Electrophysiology III

The Biological Basics of Electrophysiology

Monday, 04/21/2008

Electrophysiological Techniques

Monday, 04/28/2008

More about Patch Clamp

Today, 05/05/2008

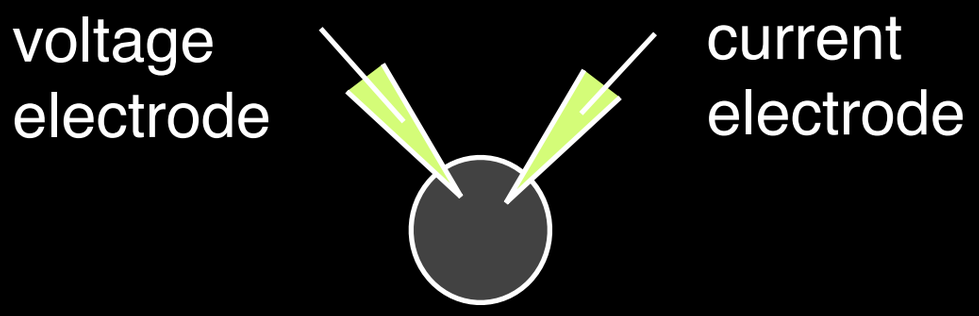
Calcium Imaging

Monday, 05/12/2008

Voltage Clamp

two main techniques to measure macroscopic whole cell current

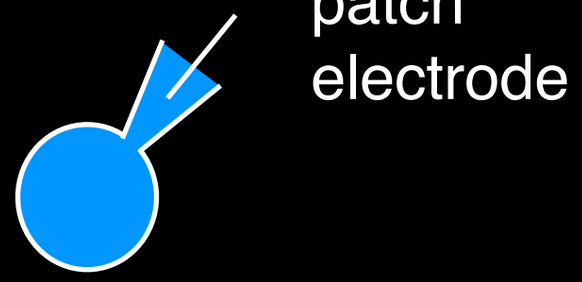
two electrode voltage clamp (TEVC)



two intracellular microelectrodes

vary external conditions

whole cell patch clamp



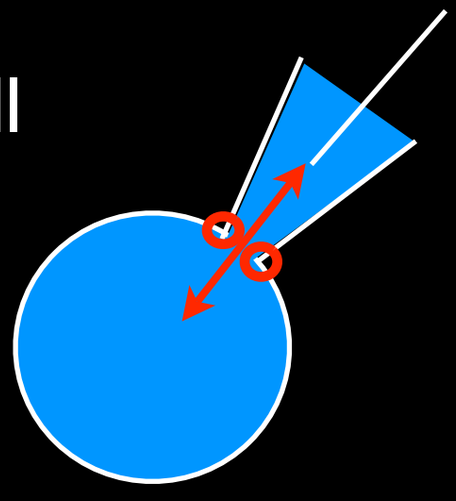
one wide tipped patch electrode

vary external and internal conditions

requirements

high resistance seal
between glass and cell
membrane (giga seal)

low resistance
access to the cell



Patch Clamp

Whole Cell Patch Clamp Experiment Step by Step

what happens at each step

what is the background of what we see

what are we (or EPC9) doing

why are we (or EPC9) doing this

Input Resistance

Offset

Voltage Ramp

C_{SLOW}

C_{FAST}

Pipette Resistance

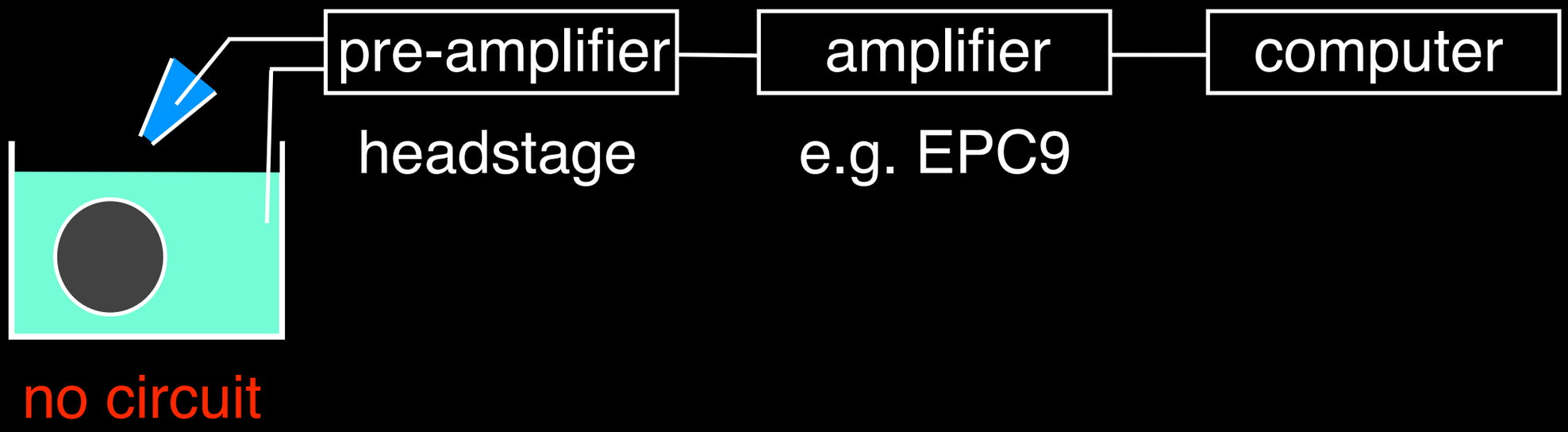
Capacitance

Series Resistance (R_S)

Compensation

START Configuration

situation - pipette connected to amplifier but not in bath yet



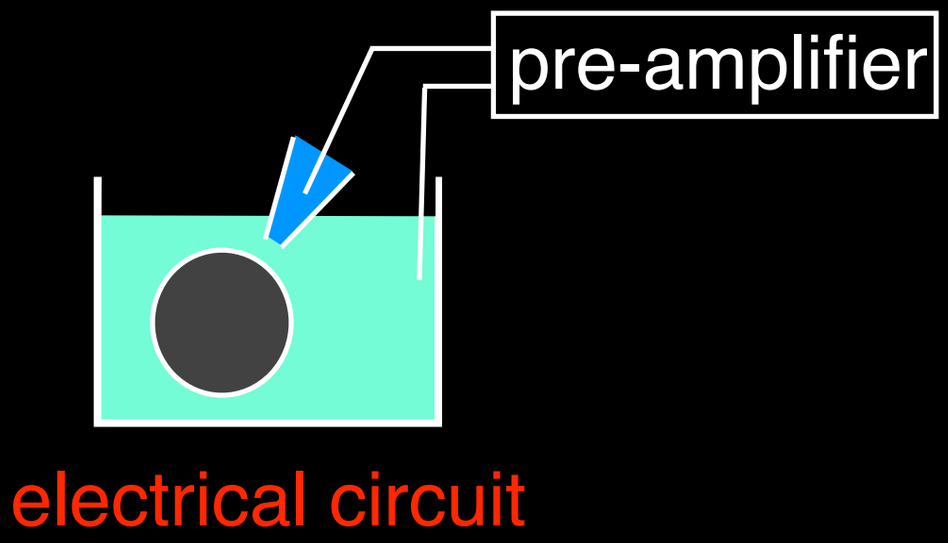
patch pipette filled with internal solution

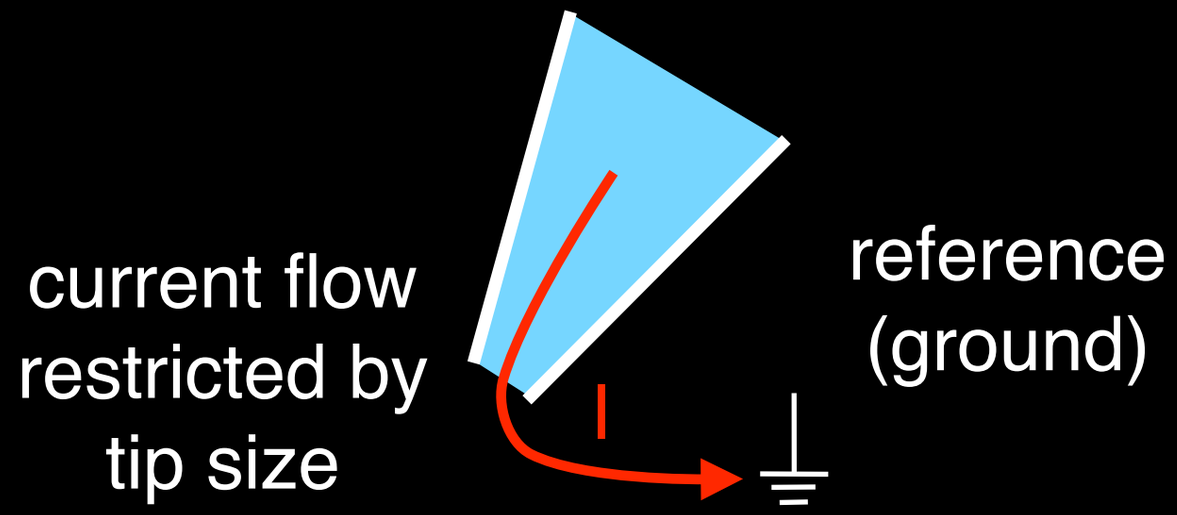
cell bathed in external solution

reference electrode (ground) in the bath

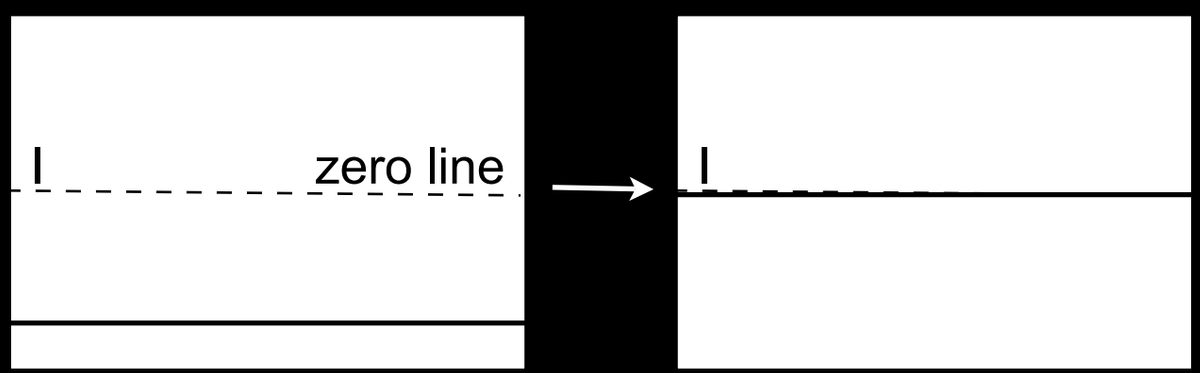
Pipette in Bath - Zero Offset

situation - pipette in the bath solution





① correct offset current to zero



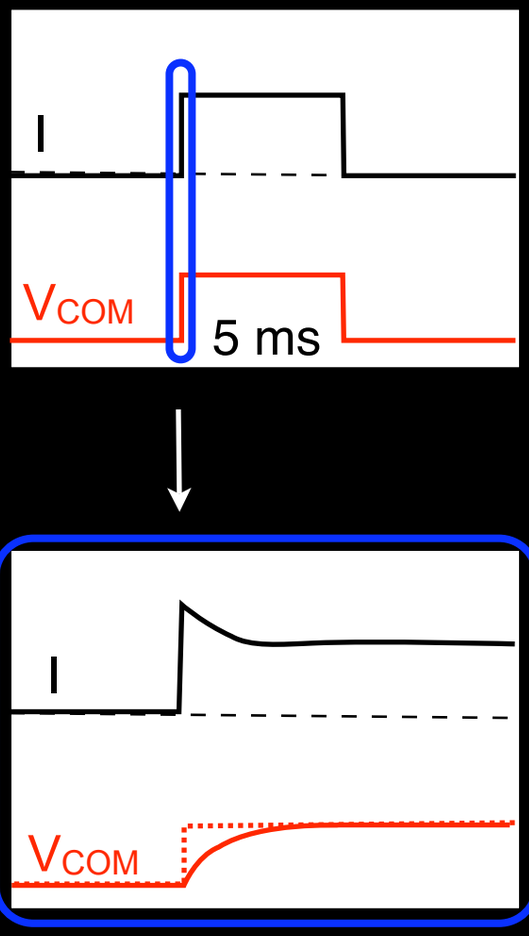
offset sources are e.g.

- junction potentials (Ag/AgCl electrodes)
- liquid junction potential
- amplifier offset(s)

Pipette in Bath - Pipette Resistance

situation - pipette in the bath solution

② measure pipette resistance by setting a defined voltage step



$\tau_P = R_P \times C_P$

(~1 μs)

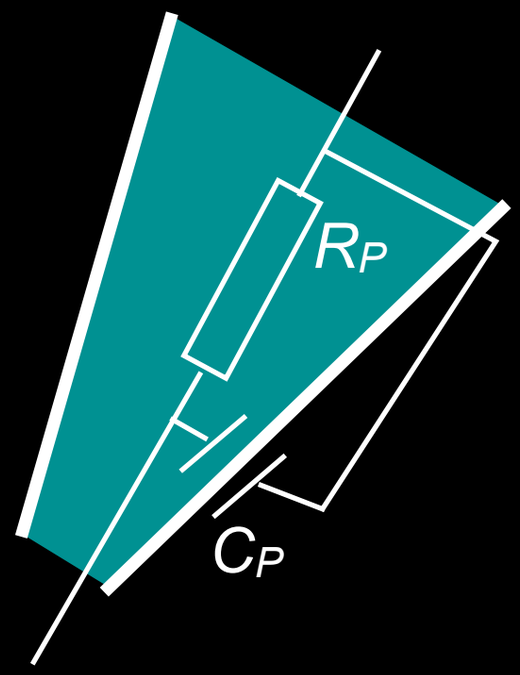
$$R = \frac{V}{I}$$

example

application of 10 mV step (command)

results in 5 nA current (reaction)

=> $R_{Pipette}$ 2 MΩ



$R_{Pipette}$

tip opening

intracellular solution

$C_{Pipette}$

glass (thickness)

shape of the tip

(surface in bath solution)

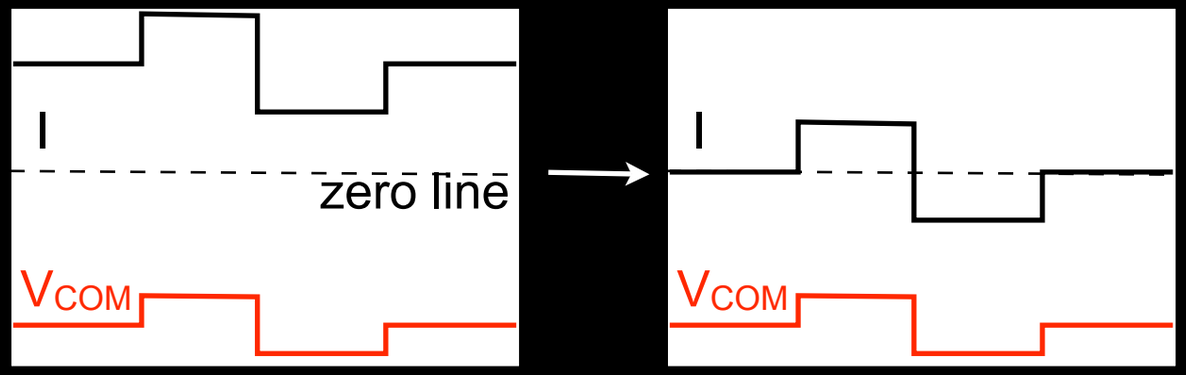
capacitor is charged first, since easiest way for current to ground

coating with hydrophobic material decrease capacitance of pipette

Pipette in Bath

situation - pipette in the bath solution

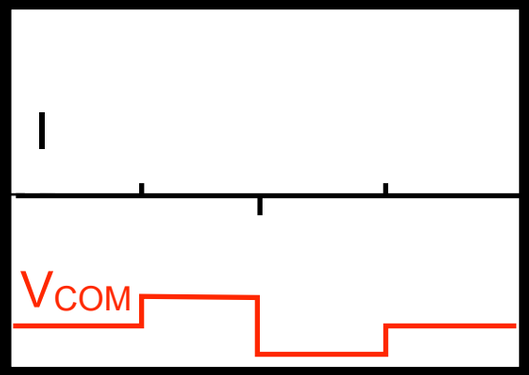
we usually zero offset and do pipette resistance check in one step



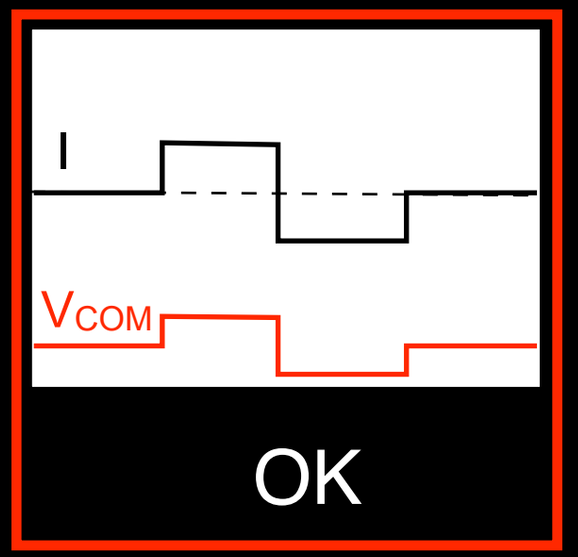
5 ms to +10 mV
5 ms to -10 mV

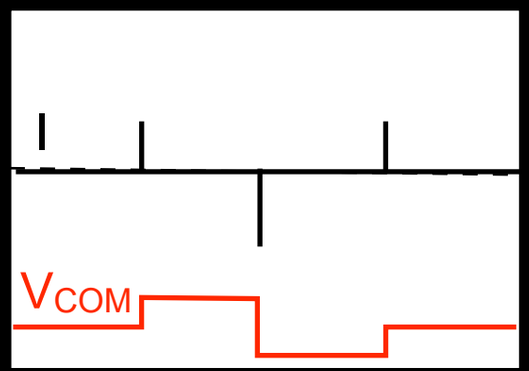
Pipette in Bath - What if ...

what happened if:

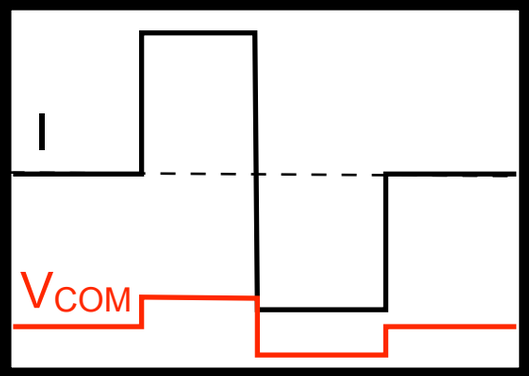


no real circuit
check reference electrode

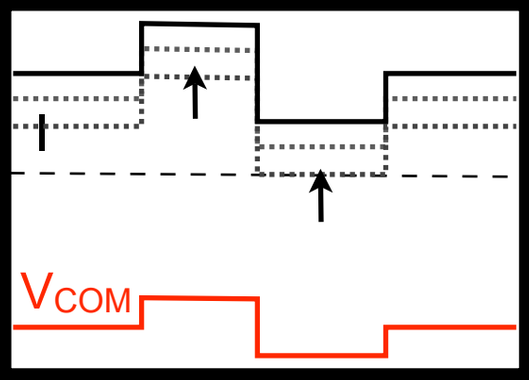




pipette clogged
high resistance
=> only capacitative current transients



probably broken pipette tip
very low resistance

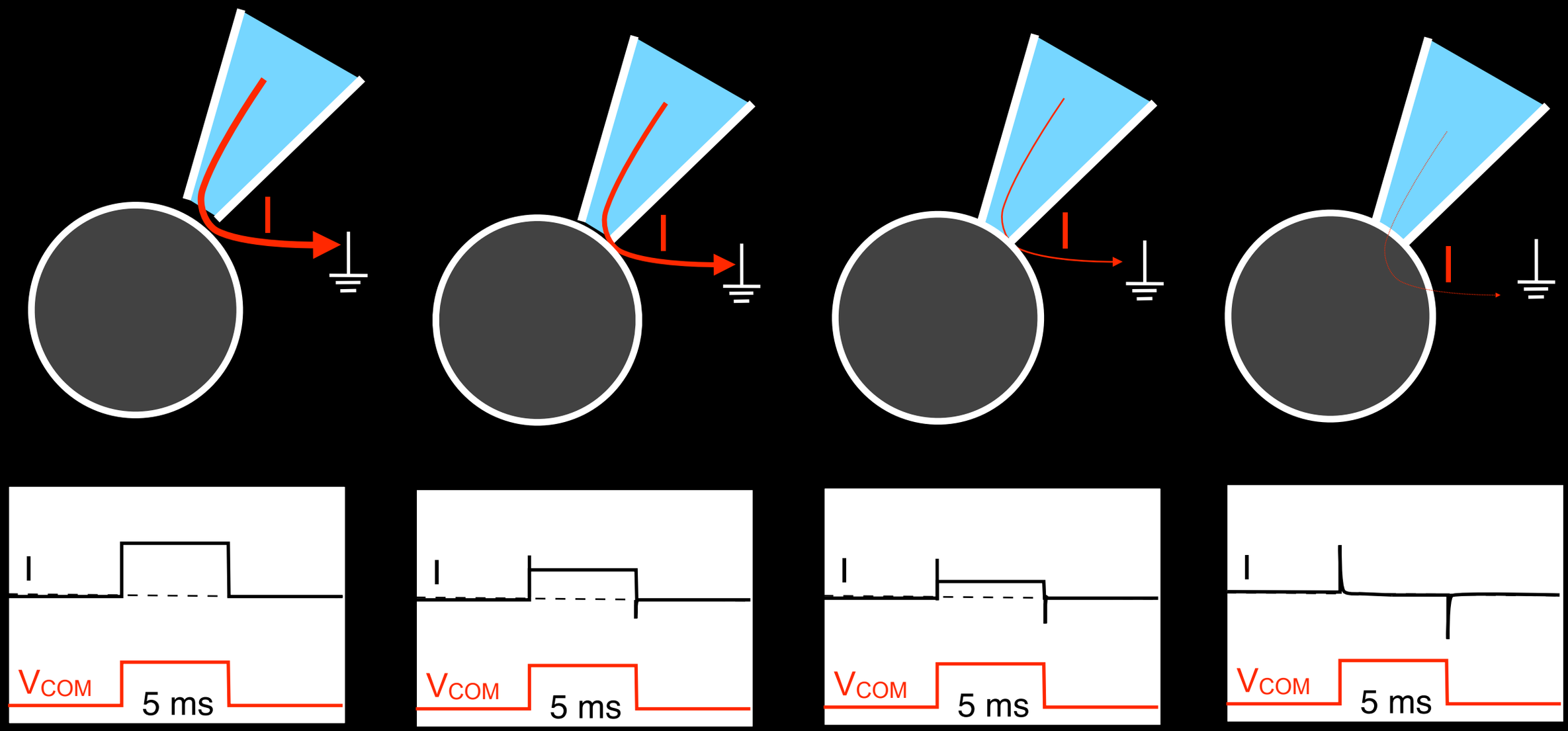


offset takes long, is impossible or baseline moves
check for chlorided Ag wires
happens sometimes with freshly chlorided wires

Getting a Giga Seal

getting a giga seal between pipette and cell membrane

approach the cell and when touching apply gentle suction to the pipette



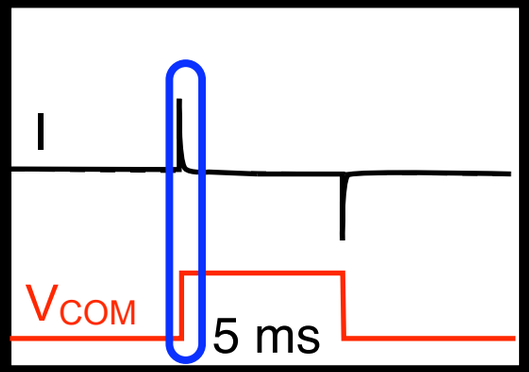
touching the cell increases the resistance,
thereby decreasing the current flow

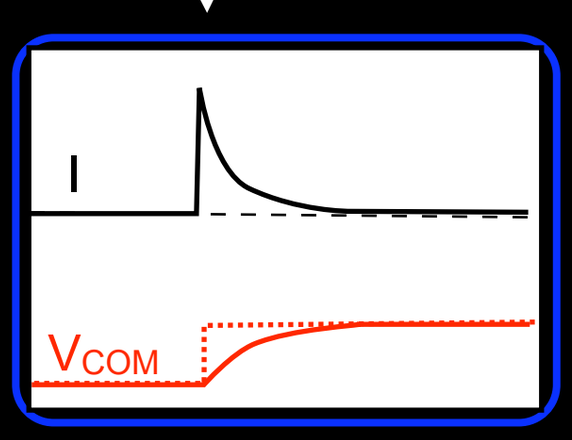
giga seal
(GΩ)

Giga Seal - On Cell

situation - giga seal between pipette and cell membrane (on cell)

cell attached (on cell) configuration





C_P and small C_{MP}
(τ ~1 μs)

main capacitance is still C_P

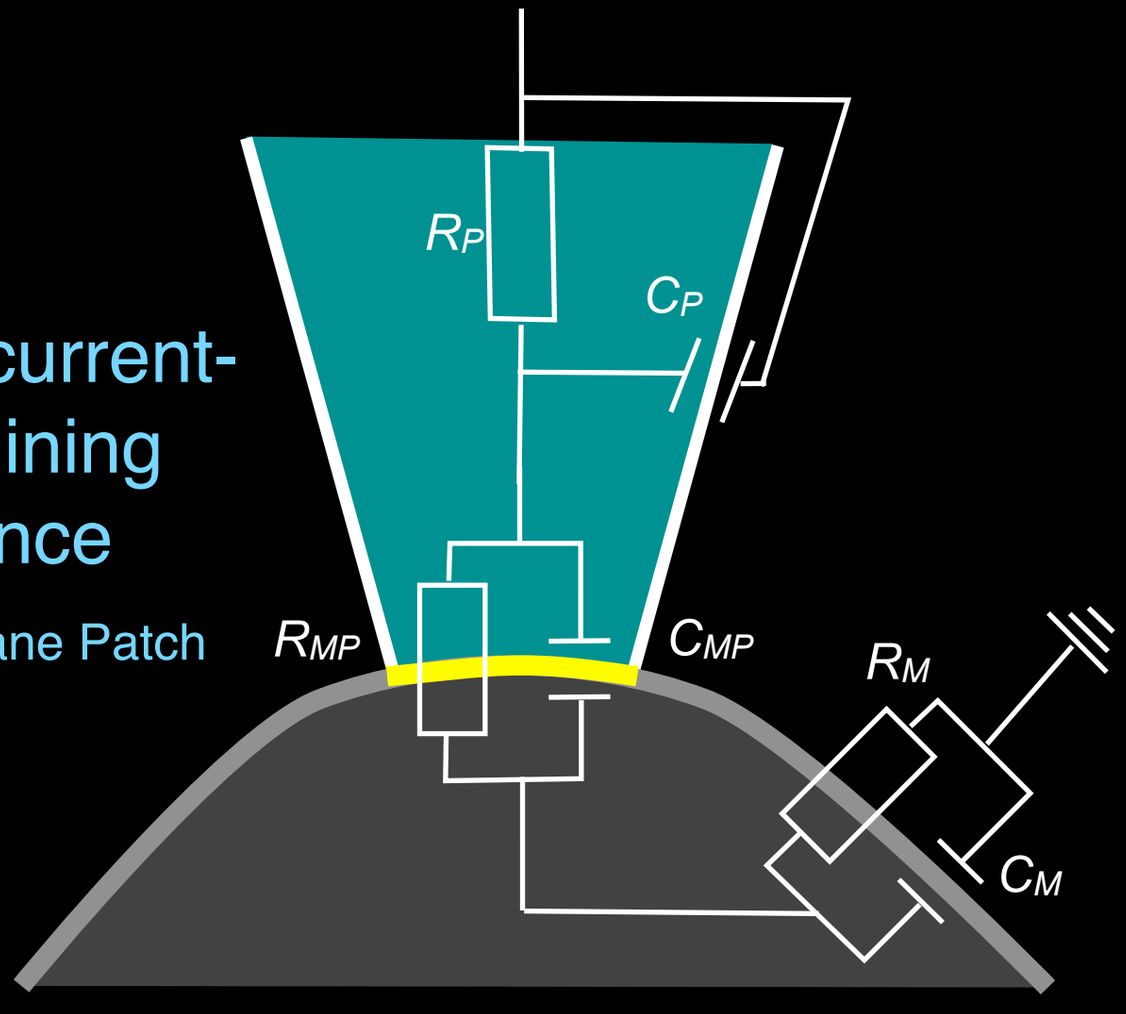
(the membrane is a slower capacitor than the pipette)

current is now forced through the cell

cell membrane is parallel combination of a capacitor and a resistor

major current-determining resistance

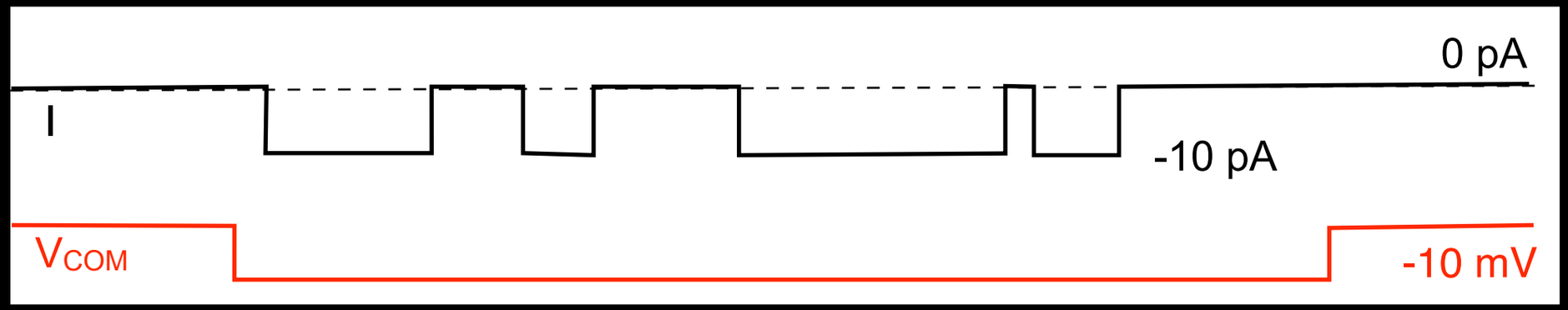
$R_{Membrane\ Patch}$



Membrane Resistance

lipid bilayer and ion channels (variable resistors) determine the resistance (conductance) of the cell membrane

conductance (resistance) of a single ion channel



this single channel conducts -10 pA at a -10 mV voltage step

Ohm's law $V = R \times I$

resistance: $R = \frac{V}{I} = \frac{1 \times 10^{-3}\ V}{1 \times 10^{-12}\ A} = 1 \times 10^{9}\ \Omega \quad (1\ G\Omega)$

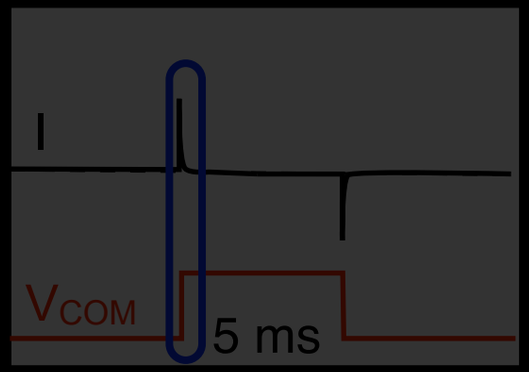
conductance: $G = \frac{1}{R} = \frac{I}{V} = \frac{1 \times 10^{-12}}{1 \times 10^{-3}\ V} = 1 \times 10^{-9}\ S \quad (1\ nS)$

the more ion channels open the lower the resistance and the higher the conductance of the membrane

Giga Seal - On Cell

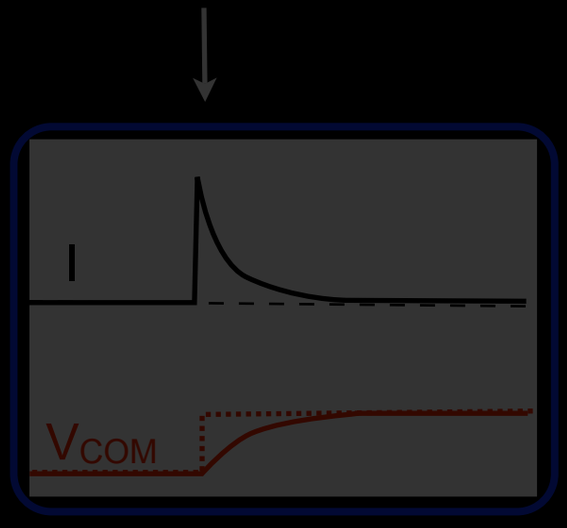
situation - giga seal between pipette and cell membrane (on cell)

cell attached (on cell) configuration



current is now forced through the cell

cell is parallel combination of
a capacitor and a resistor



C_P and small C_{MP}
($\tau \sim 1\ \mu s$)

main capacitance is still C_P

(the membrane is a slower capacitor than the pipette)

major current-determining resistance
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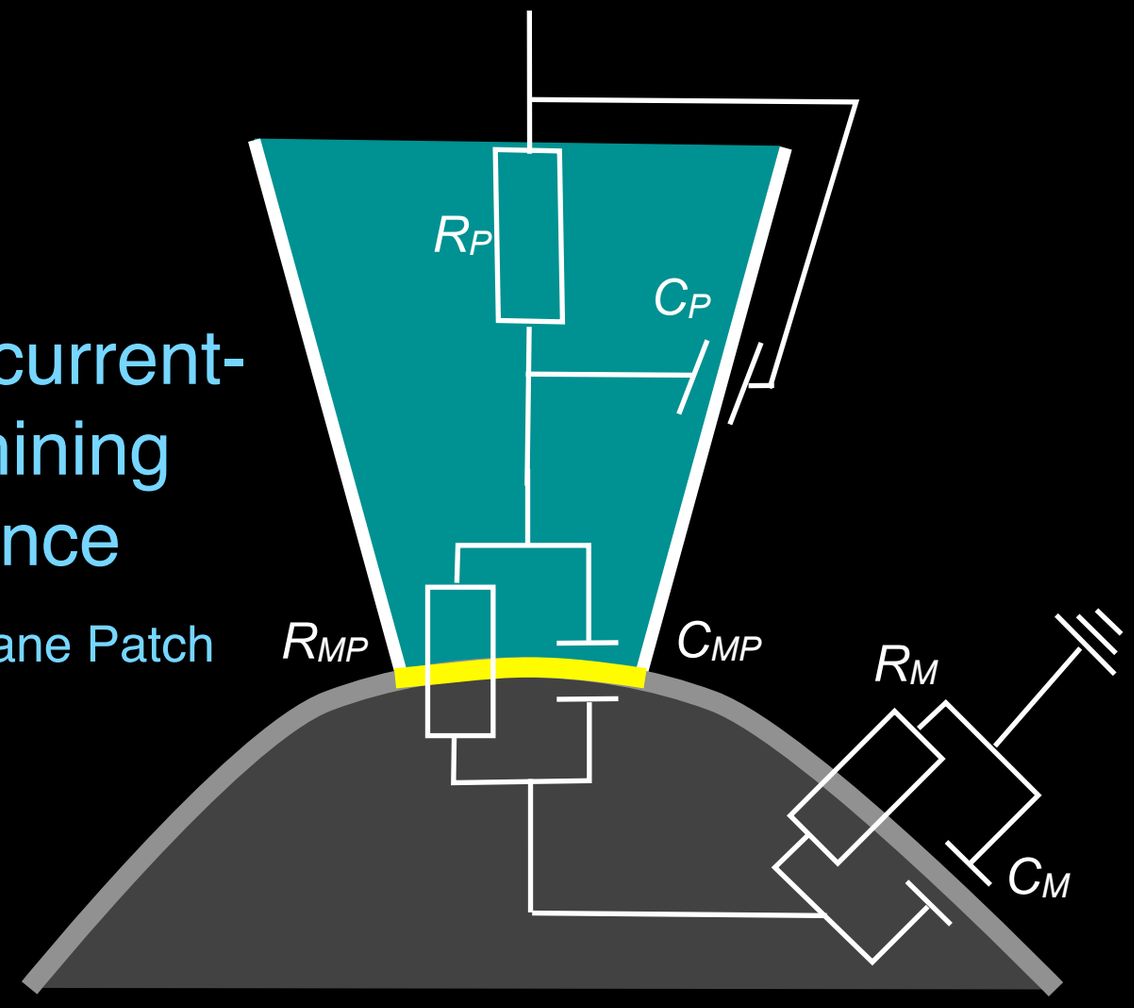
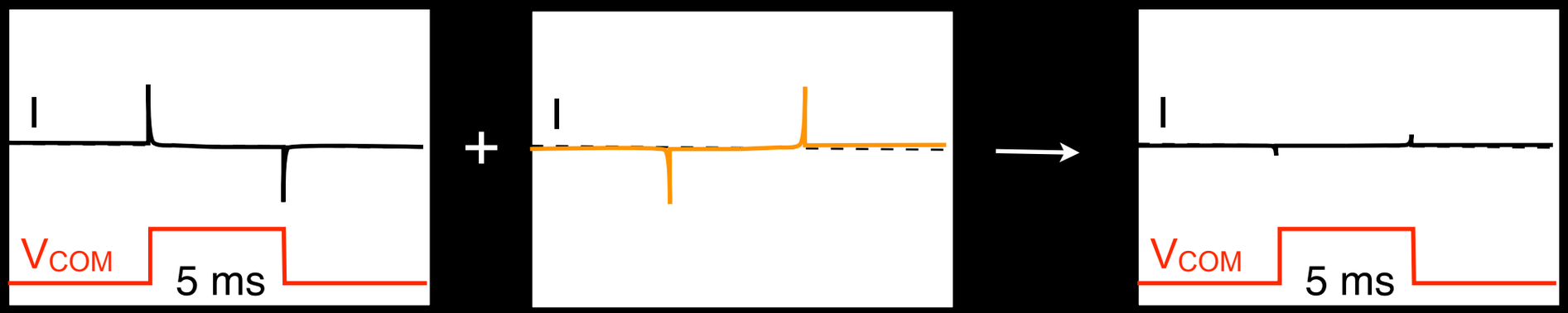


On Cell - Capacitance Compensation

capacitance compensation in the on cell mode

charging of pipette and patch can be compensated by the amplifier
=> artifact reduction



capacitative currents

amplifier delivers the same signal but inverted

capacitance compensated

during on cell mode - mainly capacitances due to the pipette (glass) occur which are much faster than membrane capacitances
=> fast capacitances

compensation of the fast capacitances = C_{FAST}

On Cell - Capacitance Compensation

reasons for the on cell capacitance compensation

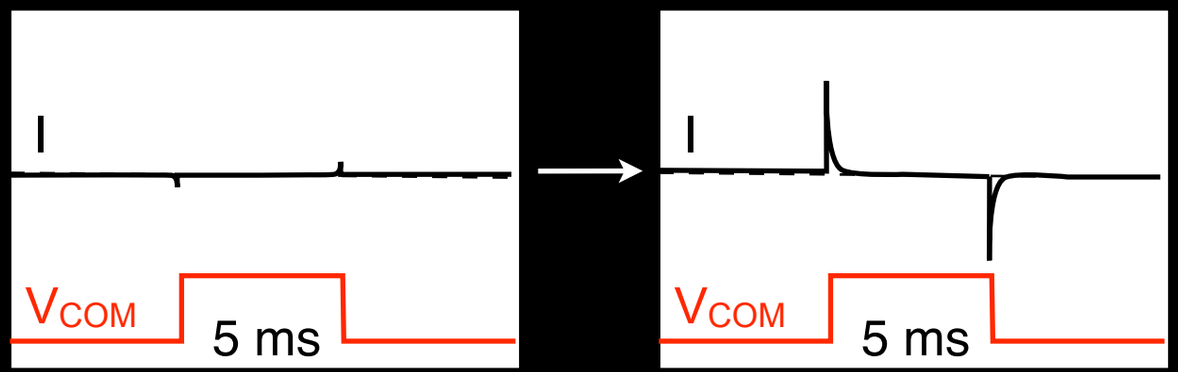
- cosmetic
- speed up voltage change on the top of the pipette
  to be able to measure rapid onset ionic currents
- non corrected fast capacitances interfere with further corrections
  especially the series resistance correction

On Cell - Attempting Whole Cell

rupturing the patch (break in) and attempting whole cell

application of short suction pulses on the pipette until transients appear (break in)

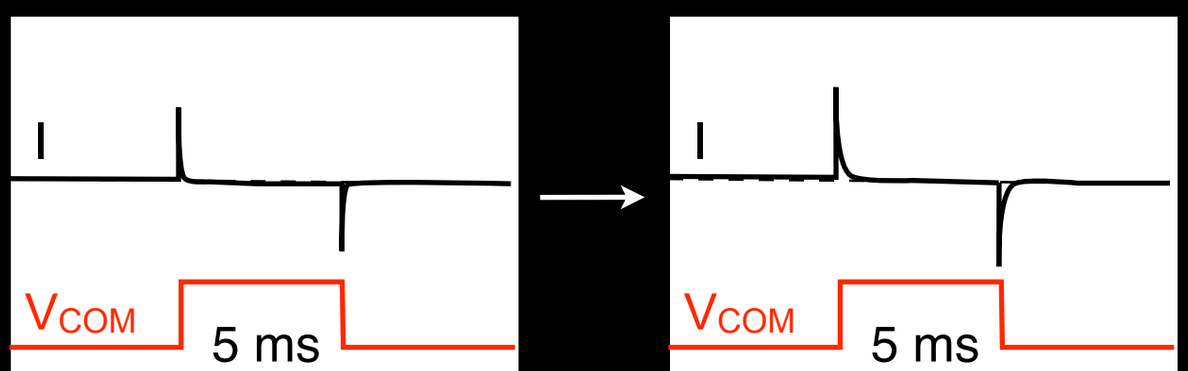
break in



transients are due to capacitance of whole cell membrane

whole cell configuration

if C_{FAST} compensation was already bad

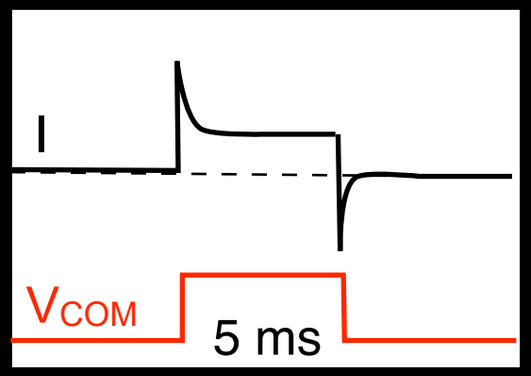


break in not always obvious
- transients increase
- transient base gets wider

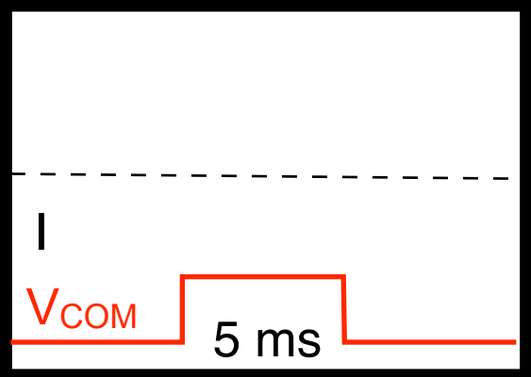
whole cell configuration

Attempting Whole Cell - What if ...

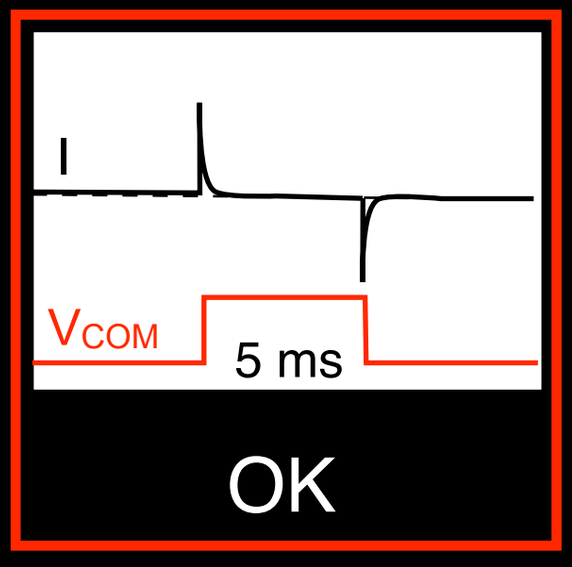
what happened if after break in:



leaky break in or already some real current

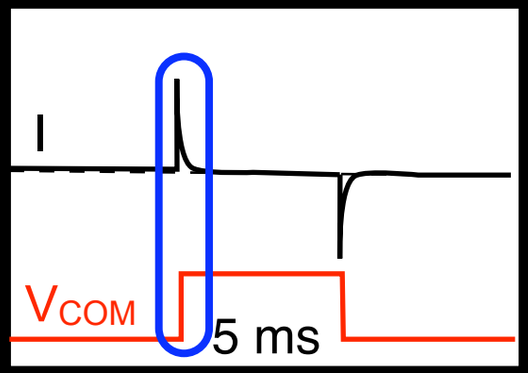


loss of the giga seal



Whole Cell

situation - whole cell configuration



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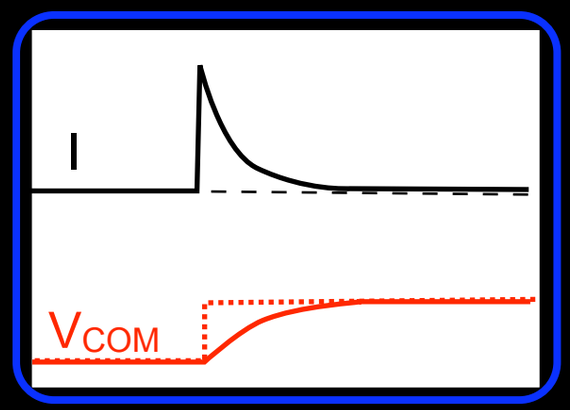
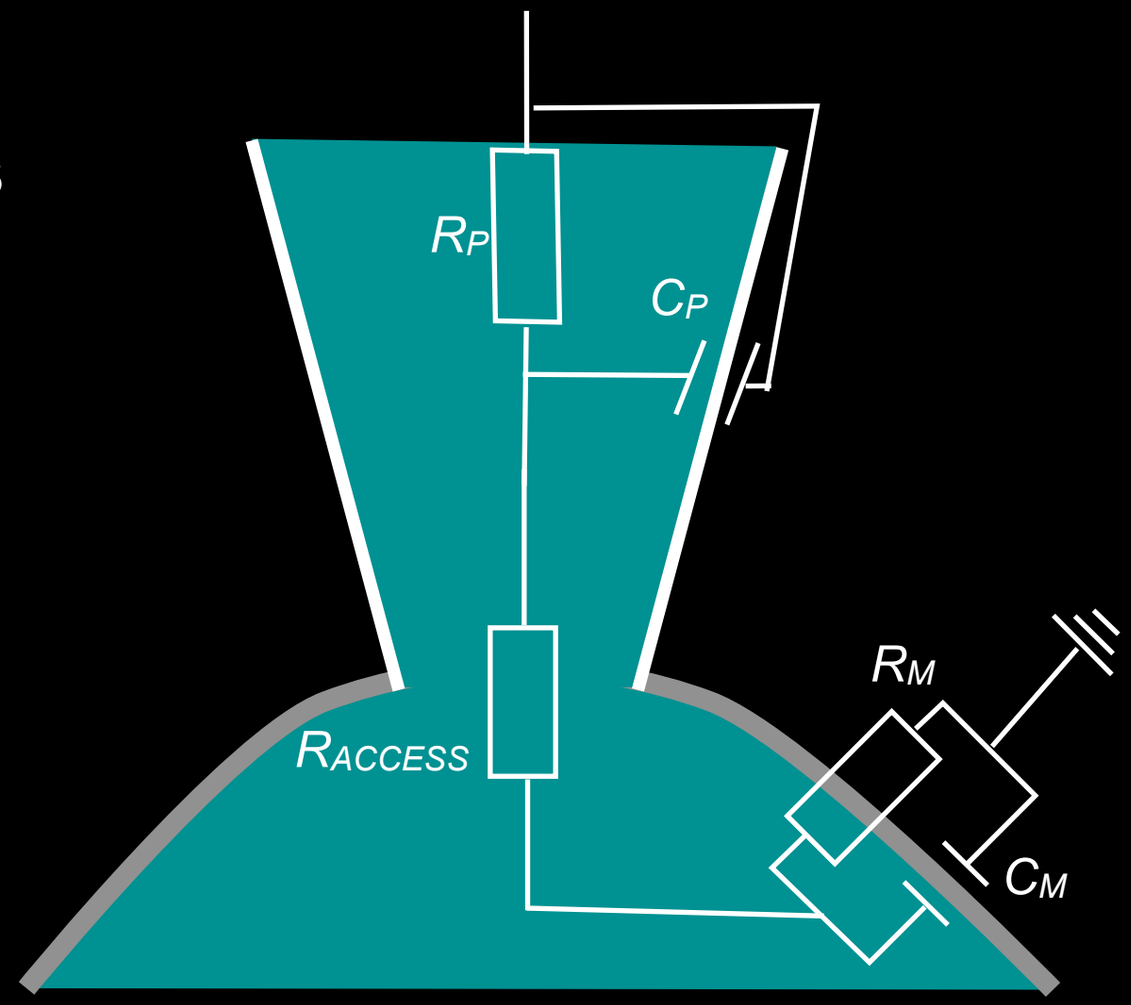


the membrane is a much slower capacitor than the pipette

τ some 100 *μs*

the bigger the cell the higher the capacitance => the slower τ



Whole Cell

situation - whole cell configuration

$$R_{INPUT} = R_{PIPETTE} + R_{ACCESS} + R_{MEMBRANE}$$

$$R_{PIPETTE} + R_{ACCESS} = R_{SERIES}$$

Kirchhoff's law

potential from sources = sum of voltage drops

$$V_{COM} = V_{Rs} + V_M$$

Kirchhoff's law

conservation of electric charge (constant I in loop)

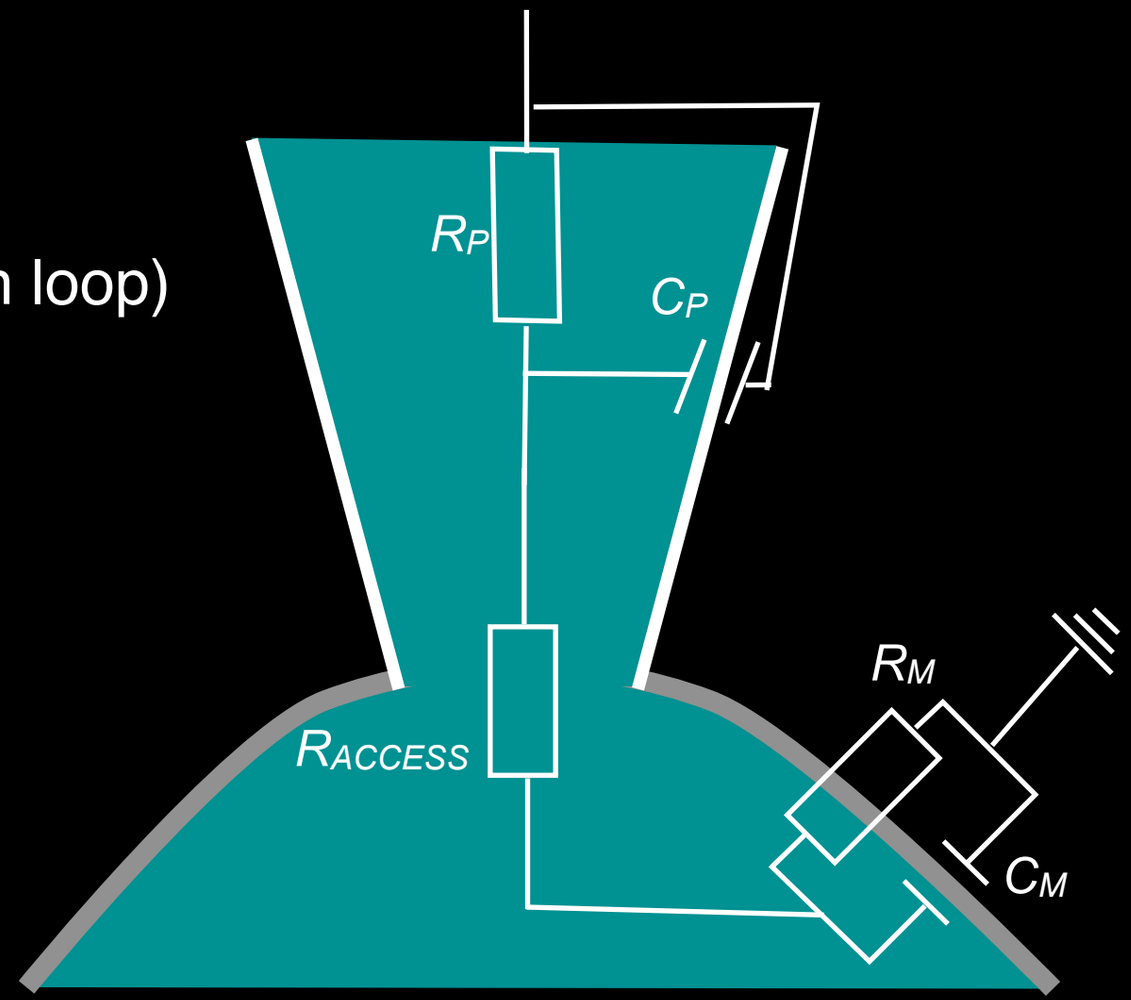
$$V_{COM} = I_M \times R_S + I_M \times R_M$$

$$V_{COM} = I_M \times (R_S + R_M)$$

$$I_M = \frac{V_{COM}}{(R_S + R_M)}$$

ideally R_S would be zero

electrical situation changed



Series Resistance - Whole Cell

whole cell configuration - voltage step

voltage error due to R_S

$$V_{STEP} = V_{Rs} + V_{M\text{-}step}$$

$$V_{STEP} = I_{M\text{-}step} \times R_S + I_{M\text{-}step} \times R_M$$

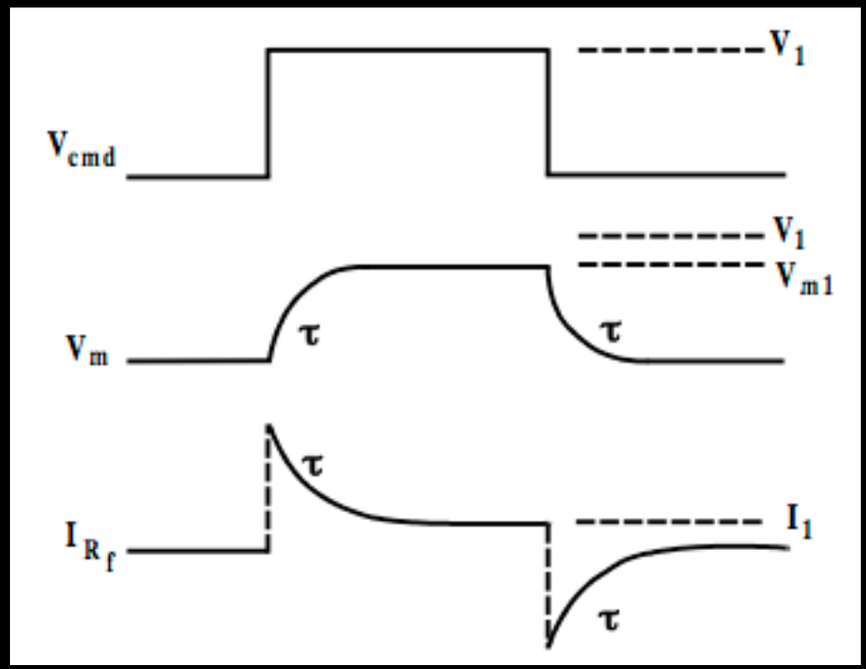
$$V_{STEP} = I_{M\text{-}step} \times (R_S + R_M)$$

$$I_{M\text{-}step} = \frac{V_{STEP}}{(R_S + R_M)}$$

$V_{STEP} = V_1$

$V_{M1} = V_{M\text{-}step}$

$I_1 = I_{M\text{-}step}$



the higher the membrane conductance
the larger the current
the bigger the error due to R_S

=> current at the membrane due to voltage step is underestimated

Series Resistance - Whole Cell

whole cell configuration - voltage step

temporal error due to R_S

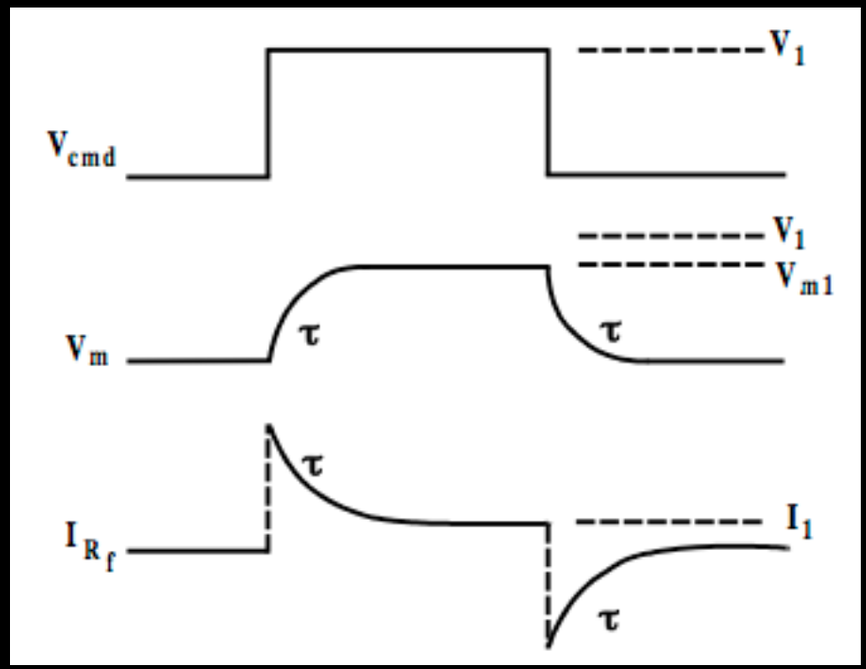
after voltage step I_M and V_M settle exponentially to steady state

$$\tau = \frac{R_S \times R_M}{(R_S + R_M)} \times C_M$$

since $R_M >> R_S$

$$\tau \approx R_S \times C_M$$

$V_{STEP} = V_1$
$V_{M1} = V_{M\text{-step}}$
$I_1 = I_{M\text{-step}}$



=> temporal error and limitations due to R_S and cell capacitance

Series Resistance and Capacitance - Limitations

example - membrane charging time limits for fast signals

- assume a cell with 20 pF and n R_S of 5 MΩ

$\tau \approx R_S \times C_M = 5\ M\Omega \times 20\ pF$

$= 5 \times 10^6\ \Omega \times 20 \times 10^{-12}\ F$

$= 100 \times 10^{-6}\ s = 100\ \mu s$ to reach 63% charging

=> to slow to measure fast Na channels (open within 20 to 100 μs)

example - membrane capacitance limits the acquisition bandwidth

- assume cell with 20 pF and an R_S of 5 MΩ

$$f = \frac{1}{2\pi \times R_S \times C_M} = \frac{1}{2 \times 3.14 \times 5 \times 10^6\ \Omega \times 20 \times 10^{-12}\ F}$$

$$= \frac{1}{628 \times 10^{-6}\ s} = 1592\ Hz = 1.59\ kHz$$

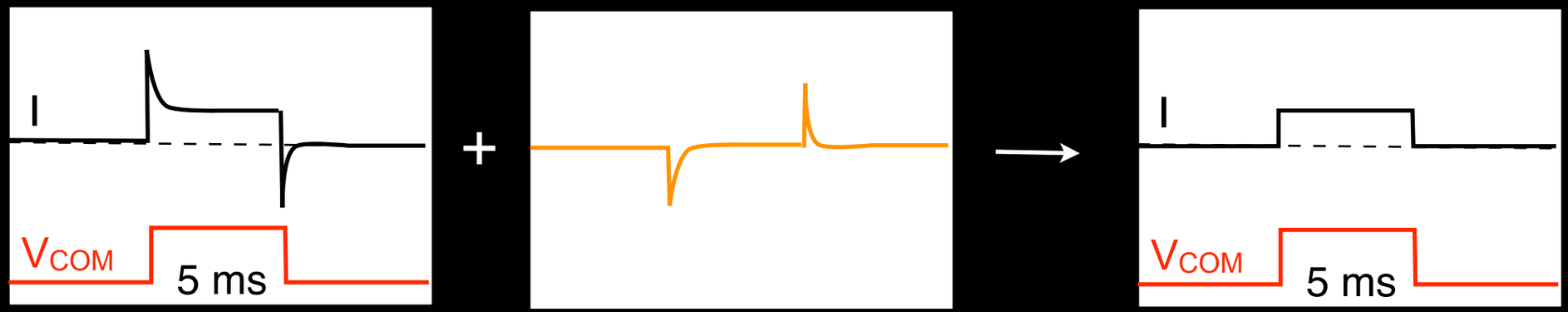
$\Rightarrow \sim 600\ \mu s$

=> no resolution of signals with a frequency faster than 1.59 Hz

Capacitance Compensation

whole cell configuration - capacitance compensation

similar to C_{FAST} compensation



capacitative currents

amplifier delivers the same signal but converted

capacitance compensated

during whole cell mode - mainly capacitances due to the cell membrane occur which are much slower than glass capacitances
=> slow capacitances

compensation of the slow capacitances = C_{SLOW}

Series Resistance Compensation

whole cell configuration - series resistance compensation

in whole cell mode the amplifier provides information about:

τ is measured by the amplifier

C_{SLOW} amplifier calculates integral below τ
-> capacitance ($C_M = C_{SLOW}$)

R_{SERIES} $\tau = R_S \times C_M$ $R_S = \frac{\tau}{C_M}$ amplifier calculates R_S

the amplifier usually corrects for R_S by applying a higher potential to compensate for the potential loss at R_{SERIES}

(requires a reasonable low $R_{PIPETTE}$ compared to $R_{MEMBRANE}$)

=> results in a more accurate V_M and thus a more correct I_M

R_S should stay constant during experiment (always check)
- if R_S goes higher than 10 MΩ it is going to be critical for experiments

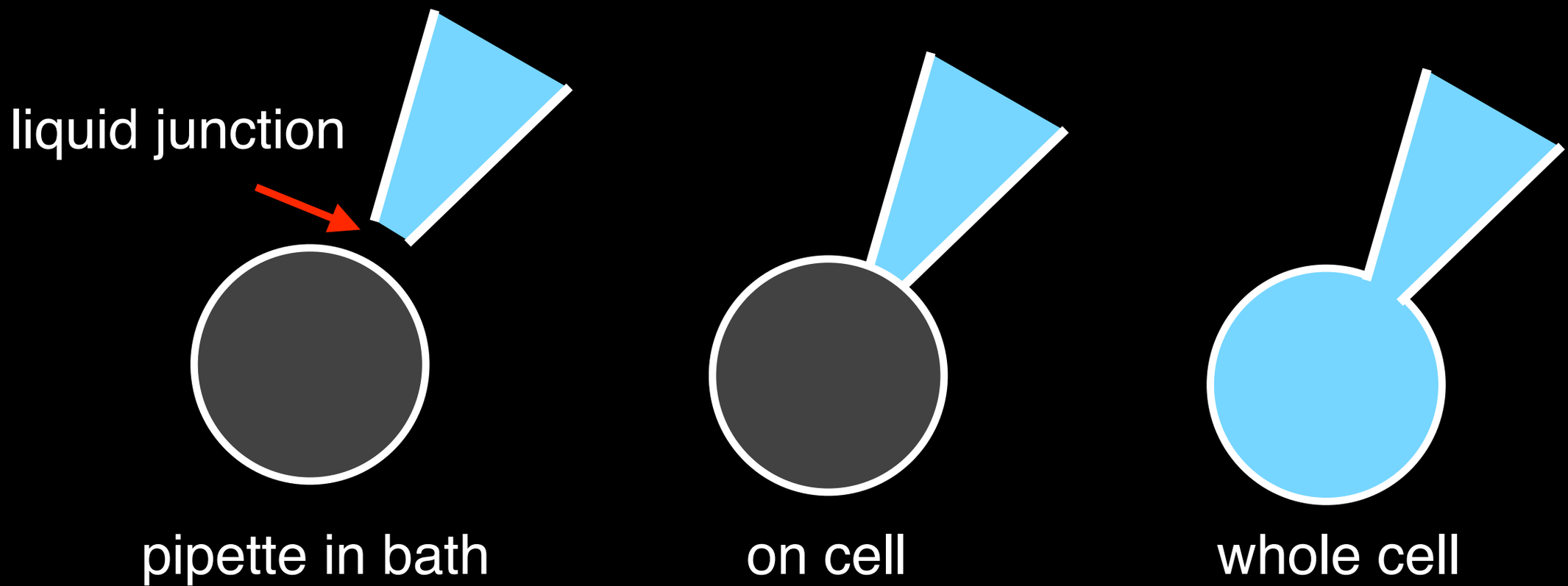
C_M can change due to exocytosis or endocytosis

Liquid Junction Potential

situation - pipette in the bath solution

zero offset also corrects for the liquid junction potential

in on cell (whole cell) mode liquid junction potential disappears



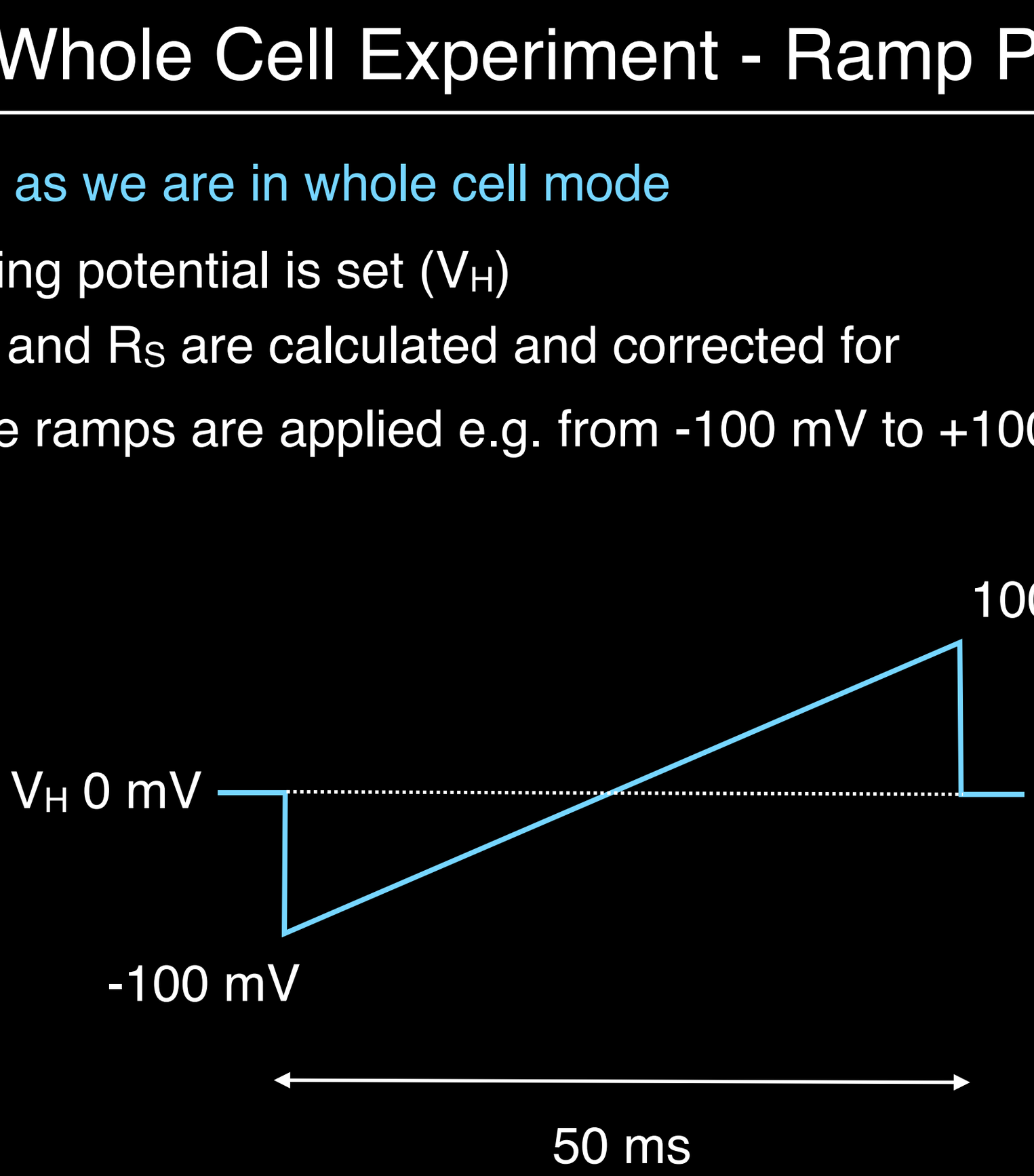
=> to much correction during zero offset leveling

=> for all patches re-correction by subtracting the corrected LJP again

Whole Cell Experiment - Ramp Protocol

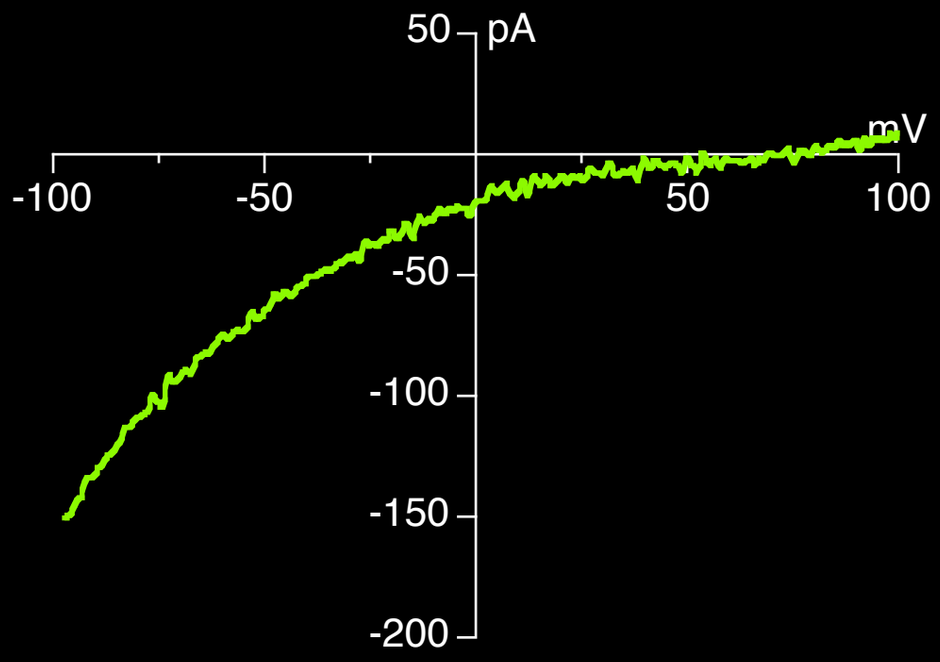
as soon as we are in whole cell mode

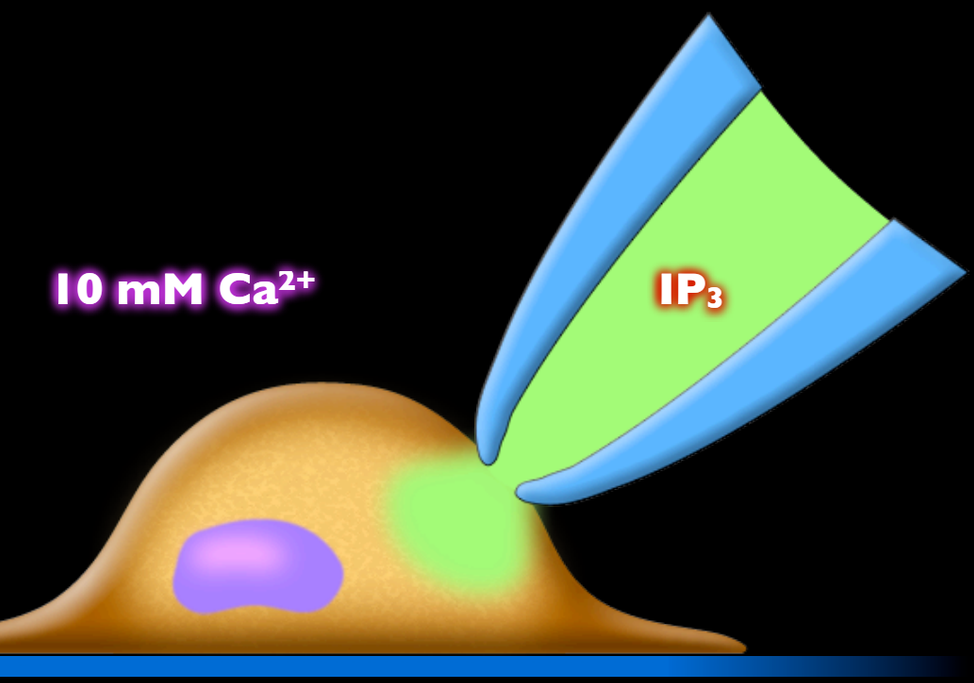
- a holding potential is set (V_H)
- C_{SLOW} and R_S are calculated and corrected for
- voltage ramps are applied e.g. from -100 mV to +100 mV in 50 ms

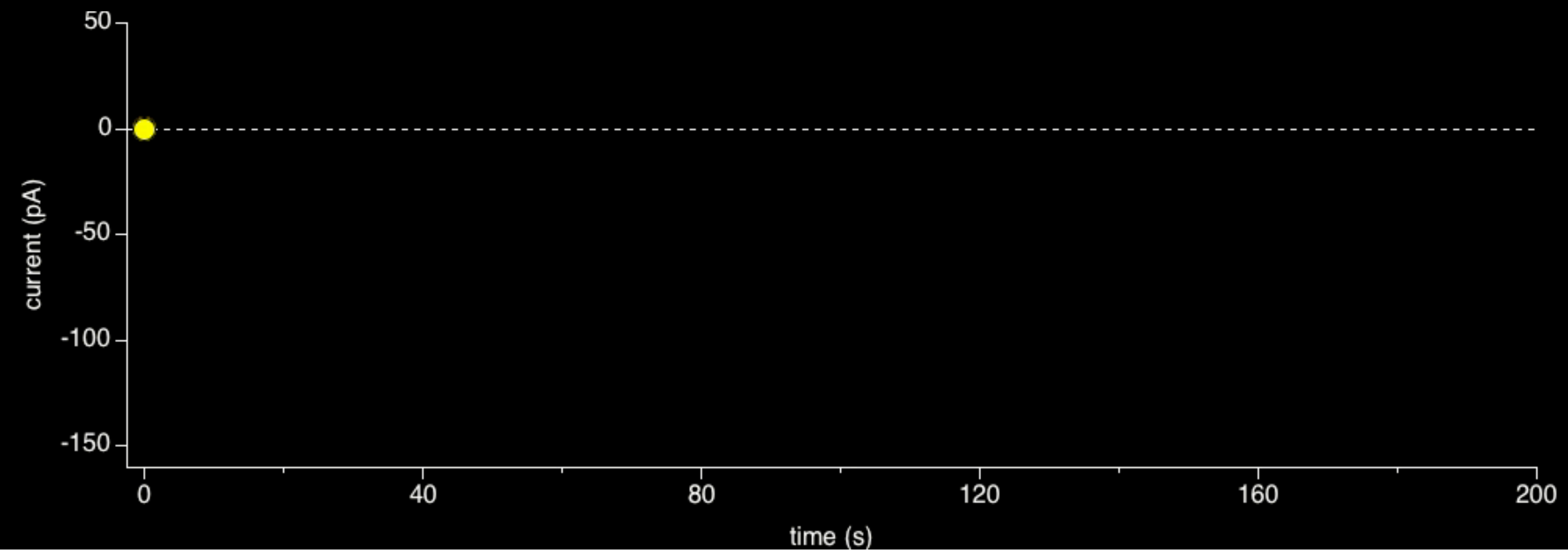


Whole Cell Experiment - I_{CRAC}

current voltage relationship (I/V)







Summary Through Whole Cell Experiment

situation 1: pipette in bath

- offset zeroing
- measuring pipette resistance

situation 2: on cell mode (giga seal)

- setting a V_H for the patch
- correction for liquid junction potential
- correction for the fast capacitances (C_{FAST}) mainly due to the pipette

situation 3: whole cell mode (after break in)

- setting V_H for the cell membrane
- calculation and correction for slow capacitances (C_{SLOW}) due to the whole cell membrane
- calculation and correction for series resistances (R_S)
- starting experiment (e.g. ramp protocol)

The Biological Basics of Electrophysiology

Monday, 04/21/2008

Electrophysiological Techniques

Monday, 04/28/2008

More about Patch Clamp

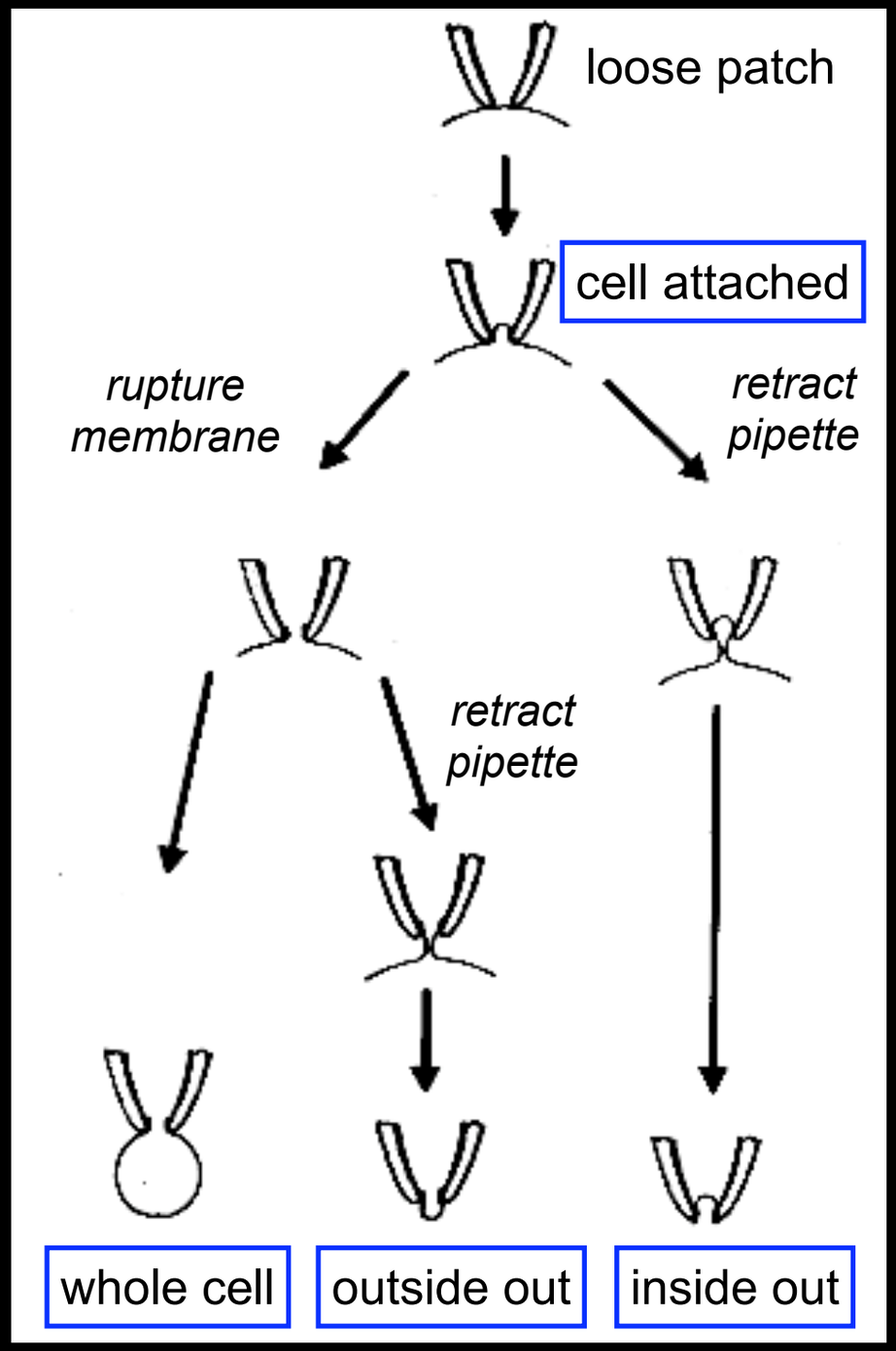
Today, 05/05/2008

Calcium Imaging

Monday, 05/12/2008

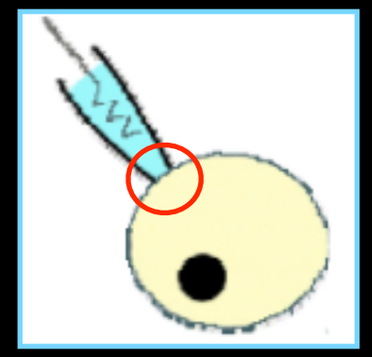
Patch Clamp Configurations

every configuration starts with the cell attached (on cell) situation



Cell Attached Configurations

cell attached patch clamp



single channel recordings from the patch

advantage
- cytosolic side intact
  allows testing for effects of cytosolic factors on ion channels in patch
- easy to obtain

disadvantage
- membrane potential unknown
- neither external nor internal solution changes

to remember
- no access to the cytosol of the cell
- pipette solution external conditions (drugs can be added)
- can only clamp the patch below the pipette to a V_H
- if V_H is meant to be negative, the V_{COM} has to be positive

Whole Cell Configurations

whole cell patch clamp

macroscopic current recording and capacitance measurements

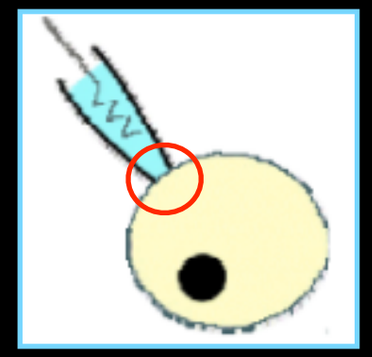
advantage
- capacitance measurements of the cell membrane
- cytosolic environment is controlled
- allows for changes of external conditions (superfusion)

disadvantage
- washout of soluble cytosolic factors (grace period ~10 min)
- series resistance and space clamp artifacts has to be considered

to remember
- access to the cytosol
- pipette solution internal conditions
- clamps the whole cell to a V_H
- if V_H is meant to be negative, the V_{COM} has to be negative

Patch Clamp Configurations

perforated patch



channel forming agent in the pipette (nystatin, amphotericin B)
macro current measurement without diluting cytosol

advantage
- reduces dialysis of the cell
- change of external conditions (superfusion)

disadvantage
- relatively difficult to obtain
- may take 10 - 30 min to obtain good access to the cell
- access resistance is higher than with whole cell
  -> decreases current resolution
  -> increases noise
  -> magnifies series resistance errors

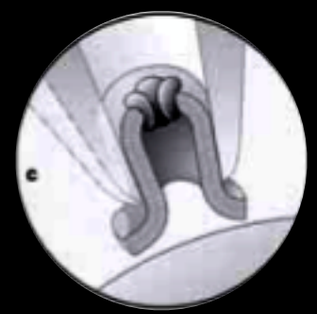
to remember
- external conditions in the pipette
- pipette solution depends on ionophore used
- if V_H is meant to be negative, the V_{COM} has to be positive

Patch Clamp Configurations

cell-free inside-out, or excised patch

measuring currents through single channels in the patch

advantage
- external environment is controlled
- cytosolic side can be superfused

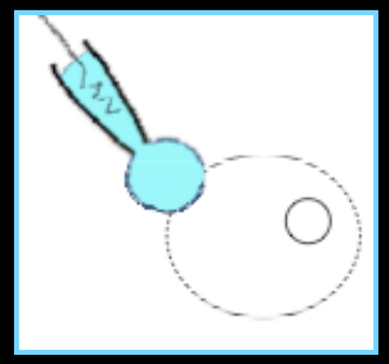


disadvantage
- loss of cytosolic factors
- bath solution has to be replaced by intracellular solution
- relatively difficult to obtain
- disruption of cytoskeletal structure

to remember
- internal side of membrane faces bath solution
- pipette solution external conditions (drug can be added)
- can only clamp the patch below the pipette to a V_H
- if V_H is meant to be negative, the V_{COM} has to be positive

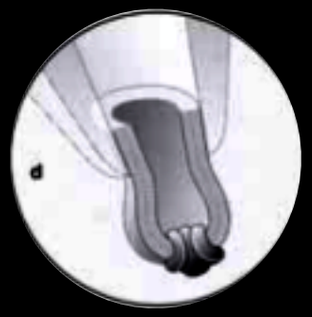
Patch Clamp Configurations

cell-free outside-out



measuring currents through single channels in the patch

advantage
- cytosolic environment is controlled
- external side can be superfused



disadvantage
- loss of cytosolic factors
- stability of the patch is difficult to obtain
- disruption of cytoskeletal structure

to remember
- external side of membrane faces bath solution
- pipette solution internal conditions
- if V_H is meant to be negative, the V_{COM} has to be negative