Physiology 416
Electrophysiology Notes

Jeremy Payne
jrpayne@uiuc.edu

January 13 1995

0.1 Introduction

These notes are intended to give you a relatively complete albeit somewhat rough background of the theory you'll need for this course. This material may seem a little daunting at first, but nobody really uses (much less thinks about) all of this on a daily basis, and most of it will quickly become second nature. You really don't need to understand Gauss's law for example to know that a Faraday cage gives you better recordings, but you should be at least passingly familiar with the concept. Further equipment and techniques will be discussed as relevant throughout the course. As always, if you don't understand something, ask. . . .

0.2 Electronics

For most people electronics is very difficult, and it's easy to convince oneself that our brains just didn't evolve with the goal of understanding Kirchoff's current law. I would tend to agree. After many years of physics and EE courses I have culled what I think are the essential concepts for a self-respecting electrophysiologist to understand into these few pages.

Voltage is potential energy caused by a charge imbalance. In a piece of everyday wire, all points have the same voltage because if you started out with an excess of charge at one end, there would be nothing to stop the charge from spreading throughout the wire, causing a homogenous potential. When you look at circuit diagrams keep this in mind. You can move nodes around on wires to help you visualize what's going on, and if all that's connecting two points on the circuit is some wire then those points *have* to be at the same voltage.

Resistance changes this fact. All a resistance does is make it difficult for electrons to move, and as they trudge through the resistor they lose a little energy in a manner analogous to friction, causing the resistor to heat up. If you now create an excess of charge on one side of the resistor, it will cause an instantaneous flow of electrons (current) as the charge tries to spread out and lower its potential energy, but since potential energy is lost as the current flows through the resistor, the potential on either side can never become equal *if a constant supply of electrons is provided to one side*. (If you started with a finite number of electrons, initially a potential would exist across the resistor, but it would quickly dissipate as the electrons equalized or were turned into heat.) Energy must be exerted to force charges through a resistance, turning potential energy into kinetic energy. This loss of potential energy effect is known as Ohm's law, and is given by $V = IR$. We quantify resistance with the eponym "Ohms" (Ω). Be sure not to confuse resistance with resistivity. The latter is a property of the *material*, so that a particular type of steel

can be said to have a resistivity of some amount, an aspect of the material along the same lines as its density. We talk about the resistance of a specific *geometry* of the material – e.g. a steel wire of a given diameter might have a resistance of 1Ω per cm.

Often we use the reciprocal of resistance (the conductance) in biology, since it can make more intuitive sense in certain situations, but it's entirely equivalent to resistance: $gV = I$. Conductance units are mhos or Siemens, and you may see conductance denoted by an upside down Ω. In figure 1, based solely on intuition,

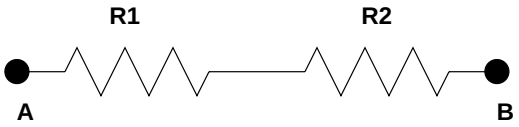

Figure 1: Series resistances.

resistors placed end to end (in series) add algebraically forming an equivalently larger resistance, since the length of the path during which the electrons are impeded is now longer:

$$R_{AB} = R_1 + R_2$$

In figure 2, when resistors are placed side by side (in parallel), the electrons have

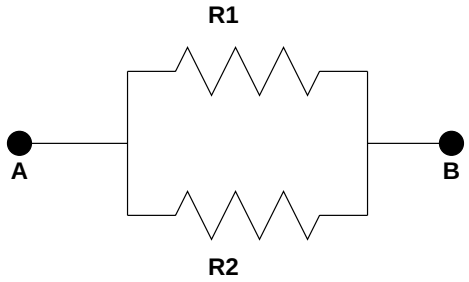

Figure 2: Parallel resistances.

much more room to cross from one side to the other, and the resistance is reduced:

$$\frac{1}{R_{AB}} = \frac{1}{R_1} + \frac{1}{R_2} = \frac{R_1 + R_2}{R_1 R_2}$$

$$R_{AB} = \frac{R_1 R_2}{R_1 + R_2}$$

Thinking more about energy, you know that it must be conserved in some form or another (first law of thermodynamics). For any circuit consisting of a single

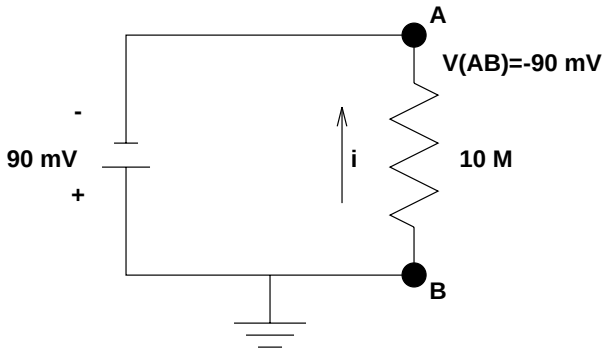

Figure 3: Simple circuit.

loop, all of the energy must be accounted for, and in figure 3 we can solve for the currents and voltages at each point based on this fact:

$$I = \frac{V}{R} = \frac{-90mV}{10M\Omega} = -9nA$$

Remember, although current is in reality carried by $\ominus$ charged electrons, we *define* $\oplus$ current as the flow of $\oplus$ charges, so in this case, out of the $+$ terminal of the voltage source, around the loop, and back into the $-$ terminal. In biology, currents can be carried by $\oplus$ and $\ominus$ ions.... Note also that V_{AB} is a free-floating potential unless we add the ground symbol. This emphasizes the point that voltages are *differences in potential*, and don't imply any particular absolute value. Take a moment to think about this.

In fact, for any circuit, no matter how complex, Kirchoff's two laws provide a way to solve for the relevant values based upon conservation principles. The first states that at any node in a circuit, the algebraic sum of all currents entering it is zero, otherwise the node would create an energy imbalance, releasing more than it is provided with, or somehow keeping some of the energy flowing into it. The second law states that as you travel around any closed loop in a circuit, the sum of all voltage drops must equal zero by the time you get back to where you started, otherwise you will have somehow gained or lost energy. These laws do not take energy dissipation by heat into account (they're *conservative*). As an example we'll derive the voltage divider equation, as shown by the circuit in figure 4:

$$KVL : V - V_1 - V_2 = 0$$
$$V = V_1 + V_2 = iR_1 + iR_2$$
$$i = \frac{V}{R_1 + R_2}$$

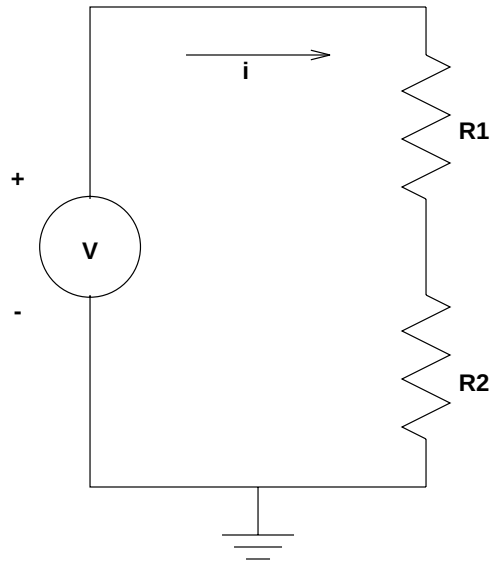

Figure 4: Voltage divider circuit.

$$V_1 = iR_1 = V\frac{R_1}{R_1 + R_2}$$
$$V_2 = iR_2 = V\frac{R_2}{R_1 + R_2}$$

figure 5 shows an example solution of a voltage divider. This type of circuit is central to understanding how electrodes and amplifiers interact, as we'll see below.

The concept of capacitance is also inseparable from electrophysiology. A capacitor is a circuit element that consists of two conducting surfaces separated by a non-conducting, or *dielectric* material. If a voltage source is connected across the capacitor, positive charges accumulate on one side, and negative ones on the other, creating an electric field. If this field is static in time and space (DC) no current flows between the two surfaces, but as described below (by Faraday's law), AC fields cause a current to flow between the plates:

$$q = CV (or C \doteq \frac{q}{V})$$
$$i = \frac{dq}{dt}$$
$$i = \frac{d}{dt}CV = C\frac{dv}{dt}$$

C refers to capacitance (measured in Faradays), and q is a measure of charge. This means that current will only flow through a capacitor if the potential across

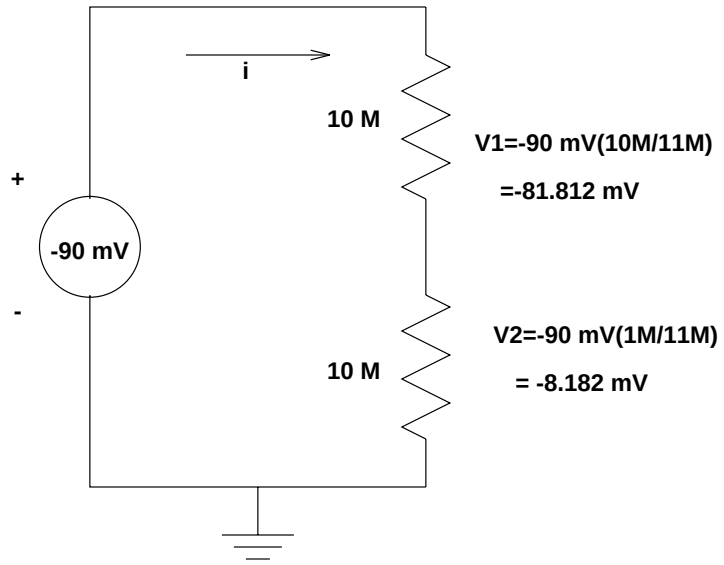

Figure 5: Solved voltage divider circuit.

it varies with time. In other words, to direct current a capacitor looks like an open circuit. An instantaneous jump in the voltage across a capacitor is not physically realizable, because it would require the movement of a finite amount of charge in zero time. Parallel capacitors add algebraically like series resistors do; since more surface is now available to store charge, larger capacitances are obtained. Series capacitors add like parallel resistors. When capacitors and resistors are added together more complex equations result which we won't solve here. As constant current is applied across a series RC combination at time zero, the resulting voltage is seen in figure 6.

The product RC turns out to be the number of seconds it takes for the voltage to rise to 63% ($1 - \frac{1}{e}$) of its final value. We refer to this as the time constant of the circuit, τ. The filtering circuits in figures 7 and 8 are also interesting.

The high pass filter only allows AC current to flow across the capacitor. In the low pass filter circuit, AC input currents will preferentially travel through the capacitor and to ground, but all DC currents will see an open circuit where the capacitor is.

A final point concerns impedance. AC electronics gets very complicated, and to simplify dealing with it, resistive and capacitative components are usually lumped together to form a single concept known as impedance, denoted with "Z". It's best thought of as a kind of resistance to current flow, both AC and DC. In the purely DC case, impedance is equivalent to resistance. You'll notice that many

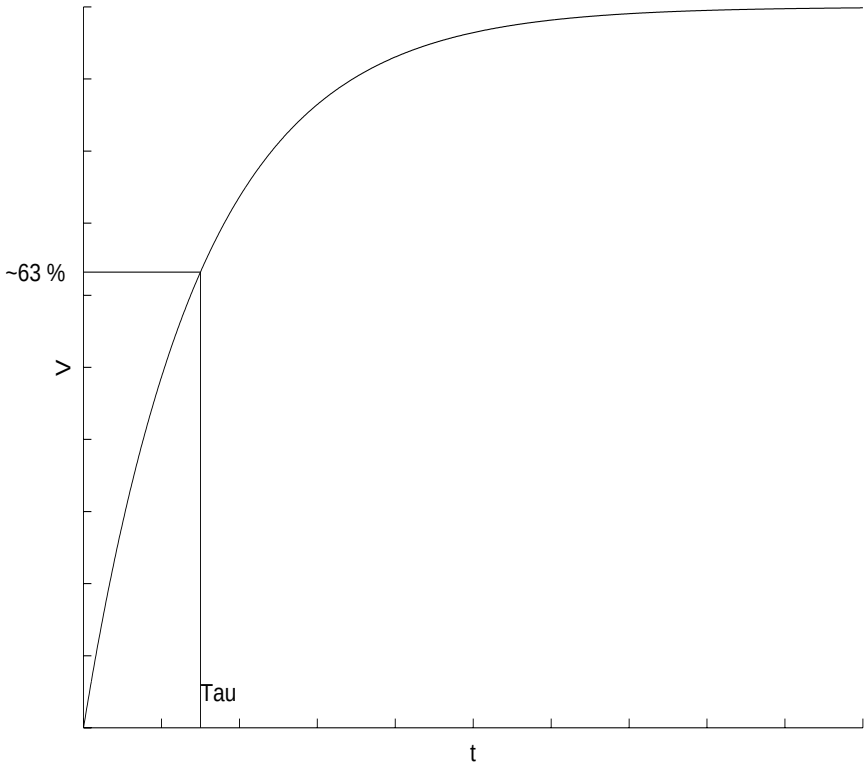

Figure 6: Voltage over time across a series RC circuit.

people use the words impedance and resistance somewhat interchangeably, but you should still understand what each *really* implies.

0.3 Oscilloscopes

Oscilloscopes are the most accurate instrument we'll use in 416 for measuring time and voltages. Other instruments such as stimulators may have knobs that appear to give accurate readings of frequency or potential, but trust only your oscilloscope....

Theory of operation is very simple: an oscilloscope is a beam of electrons directed at a phosphorescent screen. Wherever the beam hits the screen it glows. The beam's position is controlled by 2 amplifiers, one each for the horizontal and vertical axes. Time is represented along the horizontal axis by the speed at which the electron spot is moved across the screen. In order to show slow events the time base control is set to a large value (e.g. 1 second), slowing the sweep rate. In this

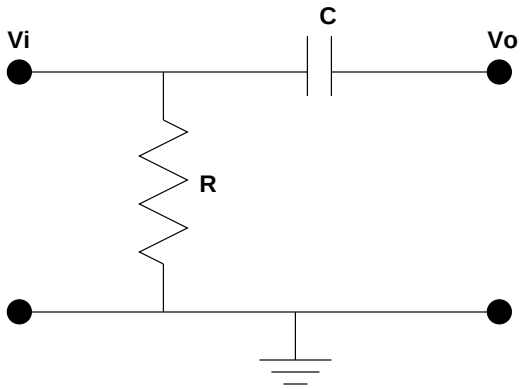

Figure 7: High pass filter.

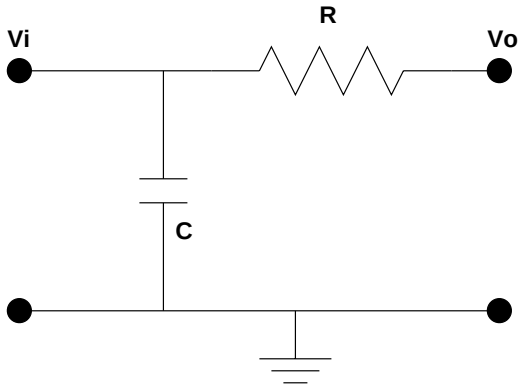

Figure 8: Low pass filter.

case for every cm the beam travels, 1 second elapses. As the time base control is set to smaller values of time per cm, more of the signal can be resolved. In general oscilloscopes can show MHz frequencies with little difficulty. The vertical axis shows voltage, again with values of volts per cm. The sensitivity of the vertical amplifier can be adjusted to view events on the order of tens of volts down to microvolts. Most signals we look at in neurobiology range from DC to a few kHz, and from μ V to several hundred mV. Most oscilloscopes have one control for the timebase, and two vertical amplifiers so that two individual signals can be visualized using the same timebase. Some scopes allow each beam to be split into two beams, effectively allowing four signals to be seen by sharing the vertical amplifiers.

Triggering is an important concept. Besides the rate at which the beam sweeps

left to right across the screen, it also needs to know when to start its sweep. Imagine a sinusoidal waveform with a period such that 10.123456... full periods fit on the screen. Without any further manipulation, if left to free run the signal would form a blur on the screen as every time it started a new sweep it would begin at a slightly different point on the sinusoid. With triggering however, you can adjust the scope to only start a new sweep when the signal reaches a certain value. So a sweep may for example only start when the signal crosses 0V (from $\ominus$ to $\oplus$), causing all of the displayed sweeps to occur in the same place on the screen, and allowing you to see the waveform. This is one of the simplest cases. In general the trigger point can be adjusted continuously from to values, and a slope control determines whether the trigger point occurs during increasing or decreasing voltage portions of the waveform. Most scopes will allow triggering from either of the input channels, or from the line voltage, which makes visualizing 60 Hz noise easy since line triggering synchronizes the horizontal sweeps with the frequency of ambient line noise.

Analog storage scopes use phosphor with 2 stable states. When enabled, in storage mode a flood beam keeps the whole screen partially excited with a low energy electron beam. When the writing gun beam hits the screen the phosphor is excited to a second, glowing state, and the flood gun provides just enough energy to keep it in that state. This feature is particularly useful for looking at spikes, since it keeps the trace on the screen until you're done looking at it, and is often used in conjunction with external triggering. In this mode a sweep is initiated only when signaled by some external device. When stimulating your prep for example, the stimulator's trigger out can be connected to the scope's trigger in, and when a stimulus is given a pulse (called a TTL pulse) is sent to the oscilloscope synchronizing the sweep with the event you're interested in. External triggering can be either AC or DC coupled. Use AC unless triggering a very slow waveform ($<$15Hz).

Connect an unshielded cable to one of the scope's channels to observe 60Hz noise. Touch ground and note that the low impedance shunt to ground reduces the current into the amplifier (i.e. noise). Compare with a shielded BNC. (Trigger on line voltage to see 60 Hz)

0.4 E/B fields

This is a very complicated subject, but a little knowledge will help you understand some later concepts. From freshman physics you should remember Faraday's law of induction stating that a changing magnetic (B) field generates a changing electric (E) field and vice versa. So if you have a changing (i.e. AC) B-field surrounding

a wire it will cause an E-field to exist, which is just a collection of various electromotive potentials, and current will flow from high to low potential energies in the wire. We call this *induction*. So far so good. The B-field itself arises elsewhere from things like unshielded power chords. Line voltages are AC potentials (E-fields), which generate ambient B-fields around them propagating across the room to your prep and into your amplifier. Shielding your equipment will stop these fields from propagating as described below. It's important to remember that only AC fields matter – static DC fields do not cause induction, so your dynamo won't generate current unless it actually moves. . . .

0.5 60Hz noise cures

1. Use differential amplification.
2. Use shielded cables.
3. Make all cables short.
4. Ground everything to a common point; fan out from there. (see 5)
5. Avoid ground loops – they form antennae. Ambient noise gets into your amplifier because E/B-fields inductively cause current flow in conductors. If one end of the conductor is connected through a minimal resistance to ground induced currents are shunted away, but if the conductor forms a nice loop connected to your amplifier they are reinforced.
6. Work on a grounded metal plate in a Faraday cage.
7. Unplug any unnecessary AC equipment – just turning it off won't do, since the power line comes up into the instrument to the panel switch, and although no current is flowing through it when the switch is off it still carries a 60 Hz varying 120 Volt potential, which generates a field.
8. Reduce mechanical vibration. It can move electrodes and destroy cells.
9. Start with the simplest possible connections when arranging your equipment, and remove unnecessary ground leads. Remember that getting rid of noise is somewhat of a black art, and sometimes you have no choice but to strip down your entire setup and put it back together step by step.

0.6 Shielding

Shielding is very important to us since we're measuring small voltages against a potentially large background of noise. Any external noise we can keep out of our recordings will allow us to see more of the signal. Shielding is a property of Gauss' law, which in a nutshell states that the current flux across a closed surface is proportional to the net difference in charge across it. Imagine a solid lump of an ideally conductive metal. Since it conducts so well, everywhere inside the lump has the same potential with respect to the outside environment – there's nothing to stop electrons from freely moving around inside the metal, evenly distributing any charge the lump has. If you then imagine a closed surface inside the lump it can't enclose any potential different from the rest of the non-enclosed lump, so since no charge difference can exist across the imaginary surface, no current flows across it either. In fact, any charge put on our lump of metal is forced to reside at its extreme surface by Gauss' law since no charge differences can exist inside a conductor. This also means that electric field gradients can have no components tangential to a conductor, since at the boundary of the conductor an equipotential surface must exist. In reality small eddy currents and charge imbalances exist, but these can usually be ignored unless you want a PhD in solid state physics.

So there's no field inside a conductor, but what about conductors with air filled cavities in them, as approximated by a Faraday cage? Since both sides of the closed conductive surface must be equipotential, no field can exist across it, and fields outside the conductor can't cause fields inside it (and vice versa). So an empty conductor has no fields inside it. If you put a field inside it by for example enclosing a wire carrying some signal in shielding, the only field that can exist inside the shielding is from the signal-carrying wire, and ideally no noise should get in from outside. If this doesn't make sense, try this thought experiment: first try to imagine how different potentials could exist on a single conductor (no resistance). Then imagine a hollow conductor, and try to think how you could get a field inside it. You can only get a field between areas of different charge (i.e. potential). Remember that here we're really talking about fields. AC and DC. Current in the conductor is what we measure, and only AC fields can induce current flow.

Sometimes "driven" shields are used. Rather than providing passive shielding, the amplifier "drives" the shield with the measured potential, so that the shield and signal carrying conductor are equipotential, reducing any capacitative effects between them. This is typically employed with high impedance headstages.

0.7 Stimulators

We use stimulators to deliver known amounts of current or to apply a particular voltage to our preparation. Typically they're only useful for rather gross work, such as whole nerve stimulation–most stimulators (and especially the ones we use for this class) just aren't precise enough to be used in single cell experiments. Most intracellular or voltage/patch clamp amps have built in stimulator type functionality.

Biphasic stimuli are used to prevent electrode polarization. We'll see later on in the course that the interface between and electrode and the medium around it forms a galvanic potential, and can quickly become ionized. We can overcome part of this effect by driving current both into and out of the electrode when we use a stimulator.

Be sure to calibrate stimulators by first calibrating the scope's vertical scale with its calibration output, then measuring stimulus voltages on the scope. Even though the stimulator may have knobs and dials with very fine control over time and voltage, *never* trust them.

0.8 Stimulus isolation

Stimulus voltage is often much larger than the signal giving artifacts. Isolation removes the common ground from the system, and current provided by the stimulator can't reach the recording site. A shunt to ground between stimulus and recording sites might also help. Since there's no path (via ground) from the stimulation site through the electrodes and amplifier to the stimulator, the current it provides shouldn't enter the electrodes. Nothing is prefect though.

0.9 Preamplifiers

Preamps provide initial signal amplification for driving the oscilloscope or another amp, and are often DC and placed in the cage to prevent noise. DC amplifiers are used when the absolute voltage level of a signal is important, for example when looking at intracellular potentials. When studying action potentials however, it's often more useful to have an AC coupled amplifier, meaning that the DC baseline that the action potentials ride on top of is filtered out. The small signal from your prep should be amplified out of the range where noise is present ASAP, preferably before leaving the Faraday cage. Their high input impedance allows efficient power transfer (from electrical engineering) and reduces current drain on the signal source

(which distorts whatever electrical mechanism is generating the current in the first place).

Early signal filtering is also very important, especially when working with discrete data, i.e. sampling continuous data at some fixed rate with a computer as we will be doing with MacLab. The Nyquist rule of sampling states that signal components above $\frac{1}{2}$ the sampling frequency will show up (as aliasing) as false components below $\frac{1}{2}$ the sampling frequency. Even if you aren't sampling the data, it's easier to manage if you filter out extraneous signals. Preamps usually have filters. While on the subject of frequencies, the Fourier theorem states that any signal can be expressed as the algebraic sum of sinusoids with an infinite number of individual frequencies (like many famous scientists, Fourier actually got credit for someone else's work). So your complicated looking electrophysiological record can be considered to contain components with frequencies from 0 HZ (the DC portion), all the way up to maybe 20 kHz. Usually the contribution of frequencies any higher than this can safely be neglected. Be aware that filters aren't perfect. Frequency responses fall off slowly around corner frequencies (the frequencies shown an your filter's knobs), and at these frequencies only a 3dB drop occurs (i.e. for a high pass filter set to 100 Hz, at 100 Hz 0.7071 of the signal is present). At 75 Hz maybe 40% of the signal is still there. The "3dB down" definition of corner frequency arises because at this value ($\frac{1}{\sqrt{2}}$) half of the signal's power is present ($power \propto signal^2$). Notch filters are often provided that reduce the signal intensity at 60 Hz to reduce ambient noise, but should only be used as a very last resort, since all but the most sophisticated digital filters have shallow falloff slopes, and will affect the signal at many neighboring frequencies. So a 60 Hz notch filter will probably corrupt your signal from 25 to 100 Hz.

DC amps record membrane potentials and slow intracellular potentials. AC amps are used for extracellular AP recording etc. Use of a particular amp depends also on the type of electrodes chosen. Glass capillary electrodes are usually used with DC amps because their tips form low-pass filters for example. Metal electrodes have good high frequency (AC) properties, but are subject to polarization leading to slow DC shifts, so they're generally used with AC amps (the reactive portion of an AC amp's input impedance tends to filter out DC portions of the signal).

Differential amplification relies on common mode rejection; the two input voltages A and B are compared and only the difference is passed. If electrode A records the desired signal, and electrode B is in A's direct proximity but in the medium or extracellular space etc., both electrodes may pick up the same interference waveform (ambient noise), but only electrode A will pick up the desired signal. So

$$A - B = (Noise_A + Signal) - (Noise_B) = Signal$$

0.10 Input impedance

Should be high ($> 10^8 \Omega$Ł). This reduces the input reactance and therefore distortion, and matches the impedance of electrodes ensuring efficient signal transfer. High impedance also limits current drawn from the source. In reality the voltage is being measured, but any voltmeter is non-ideal, and must draw a little current to make its measurement. The circuit in figure 9 should help you understand why preamps have a high input impedance.

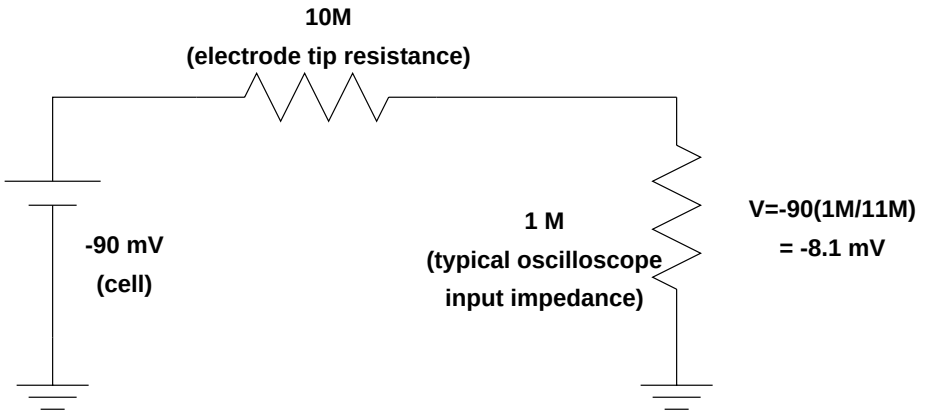

Figure 9: Electrode connected directly to the oscilloscope. Most of the signal voltage never appears across the scope (recognize the voltage divider?).

Because the voltage across the scope is obtained from the voltage divider equation, it only represents a small portion of the voltage actually appearing across the electrode. By inserting an extra impedance (a preamp) before the scope, almost all of the electrode's potential can be measured in figure 10.

Notice that in figure 10 the voltage that the electrode measures (between the two marked points) is much closer to what the voltage *really* is across the cell membrane:

$$V = V_{cell}\frac{10^8 + 10^6}{10^8 + 10^6 + 10^7} \simeq 0.91 V_{cell}$$

Some preamps just provide unity (x1) gain, but give lots of impedance and filtering capability.

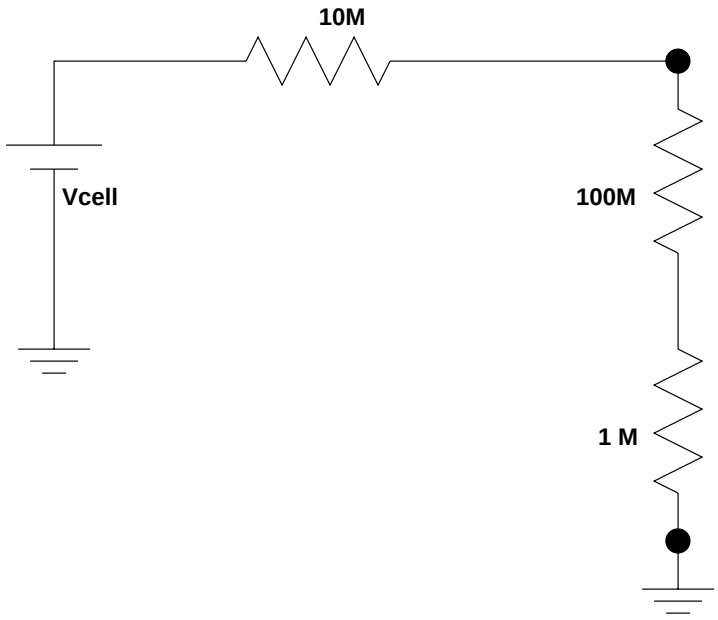

Figure 10: Electrode connected first to a preamp with a $10^8 M\Omega$ input resistance.

0.11 Johnson noise

Any resistance will generate some noise due to the thermal activity of the electrons in the resistive material, and is called Johnson noise. From freshman physics:

$$E = \sqrt{4kTR(f_h - f_l)}$$

E is in volts, k is the Boltzman constant (1.38x10-23J/ $^\circ$K), T is the absolute temperature ($^\circ$K), R is the resistance (ΩŁ), and $(fh-fl)$ is the bandwidth between the upper and lower cutoff frequencies. So noise in some form is unavoidable, and can be limited by reducing the bandwidth. For 10 MΩ at 25 $^\circ$C between 0 and 1000 Hz, Johnson noise accounts for 12.8μV of the signal. For intracellular recordings with potentials on the order of mV this isn't a big deal, but extracellular potentials are often much smaller, as are those measured when patch clamping. For good recordings its important to filter out all but the frequencies you're interested ASAP....

0.12 DC offset

Many electrodes develop DC potentials. In capillary electrodes e.g. an electrolytic junction develops between the KCl or NaCl inside the electrode and the ionic medium outside. From freshman chem, you know that this makes a little battery – the Nernst potential:

$$E = E^o - \frac{RT}{n\mathcal{F}} \ln Q$$

This potential should remain fairly stable, and can be compensated for on some amplifiers with a DC offset control. In effect this puts a second little battery before the amplification to zero the signal. It's very important especially in intracellular work to correct for DC offsets prior to entering a cell. With the electrode in the recording medium (bath), adjust the offset so that the measured potential reads zero. For ease of measurement you should also position the oscilloscope trace so that this corresponds to the ground potential. When everything's balanced, with the electrode just outside a cell the oscilloscope trace of the measured electrode potential should not move when the ground button is pressed.

0.13 Capacitance compensation

Capillary microelectrodes (the neurophysiologist's bread and butter) have an intrinsic capacitance which results in some distortion of the signal. A square wave will have its edges rounded, because the thin glass right at the electrode's tip forms a good electrolytic capacitor between the inside and outside of the electrode. Such a capacitance is parallel to the tip resistance, forming a low pass filter. This can be partially accommodated with a little good old fashioned positive feedback, or negative capacitance. The capacitance compensation provides variable gain high frequency feedback to the recording circuit, balancing the capacitance loss. Turning the knob up too far however causes high amplitude oscillations, as the positive feedback exceeds the capacitative load and becomes unstable. This will immediately kill cells, so only adjust this knob with the electrode safely in the recording medium. Sometimes this phenomenon is used to "buzz" into cells by placing the electrode (visually) on the cell with enough pressure to dimple it. A quick flick of the cap comp and back causes enough high frequency current in the electrode tip to disrupt the plasma membrane and puncture the cell. Pretty tricky.

0.14 Electrodes

Usually at least 3 electrical contacts with the prep are used: a signal electrode, an indifferent (B channel) electrode for differential recording, and a ground lead further away from the recording site to ground the prep – this provides a low resistance shunt to ground for any extraneous currents (i.e. noise) that will inevitably develop in your prep. Without a ground lead, any ambient AC E/B fields will inductively cause current to flow in anything conductive (your prep), and these currents will have no choice but to go up your electrode to get out of your dish and back to ground.

Metal microelectrodes are good for recording AC signals because they have little intrinsic capacitance. They're usually either tungsten (makes for very rigid tips) or stainless steel. Steel electrodes can be used for marking recording sites by passing enough current through them to deposit iron into the tissue (e.g. a rat brain). The Prussian blue reaction can then be used to stain this site. These electrodes have tip diameters of a few μm, and input resistance on the order of 10-20MΩ. Since all but the very tips are insulated, current can only flow into the electrode across a few microns, so although their resistivity is fairly low, a high resistance results. Metal electrodes suffer from poor DC response because they readily polarize; Current flowing through the electrode creates a shell of ions around it, impeding the flow of current between the electrode and the solution. This effect is greatest at low frequencies due to its series capacitance, causing attenuation and distortion. Polarization can be limited by treating the surface of a metal microelectrode. Silver electrodes are often coated with silver chloride, significantly reducing polarizability. This effect is not fully understood, but it is thought that the AgCl stabilizes the electrode-electrolyte interface by providing both cations and anions for exchange with the electrode and the solution. No known treatment makes metal electrodes as effective at recording DC signals as liquid filled ones.

Glass microelectrodes are commonly used for intracellular and extracellular work, and for reasons mentioned above make good DC electrodes (they don't polarize easily and shunt AC across the thin wall of the tip to ground). They are fabricated from glass capillary tubing (often 1 mm diameter), which is locally heated in the middle and slowly pulled apart producing two pieces with sharply tapered endings where they were heated. Tip diameters and resistances are approximately equivalent to those of metal microelectrodes. Intracellular electrodes are generally filled with 3M KCl because at this concentration KCl provides a low impedance (and noise), and KCl minimizes junction (Nernst) potentials since the insides of cells are loaded with K^+. Extracellular electrodes are often filled with 5M NaCl for analogous reasons. Sometimes alternative filling solutions are used in order to manipulate the cell.

An important point about these electrodes concerns the tip resistance. Several competing effects must be taken into account when fabricating and using them. A high tip resistance is desirable because of the impedance it provides, and because high tip resistances are generally directly the result of small tip diameters. Large tips look like telephone poles to the cell membrane, and will rupture the cell if you're not careful. On the other hand, large input resistances generate more Johnson noise, and make your recording much more susceptible to ambient interference.

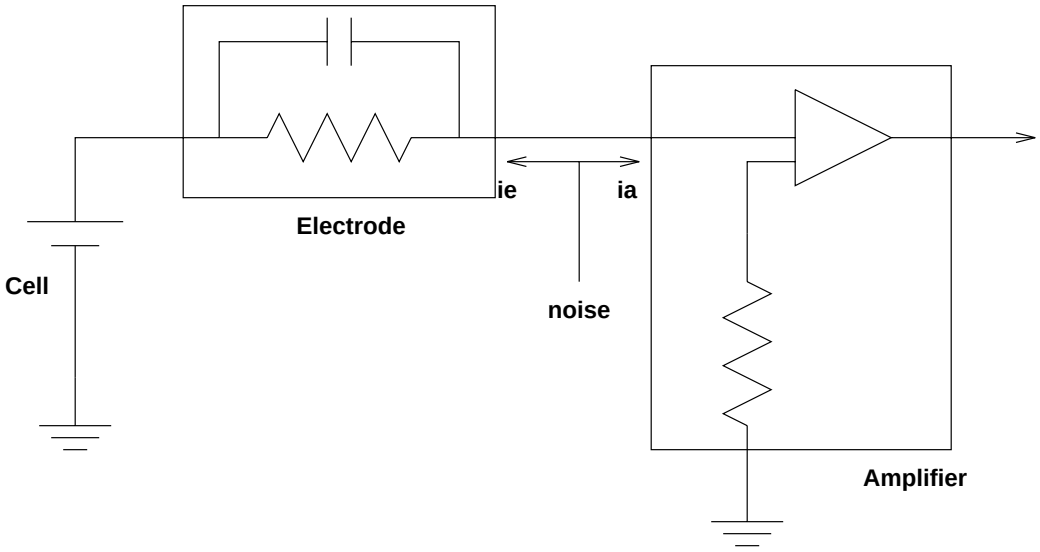

Figure 11: Noise picked up between the electrode and amplifier.

Without solving the circuit in figure 11, it should be intuitively obvious that if the tip resistance is made larger, a larger fraction of the current created by induction between the electrode and the amplifier will be forced into the amplifier. With smaller tip resistances more of this current is allowed to leak out to ground. Empirically the best electrodes tend to have resistances of 8-15 MΩŁ. Most researchers will experiment quite a bit before deciding what kind of electrode configuration they like best, and this can become black magic, but this range is a good place to start. Intracellular amps usually allow an AC current of known value to be passed through the electrode while simultaneously measuring the potential this creates at the tip. From Ohm's law you can then calculate the resistance.

filling glass electrodes is tricky at first. Try not to touch the capillary in the middle before pulling it to avoid getting your finger grease where the electrode tips will be. Once electrodes are pulled, place them in distilled water with the tip and

the first cm or so underwater. An easy way to do this is to line the edge of a small beaker with dental periphery wax, pressing the electrode shaft into it. Water should wick up the tip, and you'll avoid getting little bits of dust occluding the opening. Place a drop of your solution (e.g. 3M KCl) on the end of the electrode. Because the capillary has a fine glass rod inside, liquid will be drawn down to the tip. Do this a little at a time, and eventually the tip will be filled up to the straight shaft portion. You can now place your steel needle attached to a KCl filled syringe into the electrode to fill it. It is very important not to get any bubbles anywhere in the electrode to prevent any impedance to current flow.

Suction electrodes are a simple way to record activity in an entire nerve. They are easily fabricated from a glass capillary micropipet by breaking the tip back a little with fine forceps, initially leaving an opening of a few 100 microns. Then break the straight portion of the capillary about 2.5 cm from the tip, and use a Bunsen burner to smooth this end. Insert the large end into a length of PE (polyethylene) tubing that has a piece of silver wire inside it. We'll show you how to make these.

To use such an electrode, position it close to the cut end of a nerve, and break back the tip further to match the size of the nerve stump. Maneuver the nerve next to the tip, and provide negative pressure to the tubing with a syringe. This will suck the nerve and some of the bathing medium into the electrode. The fluid in the electrode contacts the silver wire forming the A input, and silver wire wrapped around the electrode provides the indifferent (B) input. Nerves can also be sucked up en passant without cutting them, but as you can imagine the signal recorded can be complex since at least one loop of nerve is created.

If DC potentials are to be recorded the internal Ag wire can be chlorided to correct for galvanic potentials and to stabilize slow voltage changes, but if only fast signals are of interest (eg APs), you can just record in AC mode (couple the input capacitatively), and DC shifts won't matter.

Hook electrodes are used for extremely simple recordings, such as in the cockroach ventral nerve chord. Small lengths of Ag wire are fashioned into hooks or loops and placed around the nerve. As long as an electrolyte is present, activity will be measured in each of these electrodes. Make sure that no circuit is formed between the electrodes themselves however by any extracellular electrolyte.

0.15 Extracellular recording

Usually picks up activity from several cells, and is often easier and more flexible than intracellular recording. The potentials are often smaller though, and make discrimination of individual cells difficult. The presence of multiple units in a recording does give information about interaction among neighboring neurons, and

in reasonable conditions about 10 cells can be reliably picked out of complicated records by signal analysis techniques.

0.16 Intracellular recording

Besides the fact that the electrode is inside a single cell, glass electrodes themselves are more discriminative, and are better at recording DC potentials. Metal electrodes (usually used for extracellular work) are not as well insulated. This technique provides a means of precisely measuring membrane currents and synaptic events.

0.17 Micromanipulators

These are much more expensive than they look and are very delicate. Most micromanipulators allow electrodes to be positioned to within a few microns.

0.18 Audio amplifiers

The ear is much better at discriminating temporal information than the eye, and often recorded signals are also sent to an audio amplifier. Noise provides a background of hissing interference, APs can be heard as distinct pops, 60 Hz noise forms a low hum, etc.

0.19 Selected References

- Feynman RP, Leighton RB, Sands M (1964). The Feynman Lectures on Physics Vol II. Addison-Wesley, MA.
- Geddes LA (1972). Electrodes and the Measurement of Bioelectric Events. Wiley, NY.
- Irwin, JD (1987). Basic Engineering Circuit Analysis. MacMillan, NY.
- Oakley B, Schafer R (1978). Experimental Neurobiology. U. of MI Press, Ann Arbor. (this text is the source of many of our lab procedures)
- Robinson, DA (1968). The electrical properties of metal microelectrodes. Proc. IEEE, **56**: 6, 1065-1071.
- Schanne OF, Lavalle M, Laprade R, Gagn S (1968). Electrical properties of glass microelectrodes. Proc. IEEE, **56**: 6, 1072-1082.