

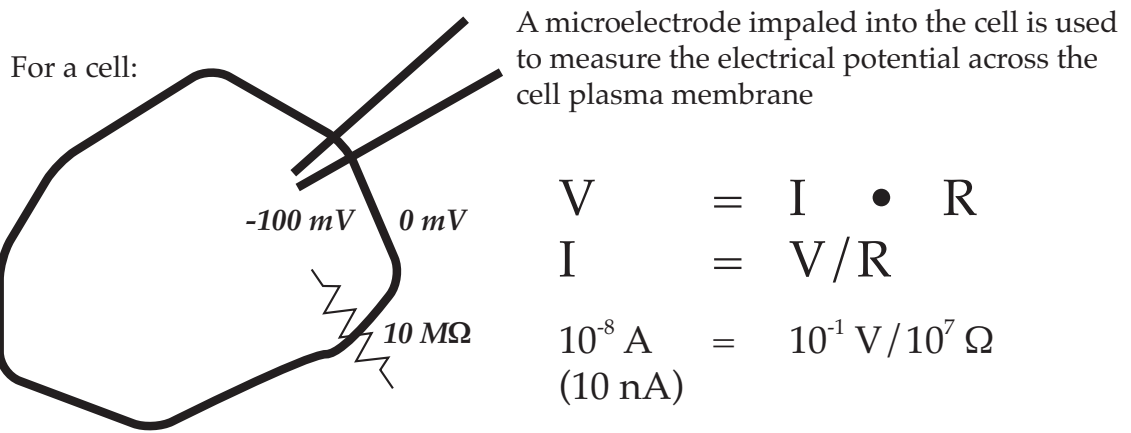
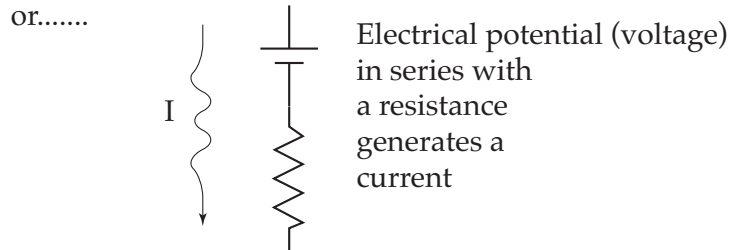
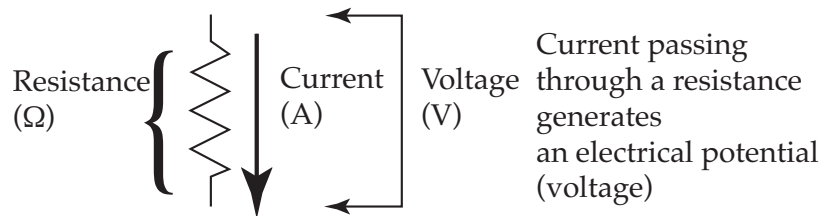
Lecture One: Electrical Properties of Cells

The electrical properties of cells include four electrical concepts: Voltage, Current, Resistance and Capacitance

Ohm's Law defines the relationship between Voltage, Current and Resistance:

$$V = I \cdot R$$

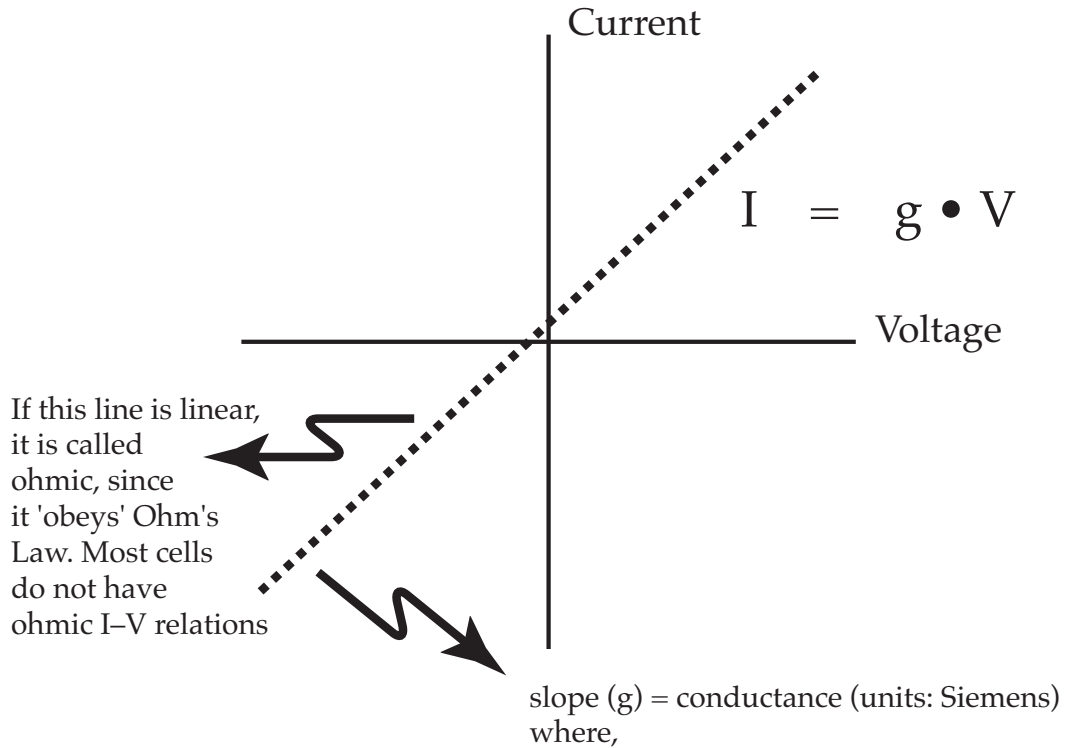
units: Voltage (V) Amperes (A) Ohms (Ω)
 (Coulombs/second) (charge transport)



There is a mental short-hand for units used in Ohm's Law:

10^{-9} A	=	10^{-3} V	/	$10^6 \Omega$		10^{-12} A	=	10^{-3} V	/	$10^9 \Omega$
nA		mV		MΩ		pA		mV		GΩ
nanoAmps		milliVolts		megaOhms		picoAmps		milliVolts		gigaOhms
(suitable for cellular impalements)						(suitable for patch clamp)				

Ohm's Law can be viewed as a current-voltage relation:

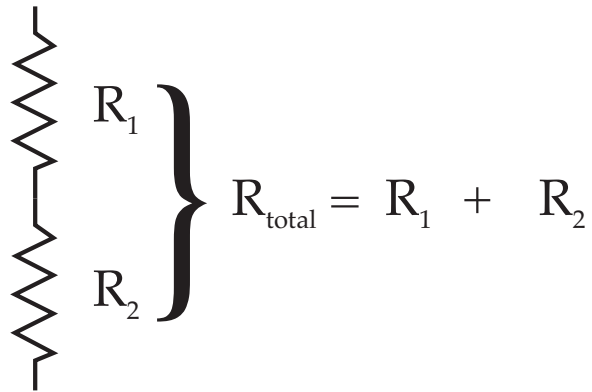


$$g = 1 / R$$

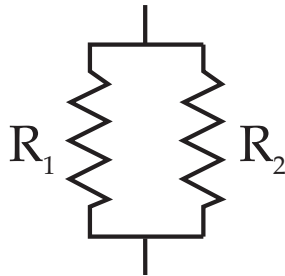
(conductance is the inverse of resistance)

In a biological system, resistances are often more complex. They may be configured in parallel or in series

Resistances in series are simpler



Resistances in parallel are more complex



$$R_{total} = \frac{1}{\left(\frac{1}{R_1} + \frac{1}{R_2}\right)} \text{ or } R_{total} = \frac{R_1 \cdot R_2}{(R_1 + R_2)}$$

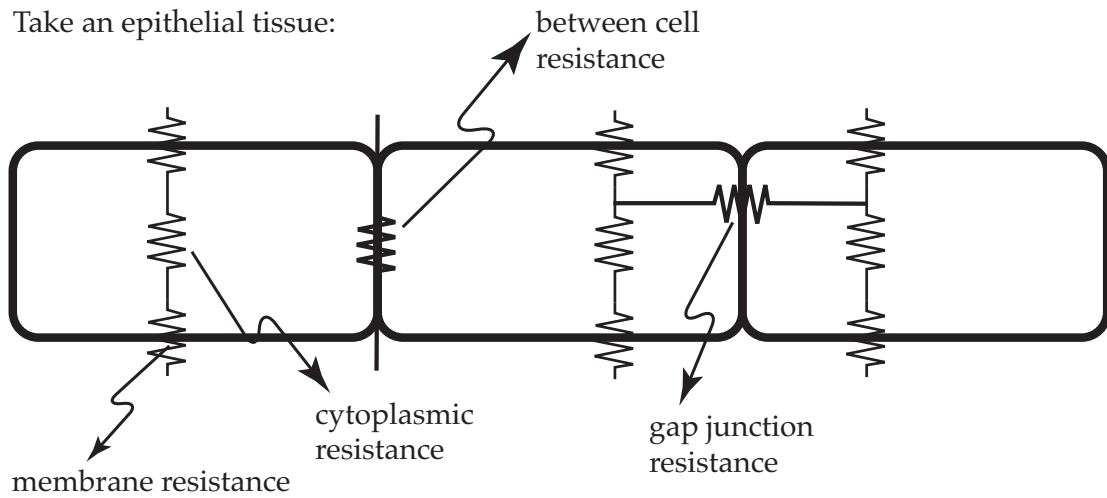
(obtained by multiplying by R_1/R_1 and R_2/R_2)

For example, if R_1 and R_2 are both $5 \text{ M}\Omega$, $R_{tot} = 2.5 \text{ M}\Omega$

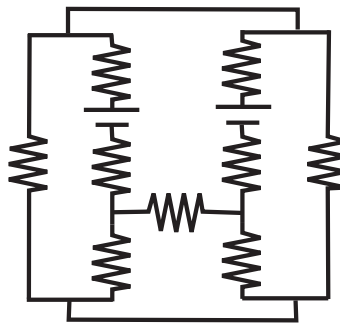
Intuitively, if you have two resistances in series, current has to flow through both of them, so total resistance is additive. But if the resistances are in parallel, the current has two alternative pathways, so total resistance is lower than either pathway alone.

Is this important? Yes.

Take an epithelial tissue:



The resistive network for two cells.....



OK, now extend this to a thousand cells.

Now, apply a voltage, where is the current going to flow?

There are some simple rules which depend upon the relative magnitude of the various resistances.

In series, if one resistance is much larger than the other: $R_1 \gg R_2$

the total resistance is approximately equal to the larger resistance: $R_{total} \sim R_1$

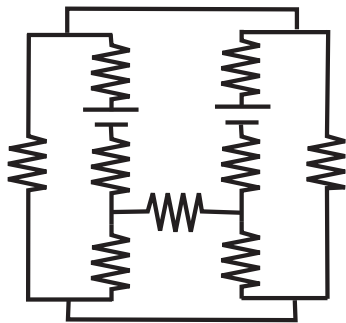
In parallel, total resistance is approximately equal to the smaller resistance:

$$R_1 \gg R_2 \quad R_{total} = \frac{R_1 \cdot R_2}{(R_1 + R_2)} \sim \frac{R_1 \cdot R_2}{R_1} \sim R_2$$

$$R_1 = R_2 \quad R_{total} = \frac{R^2}{(2 \cdot R)} = \frac{R}{2}$$

Thus, relative approximations of current flow can be made.

Formally, Thevenin's equivalent circuit theorem states that any two terminal network of resistors and voltage sources is equivalent to a single resistor in series with a single voltage source. So the epithelial network:



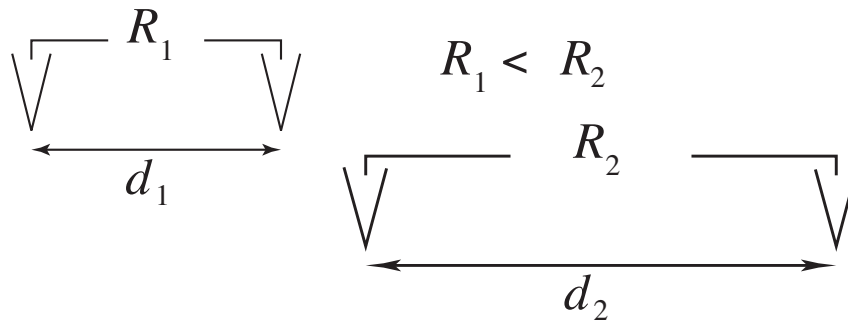
Can be simplified to:



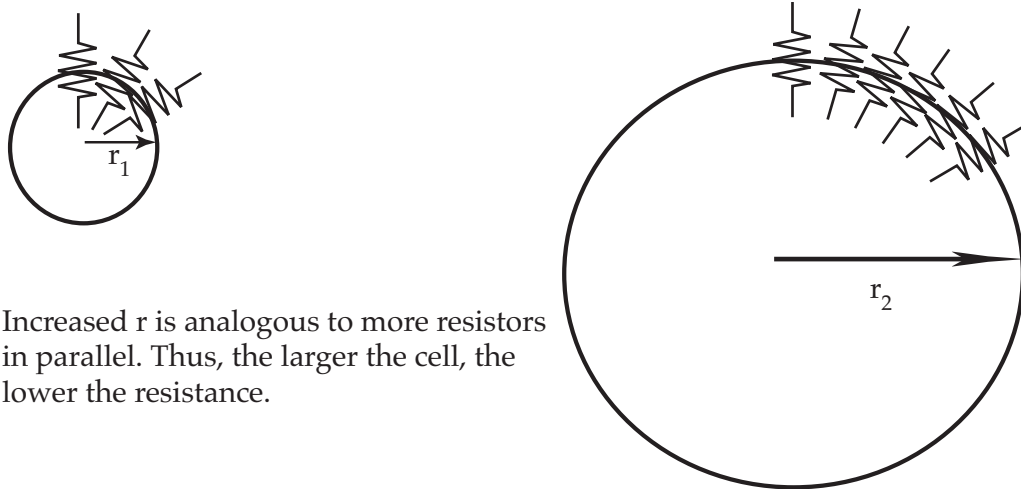
There is a problem.....

For an epithelial tissue, the simplified pathway can be measured. But if one is measuring the voltage difference and resistance across the tissue, it is not possible to dissect out the relative contributions of each current pathway.

Resistance in biological systems is a relatively simple concept. Although we focus on electrical resistance, resistive analysis is also used to research water and gas flow through complex tissues. In the context of electrophysiology, the resistance you measure in a solution will vary with the distance between the two probes of the measuring device. And, the resistance of a cell will vary with the size of the cell.



Increased distance, d is analogous to more resistors in series.



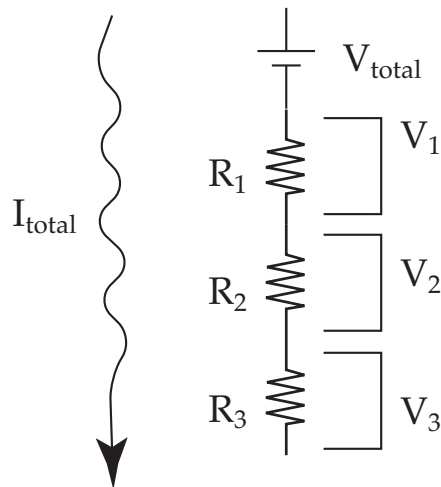
Increased r is analogous to more resistors in parallel. Thus, the larger the cell, the lower the resistance.

To normalize resistance, one uses resistivity, or specific resistance:

Material	Resistivity (units: $\Omega \cdot \text{cm}$)
Glass	$10^{12} - 10^{15}$
dH ₂ O	$25 \cdot 10^6$
0.1 N KCl	90
cell membrane	$1 \cdot 10^9$

Voltage Dividing

Suppose you have a voltage source in series with a number of resistors:



There is a total current flow through the resistive network of I_{total} which must pass through all three resistors. The voltage across R_1 , R_2 , or R_3 , would be:

$$V_1 = I_{total} \cdot R_1$$

$$V_2 = I_{total} \cdot R_2$$

$$V_3 = I_{total} \cdot R_3$$

and,

$$V_{total} = V_1 + V_2 + V_3$$

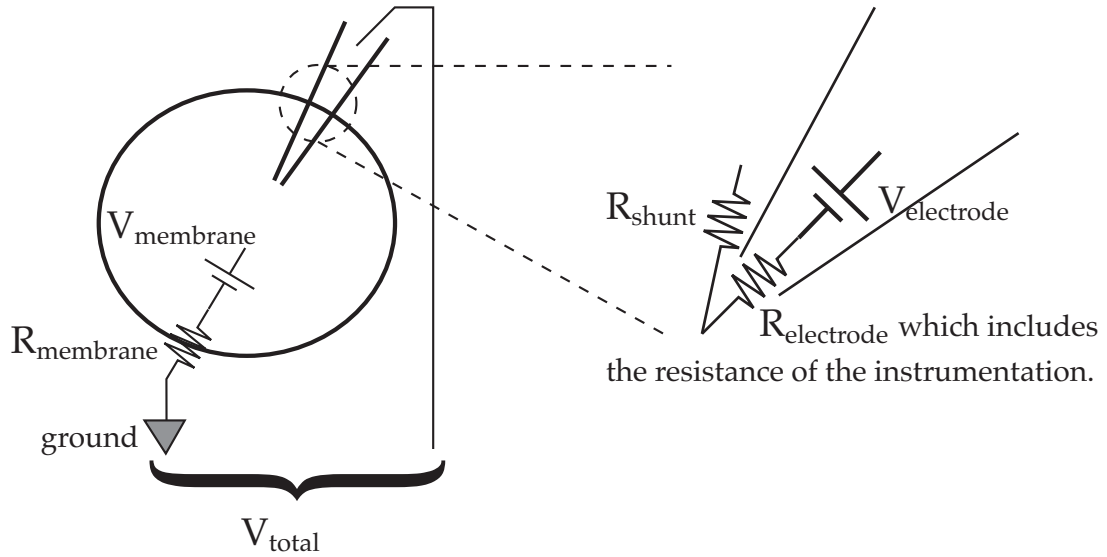
So,

$$V_1 = (R_1 / R_{total}) \cdot V_{total}$$

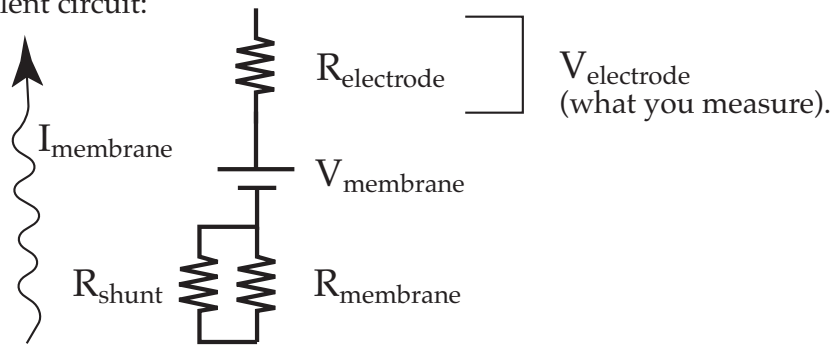
The multiple resistors act to 'divide' the voltage, hence the term voltage divider.

Is this important? Yes.

Here are some examples where voltage dividing is an experimental problem.



What you want is to measure the voltage across the membrane, V_{membrane} .
The equivalent circuit:



R_{shunt} complicates resistance measurements and can attenuate measurements of V_{membrane} .
It is most significant in small cells. Ignoring it for now:

$$V_{\text{electrode}} = \left(\frac{R_{\text{electrode}}}{R_{\text{membrane}} + R_{\text{electrode}}} \right) \cdot V_{\text{membrane}}$$

If $R_{\text{electrode}} \approx R_{\text{membrane}}$, then $V_{\text{electrode}} \approx 1/2 \cdot V_{\text{membrane}}$.

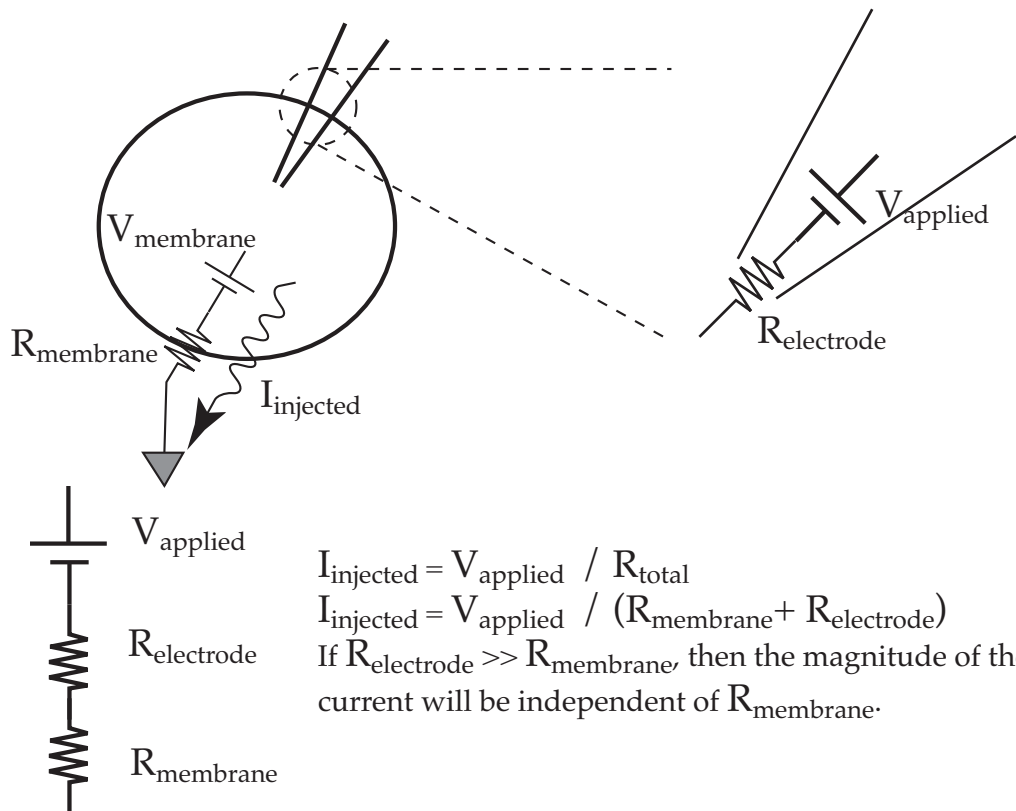
If $R_{\text{electrode}} \gg R_{\text{membrane}}$, then $V_{\text{electrode}} \approx V_{\text{membrane}}$.

In real life, the electrode is connected to an electrometer, so that $R_{\text{electrode}}$ includes not only the electrode resistance, but also the electrometer resistance, a specification commonly called the input impedance, usually about $10^{11} \Omega$.

In small cells, R_{membrane} may approach $10^{11} \Omega$, errors (significant underestimation of V_{membrane}) may result.

Current Sources and Resistance

Suppose you have a battery and some resistors, and want to inject a specified current into the cell, usually to measure R_{membrane} .



Capacitance

Capacitance is the final elementary electrical property we need to consider.

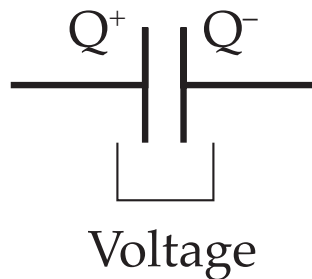
Capacitance is defined by the relation:

$$\text{units: } C = \frac{Q}{V}, \text{ or } Q = C \cdot V$$

Farads Coulombs Volts

Intuitively, capacitance can be considered the ability or capacity to hold charge.

More formally, a capacitor of C farads with V volts across its terminals has +ve Q coulombs of charge on one plate, and -ve Q coulombs of charge on the other plate.



Now, taking the derivative with respect to time:

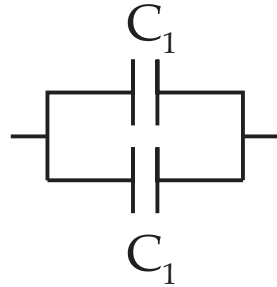
$$\frac{dQ}{dt} = \frac{d}{dt} C \cdot V = I = C \frac{dV}{dt}$$

(coulombs per second:
the same as current, I) (the capacitance,
C is constant)

That is, the current, I is proportional to the rate of change in voltage, dV/dt . Voltage must be changing to cause current to pass across the capacitor.

Analogous to resistance, capacitors sum differently when they are configured in parallel or in series:

In parallel:



$$C_{total} = C_1 + C_2$$

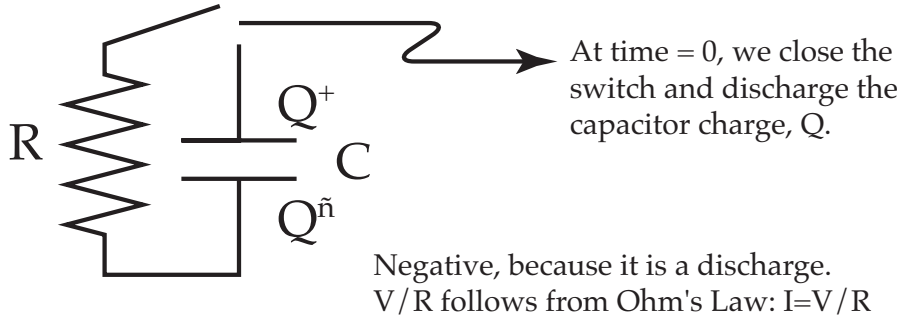
In series:



$$C_{total} = \frac{1}{\frac{1}{C_1} + \frac{1}{C_2}}$$

$$C_{total} = \frac{C_1 \cdot C_2}{C_1 + C_2}$$

Suppose we have a circuit:



Now,

$$C \frac{dV}{dt} = I = -\frac{V}{R}$$

$$C \frac{dV}{dt} = -\frac{V}{R}$$

$$\frac{1}{V} dV = -\frac{1}{RC} dt$$

$$\int \frac{1}{V} dV = -\int \frac{1}{RC} dt$$

Integrating over time, t (from 0 sec to infinity):

$$V = A_i e^{-t/RC}$$

$$\ln(V) = -\frac{t}{RC} + A$$

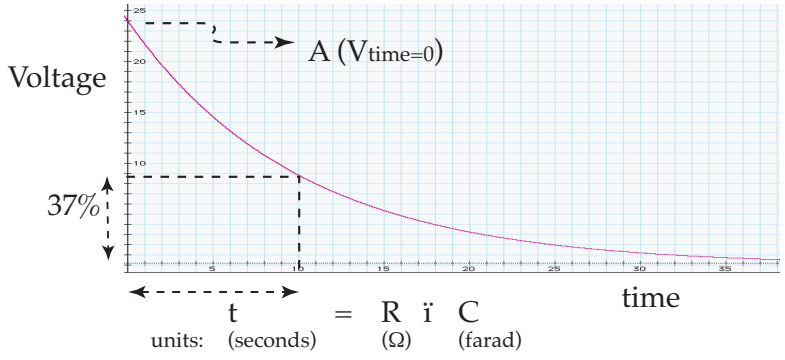
$$V(t) = e^{-t/RC} \cdot e^A$$

At time t = 0, $e^{-t/RC} = 1$

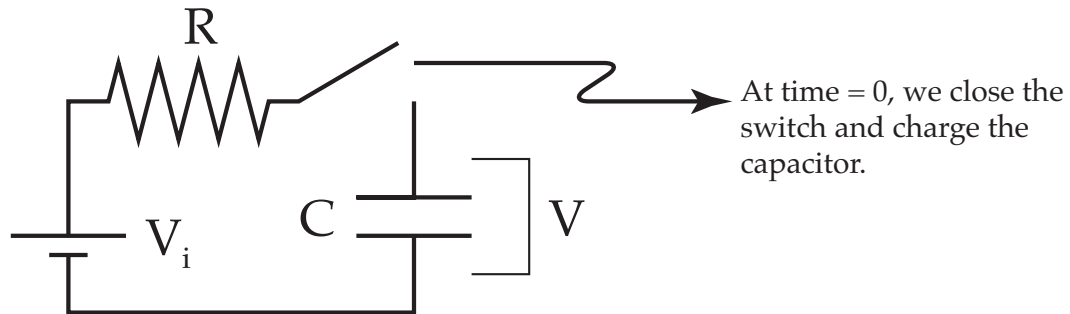
so, $e^A = V(0)$, thus

$$V(t) = V(0) \cdot e^{-t/RC}$$

which looks like:



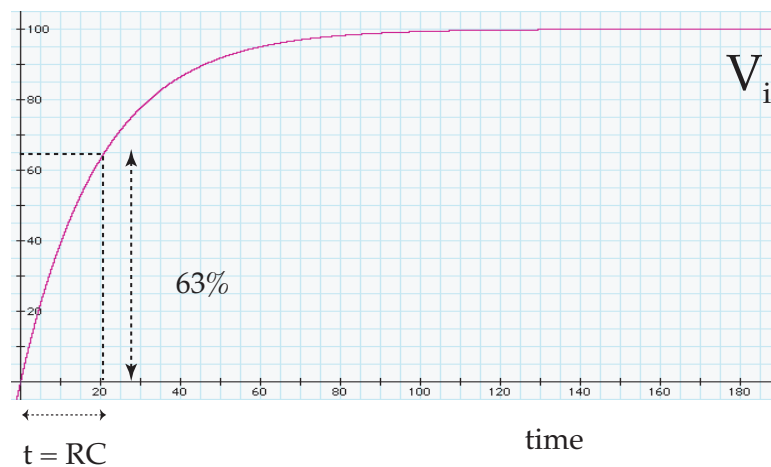
Alternatively, capacitor charging:



Now,
$$I = C \frac{dV}{dt} = \frac{(V_i - V)}{R}$$

integrating

$$V = V_i(1 - e^{-t/RC})$$

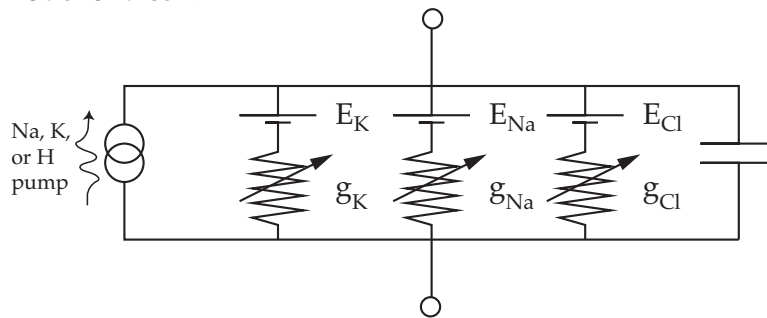


Is this important? Yes. Cell membranes have capacitance. In addition, electronic instrumentation and electrodes have capacitance. These affect the time dependence of voltage and current measurements in a variety of ways.

Lecture One: Electrical Properties of Cells (continued): Measurements

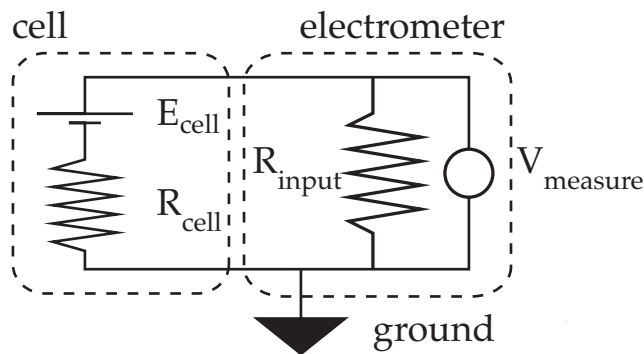
We now have an introductory understanding of the four electrical properties of cells: Voltage, Current, Resistance, and Capacitance.

A model of a cell:



The ions which are transported across the membrane include K^+ , Na^+ , and Cl^- . The ionic conductance (recall $g = 1/R$) for each of these ions is variable. A major user of cellular metabolism is the ion current pump: using ATP to transport K^+ and Na^+ , or H^+ across the membrane.

We now need to consider the tools that allow us to measure the electrical properties of the cell.

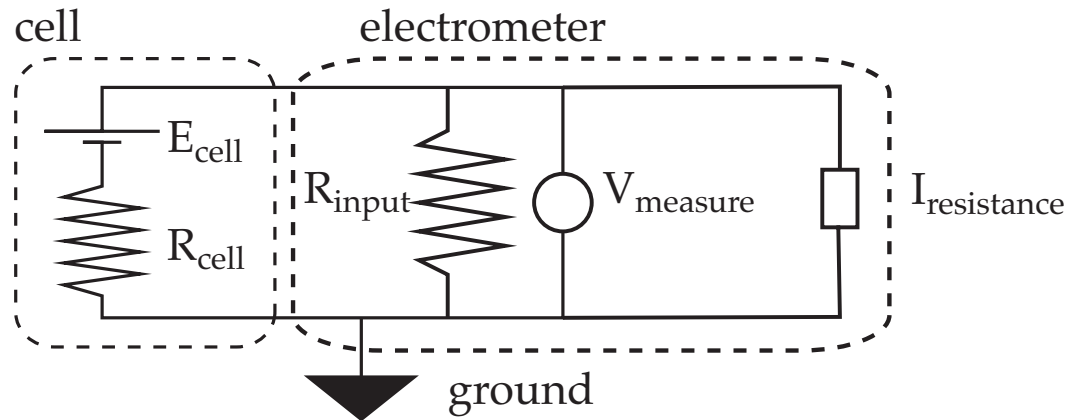


R_{input} is the input resistance (sometimes called the input impedance). During measurements, the electrical network dictates that:

$$V_{measure} = \frac{R_{input}}{R_{input} + R_{cell}} E_{cell}$$

Therefore, accuracy depends upon $R_{input} \gg R_{cell}$. Since R_{cell} is often about $20 \text{ M}\Omega$, R_{input} must be very high to measure E_{cell} accurately.

Measurements of Resistance.

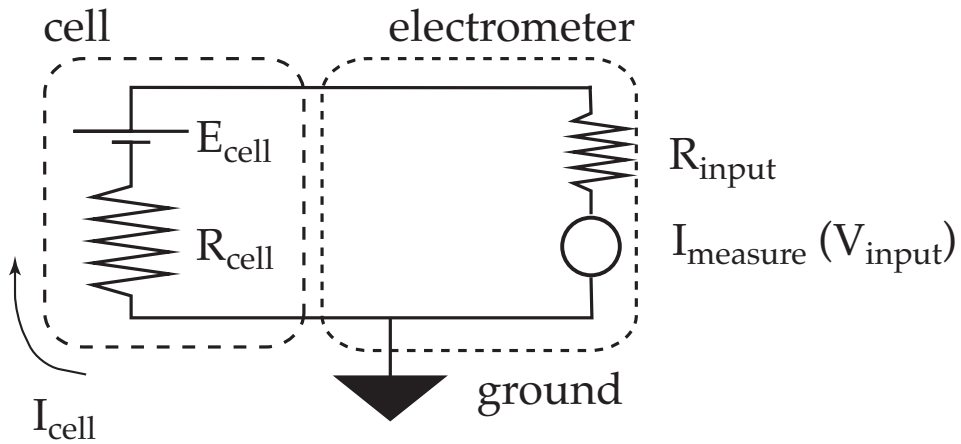


$$R_{measure} = \frac{V_{measure}}{I_{resistance}} = I_{resistance} \frac{\frac{R_{input} R_{cell}}{R_{input} + R_{cell}}}{I_{resistance}} = \frac{R_{input} R_{cell}}{R_{input} + R_{cell}}$$

If $R_{input} \gg R_{cell}$, no problem, but if $R_{input} = R_{cell}$, there are significant errors in the measurement of cell membrane resistance. It should be very clear that, at high cell resistances, a high input impedance (resistance) in the measuring equipment is essential.

Measurements of Current.

Here, things are different



In this case, the lower the R_{input} is, the better.

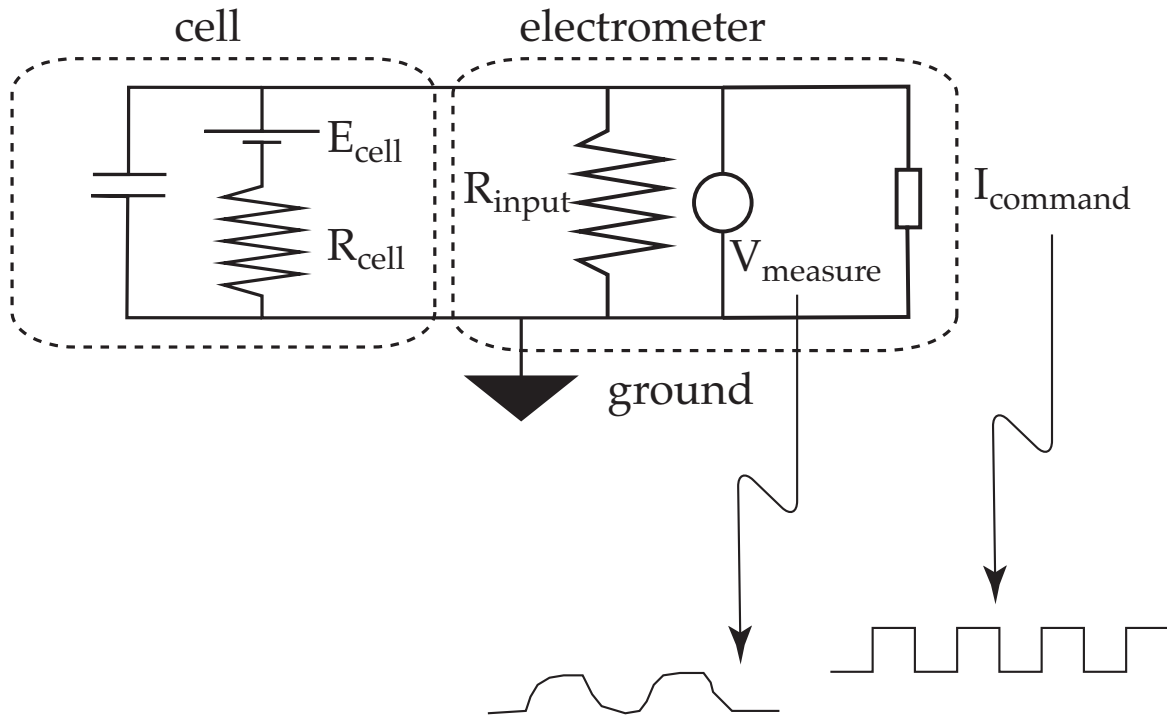
$$I_{cell} = \frac{V_{cell} - V_{input}}{R_{cell}} = \frac{V_{cell} - (I_{cell} R_{input})}{R_{cell}}$$

as R_{input} approaches 0, so does V_{input} , resulting in no error.

There is no problem with the magnitude of R_{cell} per se, but, the currents are often very low (picoAmpere to nanoAmpere), so sensitivity is a problem.

Measurements of Capacitance.

Analogous to measurements of resistance



The rise time, measured at about 2/3 maximum, is equal to $R \cdot C$, from which capacitance can be calculated if R_{cell} is known.

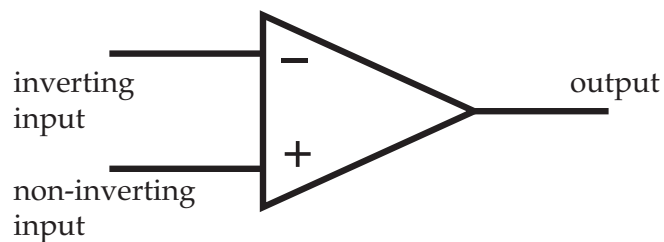
Lecture One: Electrical Properties of Cells (continued): Operational Amplifiers

The key to electrical measurements of the cell is operational amplifiers.

These are "active" devices (they commonly require ± 15 Volt direct current (DC) to run). They are used in a bewildering variety of electronics. They are analog devices. In contrast to digital devices that have 0 or 1 states and step through discrete voltage values, operational amplifiers respond continuously in output and time.

Operational amplifiers operate on the basis of some simple rules.

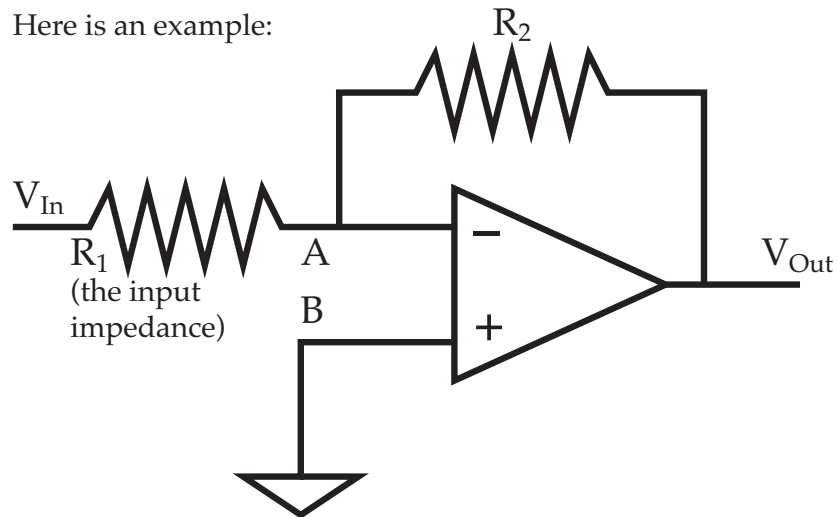
There are two inputs and one output:



Rule I: The output attempts to do whatever is necessary to make the voltage difference between the two inputs zero

Rule II: The inputs draw no current. That is, they have infinite input impedance.

Here is an example:



B is at ground, so the output will change such that A is also at ground
 For this to occur, the voltage across R_2 (V_{Out}) and the voltage across R_1 (V_{In}) must be such that A is at 0 mV (or, more accurately, ground).

$$\begin{aligned} V_{in} &= I_1 \cdot R_1 \\ V_{Out} &= I_2 \cdot R_2 \\ I_1 &= -I_2 \end{aligned}$$

so.....

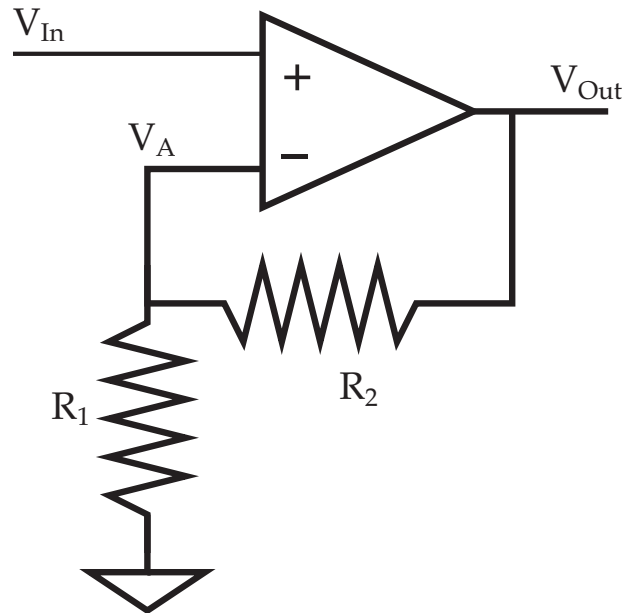
$$V_{in}/R_1 = -V_{out}/R_2$$

or.....

$$V_{out}/V_{in} = -R_2/R_1$$

Okay, let $R_1 = 10 \text{ k}\Omega$, $R_2 = 100 \text{ k}\Omega$, and $V_{in} = 1 \text{ Volt}$. Because of voltage dividing, the voltage at A will be +0.92 Volts. The output swings to -10 Volts to bring A to ground. As a consequence, the signal has been amplified 10 times, and inverted. Thus the operational amplifier is configured as an inverting amplifier.

Let's look at another configuration:



V_{In} must equal V_A , but V_A is a function of V_{Out} :

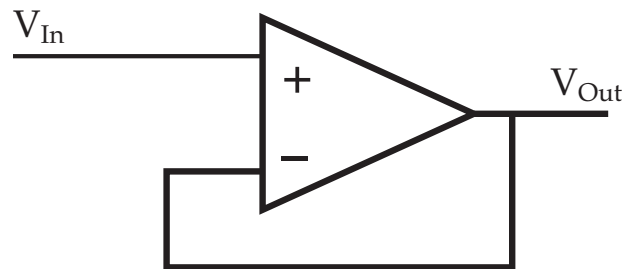
$$V_A = (R_1 / (R_1 + R_2)) \cdot V_{Out}$$

so.....

$$V_{out}/V_{in} = 1 + R_2/R_1$$

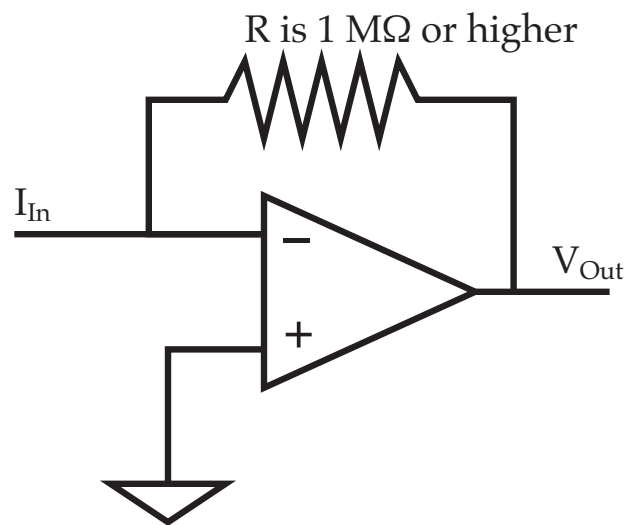
This is a non-inverting amplifier. Note that the input impedance is very high ($\sim 10^8$ to $10^{12} \Omega$) depending upon the specifications of the operational amplifier.

For a 1X gain:



You will find examples of other operational amplifier configurations in the work problems. One example will be a current source, where the operational amplifier is used to drive a current which is insensitive to the resistances of the microelectrode and cell.

A final key example of an operational amplifier circuit used in electrophysiology is the current-voltage converter (also known as the virtual ground):



The current driven across the feedback resistor will be countered by the V_{Out} . This circuit maintains the input at ground, thus the term virtual ground.

This configuration is used to monitor currents, for example in oocytes, and is a key component of the patch clamp amplifier used to measure very small ion channel currents, and, it is used in voltage clamping.

Lecture One: Electrical Properties of Cells (continued): Voltage Clamping

Many things make electrical measurements of cells distinct from ordinary electrical measurements. With cell resistance in the $M\Omega$ range, we are in a high impedance environment. This means that the instrumentation must have very high input impedance. It also means that we are very sensitive to noise.

The quantities we measure, milliVolts and nanoAmps, are very small. Thus, electrophysiologists must contend with poor signal to noise ratios.

Because of the high impedance, it is necessary to work at very low bandpass. This means it is difficult to 'see' fast events.

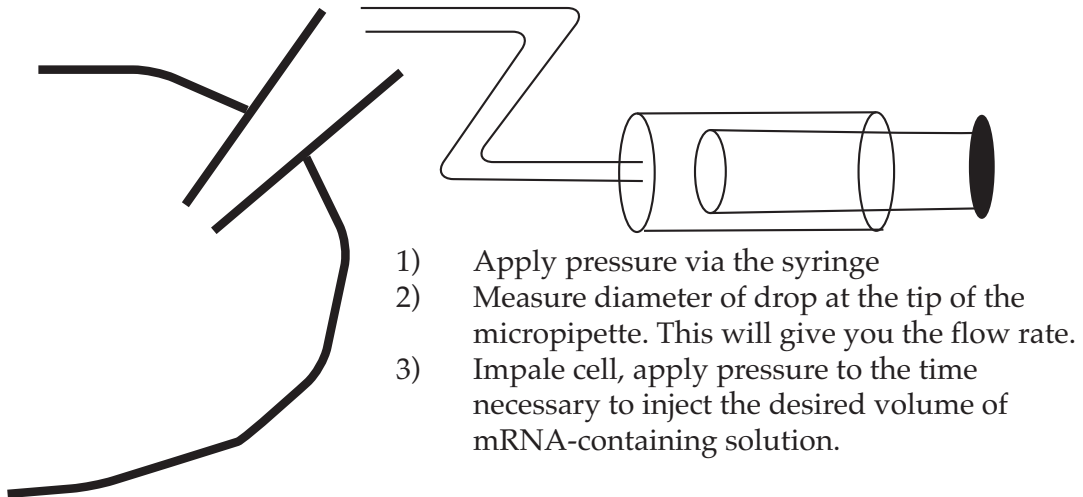
There is one major tool in the arsenal of electronic instrumentation:
The voltage clamp.

In qualitative terms, a voltage clamp holds the membrane potential of the cell at a given value. The electrophysiologist then measures the clamping current, the current required to hold the potential at the specified value. This allows the electrophysiologist to measure the current-voltage relations of the cell.

There is another alternative, the current clamp, which injects current into the cell at a specified value, but this is less useful because of cell capacitance, which 'slows' the changes in voltage induced by current injection.

To illustrate the voltage clamp, we will consider two case studies: Xenopus oocytes and patch clamp.

Xenopus oocytes are large cells which are very promiscuous, in the sense that they will express foreign mRNA injected into the cytoplasm.



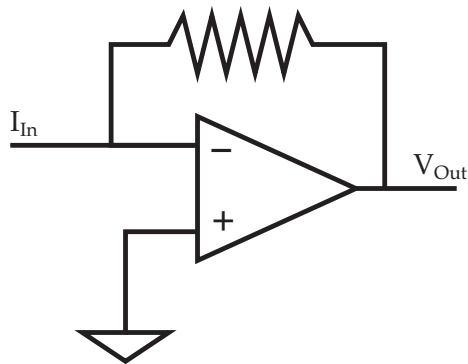
An oocyte is about 2 mm in diameter:

cell diameter	cell volume
2 mm	4.2 μ l
0.1 mm	525 pl
0.01 mm	0.525 pl

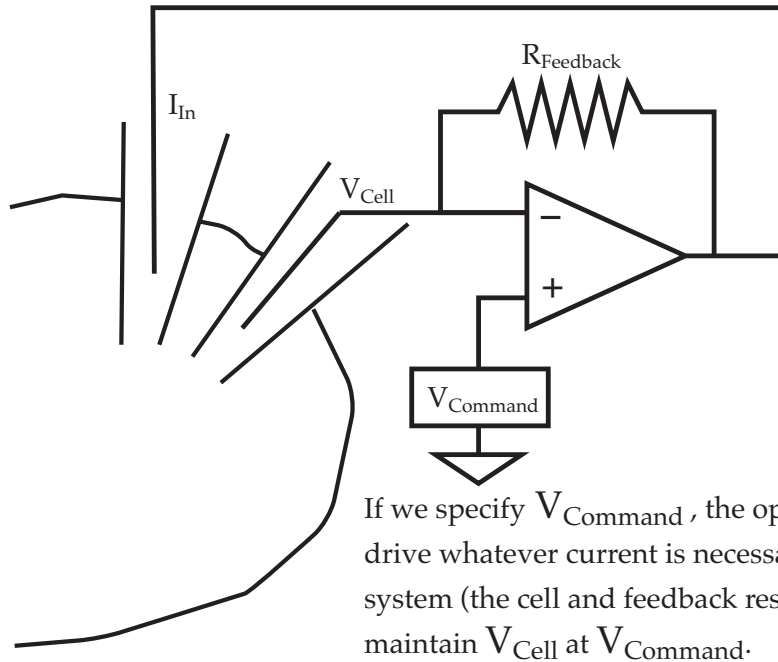
It is easy to inject a 2 mm cell with a few nanoliters of solution. But it is very difficult to inject a 100 μ m cell with a few picoliters, and virtually impossible for a 10 μ m cell. Thus, oocytes are ideal for microinjection.

Once the mRNA for, say, an ion channel, is injected and expressed, the next step is to measure the ion channel activity. For this, the voltage clamp is used.

The basic circuit:

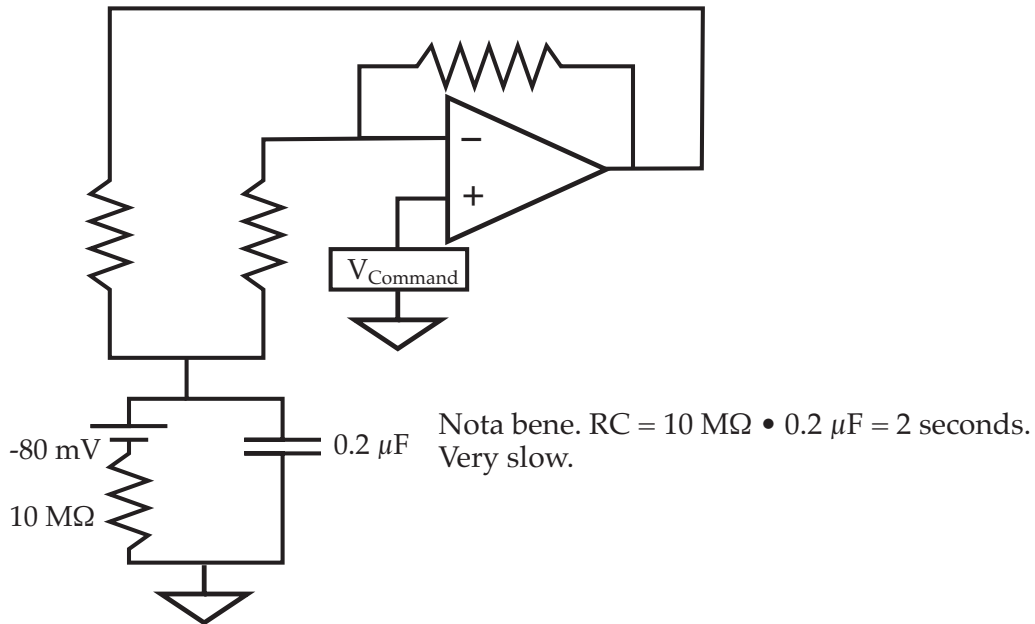


Let's include the cell in the voltage clamping network.



If we specify V_{Command} , the operational amplifier will drive whatever current is necessary into the feedback system (the cell and feedback resistor R_{Feedback}) to maintain V_{Cell} at V_{Command} .

The equivalent circuit:



Nota bene. $RC = 10 \text{ M}\Omega \cdot 0.2 \text{ }\mu\text{F} = 2 \text{ seconds}$.
Very slow.

The relationship between V_{Command} and the biological side, V_{Cell} is complicated:

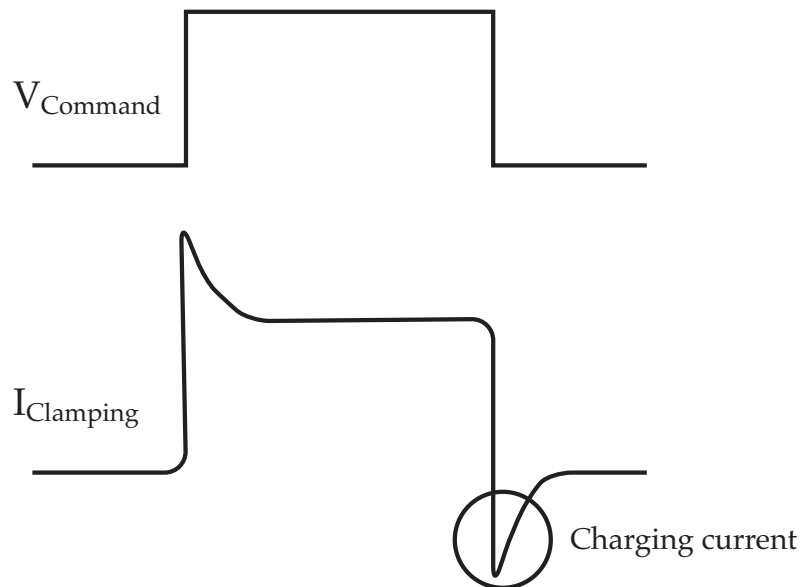
At low R_{Feedback} , V_{Cell} is attenuated compared to V_{Command} .

At high R_{Feedback} , they are comparable.

Now, in the case of an oocyte, there is an additional problem, a consequence of the large capacitance of a large cell (recall that a large cell can be modeled as many capacitors in parallel, which are added to yield the total capacitance).

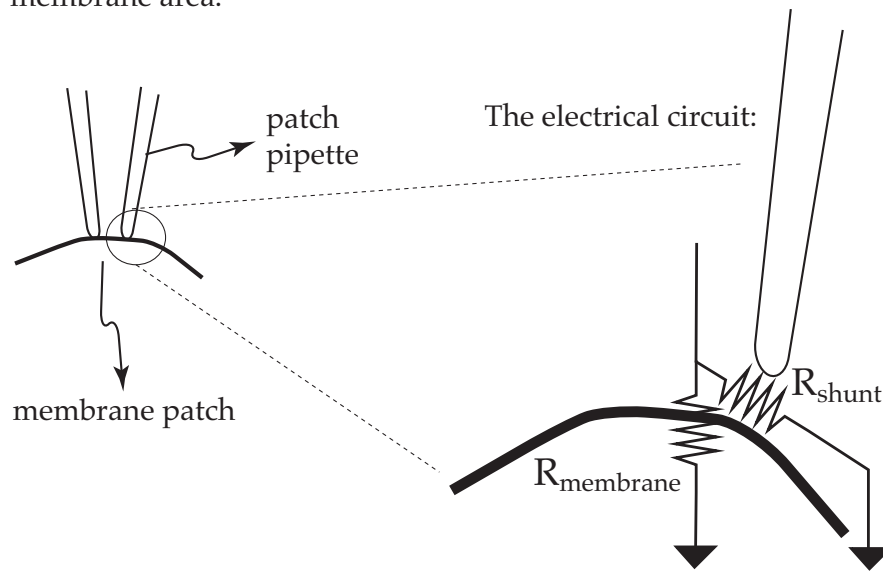
The charging time, the time required to bring the cell voltage, V_{Cell} , to the specified clamped voltage, V_{Command} is 2 seconds. The operational amplifier, as an active device, shortens the time required to charge the membrane.

To shorten the charging time as much as possible, it is necessary to maximize the magnitude of the current source.



Lecture One: Electrical Properties of Cells (continued): Patch Clamp

In patch clamp measurements of cells, the starting point is a relatively small membrane area.



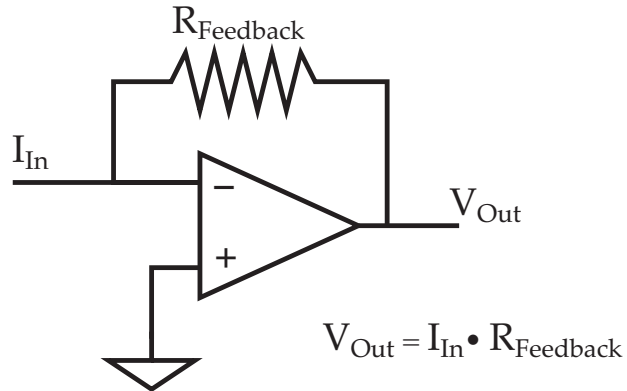
This is analogous to the voltage divider:

$$\begin{aligned} \text{If } R_{\text{shunt}} &\sim R_{\text{membrane}} \\ \text{Then } I_{\text{membrane}} &\sim I_{\text{shunt}} \end{aligned}$$

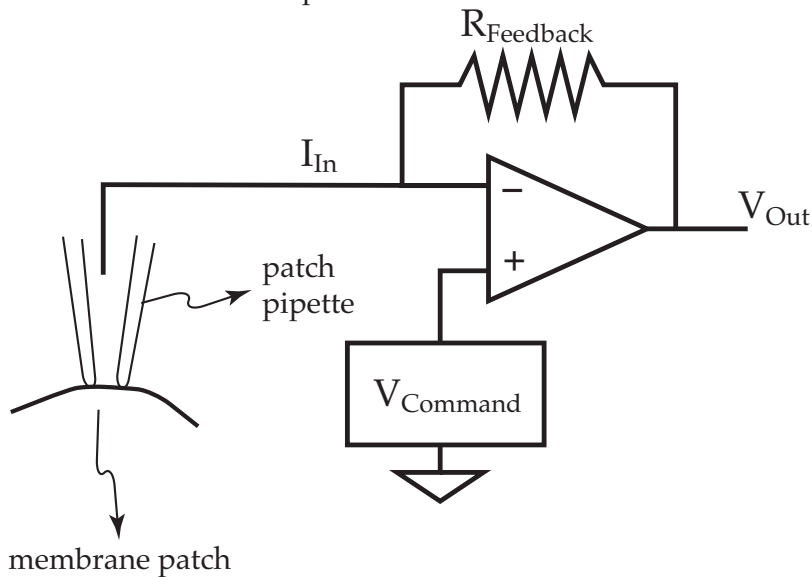
$$\begin{aligned} \text{But if } R_{\text{shunt}} &\gg R_{\text{membrane}} \\ \text{Then } I_{\text{membrane}} &\gg I_{\text{shunt}} \end{aligned}$$

With ion channels having 10^{-12} A (picoAmperes) currents, R_{shunt} must be much larger than R_{membrane} to be able to visualize the channels. R_{shunt} is known as the gigaseal, for its $G\Omega$ resistance.

How are the ion channels visualized?
With our basic circuit, the current-voltage converter.



Let's include the cell patch:



$V_{Command}$ 'clamps' the voltage, and the clamping current is monitored at V_{Out} .
 $R_{Feedback}$ must be very high to yield sufficient amplification to observe pA channels. If it is $10 \text{ G}\Omega$, the gain is $10^{10} \Omega \cdot 10^{-12} \text{ A} = 10^{-2} \text{ V}$, or 100 mV/pA .

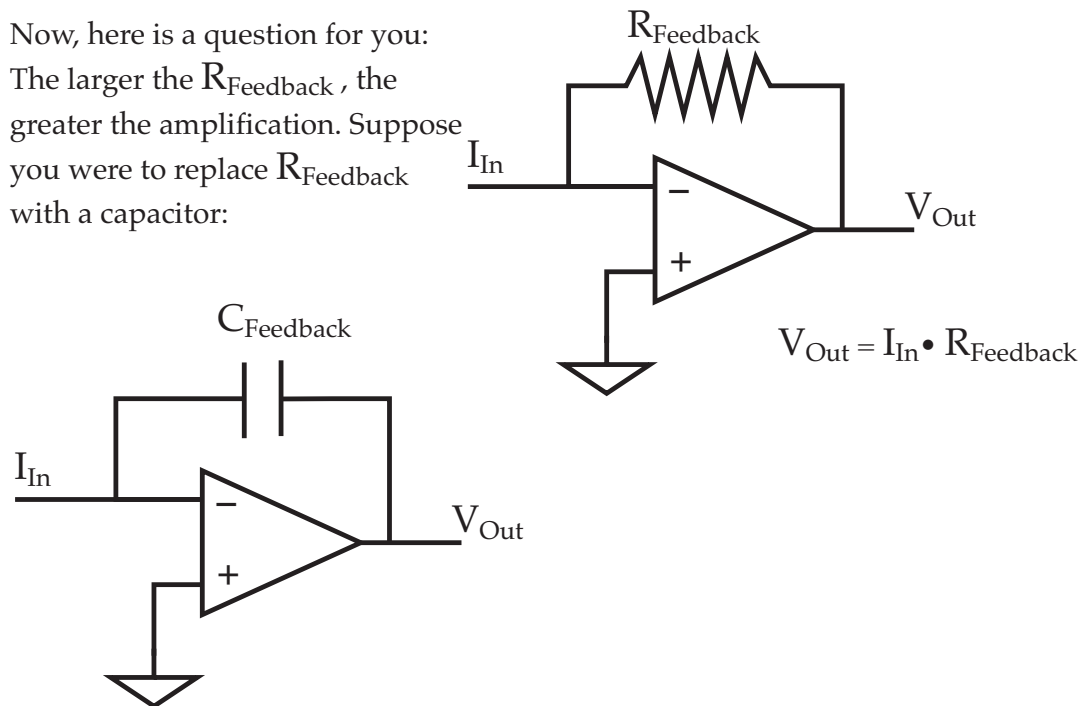
The real circuit for a patch clamp amplifier is more complex, but beyond this introductory presentation:

The presence of capacitance in the system is the major problem. With such high resistances ($10^{10} \Omega$), any small capacitance in the system can degrade severely the time response. Stray capacitance is not uncommon in electrical circuitry, even the micropipette has capacitance. PicoFarads are the common magnitude. The RC time constant will be: $10^{10} \Omega \cdot 10^{-12} \text{ F} \sim 10 \text{ msec}$, or worse.

So..... some of the complexity of the circuitry is to compensate for capacitance and boost the time response.

Now, here is a question for you:

The larger the R_{Feedback} , the greater the amplification. Suppose you were to replace R_{Feedback} with a capacitor:

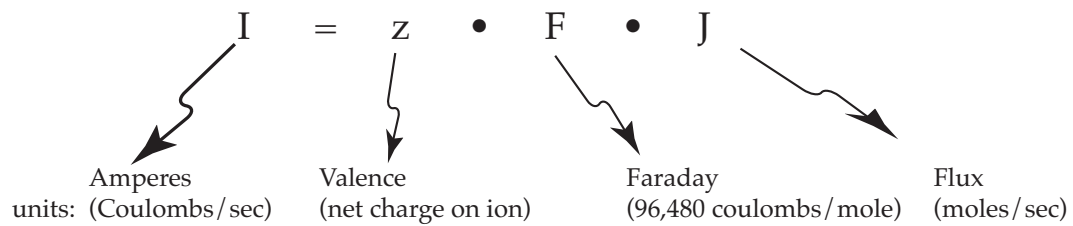


What would happen?

Hint: This is known as an integrating patch clamp.

Lecture Two: Solution and Cell Electrochemistry

The electrical properties of cells are a consequence of ion fluxes and steady state ion concentrations within and outside the cell. Therefore, current is not due to electron flow, but to ion flow:

$$I = z \cdot F \cdot J$$


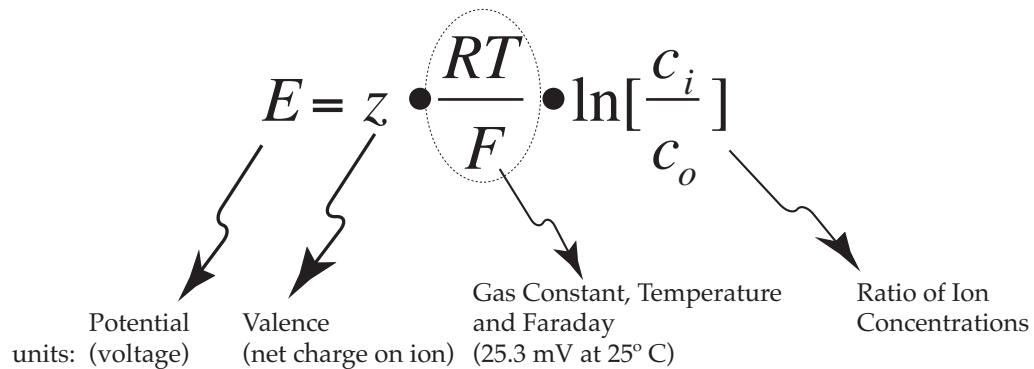
Amperes
units: (Coulombs/sec)

Valence
(net charge on ion)

Faraday
(96,480 coulombs/mole)

Flux
(moles/sec)

And voltage when the current is zero ($I = 0$)(the reversal potential):

$$E = z \cdot \frac{RT}{F} \cdot \ln\left[\frac{C_i}{C_o}\right]$$


Potential
units: (voltage)

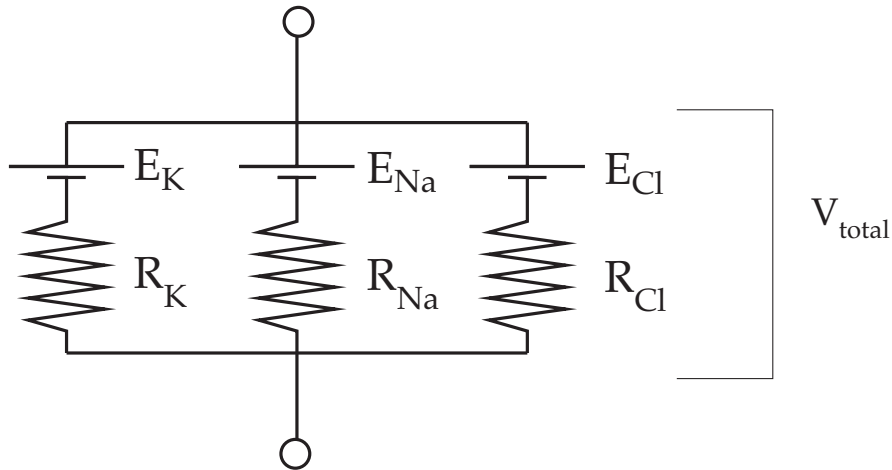
Valence
(net charge on ion)

Gas Constant, Temperature
and Faraday
(25.3 mV at 25° C)

Ratio of Ion
Concentrations

The equation holds true if one and only one ion is permeant through the cell membrane.

If the membrane is permeant to more than one ion, the equivalent circuit is:



For simplicity: $g_{Na} = 1/R_{Na}$, etc.

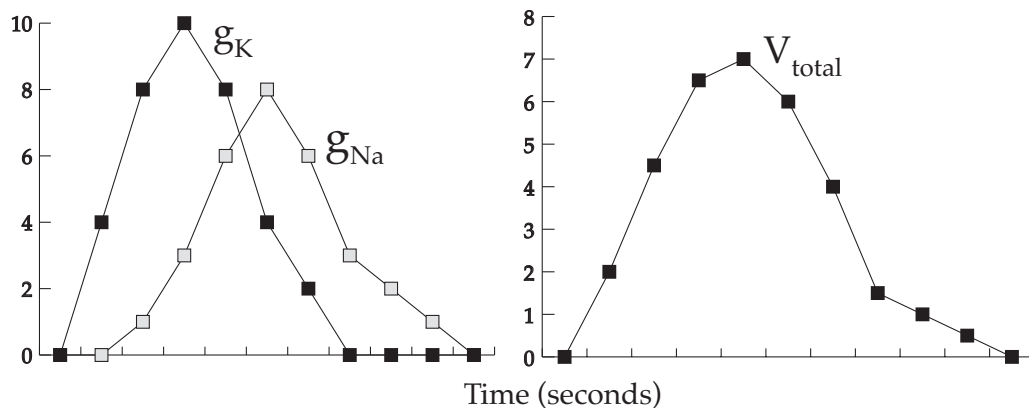
Then:

$$E_{total} = \frac{g_K}{\sum g} \cdot E_K + \frac{g_{Na}}{\sum g} \cdot E_{Na} + \frac{g_{Cl}}{\sum g} \cdot E_{Cl}$$

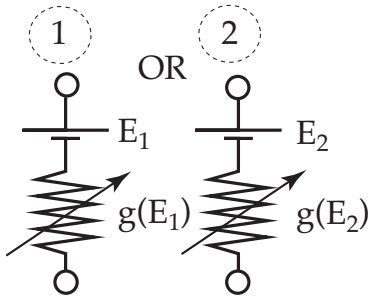
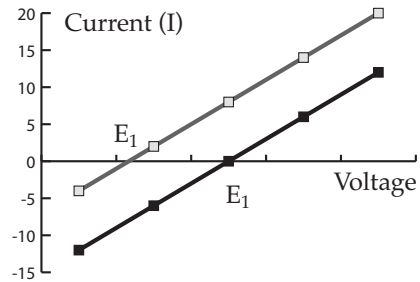
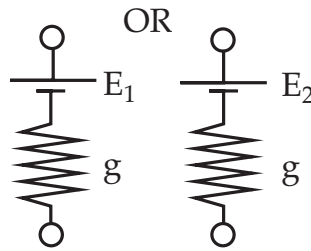
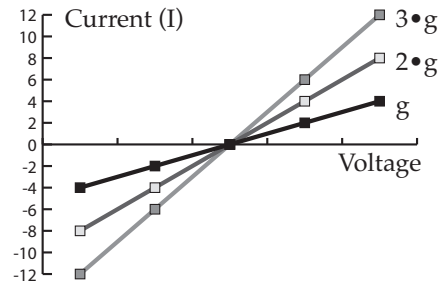
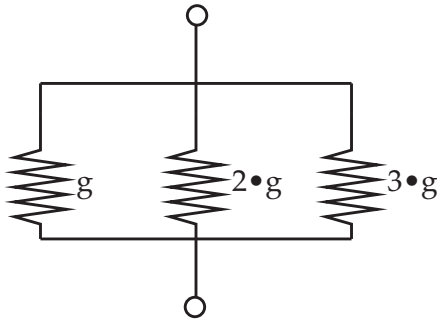
If any conductance changes, g_K , g_{Na} , or g_{Cl} , then so does V_{total} .

Is this important? Yes.

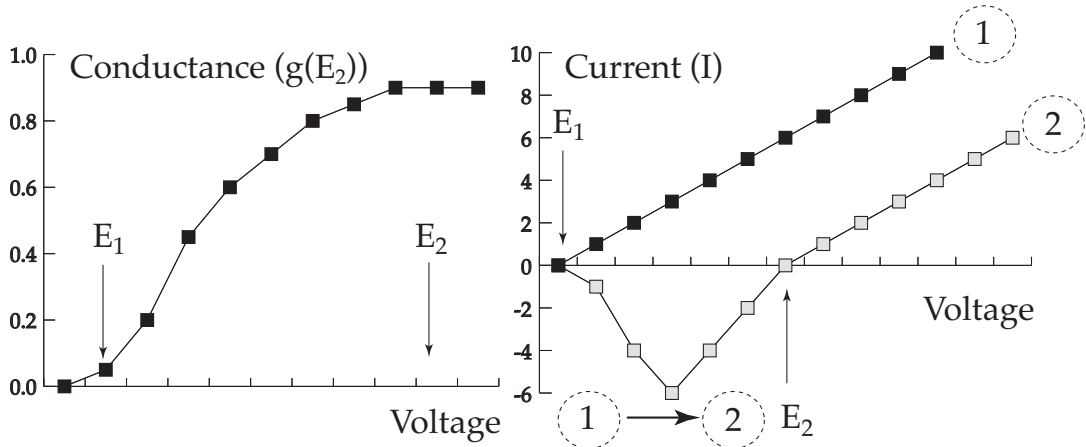
Action potentials: Changes in conductances over time, yield changes in Voltage:



Under circumstances where the conductance changes as a consequence of voltage changes, the current-voltage relation is no longer ohmic (That is, Ohm's Law no longer applies). The effect of multiple conductances under ohmic conditions:

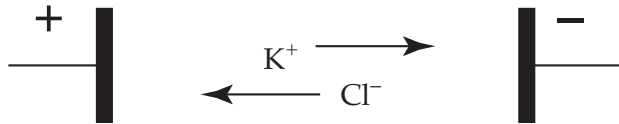


Now suppose g changes with voltage. As it changes, the reversal potential, where current is zero will change from E_1 to E_2 . But in addition, the current can exhibit an 'N' shape, a classic property of action potentials.



Back to electrochemistry...

Suppose we supply a voltage through a solution containing KCl:



The total current:

$$j = n_+ v_+ z_+ e + n_- v_- z_- e$$

Ion flux
Density
Ion velocity
Charge
 units: (Coulombs/cm² sec) (ions per cm³) (cm sec⁻¹) (Coulombs)

The ions move in opposite directions. The sum yields the total current.
 The conductivity ($\Omega^{-1} \text{ cm}^{-1}$) is very dependent on the ion concentration

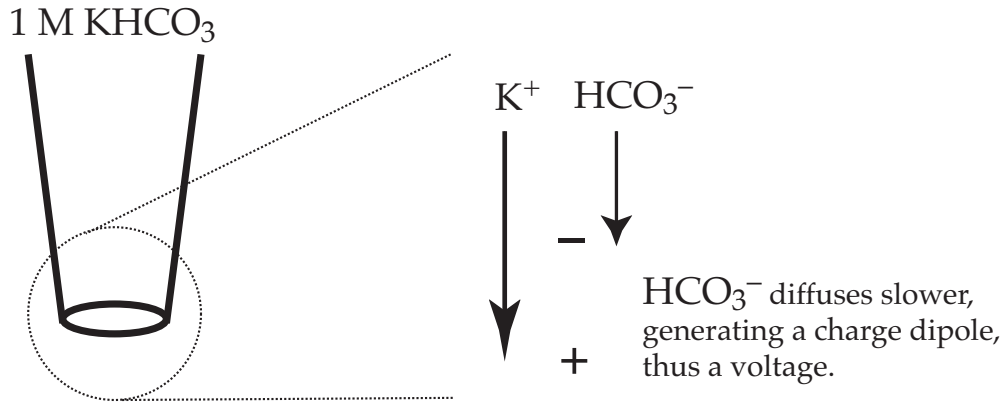
KCl (molal)	conductivity	
	0° C	25° C
1.0	0.065	0.111
0.1	0.007	0.013
0.01	0.001	0.001

What's more, the migration of ions varies greatly with ionic species:

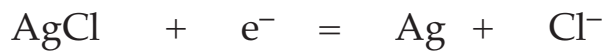
	Hydration (kcal/mole)	Atomic radii (angstroms)	Conductance	Mobility $10^{-4} (\text{cm}/\text{sec})/(\text{V}/\text{cm})$
H ⁺	-269	--	349.8	36.25
Li ⁺	-131	0.60	38.66	4.01
Na ⁺	-105	0.95	50.11	5.19
K ⁺	-85	1.33	73.52	7.62
Rb ⁺	-79	1.48	77.80	8.06
1/2Ca ²⁺	-397	0.99	59.50	3.08
OH ⁻	--	--	197.8	--
Cl ⁻	-82	1.81	76.35	7.92
Br ⁻	-79	1.95	78.20	8.09
NO ₃ ⁻	--	--	71.44	7.41
HCO ₃ ⁻	--	--	44.50	--
acetate ⁻	--	--	40.9	--

H⁺ and OH⁻ behave very differently from other ions in aqueous solution.

The ion mobility is directly related to ionic conductance. It explains one consequence of ion diffusion: The generation of the diffusion potential:



In electrophysiology, it is necessary to connect the ionic world to the electrical world. This is done using a redox reaction:

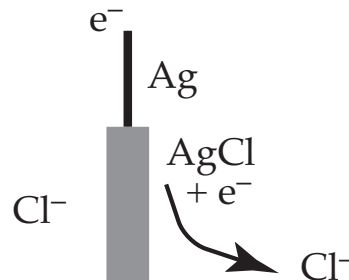


There is an electrical potential associated with this redox reaction:

$$E = E^o - \frac{RT}{F} \ln a_{\text{Cl}^-}$$

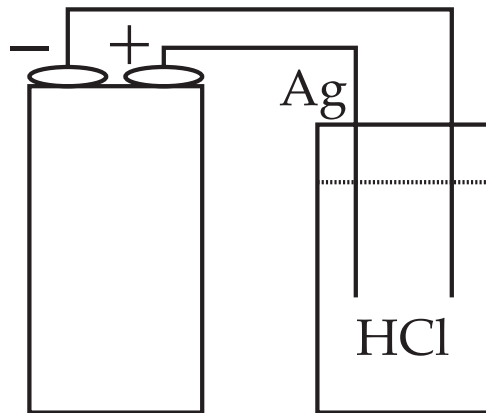
Midpoint potential of 0.222 Volts

Activity of chloride ions in solution. Approximately equal to concentration. Thus the reaction is dependent on $[\text{Cl}^-]$.

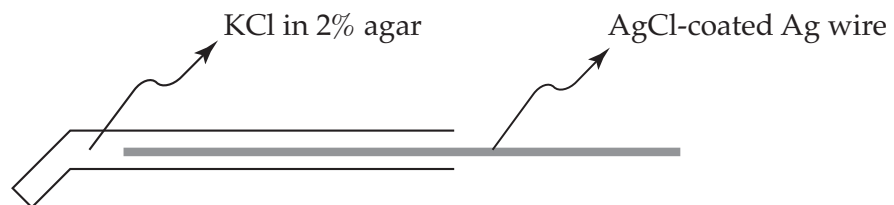


There are many redox reactions which could be used, but AgCl/Cl^- is the most common. It is freely reversible and will not become polarized, generating current on its own.

AgCl coating methods are many. By attaching wires to a battery (9 Volt will suffice), and placing the Ag wires in a solution containing chloride (for example, dilute HCl), chloriding will be rapid (in less than a minute). The AgCl coating has a black appearance. It is also possible to salvage AgCl wires from pH electrodes, since pH electrodes use the same method to convert ionic potentials to electrical potentials. Placing the Ag wire in bleach (sodium hypochlorite) also works.



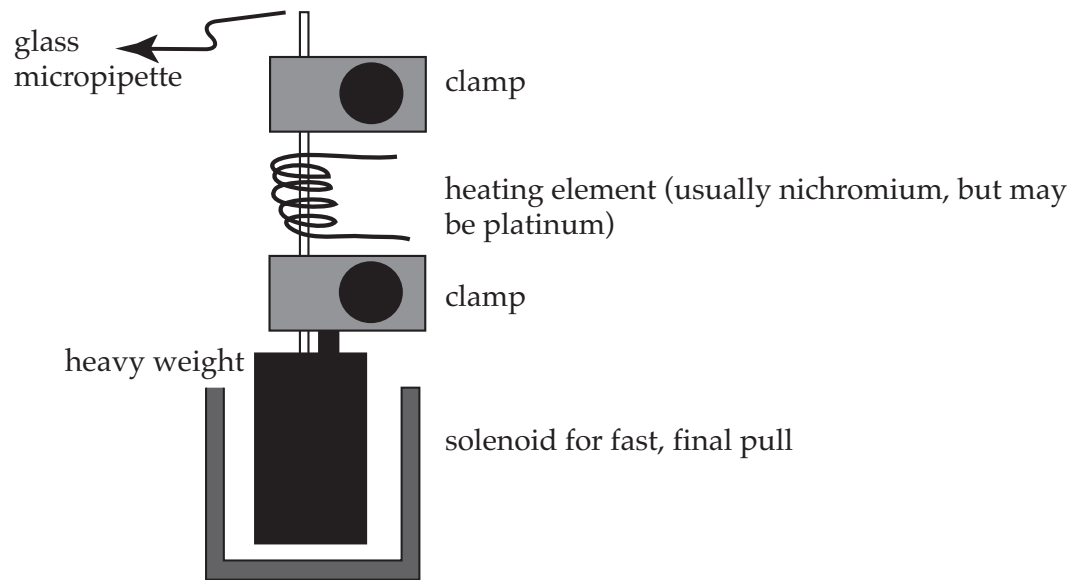
Note that both the electrode used for impalement and the electrode used for ground are AgCl/Cl⁻ electrodes. The reference, or ground electrode is usually isolated from the bathing solution using a salt bridge: the same concentration of KCl used in the impaling electrode, gelled with 2% agar.



Lecture Three: Micropipette Fabrication

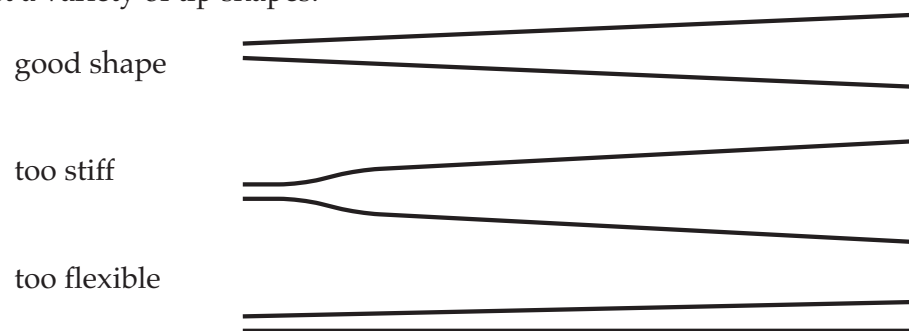
There are a variety of micropipette pullers, some horizontal and some vertical. The most recent innovations are programmability. Although I seldom use them, programmable pullers are very useful when pulling specialized pipettes. Once the parameters have been determined, it adds reproducibility of micropipette fabrication.

The key functional components of a puller are:



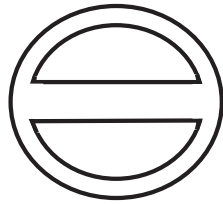
As the glass micropipette is heated, the glass softens and is stretched by the pull of the heavy weight. At some point, a solenoid is turned on, pulling the micropipette with a very strong force, causing the micropipette to break cleanly at the tip.

You can get a variety of tip shapes:

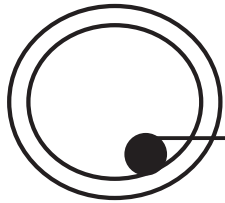


The glass tubing will have an internal filament (for use in intracellular work) or won't (for patch clamp)

There are other variants: thick-walled and thin-walled tubing, and omega tubing.

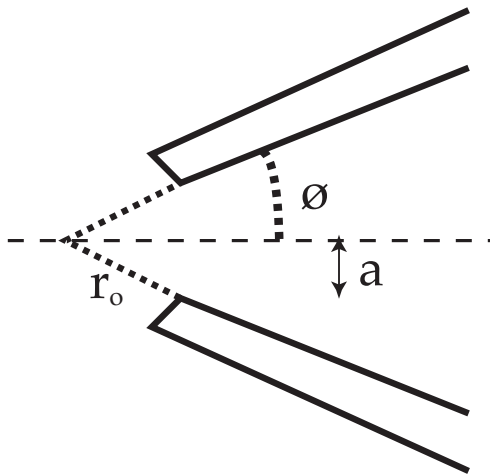


Omega tubing. Used in double-barrel micropipettes.



The internal filament is important for filling the tip of the micropipette. The filling solution 'wicks' up the interface of the filament and tubing by capillary action, allowing air to be expelled away from the micropipette tip.

The electrical resistance of the micropipette after filling is often the most practical way to assess the 'sharpness' (or tip aperture). The resistance will depend upon the size and shape of the tip, and the electrical conductivity of the filling solution (the higher the ion concentration, the lower the resistance).



The internal tip diameter is: $2a = 2r_o\theta$
 the included angle is: 2θ

$2a$	2θ	filling solution (M)	Resistance ($M\Omega$)
0.5	8°	3	1.5
0.1	6°	3	10
0.02	4°	3	76
0.5	8°	1	3.2
0.1	6°	1	22
0.02	4°	1	160

A specification for a typical capillary glass blank:

Capillary glass with internal filament made from KG-33 or Corning 7740 borosilicate glass.

Tubing dimensions: 1.0 mm outer diameter and 0.58 mm internal diameter.

Internal filament diameter of 0.095 mm.

All tolerances better than 0.015 mm.

Tubing cut into 12 inch lengths.

Cost: ~\$95/lb.

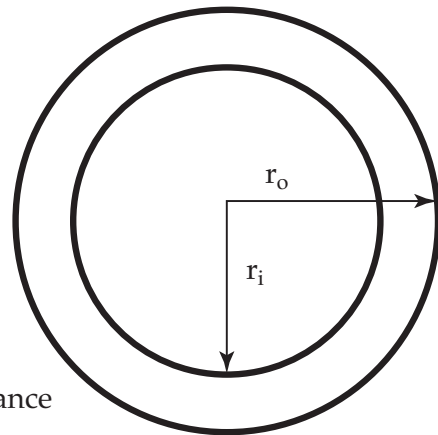
Other glass types can also be used, but are rare:

Glass Type	Manufacturer	Resistivity ($\Omega \cdot \text{cm}$)	Soft Point
Lead	Corning	$10^{10.1}$	630°C
Borosilicate	Corning	10^{15}	820°C
	Schott	10^{15}	820°C
Aluminosilicate		10^{17}	910–970°C
Soda lime		$10^{12.4}$	695°C

The resistance of a length of tubing sealed at both ends with seals of infinite resistance:

$$R = \frac{\rho \ln(r_o / r_i)}{2\pi l}$$

where ρ is the resistivity, r_i and r_o are the internal and external radii of the tubing, and l is the length. A 1 cm length of tubing of the dimensions specified above would have a resistance of about $8.7 \cdot 10^{13} \Omega$.

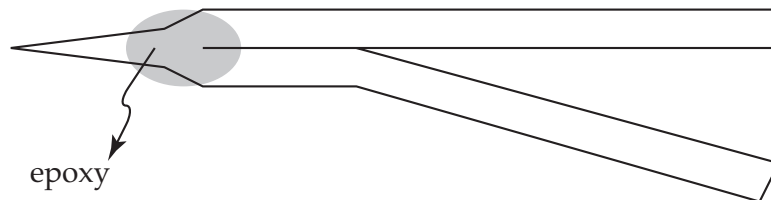


Adjusting the dimensions of the tip is an empirical process. Heat and the solenoid pull will affect the tip. In general, the higher the heat, the 'sharper' the tip. The stronger the solenoid pull, the 'sharper' the tip. There is always a trade-off: very sharp may mean too flexible.

For best results in any given preparation, it's advisable to increase heat and pull by well-defined gradations. Observing the tips under a microscope gives the best qualitative assessment of tip characteristics.

Below is a protocol for producing double or triple barrel micropipettes

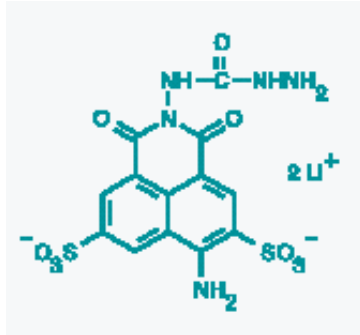
- | | |
|------------|--|
| Step One | heat two capillaries held together in the clamp |
| Step Two | when soft, rotate 360° |
| Step Three | pull |
| Step Four | apply a drop of 5 minute epoxy glue at the joint (for strength) |
| Step Five | soften one capillary just above the joint and pull it away to produce a Y-shape. |



Lecture Three: Micro-Injection

Suppose that you want to inject some substance into the cell using a micropipette. Some examples of charged molecules which can be injected by applying a current:

Lucifer Yellow:



Note that this is a lithium salt, but sodium and potassium salts are also available. If the dye is to be electrically injected (iontophoresis), the net charge on the molecule is very important. The net charge will depend upon pH.

For iontophoresis, the flux of the molecule is:

$$q = \frac{\text{transport number}}{\text{moles/sec}} = -n \cdot \frac{I}{\text{current (Coulombs/sec)}} \cdot \frac{\text{Valence}}{z} \cdot F$$

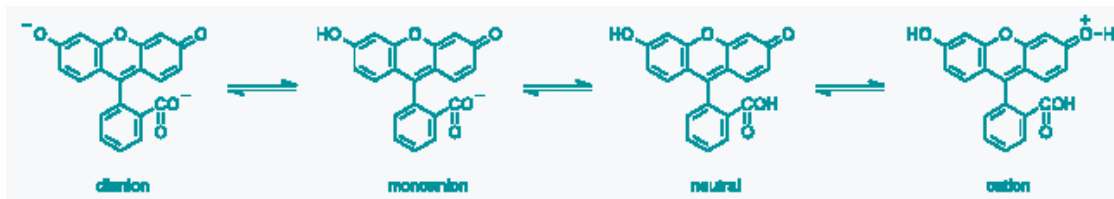
Faraday constant
(96,480 Coulombs/mole)

where n is the transport number. It corrects for the fact that part of the current, I is carried by +ve ions, and the mobilities of the ions are different.

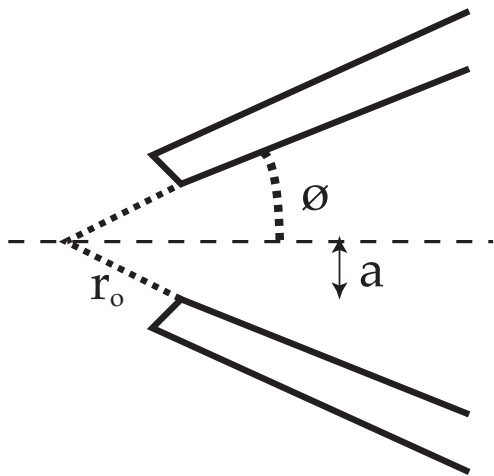
For cations, n is often about 0.1 to 0.5. For anions, it can be much less.

The transport number must be determined empirically in quantitative work.

Here is another example, in which pH dependence of the charge is very important: Fluorescein. Many fluorescent dyes use fluorescein as their 'backbone'.



There are other methods to microinject molecules into the impaled cell. One is diffusion. The rate of diffusive release from the micropipette tip depends strongly on the geometry of the micropipette tip:



The internal tip diameter is: $2a = 2r_o\theta$
 the included angle is: 2θ

Diffusional release:

$$q_d = D \cdot C_o \cdot \underbrace{\pi \cdot \theta \cdot a}_{\text{pipette dimensions}}$$

Diffusion coefficient
Concentration

The controllable factors are:

C_o , the higher the concentration, the greater the diffusional release.

$\pi \cdot \theta \cdot a$, the larger the micropipette tip aperture, the greater the diffusional release.

Another method is pressure-driven release:

$$q_p = 3 \pi \varnothing a^3 \cdot \rho g h \cdot C_o / 8f$$

pipette dimensions
density, gravitational constant and height (pressure)
concentration and viscosity

The controllable factors are:

- $\rho g h$, the higher the pressure, the higher the flow rate.
- $C_o / 8f$, higher at high concentrations, but decreased due to viscosity.
- $3 \pi \varnothing a^3$, very strongly dependent upon the tip aperture (a^3)

Comparison:

Diffusional Flow

2a	2 \varnothing	filling solution (M)	Diffusion Coefficient	q_d (n = 0.5)
0.1 μm	8°	1	1 $\mu\text{m}^2 \text{ms}^{-1}$	11 fmol sec^{-1} (2.1 nA)
0.4 μm	8°	3	1 $\mu\text{m}^2 \text{ms}^{-1}$	130 fmol sec^{-1} (25 nA)

Pressure Flow

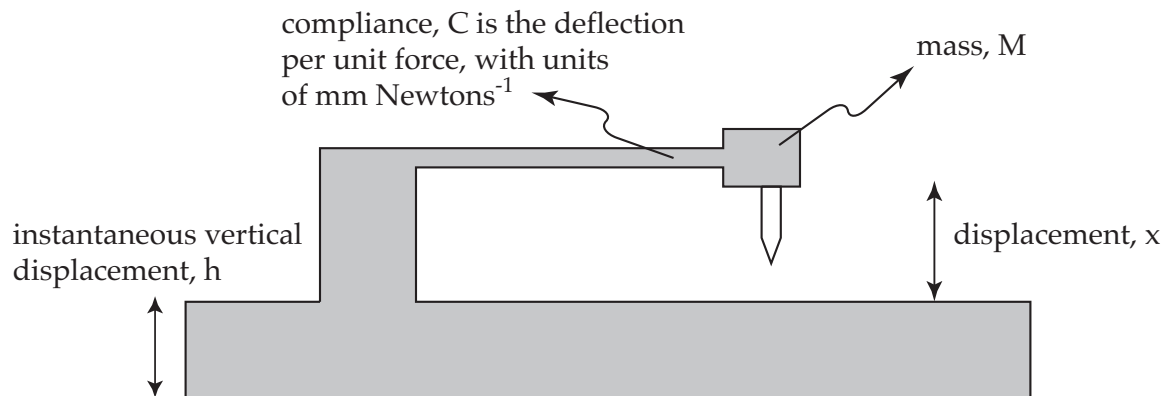
2a	2 \varnothing	filling solution (M)	Density (kg m-3)	Viscosity (Pa s)	Height (mm)	q_p
0.1 μm	8°	1	1000	0.001	20	1.8 fmol sec^{-1} (0.36 nA)
0.4 μm	8°	3	1000	0.001	20	390 fmol sec^{-1} (75 nA)

Note the much greater dependence on tip aperture for pressure flow

Lecture Five: Noise: Vibrational (mechanical)

Cell electrophysiologists measure very small voltages and currents. As a consequence, noise is a constant preoccupation. One type of noise is mechanical. Vibrations can have two effects, one is to cause the micropipette tip to vibrate, causing damage to the cell. Minute displacements may also be 'translated' into electrical signals, effectively interfering with measurements of the electrical properties of the cell.

The micromanipulators used to impale the cell will have an effect on vibration. To maximize the vibration-free nature of the set-up, we need to consider the factors which affect mechanical displacements due to vibration:



The displacement: $\Delta x = h \cdot M \cdot C$

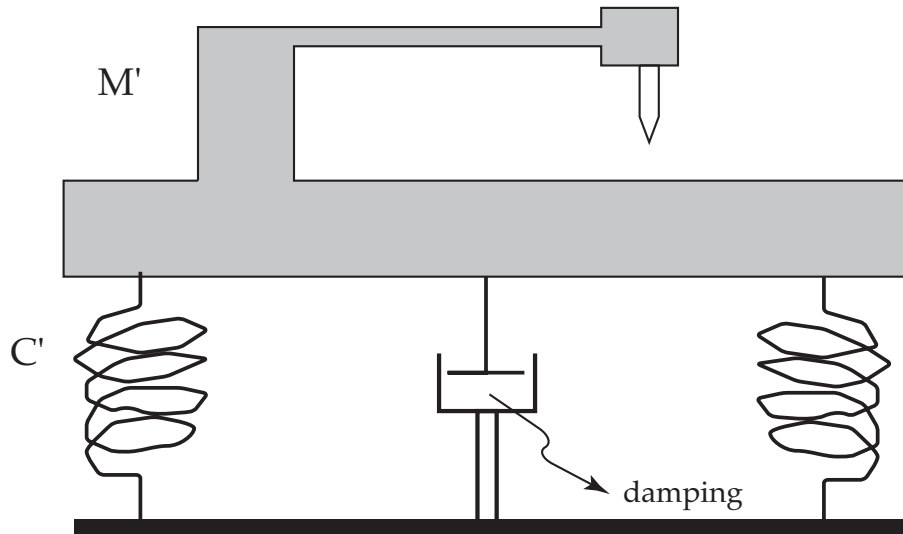
To minimize vibration, it is necessary to decrease h , M , and C

To decrease C (usually the micromanipulator), decrease the length of its support, its mass, and use materials of high stiffness (and low mass, that is, specific stiffness).

To decrease M is usually difficult (usually, M is the headstage to which the micropipette is attached).

To decrease h is to isolate the micromanipulation system from external vibrations.

To decrease h , the instantaneous vertical displacement, the system should be isolated from external vibration:



The instantaneous vertical displacement, h : $h = 1 / (M' \cdot C')^{1/2}$

That is, the larger the mass, M' , the better, and, the larger the compliance C' , the better.

The mass, M' is usually a steel plate, 50 to 100 kg.

Compliance can be provided by metal springs, inflated bicycle tires, tennis or squash balls.

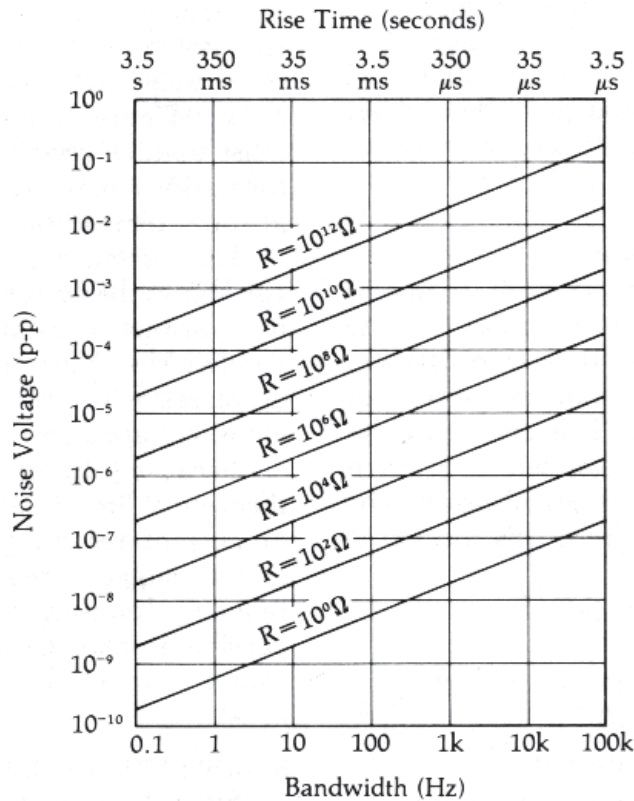
Damping is important (you don't want M' to bounce around!) Foam rubber is a common dampener.

Commercial systems usually use compressed air cylinders (4) on which is mounted a thick steel plate.

The major source of vibration problems I have experienced is a high compliance somewhere on the microscope (or base plate), micromanipulators, or most commonly, the micromanipulator to headstage mounting.

Lecture Five: Noise: Electrical

First, we need to distinguish two types of noise, voltage and current. They are very different.



Noise will vary with the bandwidth being measured, as indicated on the graph. Note that with increasing resistance, voltage noise increases. Qualitatively, a small current flowing through a large resistance generates more voltage than a small current flowing through a small resistance.

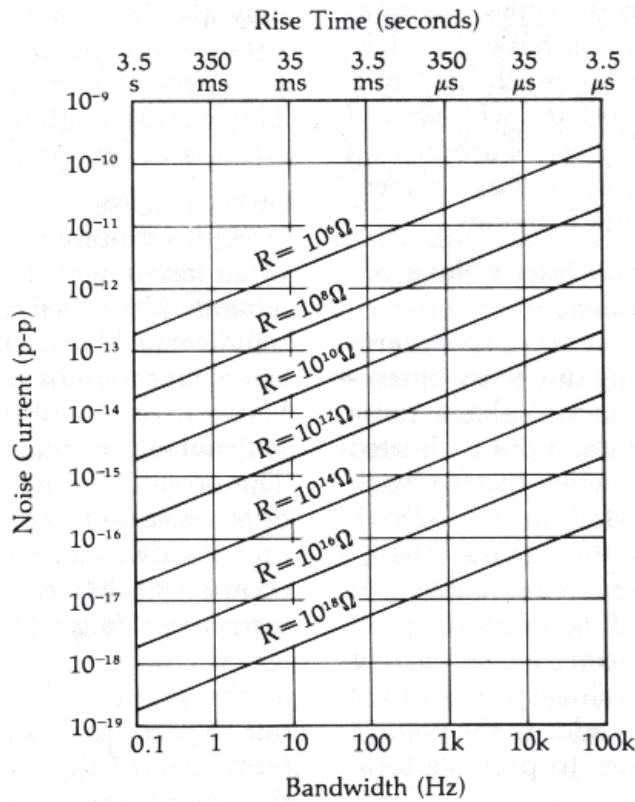
The voltage noise is called Johnson noise, and is defined by:

$$E = \sqrt{4kTR\Delta f} \quad \text{Volts rms (root mean square)}$$

where k is the Boltzmann constant, $1.38 \cdot 10^{-23} \text{ J/}^\circ\text{K}$, T is the temperature ($^\circ\text{K}$), Δf is the noise bandwidth in Hz, and R is the resistance. As the kT term implies, the noise is a consequence of thermal motion of charged ions.

This is the minimal theoretical noise level. You can't decrease the noise below this level.

Current noise behaves differently:



Noise will vary with the bandwidth being measured, as indicated on the graph. Note that with increasing resistance, current noise decreases. Qualitatively, a small voltage across a large resistance generates less current than a small voltage across a small resistance.

The current noise is defined by:

$$I = \sqrt{\frac{4kT\Delta f}{R}}$$

Amps rms (root mean square)

Again, this is Johnson noise, attributable to random thermal fluctuations, and is the minimal noise possible.

Another type of noise is called shot noise. It is a consequence of the quantal nature of charge flow:

$$I_{shot} = \sqrt{2qI_{DC}\Delta f}$$

where q is the electron charge, $1.60 \cdot 10^{-19}$ Coulomb, and I_{DC} is the current.

I_{DC}	I_{shot}
1 ampere	57 nA (10 kHz bandwidth)
1 μ A	60 pA
1 pA	56 fA

Shot noise can actually be used to tell if a channel is open, the slightly higher current of the open state will cause greater peak to peak current noise.

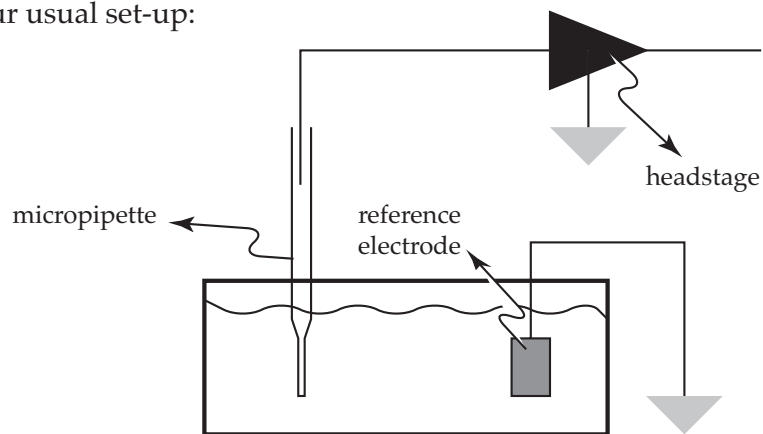
Lecture Five: Noise: Troubleshooting

Identifying and removing unwanted noise is very important to the electrophysiologist.

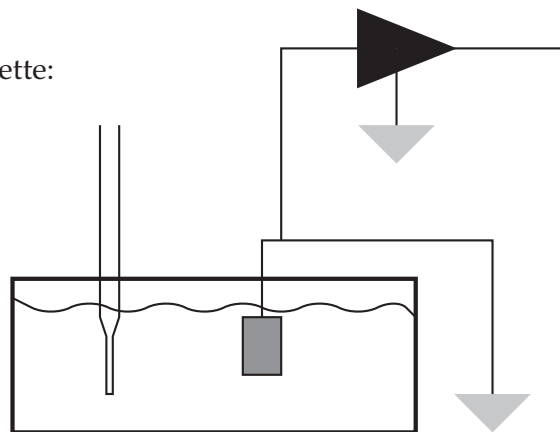
The primary source of electrical noise is 60 Hz which comes from our very electrical environment. Both electrical and magnetic fields can cause noise. To avoid electrical noise, set-ups are shielded from external electrical fields using a Faraday cage, usually a grounded aluminum box.

Purves presents a flow diagram for isolating and correcting noise problems. This forms the basis for the following troubleshooting guide.

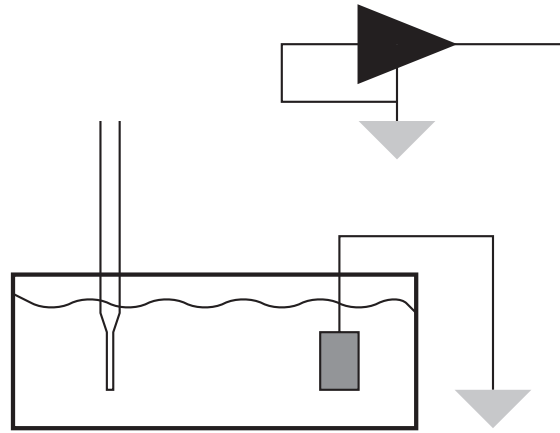
You start with your usual set-up:



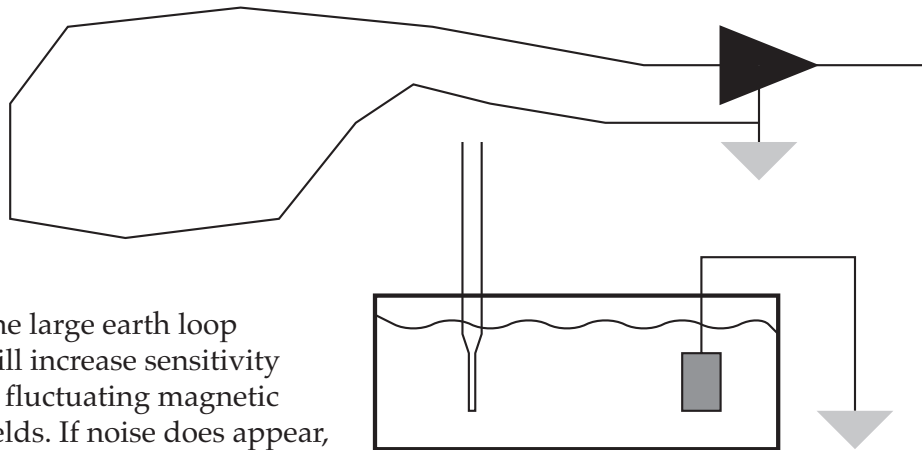
Is there noise? If not, great.
If yes, then remove the micropipette:



Is noise still present after removing the electrode from the circuit? If so, test if it is the reference electrode.

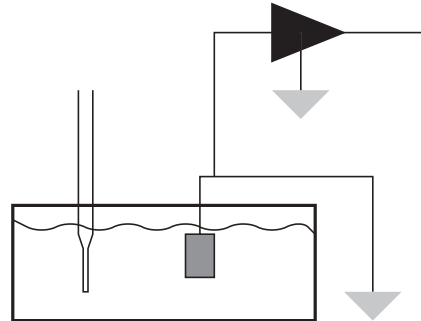


If the noise is still there, it's source is not the set-up per se, but the electrometer circuitry. If the noise is gone, test to see if the noise source is magnetic fields.

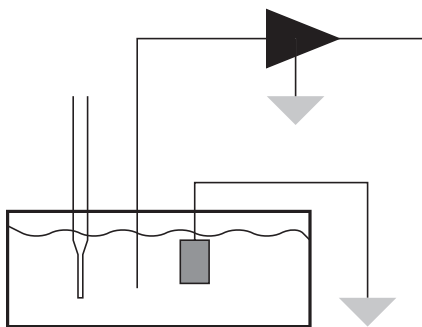


The large earth loop will increase sensitivity to fluctuating magnetic fields. If noise does appear, than a magnetic field is the cause. It could be a nearby computer monitor or other nearby power source.

What if noise is gone at this point:

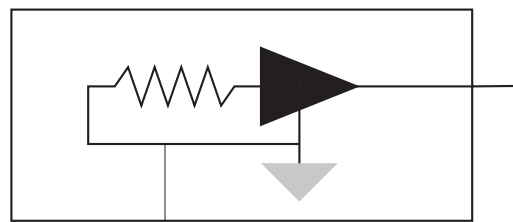


Then try:

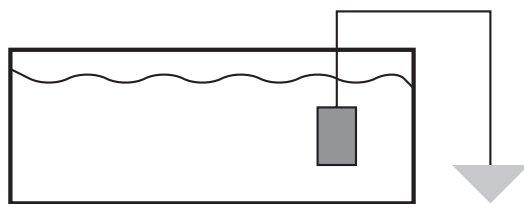


If noise re-appears, then the reference electrode is at fault.

To test for electrical interference of the instrumentation separate from the microelectrode and reference electrode, shield as follows:



If there is noise, than the circuitry is at fault.



Common problems:
faulty microelectrodes
faulty reference electrodes
microscope lamps
computer monitors
earth loops.

Purves, R.D. (1981) *Microelectrode Methods for Intracellular Recording and Ionophoresis*. Academic Press, London.

Probably out of print, this is a small book (146 pp) that introduces micropipettes and electronics for the budding electrophysiologist. I have seen Russian translations. It has a place of honour on my bookshelf, from the time I was a graduate student to now. The lecture discussions of microinjection, ionophoresis, and troubleshooting are drawn from Purves.

Horowitz, P., and W. Hill (1989) *The Art of Electronics*. 2nd edition. Cambridge University Press. Cambridge.

This is a well known textbook on electronics fabrication. It is very useful to those who design and fabricate their own instrumentation. The authors are from Harvard and MIT, and fabricate unusual measuring devices. They do an excellent job of introducing aspects of electronic design, both the good, the bad and the ugly. I have fabricated Wheatstone Bridges and differential amplifiers directly from the designs offered by Horowitz and Hill.

Horowitz, P., and I. Robinson (1981) *Laboratory Manual for the Art of Electronics*. Cambridge University Press. Cambridge.

A good opportunity to work with electronics, and get hands on experience with how to fabricate circuits, troubleshooting and testing.

Keithley Instruments, Inc. (1984) *Low Level Measurements*. For effective low current, low voltage and high impedance measurements. Keithley Instruments, Inc. Cleveland, Ohio

This handbook describes the techniques required for measurements of low amplitude signals under high impedance conditions. The handbook naturally favours the instrumentation manufactured by Keithley, who are well-known for their electrometers. The general introduction and descriptions of measuring techniques are well presented and helpful to anyone interested in measuring small signals. Some of the material on measurements of cell electrical properties is drawn from this handbook.

Ogden, D. (1994) *Microelectrode Techniques*. The Plymouth Workshop Handbook. 2nd edition. The Company of Biologists Ltd. Cambridge

Better than most compiled methods books, but the quality varies from chapter to chapter. Some are useful. Some are not.

Sakmann, B. and E. Neher (1995) *Single-Channel Recording*, 2nd edition. Plenum Press. New York and London.

A compiled methods book for patch clamp and associated techniques. Sakmann and Neher are Nobel Prize Laureates. They discovered the patch clamp technique. I would have characterized this as I do the Ogden book, but then I noticed the number of paper scraps, marking individual pages, inserted by members of my laboratory, The chapters are not easy going.