ELECTROPHYSIOLOGY

Measuring Action potential in the Cockroach leg
(Based on MIT lab course 9.02: Systems Neuroscience Laboratory)

Neurons are cells specialized for the integration and propagation of electrical signals. It is through such electrical activity that neurons communicate with each other and with muscles and other end organs. Therefore, an understanding of basic electrophysiology is fundamental to appreciating the function and dysfunctions of neurons, neural systems, and the brain.

**Neurons:** Neurons come in different sizes and shapes but all have the same basic organization:

- The cell body, which contains the nucleus and internal organelles and carries the metabolic functions of the cell.
- Dendrites, which receive signals from other neurons.
- Axon which send signal to other neurons via synapses (gaps between axon and dendrites receiving end).

The neuron conveys information through electrical and chemical signals.
These messages are passed to the cell body, which determines if the message should be passed along. Important messages are passed to the end of the axon where sacs containing neurotransmitters open into the synapse. The neurotransmitter molecules cross the synapse and fit into special receptors on the receiving nerve cell, which stimulates that cell to pass on the message.

**Electrophysiology**

Recording electrical activity in the brain is *the* key technique of modern systems neuroscience. This approach has been the source of multiple Nobel Prizes over the past 50 years, including such luminaries as Hubel and Wiesel, Hodgkin and Huxley, and Sakmann and Neher.

Electrophysiological recordings of the brain also provide the data on which most cutting edge computational models of brain function are based, and much of molecular neurobiology is aimed at determining the underlying molecular phenomena that are manifested in differences in electrical activity. In short, electrical recordings from neurons/nerves are at the heart of all systems neuroscience.

This lab will use a classic preparation designed to teach you about neural function and sensory physiology, and also the electrical ‘engineering’ principles that will allow you to conduct these techniques.
Basic concepts you should be familiar with from physics:

Charge (Q)
- two basic types of charged particles: electrons and protons.
- The net charge of an atom or a material is the difference between the number of protons and the number of electrons.
- Charges particles exert forces on each other (similar charges repel, different charges attract).
- The unit of charge is the Coulomb or the Faraday (the amount of charge on 1 mole of electrons)

Voltage (V)
- voltage is the amount of work per unit charge done when charge is moved relative to another charge. (1 volt = 1 joule/coulomb).
- Think of the voltage as the potential energy available to move a unit of charge from one point to another.
- An analogy is stones rolling off a cliff. The stones are the charges, and the height of the cliff is the voltage.

Current (I)
- current is the amount of charge moving past a fixed reference point per unit time (e.g. in a wire conductor)
- the unit of charge is the ampere (amp) (1 amp = 1 coulomb/second)
- to continue the analogy above, the current is the number of stones falling past a fixed point in one second.

Resistance (R)
- if a potential difference (Voltage) is applied to the two ends of a conductor, current will flow. Resistance is defined as the potential difference divided by the current (R = V/I).
- Resistance is a property of the conductor and characterizes how much the conductor “resists” the flow of charge. It is different for different materials.
- The unit of resistance is the ohm. (1 ohm = 1 volt / amp)
- For metallic conductors, the resistance is the same regardless of the voltage that we use. This important relationship is known as Ohm’s law. (V = I R)
- To continue the analogy above, the resistance is related to the density of the material through which the stones are falling. For example, when dropped from the same height (cliff), stones will reach a higher velocity in air than they will in water (e.g. a cliff under water). From a fixed reference point, the stone velocity determines the number of stones (charge) that will travel by the reference point in one second.
(i.e. the current).

Conductance
- Conductance is the inverse of resistance (= 1/R)

Capacitance (C)
- if there is no conductive path from two points that have a voltage potential between them (e.g. a potential difference applied to two metal plates in air), charge will accumulate at the two points.
- If 1 volt potential between two plates causes an accumulation of 1 coulomb of charge (i.e. 1 coulomb on one plate and one coulomb the other), the capacitance is defined as 1 Farad (1 Farad = 1 coulomb / volt)
- **Think of capacitance as the ability of (e.g. two plates) to store charge.**

Voltage “signals”
As you know, neurons communicate through neurotransmitters released in response to changes in membrane potential. A change in membrane potential (e.g. action potentials) is the fundamental way in which information is transmitted along nerve fibers. As a result, the measurement of potentials within and around neurons turns out to be a very useful tool for assessing information processing in the nervous system. Those potentials are typically determined relative to some distant “ground”.

**Much of this course is devoted to measuring such potentials, understanding where they come from, and understanding how they can be used to learn how the nervous system processes information and produces behavior.** (i.e. “electrophysiology”).

Given the importance of voltage potentials, it is critical that you understand what a voltage potential is, and how we measure it.

Typically, the voltage potential will be amplified and carried on a conductor (i.e. wire). A typical wire is a BNC cable. It has an inner conductive core, and an outer conductive “shield”. The recording equipment we will use in this course will be hooked up so that the measured measure membrane on the wire so that the potentials we are interested in should be measured between the BNC inner core conductor and the other shield conductor (“ground”).

One goal of the lab sessions is to teach you a common way in which these potentials are observed and measured – using an oscilloscope.

**Time**
The voltages we are recording are not constant (that would not be very interesting!). Indeed, they can change very rapidly. We often refer to the voltage measured across time as the “voltage signal”
or the “voltage waveform.”

Side bar: What does the time course of an action potential look like (draw one)? What is the relevant time scale to describe a single action potential (i.e. put a scale on the x axis)? (hours, minutes, seconds, milliseconds, microseconds?)

When working with voltage signals that vary with time, one of the fundamental concepts is that any such signal can be thought of not just a voltage signal that varies with time, but as the sum of many infinitely long sinusoids of different frequencies and phases (time shifts relative to each other).

**These two ways of describing the voltage waveform are EXACTLY equivalent.** The sinusoidal format is called the Fourier representation of the voltage signal. The Fourier representation has useful properties and advantages over the time-based representation.

**Here are some basic concepts you should know about sinusoids and other “periodic” signals:**

**Frequency and Period**

The frequency of a sinusoidal waveform is simply the number of times it moves up and down in one second. (Actually, we only count the “ups” or the “downs”, but not both). The unit of frequency is Hertz (cycles / second). A sinusoid that moves up once per second is said to have a frequency of 1 Hz. A sinusoid that moves up twice per second is said to have a frequency of 2 Hz.

Side note: Given your drawing of a single action potential above, what is the approximate “fundamental” (i.e. dominant) frequency of an action potential? (hint: think of the entire action potential as one cycle of an infinitely long sinusoid).

Side note: Computers now have clock frequencies of over 1 gigahertz. How many cycles per second does a 1 gigahertz sinusoid undergo? Compare the fundamental frequency of a computer clock and an action potential.

Closely related to frequency is the concept of “period.” The period of a sinusoidal signal is the time between each of the “ups” (or, equivalently, each of the “downs”). Period is thus the inverse of frequency: (frequency (Hz) = 1 / period (sec)). For example, a sinusoid that moves up every 1 sec has a period of 1 sec (and a frequency of: 1 cycle / 1 sec = 1 Hz). A sinusoid that moves up every 0.1 sec (100 msec) has a period of 100 msec (and a frequency of: 1 cycle / 0.1 sec = 10 Hz).

Side note: What is the period at which the earth rotates around its axis? What is the frequency?

**Amplitude**

Amplitude is simply the magnitude of the voltage signal (i.e. units of volts). Because the signal varies with time, we often refer to the “peak amplitude” (e.g. 200 mVolts). Sinusoidal amplitudes
are, by convention, often measured as the “peak-to-peak” amplitude. This is defined as the difference between the highest point (“up”) and the lowest point (“down”) of the sinusoid. The units are still in volts (or millivolts, etc.).

oscilloscope: An oscilloscope is a test instrument which allows you to look at the 'shape' of electrical signals by displaying a graph of voltage against time on its screen.

To learn more about oscilloscopes see http://www.kpsec.freeuk.com/cro.htm
Cockroach Receptive Field Lab

The cockroach senses tiny air movements using tiny hairs on two posterior appendages called cerci. It can surmise the direction of an attack and scurry away to avoid being eaten. Neural signals from the hairs converge on the terminal abdominal ganglion where the wind information is processed, and are then conveyed further by giant interneurons.

For the cockroach laboratory, you will record *receptive fields*, the pattern of sensory input that drives increase electrical activity in a given neuron. Specifically, you will record the responses transmitted through a peripheral nerve that are induced by specific patterns of contact on the leg. This lab is very simple and robust and ideal for high school students.

**Work in teams of 3.**

**Recording Equipment at each lab bench**

Audio Monitor (1)
Oscilloscope (1)
Amplifier (1)
Square of Clay on Metal Base (1)
BNC Cables (3)
Audio Cable (1)
T-junction Connectors (1)
Insect Pin “Electrodes” (3)

**Recording Connections:**

T-connector to oscilloscope channel 1.
Amplifier output to oscilloscope channel 1 via BNC cable attached to T-connector.
Audio monitor to oscilloscope channel 1 via BNC cable attached to T-connector, other side.
Amplifier G1, G2 and ground to cockroach leg via insect pin electrode.

**Other Supplies:**

Toothpick (for sensillae deflection and leg handling)
Cotton-tipped applicators (2)
Lamp
Clamp to hold lamp
Magnifying glass

**General Equipment:**

CO₂ Tank with Regulator Attached or dry ice and box- to anesthetize
Tank with Cockroaches
Small bottle/container in which to catch cockroaches
Parafilm -To cover container once roaches are caught.
Scissors and Forceps -For leg dissection.
Animals: Periplaneta americana, Cockroaches

- To catch cockroach, have second person hold tank cover open just enough while you scoop roach into bottle, covering top of bottle with your hand.
  - Second person should watch for escapees and cover the tank as soon as you are done.
  - When ready to anesthetize, cover top of bottle with parafilm. Then puncture parafilm with regulator handle and release CO\textsubscript{2} into bottle to immerse insect anesthetize.
  - While anesthetized, quickly use scissors to remove 4 largest legs above femur.
  - When done place cockroach back into tank.
  - Repeat as needed.

**Equipment Connections**

1. Connect the electrode pins to the differential inputs of the amplifier.
2. Connect a BNC from the amplifier output to the audio monitor and to the oscilloscope CH 1 simultaneously using a T junction.
3. Set the filter settings on the amplifier to 300 Hz and 2 kHz, respectively.
4. When these connections are in place, have an instructor check that the connections are correct, and then proceed to obtain a cockroach leg.
**Cockroach Leg Preparation**

It is critical that NO cockroaches escape during this process!

1. Obtain a cockroach. It will be in a vial with the top covered in parafilm.
2. Using the handheld gas trigger attached to the CO2 tank, poke a hole in the parafilm and inject CO2 into the vial until the cockroach is no longer moving
   - Be careful not to over expose the cockroach to CO2 and kill it.
3. Remove the cockroach from the vial, and lay it on its back.
4. Using a dissection scissors, disconnect a large posterior leg from the body at the upper coxa.
5. Using forceps, carry the leg and place the hind leg on the molding clay on the steel plate such that the coxa and femur are securely on the clay but the tibia and claw are freely extending off of the edge of the block.
7. Place both electrode pins in either side of the femur.

**General “Hand Mapping” of Receptive Fields**

1. Turn up the volume the audio monitor in order to hear action potential activity.
   - Be mindful that other groups are listening to their audio monitors at the same time, so try not to turn the volume up too high.

2. Look at your oscilloscope to determine if you can see action potential activity.

3. Using a toothpick, gently deflect the claw.
   - You should hear a large increase in the degree of neural activity.

4. Gently deflect individual spines on the leg, apply vibrations to the desktop, etc. to get a general sense of the evoked neural activity.
   - Be careful not to move the preparation itself.

**Qualitative “Hand Mapping” of Receptive Fields**

1. Keep electrode pins in the same position, on either side of the femur.
2. Systematically deflect each spine on the tibia on both sides of the leg, in all directions.
3. Determine qualitative responsiveness of each spine for both the direction of deflection and for spine position.

  - Was the firing rate stronger when you deflect the spine toward the claw or toward the knee?
• Was the firing rate equal across all spines?
• Did spines on the back of the leg have stronger responses than those on the front of the leg?
• Did spines near the knee have stronger responses than those near the claw?

4. Alter the electrode pin position such that both pins are on the top portion of the femur. Repeat steps #2-3.
• Do you notice any change in responsiveness when you change the electrode position?

5. Alter the electrode pin position such that both pins are on the bottom portion of the femur. Repeat steps #2-3.
• Do you notice any change in responsiveness when you change the electrode position?

You can also listen to what neurons sound like above. In this recording, you hear many action potentials (or "spikes") from neurons in the cockroach leg that sound like "popping" or "rain". After about 5 seconds, small puffs of air are made onto the leg. The increase in pressure on the leg hairs, cause an increase in spikes. This is known as rate coding.
Basic anatomy of a cockroach leg
Clean Up Instructions for Cockroach Receptive Fields

General Materials:

- Collect all of the forceps, scissors and catching container. Wash these materials thoroughly.
- Upon completing washing, leave instruments on the clean sink side to dry.
- When dry, put dissection supplies away.
- Turn off carbon dioxide tank, remove regulator and put away.
- Collect cotton tipped applicators and throw away.

Recording Equipment:

- Disconnect all of the connections between the equipment.
- Put away the oscilloscope, audio monitors and amplifiers.
- Collect and put away the connectors, BCN cables, alligator clips and insect pins
- Collect clay and put away for next use.
- Collect metal bases and put away.

Useful Links:

Recording from Cockroach Leg Mechanoreceptive Afferents
Lab from University of Illinois
http://nelson.beckman.illinois.edu/courses/physl416/roach_lab.html

Other Links about the brain

http://learn.genetics.utah.edu/content/addiction/reward/cells.html

The brain from top to bottom (http://thebrain.mcgill.ca/flash/index_a.html)
www.pbs.org/wnet/brain/3d/index.html
www.howstuffworks.com/brain.htm
thebrain.mcgill.ca/flash/index_d.html
http://brainconnection.positscience.com/