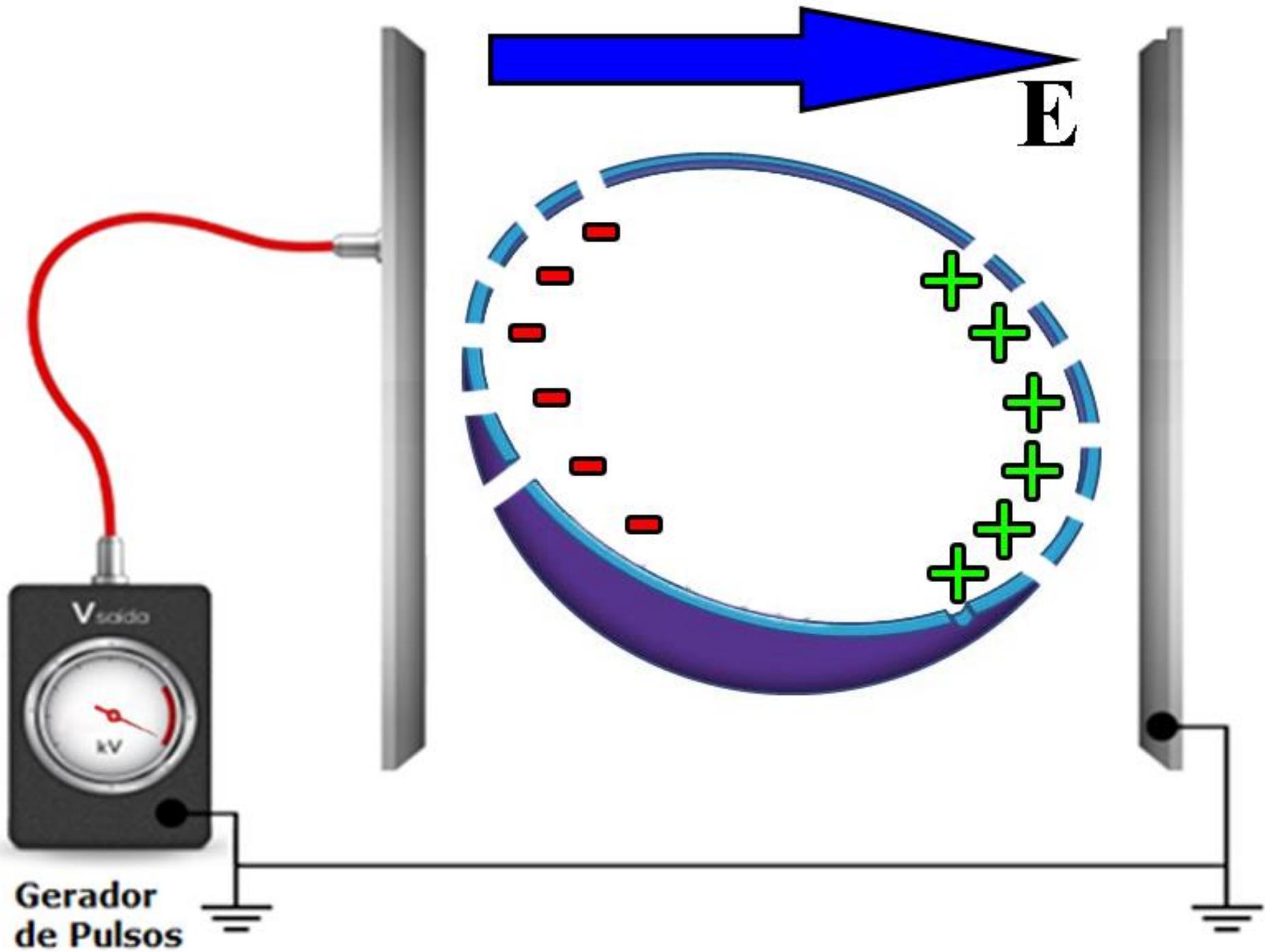
Eletroporação







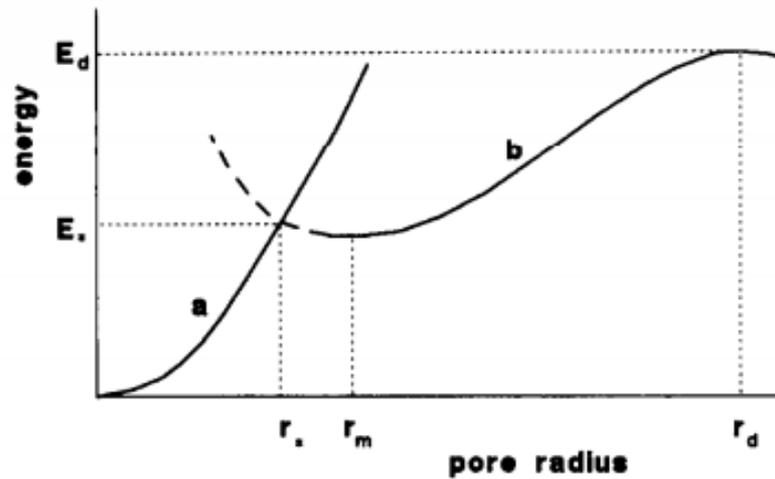
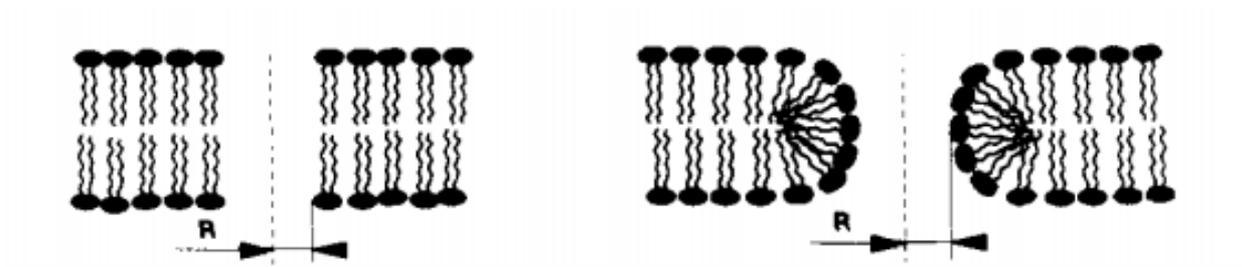




Modelo da Abertura de Poros na Membrana Celular através da aplicação de campos Elétricos (Eletroporação)



Modelo em nível de membrana



Modelo em nível de membrana

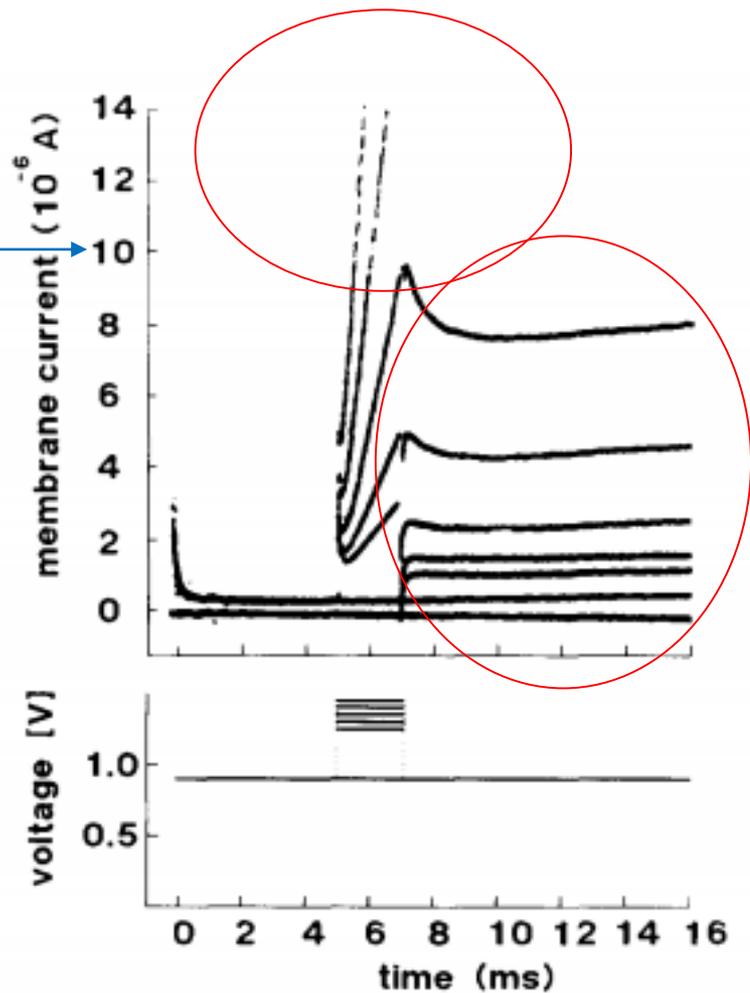


Fig. 6. Experimental determination of the rate of pore creation: oscillogram of membrane current and voltage pulse applied to the membrane.

Modelo em nível de membrana

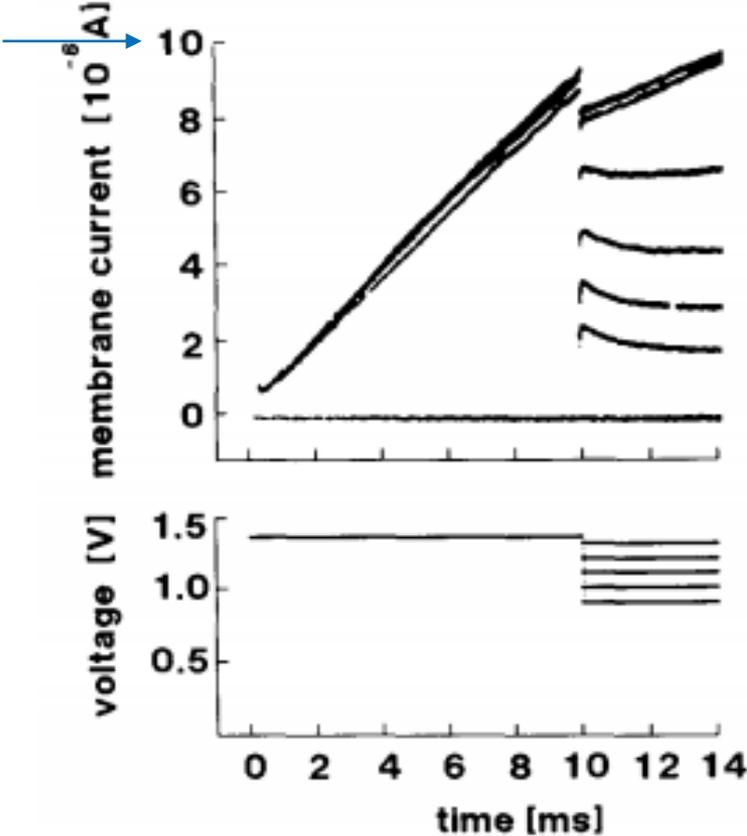
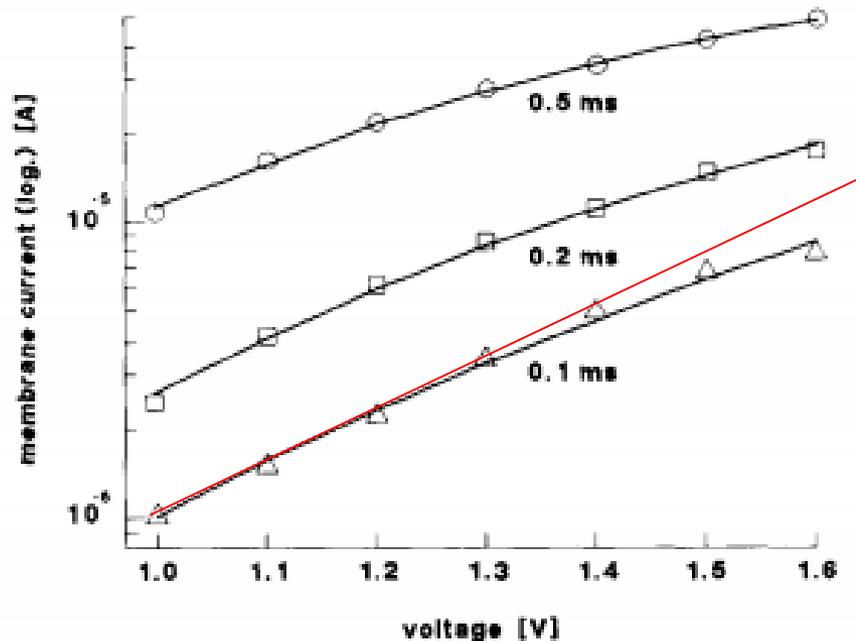


Fig. 7. Measurement of the voltage dependence of pore conductivity for the determination of pore size: oscillogram of membrane current and voltage pulses applied to the membrane.

Modelo em nível de membrana

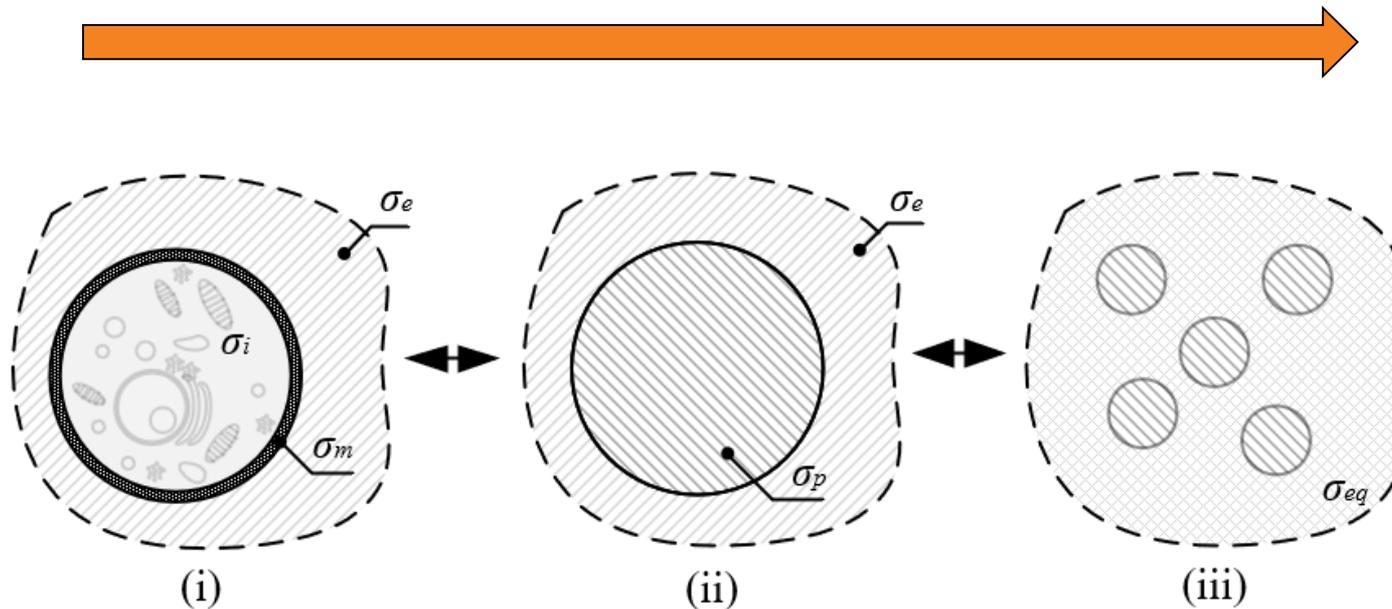


Se linear:
Independente de tensão e
tempo de aplicação.

Fig. 9. Relationship between membrane current and voltage immediately after pulses of 1.6 V and a duration of 0.1 ms (Δ), 0.2 ms (\square), and 0.5 ms (\circ) (experimental data). The experiment was performed as demonstrated in Fig. 7. —, fitted according to Eqn. 10. Parameters drawn from the fit see right column of Table I.

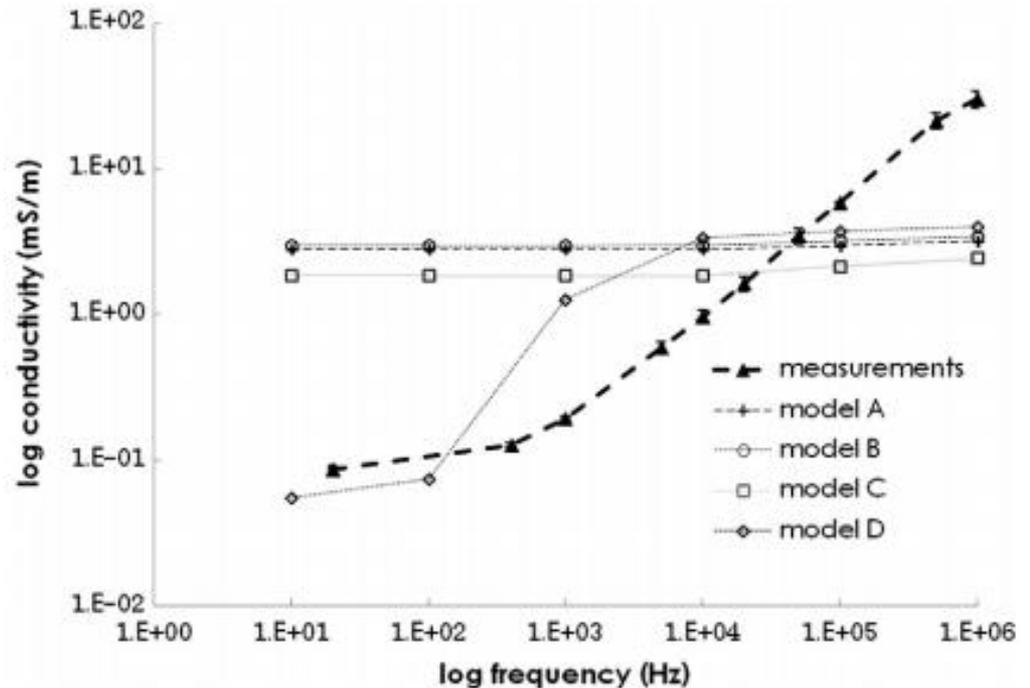
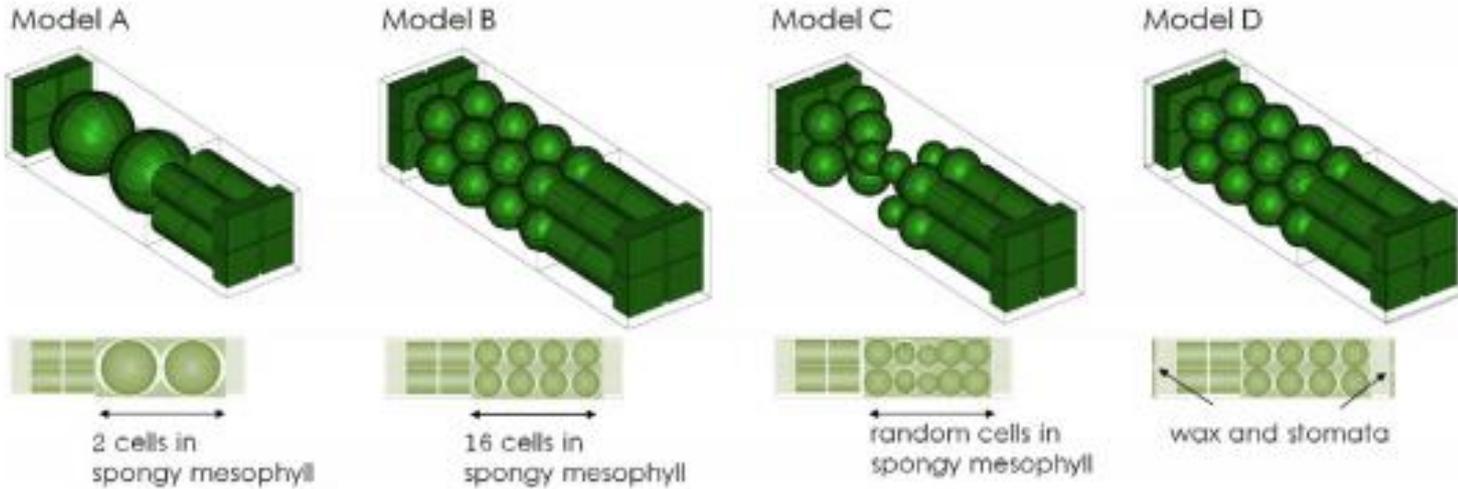
Modelo em nível celular e tecido

σ_m



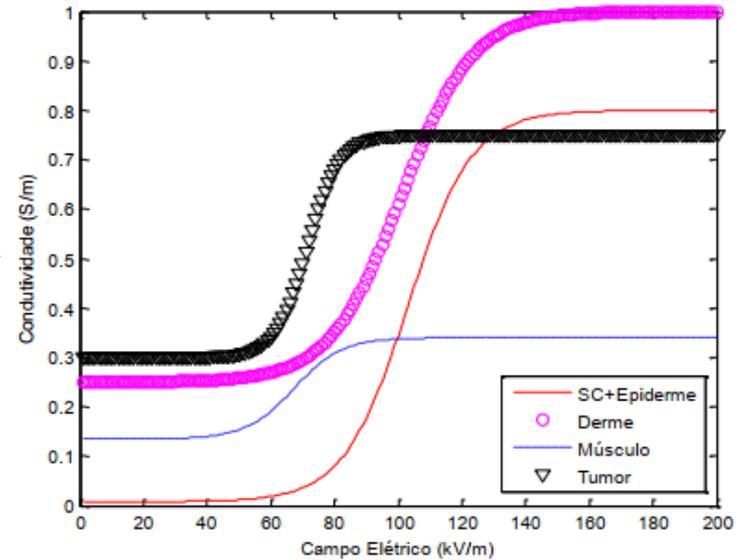
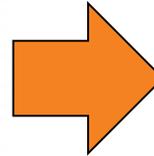
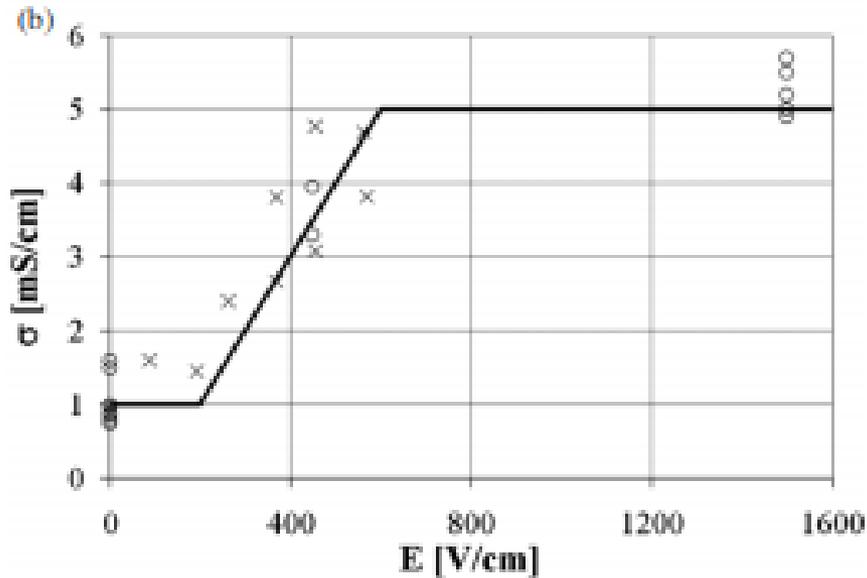
$$\sigma_p = \sigma_m \frac{2 \cdot (1 - \xi) \cdot \sigma_m + (1 + 2 \cdot \xi) \cdot \sigma_i}{(2 + \xi) \cdot \sigma_m + (1 - \xi) \cdot \sigma_i} \quad \xi = \left(\frac{1 - h}{a} \right)^3$$

Modelo em nível celular e tecido



Modeling electroporation of the non-treated and vacuum impregnated heterogeneous tissue of spinach leaves

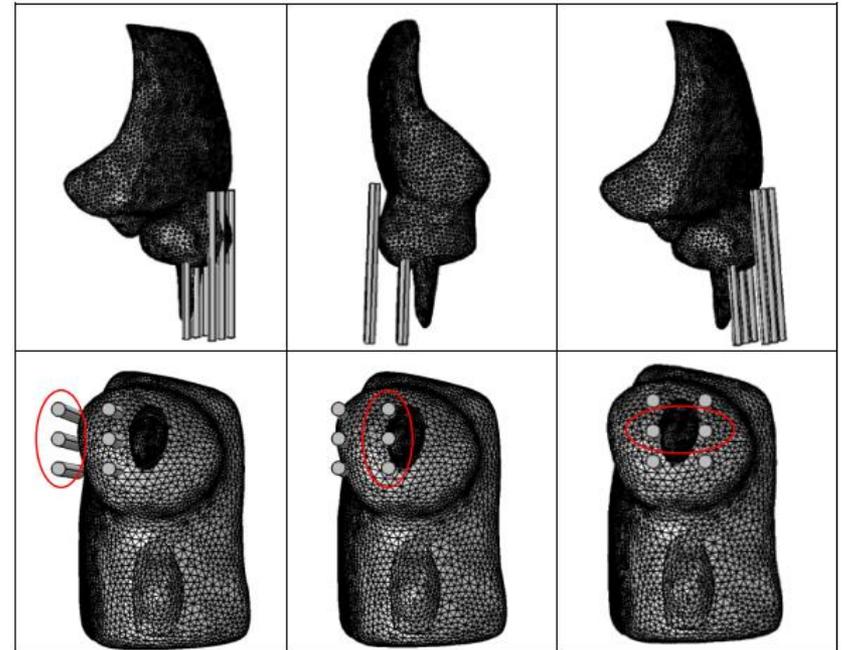
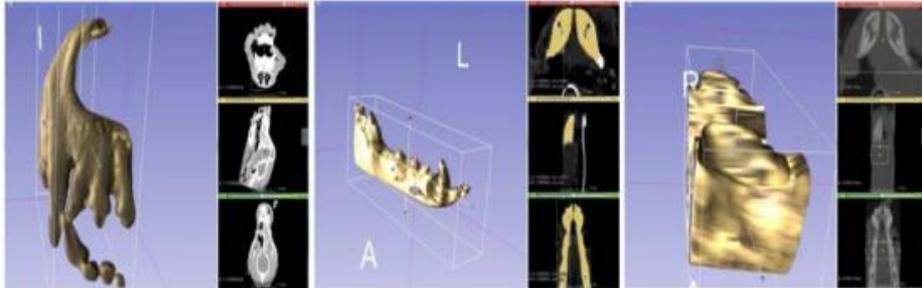
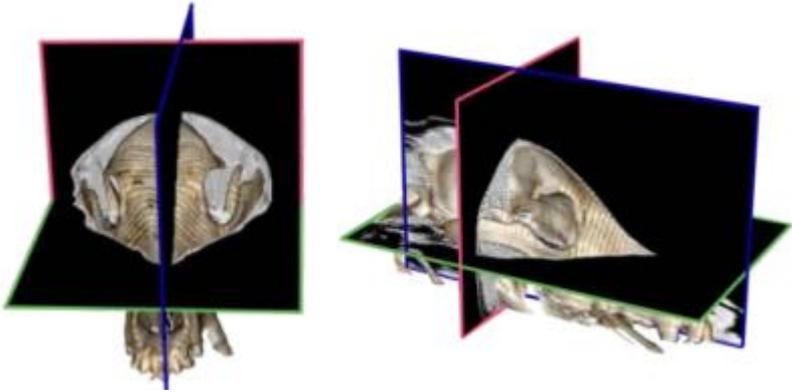
Modelo em nível de tecido



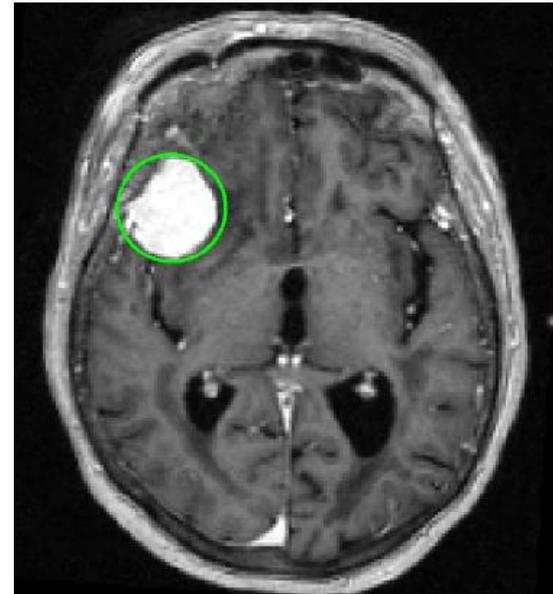
Use of conductive gels for electric field homogenization increases the antitumor efficacy of electroporation therapies

Eletroquimioterapia no tratamento de câncer: campos elétricos, eletrodos e modelos de tecidos (ex vivo, in vivo e in silico)

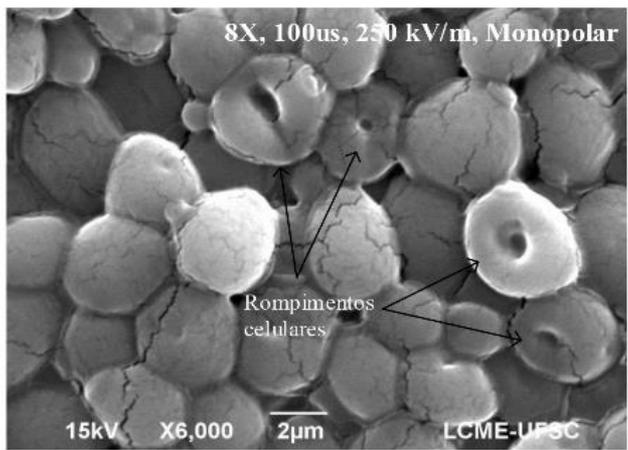
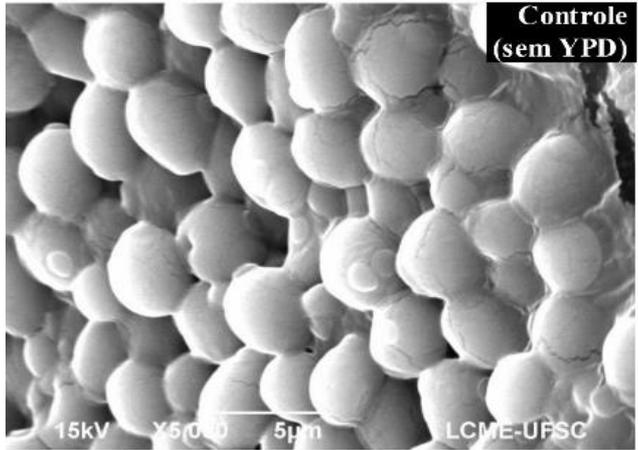
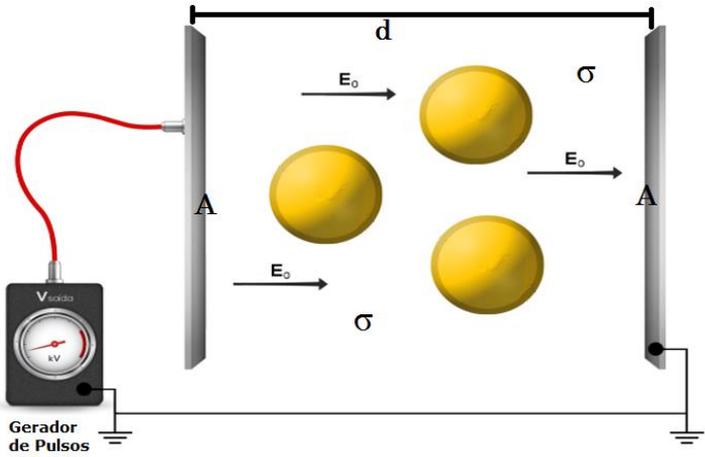
Modelo em nível de tecido



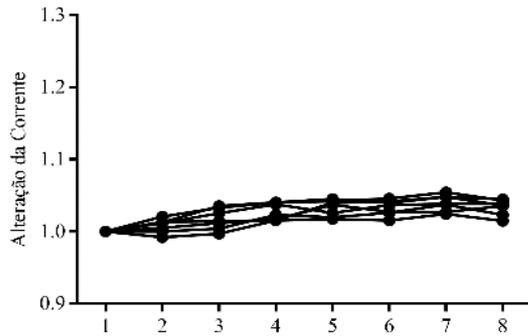
Estudo In Silico Do Uso De Modelo Tridimensional Realista Via Tomografia Computadorizada Da Eletroquimioterapia No Tratamento De Tumor Oral



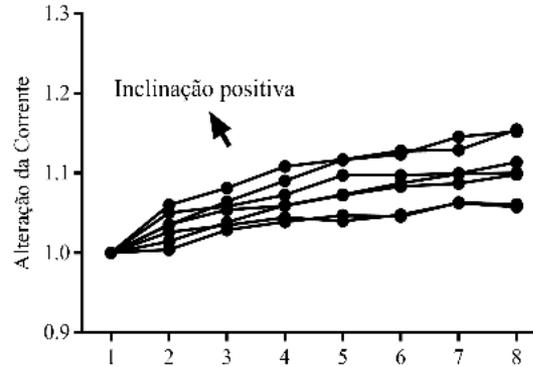
Leveduras



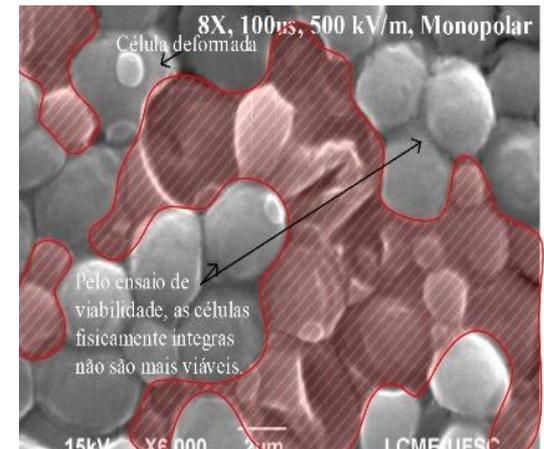
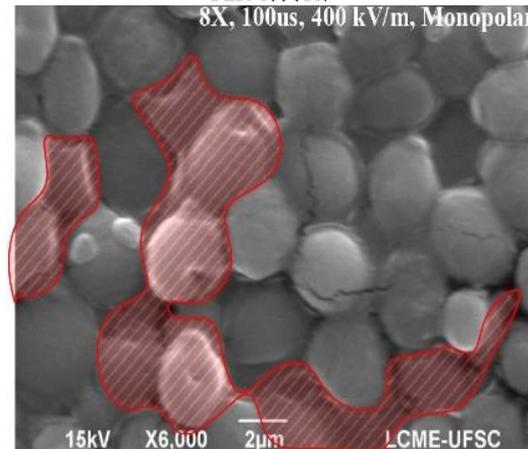
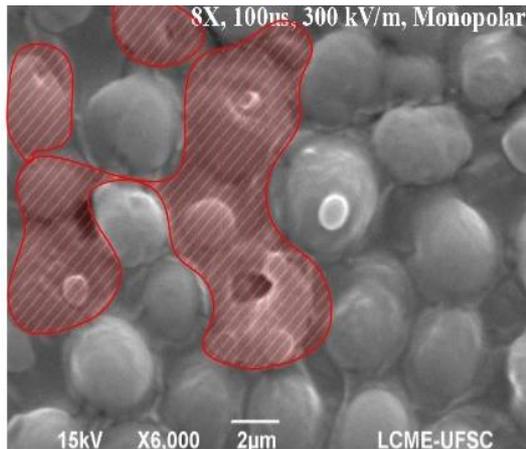
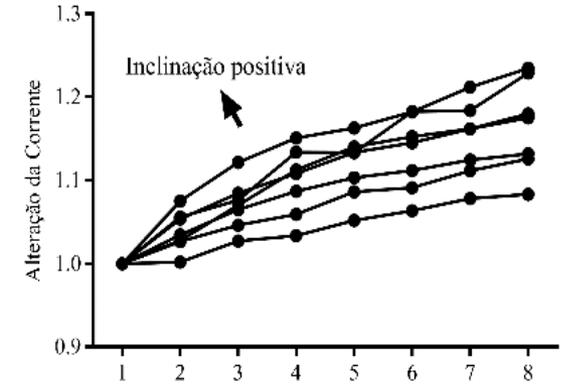
LEV 300 kV/m



LEV 400 kV/m



LEV 500 kV/m



Aplicação





PÓS-GRADUAÇÃO
ENGENHARIA ELÉTRICA
FLORIANÓPOLIS | SANTA CATARINA



Membranas Celulares: Modelagem e Leitura de Potenciais Elétricos

Profa. Daniela O. H. Suzuki
Guilherme B. Pintarelli