Electrophysiological Recording Techniques

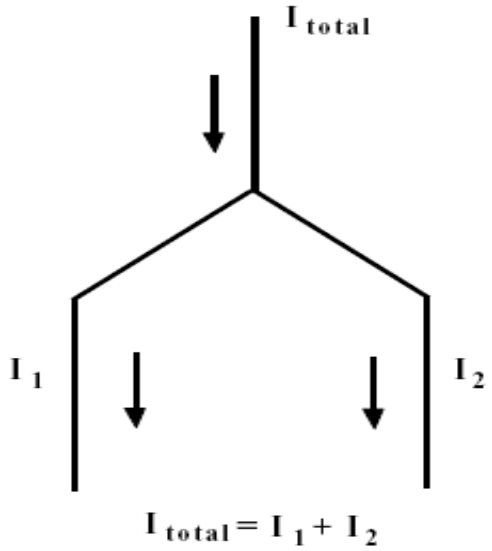
Wen-Jun Gao, PH.D.
Drexel University College of Medicine

Goal of Physiological Recording

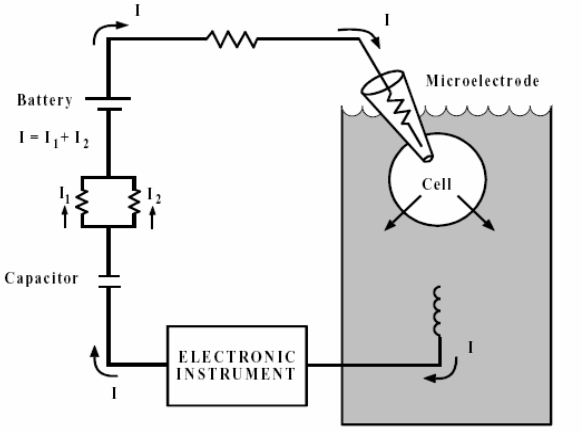
To detect the communication signals between neurons in real time (μs to hours)

- Current clamp
  - measure membrane potential, PSPs, action potentials, resting membrane potential
- Voltage clamp
  - measure membrane current, PSCs, voltage-ligand activated conductances

Current is conserved at a branch point



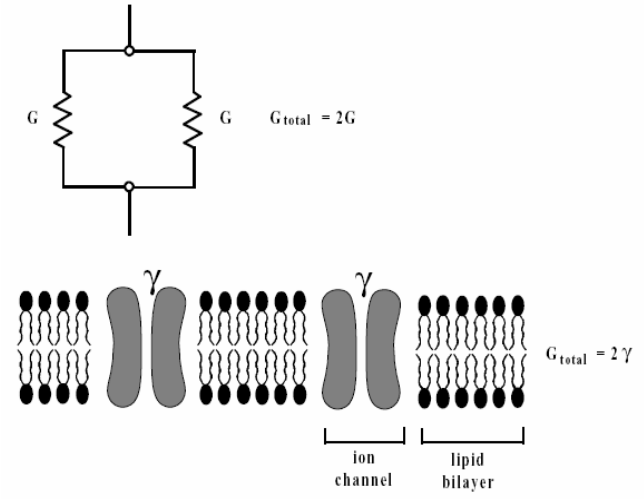
A Typical Electrical Circuit



Example of an electrical circuit with various parts. Current always flows in a complete circuit.

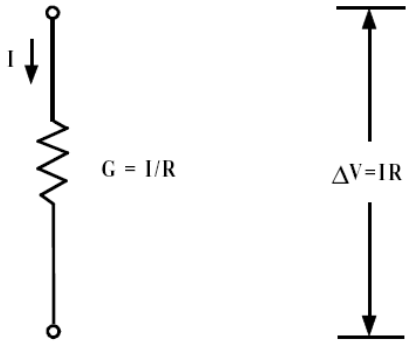
Resistors and Conductors

Summation of Conductance: Conductances in parallel summate together, whether they are resistors or channels.



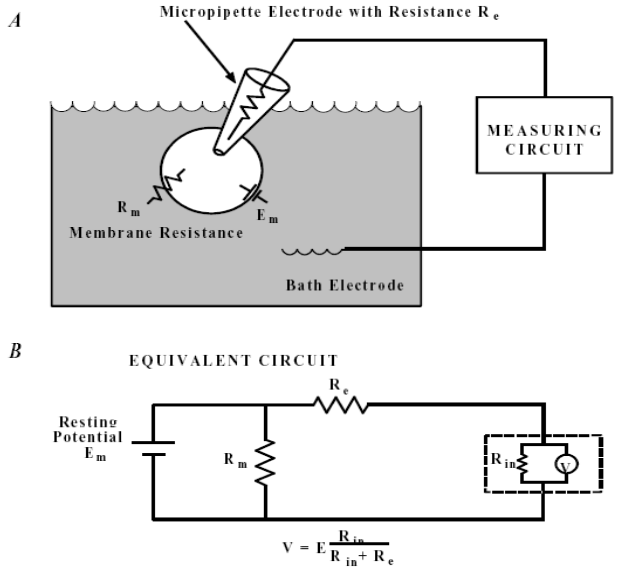
Ohm's Law

For electrophysiology, perhaps the most important law of electricity is Ohm's law. The potential difference between two points linked by a current path with a conductance G and a current I is: $\Delta V = IR = I/G$ (units: volts)



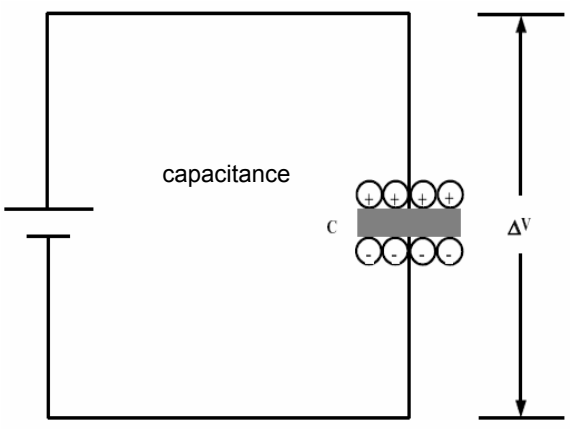
Representative Voltmeter with Infinite Resistance

Instruments used to measure potentials must have a very high input resistance Rin.

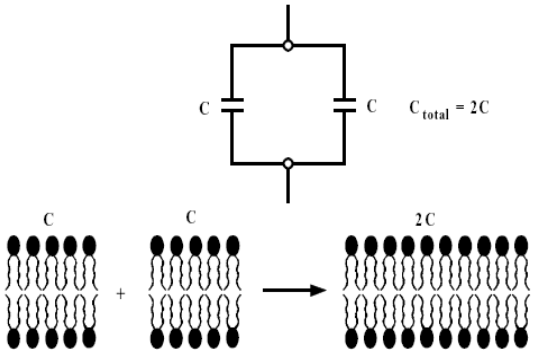


Capacitors and Their Electrical Fields

A charge Q is stored in a capacitor of value C held at a potential DeltaV. Q = C* delta V



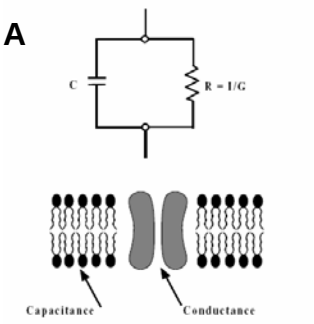
Capacitors in Parallel Add Their Values



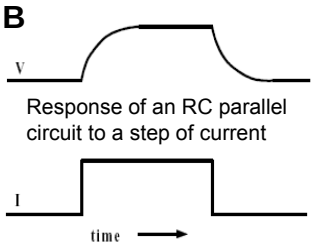
Currents Through Capacitors $I = C\frac{\Delta V}{\Delta t}$

Membrane Behavior Compared with an Electrical Current

A membrane behaves electrically like a capacitance in parallel with a resistance.



Now, if we apply a pulse of current to the circuit, the current first charges up the capacitance, then changes the voltage



The voltage V(t) approaches steady state along an exponential time course:

$$V(t) = V_{inf}(1 - e^{-t/\tau})$$

The steady-state value V*inf* (also called the infinite-time or equilibrium value) does not depend on the capacitance; it is simply determined by the current I and the membrane resistance R:

$$V_{inf} = IR$$

This is just Ohm's law, of course; but when the membrane capacitance is in the circuit, the voltage is not reached immediately. Instead, it is approached with the *time constant* t, given by

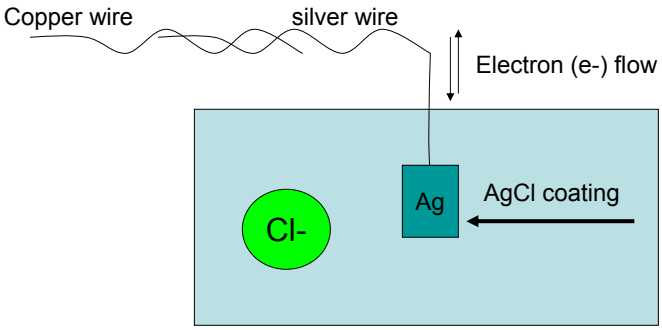
$$\tau = RC$$

- Thus, the charging time constant increases when either the membrane capacitance or the resistance increases. Consequently, large cells, such as *Xenopus* oocytes that are frequently used for expression of genes encoding ion-channel proteins, have a long charging phase.

Electrodes

- In circuits, we use wires
- In biology, nature uses liquids
- Electrodes are used to transform current flow from electrons to ions

Silver/Silver Chloride Electrode - AgCl Coating



Electrode reaction:
Ag + Cl- ⟺ AgCl + electron (e-)

The silver/silver chloride electrode is reversible but exhaustible

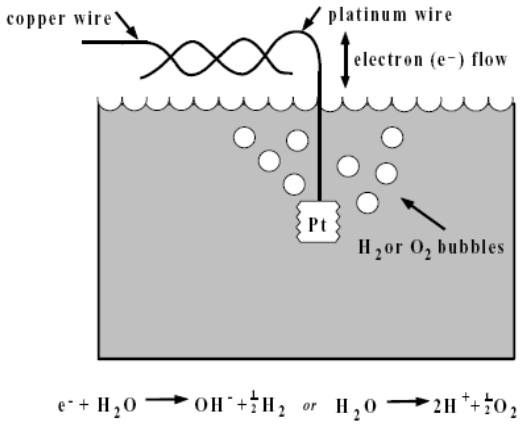
Several points to remember about Ag/AgCl electrodes

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

(2) Because current must flow in a complete circuit, two electrodes are needed. If the two electrodes face different Cl- concentrations (for instance, 3 M KCl inside a micropipette* and 120 mM NaCl in a bathing solution surrounding the cell), there will be a difference in the half-cell potentials (the potential difference between the solution and the electrode) at the two electrodes, resulting in a large steady potential difference in the two wires attached to the electrodes. This steady potential difference, termed *liquid junction* potential, can be subtracted electronically and poses few problems as long as the electrode is used within its reversible limits;

(3) If the AgCl is exhausted by the current flow, bare silver could come in contact with the solution. Silver ions leaking from the wire can poison many proteins. Also, the half-cell potentials now become dominated by unpredictable, poorly reversible surface reactions due to other ions in the solution and trace impurities in the silver, causing electrode *polarization*.

Platinum Electrode



A platinum electrode is irreversible but inexhaustible.

Electrodes and Pipettes

- Reversible electrode

– silver wire coated with Ag and AgCl

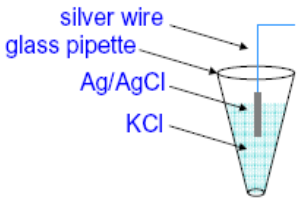
– forward flow: electrons from wire convert AgCl to Ag atoms and Cl- ions, the Cl- become hydrated and enter solution

– reverse flow: Ag atoms give up electron and combine with Cl- from solution

– **solution must contain Cl-**

– okay for some silver to be exposed

– if AgCl exhausted, Ag will leak into solution and poison cells



- Glass micropipettes

Pipette is critical for recording

- Fabrication
  - pullers (multi-stage pullers Sutter p-97)
  - glass with filament
  - tip size and shape (resistance)
  - fire polishing ?
  - sylgard? (bath solution level, dental wax or grease)
- Intracellular solutions
  - Osmolarity, pH value, blockers, dyes
  - clean and no contamination, filtered with Sterile *Membrane Filter* (0.45 μM)

Getting a recording

1. Find a “health” cell
2. Fill a pipette, place in holder and apply positive pressure
3. Put pipette in the bath
4. Get the test pulse running and make sure it works
5. Zero the offset
6. Bridget balance the pipette
7. Position the pipette above the cell
8. Verify positive pressure and advance into the slice
9. Push pipette tip onto cell surface, a small dimple would appear, and then release pressure
10. Apply slight suction and a negative holding potential (-60 mV)
11. If everything is right, you will get a gigaohm seal

Intracellular solutions

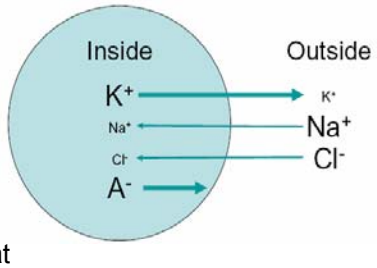
“Standard” intracellular (patch) solution:

135 K-Gluconate or K-MeSO4, 10 HEPES, 7 NaCl, 2 Na2-ATP, 2 MgCl2 (pH 7.2 with KOH)

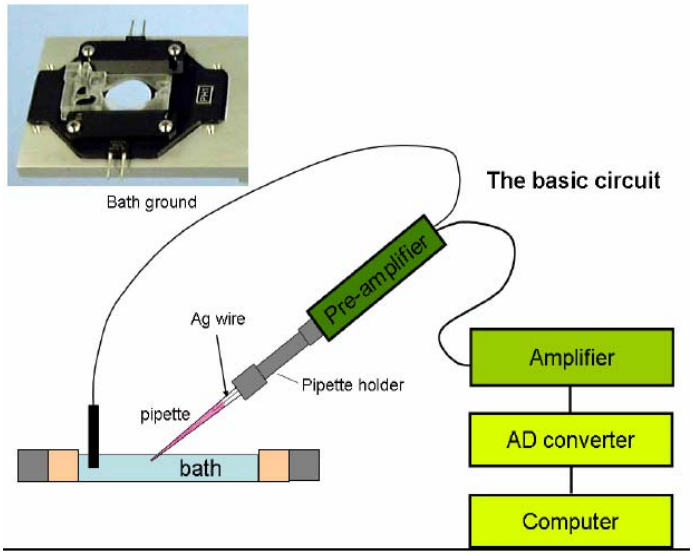
Osmolarity - ~10% lower than extracellular (higher is better for lower Rs)

- Could also add/replace:

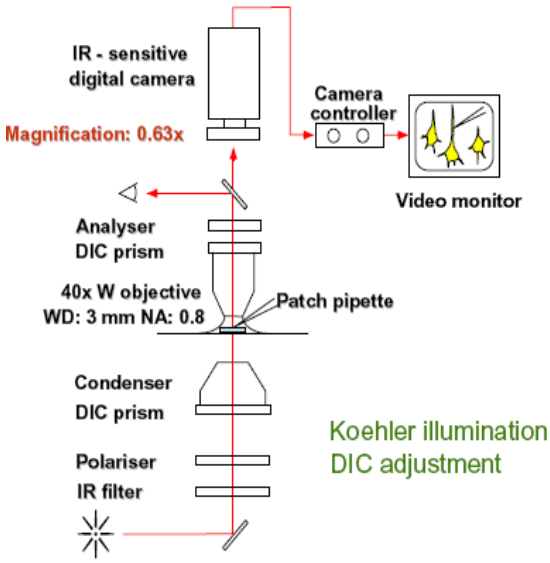
- GTP (0.3 mM)
- Phosphocreatine (10 mM)
- Fluorescence dyes or biocytin
- Cs instead of Gluconate/MeSO4 to block K+ currents
- High Cl to increase inhibitory responses at resting membrane potentials (eg. replace K-Gluconate with KCl)

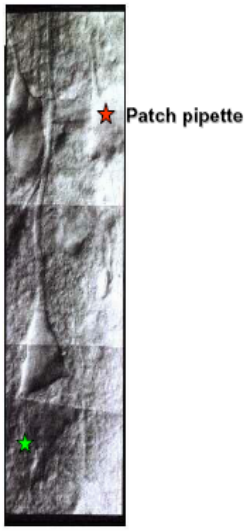


Patch clamp setup



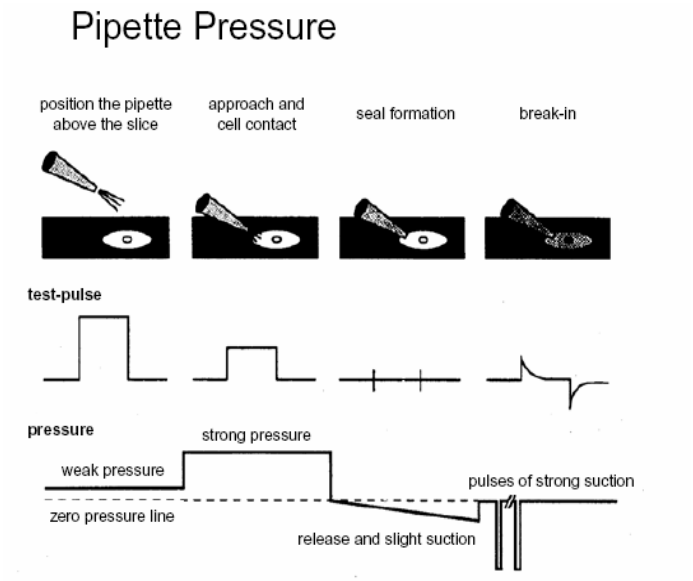
Optical Setup





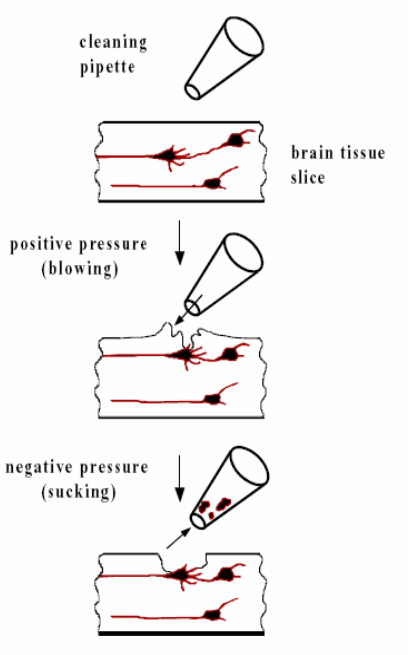
See http://www.olympusmicro.com/primer/techniques/dic/dicconfiguration.html

Steps to gigaseal



Surface cleaning is critical for successful recording

Use of a cleaning pipette to remove tissue overlying a cell in tissue slices.



Current Clamp and Voltage Clamp

- In a **current-clamp** experiment, one applies a known constant or time-varying current and measures the change in membrane potential caused by the applied current. This type of experiment mimics the current produced by a synaptic input.
- In a **voltage-clamp** experiment one controls the membrane voltage and measures the transmembrane current required to maintain that voltage.

Why record with voltage clamp

Despite the fact that voltage clamp does not mimic a process found in nature, there are three reasons to do such an experiment:

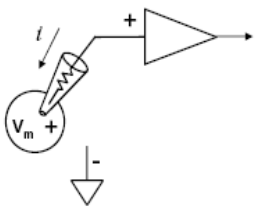
(1) Clamping the voltage eliminates the capacitive current, except for a brief time following a step to a new voltage. The brevity of the capacitive current depends on many factors that are discussed in following chapters;

(2) Except for the brief charging time, the currents that flow are proportional only to the membrane conductance, *i.e.*, to the number of open channels;

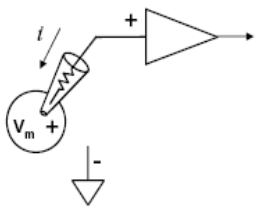
(3) If channel gating is determined by the transmembrane voltage alone (and is insensitive to other parameters such as the current and the history of the voltage), voltage clamp offers control over the key variable that determines the opening and closing of ion channels.

Record Voltage

- Positive potential
  - a positive voltage at the headstage input with respect to system ground
- Transmembrane potential, Vm
  - V_{inside} relative to $V_{outside}$
- Depolarizing potential
  - is a positive shift in Vm

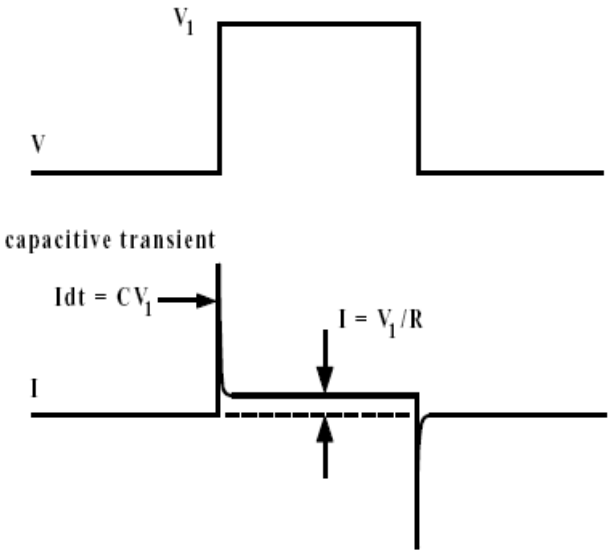


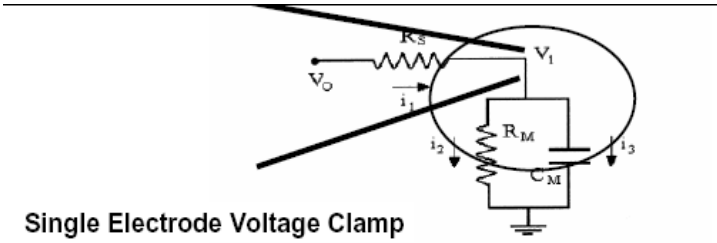
Record Current



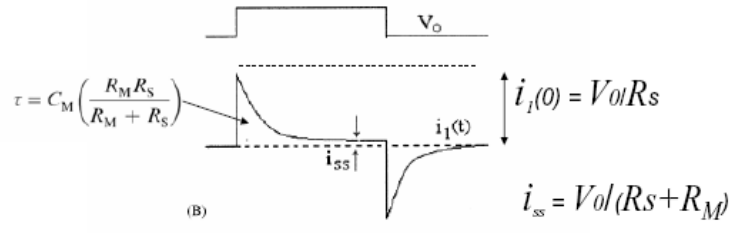
- Positive current
  - flows out of amplifier into the electrode
  - flows out of the pipette tip into the cell
- Inward current
  - flows from the outside surface of membrane to the inside surface
- Positive and Negative currents and voltages are always based on the headstage's perspective

Typical Voltage-Clamp Experiment





Single Electrode Voltage Clamp

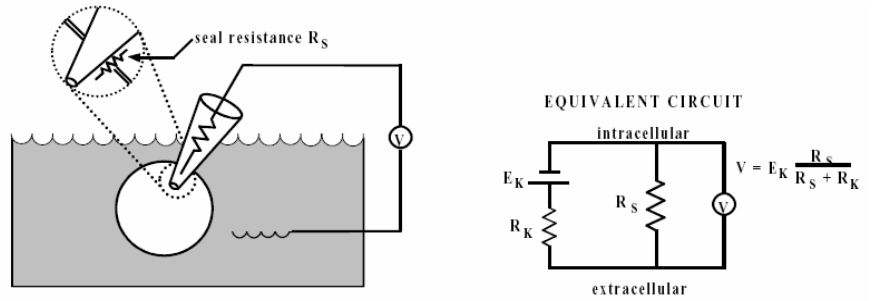


$$R_M >> R_S \Longrightarrow \quad R_M = \frac{V_O}{i_{SS}}, \quad R_S = \frac{V_O}{i_1(0)}, \quad C_M = \frac{\tau}{R_S}$$

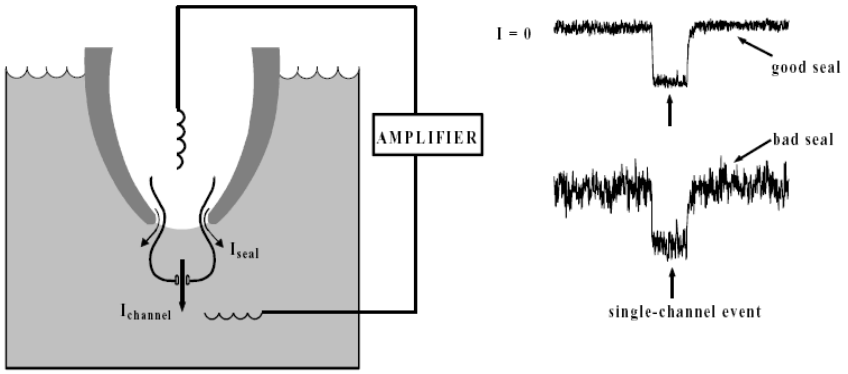
Glass Microelectrodes and Tight Seals

Intracellular Electrode Measurement

This intracellular electrode is measuring the resting potential of a cell whose membrane contains only open K+ channels. As the seal resistance Rs increases, the measurement approaches the value of EK.



Good and Bad Seals in single channel recording



In a patch recording, currents through the seal also flow through the measuring circuit, increasing the noise on the measured current.

Digitizing Analog Signal

8 bit = 2^8 = 256

12 bit = 2^{12} = 4,096

16 bit = 2^{16} = 65,536

8 bit = 278.4 mV

12 bit = 4.88 mV

16 bit = 0.305 mV

Technical issues

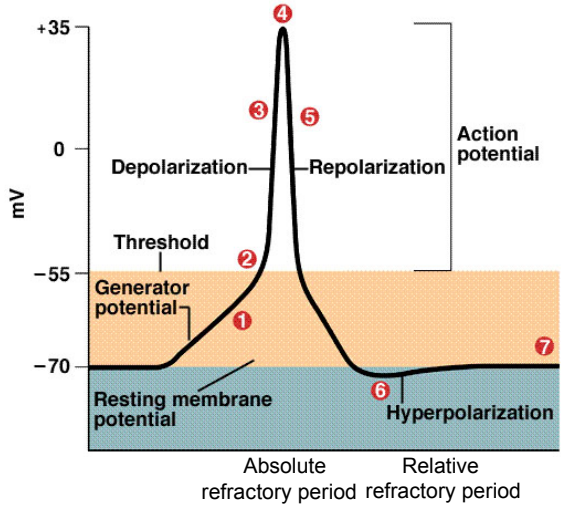
1. Loosing the seal on break-in ("leaky" cell; depolarized membrane potential, such as negative (downward) shift, "jump" in holding current,
2. Series resistance (VC/CC, filtering, voltage-drop/error, compensation),
3. Noise (60 Hz line frequency, grounding, high-frequency noise – single channel),
4. Wash-in/wash-out (perforated patch recording: Amphotericin or Gramicidin),
5. Offsets (junction potentials, Ag/AgCl electrodes),
6. Recording issues (amplifier gain, Analog/Digital boards, saturation, sample rates),
7. Space clamp.

Visually guided vs. blind?

Visually guided	**Blind**
• Expensive	• Cheaper
• Expensive microscope & camera required, better manipulators (?)	• Only need dissecting microscope & no camera
• Best for cells near the surface	• Can patch deep cells (also *in vivo*)
• Typically lower series resistance	• Typically higher series resistance
• Can record from multiple cells	• Difficult to record from multiple cells
• Can record from multiple locations on the same cell	• Impossible (?) to record from multiple locations on the same cell
• Can identify cells before recording (morphology, fluorescence)	• Identification only possible after experiment (but can use electrical cues)

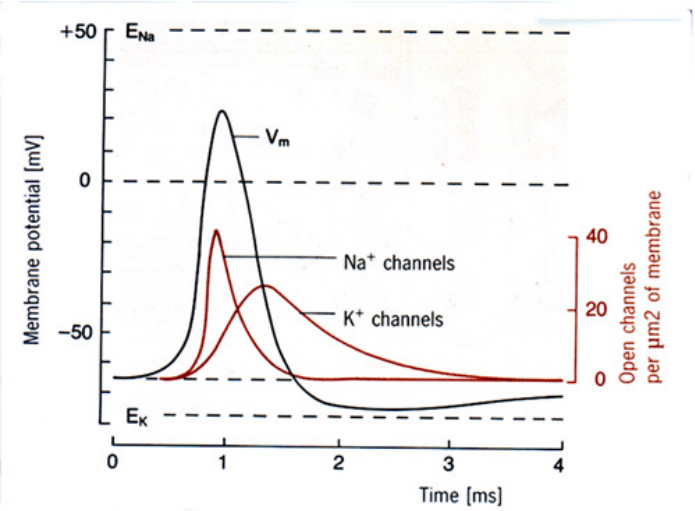
Data analysis

Phases of the Action Potential



Firing threshold is the point at which the number of activated Na+ channels > inactivated Na+ channels

Ion Flow During an Action Potential



Input resistance and time constant decay