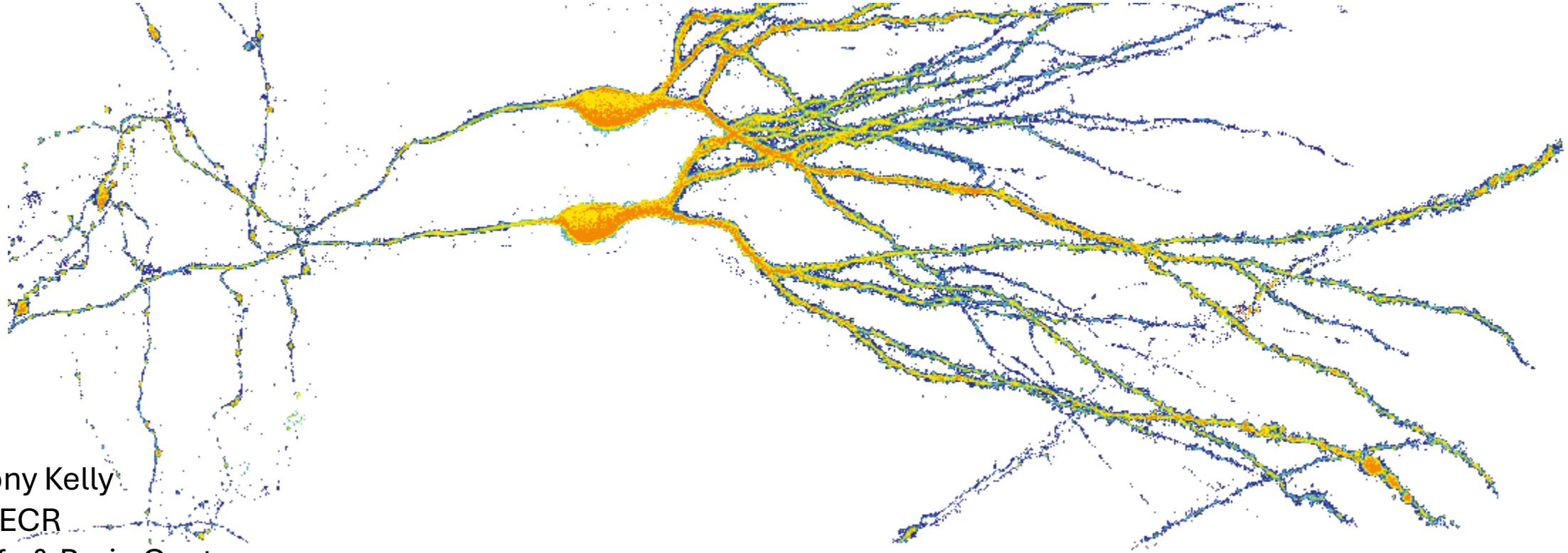
Introduction to Electrophysiological Recordings
- Current-Clamp & Voltage-Clamp -



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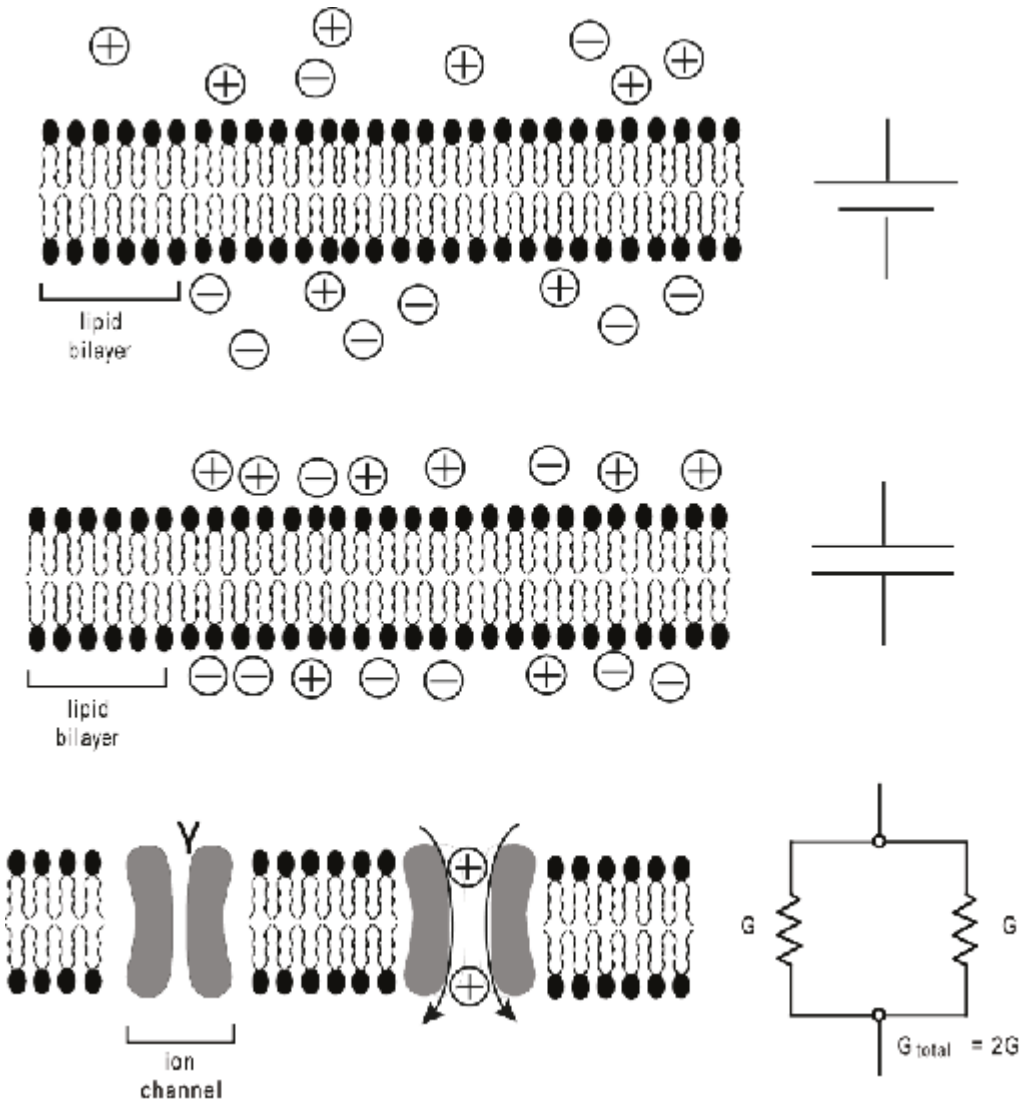
General Background

- fundamental electrical properties



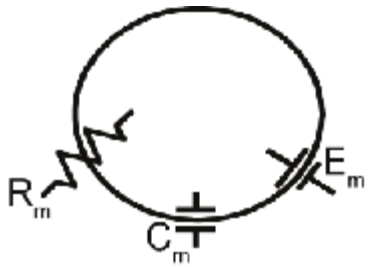
Modified from The Axon Guide 2006

An **electrical potential** (mV) difference exists between the interior and exterior of cells. The membrane potential of the cell relates the potential of the cell's interior to that of the external solution, which according to the commonly accepted convention, is zero.

The lipid bilayer is a very effective capacitor, separating charge across an insulator.
The **membrane capacitance** (C, in pF) is proportional to the area and inversely proportional to the distance separating the two conducting sheets. Capacitors in parallel sum together and nearly all lipid bilayer membranes of cells have a specific capacitance of (0.01 pF/µm2).

Ionic conductance inverse **resistance** (g in pS or 1/r in MOhms) from ion channels in the membrane These conductances allow the flow of ions down the electrochemical gradient (mV).
The flow of ions results in a **membrane current** (pA) measured as the flow of electrical charge per unit time. Sufficient Ion fluxes can also change concentration.

General Background
- equivalent circuit



Physiological properties relate to equivalent electrical properties.

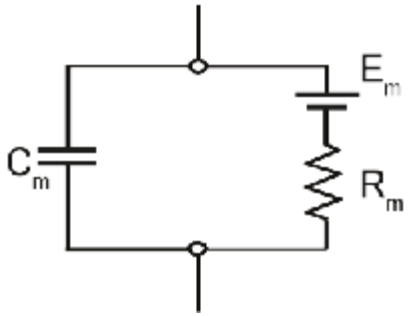
Ohm's Law - model the steady-state changes

V = IR = I/G (mV, nA, Mohms & uS are consistent units)

RC time constant - model dynamics of voltage changes

V(t) = V0(1-e^{-t/tau})

tau = RC



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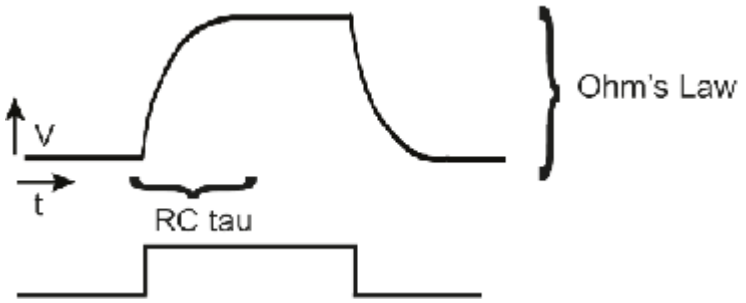
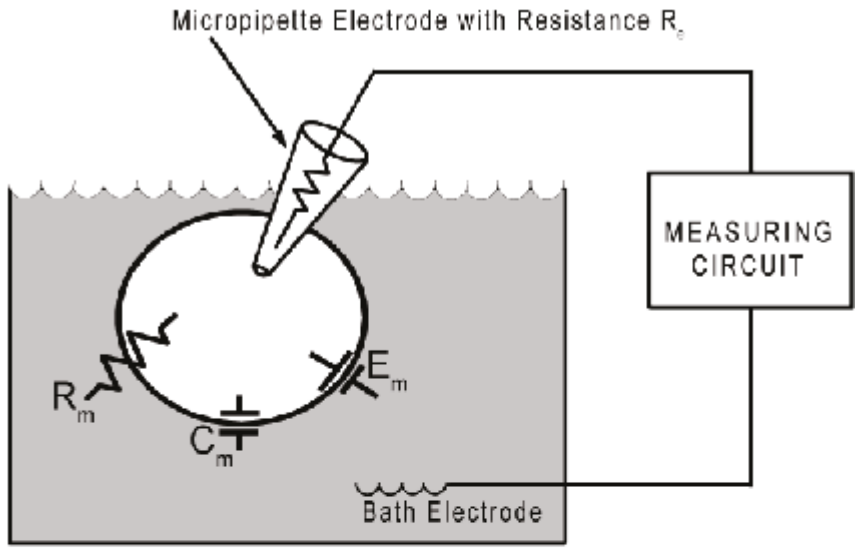
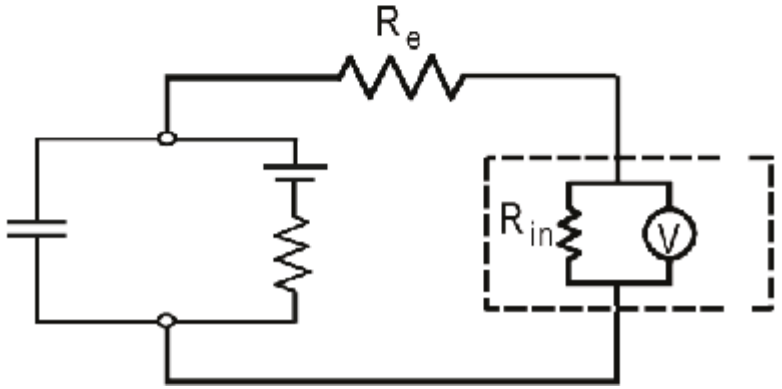


Current-Clamp Instrumentation - basic set-up





Modified from The Axon Guide 2006

What is current-clamp

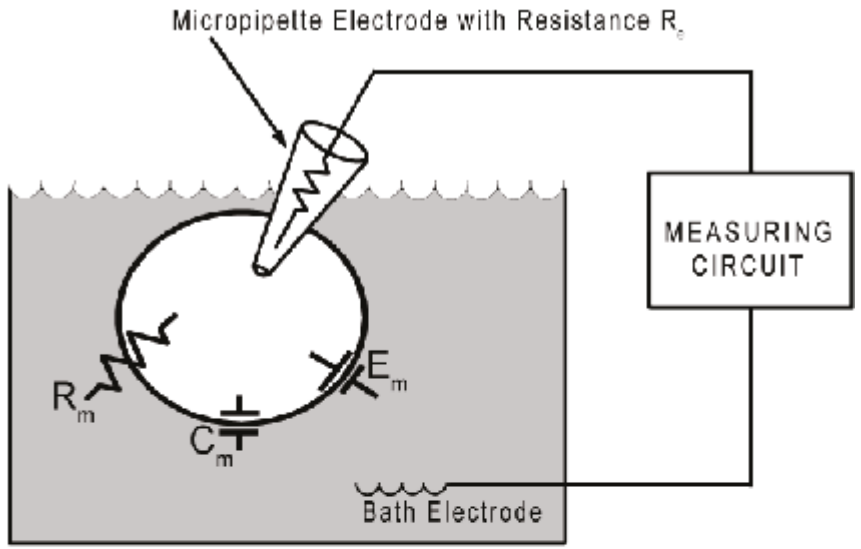
Why do current-clamp

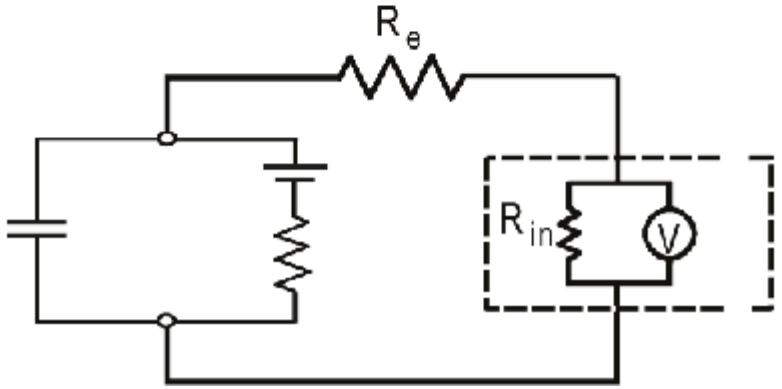
(1) Environment: the means of keeping the preparation healthy;
(2) Optics: a means of visualizing the preparation;
(3) Mechanics: a means of stably positioning the microelectrode
(4) Electronics: a means of amplifying and recording the signal.

Intracellular current-clamp recordings:
Sharp microelectrode:
- High resistance pipette (>70 MOhoms)
- Low seal resistance
- Only Blind
- No dialysis

Patch-clamp recordings:
- Low resistance pipette
- High GigaOhm seal
- Targeted cells
- Cell dialysis
- (pipette perfusion)
- (perforated patch)
- Diff Conformations

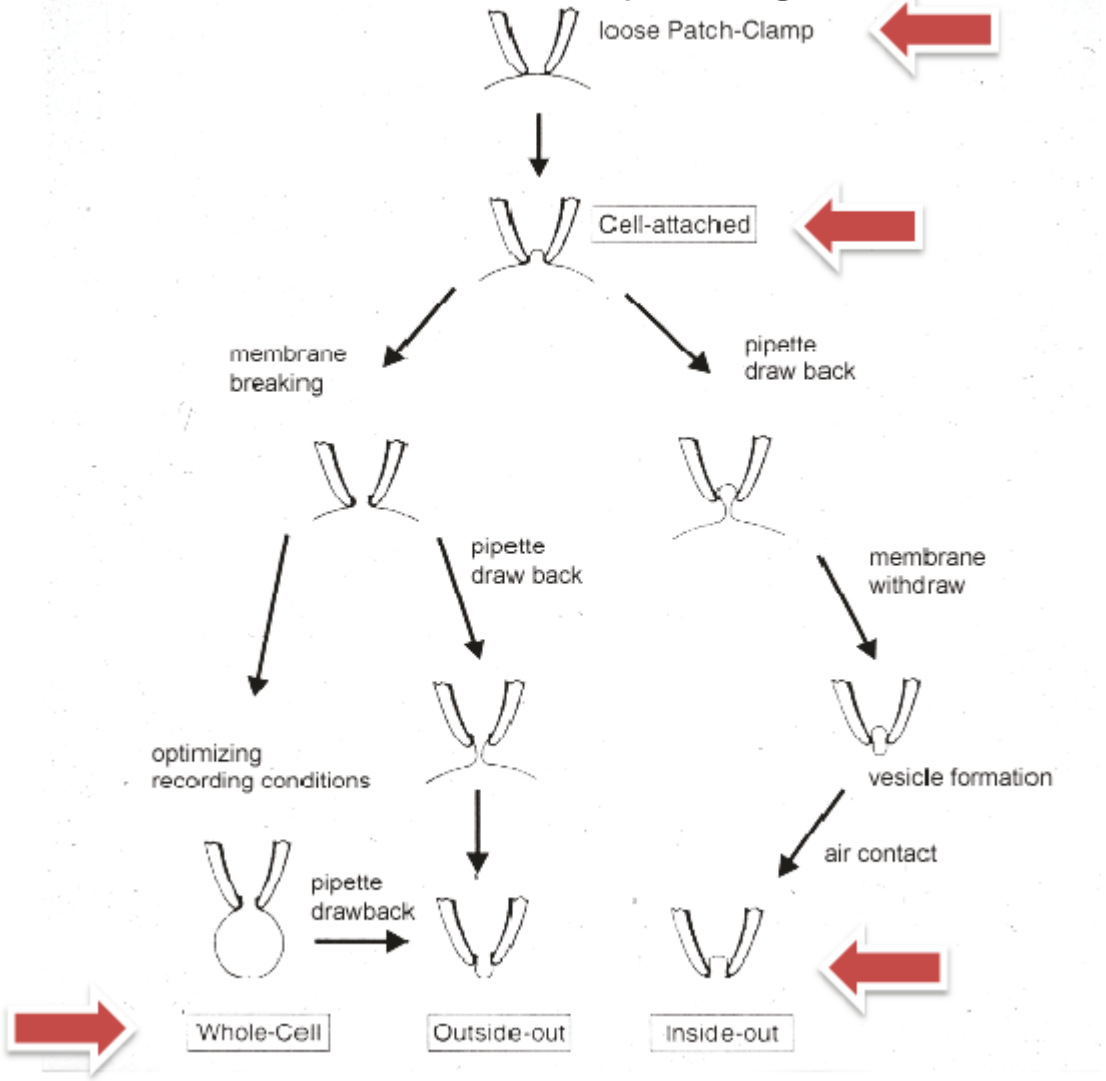
Current-Clamp Instrumentation - basic set-up



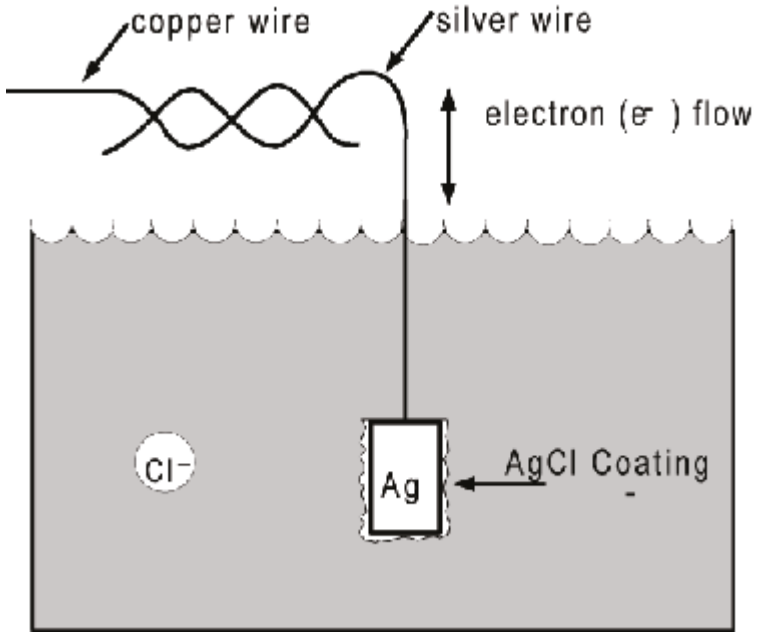


Modified from The Axon Guide 2006

Patch-clamp Configuration



Current-Clamp Instrumentation -liquid junction potential



Electrode reaction:

$$Ag + Cl^- \rightleftharpoons AgCl + electron\ (e^-)$$

This reaction can also be presented by:

$$AgCl \rightleftharpoons Ag^+ + Cl^-$$

$$+e^- \uparrow\downarrow -e^-$$

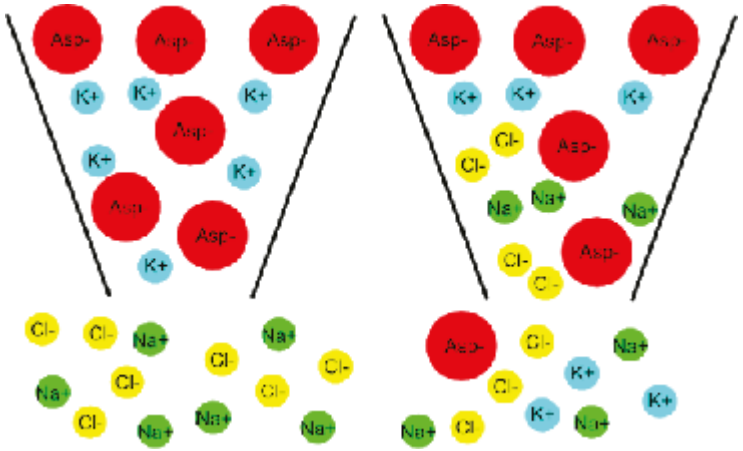
$$Ag$$

(1) The Ag/AgCl electrode performs well only in solutions containing chloride ions

(2) AgCl finite, exhaustion of AgCl exposes Ag+ and becomes unpredictable

(3) Differences in ion composition between pipette and bath solutions create potential differences (LJP)

Calculate/measure LJP
Stabilise LJP with 3M KCl agar bridge

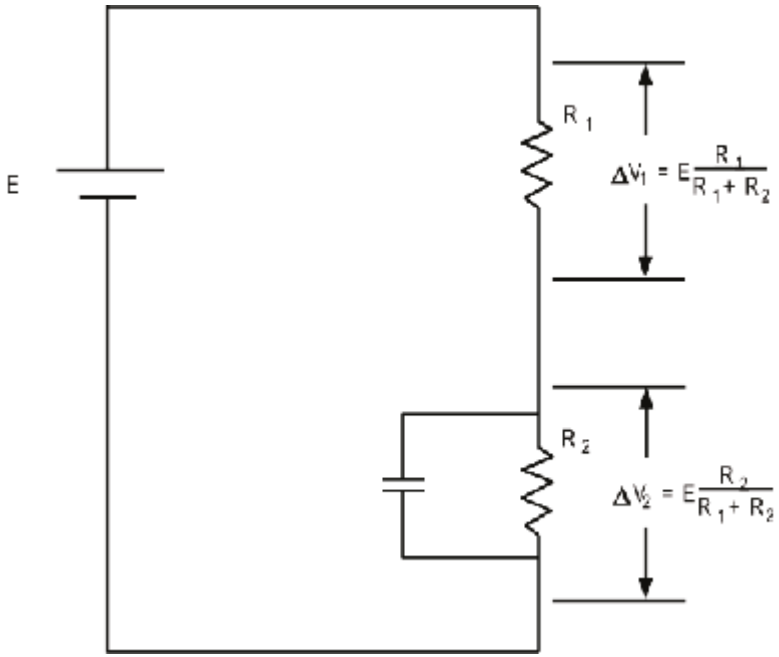


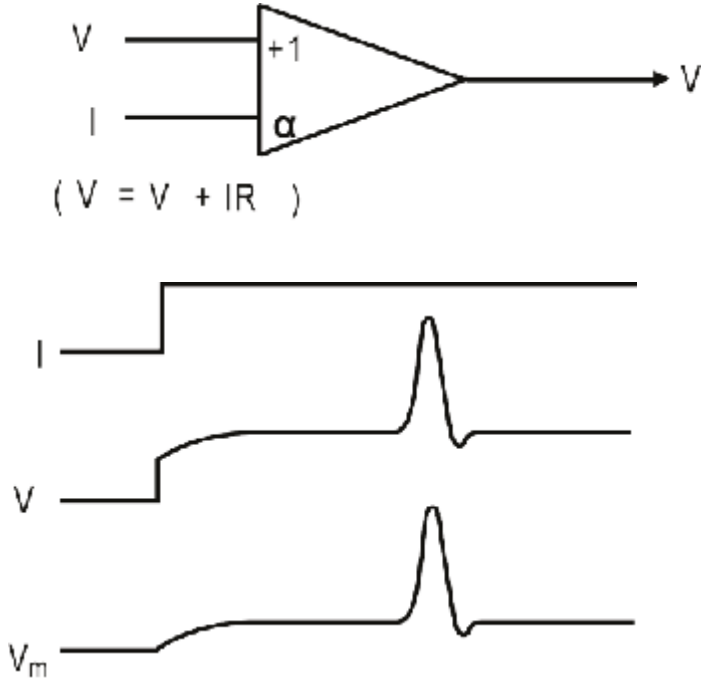
LJP = Vbath - Vpip

Whole cell configuration
Vm = V - Vljp

see Neher 1992

Current-Clamp Error
- voltage divider & series resistance





Voltage divider

When two resistors are connected in series, the same current passes through each of them, and the potential difference is split between them.

$$\Delta V_1 = E\frac{R_1}{R_1 + R_2} \;;\; \Delta V_2 = E\frac{R_2}{R_1 + R_2} \qquad (3a)$$

$$\Delta V_1 + \Delta V_2 = E \qquad (3b)$$

Bridge balance

Rs errors in CC result in shifts in membrane potentials
Rs is not parallel to Cm so instantaneous voltage drop
Rs compensation subtracts a scaled fraction of the I from Vp
continually monitor Rs throughout experiment
Rs < 30 mOhms and changes < 20%

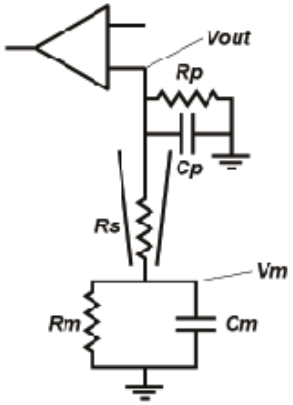
Current-Clamp Error
- series resistance as filter

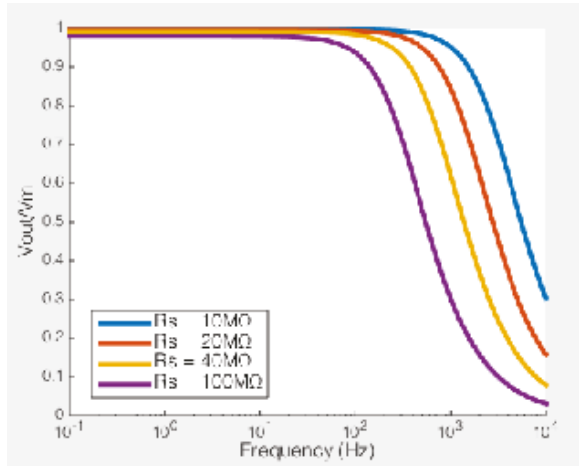
Series resistance and pipette capacitance are in parallel
- filter

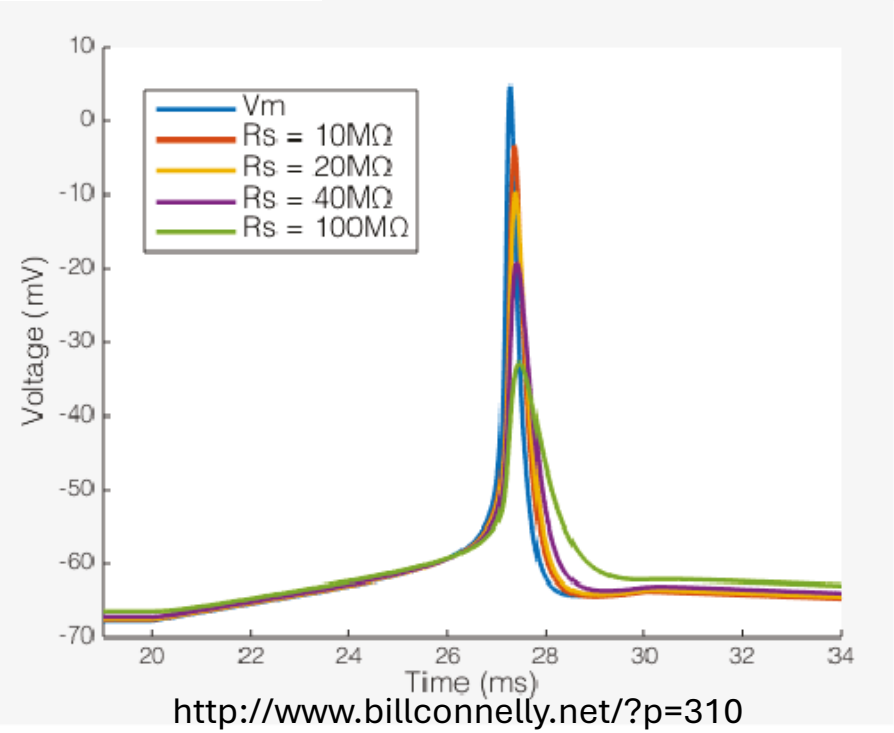
$$fc = \frac{1}{2 * pi * Rs * Cp}$$

Cut-off frequencies of >300Hz

Why would this be a problem?

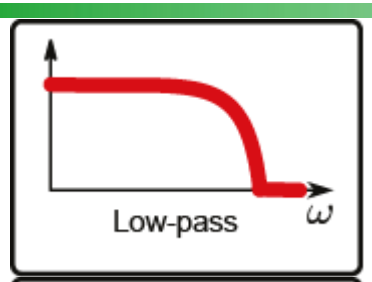


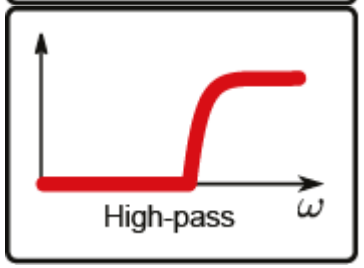


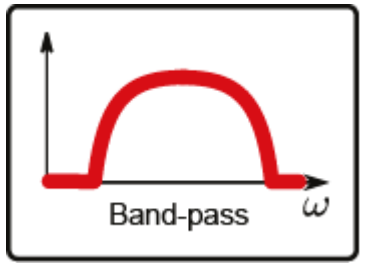


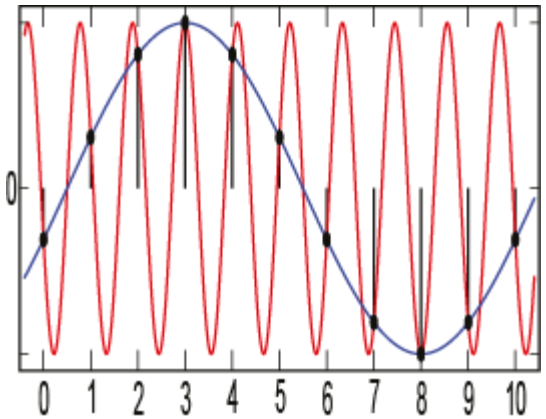
http://www.billconnelly.net/?p=310

Signal Processing
- what filtering rate?









A filter is a circuit that removes selected frequencies from the signal. Filtering is most often performed to remove unwanted signals and noise from the data. The most common form of filtering is low-pass filtering

High-pass filtering is required when the main source of noise is below the frequency range of the signals of interest.

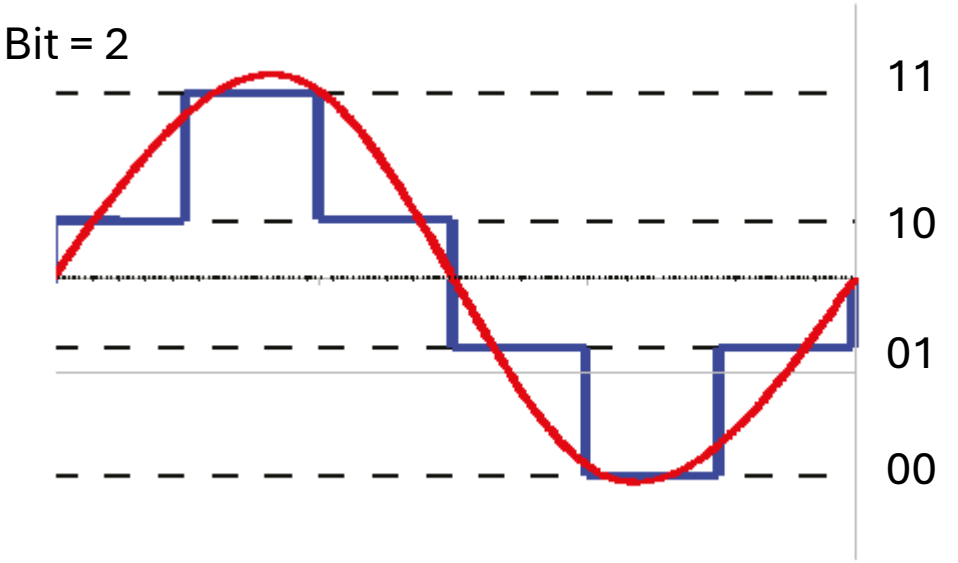
Another type of filter that is often used in biological recording is the notch filter. This is a special filter designed to eliminate one fundamental frequency and very little else. Notch filters are most commonly used at 50 or 60 Hz to eliminate line-frequency pickup.

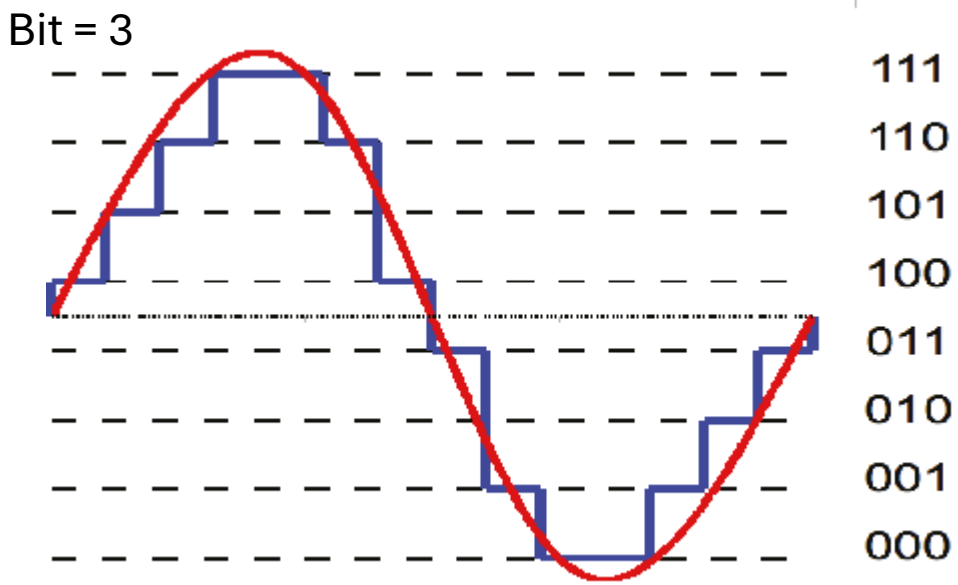
-3 dB Frequency
The -3 dB frequency (f-3) is the frequency at which the signal voltage at the output of the filter falls to ~ 0.7, of the amplitude of the input signal.

The filter you select depends on the frequencies you wish to record.
eg APs, 10kHz low pass is appropriate to measure rise at threshold.

The Nyquist Sampling Theorem states that a minimum sampling rate is twice the signal bandwidth;
Alias frequencies are sinusoids with frequencies that could fit the sampled data.
To make sure that the original frequency is the lowest, fs must be twice f.
In practice, fs x5 f. falias = f - N*fs

Signal Processing
- how to maximise A/D conversion





Digitalisation is creating a discrete signal from a continuous signal.
- Sampled at a defined freq.
- Amplitude Binned

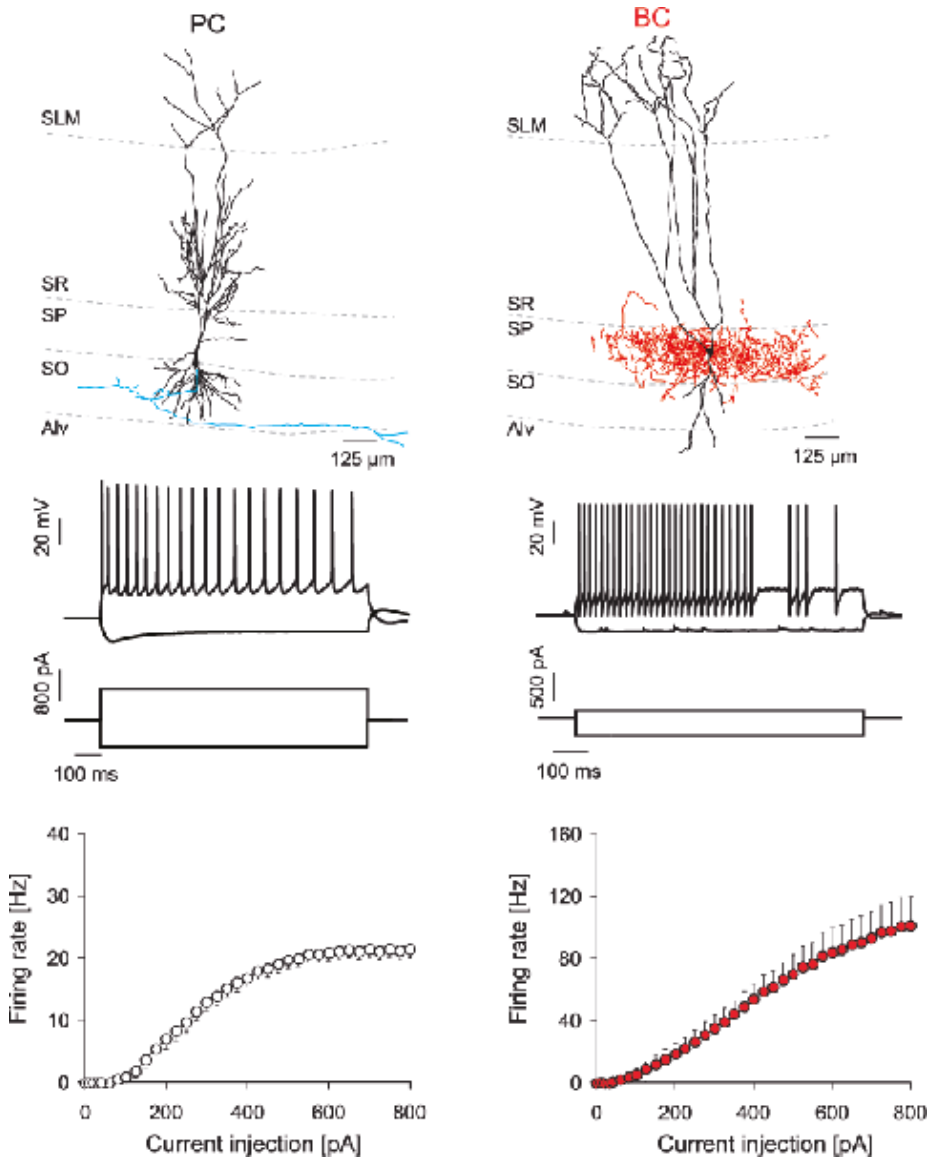
ADC usually work with Volts so all signals are presented as voltage
- max. limit usually ±10V
- bit depth

Resolution determined by the the bit depth 2^bit.
8 bit = 256 values
12 bit = 4,096 values
16 bit = 65,536 values

... and the amplification.

For a signal
1x gain +-10V signal 20V/4096 is 5mV
10x +-1V signal 2V/4096 is 0.5mV
or with 16bit is 0.03mV

Somatic Current-Clamp Recordings
- intrinsic electrical properties & cell identification



Pothmann et al. J. Neurosci. 2014

Cell identification and classification

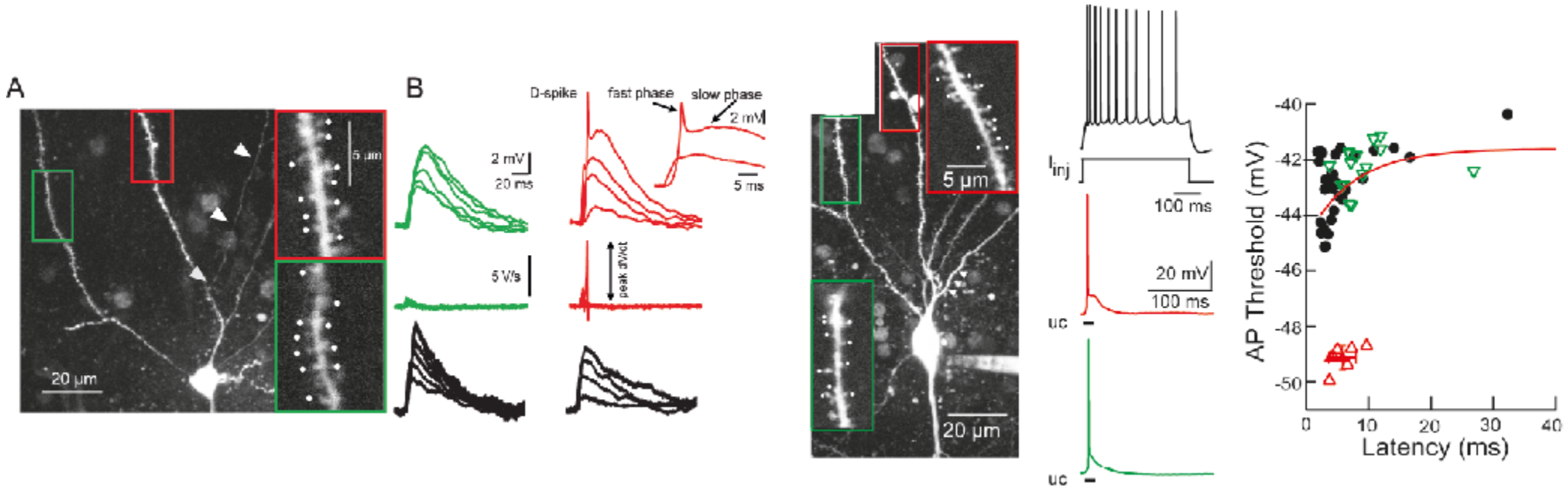
Morphology

Passive properties
- input resistance
- cell capacitance
- tau

AP properties
- Threshold
- Max. dV/dt
- Peak
- Duration
- Max. frequency
- Firing pattern

Neher & Sakmann Nature 1976
Edwards et al . Pflugers Archiv 1989.
Castaneda-Castellanos et al. Nat. Proc. 2006

Somatic Current-Clamp Recordings
- sub-threshold synaptic potentials



Thome & Kelly et al. Neuron 2014

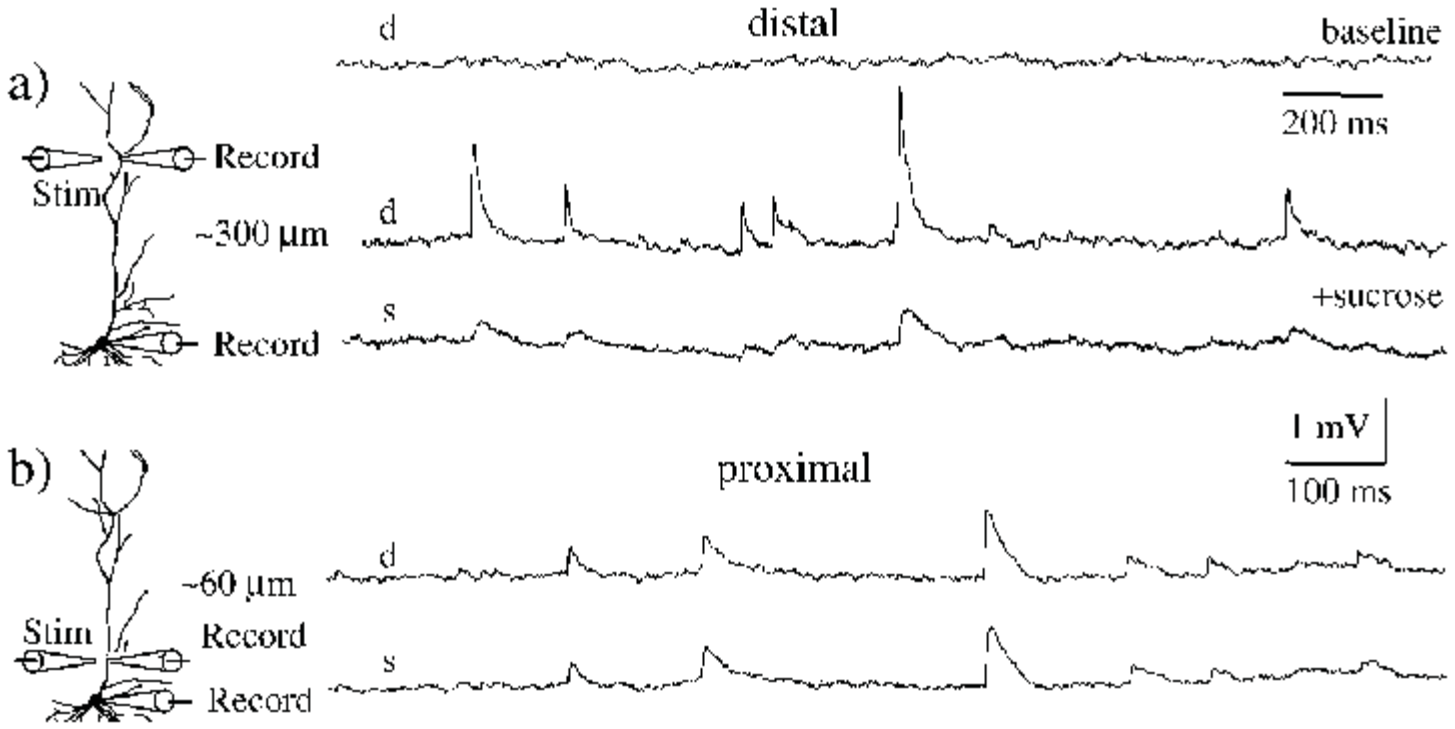
Synaptic Stimulation

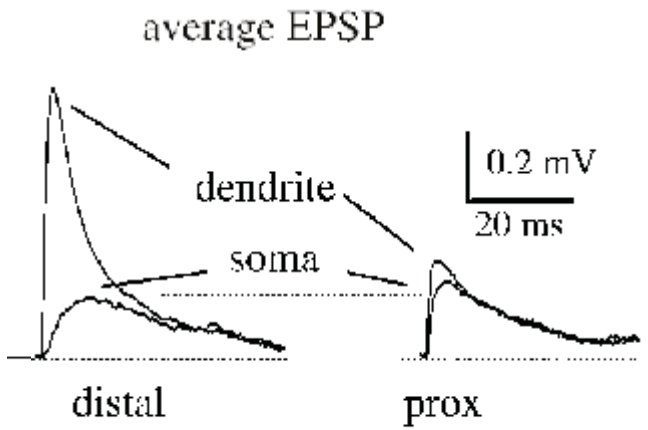
electrical, optogenetic, optical.

Probe integration of synaptic potentials.

Somatic recordings limited to somatic responses.

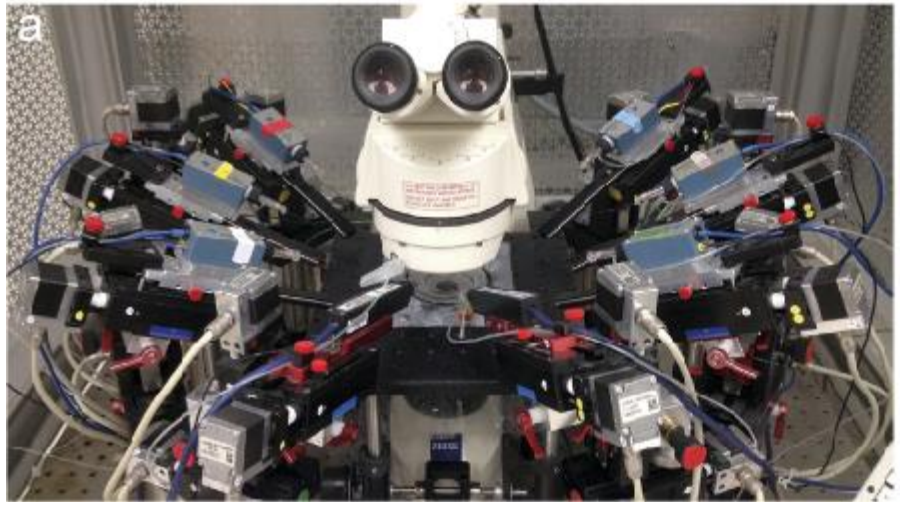
Dual Somato-Dendritic Current-Clamp Recordings - synaptic potentials

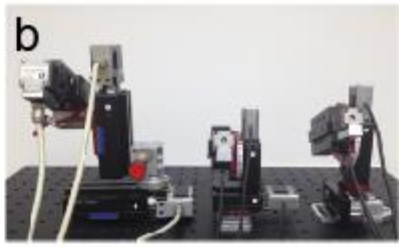


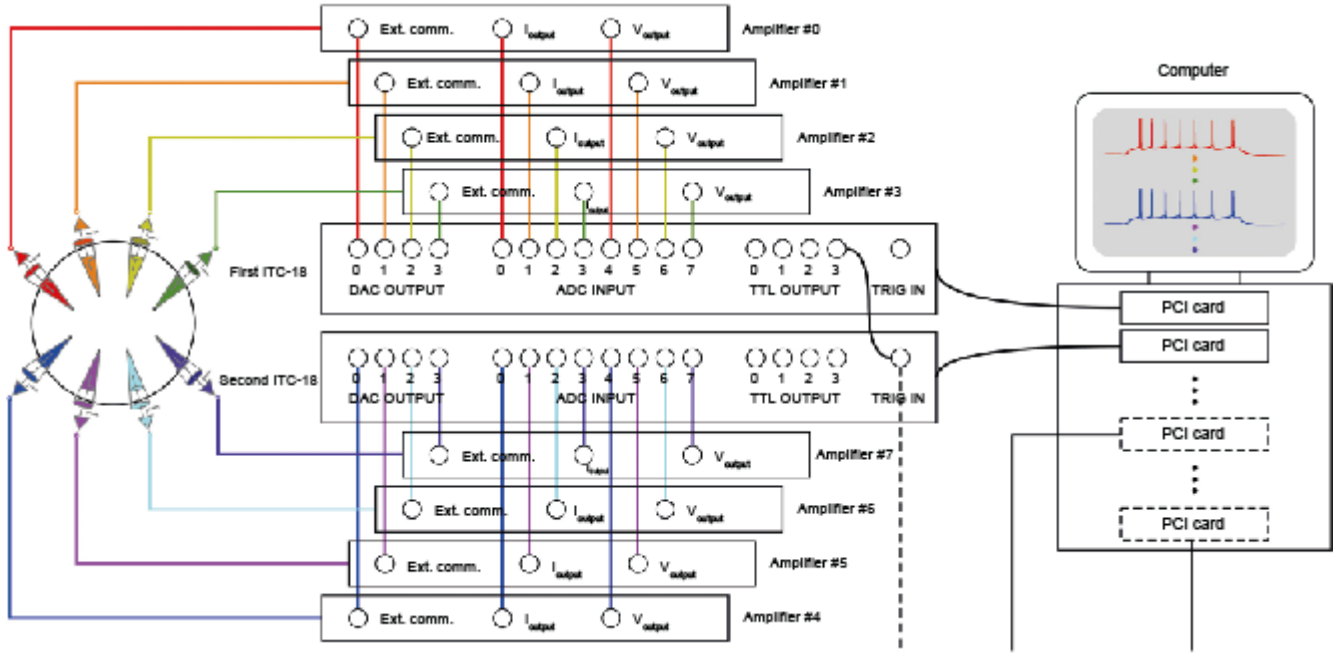


Cook & Magee Nat. Neurosci. 2000
Davie et al. Nat. Protocol 2006

Multiple Somatic Current-Clamp Recordings - set-up







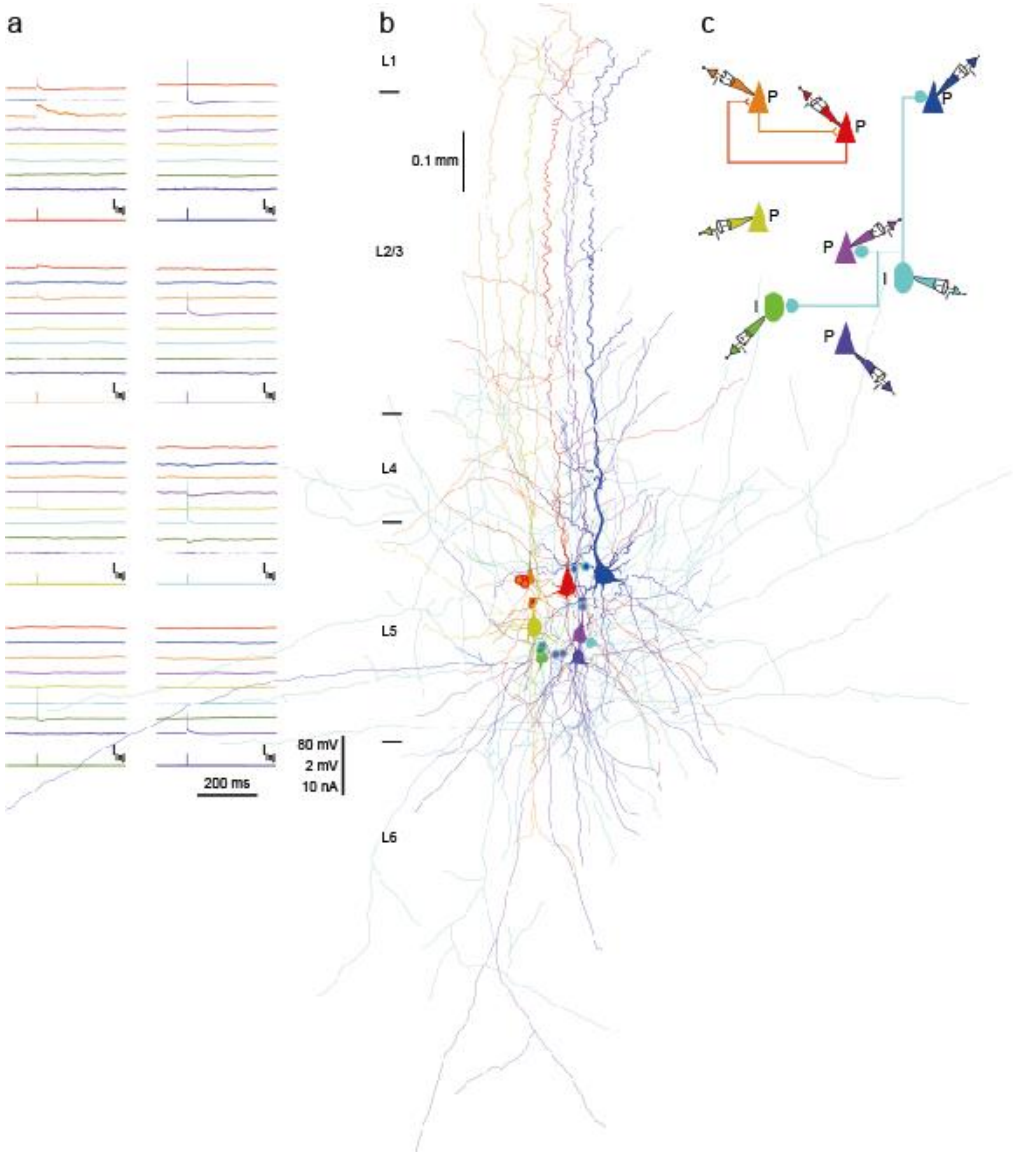
Wang et al. Nat. Protocols 2015

Functional Connections

testable connectivity increases with number of neurones. (C=4n(n-1)/2)).

limited space (no of pipettes)
expense (no of amplifiers)
synchrony between recordings

Multiple Somatic Current-Clamp Recordings - functional connectivity



Functional Connections

testable connectivity increases with number of neurones. (C=4n(n-1)/2)).

limited space (no of pipettes)
expense (no of amplifiers)
synchrony between recordings

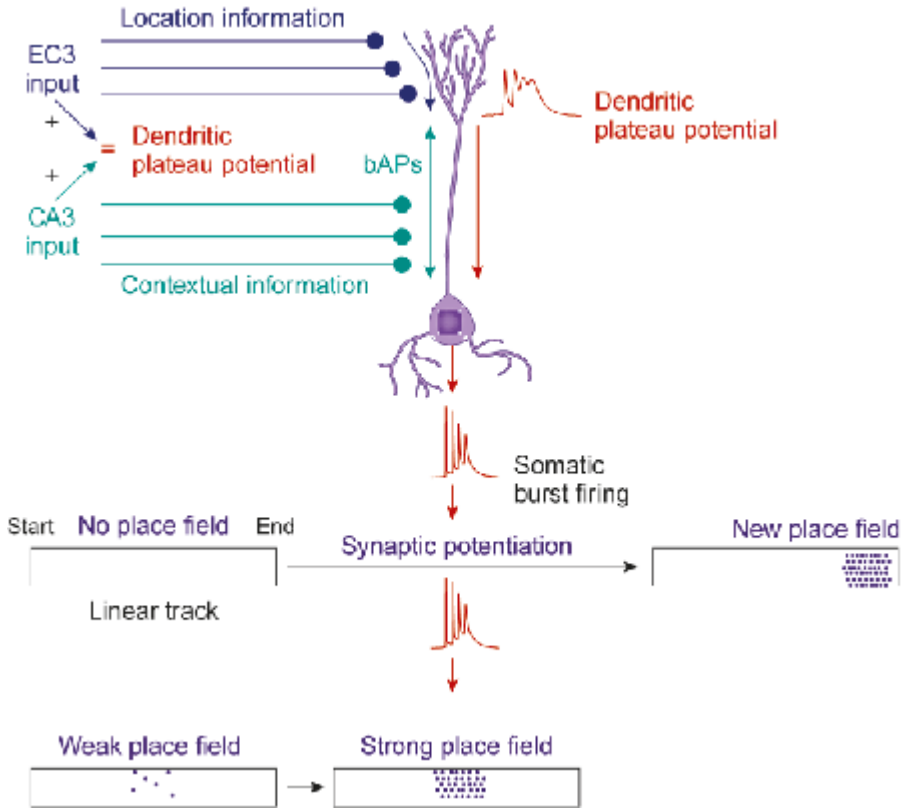
Determine

connected
strength
type (inhibitory or excitatory)

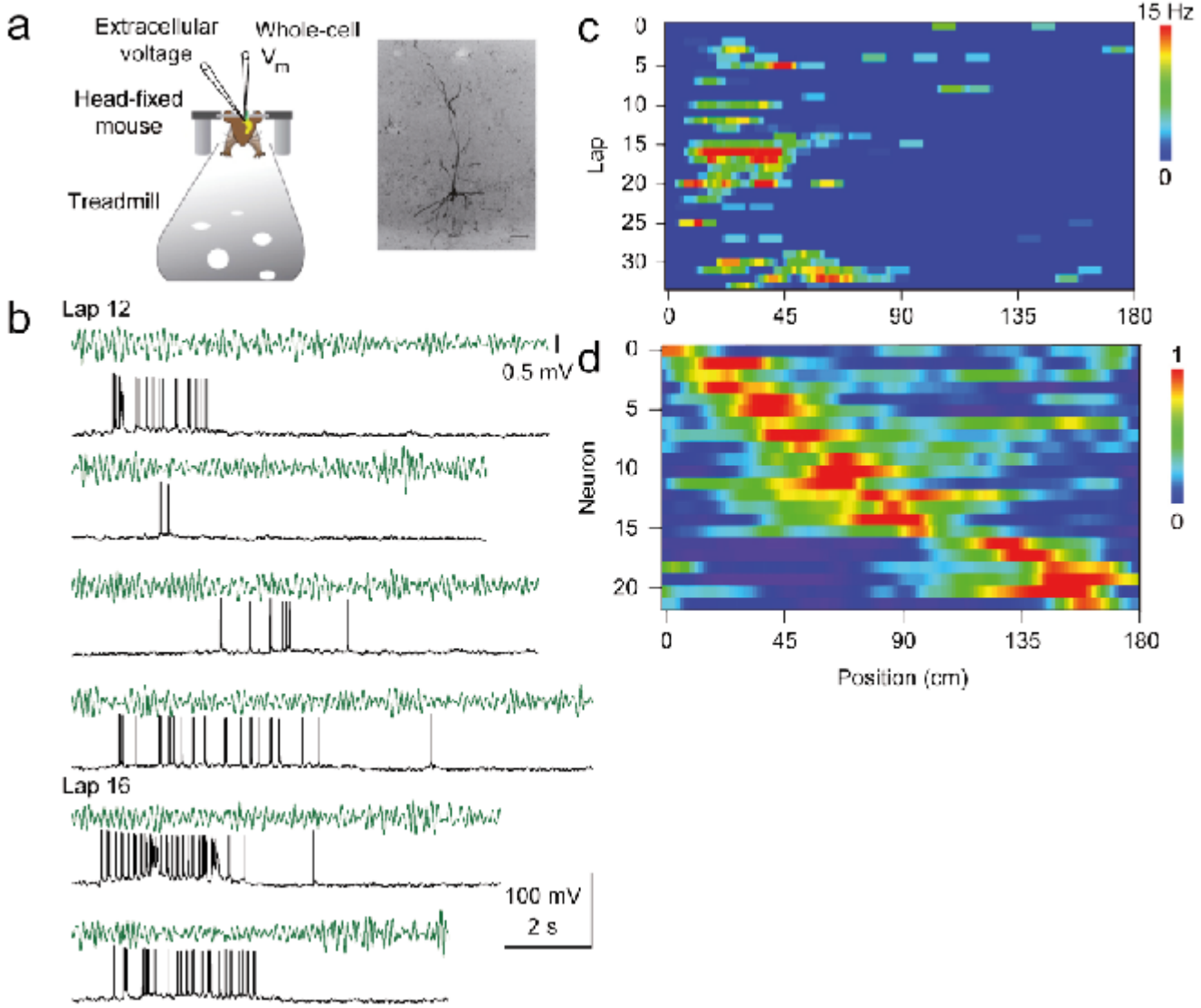
Identify cell
Identify putative synaptic sites.

Wang et al. Nat. Protocols 2015

In Vivo Current-Clamp Recordings
- record sub-threshold events and APs

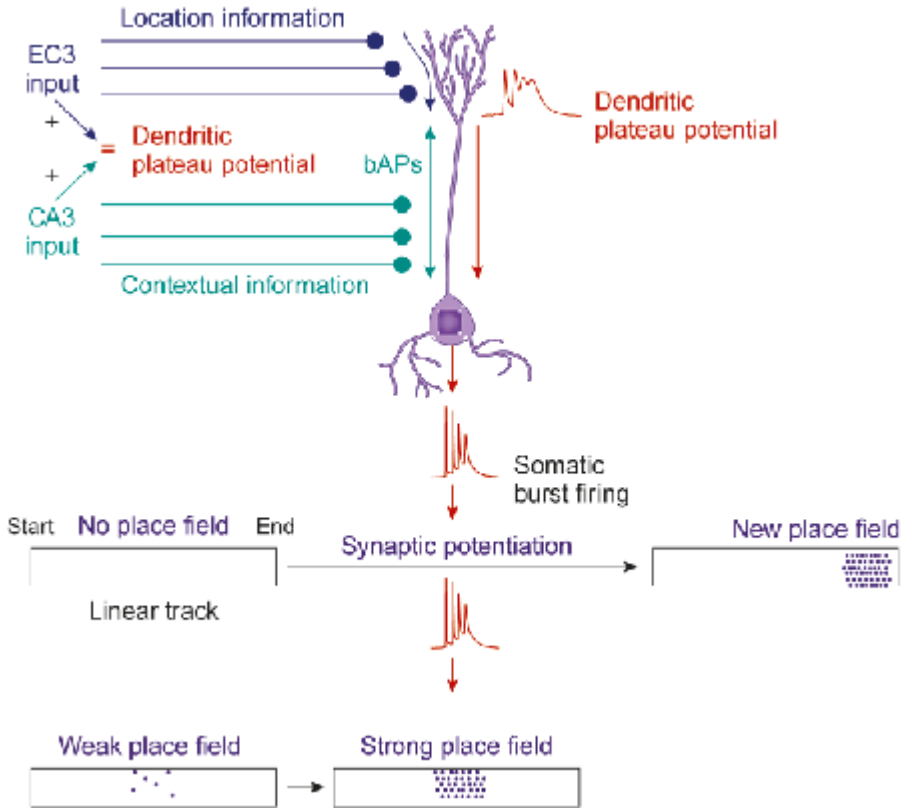


Sheffield & Dombeck Nat. Neurosci. 2015



Bittner Nat. Neurosci 2015

In Vivo Current-Clamp Recordings
- record sub-threshold events and APs



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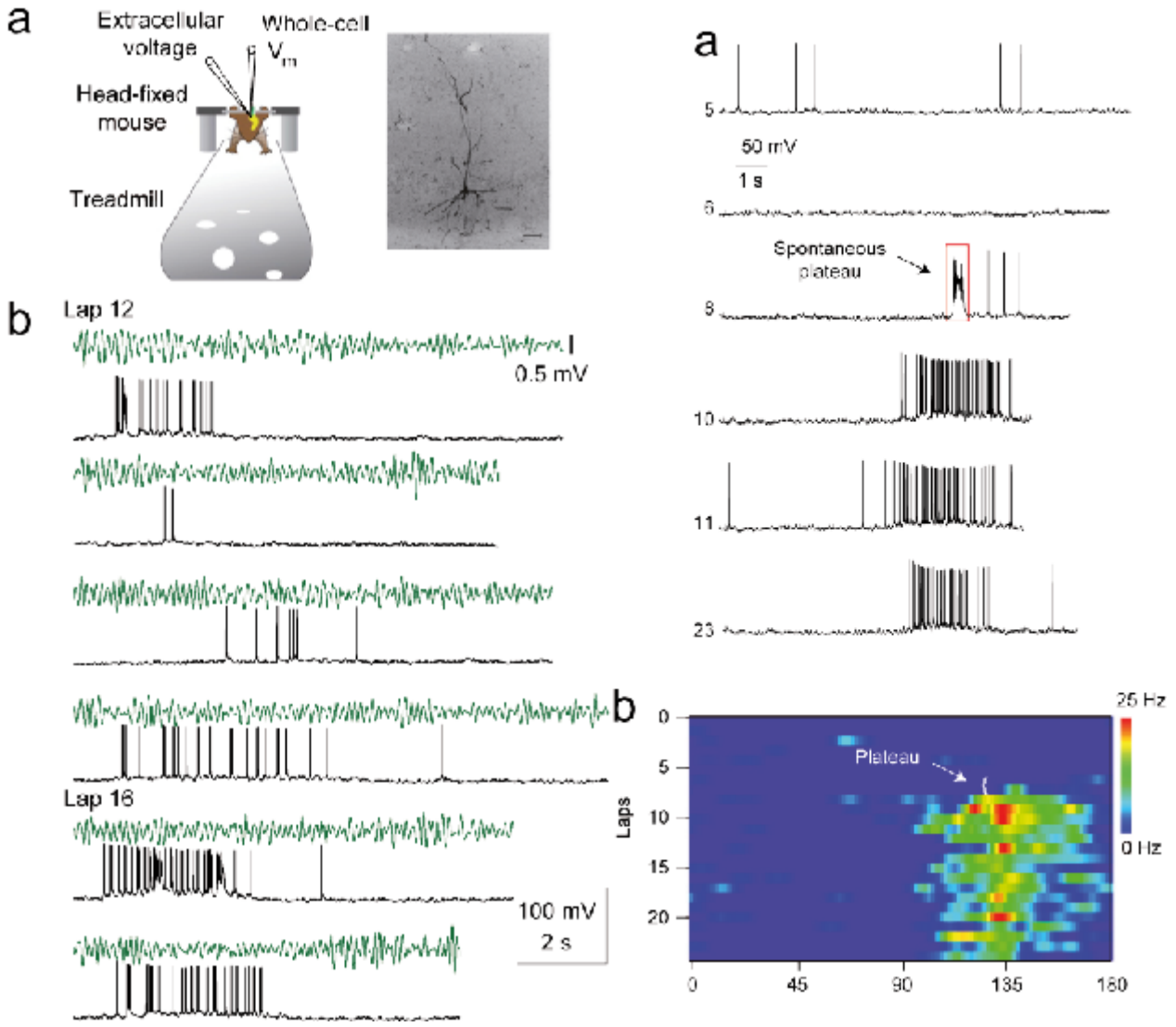


Bittner Nat. Neurosci 2015

Current-Clamp Conclusions

Current-clamp recordings measure the signals received by the cell and the responses.

Recording configurations
- Sharp Microelectrode
- Whole-Cell Patch-Clamp

Preparation
- cultured cells
- slices
- in vivo

Instrumentation
- Bath electrode - LJP, measure, compensate
- Series resistance - voltage divider
- Series resistance – filter

- What are your solutions and LJP?
- What amplifier are you using?

Signal Processing
- Filter types
- Nyquist

What is you filtering and sampling rate
What is your ADC (bits, range, gain)?

Basic Protocols and Analysis
- Passive properties (Vm Rm, tau)
- Action potential properties (Peak, dV/dt, Threshold)
- Firing pattern (Regular or Burst Firing)
- Synaptic potential (amplitude and kinetics)
- Dual-Patch (transmission of potential within the cell)
- Multiple cell recordings (connectivity and networks)
- In vivo recordings in awake behaving animals (gold standard)
- Combined with optical and optogenetic manipulations

Voltage-Clamp
- what is voltage-clamp

textbook: … in a voltage clamp experiment one controls the membrane voltage and measures the transmembrane current required to maintain that voltage …

simplest scenario: … keeping the voltage constant across a piece of membrane attached to a pipette (small membrane patch to a whole cell) and record current …

Keep control of the key variable that gates voltage-activated ion channels.

Voltage-Clamp
- why voltage-clamp

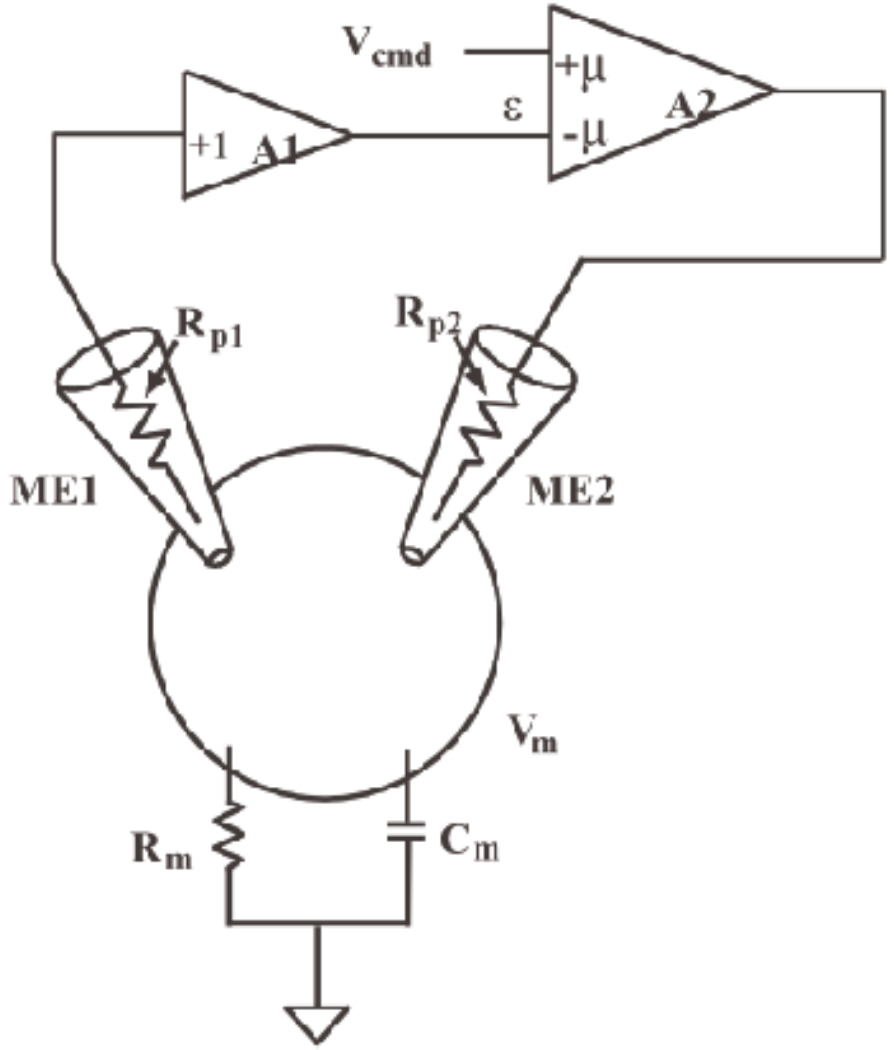
whenever transmembrane current is what you are interested in ...

- identification and observation of single channels
- studying their voltage dependence (activation, deactivation, inactivation, reverse potential)
- investigating their pharmacology
- effect of mutations/modifications on channel functions
- detect and dissect complex currents (e.g. in cells)
- monitor spontaneous and evoked synaptic currents, analyse their properties

Why record currents?

Voltage-Clamp Instrumentation
- two electrode voltage-clamp (TEVC)

TEVC equivalent circuit
- ME1 records voltage
- ME2 injects current



Voltage-Clamp Instrumentation
- single electrode voltage-clamp (SEVC)

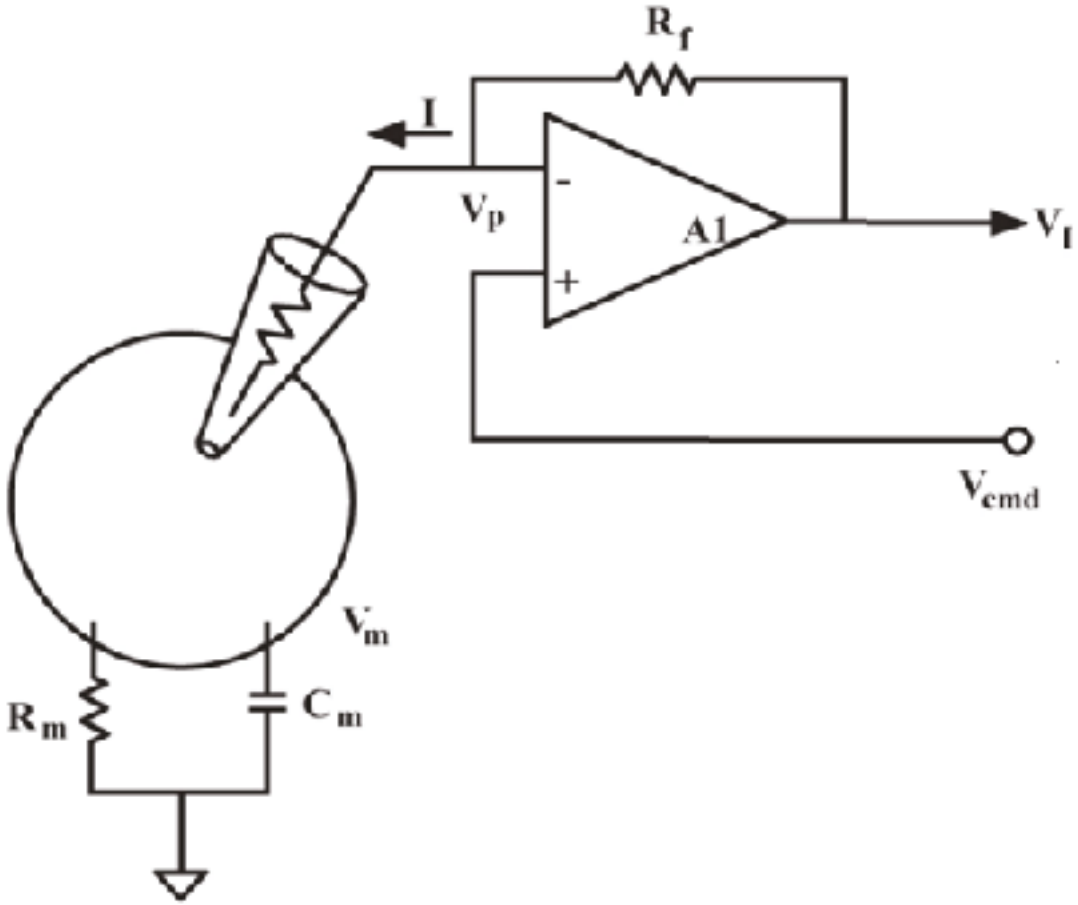
cSEVC simplified equivalent circuit

same electrode records voltage
and injects current

injects current I to keep Vp at Vcmd

Vp - Vcmd => VI is proportional to I

Rf sets ‘dynamic range’



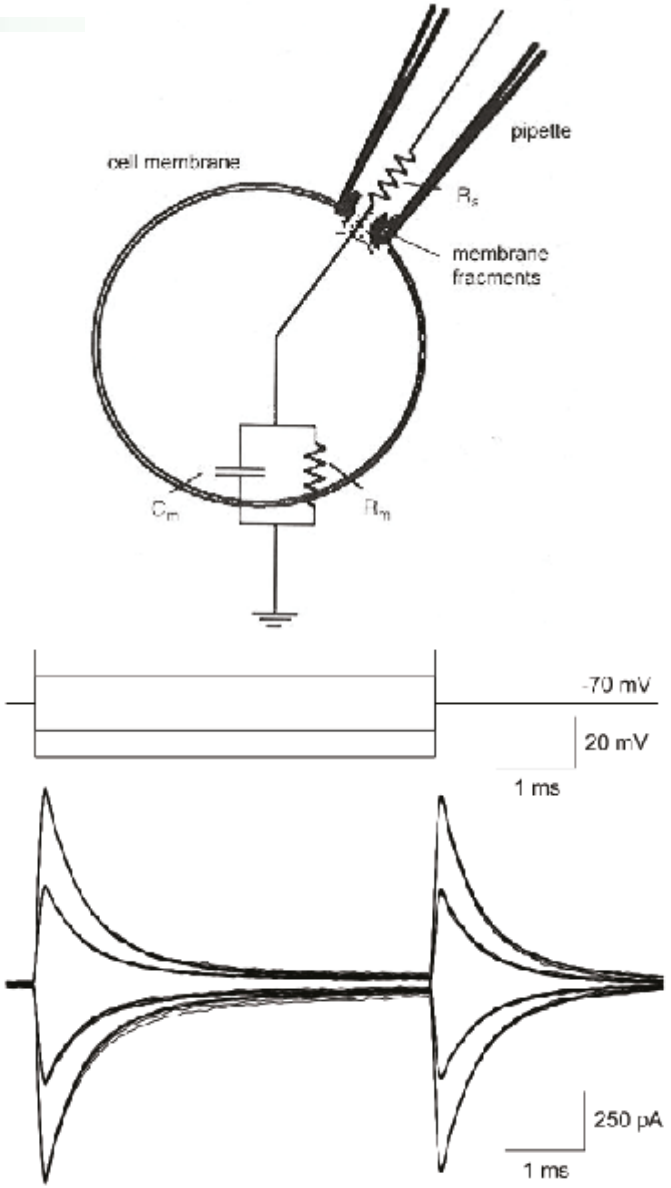
Voltage-Clamp Theory - key parameters

key parameters:

Ra series/access resistance
Rm membrane resistance,
Cm cell capacitance

key assumption:

Ra stable and << Rm



Voltage-Clamp Theory - key parameters

Ra = V1 / I0

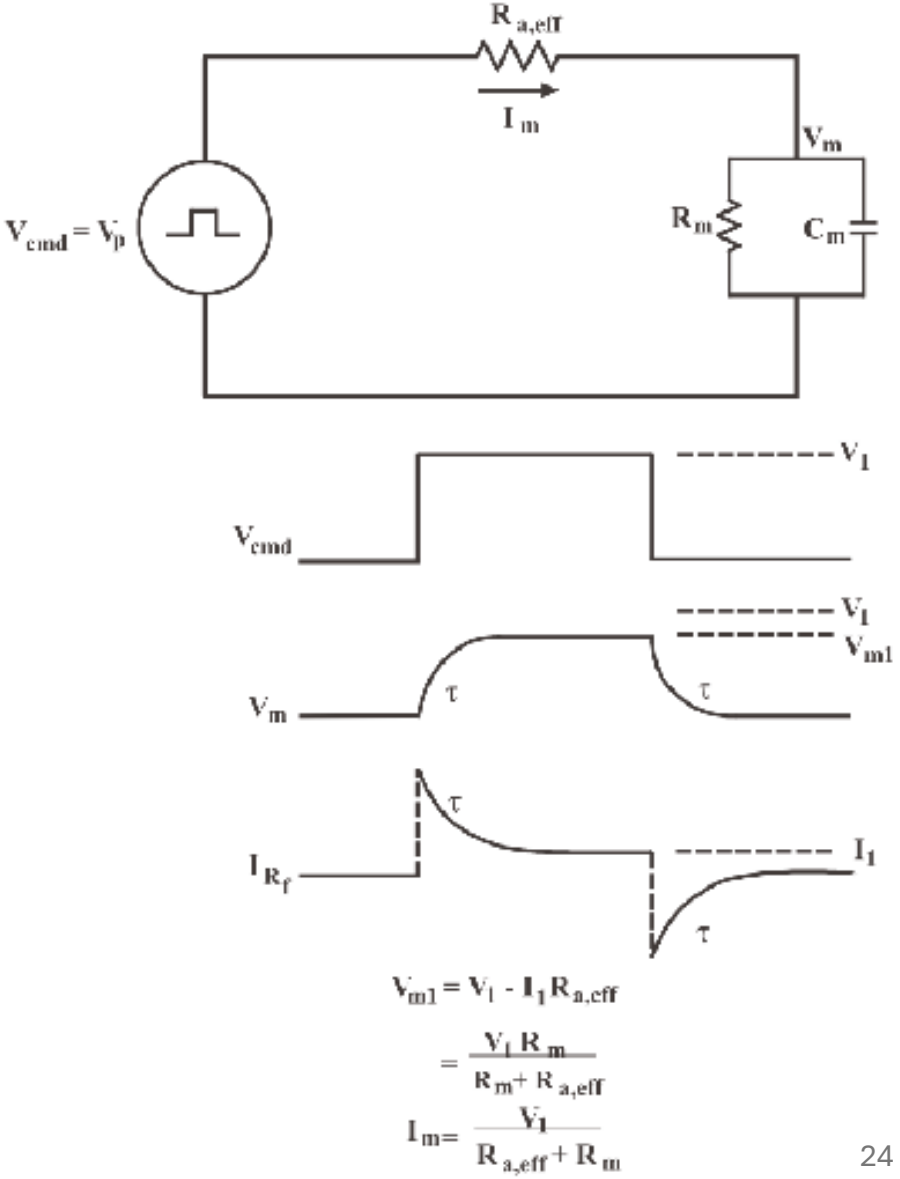
Vm1 = V1 – (I1*Ra)

Rm = V1 / I1 - Ra
if Ra << Rm then

Vm1 = V1
t = Cm × Ra

useful to extract basic information about the cell and the quality of the recording

Important to estimate for compensation.



$$V_{m1} = V_1 - I_1 R_{a,eff}$$

$$= \frac{V_1 R_m}{R_m + R_{a,eff}}$$

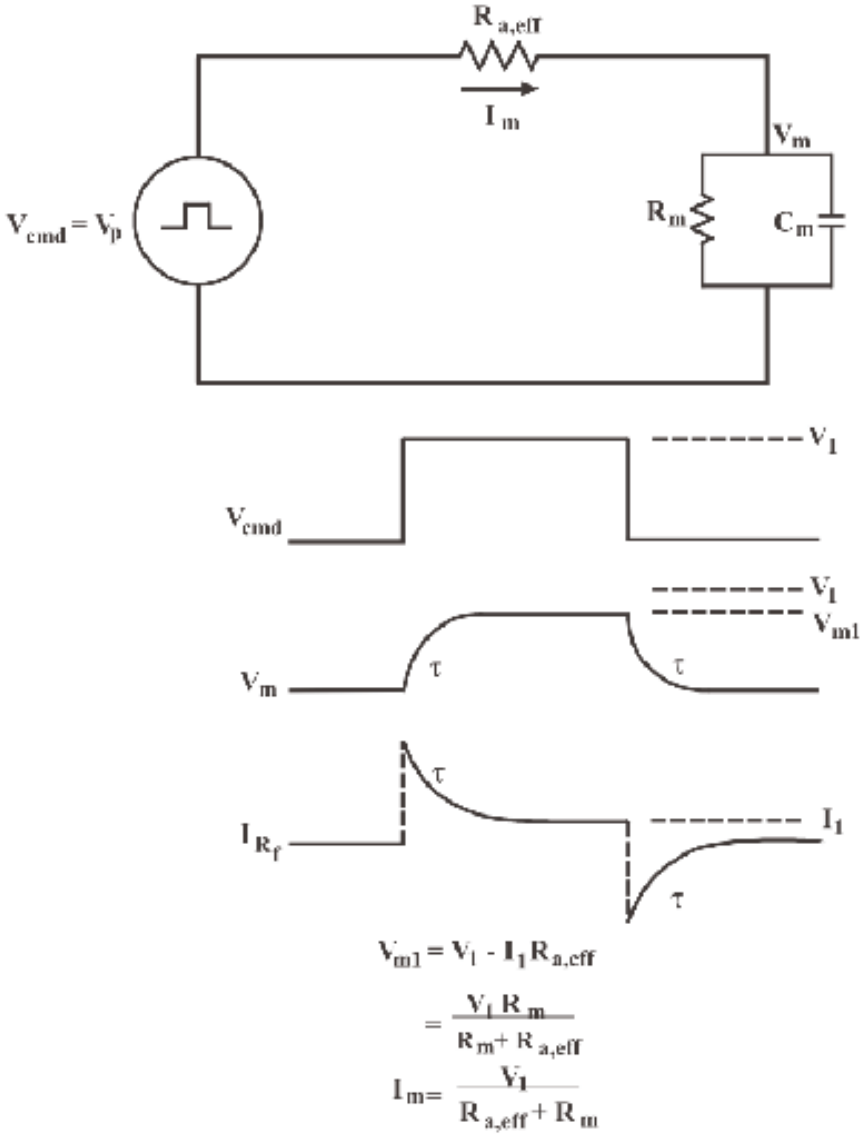
$$I_m = \frac{V_1}{R_{a,eff} + R_m}$$

Voltage-Clamp Recordings
- voltage-clamp errors

1) access resistance introduces voltage error (I × Ra).
dynamically changes with membrane current (e.g. INa)

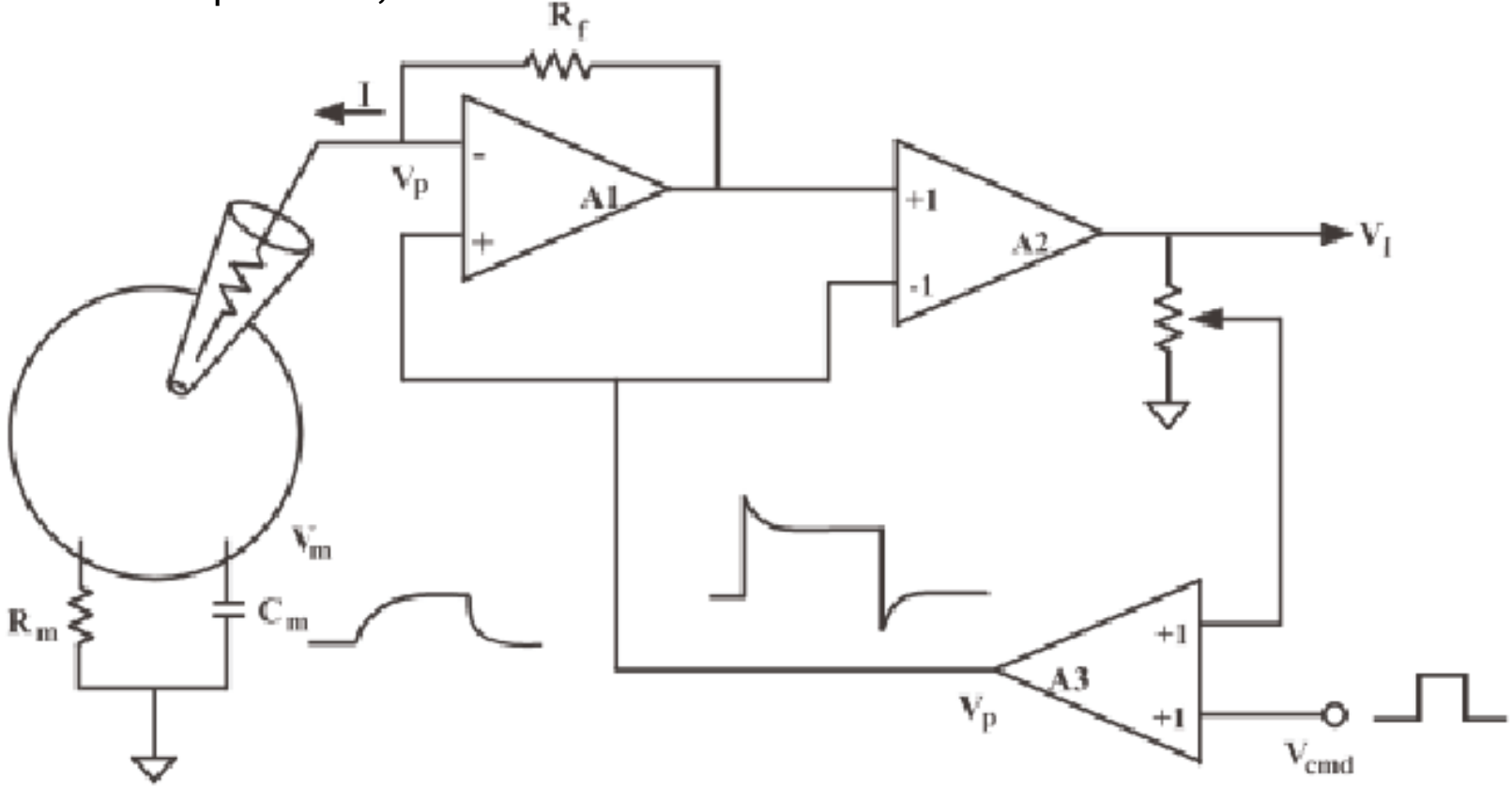
2) cell capacitance (Cm) introduces another dynamic error (voltage steps)

3) cell capacitance adds a transient current that does not correspond to a transmembrane current



Voltage-Clamp Recordings
- voltage-clamp correction

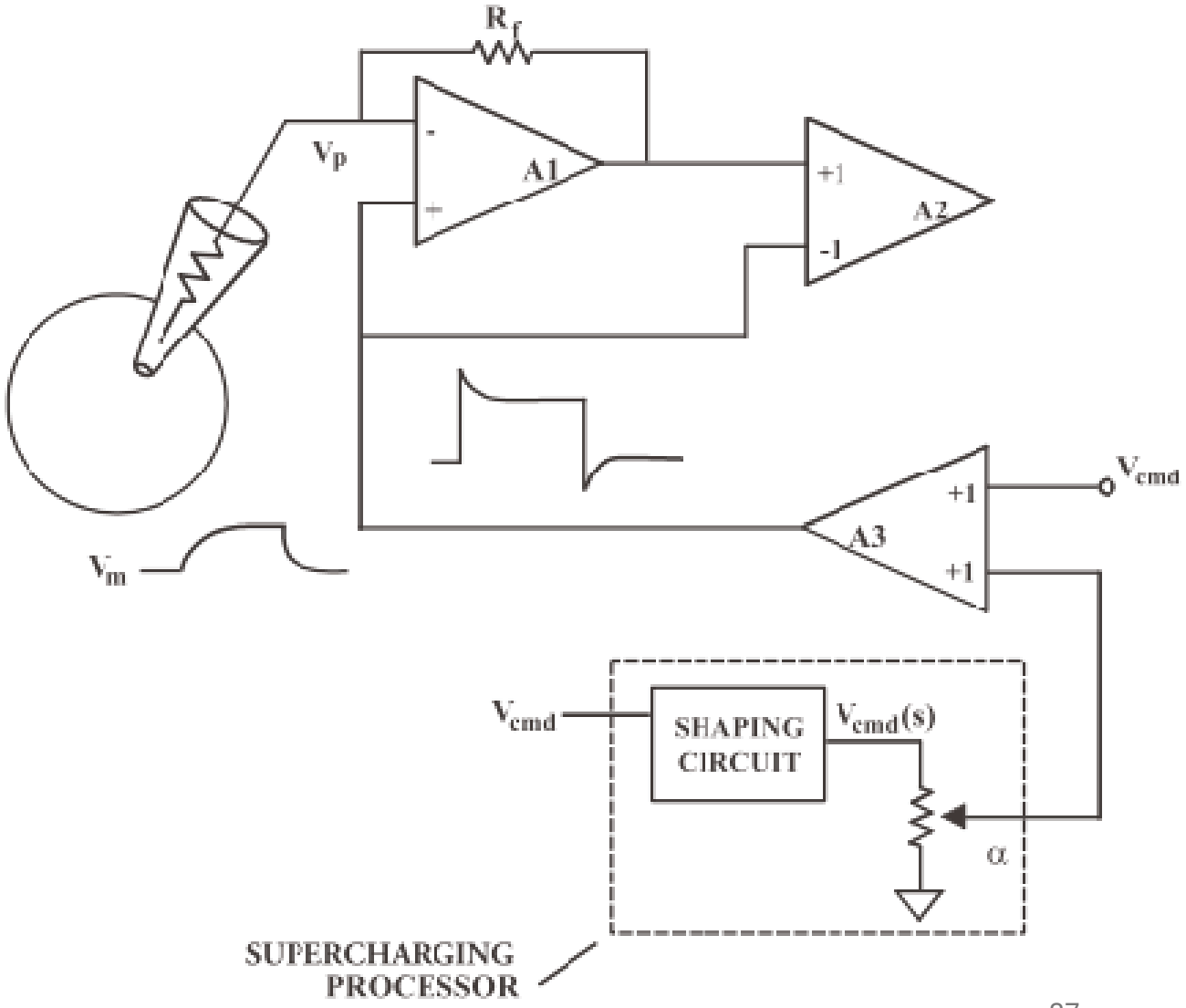
Access resistance compensation by positive feedback
(MultiClamp, % correction)
command that is proportional to the current.
corrects for both voltage errors 1 and 2.
100 % is not possible, oscillations



Voltage-Clamp Recordings
- voltage-clamp correction

Overcome slow membrane voltage change by Supercharging, added voltage command (MultiClamp: % prediction)

Calculates required additional voltage based on Known cell parameters
(no feedback = no oscillations,
no correction for steady-state voltage error
No improvement of dynamic response

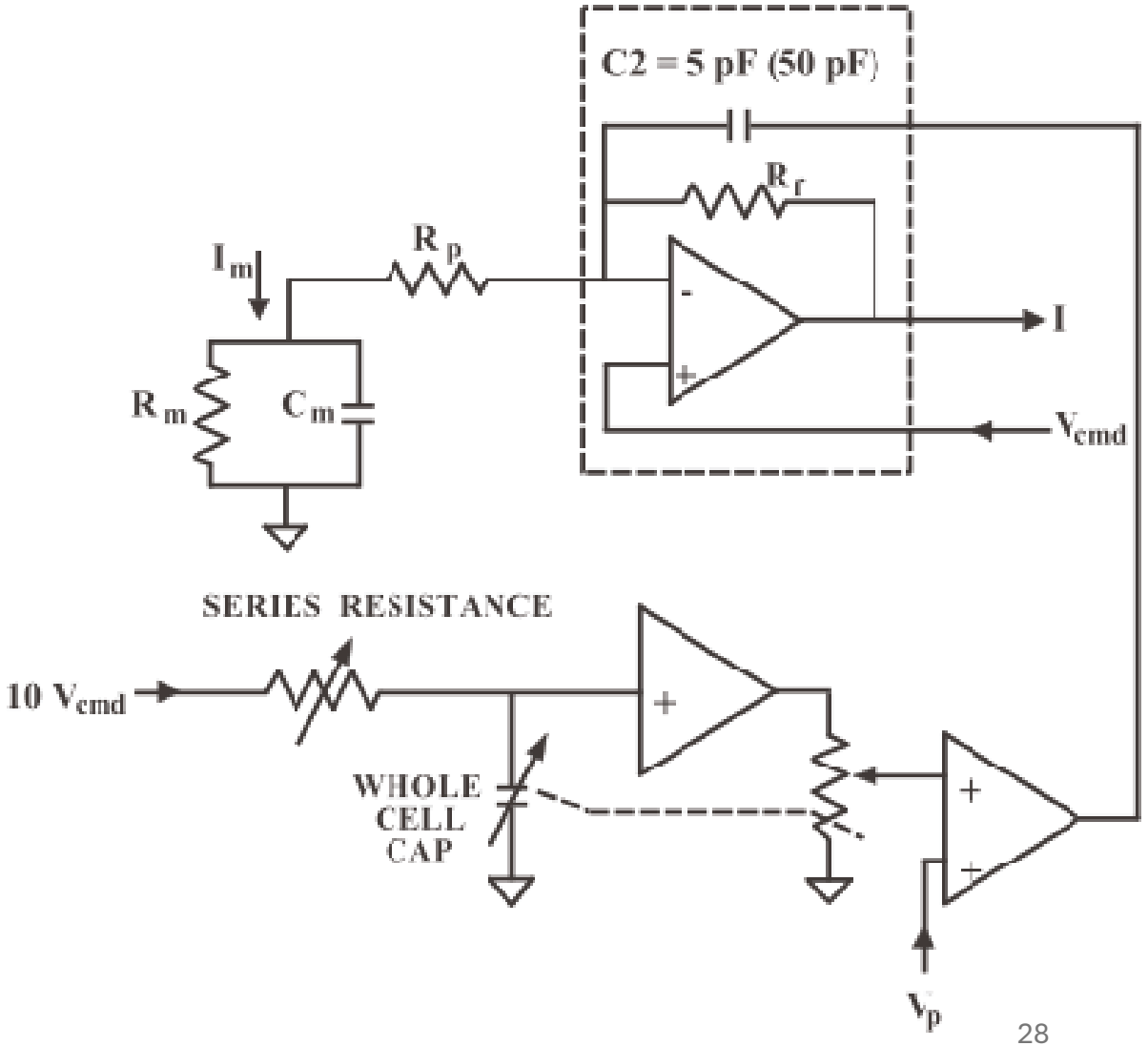


Voltage-Clamp Recordings
- voltage-clamp correction

Whole-cell capacitance compensation

MultiClamp:
Injects additional current that bypassing the feedback resistor and avoids saturation

Careful combination of methods (depends on amplifier)



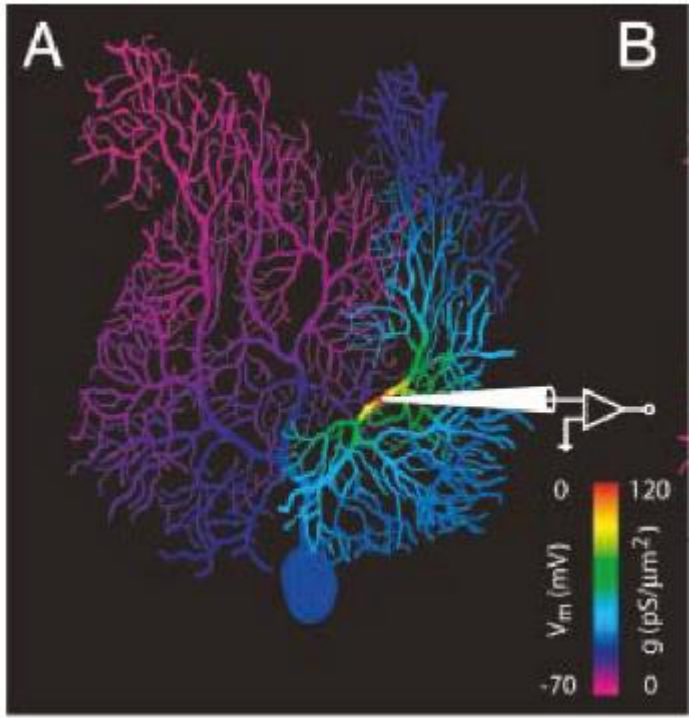
Voltage-Clamp Recordings - space-clamp errors

Ideal cell for volage-clamp,
- -small sphere
- -with moderate Rm and low Ra
- -low Cm

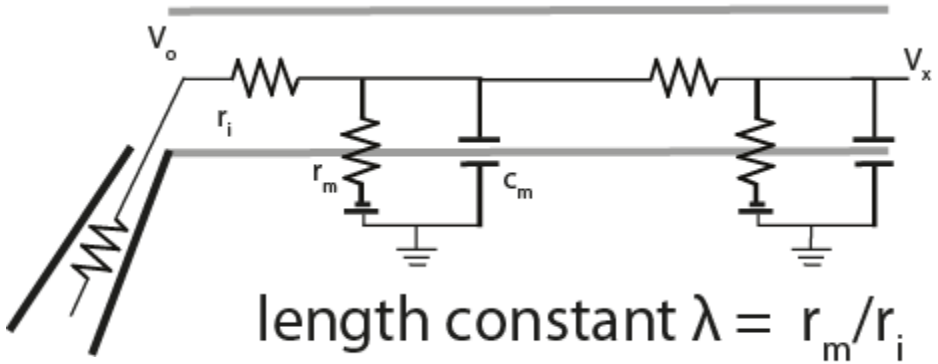
Neuones - complex cell
- -high capacitance
- -low Rm
- -high serial access resistance to distant compartments

Make neurone electrically more compact
- -Cs+ based intracellular solution

see Williams and Mitchell Nat Neurosci 2008
comment by Spruston and Johnston 2008



Häusser 2003



length constant $\lambda = r_m/r_i$

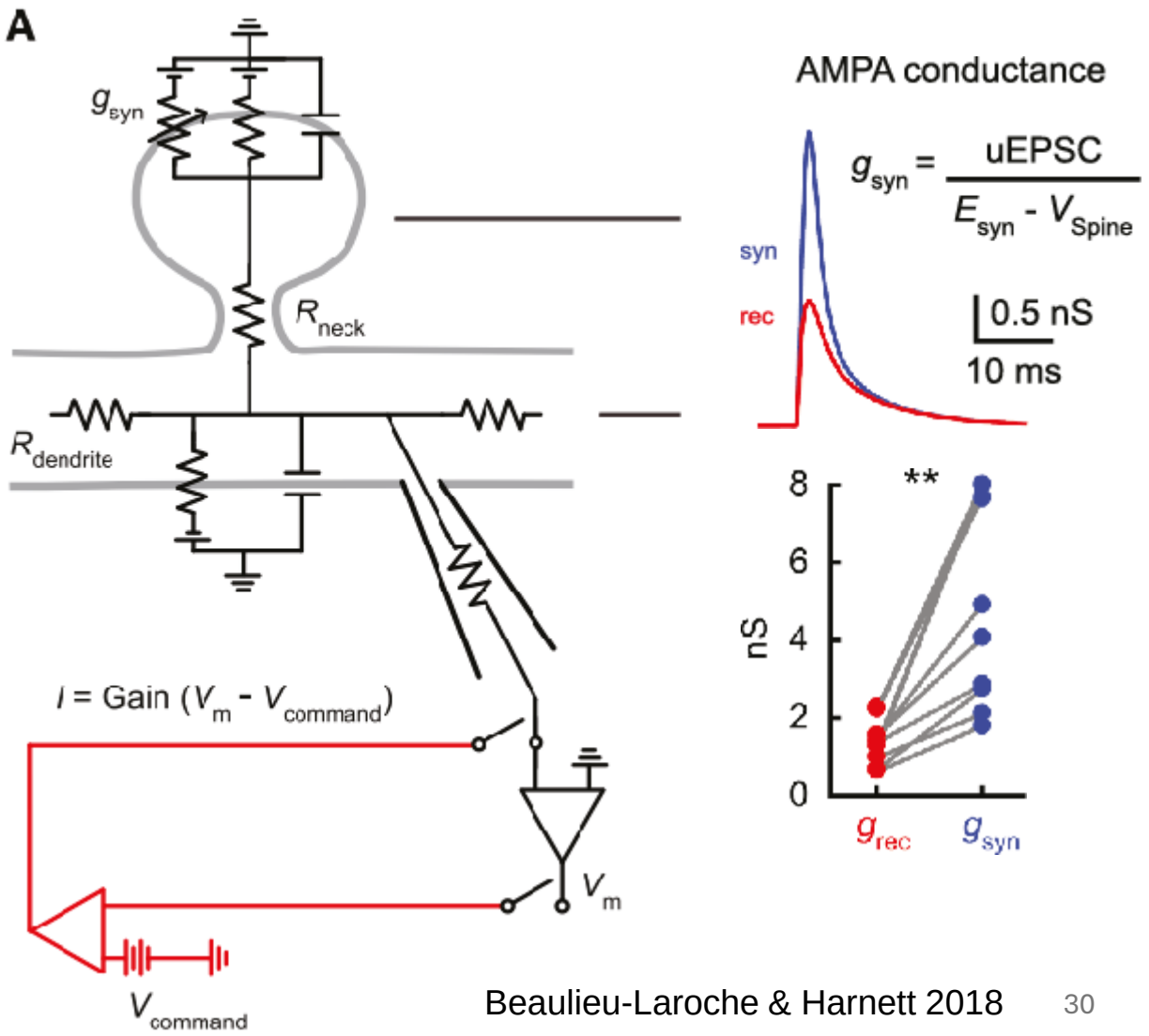
$V_x = V_o e^{-x/\lambda}$

Voltage-Clamp Recordings - space-clamp errors

spiny cells: spine neck resistance (RNeck) ~ 300-1000 MOhm

‘isolates’ dendritic spines electrically (gsyn depolarizes spine a lot more than dendrite)

voltage clamp can only affect what is ‘seen’ at the dendrite (e.g. INeck) but cannot control spine voltage



Beaulieu-Laroche & Harnett 2018

Voltage-Clamp Errors - summary

Goal: To relate the transmembrane current at a precisely controlled voltage

Key factors

Ra - the voltage across your Ra is subtracted from your Vcmd

Im - The larger the current flowing the larger the voltage error
and as channels get recruited more currents flow so the error is dynamic
So not suitable for large currents

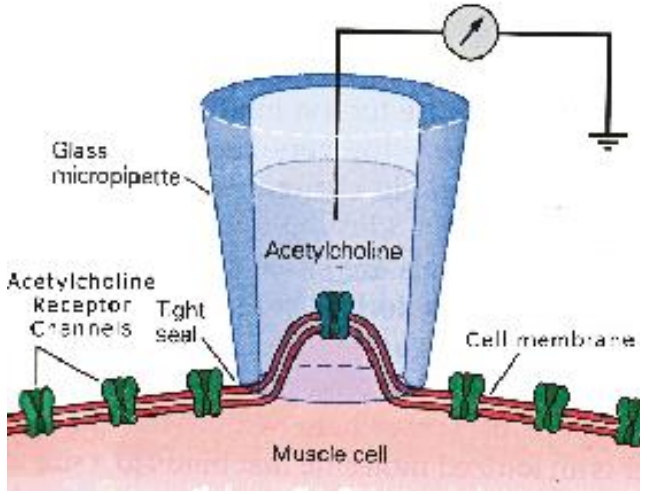
Cm - the cell capacitance determines the speed of your clamp
which is important for fast current

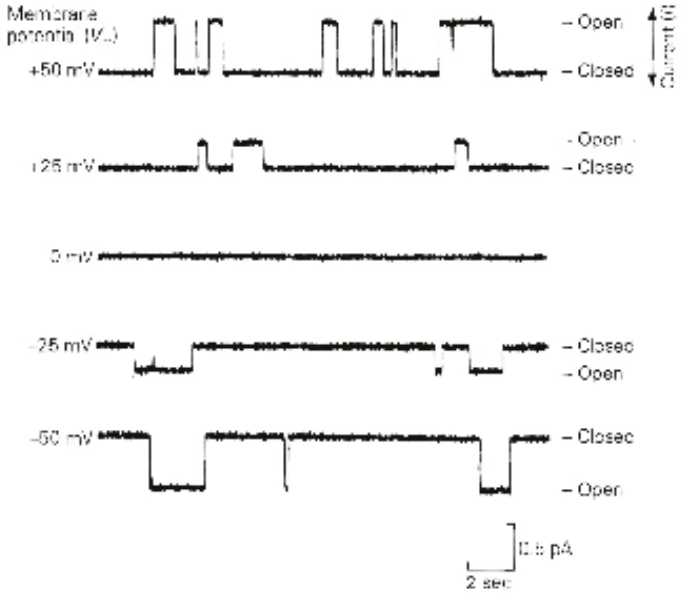
Space-Clamp - the further you are from the point of patch pipette the less voltage control you have

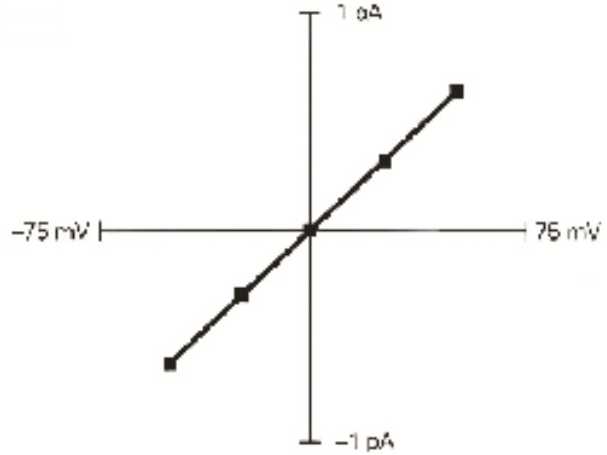
Is voltage-clamp appropriate for your question

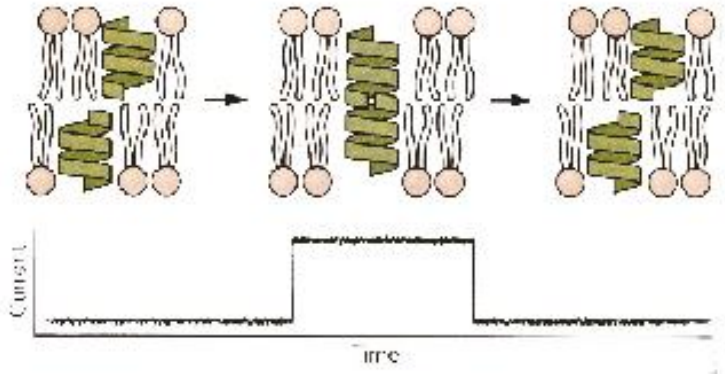
Voltage-Clamp Recordings
- single channel recordings

Neher & Sakmann, 1970-80's
Nobel prize 1991

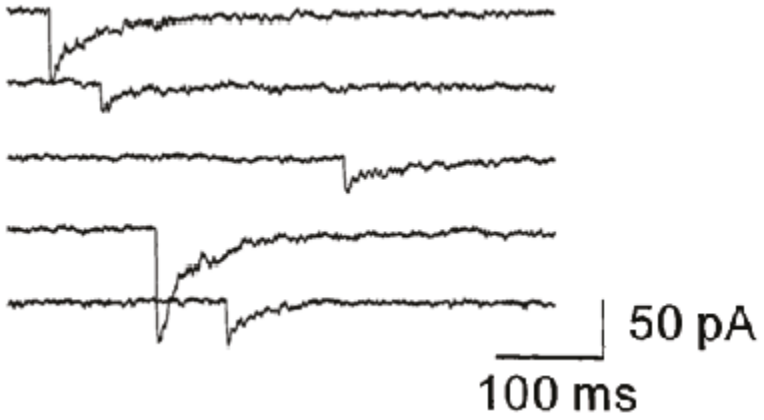




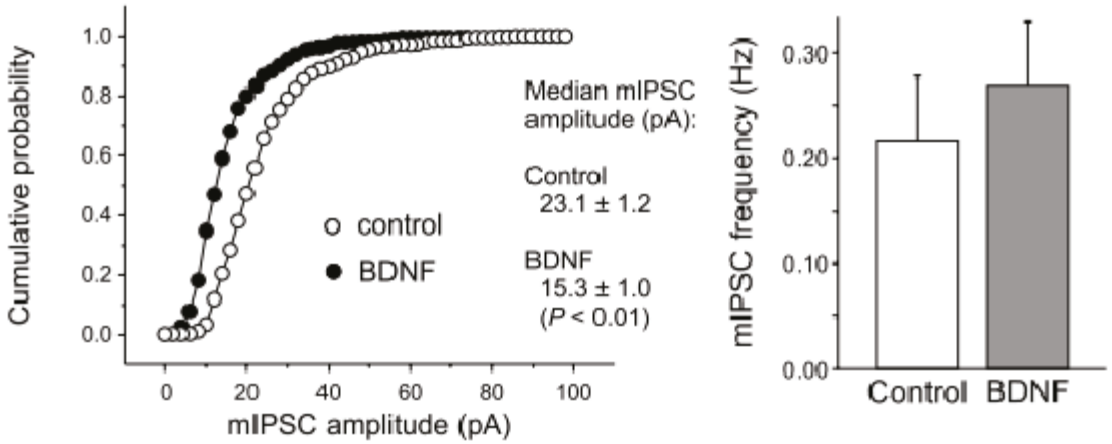




Voltage-Clamp Recordings -synaptic currents

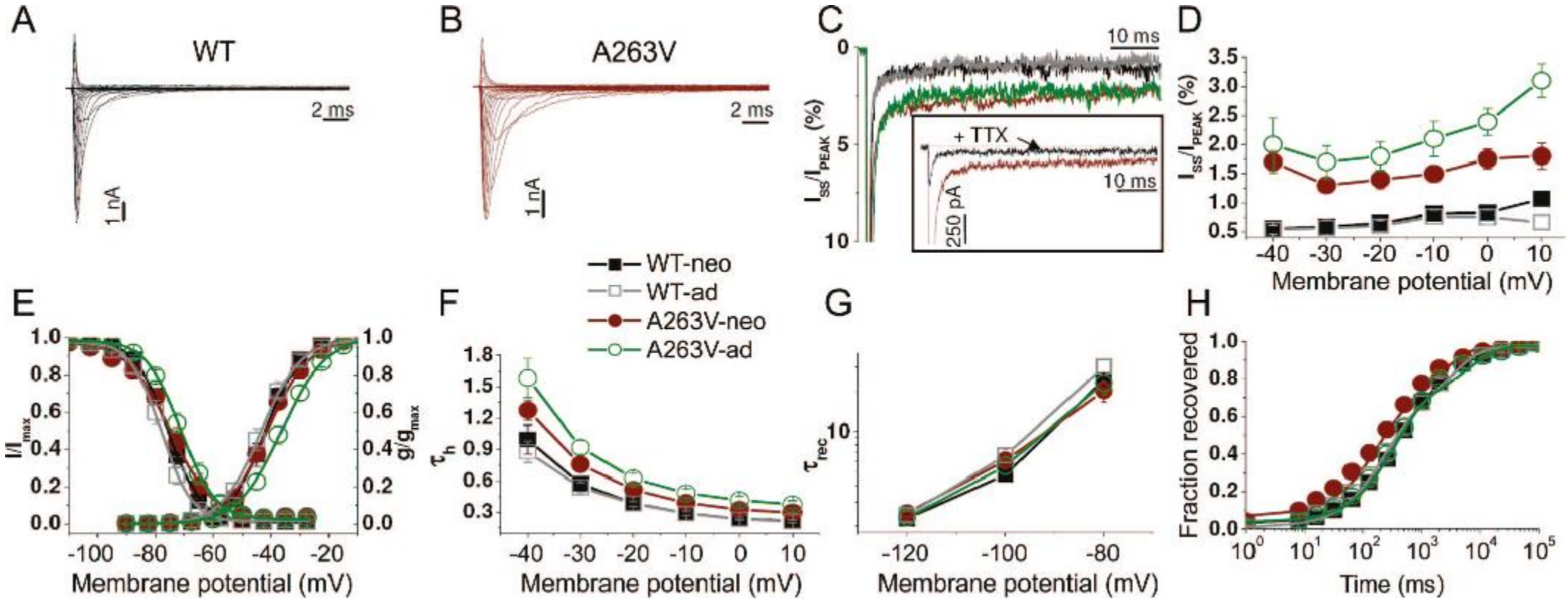


‘miniature PSC (mPSCs) action potential independent (eg in TTX), amplitude and frequency changes are used to localise an effect (pre/postsynaptic)



Jüttner et al. 2001, Henneberger et al. 2005

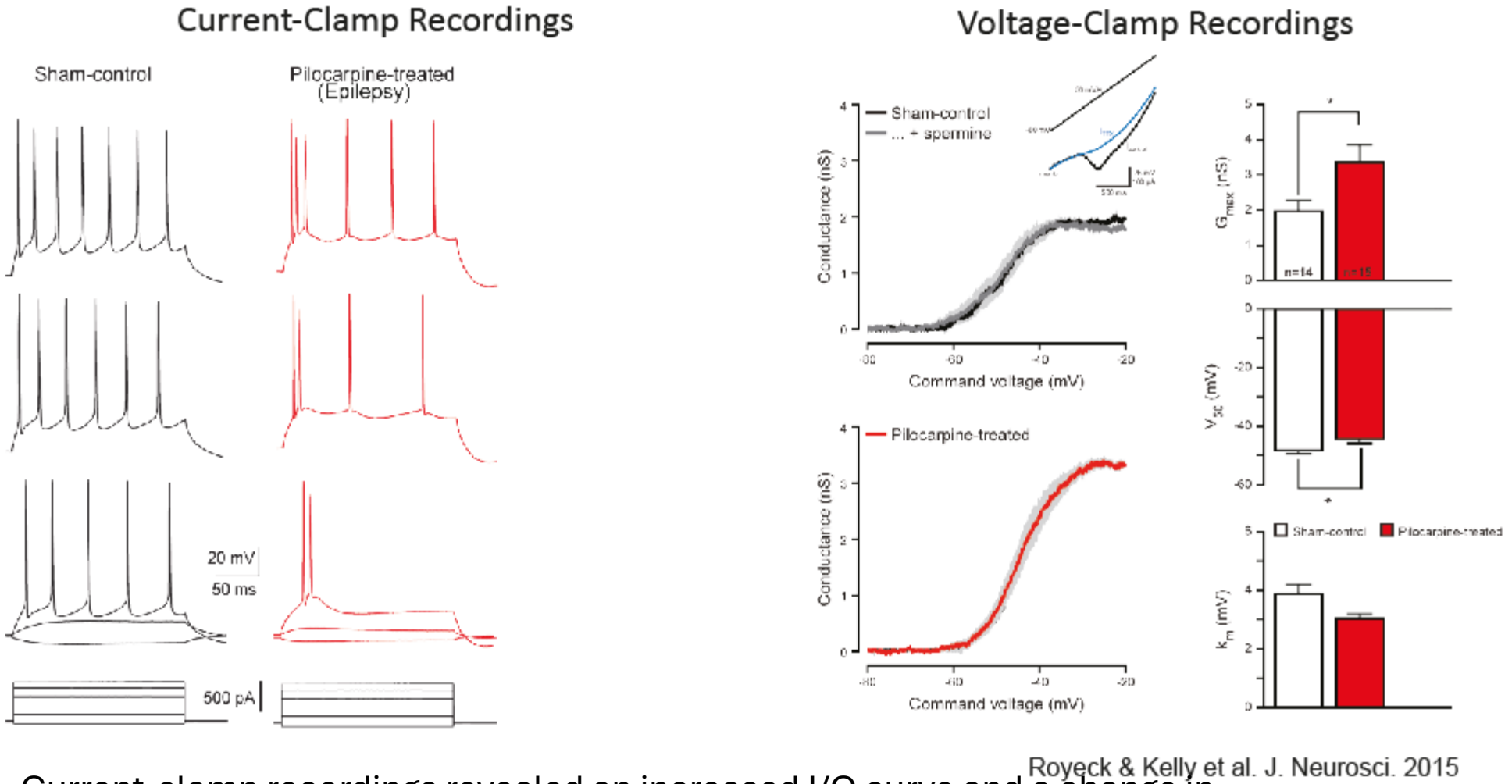
Voltage-Clamp Recordings
- mutations / pharmacology



Kinetics of activation, deactivation and inactivation
Precise biophysical understanding/model

Voltage-clamp recordings revealed increased steady-state Na+ current
Analysis of biophysical properties identified slowing of fast inactivation.

Current & Voltage-Clamp Recordings
- link ionic mechanisms and function

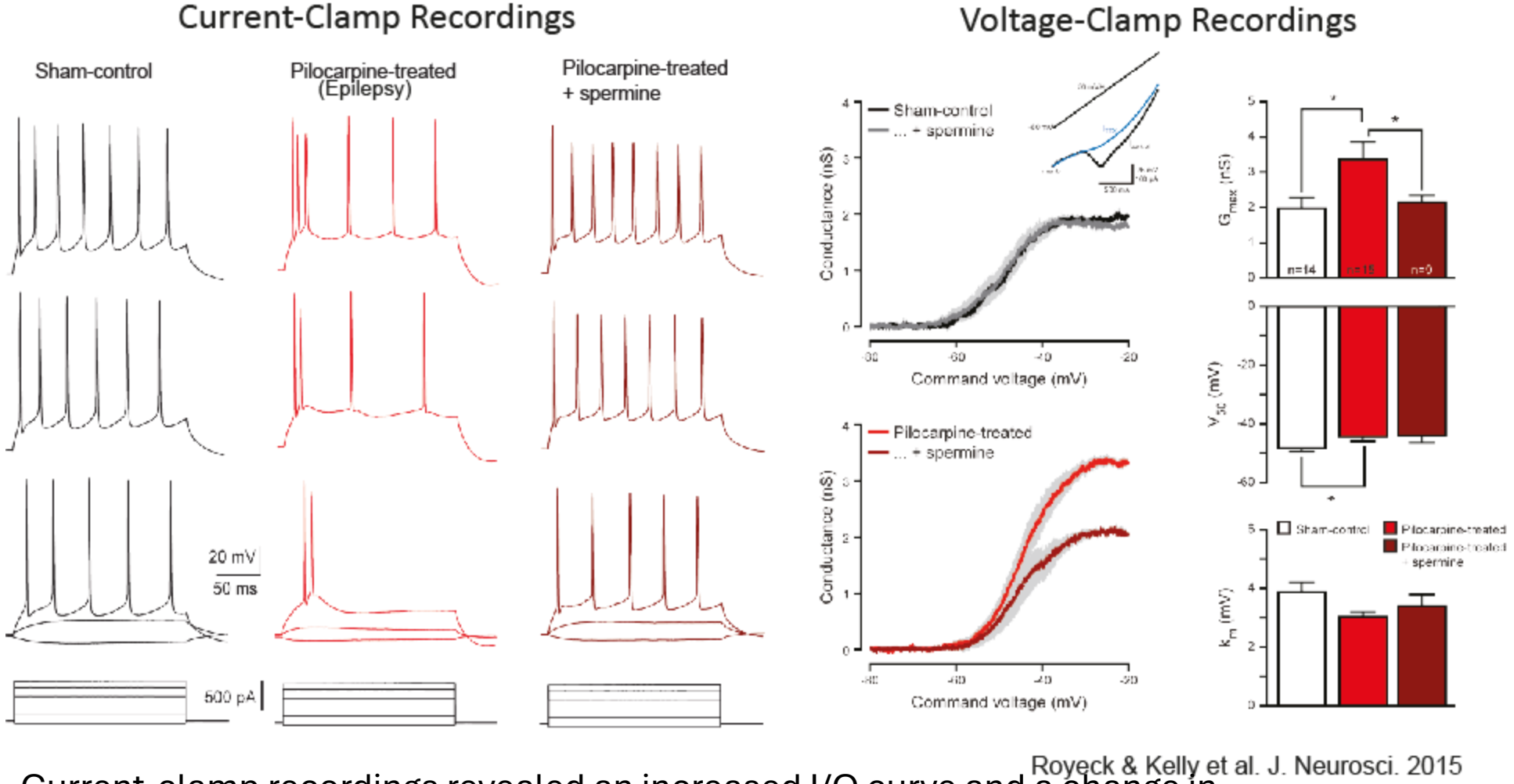


Royeck & Kelly et al. J. Neurosci. 2015

Current-clamp recordings revealed an increased I/O curve and a change in burst firing in CA1 neurones from chronically epileptic animals.

Voltage-clamp recordings identified an ionic mechanism

Current & Voltage-Clamp Recordings
- link ionic mechanisms and function



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Voltage-clamp recordings identified an ionic mechanism

Summary
- voltage-clamp

Voltage-clamp recordings measure the transmembrane currents mediated by ionic conductances.
- biophysical study of the currents/channels and their malfunction

Recording configurations
- SEVC
- TEVC

Preparation
- cultured cells
- slices

Instrumentation
- Bath electrode - LJP, measure, compensate
- Series resistance - voltage error
- Cm - voltage error and transient currents
- Space-clamp problems

Signal Processing
- Filter types
- Nyquist

- Do you need voltage-clamp and is it appropriate for your question?
- Can you improve the voltage-clamp
- Can you compensate & how

- Never ignore Rs; Rs << Rm

Basic Protocols and Analysis
- Biophysical study of the currents/channels and their malfunction
- Synaptic currents (amplitude and kinetics)
- Combined with optical and optogenetic manipulations

Conclusions

Intracellular electrophysiological recordings – patch-clamp and sharp microelectrode

Current-Clamp
- control the current and measure voltage

Careful of interpretation
voltage errors due to pipette

Use Cases
Record physiological electrical responses
characterise/identify cell
characterise/identify inputs
characterise/identify connections

Voltage-Clamp
- control voltage and measure current

Careful of interpretation
voltage errors due to pipette/Im/Cm
space clamp errors

Use Cases
Record biophysical properties
ion channel kinetics activity
ionic currents kinetic activity
mutations/pharmacology

Intracellular ephys vs LFP, probe, Ca^{2+} imaging