Advanced Electrophysiology

Lesson 3

26 March 2025

Patch-clamp recordings

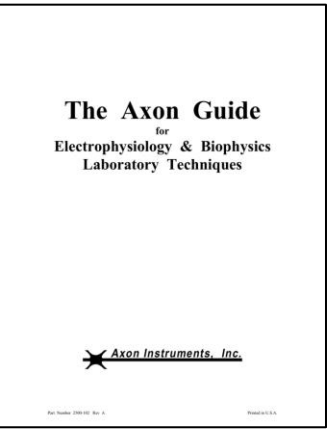
Calendar

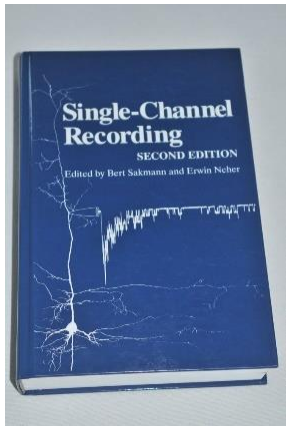
- **Th 27 March (14:00-18:00): Acute brain slice preparation (Room 309, Building Q)**
- **W 2 April (14:00-16:00): Data analysis project (ex-Cla; Bring a laptop!)**
- **Th 3 April (14:00-18:00): Patch-clamp (group 1; Room 309, Building Q)**
- **Th 10 April (14:00-18:00): Patch-clamp (group 2; Room 309, Building Q)**
- **Th 17 April (14:00-18:00): Patch-clamp (group 3; Room 309, Building Q)**

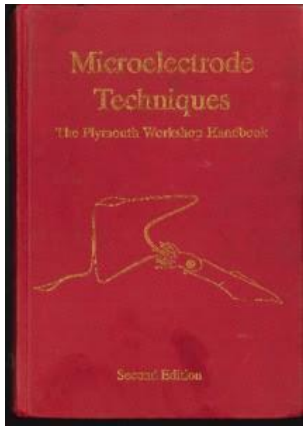
Patch-clamp recordings

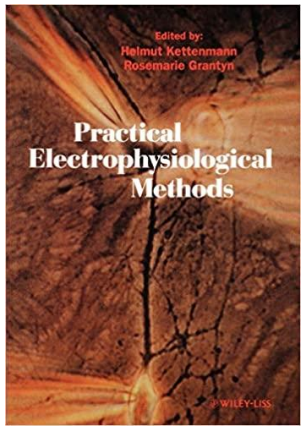
Learning objective:

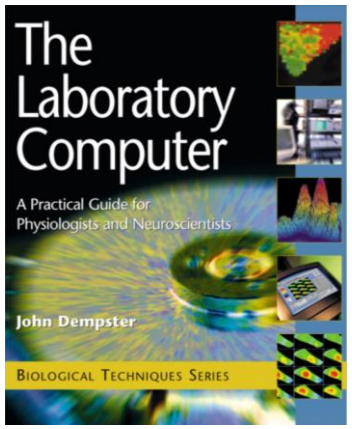
To understand why patch-clamp recordings are so important for neuroscience investigations

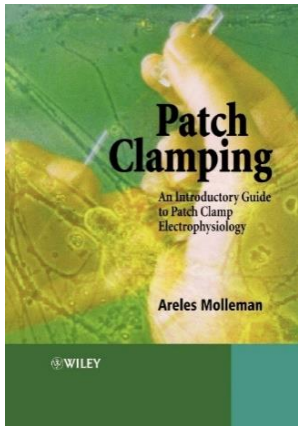












Outline

1. A bit of history

2. Patch-clamp configurations

3. Electronic aspects

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1. A bit of history

2. Patch-clamp configurations

3. Electronic aspects

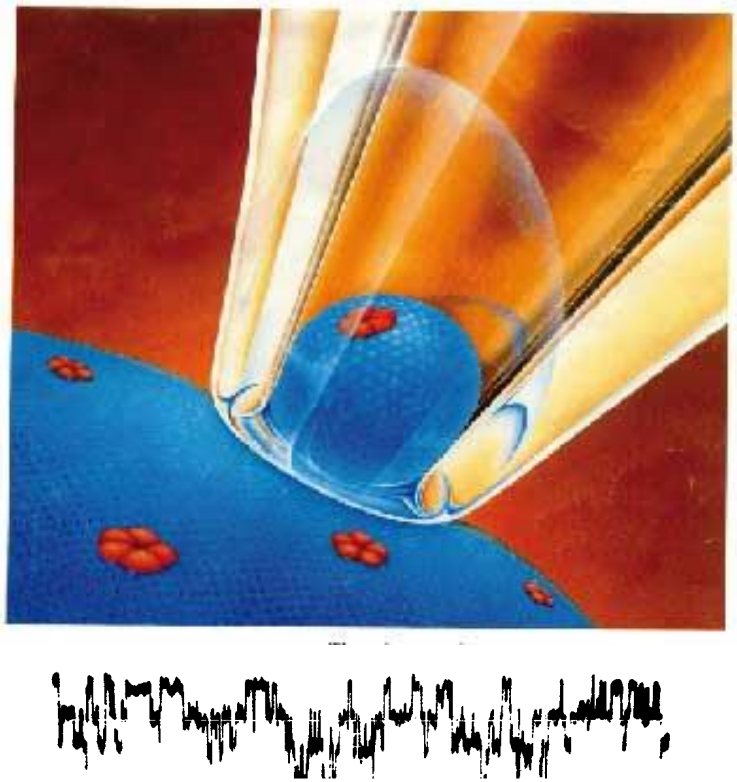
The ‘inventors’ of the patch-clamp

Erwin Neher

Bert Sakmann

Nobel Prize in Physiology or Medicine 1991

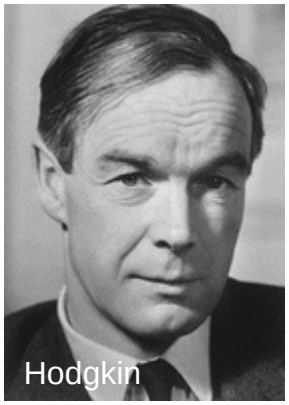
"for their discoveries concerning the function of single ion channel in cells"

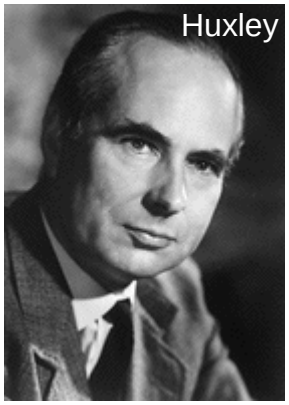


Inventors of the patch-clamp technique which for the first time allowed for direct recording of the current flowing through a single ion channels

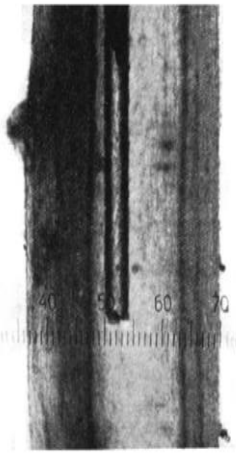
‘Pre-patch-clamp’ single cell electrophysiology

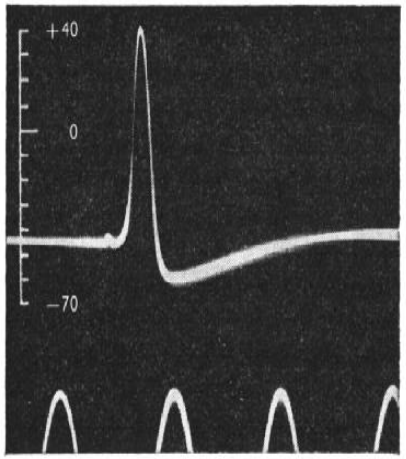
The Nobel Prize in Physiology or Medicine 1963





From 1939 to 1952, with a 5-year break due to the war, Hodgkin and Huxley, exploiting the work of Young (who found the best way to insert electrodes into the squid axon), gave an elegant and (still) correct explanation of the AP without ever mentioning the word channel. The concept of channel gained acceptance from the early 1970s thanks to the development of the patch-clamp technique (Neher and Sackman).

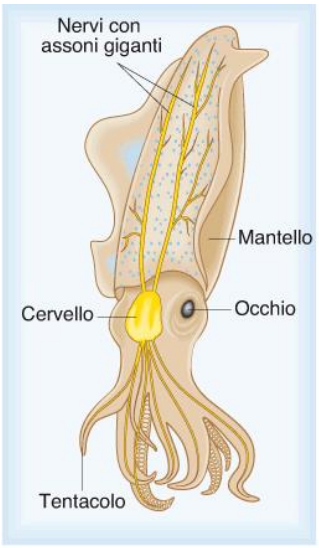




The first AP recording

Why the axon of the squid?

Because it is giant, the axon not the squid



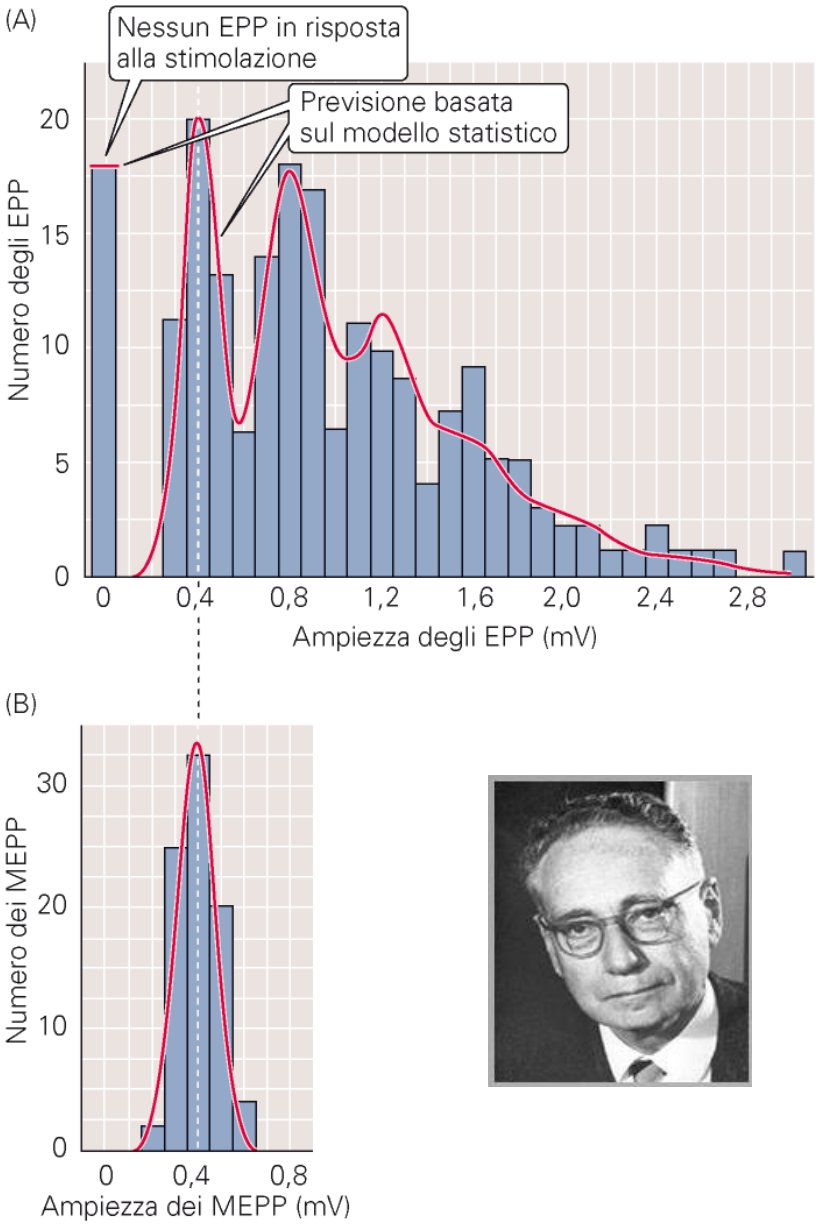
‘Pre-patch-clamp’ single cell electrophysiology

Much of the pioneering research on **quantal synaptic transmission** was conducted in the **frog neuromuscular junction** by **Bernard Katz** and collaborators at UCL in the **1950-1960s** .

Synaptic transmission either does not occur or if it does occur it does so as a multiple of an indivisible basic unit (the quantum)

Synaptic strength = N * P_r* q

N = number of release sites
P_r = release probability
q = quantal size (amplitude of 1 quantum, i.e. of 1 mEPP)

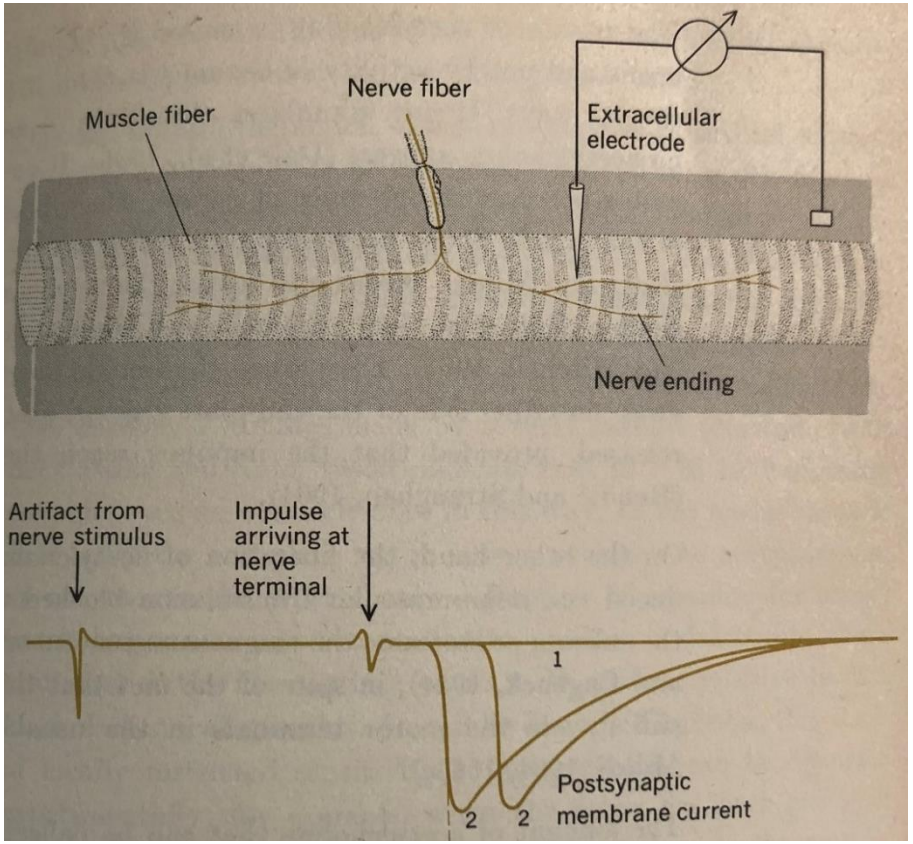


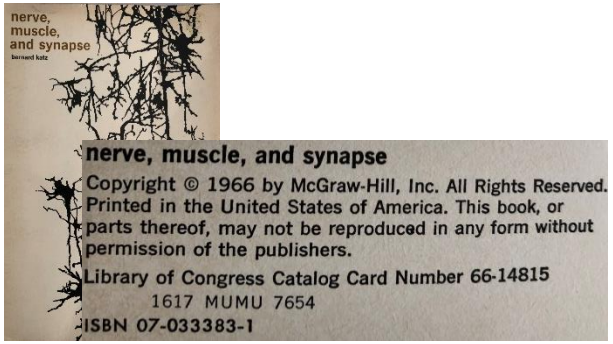
Sharp microelectrodes

Much of the pioneering research on **quantal synaptic transmission** was conducted in the **frog neuromuscular junction** by **Bernard Katz** and collaborators at UCL in the **1950-1960s** .

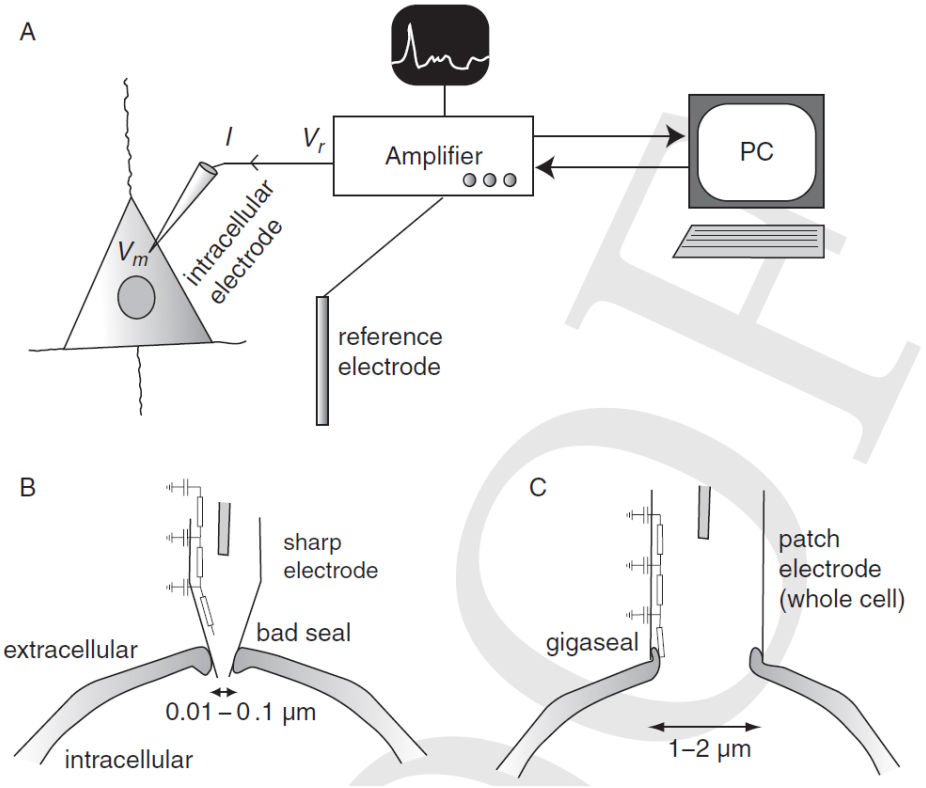
Neuromuscular junction: highly specialized synapse that convey a potent electrical signal to a very large post- synaptic cell.

The size of the end-plate current typically reaches 100 nA, roughly 3 to 4 orders of magnitude larger than synaptic currents at small central mammalian synapses

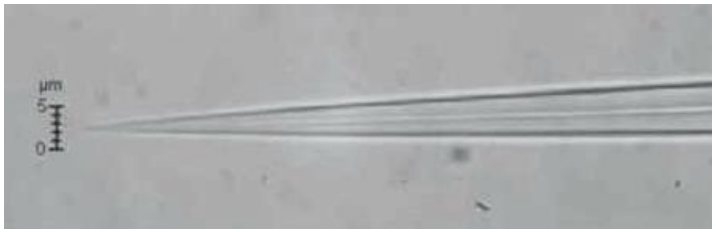


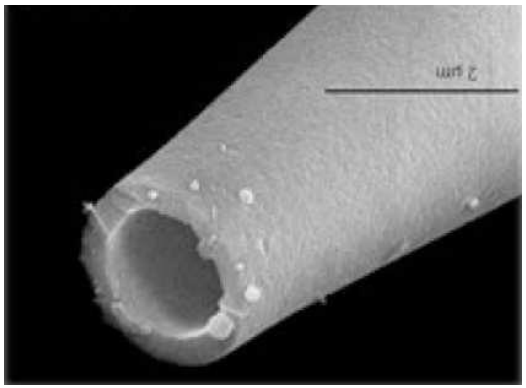


Sharp microelectrodes vs. patch-clamp electrodes



Patch-clamp electrode





	Sharp	Patch
Tip geometry	Thin	wide
Resistance	High (25-125 MΩ)	Low (<20 MΩ)
Dialysis	No	Yes
Seal	Bad (no seal)	Good (Giga Ω)
Noise	High	Low

Outline

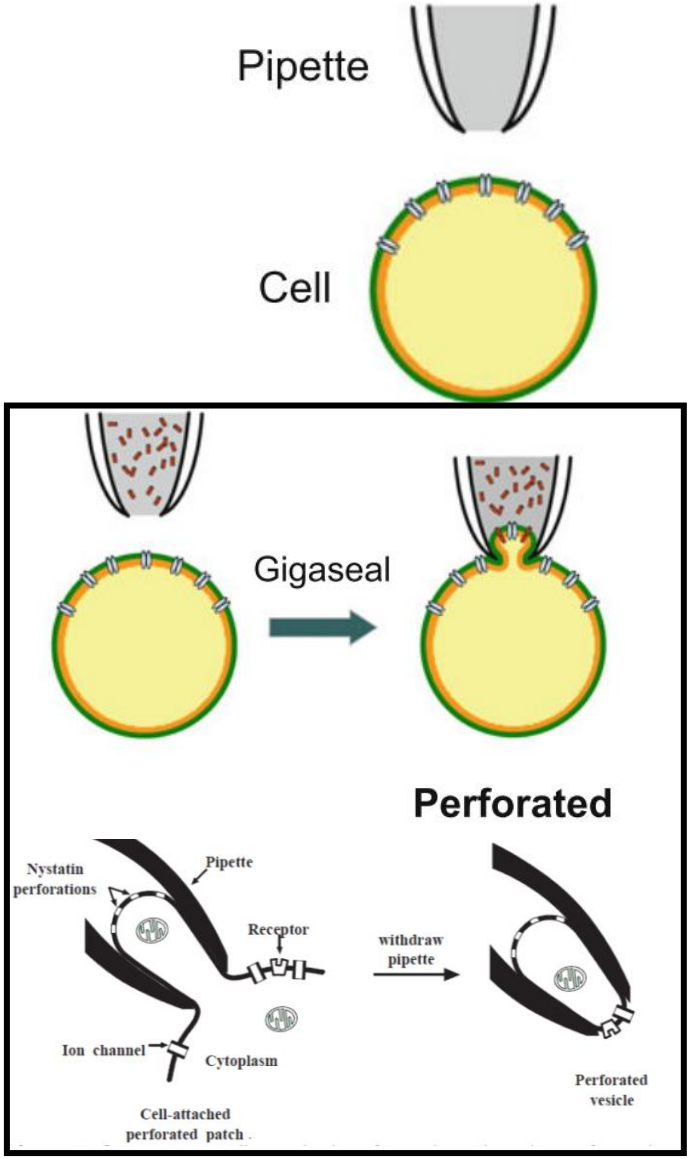
1. A bit of history

2. Patch-clamp configurations

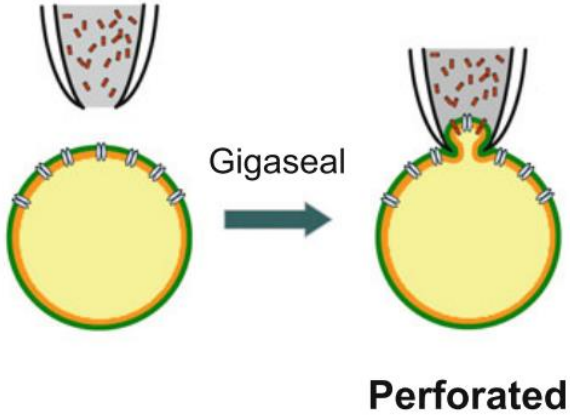
3. Electronic aspects

Patch-clamp methods

(Loo



Perforated-patch configuration



Gramicidin = impermeable to Cl^-

$$E_{eq} = \frac{RT}{zF} \ln \frac{[Cl^-]_0}{[Cl^-]_i}$$

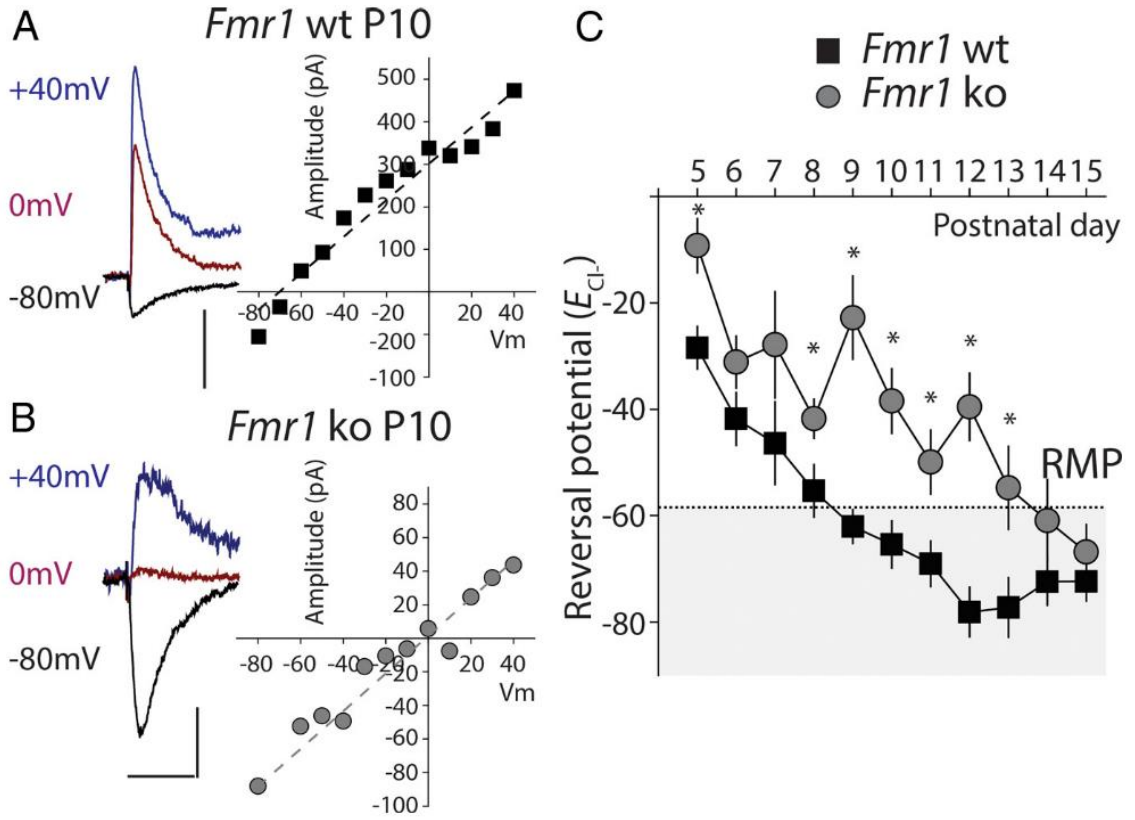


Figure 1. E_{Cl^-} remains depolarized in Fmr1 ko mice during cortical development. ***A***, Representative example of a perforated patch-clamp recording from a layer IV neuron in the somatosensory cortex of a P10 *Fmr1* wt mouse. Recordings were made at several hold potentials and the E_{Cl^-} calculated from the linear fit of the current–voltage relationship. GABA responses shown at −80, 0, and +40 mV were evoked by extracellular stimulation in the presence of glutamate blockers D-APV (50 μM) and CNQX (10 μM). Calibration for current traces: 50 ms, 200 pA. ***B***, Representative recording from *Fmr1* ko at P10 and current–voltage relationship of GABA-mediated currents. E_{Cl^-} is significantly more depolarized at this age in recordings from *Fmr1* ko mice. Calibration for current traces: 50 ms, 50 pA. ***C***, Grouped data from all recordings. The average E_{Cl^-} calculated from each individual recording is plotted against the age of the mouse (postnatal day). The RMP measured at P10 is denoted by the dashed line and shaded area represents points at which GABA would have a mature hyperpolarizing response. *$p < 0.05$ (P5: wt, $n = 13$; ko $n = 4$. P6: wt, $n =$

He et al., 2014

Loose-patch and cell-attached configuration

In common: **extracellular recordings of single cell activity**

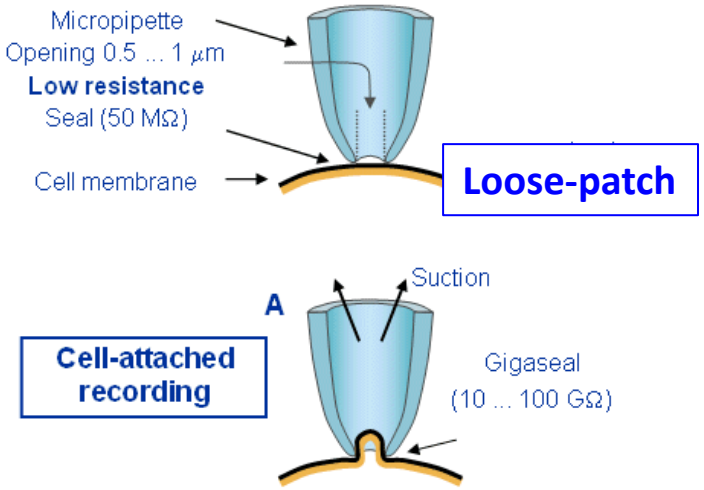
Differences: loose vs. tight contact

of a glass micropipette onto the cell membrane

(R_{seal} = MΩ vs. GΩ)

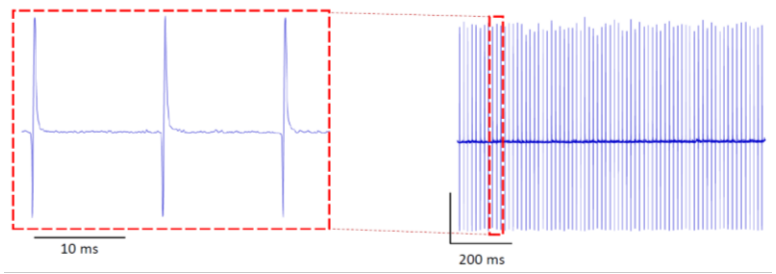
Loose-patch pro: **less invasive**

Cell-attached pro: **more sensitive**



For both configurations:

- **Pros**: easy (high success rate),
  no wash-out of ions and metabolites
- **Cons**: no control on V_m
  no possibility to use intracellular drugs

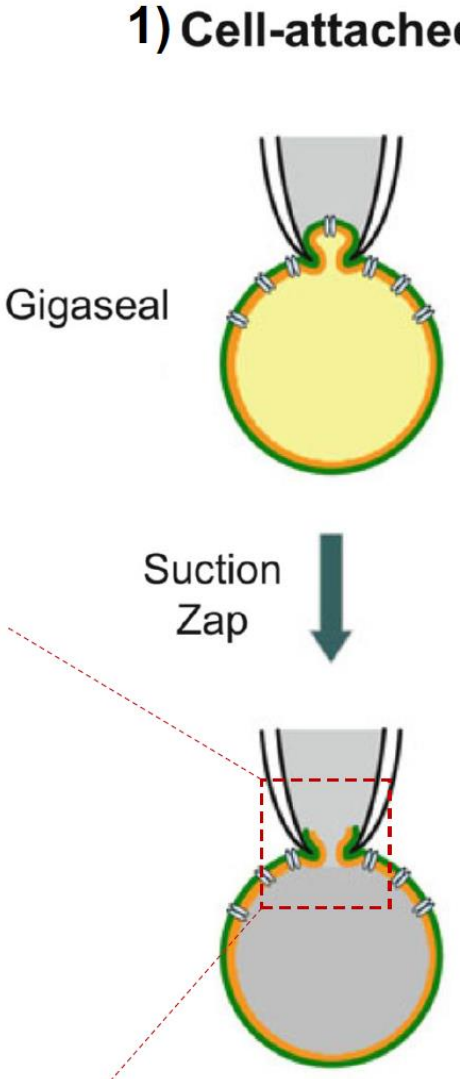


Both are suitable for the recording of action potentials from single neurons

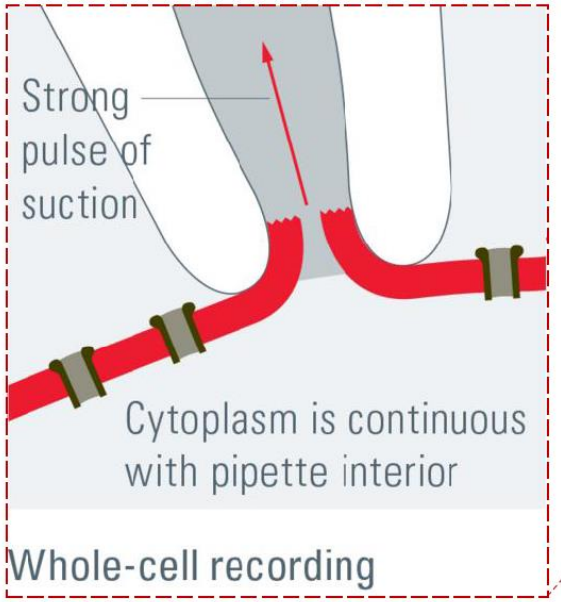
Whole-cell patch-clamp

Rupture of the membrane under the pipette without rupture of the giga-seal to obtain **chemical and electrical access to the cell**

- **Pros:** - control on V_m (**voltage-clamp**) or current (**current-clamp**)
  - possibility to use intracellular drugs
- **Cons:** - relatively skill-demanding
  - invasive (wash-out of ions and metabolites)

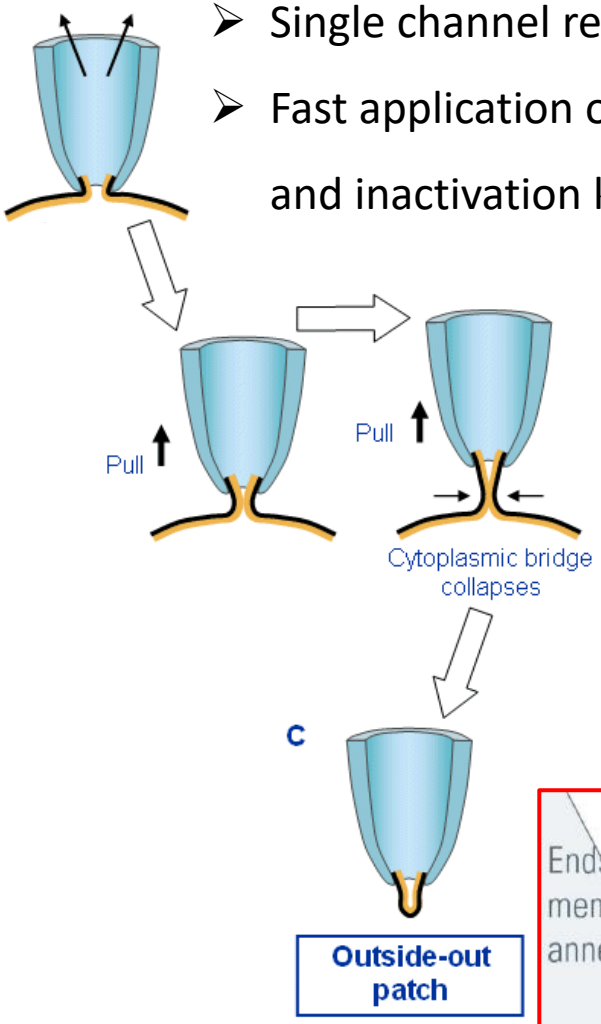


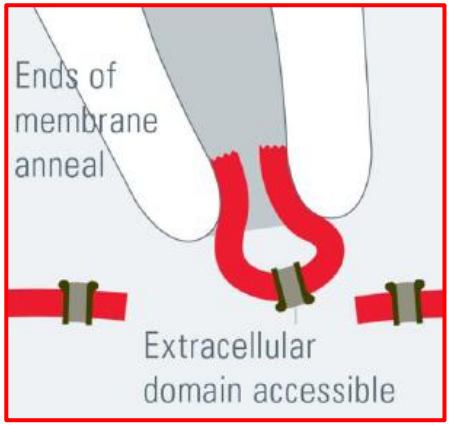
- In **current-clamp** configuration suitable for recording of APs and synaptic potentials (E/IPSPs)
- In **voltage-clamp** configuration suitable for recording **macroscopic** currents (voltage-gated or synaptic; e.g. E/IPSCs), but generally not suitable for single-channel recordings

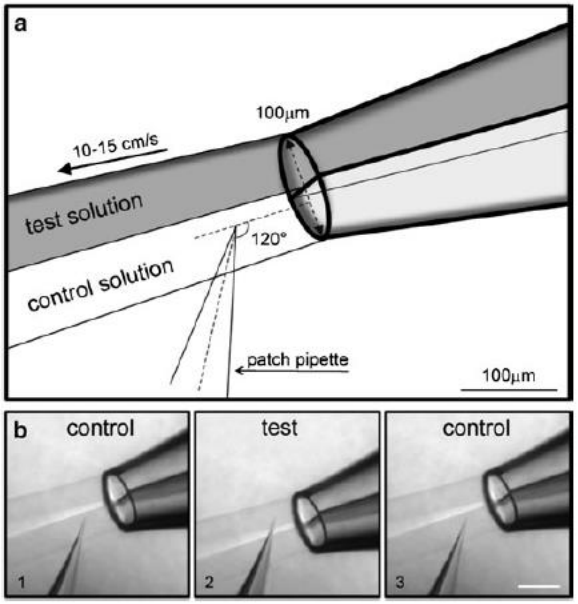


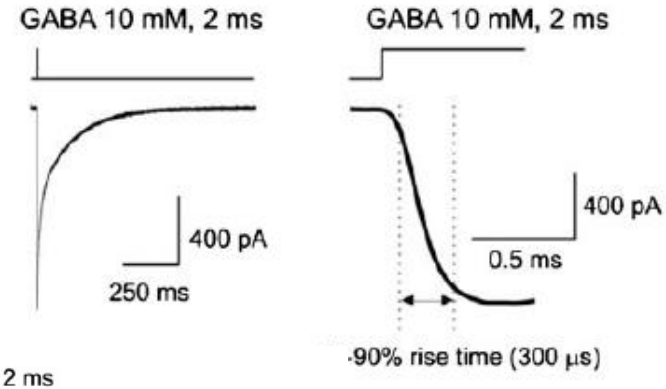
Outside-out

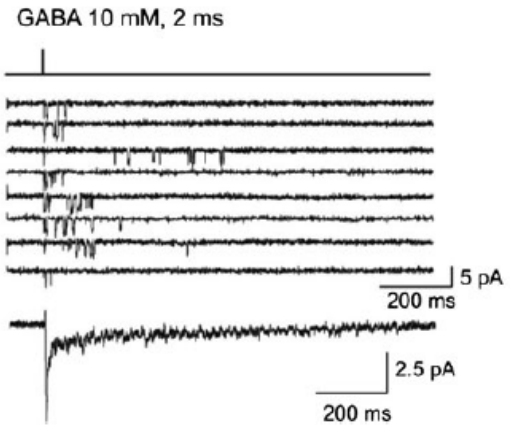
- Single channel recordings
- Fast application of drugs (e.g. to study activation and inactivation kinetics of channels)





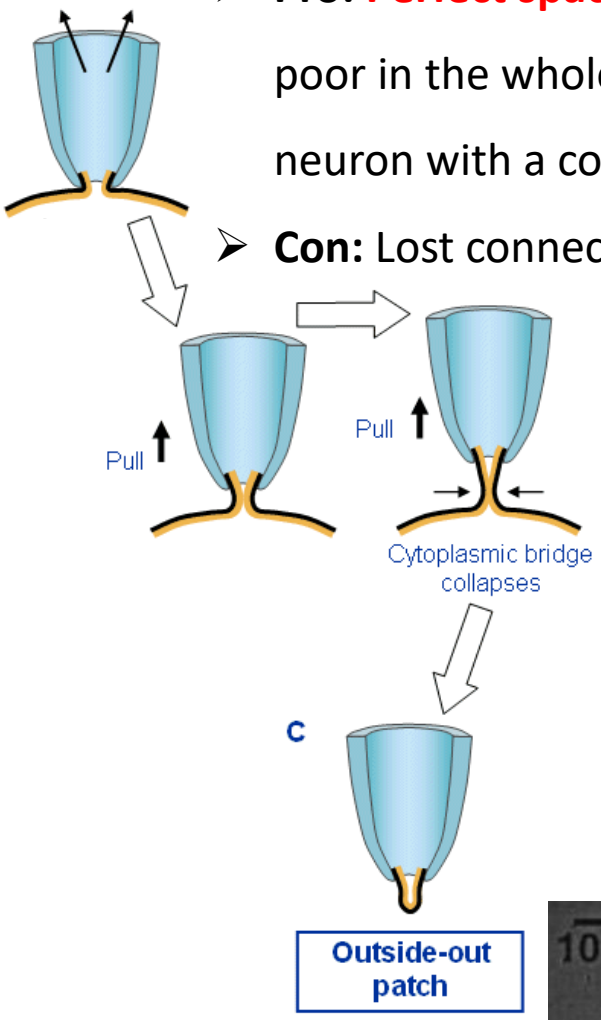


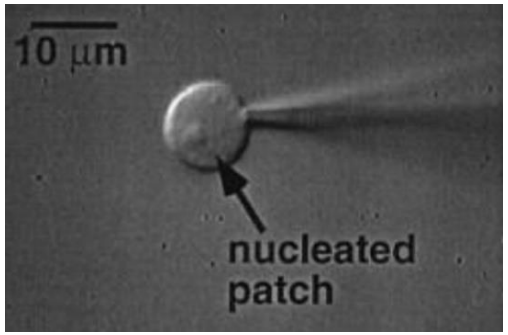


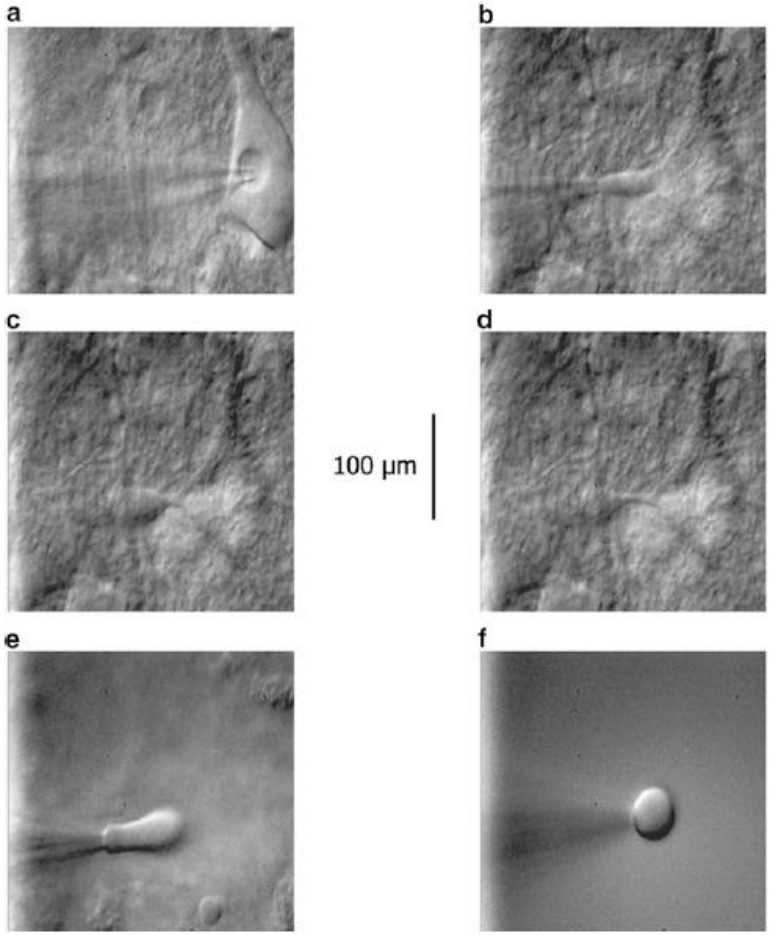


Outside-out nucleated patches

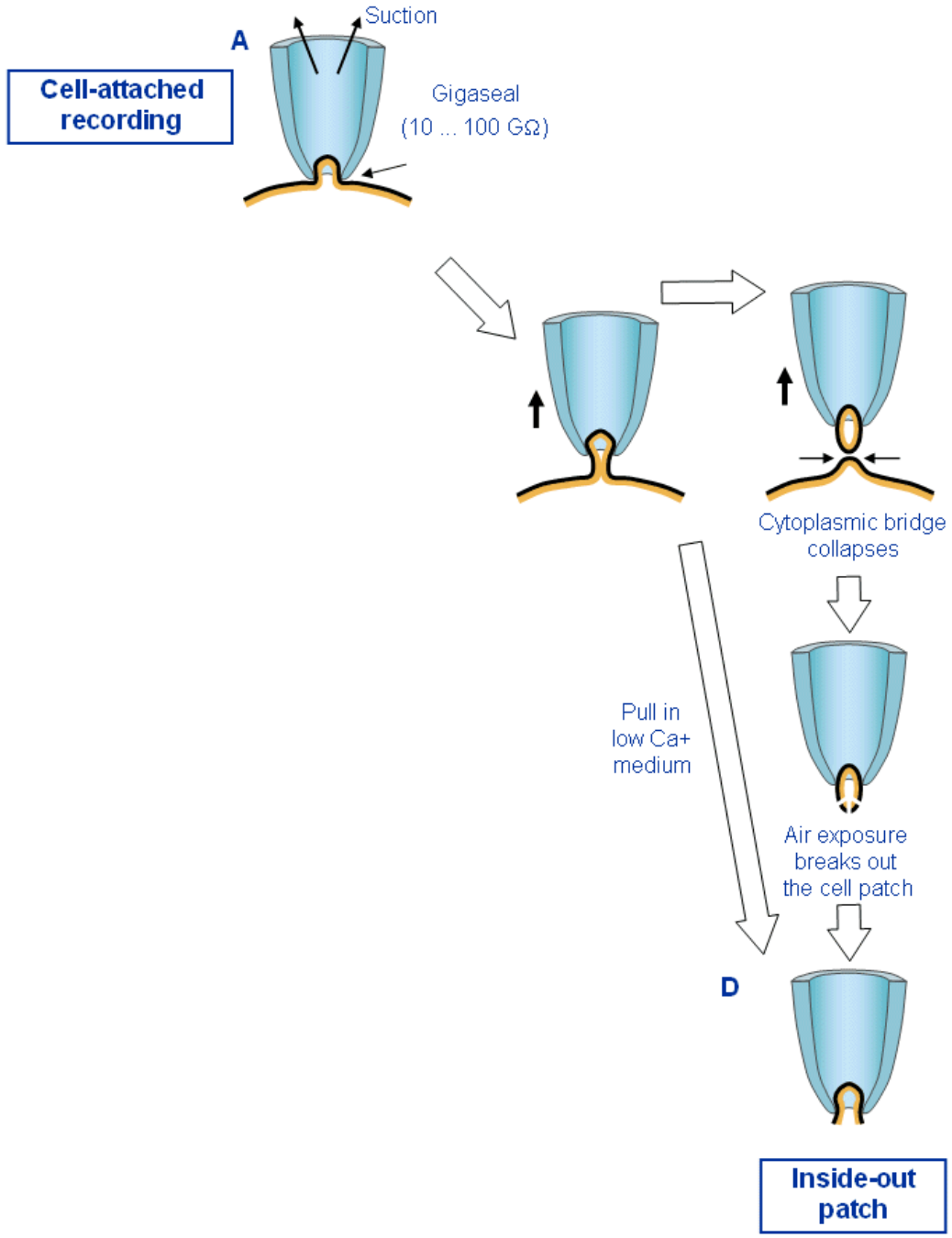
- **Pro:** **Perfect space-clamp** (space-clamp is poor in the whole-cell configuration for a neuron with a complex dendritic tree)
- **Con:** Lost connectivity







Inside-out


A
Suction
Cell-attached recording
Gigaseal
(10 ... 100 GΩ)
Cytoplasmic bridge collapses
Pull in low Ca+ medium
Air exposure breaks out the cell patch
D
Inside-out patch

Outline

1. A bit of history

2. Patch-clamp configurations

3. Electronic aspects

Current-clamp vs. voltage-clamp

In **current-clamp**, one applies a known constant or time-varying current and measures the change in V_m caused by the applied current. **This type of experiment is 'more physiological' because it mimics the currents produced by synaptic inputs.**

Current-clamp vs. voltage-clamp

In **voltage-clamp**, one holds constant (= clamps) V_m and **measures ion channel currents.**

Although voltage-clamp does not mimic a process found in nature, there are 3 reasons to do such an experiment:

1) **Usually, one has no interest in membrane currents *per se* but in the activity of a (homogeneous) group of ion channels (Conductance (G_{macro}) = number of open ion channel (N_{open}) times their single channel conductance (G_i)).**

   By holding V_m (i.e. the driving-force) constant, one ensures that $\mathbf{G_{total}}$ is proportional to the recorded macroscopic current (i_{macro})

We measure it (for each V_m)

$$\mathbf{G_{macro} = \frac{i_{macro}}{(Vm - Erev)}}$$

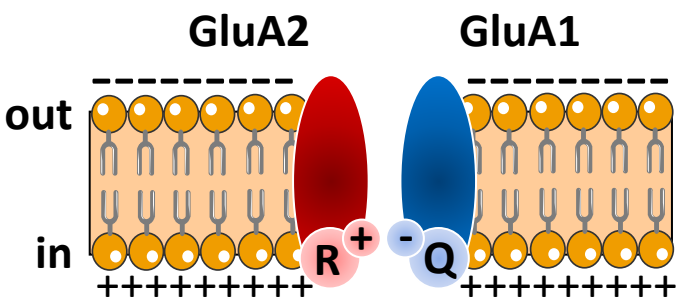
(Ohm's (first) law)

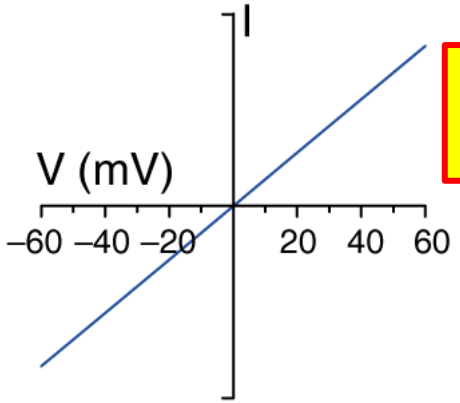
We hold it constant (or we change it step-wise as we please)

We estimate it using the Nernst equation and/or we empirically determine it

Current-clamp vs. voltage-clamp

Classical AMPAR currents





Linear
I–V curve

G = slope of the linear (= ohmic) I/V plot

We measure it (for each V_m)

$$G_{macro} = \frac{i_{macro}}{(V_m - Erev)}$$

(Ohm's (first) law)

We hold it constant (or we change it step-wise as we please)

We estimate it using the Nernst equation and/or we empirically determine it

Current-clamp vs. voltage-clamp

In **voltage-clamp**, one holds constant (= clamps) V_m and **measures ion channel currents.**

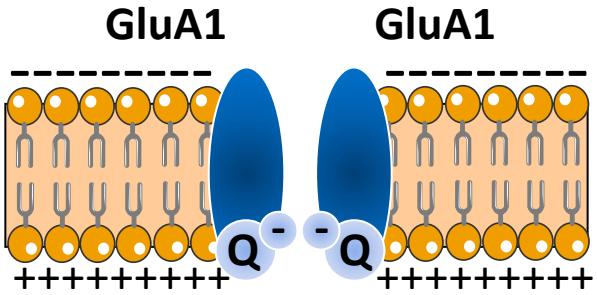
Although voltage-clamp does not mimic a process found in nature, there are 3 reasons to do such an experiment:

2) **If the gating of the channel is voltage-dependent (i.e. G_i itself depends on V_m), voltage-clamp offers control over a key variable that determines opening and closing of the ion channel.**

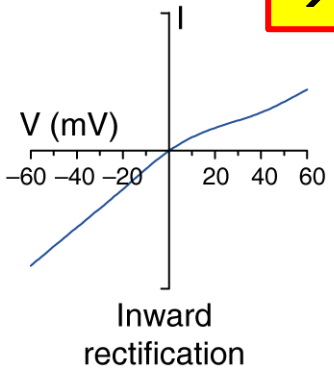
$$G_{macro} = \frac{i_{macro}}{(Vm - Erev)}$$ (Ohm's first) law)

Current-clamp vs. voltage-clamp

GluA2-lacking AMPAR currents



The I/V plot is not linear
→ G_i is voltage-dependent



Spermine

$G_{macro} = \frac{i_{macro}}{(Vm - Erev)}$ (Ohm's first) law)

Current-clamp vs. voltage-clamp

In **voltage-clamp**, one holds constant (= clamps) V_m and **measures ion channel currents.**

Although voltage-clamp does not mimic a process found in nature, there are 3 reasons to do such an experiment:

3) **Clamping V_m eliminates the capacitive current, except for a brief time following a step to a new voltage.**

$$i_T(t) = C\frac{dV_m}{dt}$$

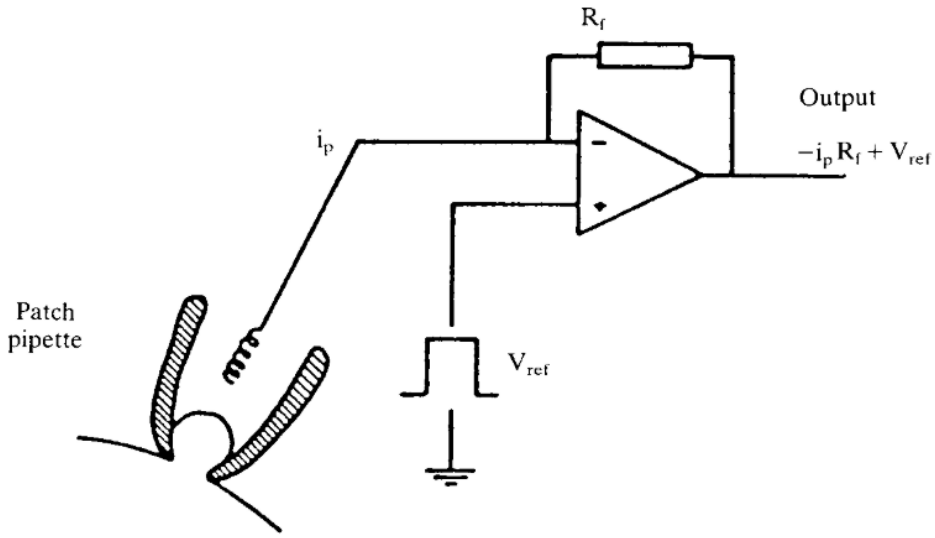
Voltage-clamp configuration

In **voltage-clamp**, one holds constant (= clamps) V_m and '**measures ion channel currents'.**

How?

The voltage-clamp circuit is a negative feedback device designed to monitor V_m and inject current into the cell to hold V_m constant ⟹ The amplifier 'knows' the ion channel currents by the current it needs to inject to keep V_m constant!!!

In the whole-cell patch-clamp configuration, one single pipette is used to simultaneously monitor V_m and inject current!!!!

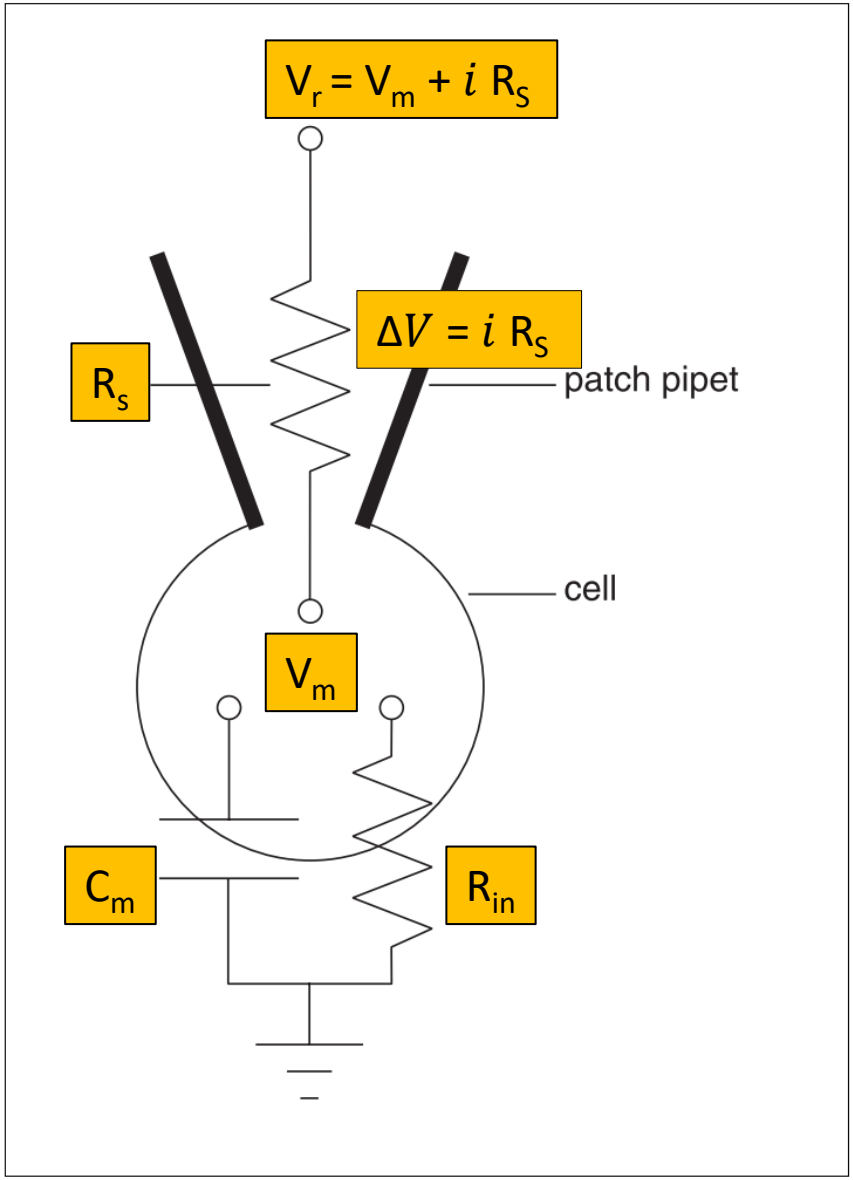


Voltage-clamp configuration

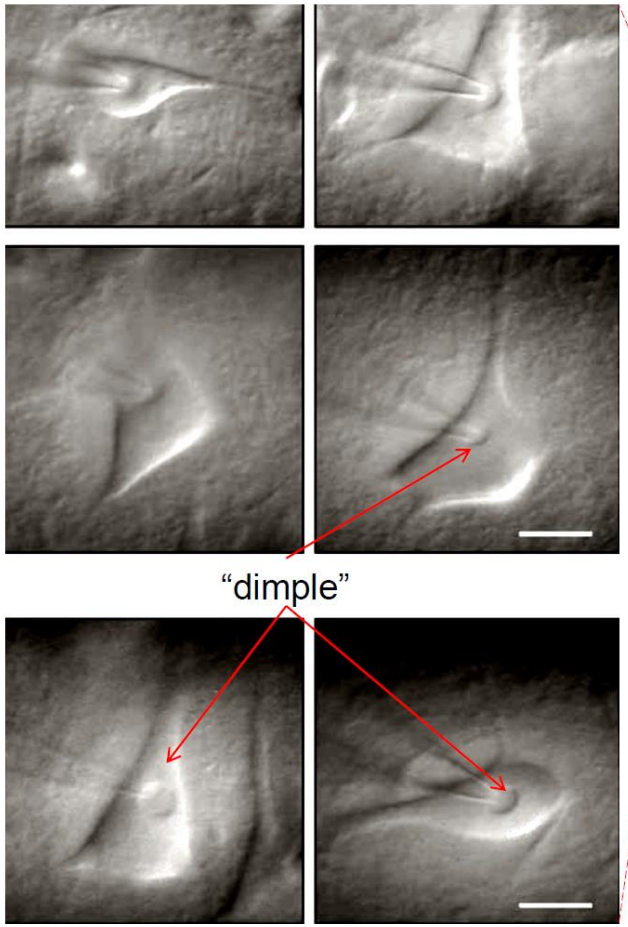
Problem of using one single pipette to simultaneously monitor V_m and inject current: **V** is recorded by the amplifier at the top of the pipette (**V_r** rather than **V_m**); this is the sum of V_m (which we wish to control) and the ΔV drop across the pipette resistance (**$\Delta V = i\ R_S$**).

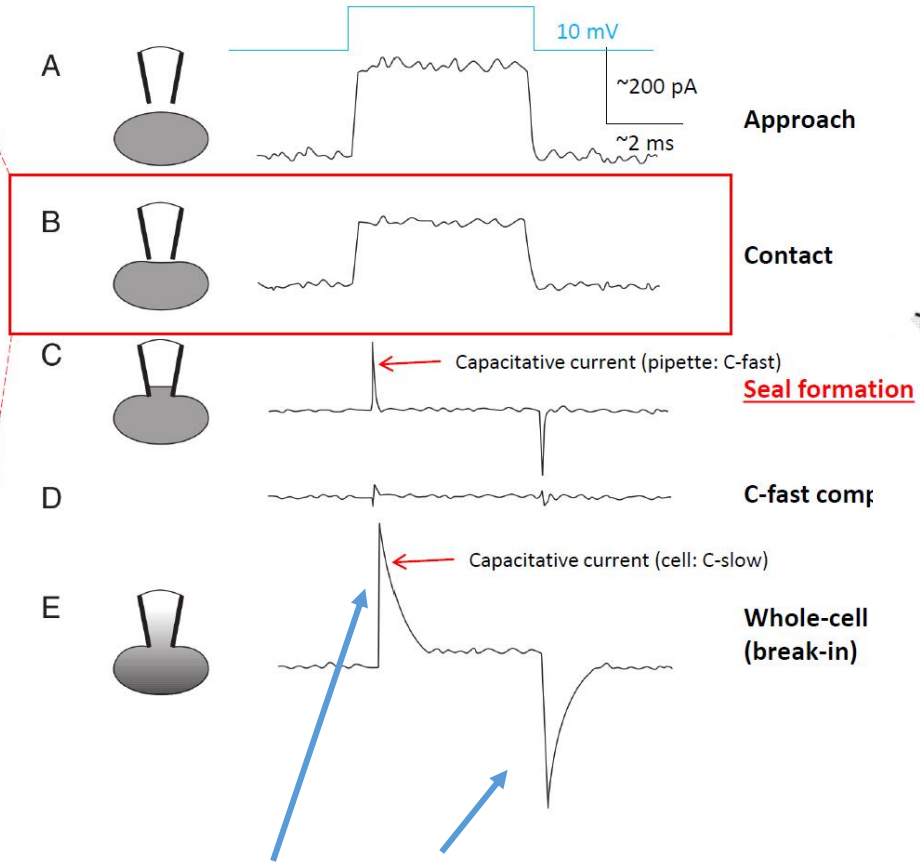
To avoid introducing voltage errors:

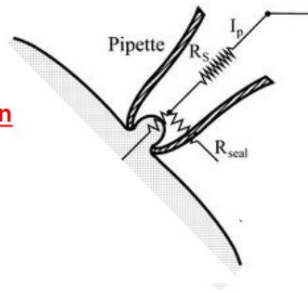
1) R_s must be as small as possible (<20 MΩ, **this is possible only with patch-clamp electrodes!!!**); R_s must also be as constant as possible;
2) Recorded currents must be as small as possible (tens of pA range): **the amplifier does not measure directly ion channel currents but the current it needs to inject to keep V_m constant!!!**)



The formation of a giga-seal







3) **Clamping V_m eliminates the capacitive current, except for a brief time following a step to a new voltage.**

Voltage-clamp configuration

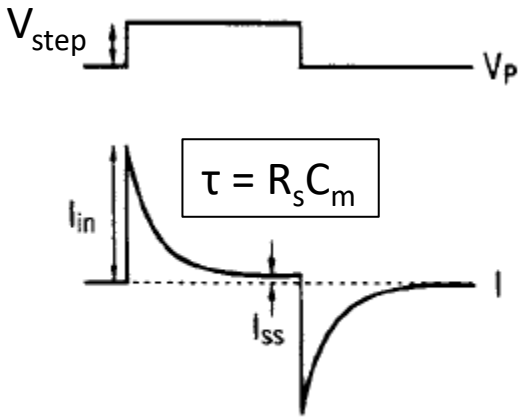
3) **Clamping V_m eliminates the capacitive current, except for a brief time following a step to a new voltage.**

If $R_s << R_{in}$

$$R_s = \frac{V_{step}}{i_{in}}$$

$$C_m = \frac{\tau}{R_s}$$

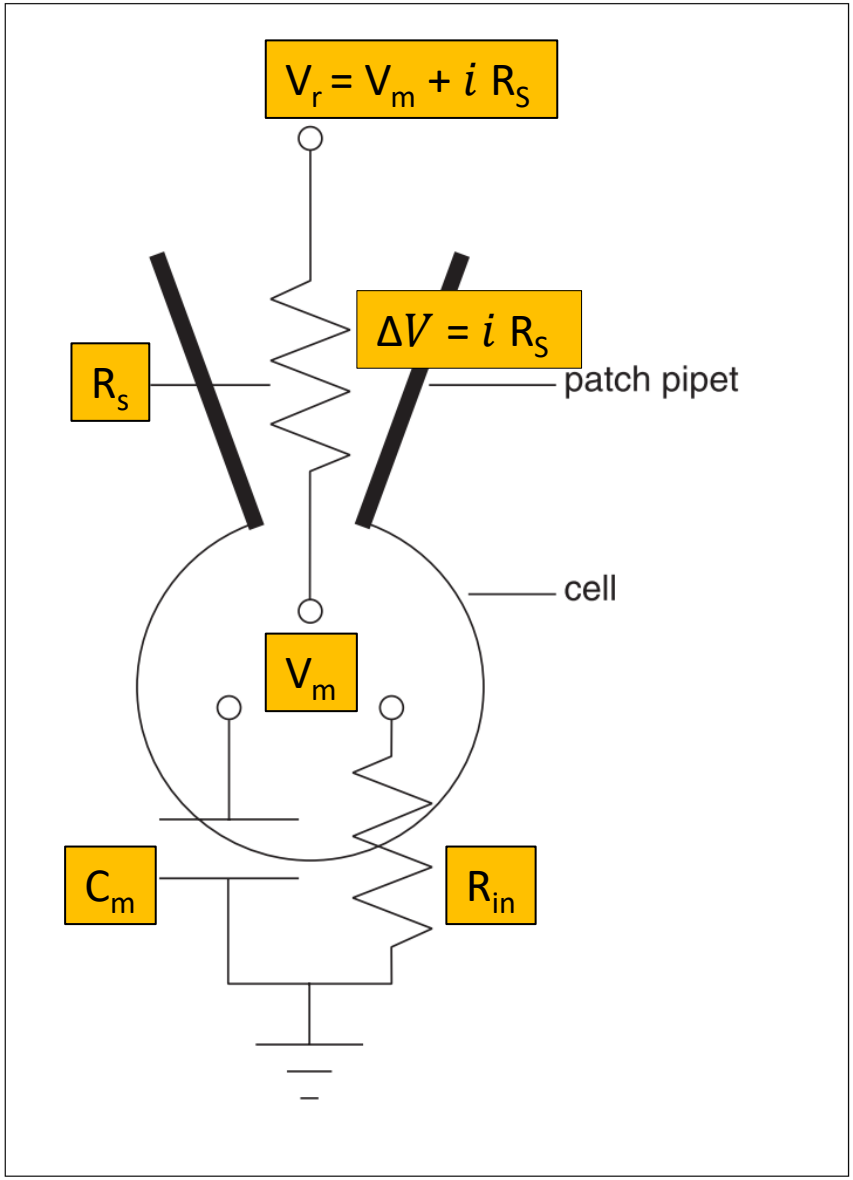
$$R_{in} = \frac{V_{step}}{i_{ss}}$$



$$V_{step} = R_s \frac{dQ}{dt} + \frac{Q}{C_m}$$

$$Q = V_{step} C_m (1 - e^{-\frac{t}{RsCm}})$$

$$i = \frac{dQ}{dt} = \frac{V_{step}}{R_s} e^{-\frac{t}{RsCm}}$$



Learning objectives

1. **To know the major differences between sharp and patch-clamp electrodes**
2. **To know the major patch-clamp configurations and what they are good for**
3. **To understand the differences between current-clamp and voltage-clamp**
4. **To understand advantages (and limitations) of the whole-cell voltage clamp configuration**