

**SUPPLEMENTAL MATERIAL AND METHODS FOR
EXTRACELLULAR RECORDINGS FROM THE COCKROACH FOR
NEUROPHYSIOLOGICAL INSTRUCTION AND DEMONSTRATION**

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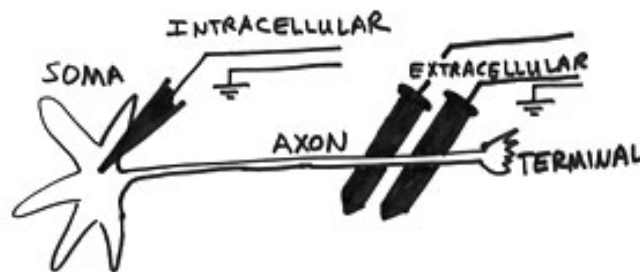
ACTION POTENTIALS & EXTRACELLULAR RECORDING IN THE COCKROACH

Neurons generate *action potentials* to transmit information over long distances. The *all-or-none* characteristic of the action potential is fundamental to way that neurons distribute information throughout the nervous system. Additionally, the all-or-none nature of the action potential can provide us with a powerful tool to characterize extracellular nerve recordings.

Intracellular vs. Extracellular Neural recordings

The electrical activity of a neuron can be recorded several different ways. Two common techniques are referred to as *intracellular* and *extracellular* recording. *Intracellular* recordings rely on a microelectrode (typically an ultra sharp glass pipette filled with an electrically conductive solution) that is inserted inside a single neuron. This intracellular electrode can record the neuron's resting membrane potential and the action potential. Intracellular recordings are difficult to carry out in the student lab so we will use *extracellular* recording techniques.

Extracellular recordings rely on electrodes that are placed close to, or in contact with, the outside of nerve bundles. These electrodes will be sensitive to the flow of current that occurs around neurons when they generate action potentials.



When recorded using intracellular electrodes, the resting potential of a neuron is in the vicinity of -70 mV and the maximum action potential amplitude approximates the sodium

reversal potential (approximately +40 mV). However, when recorded extracellularly, the resting potential cannot be recorded but action potentials can be recorded. Action potentials recorded extracellularly will typically be less than 1000 microvolts (μV) unlike intracellular action potentials which span nearly 100 mV.

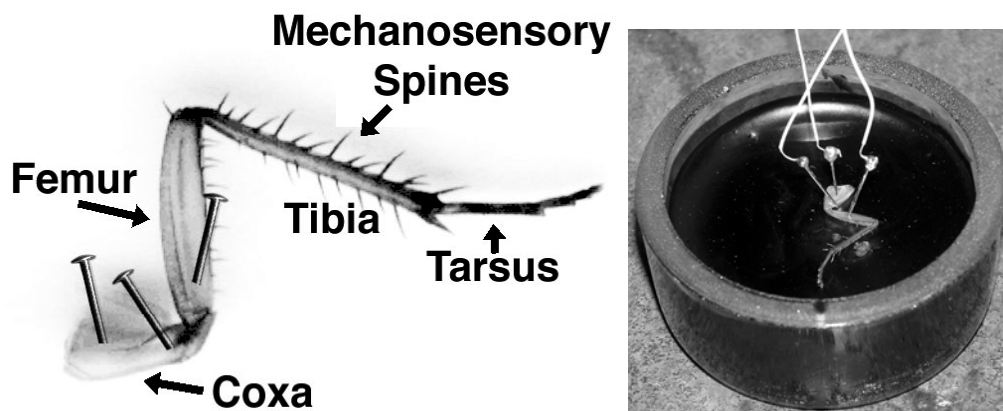
EXERCISE 1: Extracellular recording of spontaneous neural activity in the cockroach leg.

The objective of this lab is to record spontaneous neural activity from the leg nerve of a cockroach using extracellular pin-electrodes. Action potentials will be recorded, digitized and stored in the computer for later analysis. You will measure the amplitudes of action potentials and display your results as a histogram showing the frequency of each action potential amplitude.

Before beginning construct pin electrodes as described in the Addendum. In addition, familiarize yourself with the operation of the amplifier, oscilloscope, and audio monitor.

General Methods:

- Anesthetize a cockroach by placing it in the refrigerator until it is immobile. This typically takes 3 – 5 minutes.
- Remove a meso- or metathoracic leg by cutting the leg as closely as possible to the body ensuring that you leave the coxa, femur, tibia, and tarsus intact . (*Meso* position corresponds to the middle leg; *Meta* position corresponds to the large rear leg.)
- Place three pin-electrodes into the isolated leg as shown below - 2 pins in the coxa and one in the femur.



- Viewing this picture from left to right, the two electrodes placed in the coxa will be used as ground and indifferent electrodes. One of these should be connected to the ground connector of the amplifier and the other should be in the indifferent (-) connector of the amplifier. The electrode placed in the femur will be the active recording electrode (+). This pin should be connected to the active input found on the amplifier.
- Once electrodes are in place and the amplifier is turned on and in REC mode, spontaneous action potentials should be evident as fast deflections on the oscilloscope. You should also be hearing ‘pops and crackles’ coming from the audio monitors.
- Change the sweep speed on the oscilloscope in order to look at the action potentials more closely and pay special attention to their sizes and shapes.
- Record 2 minutes of spontaneous activity.

Reviewing the data from this exercise

Can you identify the waveforms that correspond to extracellular action potentials? What criteria can you use to distinguish action potentials from ‘background’ activity?

What are the characteristics of the shape of the extracellular action potential (EAP)? What range of EAP durations do you see?

EAP amplitudes should be measured as peak-to-peak voltages. Measure the maximum and minimum voltages of a given EAP. The peak-to-peak voltage is equal to the $V_{max} - V_{min}$.

Measure the peak amplitudes of the action potentials in your data set. Plot a frequency histogram of the action potential peak-peak amplitudes.

Creating a frequency histogram of action potential amplitudes in EXCEL

Copy each action potential amplitude value into a separate cell in a single column (e.g. Column A). Sort your data using DATA → SORT so that your amplitude values are in ascending order. Identify the smallest and largest action potential amplitudes in your data set. Subtracting the smallest value from the largest one will give you the range of amplitudes in your data set. Divide the range of amplitude values by ten. We will use this number/interval in creating our frequency histogram. Thus if the smallest amplitude value observed was $125\mu\text{V}$ and the largest $550\mu\text{V}$, the range will be $325\mu\text{V}$ and the interval/bin size we will use for our histogram will be 32.5 .

In the first cell of a second column (e.g. Column B) place the smallest action potential amplitude value found in your data set ($125\mu\text{V}$, in our example). In each subsequent cell, add the interval value you determined by dividing the range by ten to the value in the previous cell (32.5 in our example). Thus in cell 2, you will have the product of the lowest amplitude value and the interval value ($157.5 = 125 + 32.5$). Cell 3 will have the product of the value in cell 2 and the interval value ($190 = 157.5 + 32.5$). Once you reach the cell 10 you should notice that you have the value of the largest action potential amplitude in your data set.

You are now ready to construct a frequency histogram of you data. The values in Column B will create a histogram with ten evenly spaced bins ($32.5\mu\text{V}$) which span the range of action potential amplitudes recorded. Create your histogram by going to TOOLS → DATA ANALYSIS → HISTOGRAM. The INPUT RANGE includes the action potential values in Column A. The BIN RANGE includes the values in Column B. Select a location to display your OUTPUT RANGE results (e.g. Column C, cell 1) and select CHART OUTPUT to graphically display your results.

Interpreting your frequency histogram

The concept of ‘all-or-none’ is a useful way to think of the action potential amplitude for any individual neuron keeping in mind that all neurons are not equivalent. For example, different axons may have different diameters. When an action potential is recorded *extracellularly*, large diameter axons produce higher amplitude extracellular action potentials than small diameter

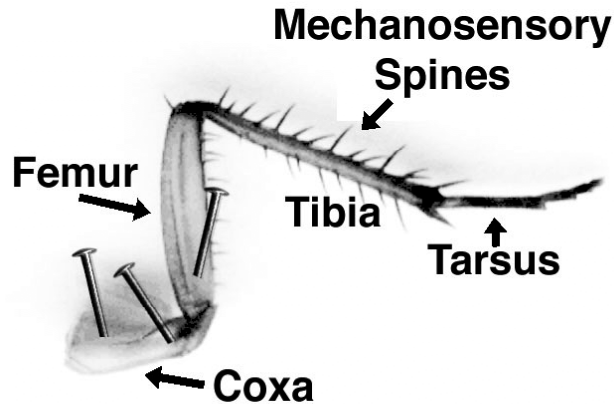
axons. Thus, if we record from a bundle of nerves containing axons of different diameters, we *may* record action potentials of different amplitudes. In general, we will assume that *action potentials of different amplitudes come from different axons* (having different diameters). This provides a powerful tool for identifying individual nerve axons.

Look at your frequency histogram and characterize the shape of the distribution of amplitudes. Is there one very broad peak distributed over many bins? Are there multiple peaks?

Multiple peaks in the frequency histogram are indicative that the recorded data set contains action potentials from more than one spontaneously-active axon. Note that more than one axon may have action potentials with similar amplitudes in which case individual peaks in the frequency histogram give the false impression that only one axon has been recorded.

Make a new frequency histogram with 25 and 50 intervals (i.e. divide the range of amplitudes by 25 or 50). How does this change the shape of the distribution? How does this affect the number of axons that you think you recorded from?

EXERCISE 2: Extracellular recording of evoked neural activity in the cockroach leg.



The mechanosensory spines (hairs) on the cockroach leg are sensitive to touch. At the base of each hair is a flexible socket. Movement at the socket can cause distortion of sensory receptors at the base of the hairs. This, in turn, generates action potentials in axons located in the leg.

How do we 'stimulate activity' in a neuron? In this exercise we will record *spontaneous* or *evoked* neural activity from isolated legs. Spontaneous activity is non-specific activity that is produced without intentional stimulation. Evoked activity is produced in response to specific, intentional stimulation. For example, when you bend/flex the leg you evoke neural activity similar to the activity changes that signal changes in leg position while the cockroach is walking/running. When you press on a leg hair/spine (this is the *stimulus*) you also evoke neural activity. The evoked response may co-exist with spontaneous activity. The spontaneous activity may come from the same cell as the response, different cells, or both. You must be able to identify which action potentials were evoked by the stimulus (and therefore represent the response) and which action potentials are unrelated to the stimulus (i.e., are spontaneous activity).

Applying these concepts to exercise #2 we can develop an experimental plan. Basically, you need to record the extracellular response to stimulation of **2 different** identified sensory hairs. **Quantify** the action potential amplitude(s) associated with stimulation of each hair. (Does the response to an individual hair contain action potentials of a single amplitude?) **Compare** the responses from the 2 different hairs. (Do they produce action potentials of the same, or different, amplitudes?) Finally, you must **interpret** your results.

General methods

- Place 3 pin electrodes into the cockroach leg as described for exercise #1.
- With the pin electrodes positioned appropriately, and the leg carefully positioned and stabilized, you should observe *spontaneous* activity (neural activity without any obvious stimulus.) If there is too much spontaneous activities try repositioning the leg. Hint: Try to avoid pressing the leg against the dissecting dish. Position pins around the femur and tibia, not touching sensory hairs, to immobilize and stabilize the leg.
- Look at the leg through the dissecting microscope. Use a probe like a glass Pasteur pipette to carefully wiggle *individual* spines, one at a time. As you wiggle some spines, you will hear action potentials on the audio monitor. Simultaneously, you will be able to visualize the action potentials on the oscilloscope. Once you have confirmed that you can stimulate individual sensory spines and record identifiable action potentials in response to stimulation, you may begin the experiment.
- Record 20 seconds of spontaneous activity.
- Record for another 20 seconds while stimulating an individual spine repetitively.
- Record for another 20 seconds while stimulating another individual spine repetitively.
- Repeat with two more additional spines.

Data Analysis:

- Measure the number and amplitude of action potentials for each trails including unstimulated/spontaneous. Create a frequency histogram of these results.
- Compare the number of action potentials you recorded for each trial. Did the trials with stimulation always produce more neural activity than that observed during spontaneous recordings? Were different stimulation trials characterized by different numbers of action potentials?
- Compare the frequency histograms between stimulated and spontaneous recordings. Are there more or less peaks in stimulated trails? How are the shapes of these distributions different? Do different spines recruit activity from different axons?

EXERCISE #3 – Coding of Peripheral Stimuli by Action Potentials in the Cockroach Leg (Receptive Fields)

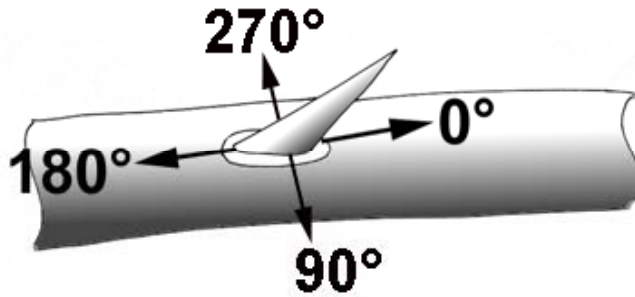
In the previous exercise you recorded action potentials from individually stimulated mechanosensory hairs and on a cockroach leg. This week you will concentrate on the detailed response properties of individual hairs.

What capabilities do these hairs have to encode information about the environment? We can make a few assumptions and see where they take us. First, most sensory systems have the ability to encode the *intensity* of a stimulus. Can intensity information be encoded in a *single axon*? We already know that the action potential for a single axon is *all-or-none*; it is always the same amplitude. If the amplitude cannot be changed, how do we indicate increasing stimulus intensity? One obvious parameter is *action potential frequency*.

If you looked closely at the hairs, you may have noted that they seem to be oriented in specific directions. Is the orientation of a specific hair significant? Maybe the orientation of a hair allows it to be more selective to stimuli from a certain direction.

General methods

- Use the same electrode and electronics setup as the previous exercise. Try to position the leg so that you have easy access to the mechanosensory spines and can easily stimulate them. You will want to use the dissecting microscope to be certain you are stimulating an individual hair.
- Sketch the cockroach leg showing the positions of the mechanosensory spines. Notice that the spines form an angle with the surface of the leg. Use an arrow to indicate the direction that each spine is 'pointing' as shown below. This direction will be defined as 0°.



- Use a ruler or other object of known size to establish a **size scale** for the sketch. Your sketch should reflect obvious differences in the sizes and shapes of spines that you see. You will use this sketch to record the position of spines that you stimulate, below.

When we use the term *direction* in this exercise, we are referring to the orientation as if we were reading a magnetic compass. Now that we have defined the 0° direction, we can use the compass-like directions of 0-360° to describe the direction in which we are pushing the hair. This is different from the *magnitude* or *strength* of the applied stimulus. Make sure you do not confuse *direction* or *orientation* with the *magnitude* or *strength*, which can also be measured as an angle (i.e., the number of degrees you deflected the hair).

Simple Characterization of Stimulus/Response

Identify a mechanosensory spine that elicits well-defined action potentials when stimulated and *identify it on your sketch*. Press the spine down (0° direction) a small distance with a glass Pasteur pipette or other non-conductive probe. **Use the dissection microscope! You need to be able to repeat this movement accurately.**

In response to deflection in the 0° direction:

- Estimate how far you are moving the spine. (You can estimate this as either an angle or the distance the tip moves.)

- Identify a specific action potential (by its amplitude) that is stimulated by the specific spine. Confine your analysis to action potentials of this amplitude.

- What is the action potential frequency generated during the stimulus?¹ Determine the relationship between stimulus magnitude (i.e., how far you deflect the hair) and action potential frequency?

Make the same measurements in response to a deflection of the same mechanosensory spine in the 180° direction.

- Does deflection in the opposite direction elicit activity in the same axon? (Perform the appropriate statistical test to determine whether this stimulus is stimulating action potentials of the same amplitude as the 0° direction)

- Is the sensory structure equally sensitive to 180° stimulation as it is to 0° stimulation? (Compare the number of action potentials resulting from similar magnitude deflections in the tested directions.)

Repeat these measurements for deflections in the 90° and 270° directions.

Some added complexity

Tonic or Phasic Coding

A tonic neuron responds to a sustained (continuous) stimulus with a constant frequency of action potentials.

¹ There are two basic ways to measure the firing frequency for an action potential. One way is to count the number of action potentials generated during a particular time interval, typically the duration of the stimulus. This would result in a frequency with units of action potentials per second. For example, if you recorded 5 action potentials over an duration of 50 msec, the frequency would be 100 action potentials/sec.

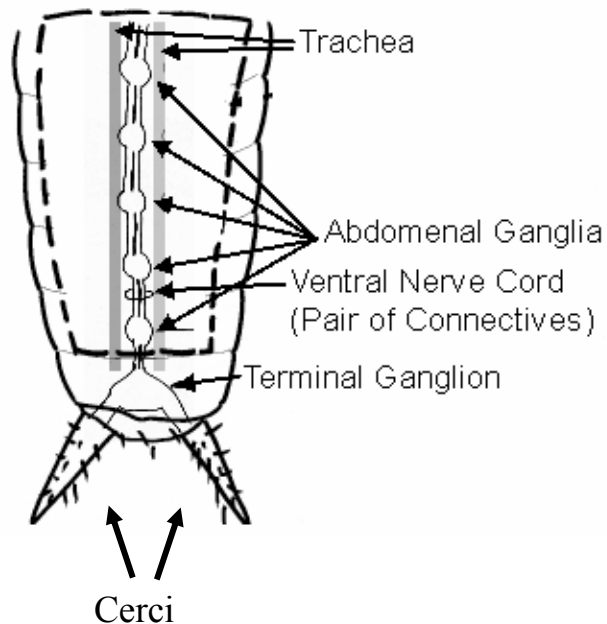
A *phasic* neuron responds to a sustained (continuous) stimulus with a short burst of action potentials. The burst does not continue for the entire duration of the stimulus.

Habituation

As a stimulus is repeated, a neuron's response may decrease or cease completely. By waiting a longer time before restimulating you can usually regain the original response. You may also be able to regain the response by stimulating other receptors before restimulating.

Review your data and determine whether the response you measured was phasic or tonic, and whether it habituated.

EXERCISE #4 – Recording and Stimulation of Ventral Nerve Cord



Cerci are complex sensory organs found on the 'tail end' of many insects. Sensory information from the cerci is *integrated* in the *terminal ganglion* and passed to other areas of the nervous system by *interneurons*. The cockroach's nervous system runs along its *ventral* side and is known as the *ventral nerve cord*. The ventral nerve cord is composed of *connectives* that contain a multitude of nerve axons, and *ganglia* which contain cell bodies, dendrites, terminals and axons.

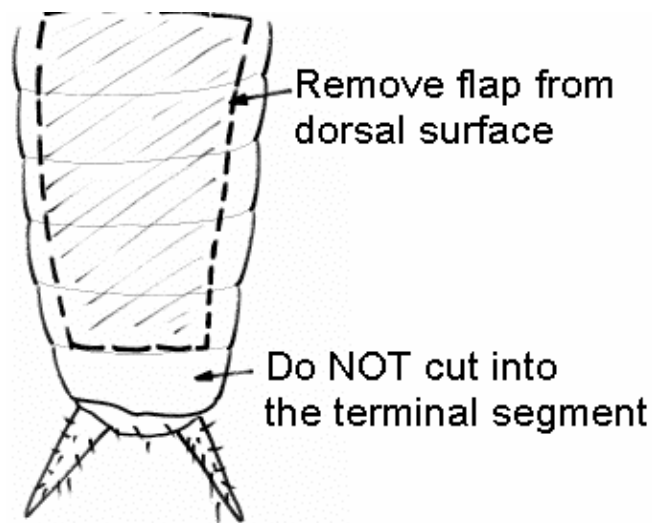
If you look at the cerci through the dissecting microscope you will notice that each cercus (which is the singular of cerci) is covered with fine hairs. Close inspection of these hairs will reveal many specializations in size, shape and spatial distribution. If you compare the size and morphology of the cercal hairs to the mechanosensory spines on the leg (that you have been working with during the previous labs) you'll see that the cercal hairs are a lot finer. Blow a puff of air through your lips as you look at the cerci through the microscope. You should see some of the hairs bend as the pulse of air passes over them. The bending hairs stimulate receptors and send information towards the head along the ventral nerve cord.

When you set up this exercise you will see that the cerci are extremely sensitive to wind as measured by extracellular activity recorded from the ventral nerve cord.

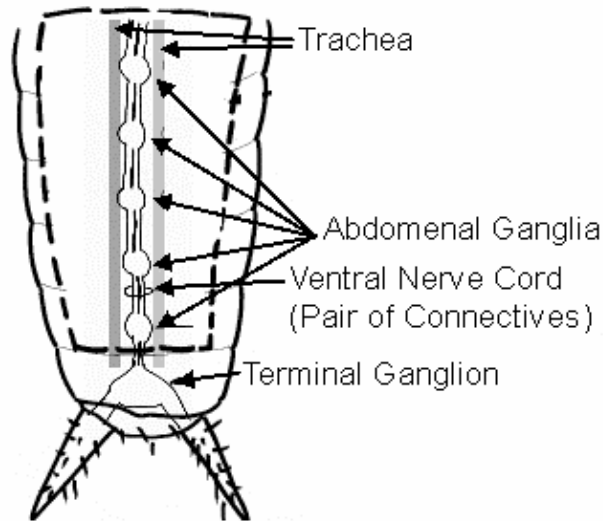
General methods

Preparing to Record from the Ventral nerve Cord

- You will record from the *ventral nerve cord* using the same pin electrodes used for recording from the leg. After anesthetizing the cockroach you need to remove its head, wings and legs. Place the body ventral side down on the dissecting tray and pin the thorax to the tray (pins should be placed along laterally and away from the nerve cord).
- Place **two pins at the anterior portion** of the abdomen and **two pins as far posterior** as possible *before* you begin your dissection.
- Using scissors, make two shallow cuts in the exoskeleton from the rear of the abdomen to the most anterior abdominal segment. Make two transverse cuts with the scissors and remove the resulting rectangular flap from the dorsal surface of the abdomen.



Grab the anterior-most section of the gut with forceps and cut it using the scissors. Use the forceps to lift the gut and other internal organs and cut them as far posterior as possible. As you lift the gut you should be able to see the ventral nerve cord (VNC) on the ventral surface of the body. The VNC will be on the ventral *midline*, flanked by two shiny white tube-like structures. (The tubes are trachea – the breathing apparatus of insects.) The nerve cord itself is translucent and more difficult to see than the trachea.



After the internal organs are removed you need to place your recording electrodes into or touching the ventral nerve cord. The single ground electrode can be placed in the abdomen or thorax, at any convenient location.

You must periodically moisten the nerve cord so that it does not dry out. However, you *must not have fluid pooling around the electrodes.* If there is too much saline or fluid around the electrodes, the fluid will short-circuit the electrodes and you will be unable to record neural activity. Excess fluid can be removed from the body cavity using a pipette and/or a small piece of paper towel rolled up into a small cylinder. Cerci must be kept dry.

Spontaneous action potentials are evident in almost all recordings. If you don't see or hear spikes, make sure all equipment is turned on and all connections are made correctly. Inspect

your VNC preparation under the dissection microscope and make sure the VNC remains intact and was not severed or damaged during the course of dissection. Try snapping your fingers close to the preparation or blowing gently on the preparation, as these are two stimuli that reliably elicit vigorous activity. If you see and hear spikes following these two stimuli then you have an unusually 'quiet' but still useable prep.

Spontaneous action potentials in the VNC

- Record 2 minutes of spontaneous activity.

Can you identify action potentials in your data? What criteria can you use to distinguish action potentials from 'background' activity? How are these spikes different from those we recorded from the leg?

What are the characteristics of the shape of the extracellular action potential (EAP)? What range of EAP durations do you see?

EAP amplitudes should be measured as peak-to-peak voltages. Measure the maximum and minimum voltages of a given EAP. The peak-to-peak voltage is equal to the $V_{max} - V_{min}$.

Measure the peak amplitudes of the action potentials in your data set. Plot a frequency histogram of the action potential peak-peak amplitudes.

Stimulation of the cerci with manual air puffs

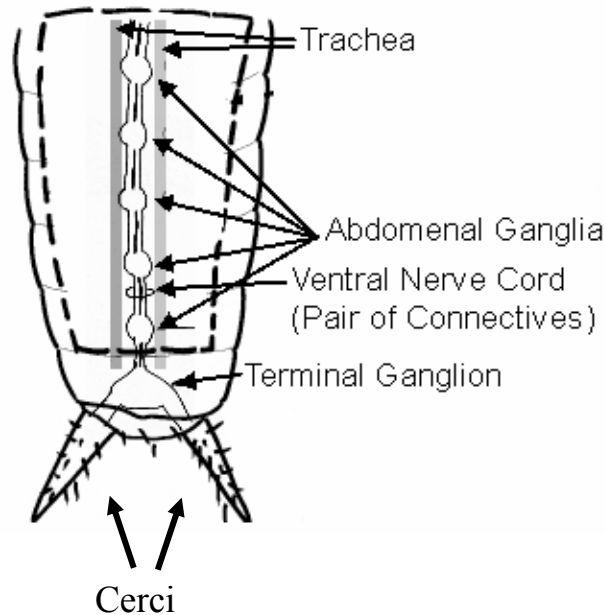
- Take a Pasteur pipette and attach a rubber bulb to the end. Apply wind stimuli to the cerci and record any changes in activity.

How do wind stimuli change VNC activity? Do wind stimuli increase the number of axons that fire action potentials?

- Try rhythmically stimulating the cerci with wind stimuli. Try slow and rapid frequencies.

Do VNC responses to repeated wind stimuli change as a function of time (Habituation)?

EXERCISE #5 – Action Potential Conduction Velocity In the Ventral Nerve Cord

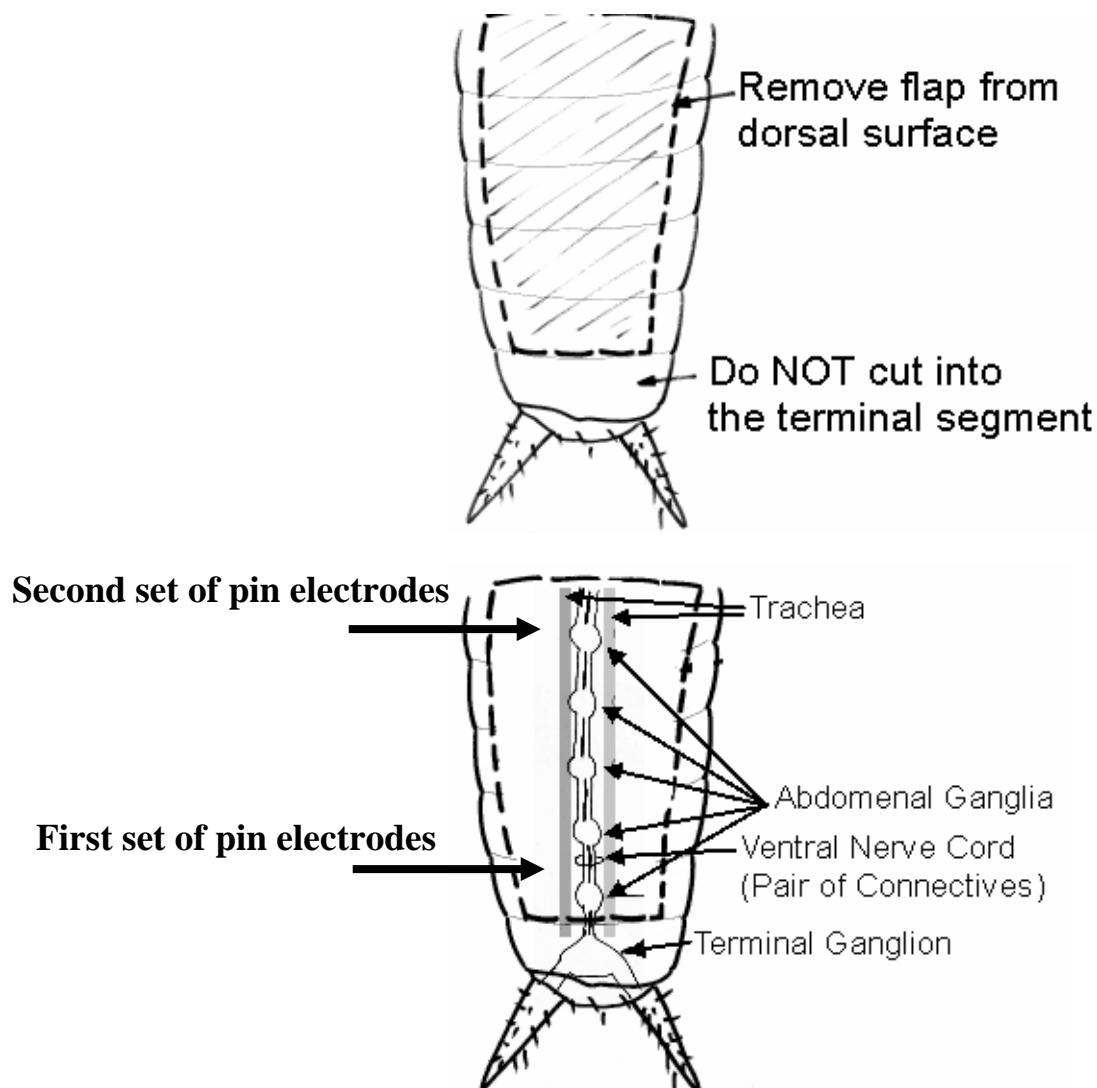


Cerci are complex sensory organs found on the 'tail end' of many insects. Sensory information from the cerci is *integrated* in the *terminal ganglion* and passed to other areas of the nervous system by *interneurons*. Some of the most effective natural stimuli of the cerci are associated with predators. If the cockroach can respond to these stimuli quickly enough, the cockroach may be able to escape from the predator. Therefore, there is selective pressure for the interneurons that receive this information to transmit it to the brain as quickly as possible.

For this exercise we will use extracellular (pin electrode) recordings to measure the conduction velocity of the interneurons that carry information away from the terminal ganglion. We will use two techniques to measure the conduction velocity, 1) *en passant* recording of action potentials generated from cercal stimulation (sound or wind), and 2) action potentials generated by electrical stimulation of the ventral nerve cord.

Preparing to Record from the Ventral nerve Cord

Prepare the cockroach for recording the same way as you did last week. Remember to place **two pins at the anterior** end of the abdomen and **two pins as far posterior** as possible *before* you begin your dissection. The pins will be most effective if you stretch the abdomen as far as possible without ripping any of the body tissue.



After the internal organs are removed you need to place two pairs of *pin electrodes* in the nerve cord. One pair should be at the posterior end of the cord; the other pair should be at the anterior end of the cord. Try to keep the two anterior electrodes close together, but not touching,

of course. Try to do the same with the posterior pair of electrodes. A *single* ground electrode should be placed lateral to the connective *between* the anterior and posterior pair.

Placing the electrodes

Place one set of electrodes at a time and confirm that they are working *before* you place the second pair – that is, you can see and hear spontaneous action potentials. Next, place the pair of pin electrodes in contact with the posterior end of the ventral nerve cord. *Confirm that you are recording responses to cercal stimulation* by monitoring the response as you puff on the cerci. Reposition the electrodes, if necessary, until you have an adequate recording.

Once your first pair of electrodes is working, you should connect two additional pin electrodes to a second amplifier. (Connect to the black and red connectors only. Use the banana-banana plug to connect the green connector to the green connector of your other preamp.) The output of this preamp should be connected to CH-B of the oscilloscope. Make sure your oscilloscope is set to display both CH-A and CH-B. Place this pair of electrodes as anterior as possible. *Confirm that you are recording responses to cercal stimulation* just as you did for the other pair of electrodes. Reposition the electrodes, if necessary, until you have an adequate recording.

If everything is working you will be able to record simultaneously from the two electrode positions in response to cercal stimulation.

IMPORTANT: Carefully sketch the positions of all of your electrodes and measure the distances using the rulers. These measurements will be needed to calculate conduction velocity.

Conduction Velocity

Conduction velocity (cv) is defined as the distance (s) the action potential travels divided by the time (t) it took it to cover that distance.

$$cv = \frac{s}{t}$$

Direction of Action Potential Travel

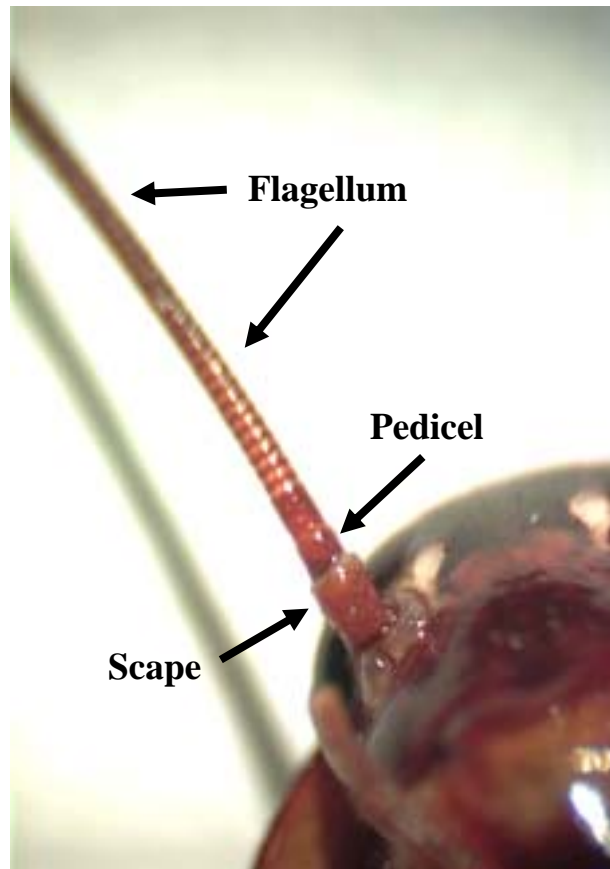
Once you identify action potentials that are *common* to both channels and demonstrate that they are from the same axon, you can easily determine the direction of travel of the action potentials. Action potentials in one of the oscilloscope traces will occur earlier than the correlated action potentials in the other trace. This is an unambiguous indication of the direction of travel.

Lab Exercise

° Calculate the conduction velocity from the two pairs of recording electrodes in response to sound or puff stimulation of the cerci.

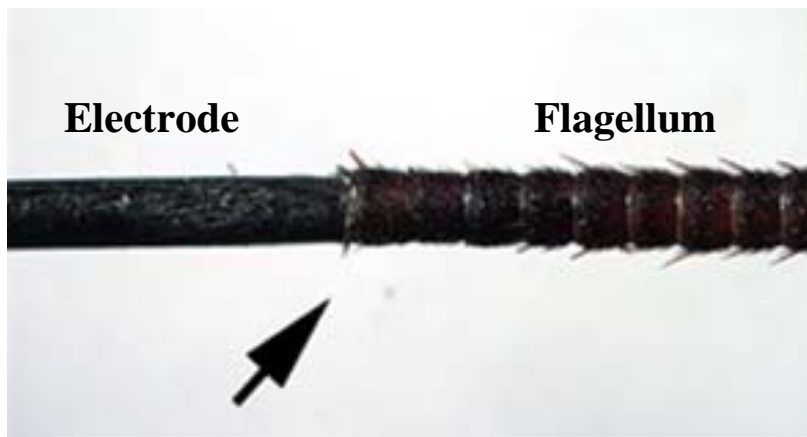
EXERCISE #6 – Recording from the antennal nerve

The antennae of most insects serve a number of different sensory functions including chemoreception, somatosensation, and hydroreception. These functions naturally implicate antennal processing in behaviors such as food localization, mate and con-specific recognition, as well as escape behavior. The observation that antennae can display quite dynamic movements is also indicative of the role they likely play in texture and shape discrimination.



A number of anatomical subdivisions of the antennal system will be evident upon examination of the head of the cockroach under the dissection microscope and are illustrated above. Immediately emerging from the head is the first segment of the antenna known as the scape which is the only part of the antenna containing muscles and therefore participates in movement of the antenna. The pedicel is located above the scape and is the first sensory segment of the antenna. Above the pedicel is the segmented portion of the antenna known as the flagellum consisting of ~140 segments in adult cockroaches. Like the legs and cerci,

bristles/hairs are distributed along the flagellum and serve as chemoreceptors and hydroreceptors.



An anterior and posterior nerve is located on dorsal and ventral (respectively) positions along the length of the antenna. You will be recording from axons that make up these nerves.

General methods

- Cockroaches should be anesthetized and all legs and wings removed. Stabilize the thorax of the roach with pins. Pins can be placed in the cuticle near the head as well.
- Cut one antenna in half. Leave the other antenna intact.
- Insert a pin electrode into the cut end of the antenna lengthwise. Until the electrode cannot advance further into the antenna. The dissection microscope will facilitate insertion of the electrode into the antenna. If the open-end of the antenna is too small to fit any part of the electrode, cut another 2-3 mm off of the end of the antenna and try inserting the electrode again.
- Let the electrode rest on the surface of the recording dish or raise the electrode slightly with some modeling clay so that the antenna is not touching the recording dish.
- Spontaneous action potentials from antenna axons are evident in almost every preparation. If you do not see or hear spikes, make sure everything is connected properly.

ASSIGNMENT

Record 3 minutes of spontaneous action potentials. Create a frequency histogram of action potential amplitudes as you had done for recordings in the leg and VNC. How do these recordings differ from those of the leg and VNC? How many axons do you think you recorded from?

If possible Isolate large spikes from an individual axon and quantify the temporal firing properties of these spikes including interspike-interval distribution, firing frequency histogram, max & min firing frequency.

OLFACTORY STIMULATION

Odorants elicit strong activation of antennal axons. Used cotton-tipped swabs soaked in an odorant in antennal axons and record changes in activity. As a control, record spontaneous activity as well as activity to a cotton-tipped swabbed soaked in water.

Odorants that produce strong activation include:

10% glacial acetic acid, 50% ETOH, lemon oil.

ASSIGNMENT

Prepare cotton swabs soaked in a particular odorant.

Record neural activity to olfactory stimulation of the same concentration odorant but for varying durations. Describe how activity codes for stimulus duration. Do we see habituation to prolonged exposure to odorants?

Record neural activity to olfactory stimulation of similar duration but at different concentrations. Describe how neural activity codes for stimulus duration? As the concentration of the odorant increases do responses increase in duration?

ADDENDUM: Use and care of laboratory instruments.

Differential Amplifier

Theory of Operation

Most biologically generated electrical signals are relatively low voltage. Nerves and muscles, for example, usually generate voltages of about 50 to 100 mV. (By comparison, a typical flashlight runs at 3 volts and a room lamp runs at 110 volts). The voltages we measure, however, may be significantly less than the animal actually generates. The farther away we are from the generator, the lower the voltage. This is further complicated by the resistive properties of the environment and our electrodes. Thus, we may have to monitor voltages of 100 μ V (microvolts) or less. If you glance at the oscilloscope GAIN control you will note that at maximum sensitivity, the oscilloscope is scaled to 2 mV/division. A 100 μ V signal would only deflect the beam by 1/20 of a division— an imperceptible amount. The solution to this problem is to *amplify* the signals we wish to monitor.

Differential amplifiers enable us to amplify a signal by an amount sufficient to raise it to voltages compatible with other equipment. The amplification usually ranges from 100 to 1000 times. Unfortunately, amplifiers amplify *noise* as well as the signal. The noise comes from several sources such as interference from nearby power lines. To reduce the effects of noise, most amplifiers also have built-in filters.

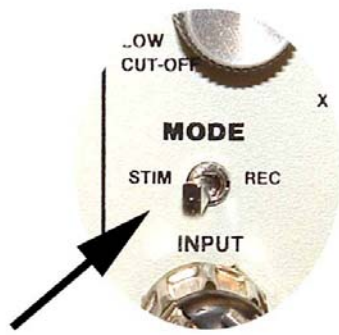
Familiarization with the AC amplifier



For most of our exercises, we will only be using one channel of the amplifier. Channel-1 controls are on the left side of the instrument. The on-off power switch is on the right side of the amplifier. **The power switch should be OFF when the amplifier is not being used.**

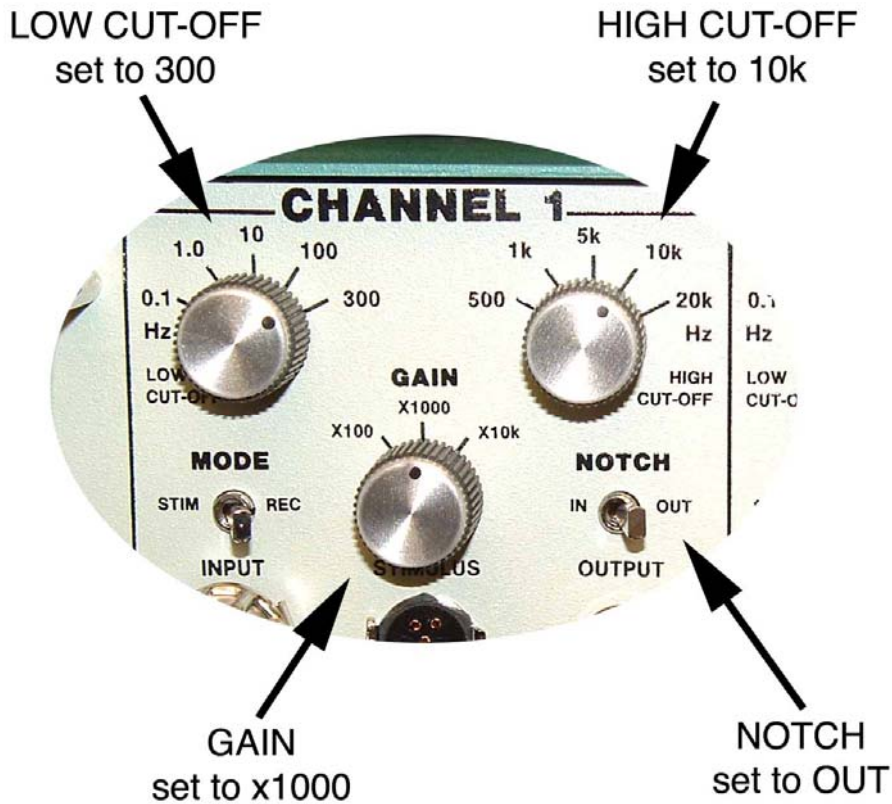


Set the **MODE** of all channels, including the ones not being used, to STIM.



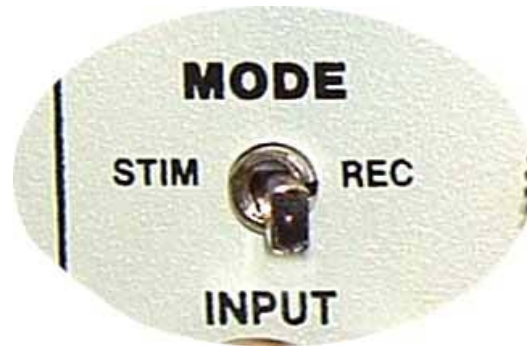
Set MODE of unused channels to 'STIM'

Set the filter and gain controls as shown in the following figure:



WHEN YOUR ELECTRODES ARE IN POSITION AND YOU ARE READY TO RECORD NERVE ACTIVITY

- 1) TURN ON THE POWER
- 2) Switch the **MODE** for the channel you are using to **REC.** (The MODE for all unused channels should be kept at STIM.)



Oscilloscope

Theory of Operation

Many physiological processes can be described by variations in voltage as a function of time. In some cases, such as the electrocardiogram, the voltages are the direct result of the physiological process being observed. In many other cases, the actual physiological effect is converted into a voltage by a transducer. An example of this is the measurement of strength of muscle contraction; a force transducer converts the force produced by the muscle into voltages.

Digital Oscilloscope

Digital oscilloscopes operate with the same basic principles as analog oscilloscopes. Analog oscilloscopes convert the voltages present in a signal into deflections of an electron beam 'drawing' an image of the waveform on a screen. Without special equipment the image drawn by the analog oscilloscope will fade and disappear in, at most, several seconds. Digital oscilloscopes convert the voltages that they receive into numbers that are stored in a computer-like memory. The contents of the memory are, in turn, reconverted into voltages that control an electron beam and 'draw' the signal on a screen. Since the signal is being drawn is from a voltages stored in memory, the same signal can be redrawn indefinitely! The stored voltages can also be transferred to printers, or other computers for analysis and storage.

Whenever a signal is *digitized*, that is, converted from a *continuous analog signal* to a *digitized signal* we must be wary of potential errors. The most pervasive error is *aliasing*.

To digitize a continuous analog signal we must obtain a sample the signal at a fixed repeating interval. Imagine a car tire rotating continuously at the rate of 1 revolution per second. If we digitize this rotation (e.g., make a movie) we can illustrate aliasing. If we sample at exactly 1 frame per second, what will the movie show? Since the tire is rotating at 1 rpm, we will always see it in the same position and it will appear stationary. This image does not accurately reflect the tires movement. Similarly, at certain *sampling rates* we the tire will appear to move backwards, even when it is actually moving forwards. These errors are prevented by

determining the *highest frequency present in the signal* and sampling at *greater than two times* this frequency.

The oscilloscope is the primary instrument used to visualize many physiological processes. You should spend time familiarizing yourself with the oscilloscope and become proficient with its operation!

Audio Monitor

Theory of Operation

Our ears have evolved to detect subtle patterns of sounds. Neurophysiologists exploit this capability by *listening* to the electrical signals recorded from the nervous system. Individual action potentials are heard as a *popping sound*. Simultaneous action potentials from multiple neurons are heard as a *whoosh sound*. One use for the audio monitor is to listen to the neural signals while stimulating the neurons- you can easily determine whether some sounds are correlated to your stimulation. You can then look at the oscilloscope to see whether you can pinpoint the features in the signal that correspond to the sounds you heard thus identifying the relevant activity within the recording.



Use the volume, bass, and treble controls on the Audio Monitor to adjust the signal level and sound quality; these controls only affect the speakers and will have no effect on the signal that is recorded by the computer. Both speakers will deliver the same sound.

Physiological Stimulator

Theory of Operation

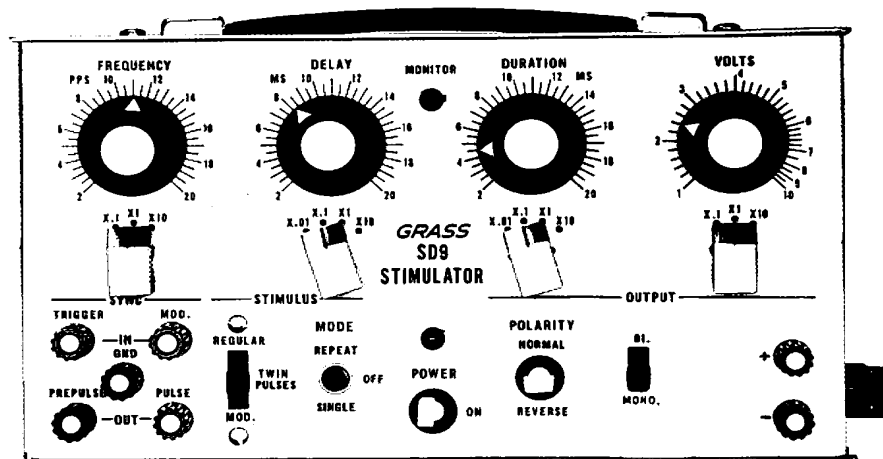
Nerve and muscle activity can be stimulated electrically. The STIMULATOR is used to generate electrical pulses that can be directly applied to living tissue. The voltages produced are described by several parameters. VOLTS, the strength of the stimulus; DURATION, how long the particular voltage is present; and FREQUENCY, the number of electrical pulses that are delivered, per second. Each of these parameters can be independently controlled with the stimulator.

Note: The stimulator can produce high voltages (in excess of 100 volts). Be sure you know what the voltage setting is BEFORE delivering pulses during experiments.

High voltages can destroy the tissues you will be experimenting on.

Familiarization with the Grass Stimulator

Most of the controls on the stimulator are self explanatory. You should, however, familiarize yourself with the *units* and *multipliers* that are used to describe the output of this device.



The four large knobs control the magnitude of the stimulus FREQUENCY, DELAY, DURATION and VOLTAGE. To read the value of any of these parameters, first look at the

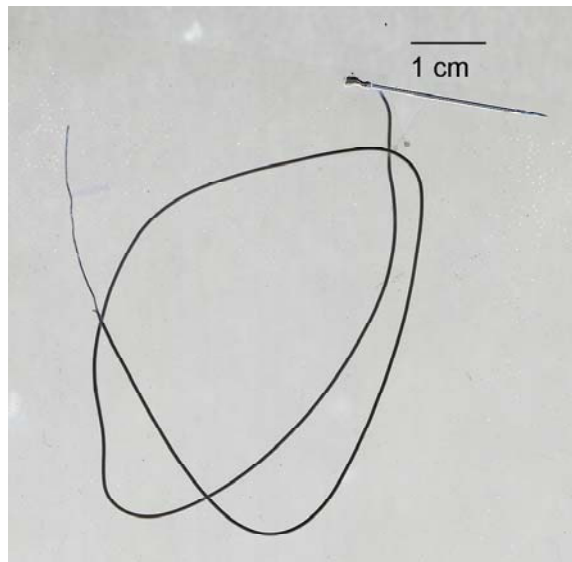
number that the knob's indicator points to (e.g., lets say the VOLTS knob pointed to 5); then, and most important, read the value that the pointer on the lower knob indicates (e.g., X.1). The expected output should be 5 VOLTS times the multiplier X.1, or 0.5 VOLTS output (which is the same as 500 mV or millivolts). Each of the other settings is calculated in a similar manner

Extracellular Electrodes

There are many different types of electrodes that are used to record the electrical activity of the nervous system. We use one of the simplest types due to their ease of use and their mechanical simplicity.

Pin Electrodes

Just as the name implies, these electrodes are constructed out of metallic pins and are connected to a wire by solder. The electrodes are indefinitely reusable provided the connection between the wire and pin remains strong. Recordings will degrade with each use of these electrodes unless pins are cleaned with soap and water periodically..



We will typically use 3 pin electrodes for a recording. One connected to the active (+) connector on the amplifier, the second connected to the indifferent (-) connector, and the third going to *ground*. Ideally, the (+) and (-) electrode will be in contact with the nerve and close together, while the *ground* electrode is not touching the nerve, but rather is in the animals non-neural tissues (and avoiding muscles if possible). Although the tissues must remain moist to avoid drying out and becoming damaged, the saline and/or body fluids (which are electrically conductive) must not *short circuit* the electrodes.

Electrical Connectors

You will be using several types cables and connectors. Remember that at least two wires are needed to complete any circuit.

A **WIRE** is a single electrical conductor. Wires come in many diameters, ranging from extremely fine wires (in the range of 100 μ m diameter) used to make electrical connections directly to nerve or muscle tissue, to large wire (>1-2mm diameter) used to connect instruments. Wires may be bare, or insulated.

A **CABLE** is an assembly of more than 1 wire.

Several **Connector** types will be used throughout the semester.

Connectors are often color coded. The conventional color codes are:

RED Voltage or Signal will be carried. When referring to amplifiers or power supplies, the **RED indicates POSITIVE voltage**.

BLACK Voltage or Signal will be carried. When referring to amplifiers or power supplies, the **BLACK indicates NEGATIVE voltage**.

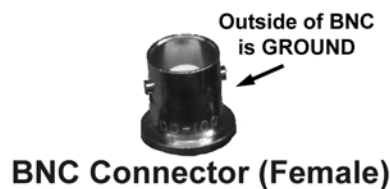
GREEN Indicates the circuit **GROUND**, which is the reference for all other voltages and/or signals.



Banana Plug

The Banana Plug connects a *single* wire. Banana plugs often contain a socket so that multiple plugs can be stacked.

BNC connectors allow cables (containing 2 wires) to be connected. The metal shell is electrically connected to one of the wires and typically is connected to GROUND. Inspection of the female connector will reveal two studs; these studs fit into the slots on the male connector and allow you to lock the connectors together. To lock, twist clockwise after pushing the connectors together. To remove the connectors, unlock by turning counterclockwise, then pull straight back. *Pull and twist the connector, NOT THE CABLE!*

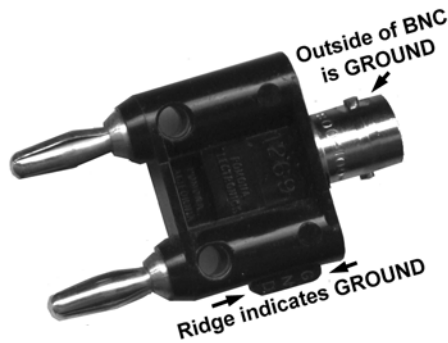


The **BNC-T** Connector has a male connector and two female connectors. This allows you to connect two BNC male connectors to a single BNC female connector.

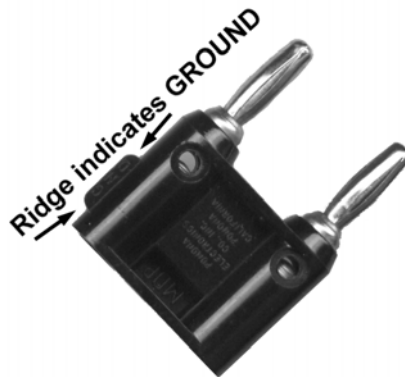


You may have to connect a BNC Cable to other instruments that have banana plug connections. A BNC-Double Banana plug adapter will be used to make such connections. Note that one side

of this connector has a ridge. The banana plug adjacent to the ridge is electrically connected to the outer shell of the BNC connector. Typically, it is at GROUND potential.

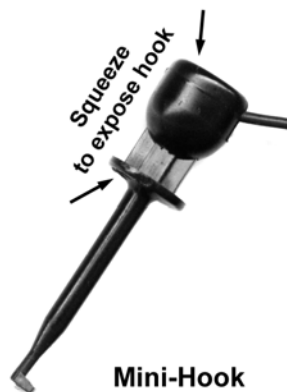


BNC (Female) – Double Banana



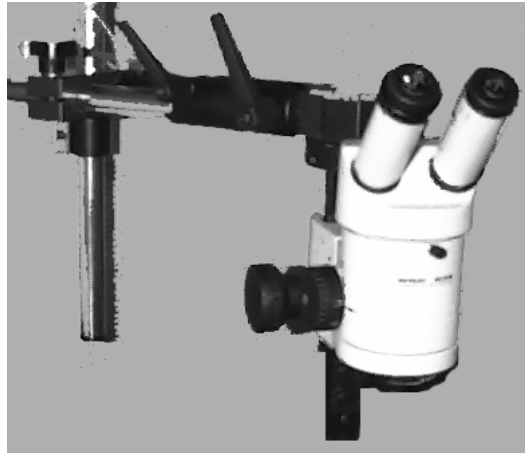
Double Banana Plug

Mini-Hooks provide a simple way to attach a wire to electrical circuits for testing and monitoring signals and voltages. Squeezing the top will expose a hook at the bottom of the mini-hook that can then be placed around the wire that will be monitored. The spring loaded hook will remain attached after you release the top.



Mini-Hook

Dissecting Microscope



You will be using extremely high quality research-grade dissecting microscopes. The distance between the oculars is adjusted to match the distance between your eyes by pulling apart, or pushing together, the eyetubes. Both oculars are adjustable to compensate for focusing differences between your eyes. The scopes can be used with or without eyeglasses; you should experiment to see which is more comfortable. The large knobs towards the rear of the scope raises and lowers the scope to allow you to focus the image. The knobs on the sides of the scope adjust the magnification.

The microscope lenses are **very** expensive! **Do NOT clean these lenses** unless you know what you are doing– if damaged they cannot be replaced. Please ask your instructor for assistance if your lenses need to be cleaned.

These microscopes are designed to have a *long working distance*, or a long distance from the bottom objective and the object being viewed. The working distance will be several centimeters. This provides extra room for lighting the area, placing experimental apparatus on the subject, and increases the access to the subject for dissection. It takes a bit of practice before you can work with instruments while viewing them under the microscope. Once you learn this skill you will find that you will be able to do extremely fine surgery and manipulation while looking through the dissecting microscope.

Dissection Instruments

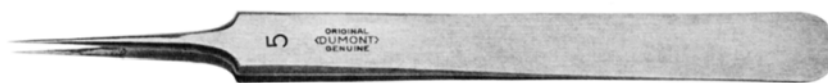
You will have an assortment of surgical instruments available for your use.

Standard Instruments:

Assorted scissors and forceps are available. *Use the coarse (large) instruments to do rough cutting, pulling, setting pins, etc.* You should almost never have to touch the biological preps with your hands (gloves, which will be available, should be used when handling the material)– whenever possible, use surgical instruments.

Fine Instruments:

Delicate manipulation will require the use of fine instruments. Shown below are **fine forceps**. Fine forceps will typically be labeled with a number, in this case #5, that indicates the style of the forceps; #5's are characterized by the long, slender, sharply pointed tips. Other styles are also available.



#5 Forceps

These forceps can be very sharp. Care should be taken to avoid poking yourself (or your lab partners) with the tips! The fine tips are easily damaged by misuse. Do not stick the ends into the dissecting dish, the table, or drop them on the floor. They are also easily damaged by applying too much force (tips will bend) or using these for jobs that belong to larger forceps such as placing insect pins into the dissecting dish.

Clean the forceps after use so that tissue does not dry onto the tips.

Certain procedures may require the use of **microdissecting scissors**. These scissors are very delicate and are susceptible to damage. They should only be used to cut the most delicate nerve and connective tissue. They should never be used to cut the skin, paper, or suture. As with all delicate instruments, do not drop them and be aware that they may be sharp enough to pierce your skin, so use them carefully.



Microdissecting Scissors