

# Misure di attività elettrica in cellule viventi



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## Perché elettricità?

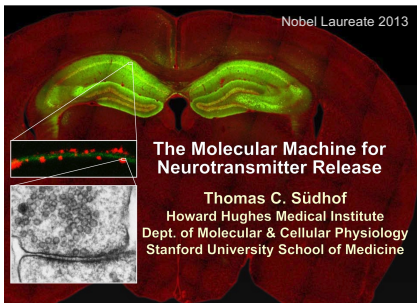
MINISTERO DELLA SALUTE, DECRETO 11 aprile 2008  
Aggiornamento del decreto 22 agosto 1994, n. 582 relativo al: «Regolamento recante le modalità per l'accertamento e la certificazione di morte». (GU Serie Generale n.136 del 12-06-2008)

**Art. 1. Accertamento della morte e arresto cardiaco**  
In conformità all'art. 2, comma 1, della legge 29/12/1993, n. 578, l'accertamento della morte per arresto cardiaco può essere effettuato dal medico con il rilievo continuo dell'elettrocardiogramma protratto per un periodo di almeno 20 minuti primi, registrato su supporto cartaceo o digitale

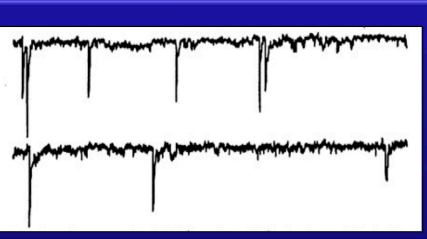
**Art. 2. Requisiti clinici per l'accertamento della morte nei soggetti affetti da lesioni encefaliche sottoposti a trattamento rianimatorio**  
1. Nei soggetti con lesioni encefaliche sottoposti a trattamento rianimatorio, salvo i casi particolari di cui all'art. 2, comma 2, le condizioni che, ai sensi della legge 29/12/1993, n. 578, art. 3, impongono al medico della struttura sanitaria di dare immediata comunicazione alla Direzione sanitaria dell'esistenza di un caso di morte per cessazione irreversibile di tutte le funzioni dell'encefalo, sono:  
a) assenza dello stato di vigilanza e di coscienza, dei riflessi del tronco encefalico e del respiro spontaneo;  
b) assenza di attività elettrica cerebrale; c) assenza di flusso ematico encefalico, .....

*elettricità = vita*

# Misure di attività elettrica in cellule viventi

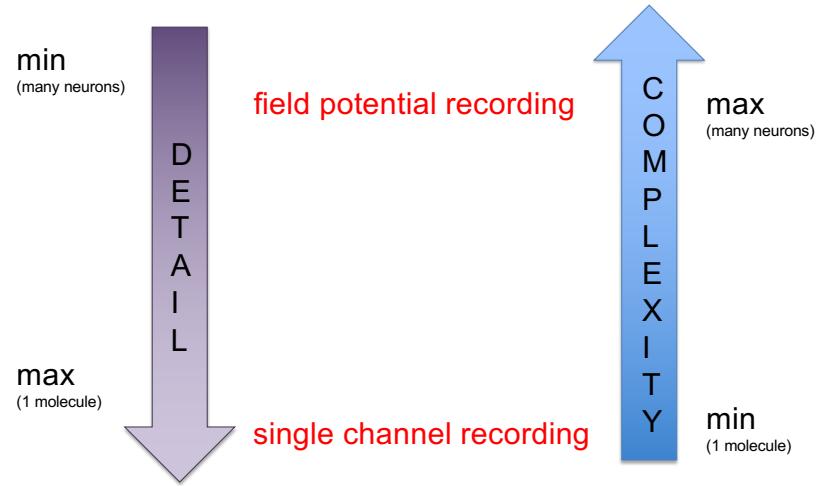


**Approach:**  
Synaptic function is measured electrophysiologically via excitatory or inhibitory postsynaptic currents (EPSCs or IPSCs)



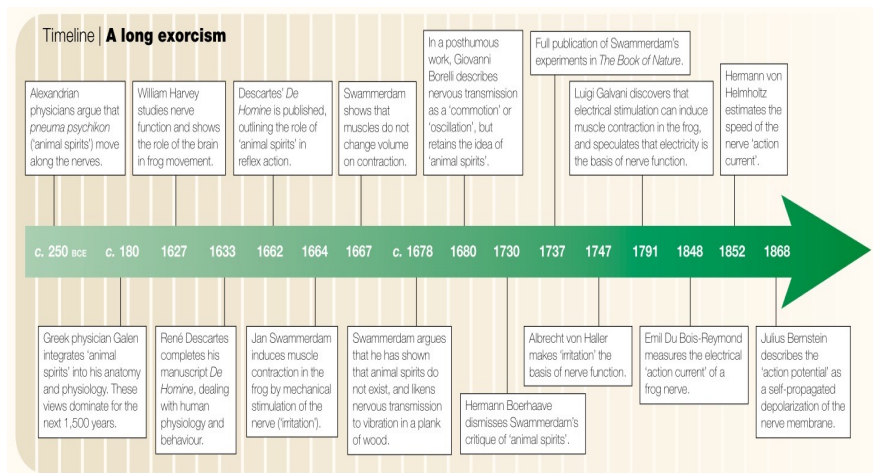
Notes by Francesca Grassi for the students of IMS/ CLM Medicina e Chirurgia Sede S. Andrea at Sapienza University, Rome. Reproduction not allowed

# Misure di attività elettrica in cellule viventi



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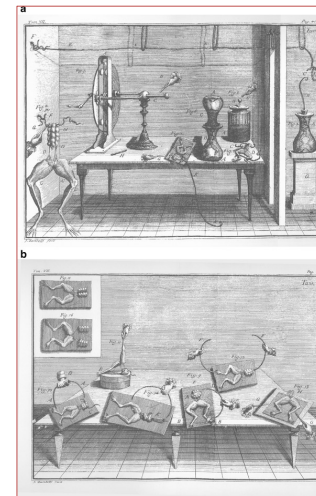
# Breve storia dell' elettrofisiologia



Cobb M., Nature Reviews Neuroscience, 2002

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# Breve storia dell' elettrofisiologia



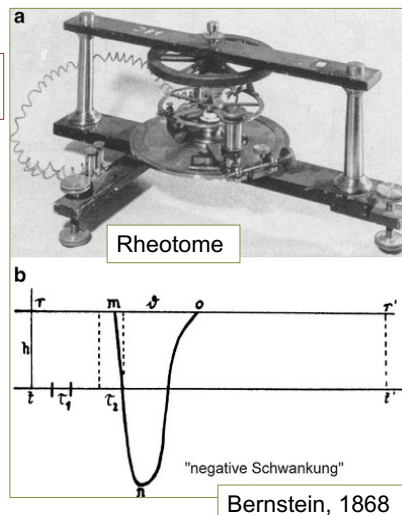
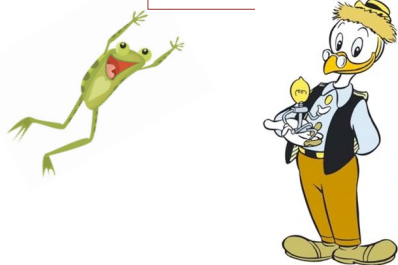
Galvani, 1794

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# Breve storia dell' elettrofisiologia

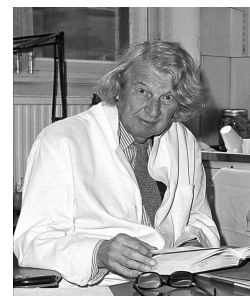
Cell membrane

Thermionic vacuum

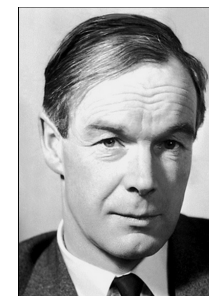


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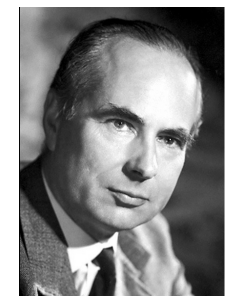
# L'assone gigante del calamaro



J.Z. Young, scopre l' assone gigante del calamaro e la corrispondente sinapsi gigante (1930-40)

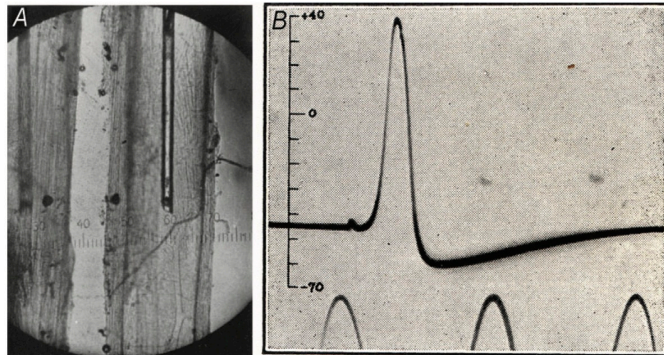


Hodgkin (a sinistra) e Huxley (a destra), premi Nobel nel 1963 per " le loro scoperte riguardanti i meccanismi ionici coinvolti in eccitazione e inibizione delle porzioni periferiche e centrali delle membrane delle cellule nervose"



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# L'assone gigante del calamaro

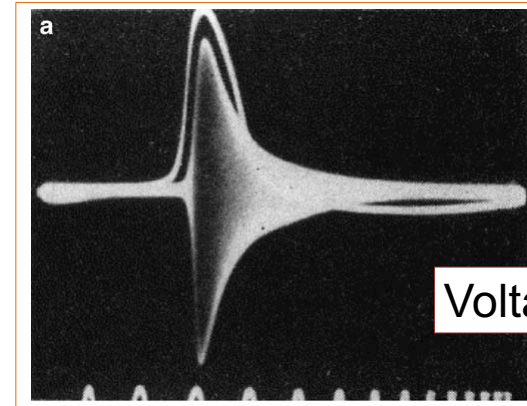


Membrane potential

**Figure 3. Intracellular recording of the squid giant axon action potential**  
 A, photomicrograph of an electrode inside a squid giant axon (diameter ~500 μm). Two views of the same axon are visible from an ingenious system of mirrors devised by Huxley. This allowed simultaneous viewing of the electrode from both front and side and was essential to avoid the electrode damaging the nerve membrane as it was threaded down the axon. Image taken from Hodgkin & Huxley (1945). B, the first intracellular recording of an action potential. The sine wave time marker has a frequency of 500 Hz. Reproduced from Hodgkin & Huxley (1939) by permission from Macmillan Publishers Ltd: *Nature* ©1939.

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# Breve storia dell' elettrofisiologia



Action potential & Conductance increase

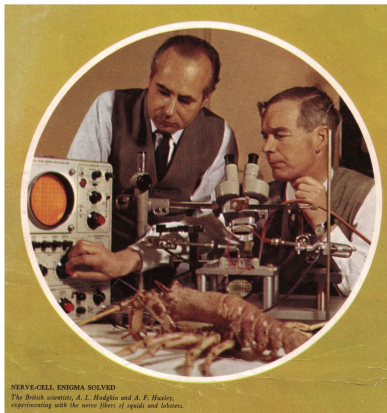
Voltage clamp

Cole and Curtis, 1939



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# Breve storia dell' elettrofisiologia



J. Physiol. (1952) 117, 500-544

A QUANTITATIVE DESCRIPTION OF MEMBRANE CURRENT AND ITS APPLICATION TO CONDUCTION AND EXCITATION IN NERVE

By A. L. HODGKIN AND A. F. HUXLEY

From the Physiological Laboratory, University of Cambridge

Ion channels



1970s

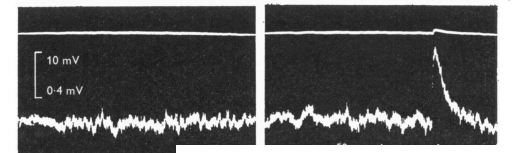
**Figure 1. The cover of the 1963 Nobel Prize Programme**  
 Huxley, left, looks on as Hodgkin adjusts a brand-new Tektronix 502A oscilloscope. Together with John Carew Eccles (27 January 1903-2 May 1997), Andrew Fielding Huxley (22 November 1917) and Alan Lloyd Hodgkin (5 February 1914-20 December 1998) won the 1963 Nobel Prize in Physiology or Medicine 'for their discoveries concerning the ionic mechanisms involved in excitation and inhibition in the peripheral and central portions of the nerve cell membrane'. Programme provided by Deborah Hodgkin.

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DOI: 10.1113/jphysiol.2012.230458

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# Breve storia dell' elettrofisiologia



"ACh noise" or "endplate noise" used to estimate single channel conductance and open time

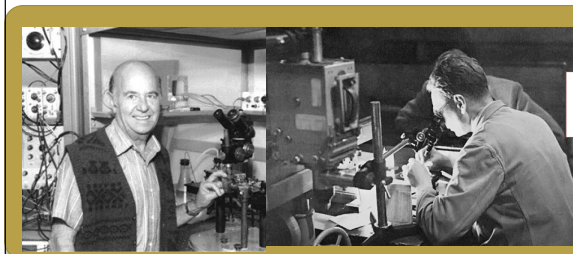
J. Physiol. (1973), 224, pp. 665-699  
 With 16 text-figures  
 Printed in Great Britain

665

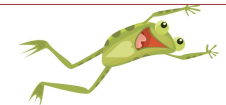
THE STATISTICAL NATURE OF THE ACETYLCHOLINE POTENTIAL AND ITS MOLECULAR COMPONENTS

By B. KATZ AND R. MILEDI

From the Department of Biophysics, University College London, Gower Street, London WC1E6BT

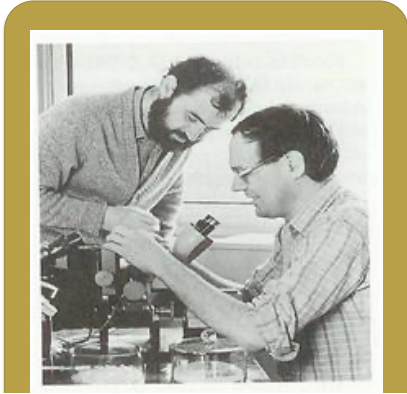


Indirect approach



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# Breve storia dell' elettrofisiologia

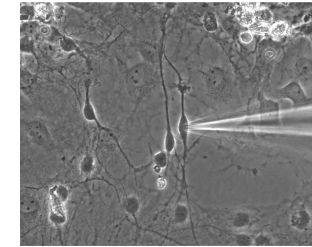


Single channel recording

Erwin Neher and Bert Sakmann  
 "for their discoveries concerning the function of single ion channels in cells" Nobel Prize motivation, 1991

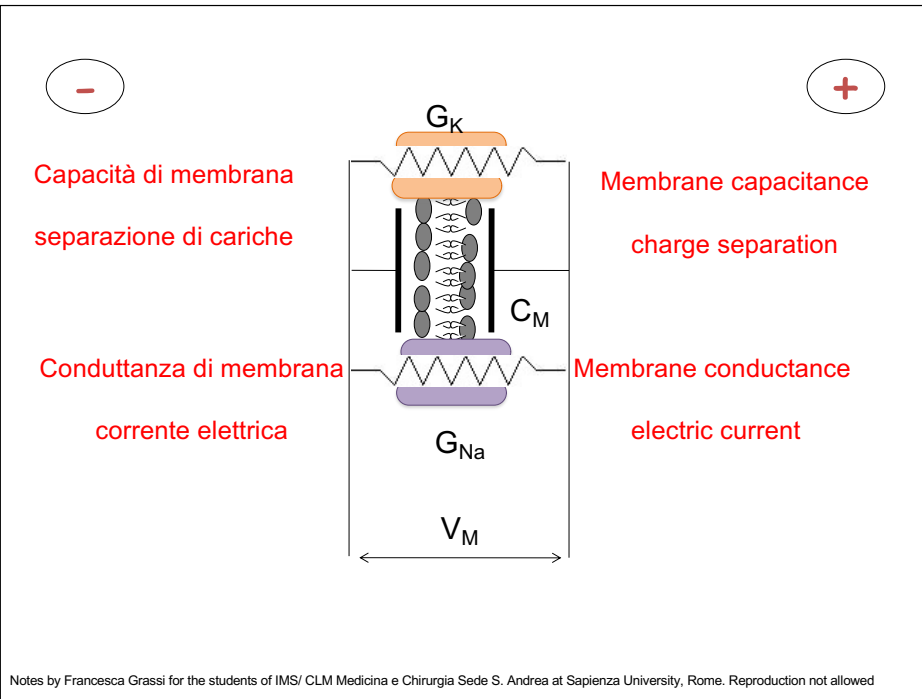
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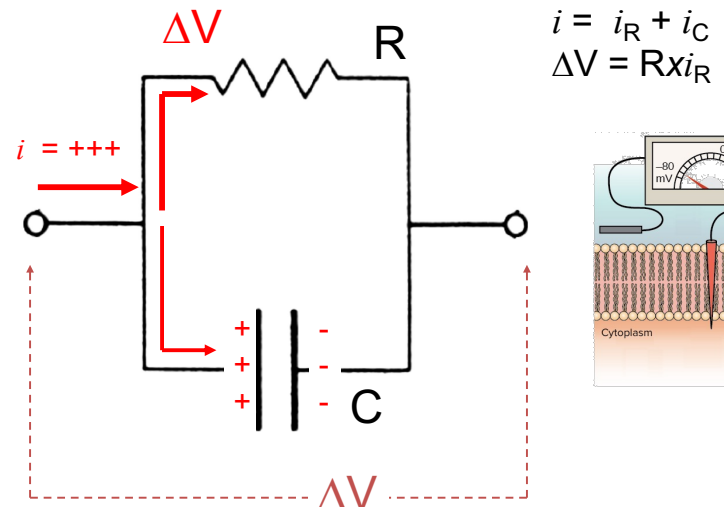
Voltage clamp

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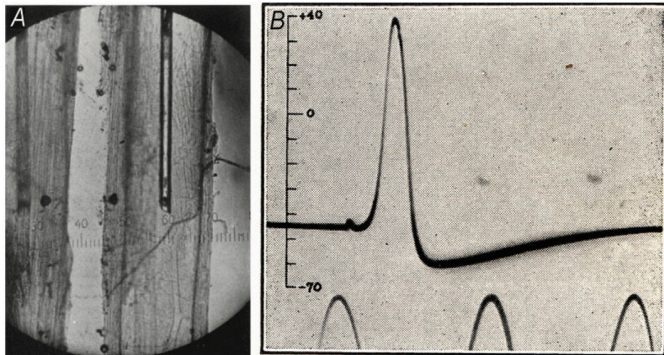
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# Misure di differenza di potenziale



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## Assone gigante di calamaro



Membrane potential

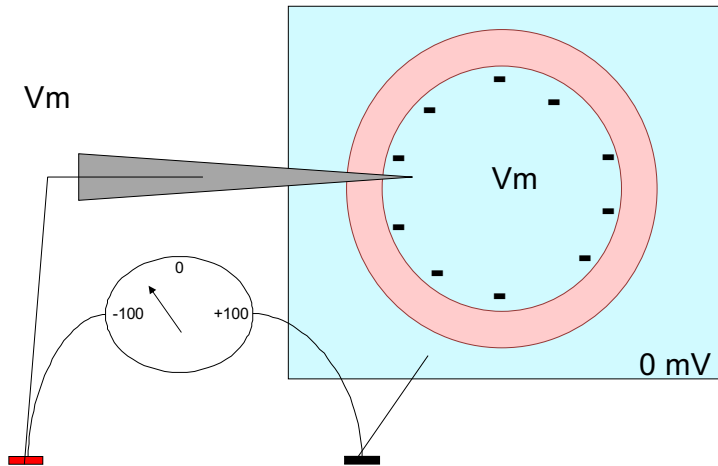
Figure 3. Intracellular recording of the squid giant axon action potential

A, photomicrograph of an electrode inside a squid giant axon (diameter  $\sim 500 \mu\text{m}$ ). Two views of the same axon are visible from an ingenious system of mirrors devised by Huxley. This allowed simultaneous viewing of the electrode from both front and side and was essential to avoid the electrode damaging the nerve membrane as it was threaded down the axon. Image taken from Hodgkin & Huxley (1945). B, the first intracellular recording of an action potential. The sine wave time marker has a frequency of 500 Hz. Reproduced from Hodgkin & Huxley (1939) by permission from Macmillan Publishers Ltd. *Nature* ©1939.

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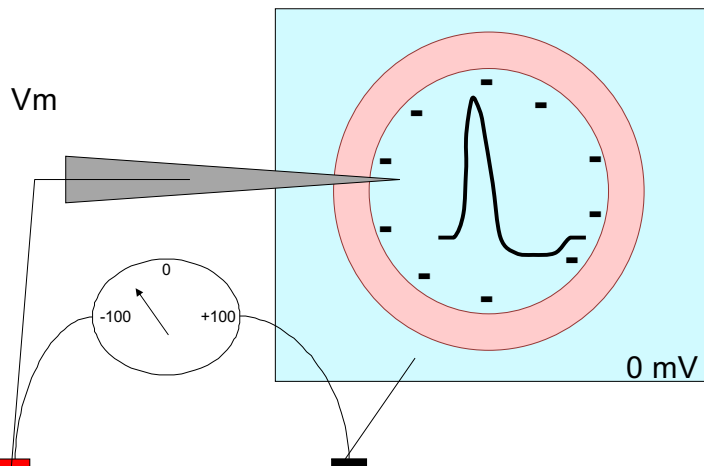
## Misure di differenza di potenziale

Voltmetro



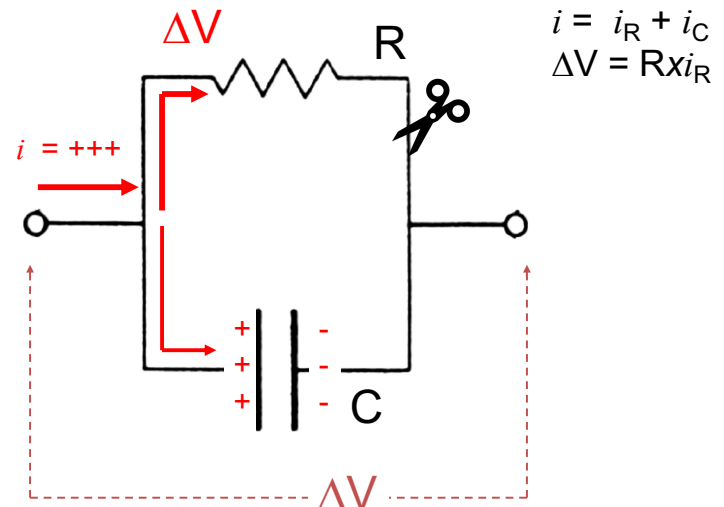
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## Misure di differenza di potenziale



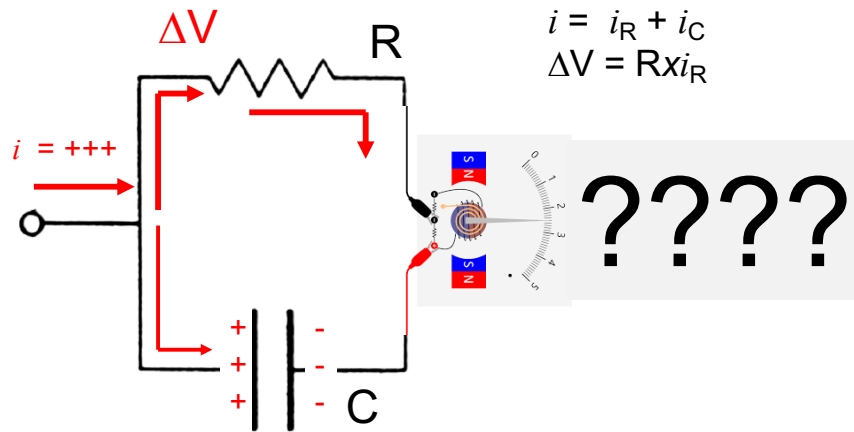
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## Misure di corrente



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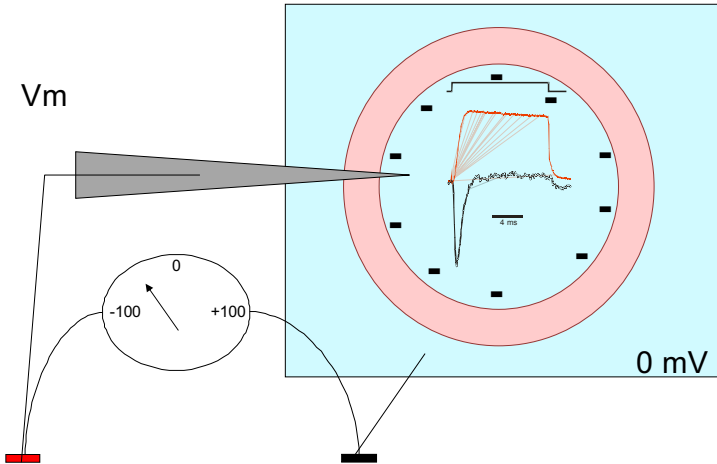
## Misure di corrente



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## Misure di corrente

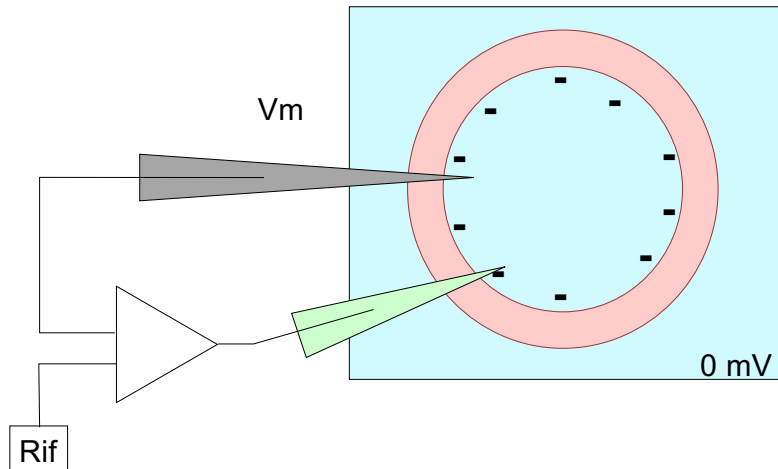
E allora?



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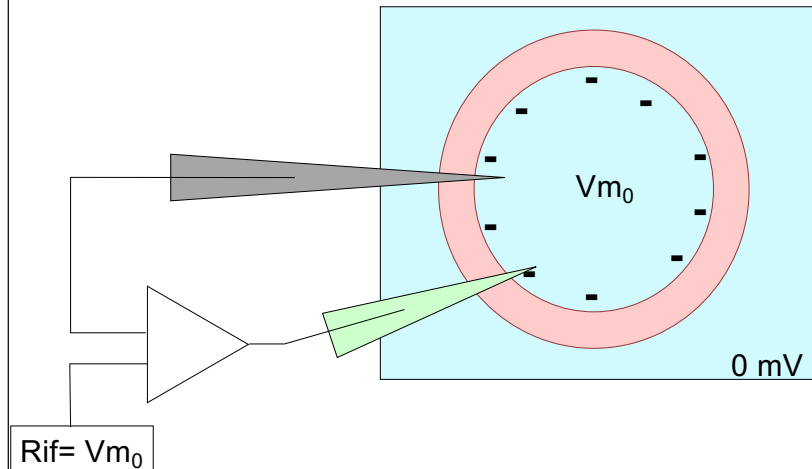
## Misure di corrente

Voltage clamp (= blocco del potenziale)



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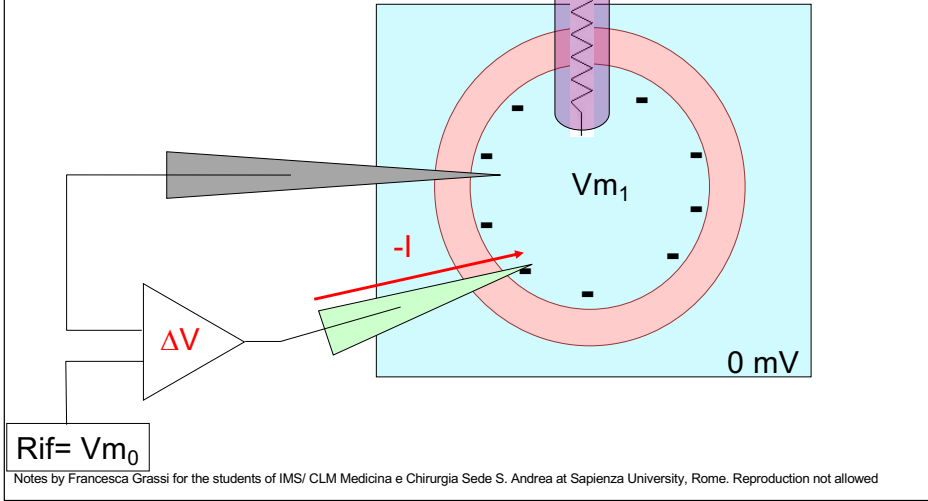
## Voltage clamp



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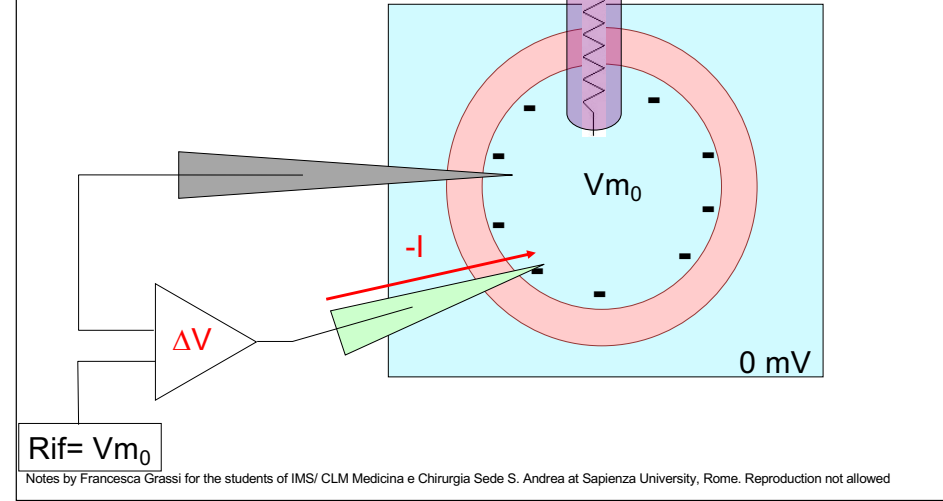
# Voltage clamp

$$\Delta V = Vm_1 - Vm_0 = I \cdot R$$



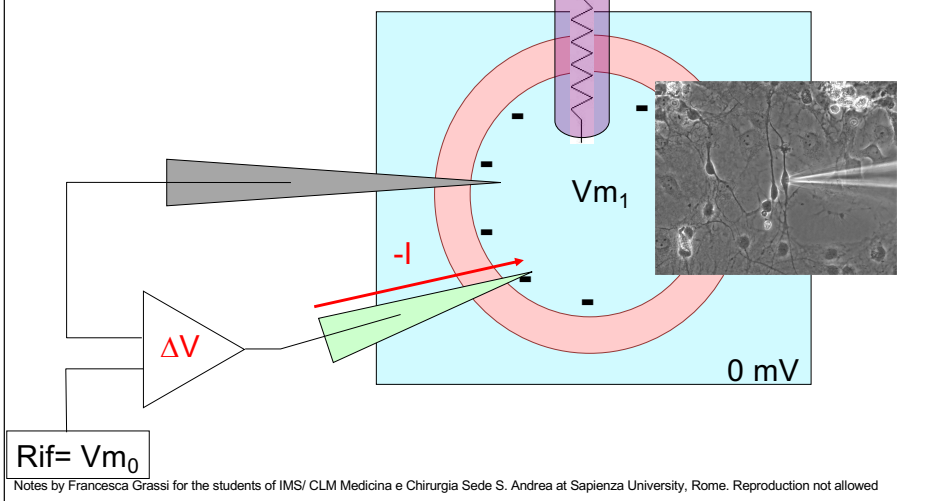
# Voltage clamp

$$\Delta V = (I - I) \cdot R = 0mV$$

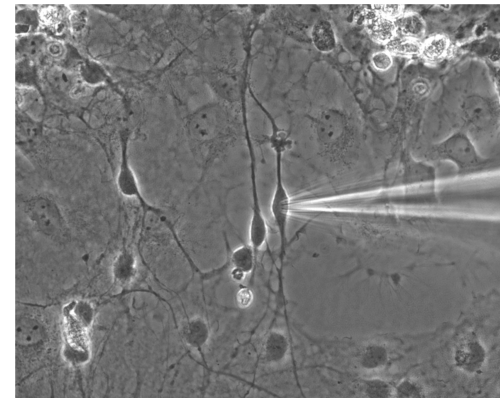


# Voltage clamp

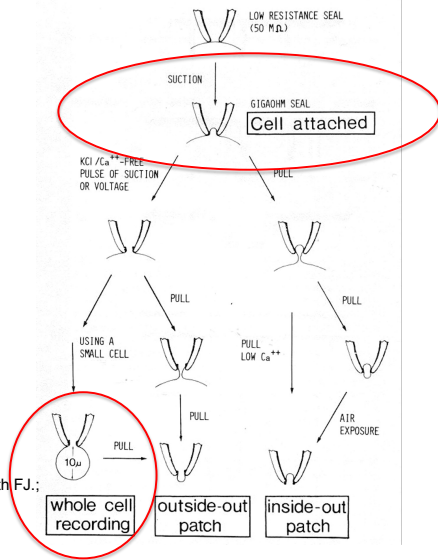
Problema!!!!



# Patch clamp



# Patch clamp: varie configurazioni



Hamill OP, Marty A, Neher E, Sakmann B, Sigworth FJ.; Pflugers Arch. 1981 Aug;391(2):85-100.

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# Patch clamp: varie configurazioni

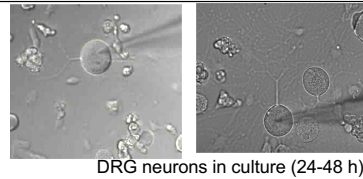
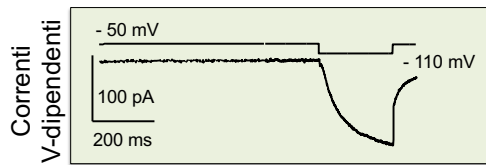


Cell attached:  
correnti di singolo canale

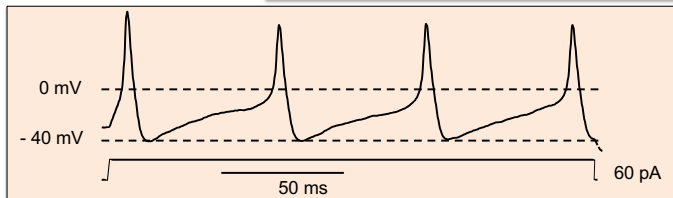
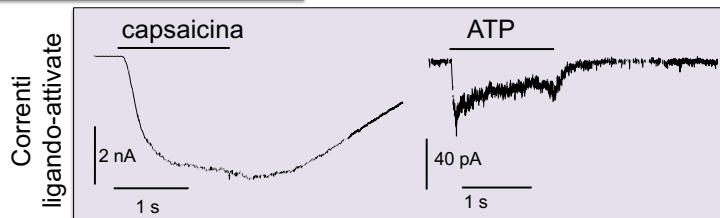
Whole Cell:  
correnti totali con 1 elettrodo

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# Whole cell



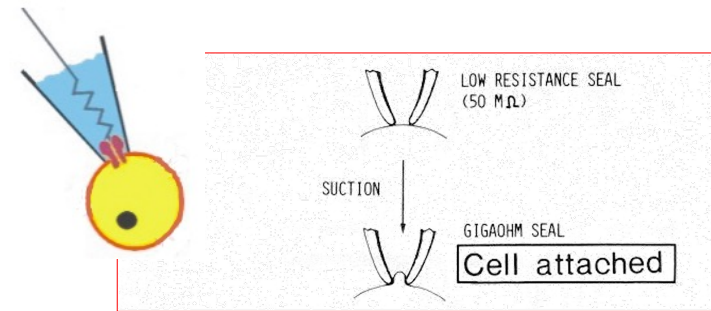
DRG neurons in culture (24-48 h)



Potenziali d'azione (current clamp)

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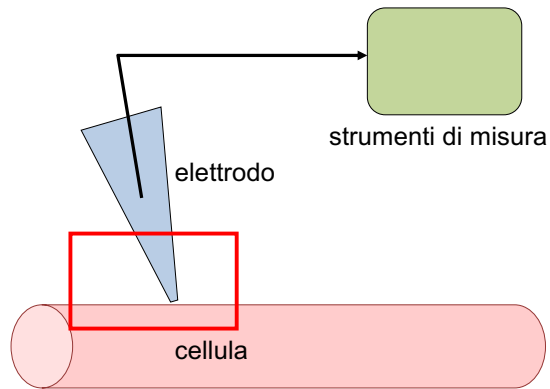
# Cell attached



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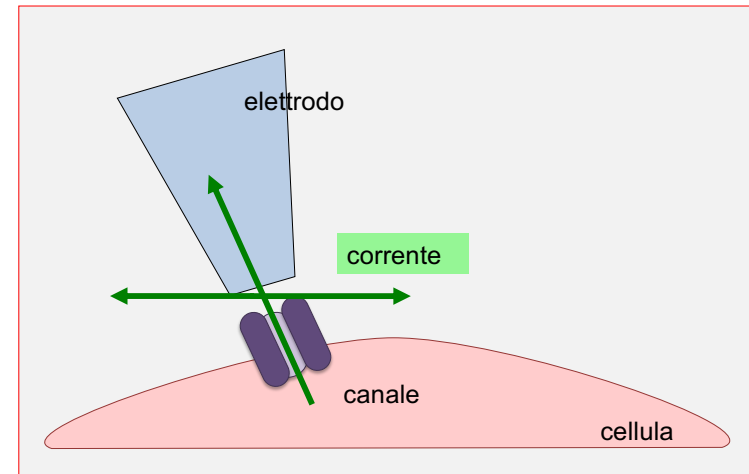


# Correnti di singolo canale



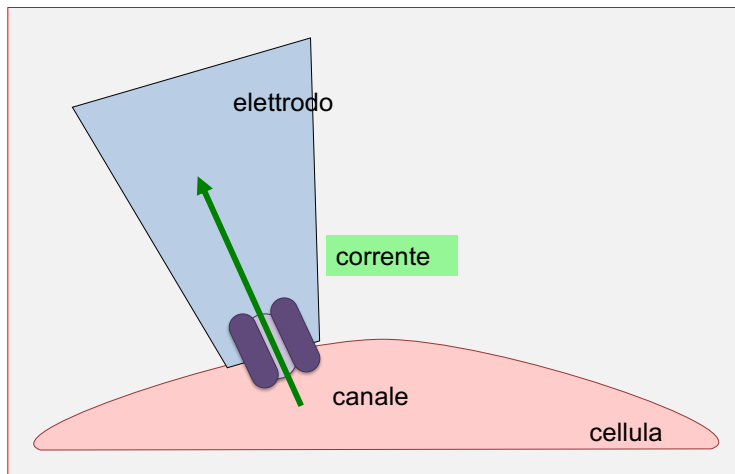
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# Correnti di singolo canale



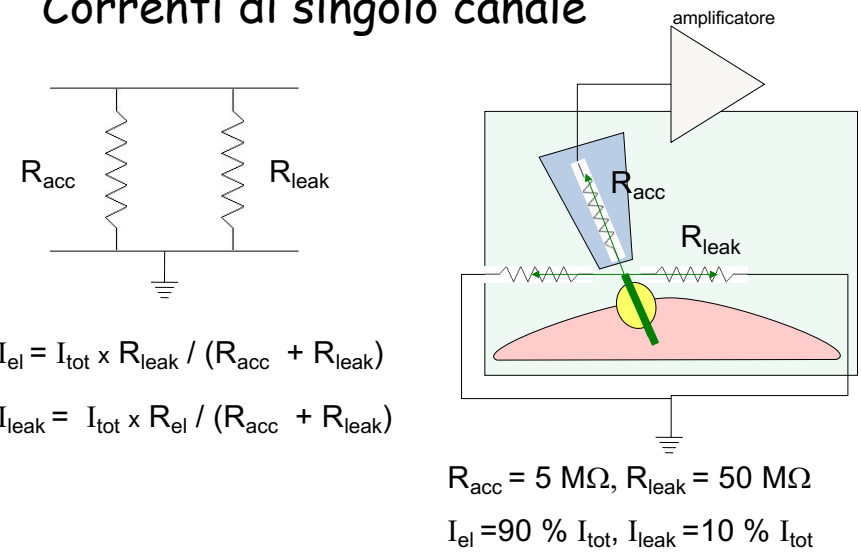
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# Correnti di singolo canale



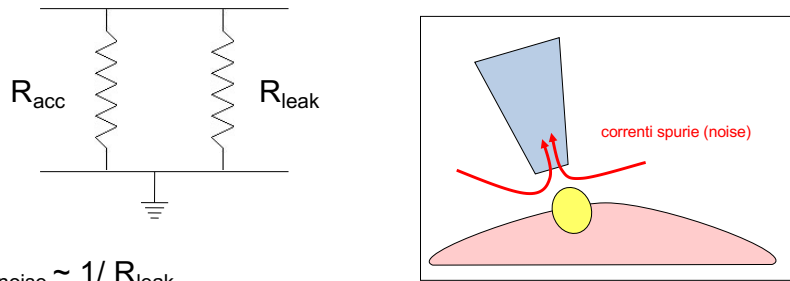
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# Correnti di singolo canale



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# Correnti di singolo canale

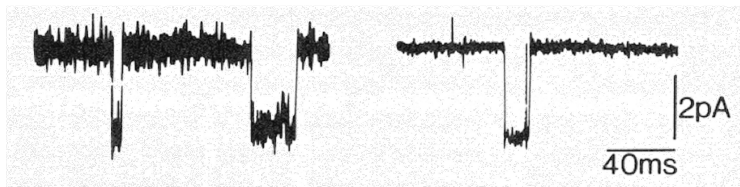
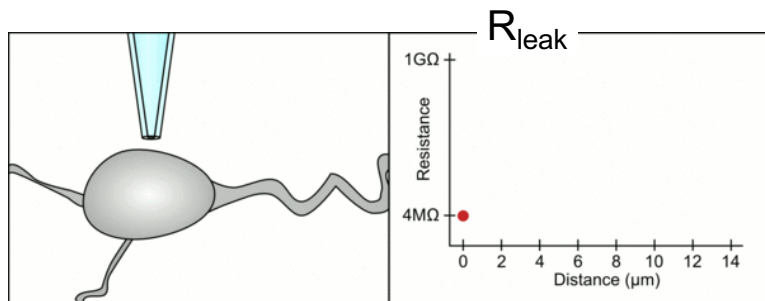
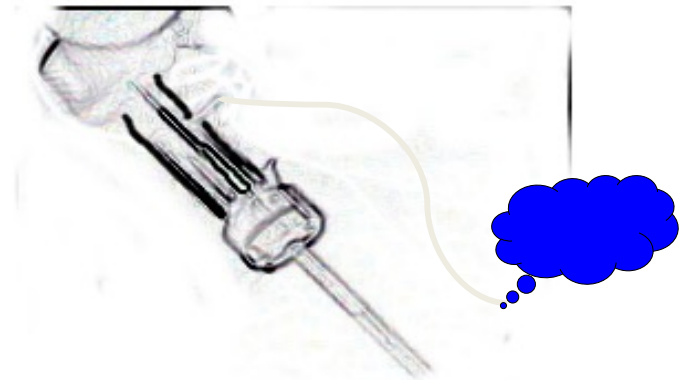


$$I_{\text{noise}} \sim 1/R_{\text{leak}}$$

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# La pressione negativa

Una pressione negativa induce una saldatura ("seal") tra elettrodo e membrana

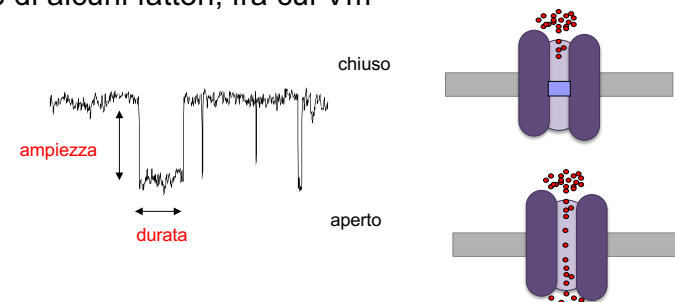


# Parametri caratterizzanti le correnti di singolo canale

**Ampiezza:** dipende dalla conduttanza ( $\gamma$ , in pS) del canale e da  $V_m$  secondo la **legge di Ohm:**

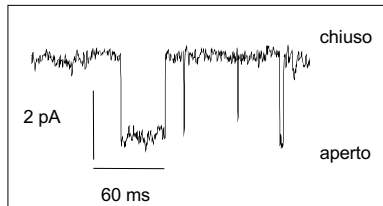
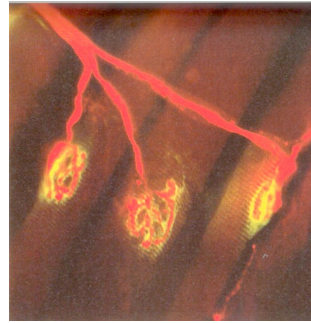
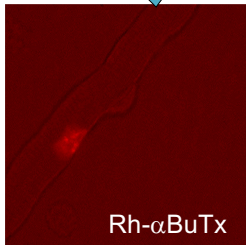
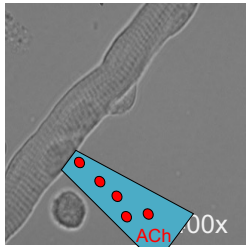
$$i = \gamma (V_m - V_0)$$

**Durata:** riflette proprietà intrinseche del canale e varia in funzione di alcuni fattori, fra cui  $V_m$



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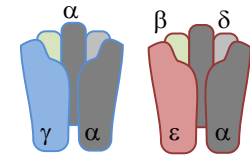
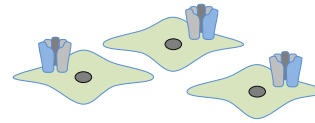
# Giunzione neuromuscolare



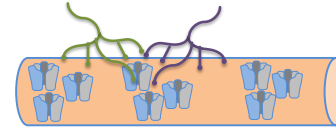
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# AChR

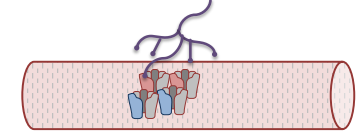
differentiating myoblasts



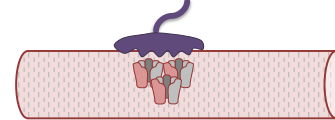
developing muscle



developing NMJ



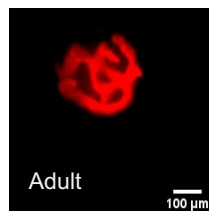
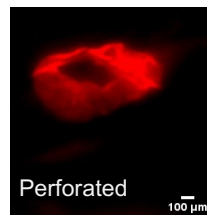
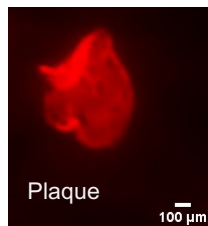
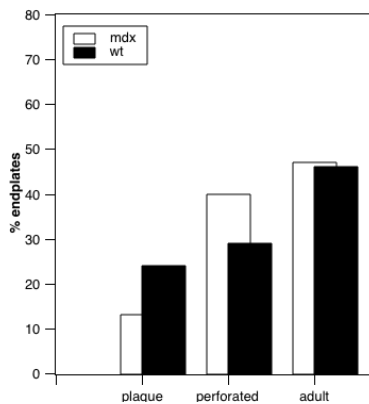
mature NMJ



Tra la seconda e la terza settimana postnatale nel topo; intorno alla 30<sup>a</sup> settimana gestazionale nell'uomo

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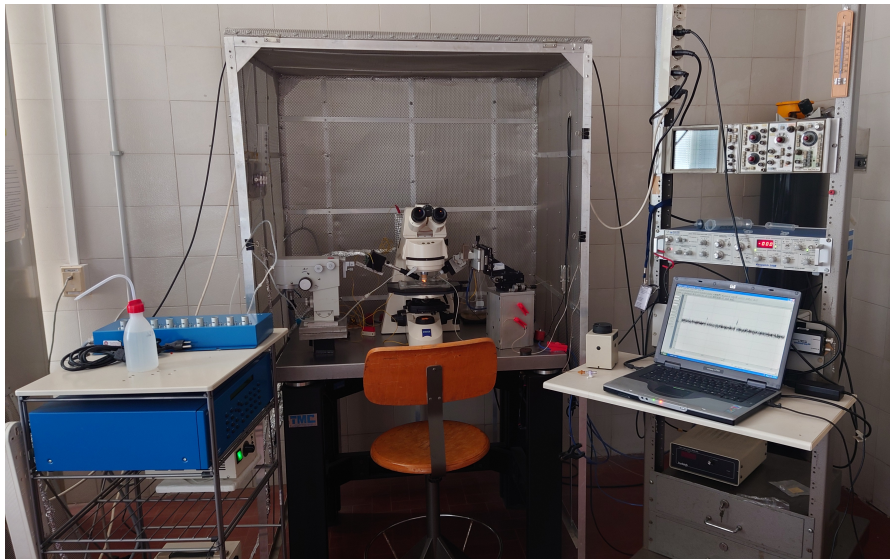
# NMJ @ P14



# NMJ @ P14

E come funziona?

## il lab

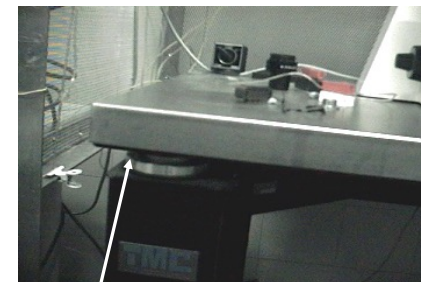


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## Gabbia di Faraday



## Tavolo Antivibrazioni

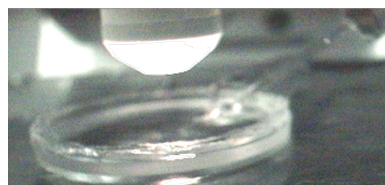
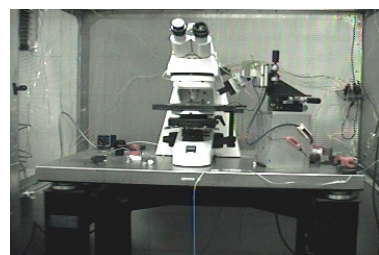


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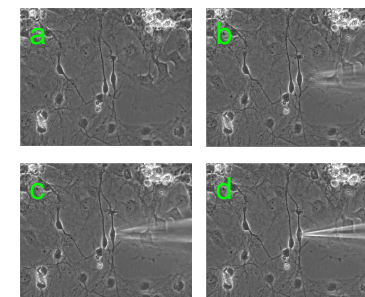
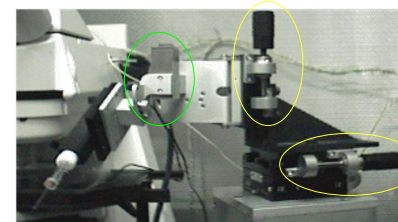
## Micromanipolatore



## Microscopio



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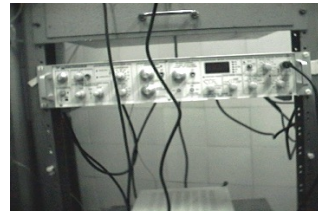


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## L'amplificatore



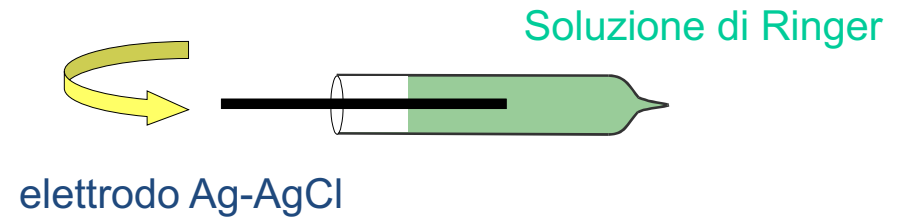
Stadio 1 (sonda)



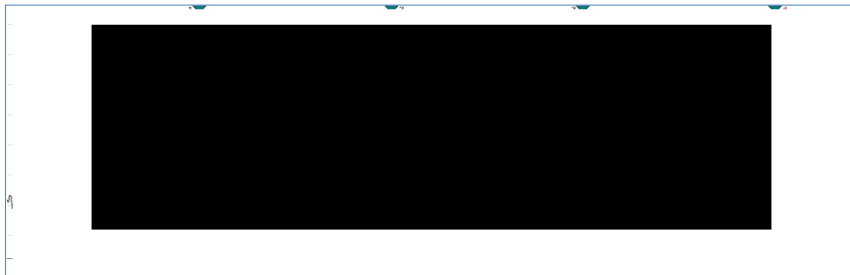
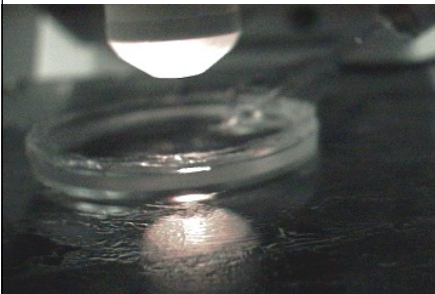
Stadio 2

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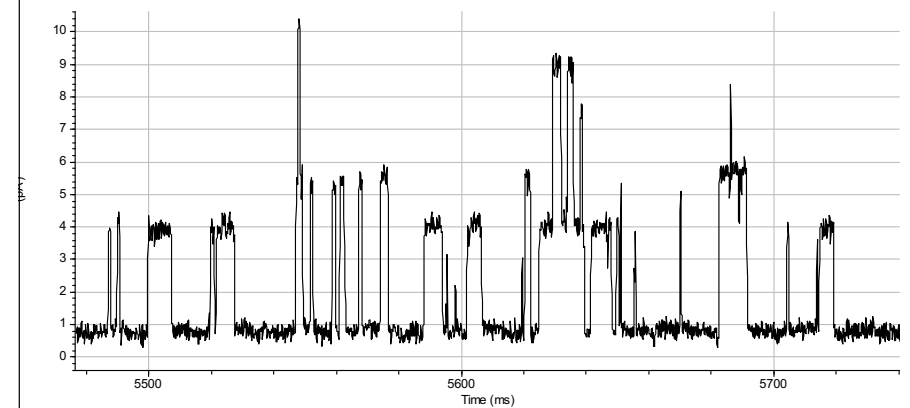
## Come è fatto un microelettrodo

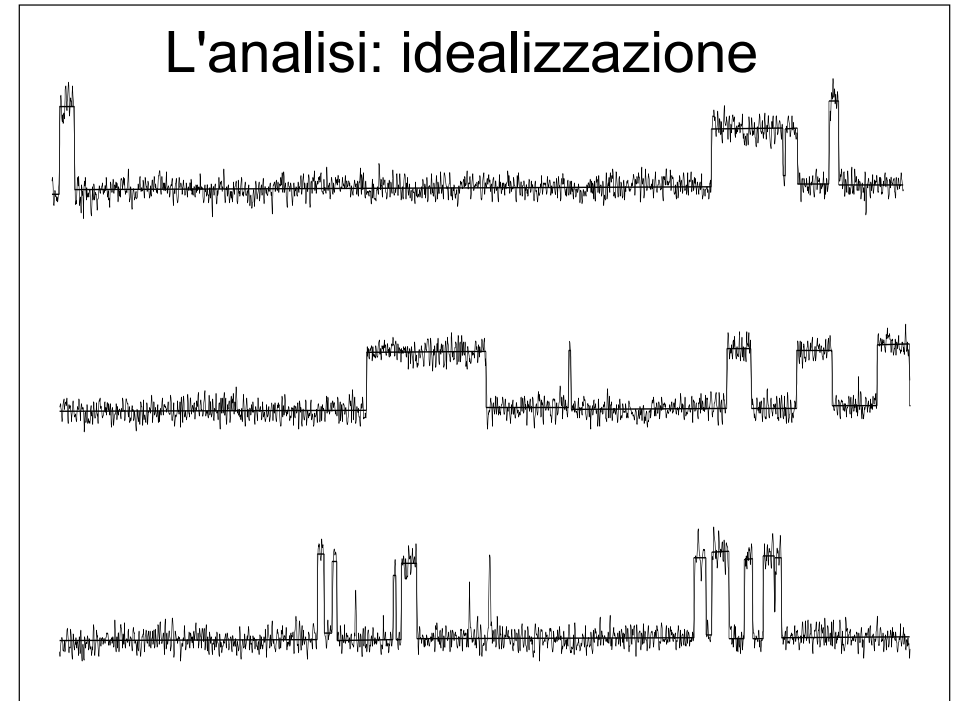
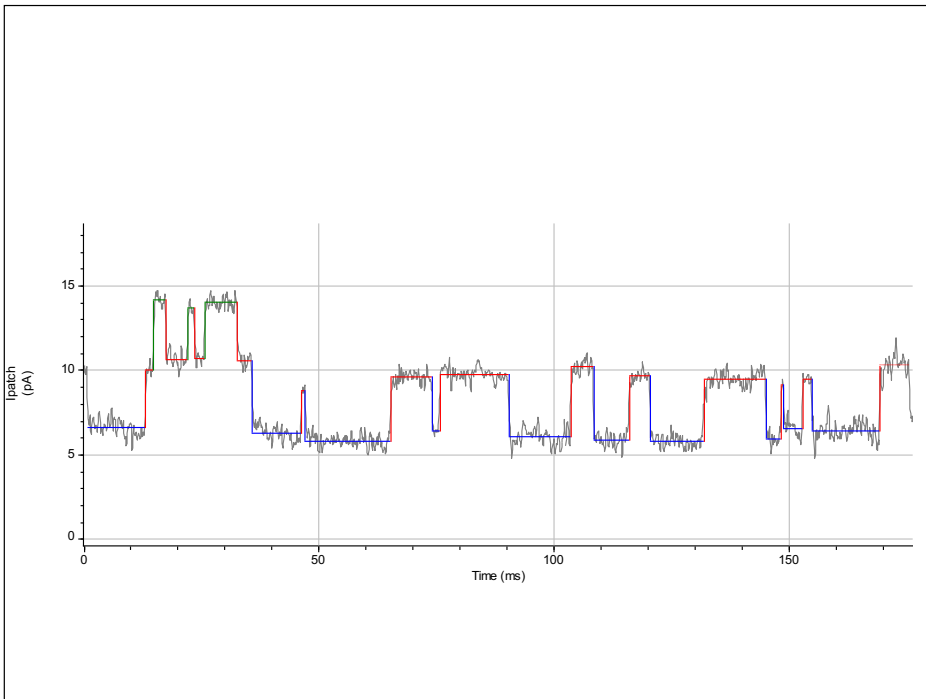
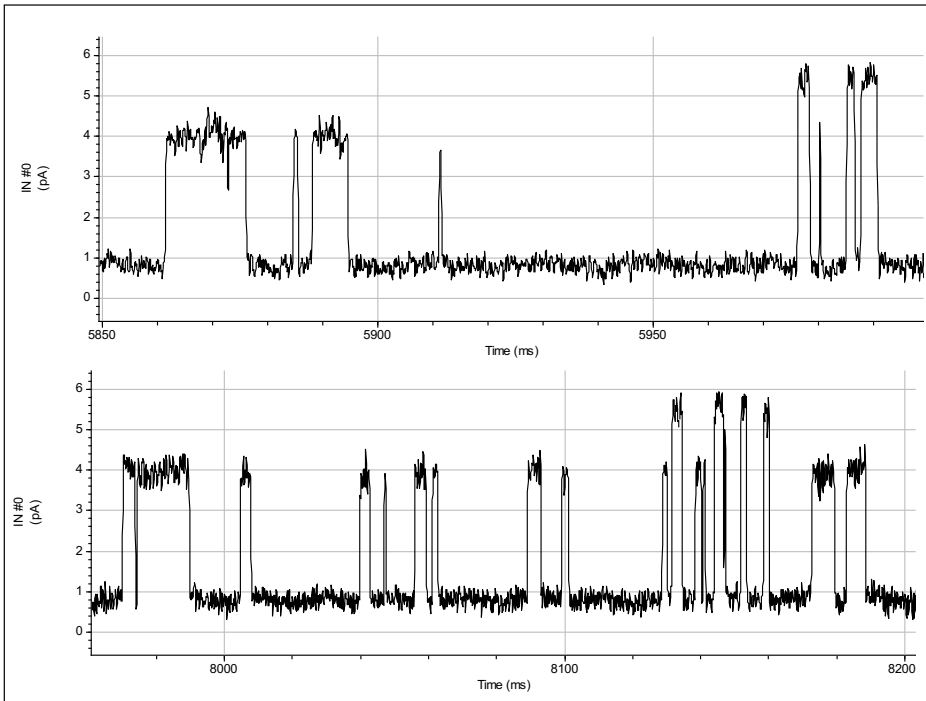


## muscolo scheletrico



## I dati: muscolo di topo P14

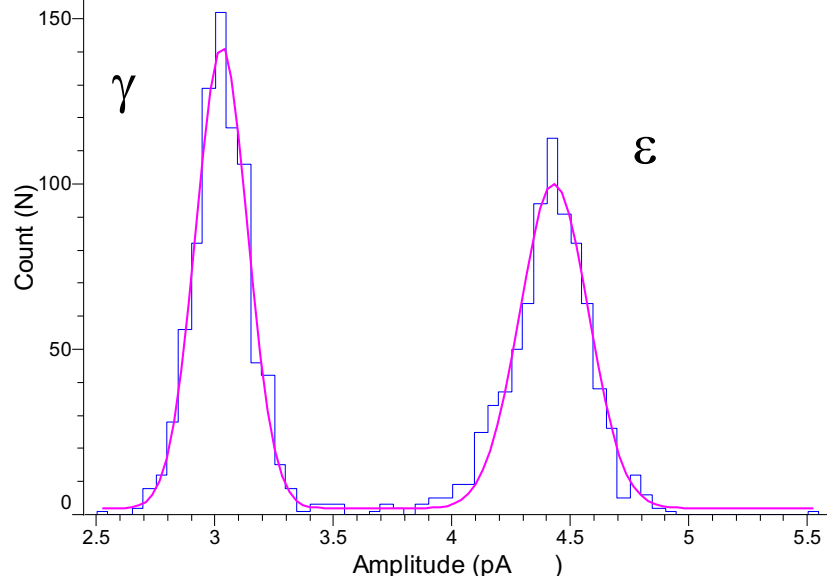




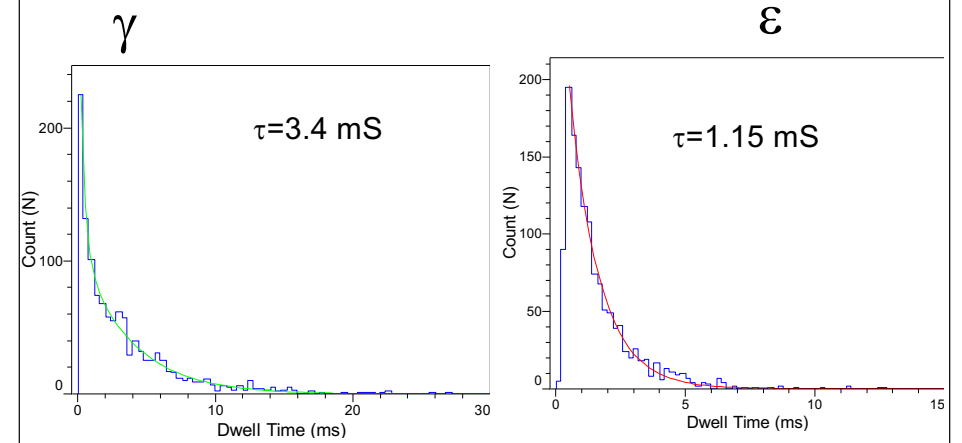
## L'analisi: lista degli eventi

Level	Event	Start	Event End	Ti	Amplitude (	Amp S.D. (p	Dwell Time	Inst. Freq. (
0	0.000	23.720	-0.01637	0.81937	23.720	N/A		
1	23.760	24.160	3.82924	0.60533	0.400	N/A		
0	24.160	25.960	0.07956	0.85250	1.800	41.39073		
1	25.960	27.200	4.00448	0.68911	1.240	454.54568		
0	27.200	44.880	0.01128	0.80750	17.680	328.94727		
1	44.920	45.080	-3.21125	0.55025	0.160	52.74262		
0	45.080	374.120	-0.33266	1.22187	329.040	55.92841		
1	374.760	375.160	3.69576	0.67444	0.400	3.03177		
0	375.160	375.240	0.55625	1.23625	0.080	3.02957		
1	375.240	376.320	4.16298	0.47264	1.080	2083.41821		
0	376.320	396.200	-0.06434	1.13125	19.880	862.06622		
1	396.240	397.240	4.22310	0.58946	1.000	47.61905		
0	397.240	398.640	0.04665	1.14812	1.400	47.80119		
1	398.640	399.160	3.45062	0.75509	0.520	416.66241		
0	399.160	407.000	-0.06364	0.93875	7.840	520.82971		
1	407.000	408.520	4.42959	0.62503	1.520	119.61743		
0	408.520	420.680	0.03177	0.87375	12.160	106.83778		
1	420.720	421.200	4.17892	1.03711	0.480	72.88629		
0	421.200	422.160	0.00948	0.88063	0.960	78.86421		
1	422.160	423.640	4.35113	0.48691	1.480	694.44324		
0	423.640	424.480	0.05372	0.89687	0.840	409.83566		

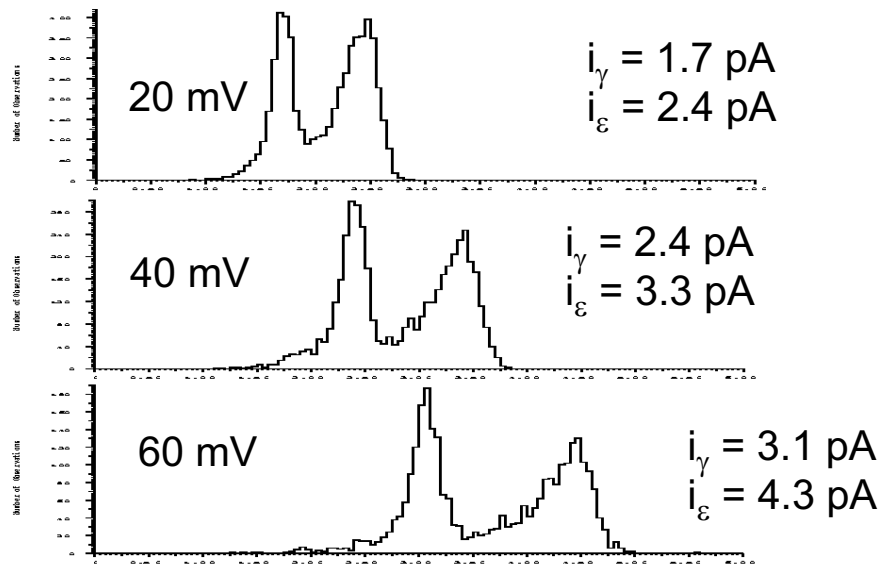
## Gli istogrammi: ampiezza



## Gli istogrammi: open time



## La curva corrente-voltaggio



## La curva corrente-voltaggio

