Introduction

Welcome to Bi/CNS 162! In the first half of this course we will focus on electrophysiology: the art and science of recording the electrical signals that living neurons use to communicate to one another. This is a nontrivial enterprise, because a typical mammalian neuron is about 20 micrometers in diameter, and its ability to drive electrical currents is correspondingly small: on the order of 100 picoamperes. Thus, if you want to record its electrical activity, you need a very sensitive recording instrument. (You obviously also need a very small electrode; we’ll come to that later.) In this class, we will use the AxoClamp-2B amplifier by Axon Instruments. This tutorial will teach you the basics of how to use this amplifier to get clean recordings from neurons, that is, recordings that reflect the unperturbed activity of the cells most accurately.

A lightning-fast overview of a neuron from an electrical perspective

A neuron can be roughly modeled as a conductive sphere with an insulating membrane around it. The membrane is very thin and therefore has an appreciable capacitance, $C_m$, of about 10 pF. The membrane is also not a perfect insulator: it has some leak conductance, $R_m$, of about 50 MΩ. Finally, the membrane is not electrically passive: it contains ion pumps that maintain a difference between the concentration of various ions on the inside versus the outside of the cell. This concentration difference causes the resting potential of the cell’s cytoplasm (its inside) to be negative relative to the extracellular medium, by about 70 mV in mammalian neurons.

There are other voltage-gated ion channels in the membrane that enable the cell to produce action potentials: brief depolarizations of the membrane potential that can propagate from the cell body along the axon, a long and thin appendage of the neuron, sometimes with multiple branches, that can project to nearby cells or all the way across a brain or even to distant parts of an animal’s body. The end of the axon is marked by an axon terminal, where arriving action potentials lead to the release of chemicals known as neurotransmitters into the extracellular space. Typically, axon terminals are located in immediate proximity to other neurons’ dendrites—shorter appendages of neurons that relay electrical signals to the cell body—and form synapses with those other neurons. At the location of the synapse, the postsynaptic neuron has a specialized structure known uncreatively as the postsynaptic density that contains ion channels that open specifically in response to the neurotransmitters released by the presynaptic neuron and that cause a small amount of current to flow into or out of the postsynaptic cell. This current ultimately leads to a depolarization or hyperpolarization of the cell body. If a neuron receives sufficiently many depolarizing inputs in a short enough time, its cell body may depolarize so much that an action potential is triggered. In this fashion, signals can propagate from cell to cell.

If the previous paragraph contained a lot of unfamiliar words, you should take a class like Bi 150 or Jerry Pine’s Ph/Bi 103, or ask me for advice on a suitable textbook. For the purpose of today’s class, however, we don’t need to worry about axons and dendrites: we simply want to understand how recording from cell bodies works.

We can visualize the cell body, or soma, as follows:

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1Don’t quite remember what a capacitor or a resistor is? The “Recap of Basic Electronics” will refresh your memory.
Besides the membrane capacitance, the figure shows two batteries and corresponding resistors, for the two main ionic contributions to the membrane resting voltage. (Note that these are not meant to represent the (voltage-dependent) channels that give rise to action potentials; instead, these two resistors together are responsible for the major part of the leak conductance mentioned earlier.)

Connecting to neurons electrically

As electrophysiologists, we want to measure the membrane voltage ($V_m$) when the cell is at rest, as well as when we inject different amounts of current into the cell. This would be easy if we could just stick an alligator clip onto the cytoplasm, but of course the cell is much too small to contemplate such a thing. Instead, we have to resort to a sticking a very tiny electrode into the cell. In practice, one uses a chlorided silver wire inside a glass micropipette filled with a strong electrolyte. This affords us a way to electrically contact the cytoplasm, albeit not quite directly:

The connection is indirect, because making an electrode small and sharp enough to penetrate a cell without doing major damage inevitably leads to a relatively large $R_{\text{elec}}$, typically at least 30 MΩ, that is, rather similar to the input resistance ($R_m$) of the cell. In addition, because the glass is so thin, there is an appreciable amount of stray capacitance between the inside of the micropipette and the bath ($C_{\text{elec}}$) which can be almost as large as the cell's capacitance ($C_m$).

In today's tutorial, you will gain an understanding of the properties of electrodes on intracellular recordings, and you will learn how to operate the AxoClamp-2B, a sophisticated piece of equipment that enables us to compensate for the effects of the electrode and obtain a nearly pure recording of the actual voltage inside a neuron. In order to decouple the challenges of learning to operate this
machine from the challenges of sticking microelectrodes into living cells (and keeping those cells alive), we will construct model cells and model electrodes on a breadboard. Next week you will learn to record from actual cells.

The experimental setup

Most of the experiments you will do in the first few weeks will be controlled through computer software. There is one piece of software for acquiring, displaying, and storing data, and another piece for providing stimulation.

Recording and displaying data: escope

For those of you who grew up with oscilloscopes, it will be comforting to know that the recording software, escope, behaves very much like a virtual oscilloscope. For all others, I hope that the user interface will prove intuitive anyway. For our first test, we will build a simple circuit of a battery and two resistors in series:

![Circuit diagram]

Suitable resistor values would be $R_1 \approx 2 \text{ M}\Omega$; $R_2 \approx 1 \text{ M}\Omega$.

As you may recall\(^2\), the voltage at point $B$ should be

$$V_B = V_A \frac{R_1}{R_1 + R_2}. \quad (1)$$

**Exercise 1** — Build the circuit, and connect points $A$ and $B$ to channels 0 and 1 of your BNC-2090 data acquisition board. It is essential to connect the ground point too. (Why?) Make a drawing of your circuit with actual resistor values in your lab journal. Start escope set it running, and check that relation (1) holds. (Write down the expected (calculated) values for $V_A$ and $V_B$ as well as the measured values in your journal.) You may find it instructive to pull out the connection to either or both points $A$ and $B$ and see the traces on the screen jump.

The BNC-2090 data acquisition panel. The arrows in the schematic on the right point out several important features: The analog input channels (ACH) 0 and 1, the analog output channel DAC0OUT (which we will use later), and the “ground mode” switches, which all need to be in the “up” position.

\(^2\)If not, ask me to prove it to you based on the equations in the Electronics Recap.
The *escope* data acquisition program. The two lines represent live data from two recording channels. The “ball” handles on the left indicate the zero-volt level for each channel. You can drag them around. To change the vertical scale of any channel, drag the scale bars (on the right). The time scale can be changed by dragging the bottom scale bar. To select which channels to display, click on the “Channels” button.

**Stimulating with *espark***

To make the circuit slightly more interesting, let’s replace the battery by a variable voltage source. The BNC-2090 provides two convenient sources: DAC0OUT and DAC1OUT.

**Exercise 2** — Remove the battery from the circuit, and connect DAC0OUT to point A:

![Circuit Diagram]

Start *espark*, and program in some pulses of your choice. While *escope* is also running, click “Run” on *espark* and watch the *escope* screen to see if you can see the stimuli. (If they flew by too fast, set *escope* to “Trigger mode.”) Draw the results in your journal. Does relation (1) still hold?

The *espark* pulse generation program. The program can generate synchronized pulse trains on two separate outputs, but for now, we only use one output (labeled “A”, corresponding, by default, to DAC0OUT). All aspects of the pulse (train) can be changed, and the little graphs give you an indication of what output to expect.
As a last non-biological example, let’s try a simple RC circuit:

![Diagram of RC circuit]

**Exercise 3** — Replace $R_1$ with a capacitor ($10 \text{ nF}$ would be a fine value), and apply the same pulses as before. Draw the circuit and the voltage traces at points $A$ and $B$ in your journal. You may remember that following a voltage step, a capacitor in an RC circuit like this slowly charges up. Do you see that happening here? The time it takes for the capacitor to charge up to $1 - \frac{1}{e} = 0.63$ times its final value is known as the “time constant” of the circuit, and should equal $R \times C$. Write down the calculated and measured time constants in your journal. Do they match?

**Injecting current with the AxoClamp**

Before we start on building up our model of a neuron, we need to learn one more thing: how to use the AxoClamp as a current source. (We will want to inject known quantities of current into our model cells, and, as yet, we have only learned how to apply a known voltage, which is not the same. Do you see why?)

**Exercise 4** — Build the following RC circuit on your breadboard:

![Diagram of RC circuit]

where $R_2$ is $1 \text{ M}\Omega$ and $C_1$ is $10 \text{ nF}$ as before.

We will use the AxoClamp to inject current into this circuit. The AxoClamp has two “head stages” that can be used for this purpose, and they are not equivalent. In particular, one is capable of driving 10x more current than the other, which is quite useful for our purposes today. Find the head stage that is labeled “1x” and not “0.1x,” and connect its front end (the center hole within the white sleeve) to point $A$ in the circuit. Also connect the back end of the head stage to ground:

![Diagram of AxoClamp connection]

Also hook up point $A$ to an analog input (e.g., $ACH0$) so you can measure its voltage directly with $escope$. At all times, leave the “Mode” of the amplifier set to “Bridge”. (“DCC” mode is not “DC Current mode.” I will be happy to talk to you about “DCC” mode later, or you can read about it in the AxoClamp user guide.)
The AxoClamp-2B. Several features relevant for current injection are indicated: the “DC Current Command” for electrode #1 (arrows on bottom right), the “Step Command” (circle on left), and the “I Display Select” (arrow on top). Note that there is also a “DC Current Command” dial for electrode #2. Check your wiring to figure out whether the head stage you are using is #1 or #2.

There are multiple ways to make the AxoClamp output a current. The easiest is to use the “DC Current Command” dial and switch. That allows you to directly dial in an approximate current.

**Exc. 4a** — Try it. Noting that $V = I \times R$, use the voltage that you measure at point A to figure out what the numbers on the DC Current Command dial mean (or look it up in the Quick Guide). Draw what you see and write down whatever sentences you think you will need to remember the multiplication factors.

Slightly more advanced is to use the “Step Command” manually, which allows you to inject a more precisely defined amount of current.

**Exc. 4b** — Try it. Note that you need to set the “Step Command” destination before doing anything else. Again, use Ohm’s law to figure out what the numbers on the AxoClamp’s front panel mean. (Answer: for ME2, i.e., the 1x headstage, “+050.0” means a positive current of 50 nA.) Write down in your journal exactly what you did and what the results were. A drawing of pulses is always helpful.

A much fancier way to inject current is to use espark to provide a timing signal to the AxoClamp. To do that, you connect DAC0OUT on the BNC-2090 to the connector labeled “Step Activate” on the back of the AxoClamp, and program espark to deliver 5 V pulses. Whenever the “Step Activate” input is driven high in this manner, the current selected on the front of the AxoClamp is delivered to the head stage.

**Exc. 4c** — Try it. Write down what you told espark to do, what you set “Step Command” to, and what the results were. (Include a sketch of the pulse waveforms.)

The most fancy way to inject current is to have espark directly program the current waveform. To do that, you would connect DAC0OUT to either the “Ext ME1 Command” or “Ext ME2 Command” inputs on the back. But we don’t need to do that today.

**Modeling the cell membrane**

In the next several exercises, you will build up on your breadboard the model of the cell membrane introduced in the beginning of this tutorial. Let’s begin with the “ion channels,” represented by batteries and resistors, and worry about the membrane capacitance later.

**Exercise 5** — Build the following circuit on your breadboard:
where $R_1$ is about 50 kΩ and $R_2$ is a variable resistor or “potentiometer.” Note that the two batteries are placed with opposite polarity.

Point $A$ represents the inside of the cell, so that $V_A$ will be $V_m$. Can you predict, a priori, what value $R_2$ should take so that the voltage at point $A$ is zero? (Write down your prediction in your journal.) Now, turn $R_2$ such that the voltage at point $A$ is a realistic value for $V_m$. (How are you measuring that voltage? Write it down in your journal.\(^3\))

To turn this into a realistic model of the cell membrane, all we need to do is to add the membrane capacitance back in:

where $C$ is 10 nF.

For our first pseudobiological example, we will use the AxoClamp to inject current into this circuit, in a manner analogous to our previous RC circuit example:

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**Exercise 6** — Build this circuit and draw it in your journal. Try injecting a pulse of 50 to 100 nanoamperes into the circuit and measure the voltage at point $A$ by connecting it to ACH0.

**Exc. 6a** — Calculate the effective membrane resistance (again using Ohm’s law). Also calculate the parallel combination of $R_1$ and $R_2$. Do the two calculations yield the same number? They should, because batteries have no internal resistance.

**Exc. 6b** — At the onset and offset of the pulse, you can see the capacitor gradually charging up. Does the time constant make sense? (Remember, it should equal $R \times C$.)

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\(^3\)As these exercises progress, I will be less and less specific about what you should write or draw in your journal. Regardless, you should keep taking detailed notes, as always.
**Modeling a cell and an electrode**

If we could record from actual cells in the way we recorded from the circuit in the previous example, life would be good. Unfortunately, we cannot ignore the electrical properties of the microelectrodes needed to record from living cells. In the next exercise, we will study a more or less realistic model of an electrode stuck into a cell.

**Exercise 7** — Based on the two drawings on page 2, can you add a model of an electrode to the circuit diagram above? (Don’t do it on the breadboard, just make a sketch in your journal. My solution is on the next page so don’t look yet.)
Did you draw something like

? Building that whole circuit up on our little breadboard would involve quite a tangle of wires, so I propose we simplify it to:

This circuit behaves exactly like the previous one, except that the resting potential of the model cell is now 0 V.

**Ex. 7a** — Build this circuit. Start with $1 \text{ M}\Omega$ for $R_m$ and $R_{elc}$, $10 \text{ nF}$ for $C_m$, and $470 \text{ pF}$ for $C_{elc}$. These are not realistic values, but they do result in more or less realistic time constants, and will allow for some interesting measurements. Connect points $A$ and $B$ to the analog inputs of your BNC-2090. Use the AxoClamp to inject $50 \text{ nA}$ of current into the “electrode.” Is the voltage response of the cell (as measured at point $A$) affected by the presence of the complex electrode? (Consider both the steady-state result and the capacitive transient.)

It is worth repeating that in an actual biological experiment, we have no direct access to point $A$; only to point $B$. If you compare, on the *scope screen, the voltage trace measured at point $B$ to the trace measured at point $A$, you will notice two differences: the most obvious difference is that point $B$ experiences a larger steady-state voltage deflection than point $A$, as a result of the series resistance of the electrode ($R_{elc}$). A more subtle difference, which may be hard to see, is due to the fact that an electrode has a non-negligible capacitance between its inside and its outside. In our circuit, this is modeled by $C_{elc}$, and it affects the shape of the initial transient at point $B$. Fortunately, the AxoClamp contains circuitry to overcome both of these problems. As the final exercise before we advance to actual living cells next week, we will learn how to operate this circuitry.
Recording voltages with the AxoClamp

Thus far, we have only used the AxoClamp as an expensive current source. Now, we will put it to some real use. The AxoClamp contains two entirely independent modules for amplifying voltages from the two headstages. It also sports a dazzling array of knobs and dials on its front panel. I created a “Brief Guide to the Front Panel of the AxoClamp” to help you see through the thicket. Please read it before continuing.

Exercise 8 — Connect a cable between the appropriate voltage output of the amplifier (the BNC labeled V2 on the top right of the front panel) and a third input of the BNC2090, and enable the corresponding trace in escope. When you inject a current (as before), does the V2 trace match the recording from point A? Point B?

Compensating for the electrode’s properties

Probably, the answer was: neither. We will now use the three knobs “Output Offset,” “Bridge,” and “Capacitance Neutralization” to ameliorate the situation.

First off: Real electrodes feature an electrode–electrolyte interface that can act like a small battery. Therefore, reading absolute voltage values is not trivial: any measurement you take may be corrupted by a (hopefully constant) offset. The “Output Offset” button allows you to compensate for it.

Exc. 8a — Try turning it left or right until (in the absence of stimuli) the voltage from V2 matches the voltage at point A and B, which should be zero. Make sure the “Capacitance neutralization” and “Bridge” knobs of the AxoClamp are all the way off (turned counter-clockwise). Use either the “DC Current Command” or the “Step Command” to inject a current pulse into your model. If all is well, the output from V2 should match exactly the direct measurement from point B. Check that it does.

What we want, is for the output of the AxoClamp (i.e., V2) to match the voltage at point A instead. There are two knobs on the AxoClamp to approximate that goal. The first is the “Bridge” dial. By turning that up, you can compensate for the electrode’s series resistance.

Exc. 8b — Try it. Can you set the dial such that the steady-state output of the AxoClamp matches the steady-state voltage at point A? Draw the resulting waveforms in your journal. Does the number on the dial match the actual resistor value that you used for $R_{elc}$?

If you successfully got the steady-state output to match, you can probably see the transient due to the electrode capacitance more clearly. The AxoClamp contains specialized circuitry to get rid of that.

Exc. 8c — Try it, by turning up the “Capacitance Neutralization” knob. Draw the resulting waveforms in your journal.

Probably, turning the “Capacitance Neutralization” knob didn’t have much of an effect. That is because the value we used for $C_{elc}$ is unrealistically large. To see the effect of capacitance neutralization, we need to use more realistic component values. Reasonable values for the components are: $R_{elc}$: 30 MΩ; $C_{elc}$: 10 pF; $R_m$: 30 MΩ, and $C_m$: 470 nF. Unfortunately, with such large resistors, we will not be able to use the BNC-2090 for a direct measurement from points A or B. (Its internal circuitry would too strongly influence the delicate circuit on your breadboard.)

So, we will only use the AxoClamp. Unfortunately, that means that we will not be able to directly compare the “real” voltage at points A and B with the measured value for V2. How then can we know that we are setting the knobs to the appropriate position? We will find out in the next series of exercises.
A realistic model electrode

Let’s start out with only a model electrode on our bread board, and no cell, because then we know what we want to see at $V_2$: absolutely nothing, even when we stimulate. This situation is captured by the following circuit:

![Circuit Diagram]

where $R_{elc}$ is 30 MΩ and $C_{elc}$ is 10 pF. This corresponds to the situation when a real electrode is dipped into saline, but not stuck into a cell.

Exercise 9 — Build the circuit. Then, first turn the “Bridge” and “Capacitance Neutralization” knobs all the way off. After, tune the “Output Offset” until the LED display and escope indicate zero volts for $V_2$ in the absence of stimuli. Now, get the stimuli going again.

Exc. 9a — Can you balance the bridge? Try doing that by looking only at the escope screen. Once you are satisfied, look at the “Bridge” knob, and see if the numbers make sense. (They should match $R_{elc}$.)

Exc. 9b — Next, use escope to zoom in on the electrode transient, and gradually turn up the “Capacitance Neutralization” knob. Do you see the transient getting shorter in duration? Can you tune it all the way away? If not, turn the knob all the way back down, and estimate, from the screen, the time constant of the transient. Remembering that $\tau = RC$, does the time constant make sense?

Probably, your time constant is larger than what you would expect from the component values in your circuit. That is because there is a fair amount of “stray capacitance” in circuits like this. If you pull the $C_{elc}$ capacitor out altogether, you should see the time constant get shorter (by $\Delta \tau = R_{elc} C_{elc}$), but not go away. What remains is due to the stray capacitance, which is typically around 10 pF. (Calculate the stray capacitance in your circuit, and see if it similar.)

Exc. 9c — If you couldn’t compensate for the full capacitance earlier, try turning up the “Capacitance Neutralization” now, to see if you can compensate for the stray capacitance. You probably can. What happens when you overcompensate?

This “ringing” can easily kill a cell if you make it happen when your electrode is inside one, but you can also use it to your advantage: briefly ringing often helps an electrode get into a cell. In fact, that is just what the “Buzz” button does: it temporarily increases the capacitance neutralization beyond its stable range.

Electrode traces in response to a 100-ms long current pulse of 1 nA, when the electrode is not in any cell.
A realistic model cell

Finally, let’s return to our full electrode-plus-cell model:

but be sure to turn the “Bridge” and “Capacitance Neutralization” knobs all the way down before you change anything on the breadboard.

**Exercise 10** — Build this circuit using the component values suggested earlier, and if you previously had to pull \( C_{elc} \) out to achieve capacitance neutralization, leave it out. The same stray capacitance will still be there.

**Exc. 10a** — Can you balance the bridge? How do you know which part of the signal is due to the electrode and which is due to the cell? This can sometimes be a bit of an art, but in favorable circumstances, the time constant of the electrode is much shorter than the time constant of the cell. You can use that to your advantage, by turning the “Bridge” balance up until the fast transient is just barely compensated for, but the slow part is still left in place. As before, see if the numbers make sense.

**Exc. 10b** — Can you compensate for the capacitance?

Electrode traces when the electrode is inside a neuron.

**Conclusion**

Congratulations! You have successfully recorded from a realistic model neuron. You are now ready to do the real thing. Next week, we’ll have leech ganglia ready for you.