Equivalent electrical circuit

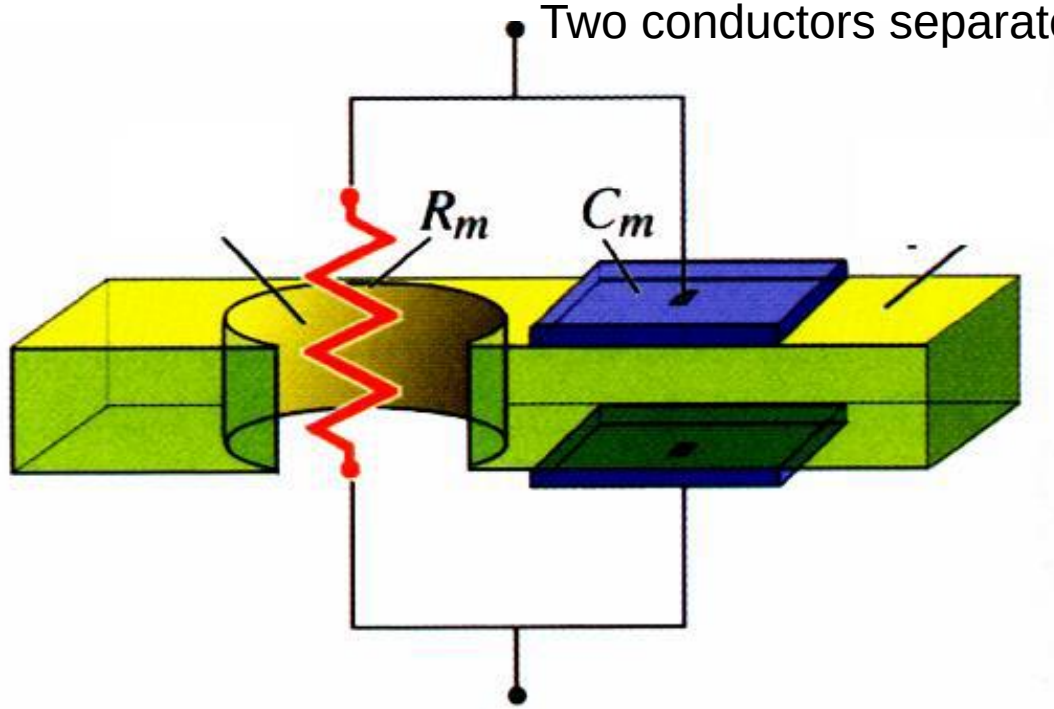
The membrane can be considered a capacitor accumulating opposite charges inside and outside the cell

$Cm = Q / V$

$I = dQ / dt = CdV / dt$

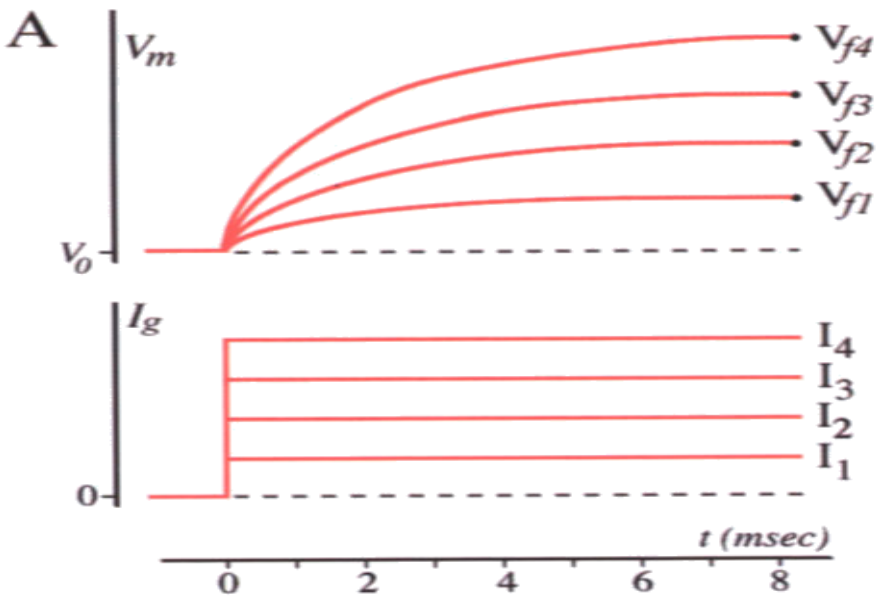
$dV = R\,I \qquad I = dV/R$

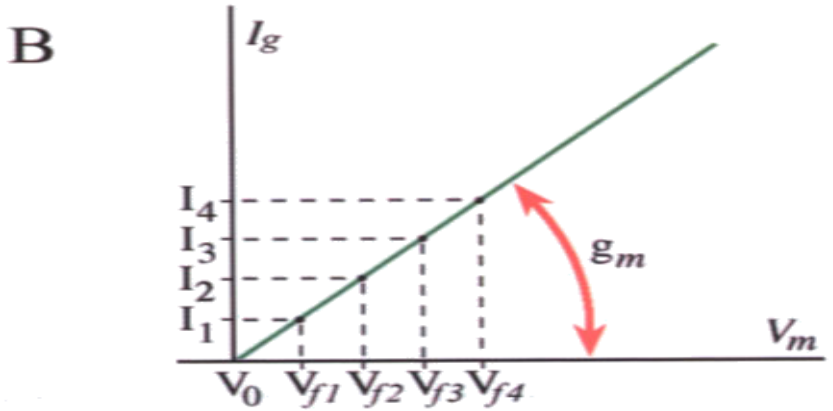
the higher the membrane resistance, the lower the current required to maintain a given Vm



The membrane is indicated as a parallel resistance (R_m) and capacitance (C_m).
R_m or conductance ($G = 1/R_m$) results from the ion channels, proteins that reduce R_m.
C_m results from the lipid bilayer matrix

Passive variation of V_m





Ohm Low

$\Delta Vm = R\ I$

$R=V / I$

G = 1 / R

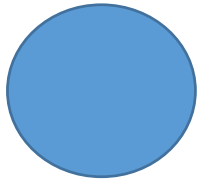
G = I/V is constant,
it does not depend on Vm!!!

Taglietti, Casella, Principi di fisiologia e biofisica della cellula

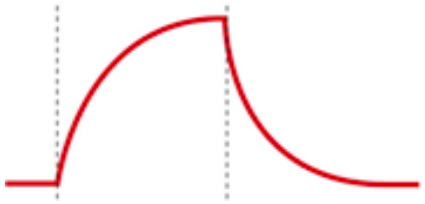
R_m or conductance ($G = 1/R_m$) results from the ion channels,

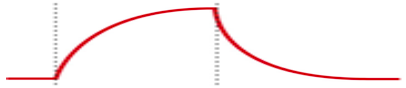
more ion channels, lower R_m.

Small cells big resistance



voltage response

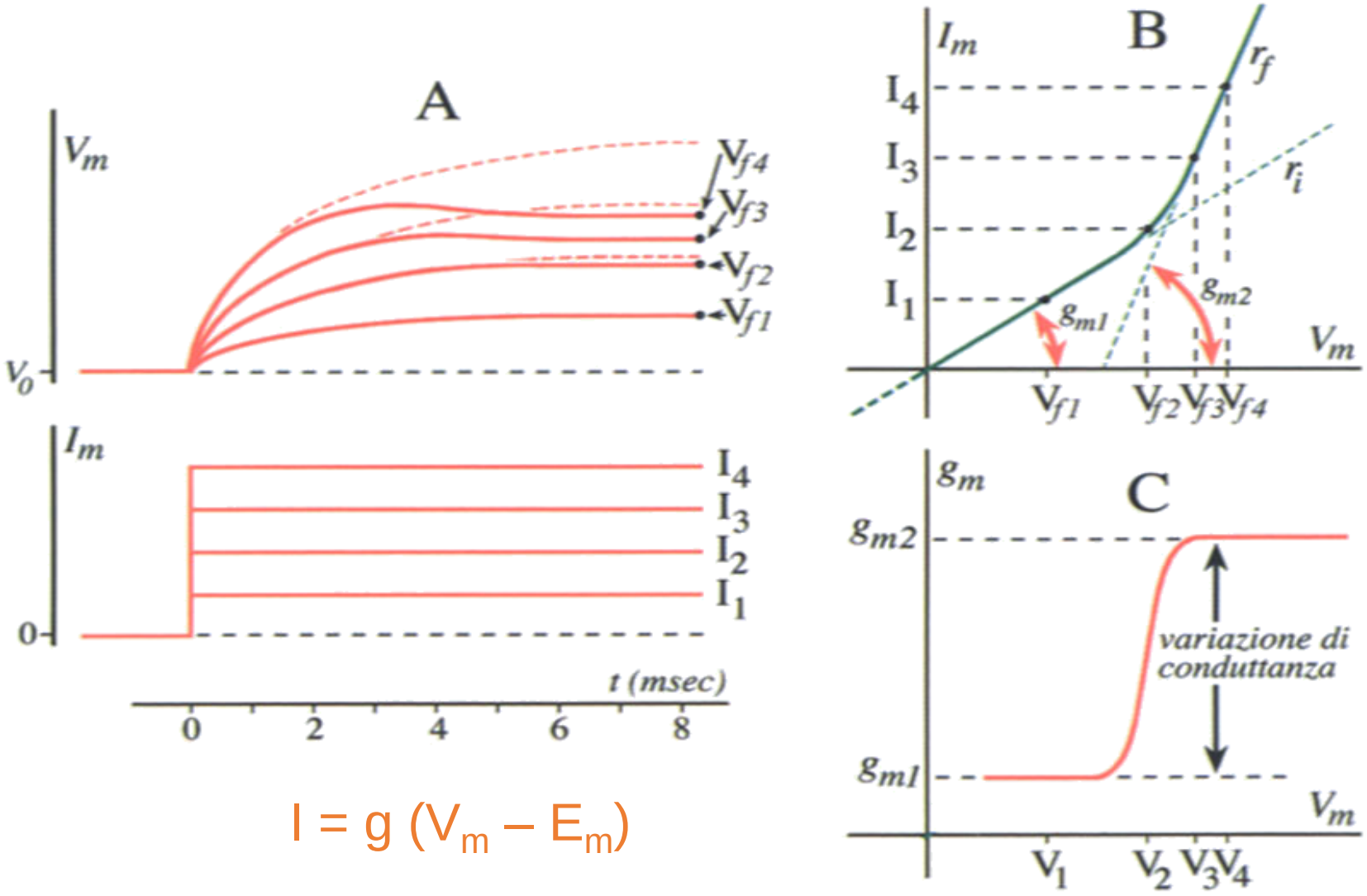




current pulses

$\Delta Vm = R\ I$

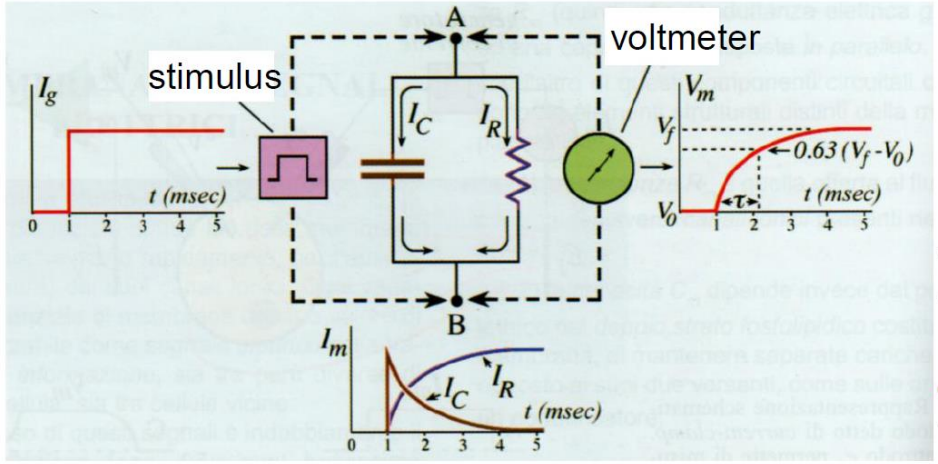
Time-dependent rectifications when the ion channels are active, See voltage-gated K^+ channels

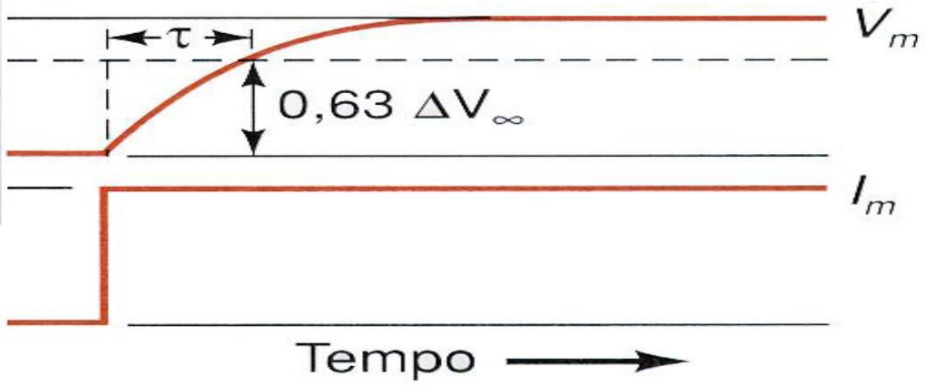


$$I = g\,(V_m - E_m)$$

Taglietti, Casella, Principi di fisiologia e biofisica della cellula

Membrane time constant



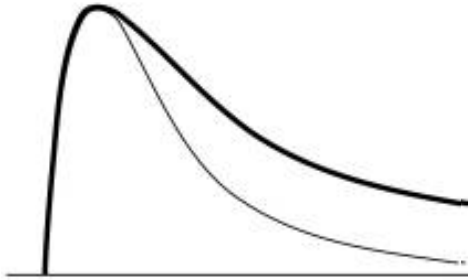


$$\tau = R_m C_m$$

Time required to charge the capacitor

63 % (1-1/e) of full charge.

The values of Rm and Cm determine how quickly the steady state is reached: the larger the R_m or the C_m, the larger the charging will take.

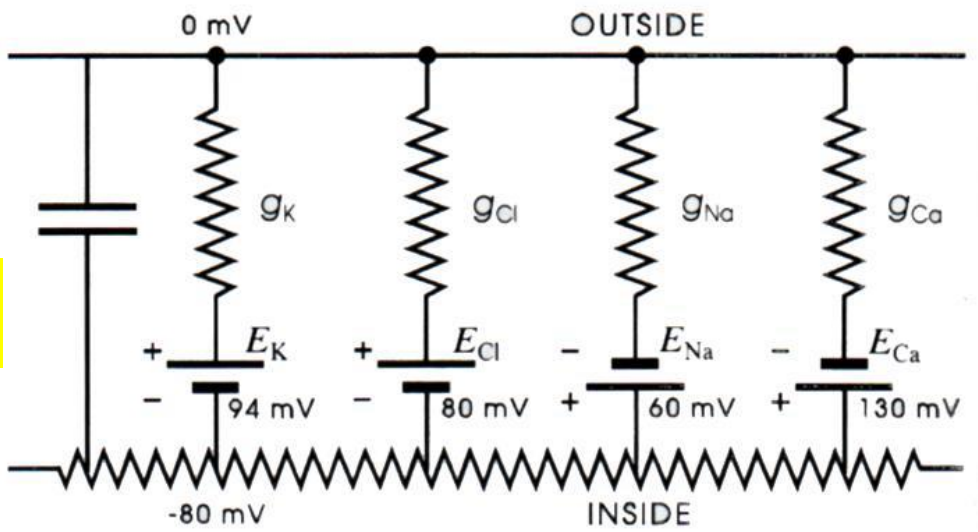


Passive electrical properties affect the time-course of synaptic potentials

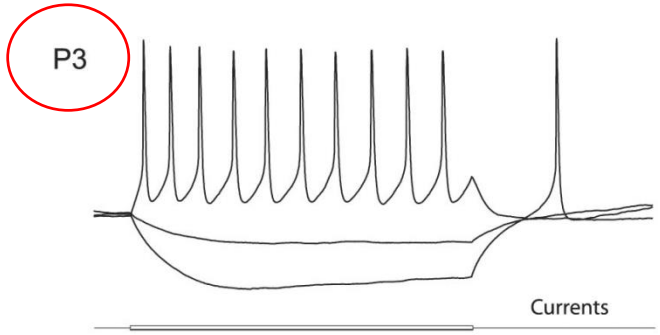
More components in the circuit

$I = V/R$

$I_x = g_x (V_m - E_x)$



In each component there is a R (ion channel) in series with a bactery given by the E_x of the specific ion that across the channel. A concentration gradient exists for each ion that creates an electromotive force, a force that drives that ion through the channel.

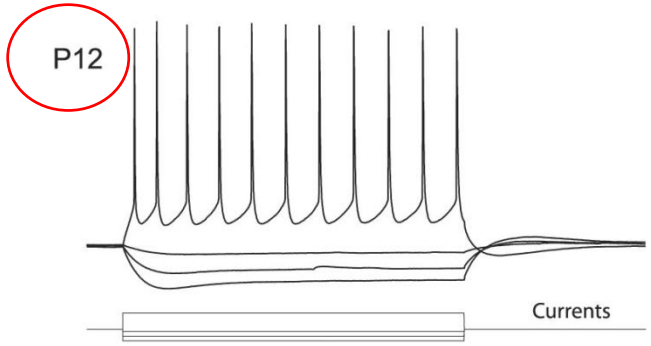


V_{rest} = -65 mV

R_m=1139 M Ω

T = 69 ms

Vthresh - 42 mv

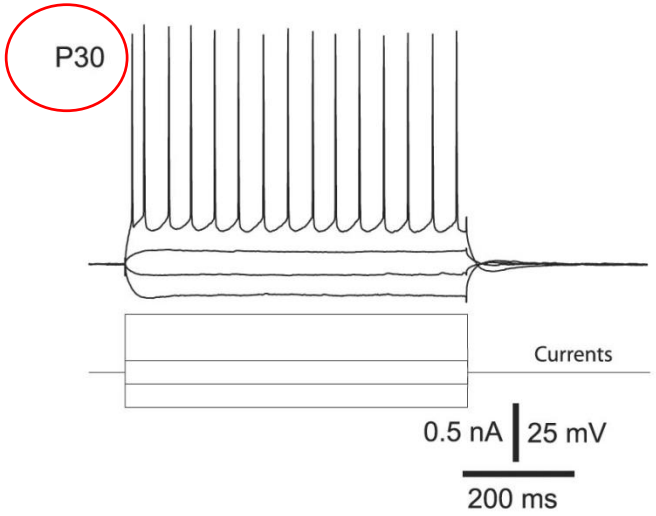


V_{rest} = -76 mV

R_m=168 M Ω

T = 28 ms

Vtresh -47 mV



R_m=70 MΩ

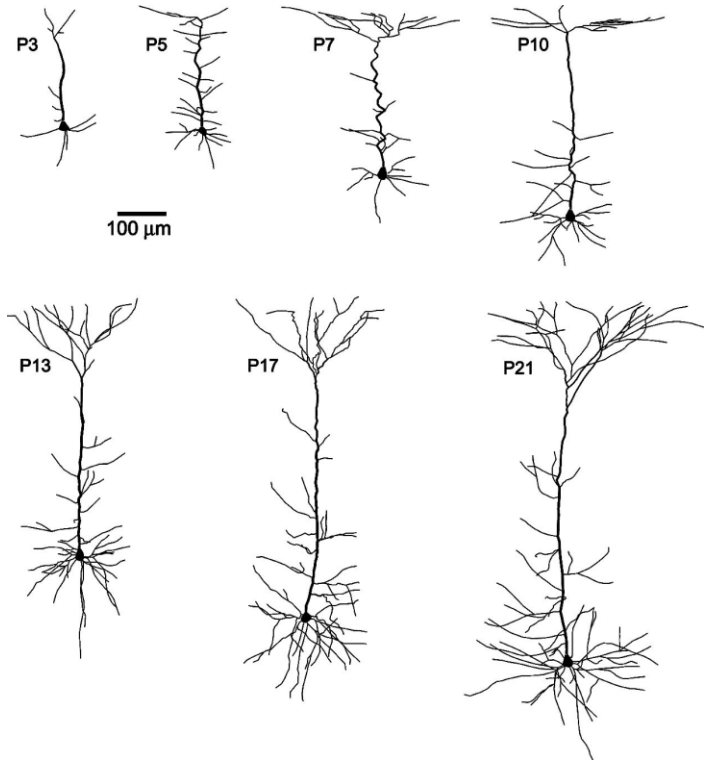
T = 13 ms

Vthresh -55 mV

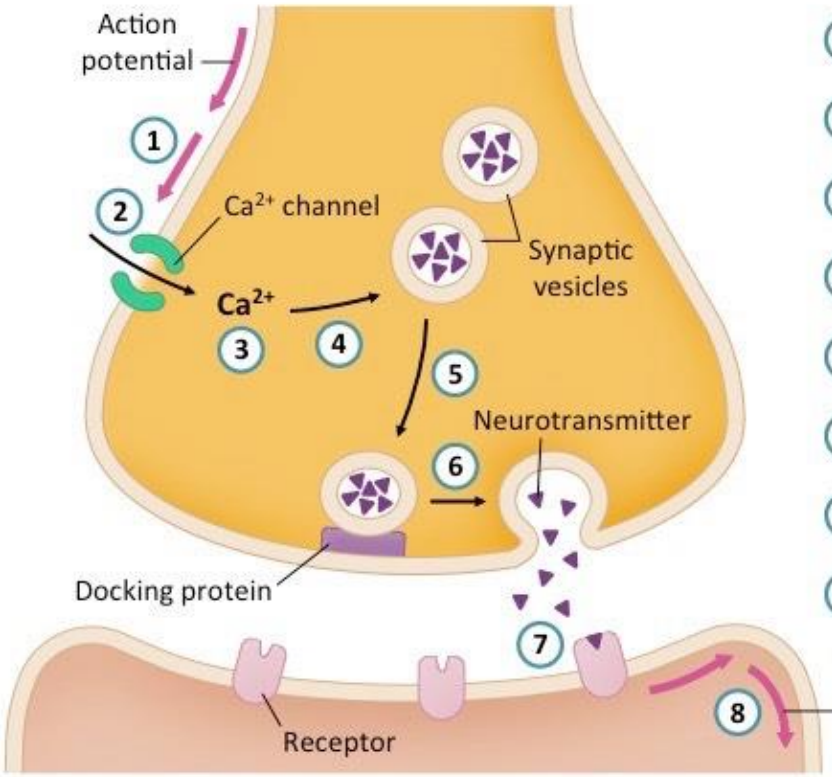
Zhang, J Neurophysiol 91: 1171–1182, 2004.

Firing of Layer V pyramidal neurons in the PFC at various ages

Look at the duration of action potential

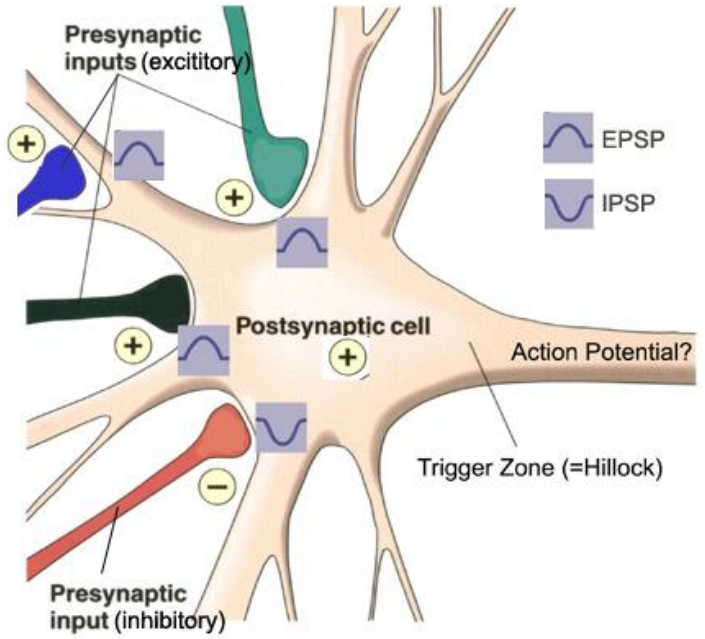


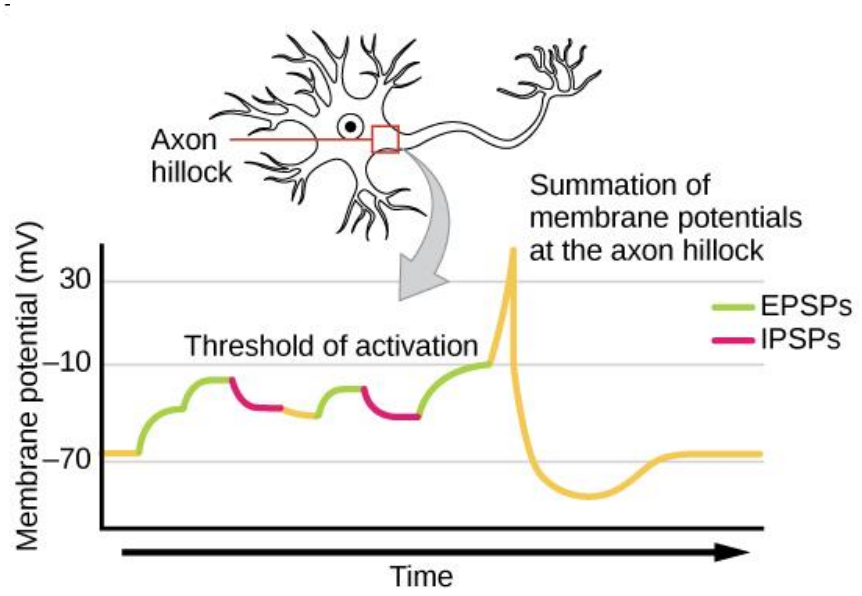
At each chemical synapse



1. Action potential arrives at axon terminal
2. Voltage-gated Ca^{2+} channels open
3. Ca^{2+} enters the presynaptic neuron
4. Ca^{2+} signals to neurotransmitter vesicles
5. Vesicles move to the membrane and dock
6. Neurotransmitters released via exocytosis
7. Neurotransmitters bind to receptors
8. Signal initiated in postsynaptic cell

Chemical synapses generate post-synaptic potentials

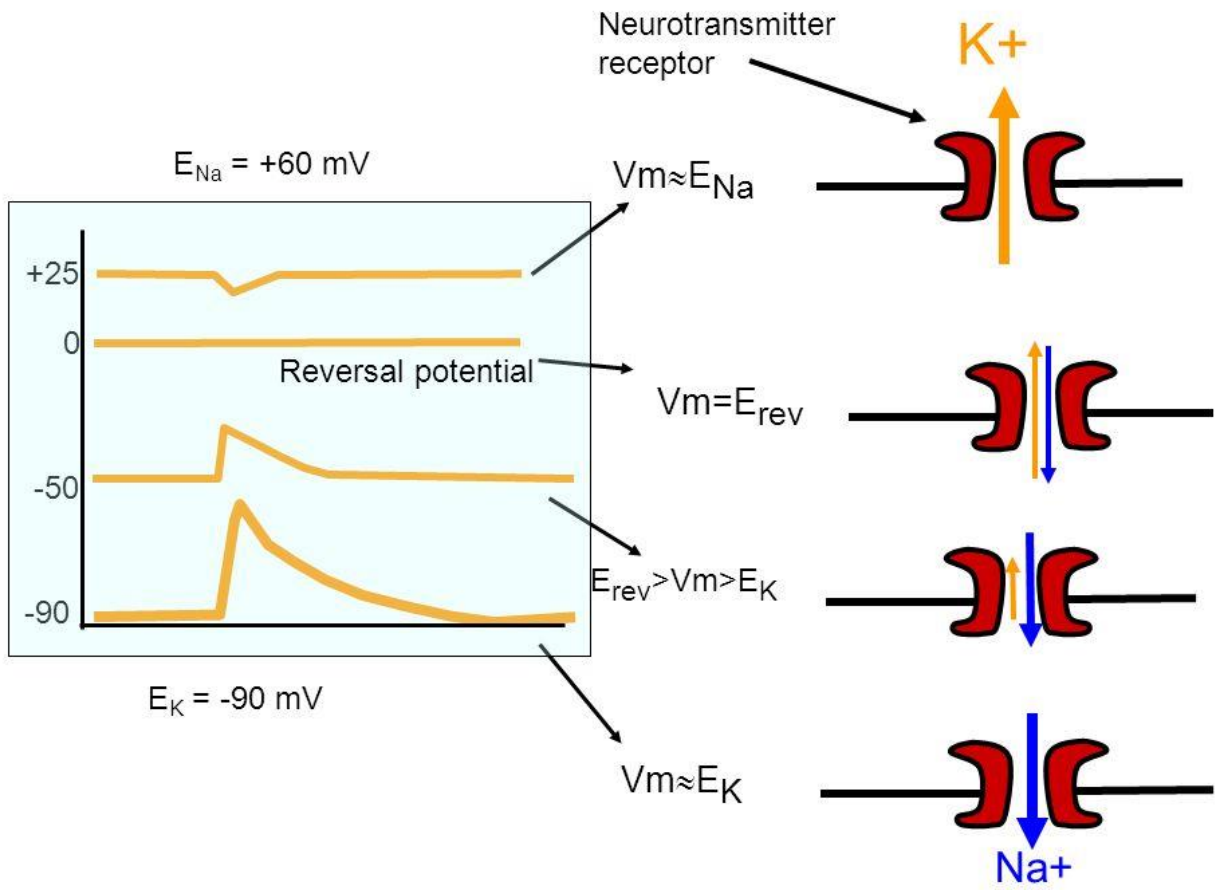




Fast glutamatergic and colinergic transmissions give origin to EPSP

Ligand gated cation channels permeable to Na^+ and K^+

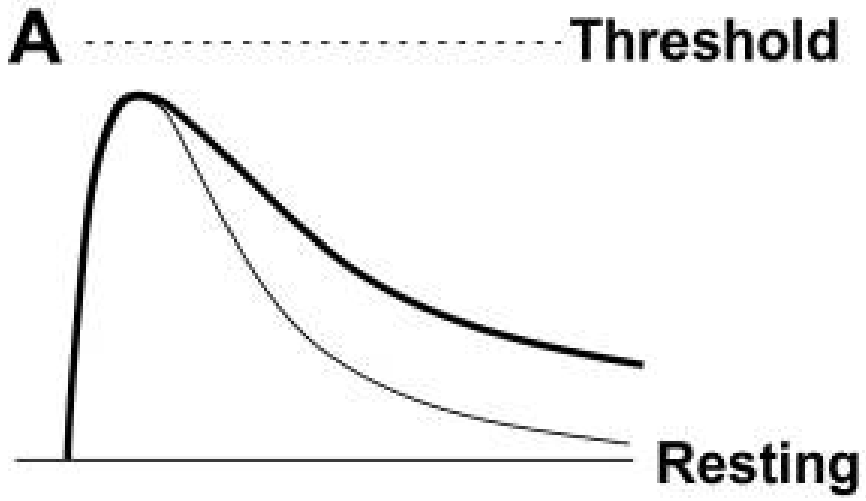
Reversal potential is the V_m in which inward current = outward current



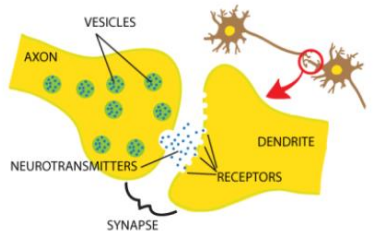
$V_m = E_{rev}$ → net current = 0

EPSP amplitude and time-course depend on:

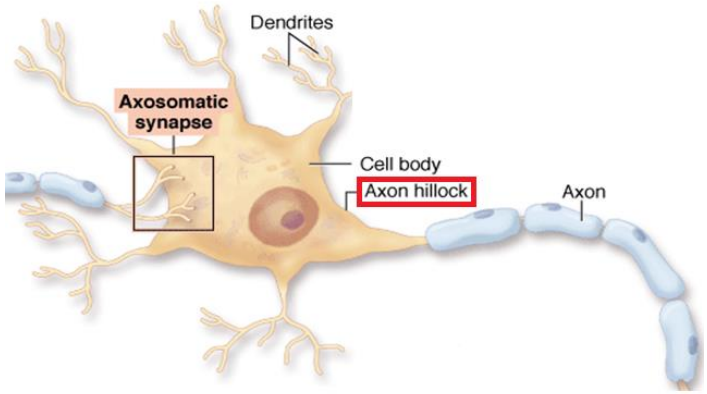
- Resistance and Capacitance of the postsynaptic cell
- Kinetics of post-synaptic channels
- Reversal potential
- Diffusion/synaptic geometry
- Enzymatic degradation
- Re-uptake

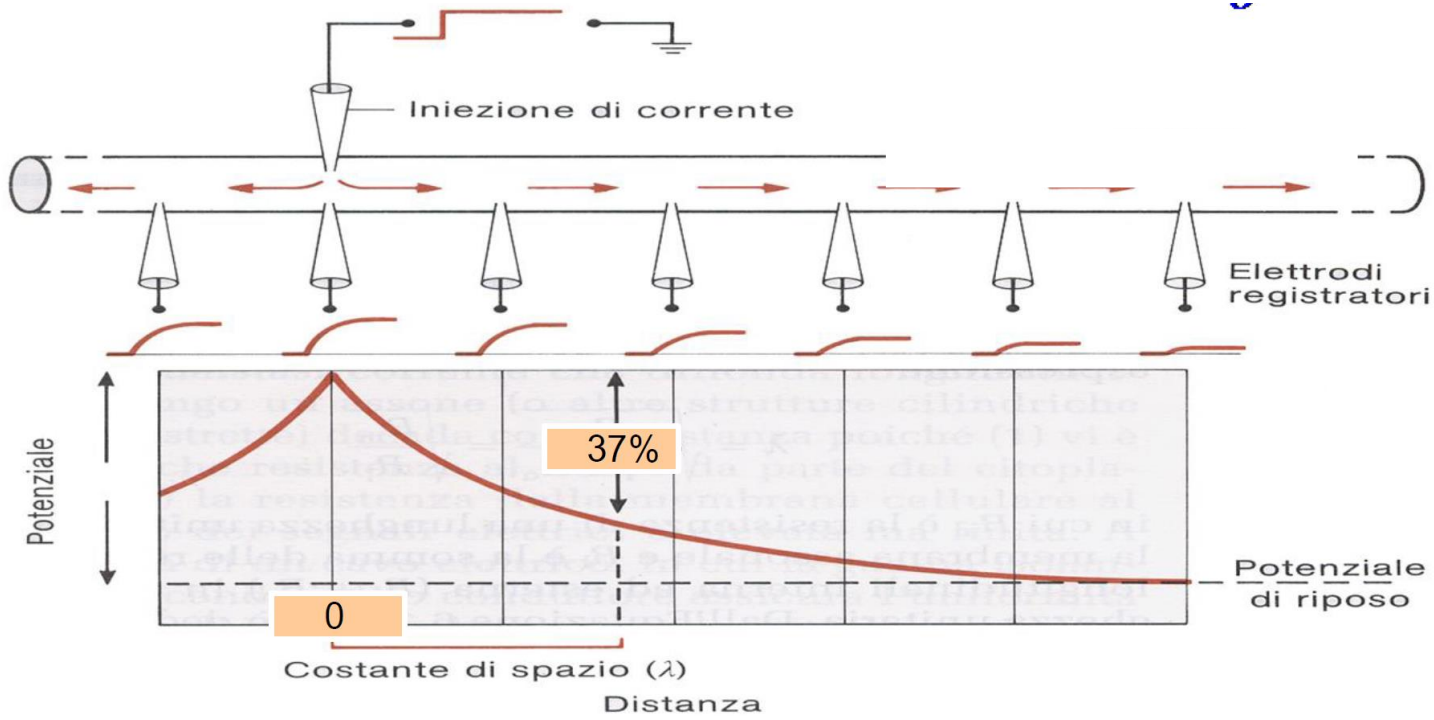


A potential problem in the signal integration: **DENDRITIC FILTERING**



Because of the leaky cable structure of dendrites, inputs fade away with distance.



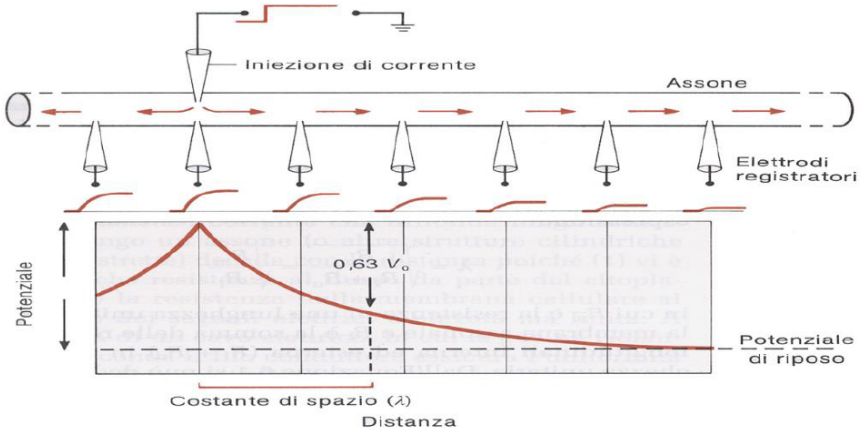


Passive membrane properties affect the efficiency to conduct PSP from their site of origin to the hillock zone

Vm dacays exponentially with distance along the length of the dendrite

$$V_x = V_o\, e^{-x/\lambda}$$

$$\lambda = \sqrt{\frac{r_m}{r_i + r_{ext}}}$$



The length constant, λ, is the distance along the dendrite to the site where ΔV_m has decayed to 1/e, or 37 % of its value at x = 0.

e = 2.7 if x=λ e^{-1} = 1/e = 0.37 V= 37% V_o

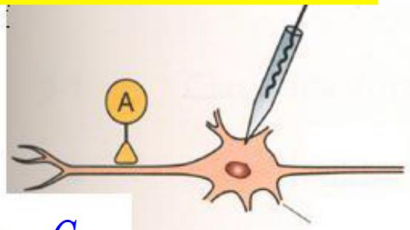
The longer the length constant, the greater the effect of a dV in the cell

r_i is the **Intracellular resistance per units length**
(decreases with diameter, increases with length)
Units: kΩ / cm

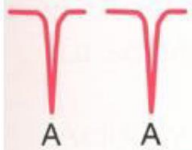
r_m is the membrane resistance of a unit length
(increases with diameter)
Units: kΩ cm

$$\lambda = \sqrt{\frac{r_m}{r_i}}$$ **Units: cm**

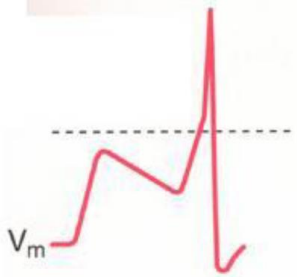
Temporal summation



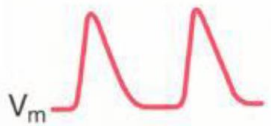
$\tau = R_m C_m$



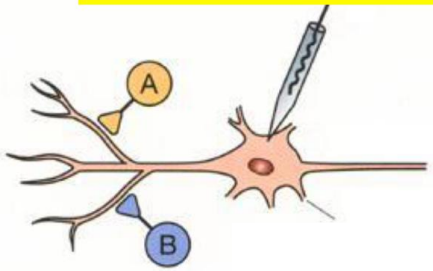
τ = 100 ms



τ = 20 ms

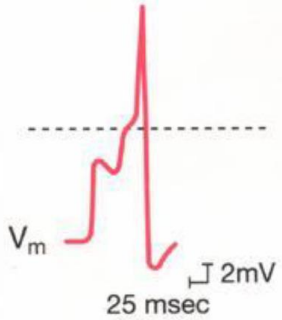


Spatial summation



$\lambda = \sqrt{\frac{r_m}{r_i}}$

λ = 1 mm

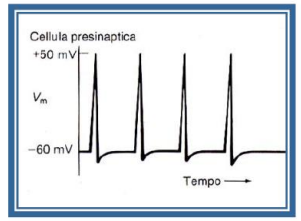


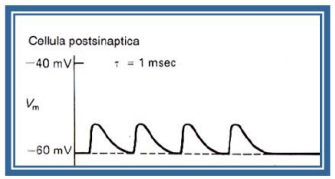
λ = 0.1 mm

Effects of τ on postsynaptic potential integration

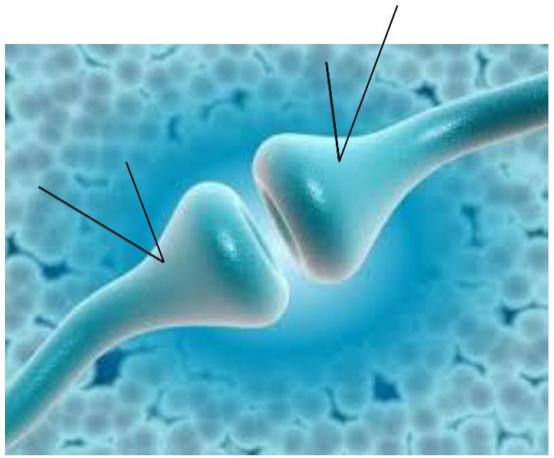
TEMPORAL SUMMATION???

presynaptic
action potential

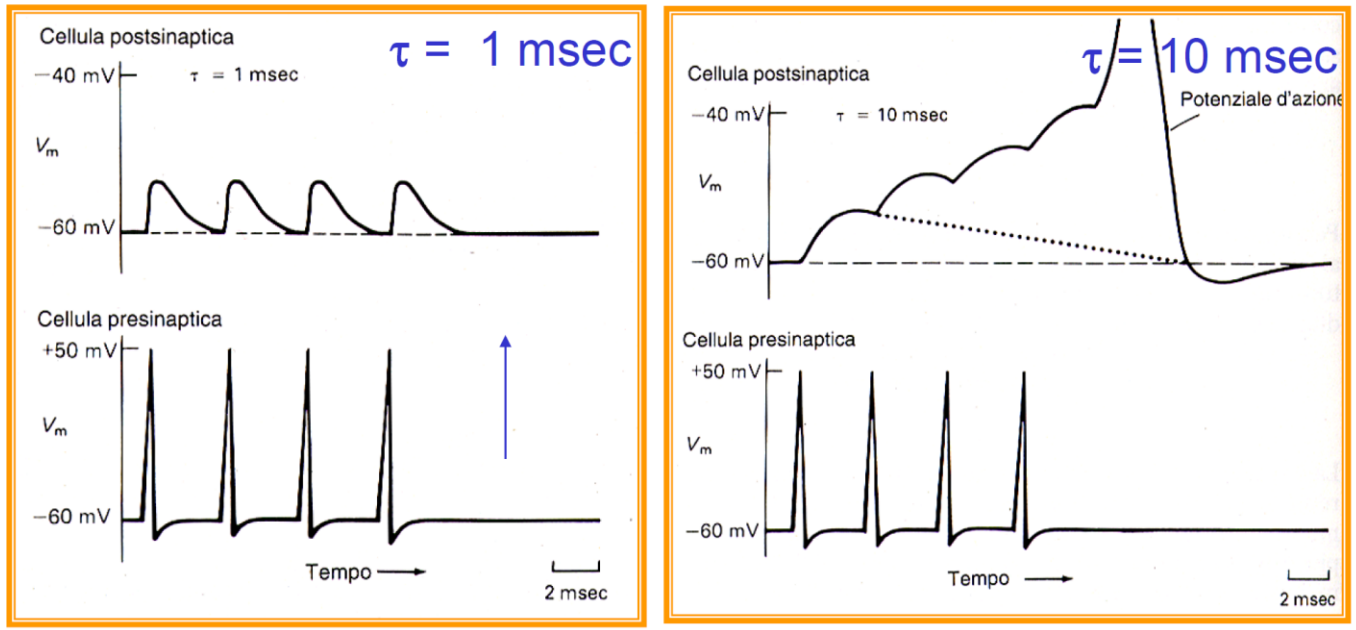




postsynaptic
graded potentials



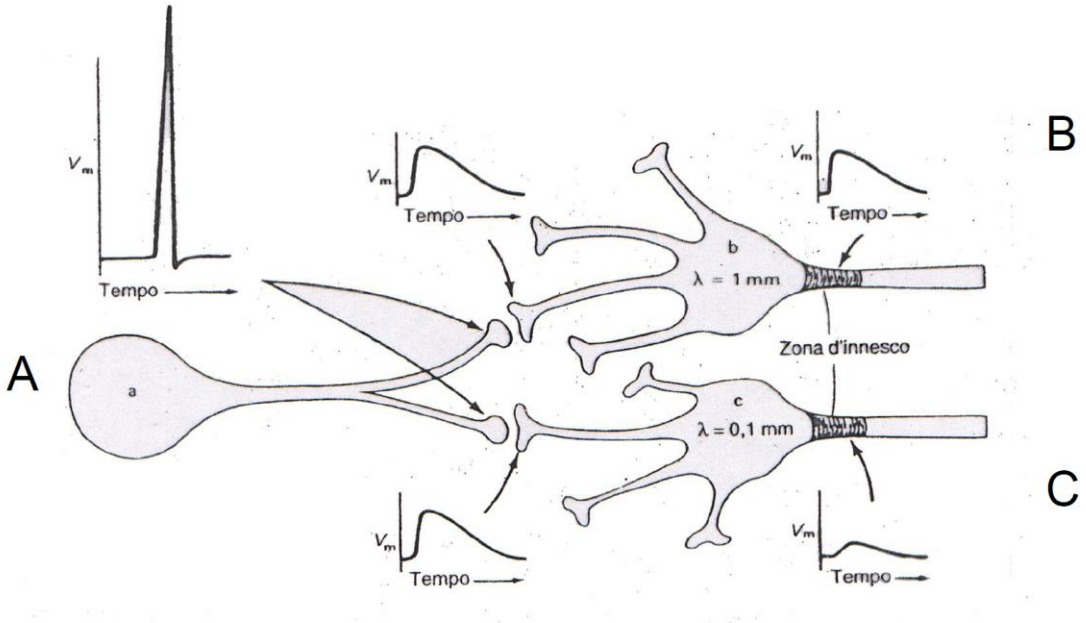
The larger the membrane time constant, τ, of the postsynaptic cell, the longer the postsynaptic potential lasts



and greater the extent of temporal summation

The length constant λ, affects the efficiency of electrotonic conduction of synaptic potentials

SPACIAL SUMMATION ??



The two EPSP are equal in amplitude at their sites of initiation.
The amplitude of EPSP that arrives at the trigger zone in cell B (λ = 1 mm) is much larger than in C (λ = 0.1 mm).

The longer is the length constant of the postsynaptic cell, the higher is the postsynaptic potential peak for long distances and greater will be the extent of spatial summation

Problems with chemical transmission-electrotonic filtering

Possible physiological solutions:

Passive membrane properties:

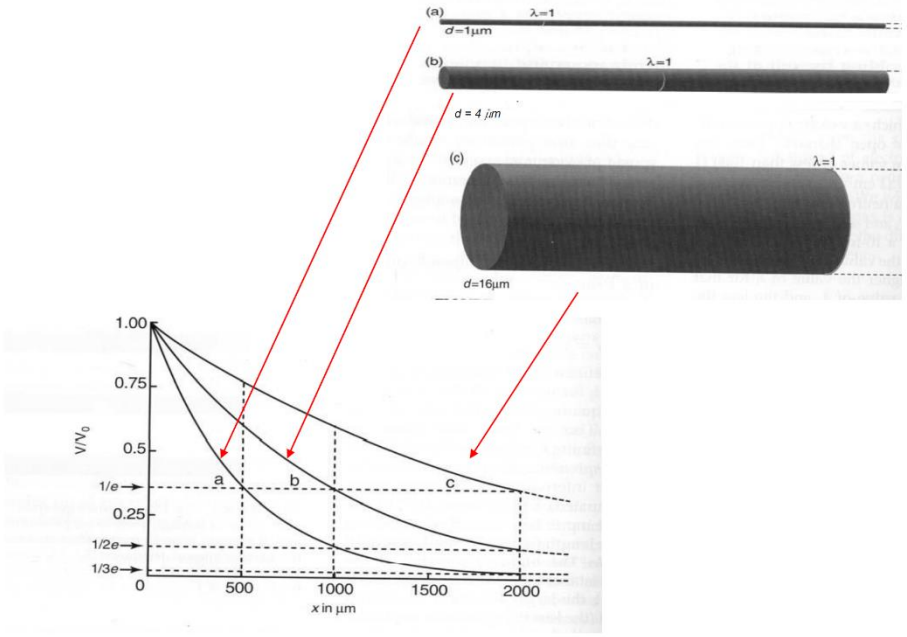
-High length constant to increase the size of EPSPs distally

Active properties:

-Voltage-dependent ion channels could boost the signal along the way.

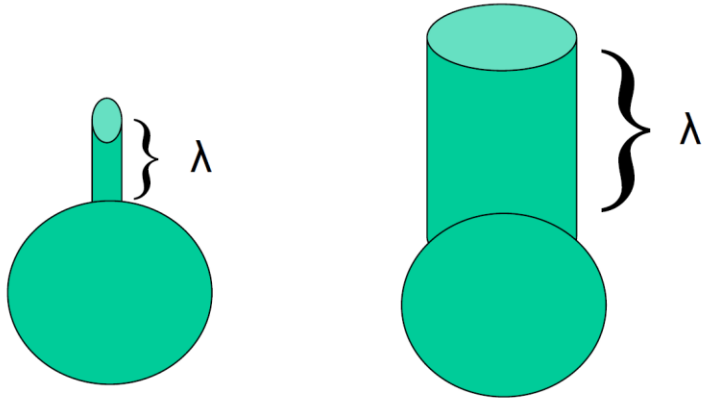
Higher process diameter / Decrease in internal resistance

Length constant is proportional to square root of process diameter



$$\lambda = \sqrt{\frac{r_m}{r_i}}$$

Problem: changes in morphology are not always practical:



In order for the length λ constant to double, the diameter of the dendrite has to increase by a factor four.

Increase strength of distal synapses features

Use voltage-gated channels to boost distal inputs

Active properties vary within and between neurons

Purkinje cells

- P-type calcium channels
- Few sodium channels

Cortical pyramidal cells

- Calcium and sodium channels

- Some neurons have minimal active properties

Propagation of action potential

Factors influencing
Conduction Velocity

- Axonal diameter
- Myeline

Faster propagation of action potential..

One adaptive strategy for rapid propagation of action potential is increasing the axon diameter.

Axons with larger diameters have bigger length constant, the current will propagate faster

But …the larger the membrane surface, the larger the capacitance, the more charge must be deposited on the membrane to change the Vm.

$$\lambda = \sqrt{\frac{r_m}{r_i}}$$

Length constant is proportional to square root of process diameter

A second mechanism for increasing conduction velocity is "Myelination"

wrapping of glial membrane around the axons

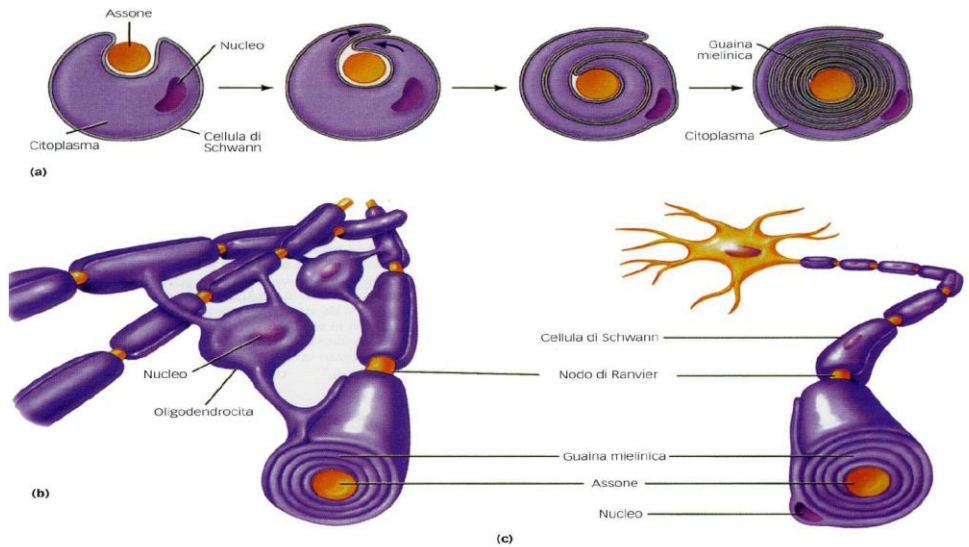


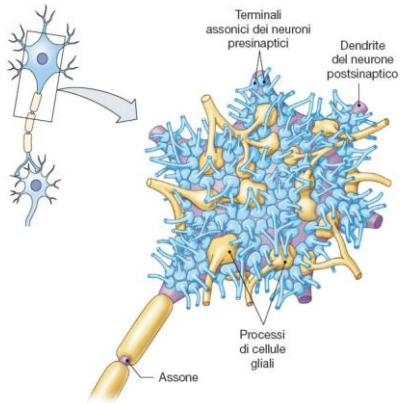
FIGURA 6.5 **Formazione ed origine delle guaine mieliniche.** **(a)** *Formazione della guaina mielinica da parte delle cellule di Schwann. La mielina, che è formata da strati concentrici di membrana citoplasmatica forniti dalle cellule di Schwann oppure dagli oligodendrociti, forma uno strato isolante intorno all'assone.* **(b)** *Formazione delle guaine mieliniche ad opera degli oligodendrociti nel SNC. Un singolo oligodendrocita invia processi citoplasmatici che formano rivestimenti di mielina attorno a molti cilindrassi. Notate i nodi di Ranvier, che rappresentano zone sprovviste di mielina.* **(c)** *Avvolgimento degli strati di mielina formati dalle cellule di Schwann nel sistema nervoso periferico. Una singola cellula di Schwann avvolge soltanto un singolo assone.*

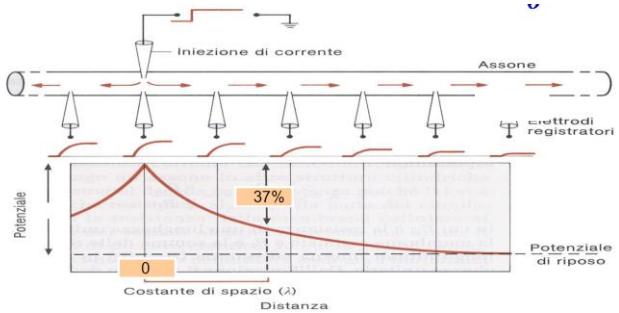
The CONDUCTANCE of a capacitor is in inverse proportion to the thickness of the insulating material, therefore ----
MYELINATION DECREASES Cm

The smaller the Cm, the less charge must be deposited on the membrane to change the Vm

-MYELINATION INCREASES Rm.

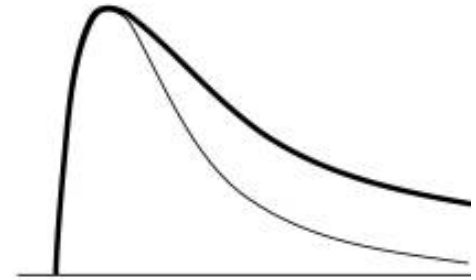
Passive electrical properties of nerve membranes



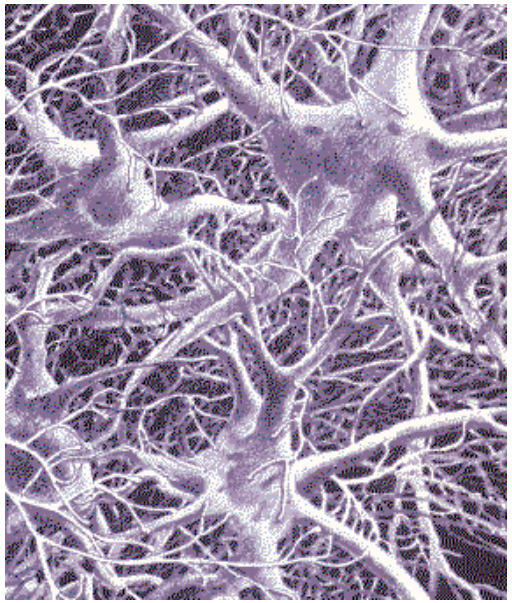


The passive electrical properties, specifically the Rm and Cm of the neurons and the resistance of the cytoplasm, play a major role in signaling.
At the synapses they contribute to synaptic integration to generate or not an action potential.

-They affect the time course of synaptic potentials as well as how efficiently they are conducted from their site of origin to the trigger zone.
- Once an action potential is generated, the speed of conduction depends on the passive electrical properties of the axon



The ability of the brain to produce complex behaviour is related to the interconnection of neurons into networks or circuits.



Organization of neural networks :
objective of modern Neuroscience

The operation of a neural network depends upon interactions among multiple non-linear processes at the cellular, synaptic and network level. Modulation of BUILDING BLOCKS can change the network operation.

Cellular	Synaptic	Connectivity
Phosphorylation	Sign	Mutual
Threshold	Strength	or recurrent inhibition
F-I relationship	Time course	Reciprocal or
Spike frequency adapt.	Transmission	recurrent excitation
Post-burst hyperpol.	Electrical	Recurrent cyclic inhib.
Delayed excitation	Chemical	Parallel excitation /inhib.
Post-inhibitory rebound	Release mechanism	
Plateau potentials	Graded	
Bursting	Spike	

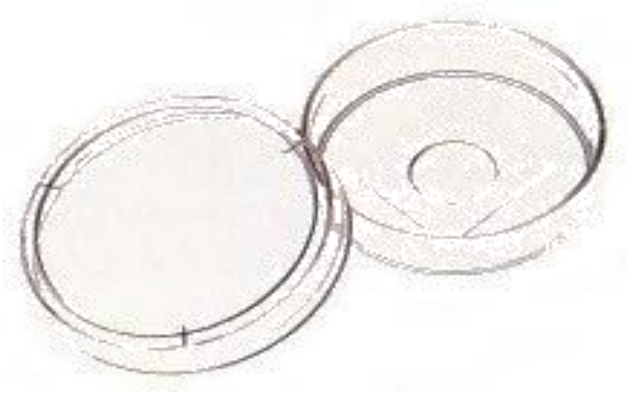
Ann. Rev. Neurosci. 12: 185-204 (1989)

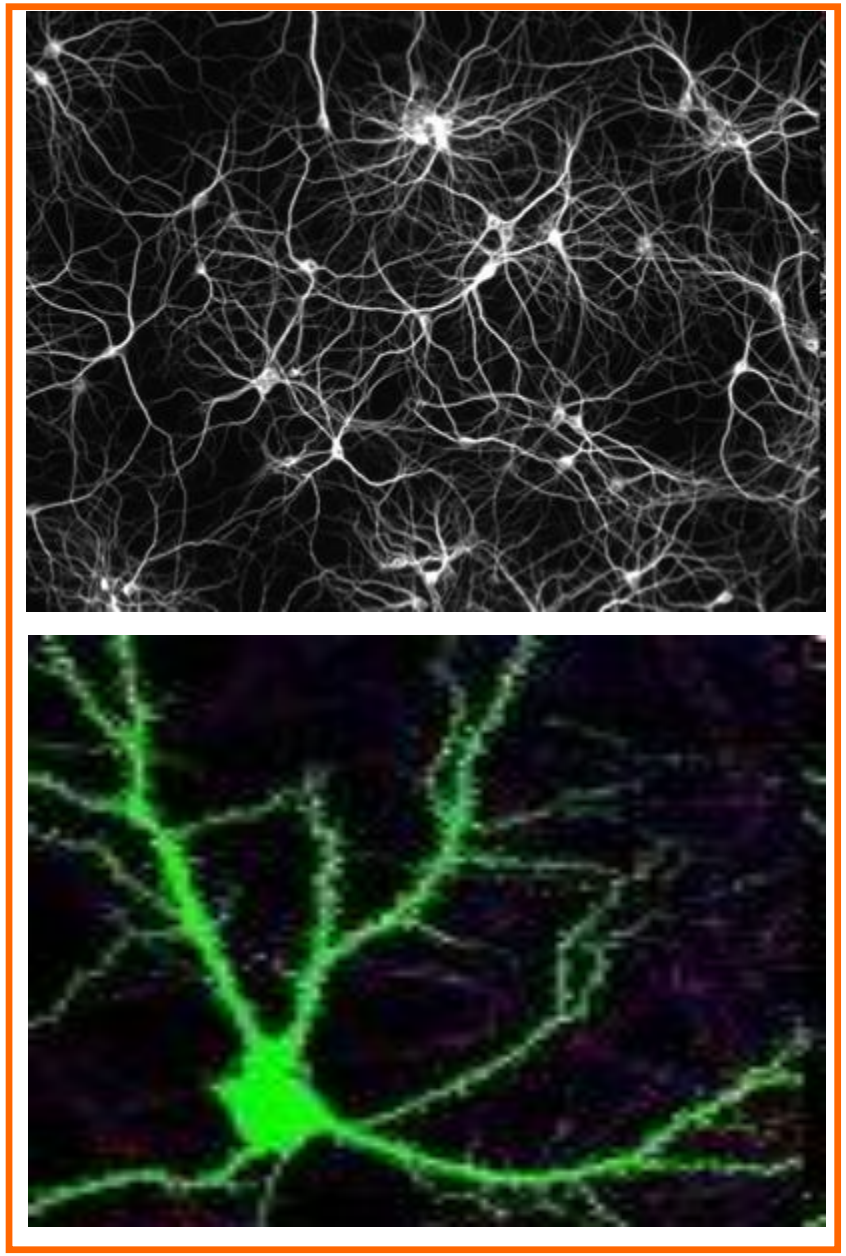
Functional properties of ion channels are identified with electrophysiological studies using the *voltage-clamp* and the *current-clamp* modes.

Electrophysiological properties studied in **neuronal cultures**

-Better control of extracellular behavior
-Better control of V_m

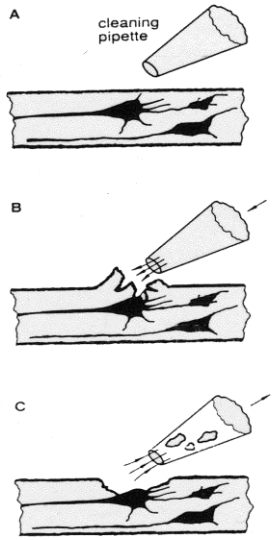
-Emphasize the somatic membrane properties
-Biophysical mechanisms can be altered
-Not easy to identify the nature of single cells

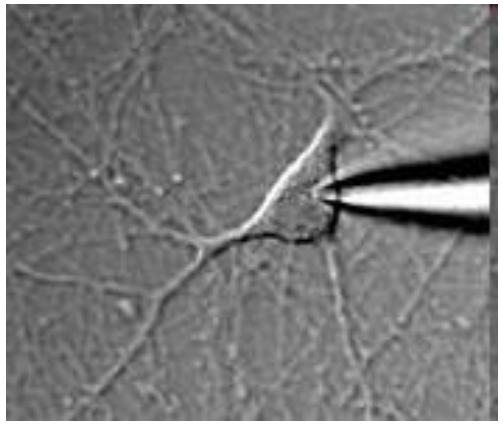




What about brain slices?

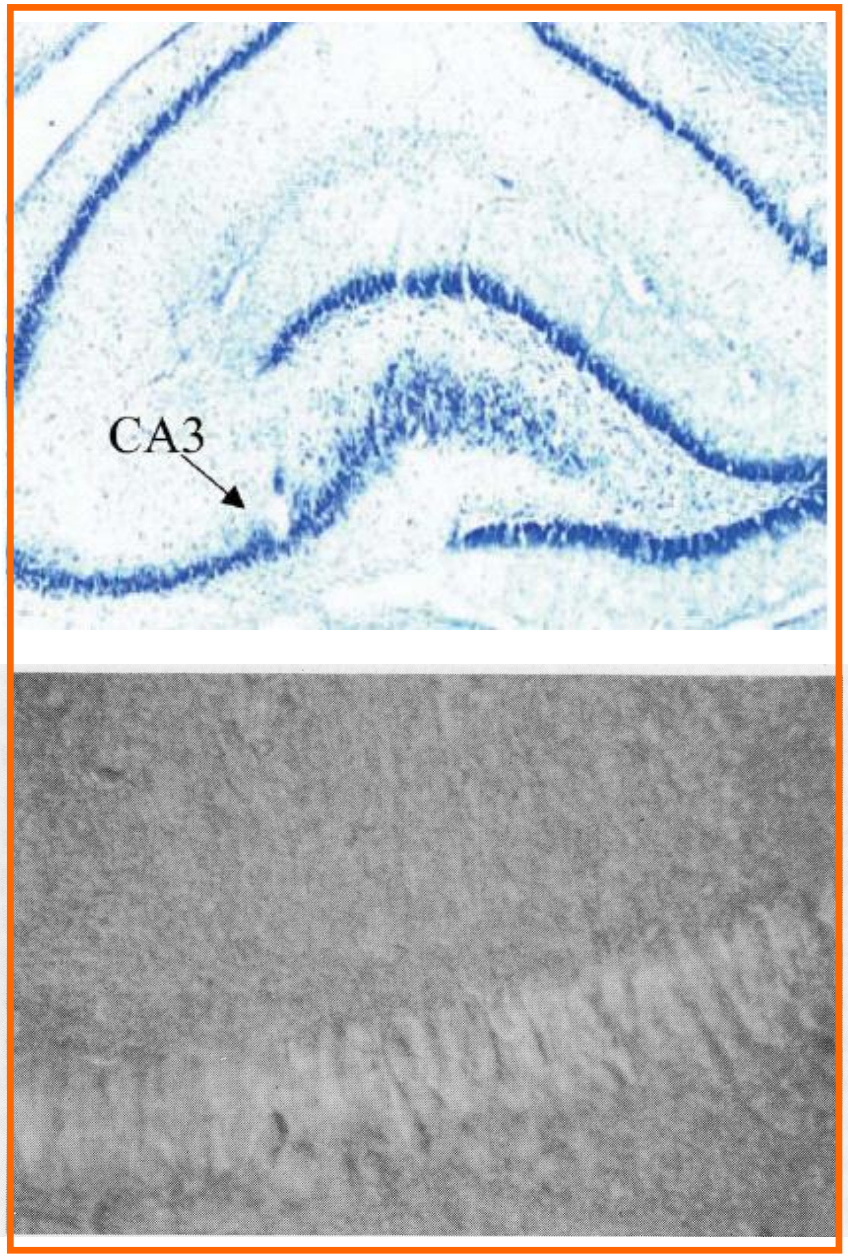
-Minimal loss of cell morphology





-Low access to pharmacological manipulations.

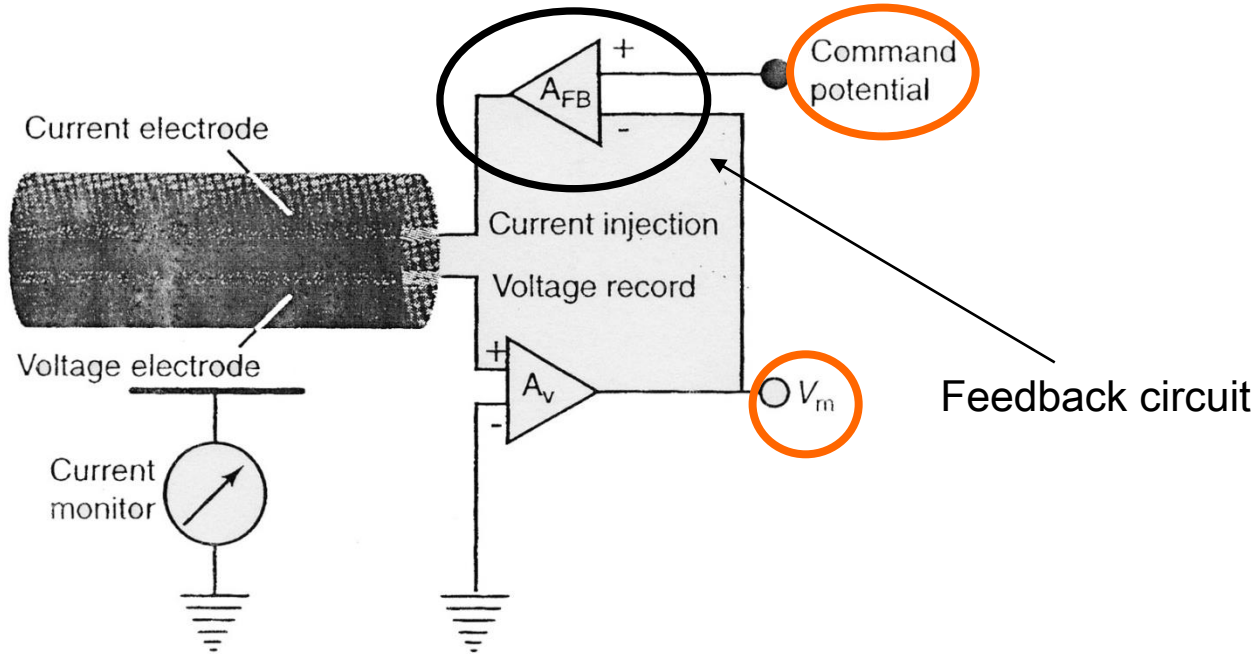
-Low control of V_m



As we have already said…..

Experimental arrangement

Two-electrode voltage-clamp experiments on squid axons

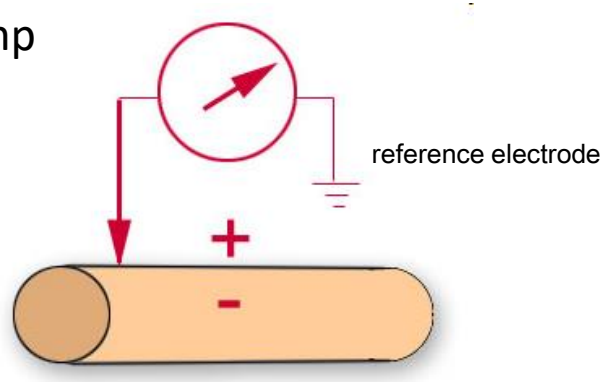


Two silver wires are inserted longitudinally. One of the wires provides a measure of the membrane potential, Vm inside the fiber with respect to that of the seawater (which is grounded); the other to inject current in the axon. These electrodes are connected to a feedback circuit to compare the Vm with the Vc, set by the person doing the experiment. If Vm is different by Vc, output current is injected in the axon to remove the voltage difference between the two inputs.

If the circuitry is properly designed, the change in Vm is achieved within a few microseconds. The delivered current is equal to the current flowing through the channels and it is that measured by the experimenter.

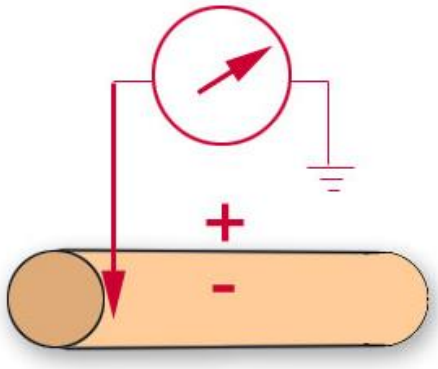
Extracellular *versus* Intracellular recordings

Current clamp or voltage clamp



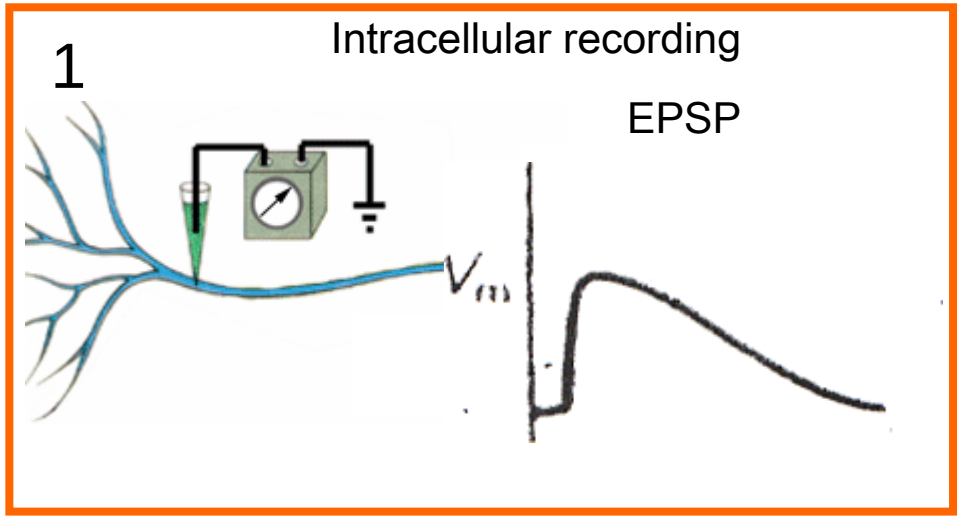
Extracellular positioning of the recording electrode

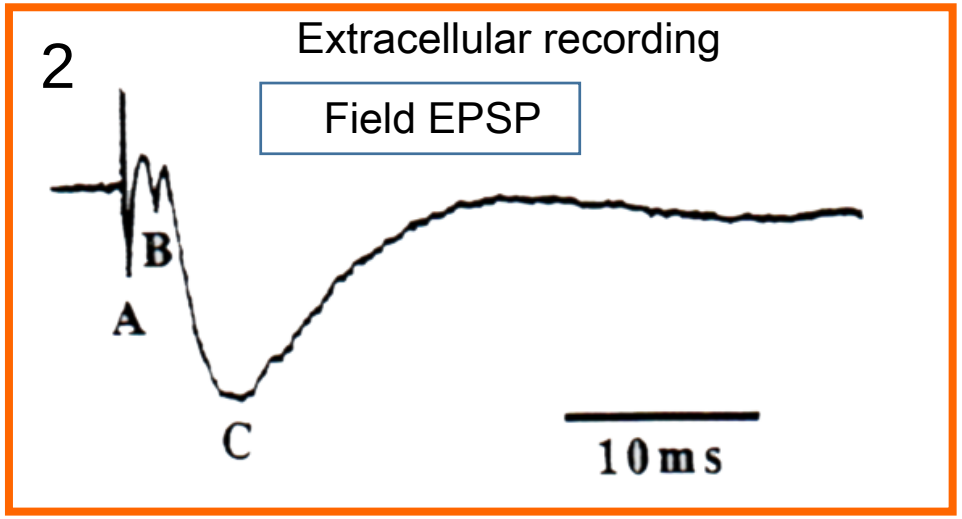
This arrangement **records potential changes at the membrane surface** rather than across the membrane. Extracellular techniques are therefore better suited where you only want to record the activity of an entire population of cells.



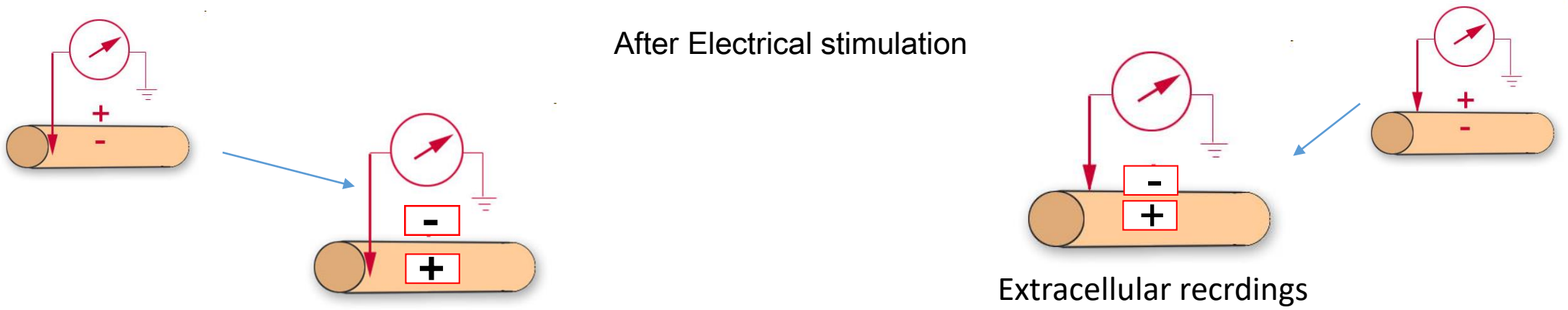
Intracellular positioning of the recording electrode

One can measure a **single trans-membrane potential** by inserting a glass pipette into one cell and recording the potential changes with respect to an extracellular reference electrode.





Typical EPSP obtained by intracellular recording (1) and the field EPSP obtained by extracellular recording (2) after an electrical stimulus.



Intracellular recording

Extracellular recordings / summary

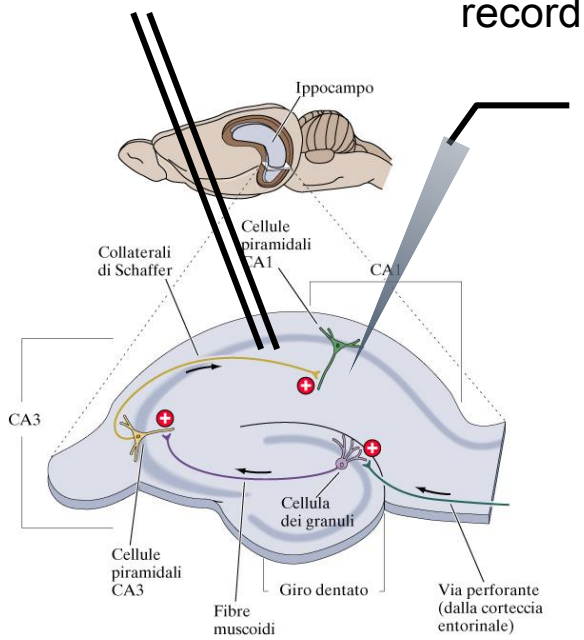
Field potentials reflect the linear sum of fields generated by e.g. EPSPs and IPSPs. The recordings can reflect the synchronized synaptic activity of the population of neurons in the local area around the recording electrode.

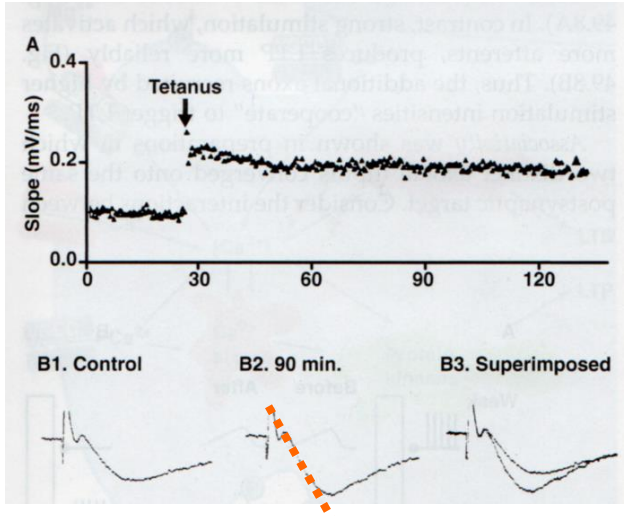
Extracellular recordings suffer from low signal-to-noise ratio, precluding detection of subthreshold synaptic potentials.
Since the fluctuations in the local field potential that occur in the brain are commonly less than 1 millivolt, the signal must be amplified so that it may be detected and recorded.

Extracellular recordings - plasticity

stimulating electrode

recording electrode





Hippocampal LTP: Electrical stimulation of Schaffer Collaterals (SC) gives origin to f EPSP in CA1 region. A tetanic stimulation of SC gives origin to LTP.

Slope is proportional to the amplitude of the synaptic response.
Often the amplitude can be disturbed by population spikes!!

Multi- electrode array

Devices with multiple (ten to thousands) electrodes
Spatial resolution!!!

Electrode diameter 30 µm

distance between the electrodes 200 µm

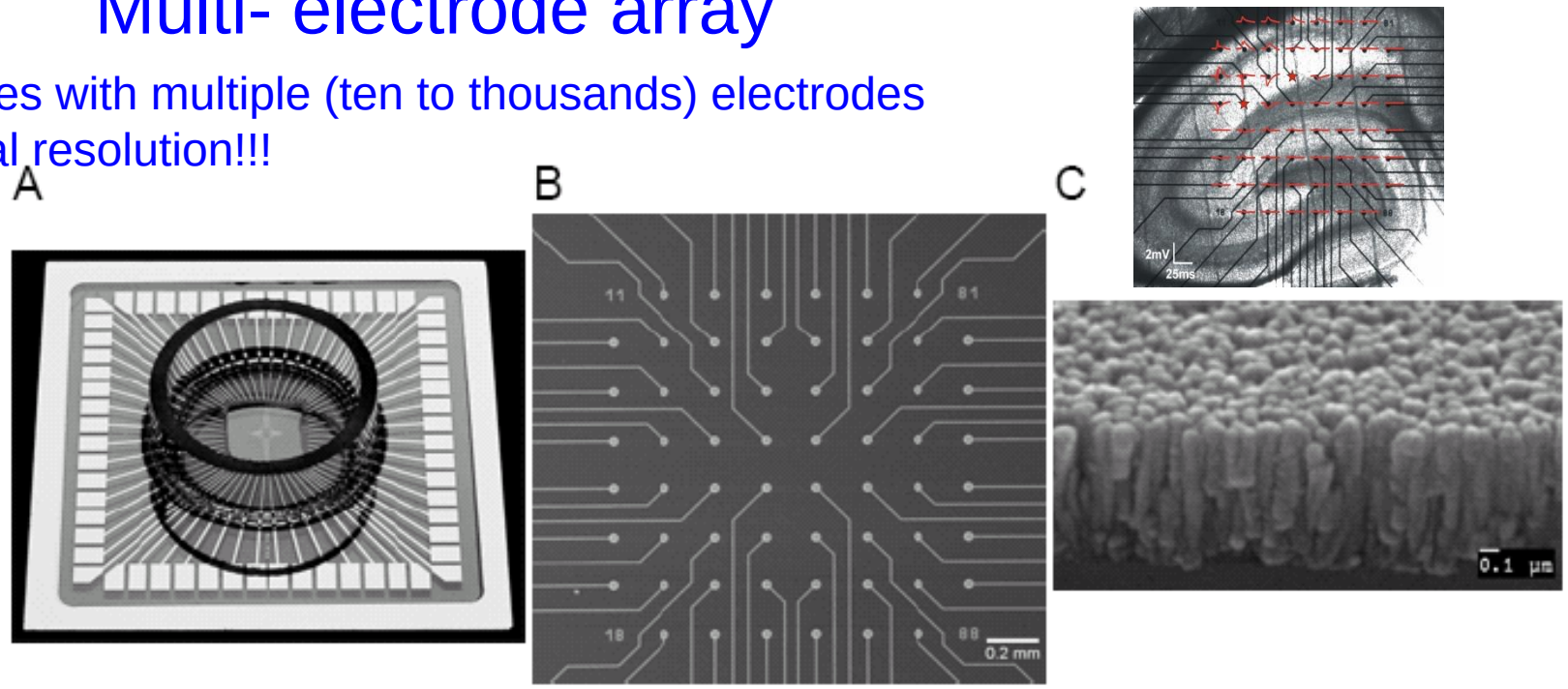


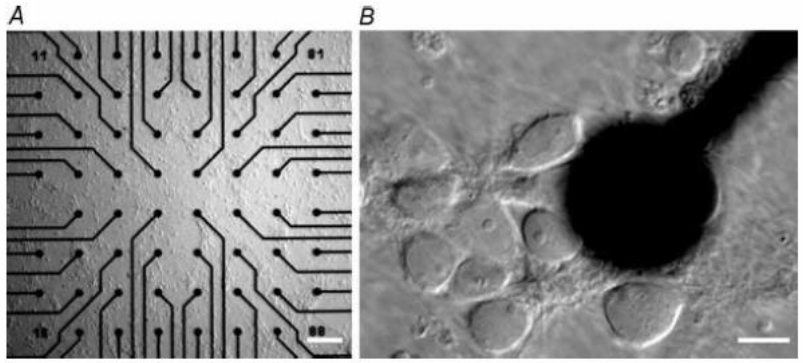
Figure 10-1. Design of a substrate integrated microelectrode array as used in our laboratory. (A) This version is on a 5×5 cm glass plate with conducting gold leads. A glass ring forms the recording chamber. (B) SEM micrograph of the recording area. The leads are insulated except for the center of the circles at the end of each lead. (C) SEM micrograph of a break-away of the electrode surface. Titanium nitride sputtered onto the electrode forms a columnar structure with a large inner surface, increasing the capacitance of the electrode area. MEAs from other producers are similar in their basic layout (Images by courtesy of Multi Channel Systems, Reutlingen and NMI Reutlingen, Germany).

M. Heuschkel et al., *J. Neurosci. Methods* **114** (2002) 135-148

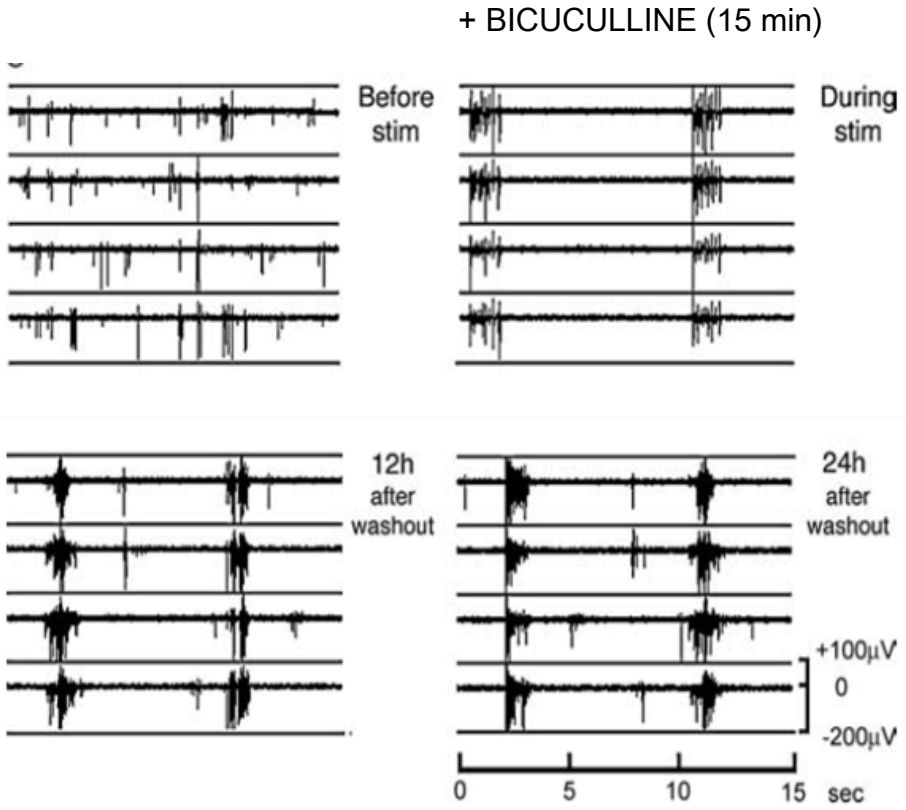
Hippocampal cultures on MEA

After exposure to bicuculline (GABA receptor blocker):
a change in network activity from uncoordinated firing of neurons to a highly organized periodic and synchronous burst pattern

Arnold et al., J Physiol, 2005



Electrode diameter 30 μm
distance between the electrodes 200 μm



Benefits to recording neuronal activity from extracellular recording electrodes.

-Extracellular electrophysiology is currently the best tool for performing high-resolution **recordings from neural tissue in an awake animal**.

-Relative ease and long duration of recordings.

-Improving micro-machining techniques and personal computers, is possible to record simultaneously from hundreds of neurons in a population.

Major problems

The technique obscures differential functions within the network. There is no way to know the anatomy of the recorded neurons. **Details on the transmission, on the processing of the stimulus information (intensity, quality, duration, velocity) can not be assessed**.

Unlike traditional two-electrode voltage clamp recordings,
sharp intracellular recordings and patch-clamp recordings use a single electrode to record voltage and current and allow to record transmembrane potentials.

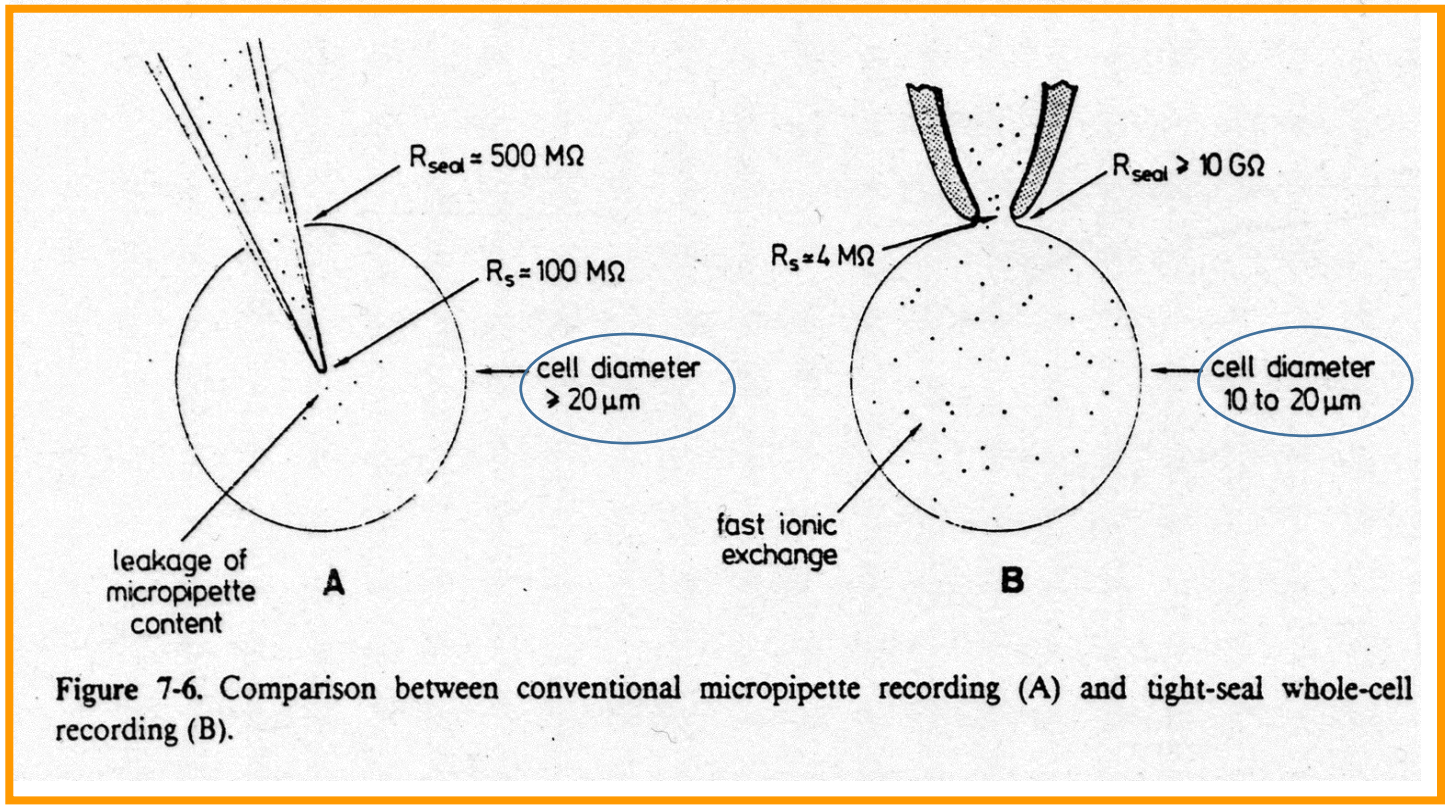
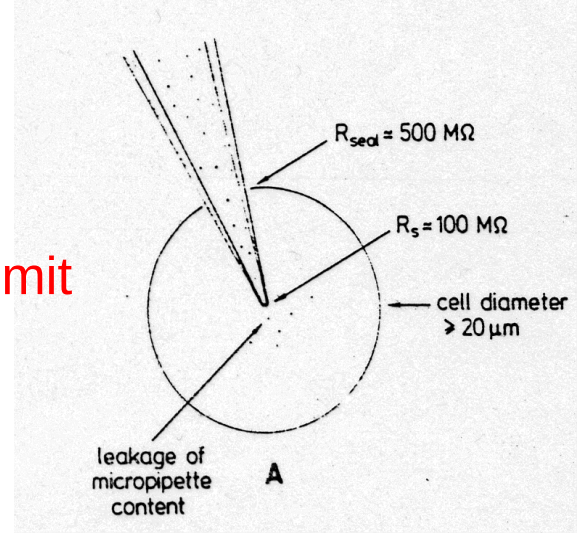


Figure 7-6. Comparison between conventional micropipette recording (A) and tight-seal whole-cell recording (B).

Sharp intracellular recording technique

no age limit



(pipette resistance 150-180 MΩ)

Sharp recordings cause a leakage resistance in the neuronal membrane, therefore they produce a drop in neuronal Rm

DG granule cells:

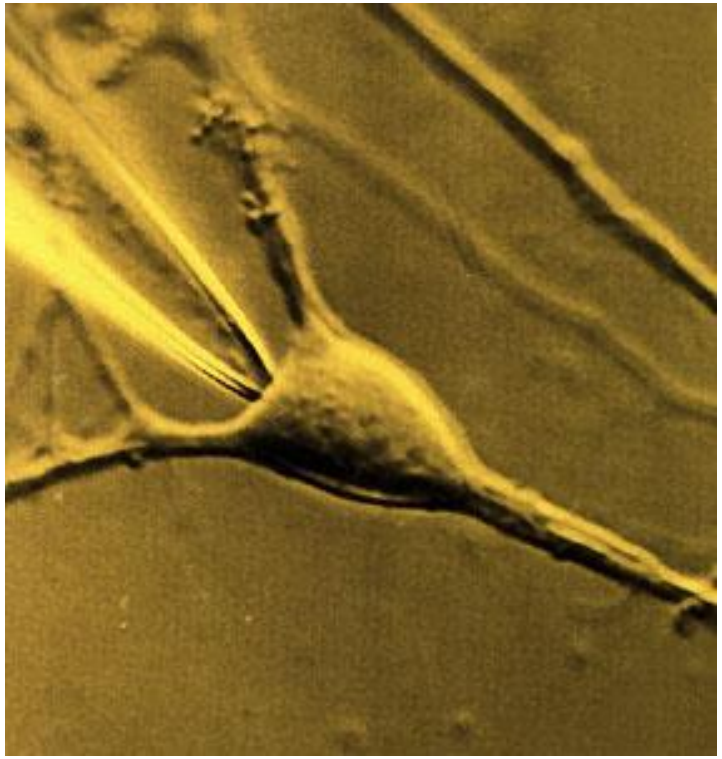
R_m = 107-228 MΩ with whole-cell recordings,

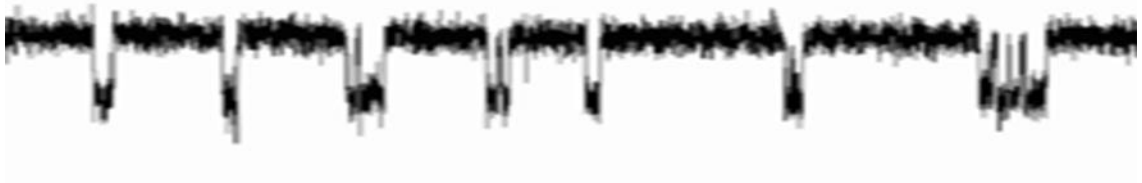
37-54 MΩ with the sharp electrode recordings

Patch-Clamp technique

The Nobel Prize in Medicine 1991

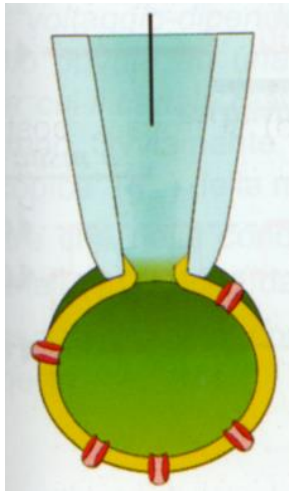
Bert **Sakmann** Erwin **Neher**



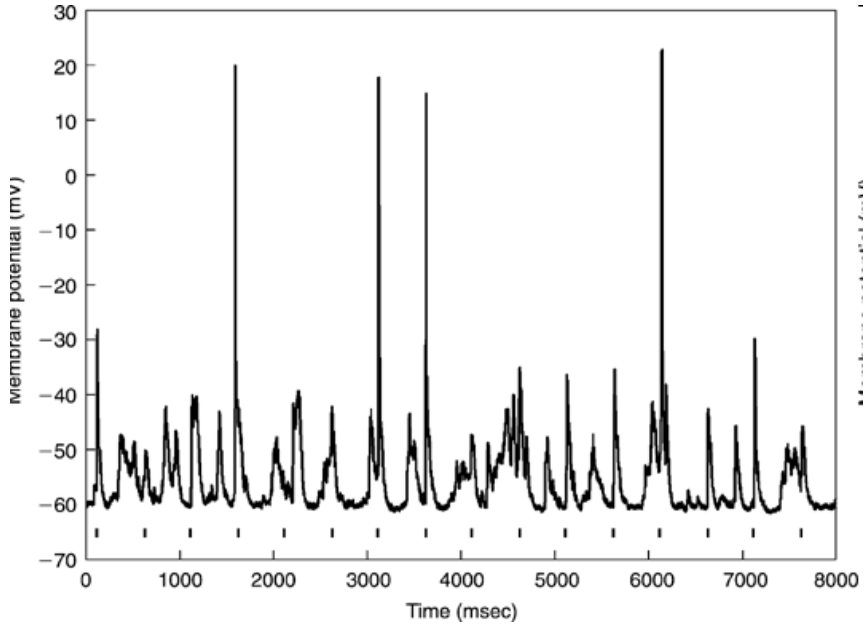


The real time movement of a single protein!!

Whole-cell recordings

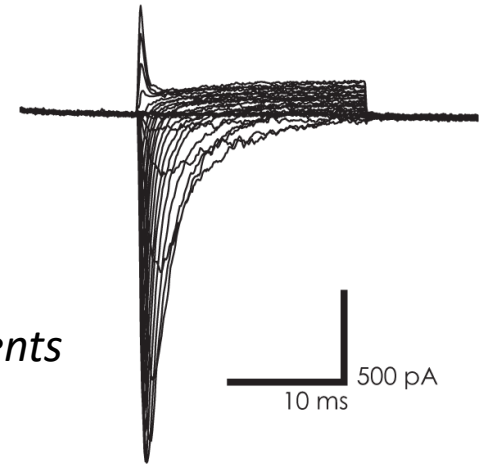


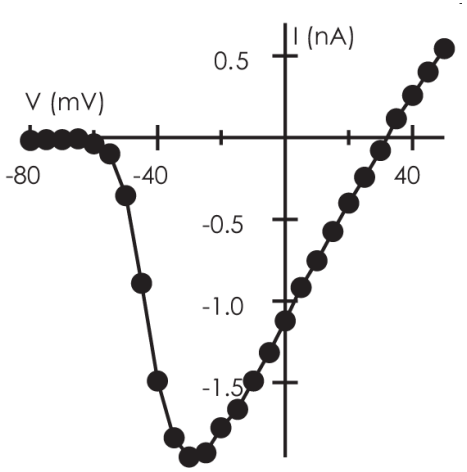
Current-clamp recordings

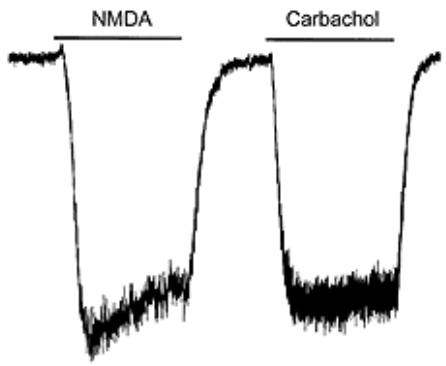


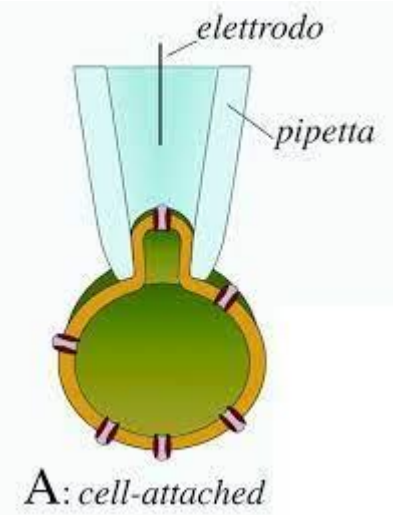
Voltage-clamp mode

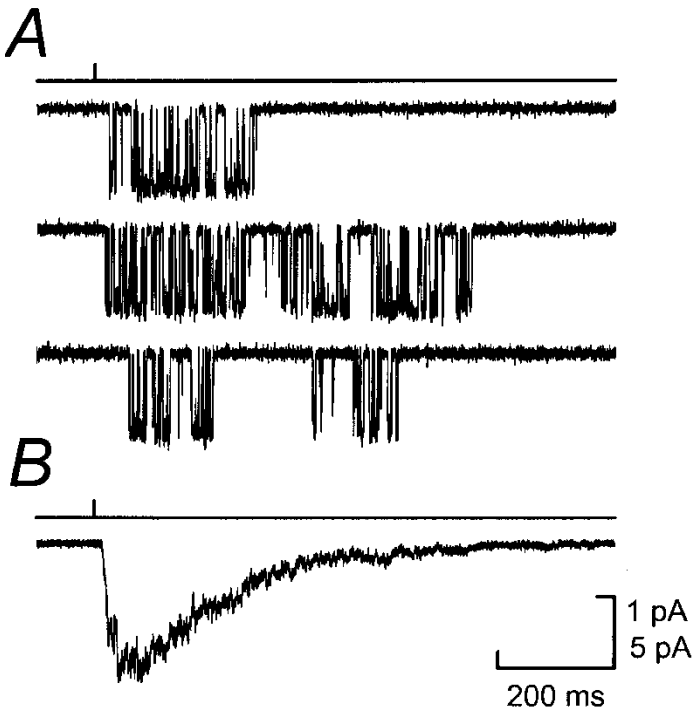
Na⁺ currents











The whole cell current recorded in B is the result of single channel openings in A

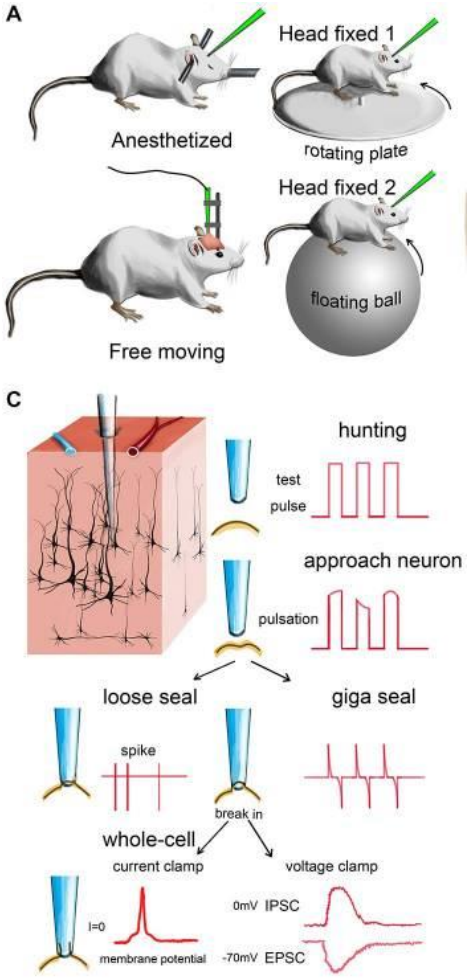
The whole-cell recording technique rapidly took over from conventional sharp electrode intracellular recording in preparations of cultured cells and *in vitro* studies.
The whole-cell technique is believed to result in less damage to the recorded cell and lead to a more faithful recording of the cell's electrical signals.

With the development of ***in vivo*** **patch-clamp recording**, researchers can not only directly measure neuronal activity, such as spiking responses or membrane potential dynamics, but also quantify synaptic inputs from excitatory and inhibitory circuits in living animals.

Patch clamp *in vivo*

Heart rate and breathing is relatively stable and smooth

To study higher brain function such as cognition

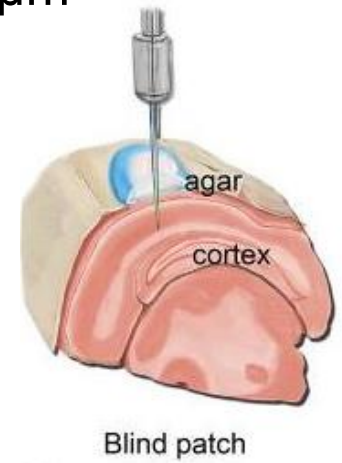


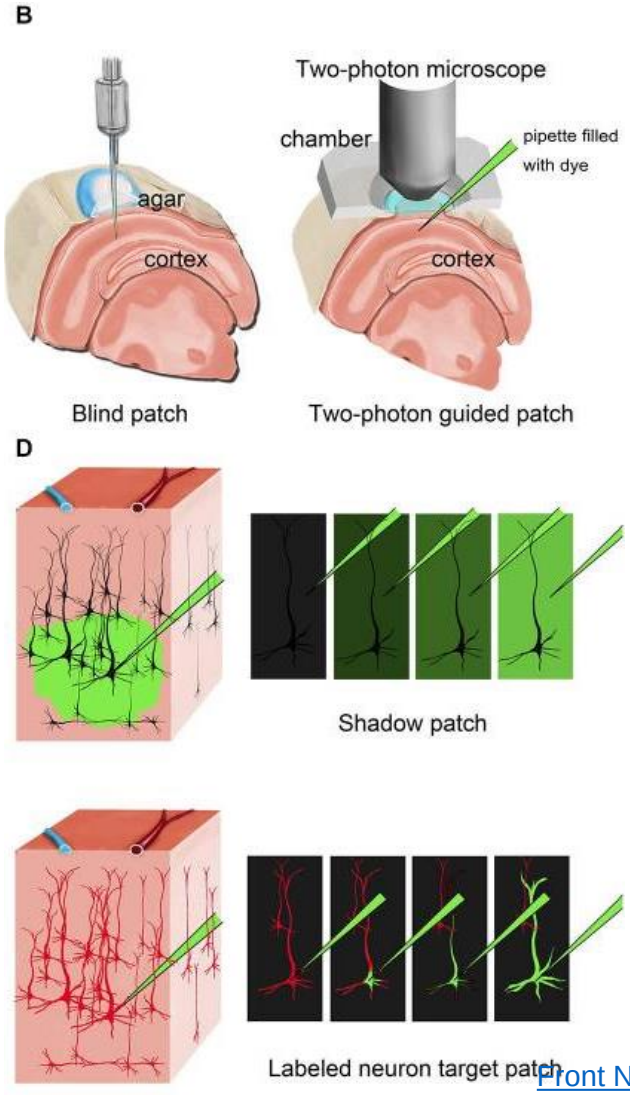
A stereoscope is used

Front Neural Circuits. 2015; 9: 23.

Imaging

Possible depth of imaging is generally less than 500 μm

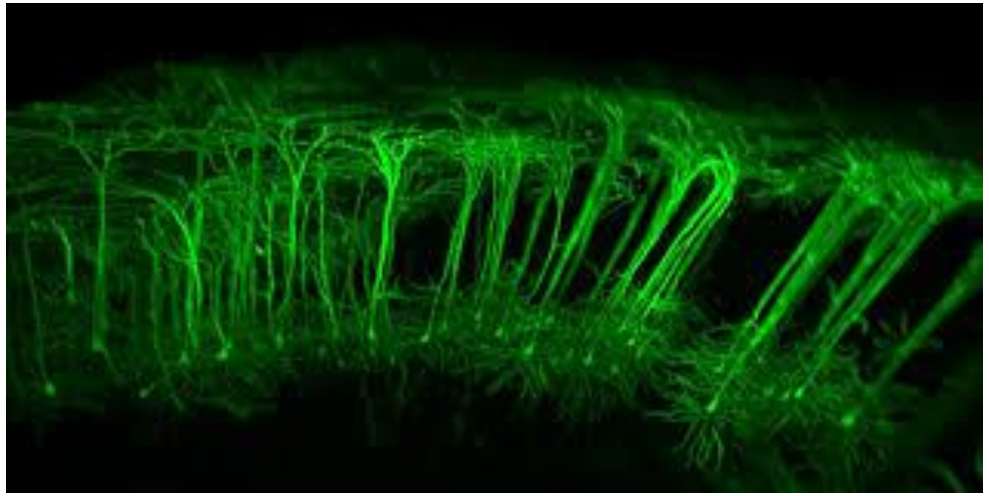


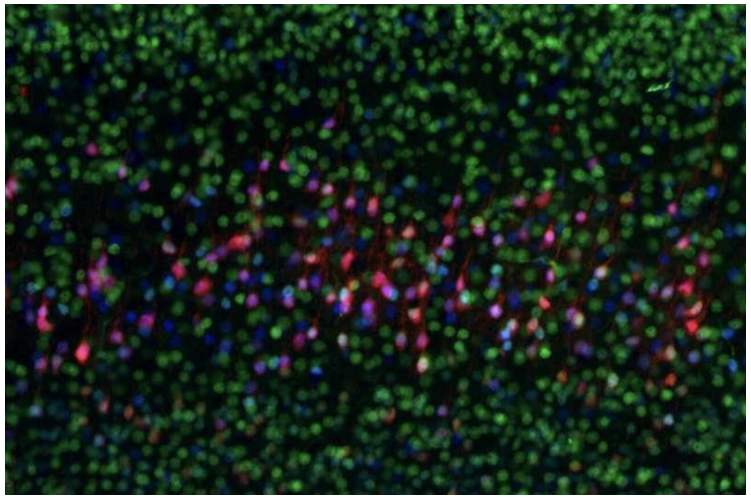


Combining *in vivo* patch-clamp recording with other techniques, such as two-photon imaging or optogenetics, can provide even clearer functional dissection of the synaptic contributions of different neurons or nuclei.

Front Neural Circuits. 2015; 9: 23.

It would be interesting to map the brain on the chemical identity of neurons



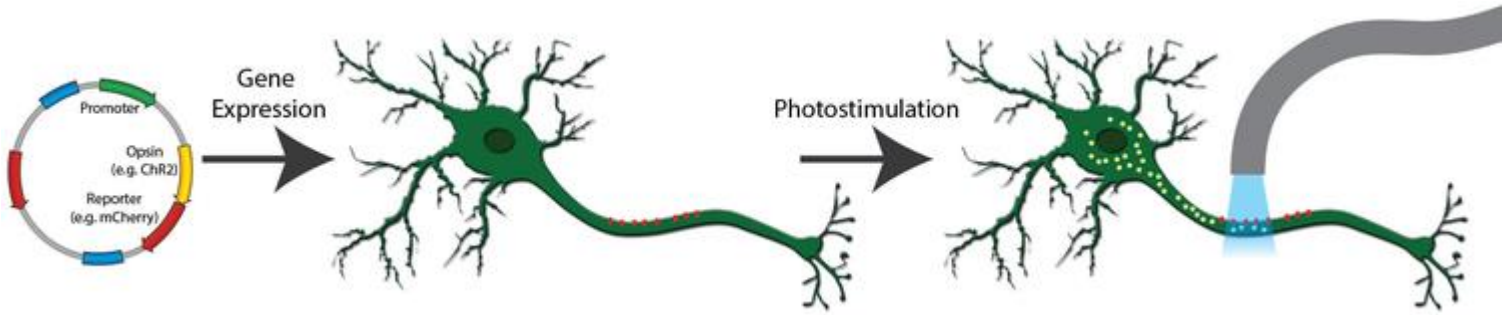


It is possible to install in neurons proteins (see channelrhodopsin) able of converting light into electricity and use laser light to stimulate specific cells

This allow to turn on or off the cells to control their participation to a neuronal network and stimulate them with magnetic transcranic stimulation

Optogenetics controls the activity of neurons with light

Genetic targeting strategies are developed such as cell-specific promoters or attenuated virus, to deliver the light-sensitive probes to specific populations of neurons.
Shining light on the specific neurons, light get converted to electricity.



Efforts have been made to confer light sensitivity onto ion channels to switch their activity

Optogenetics: A Light Switch for Neurons - YouTube

The major limitation of *in vivo* patch-clamp recording is its technical difficulty, which requires experienced personnel and a large amount of patience.

A completely automated *in vivo* patch-clamp setup is expected to be available in the near future as more research groups become interested in applying this tool to facilitate their research work.