

Section 5. The Compound Action Potential

5.1 The Compound Action Potential (Frog Nerve)

Overview

In the last chapter, you learned about action potentials how to record them in the lab, and how they relate to receptive fields in cockroaches. In session 5.1 (Frog nerve), you will consider how the action potential relates to what you have previously learned about underlying ion channel mechanisms. Building upon the techniques you have already learned, you will generate and record action potentials in the sciatic nerve of a frog.

5.1 Frog Compound Action Potential Stimulation

Below, you will find the protocol for the 9.02 frog lab. Before proceeding, please review the chapter describing the original setup of this experiment (attached after this protocol) to understand the experimental concepts.

Set up the nerve chamber and connect the equipment

1. Fill the nerve chamber with saline to a level just below the cross wires.
2. Use alligator clips to connect the cross wire leads from the nerve chamber to the amplifier; these MUST be good connections.
 - That is, grab the metal cross wire leads on the nerve chamber with alligator clips.
3. Info to help you:
 - The trigger pulse from the stimulator is provided on the BNC connector labeled “pulse.”
 - The stimulator voltage is provided from the red and black terminals at the lower right of the front panel.
 - The output of the amplifier is provided by a BNC connector (“output”).
 - The recording amplifier input connectors you should use are labeled G1 and G2.

****You will be recording the potential difference between these two leads. This is called “differential amplification” because you are amplifying only the **difference** in potential between two points in the preparation rather than the potential of a single point relative to a common instrument ground (called “single-ended amplification”).**

Set up the equipment parameters

1. Amplifier:
 - Set to “use” (rather than calibrate)
 - 100x gain (What does this mean?)
 - 100hz-10kHz (Why are we using these filter settings?)
2. Oscilloscope:
 - CH1 sensitivity set to 0.5-1 V/div (amp should be connected to Channel 1)
 - Time base: ~1 ms/div
 - Trigger menu: source: external, mode: normal
3. Stimulator: **DO NOT TOUCH THE STIM. LEADS WHEN YOU ARE STIMULATING.**
 - Pulse shape “normal”
 - Frequency = 0.5 s.
 - Delay = 1 ms (will not matter for now)
 - Duration = 0.2 ms
 - Amplitude = 100 mV (0.1V) (LOWEST SETTING)
 - Before turning on the stimulator, set to single pulse.

4. Confirm that your scope is triggered every time you press the single pulse button on the stimulator. You may want to connect the stimulator output to channel 2 on the scope so that you can observe the width and amplitude of the pulse.
-If you do this, connect just ONE of the stimulation leads using the BNC probe connector.

Extract the frog sciatic nerve

**After you have setup your equipment, let your instructors know that you are ready for the frog. The teaching staff will anesthetize, decapitate and spinally pith the frog for you.

1. Cut the skin in a circle around the abdomen and pull the skin off the legs.
-Like removing a pair of pants.
2. Place the frog dorsal side up. Lift up the urostyle or a long tail bone and cut along both sides of the urostyle as far as you can. As you progress, keep lifting the urostyle and keep the scissors close the urostyle to prevent cutting the nerves below.
-It is best to try to visualize the nerves before making these final cuts.
3. Remove the urostyle by cutting it at its base.
4. Begin dissecting the sciatic nerve on one side from its roots at the spinal cord, into the pelvis, through the thigh, and beyond the knee.
-Take your time when performing this step.
5. While removing the sciatic nerve, NEVER grab the nerve itself with forceps. NEVER put a large amount of tension along the nerve (i.e. do not stretch it). Try to avoid touching the nerve with any metal objects. Instead, use the rounded glass pipette to lift and manipulate the nerve.
6. The best way to extract the nerve is to pull the tissue apart laterally and then lift the nerve out of the fascia tissue using the glass pipette. Always visualize the nerve before pulling or grabbing anything.
7. During the dissection, drip saline along the nerve every few minutes to keep the nerve moist.
8. Once you have freed the nerve at the proximal end, you can tie a tight ligature around that end with the thread provided. Leave a tail on your ligature to help you lift the nerve later.
-As you tie the ligature, the nerve will discharge and you should see some muscular twitching of the leg below the nerve. This is a good sign that the nerve is healthy.
-This is the end of the nerve that you will want to stimulate.
9. Once you dissected the nerve down to the knee or slight below, tie another ligature at this end.
-The longer the nerve the better.
-You should see twitching of the most distal muscle of the leg.
10. Lift the nerve with forceps by grabbing the ligature tail, and place it in a saline bath.

Generate and observe a compound action potential (CAP)

1. Place the nerve in the nerve chamber while keeping the ends of the nerve out of the saline bath.
2. Adjust your recording and stimulation connections as required.
3. Place the glass cover over the chamber.
4. Try to generate a CAP in the nerve using a single stimulation pulse.

5. Be sure to distinguish the CAP from the stimulation artifact.
 - When doing this step, it is helpful to observe the time course and amplitude of the actual stimulation pulse on channel 2 of your oscilloscope.
6. To distinguish the CAP from the stimulation artifact, slowly increase the stimulation voltage each time you generate a CAP. Do not exceed 5V. Changing the scope display persistence to “infinite” can help.
 - The CAP has a stimulation threshold and reaches a maximum amplitude. After an initial rise in amplitude with stimulation voltage, it stops increasing.
7. To distinguish the CAP from the stimulation artifact, slowly increase the stimulation duration each time you generate a CAP.
 - The CAP duration does not increase with increasing stimulation duration.

Determine the effect of stimulation amplitude on CAP amplitude

**The approximate range of amplitudes is 0.1 V (lowest stimulator setting) to 5V (DO NOT EXCEED 5V).

1. Stimulate the nerve at each stimulator amplitude along its range, stopping at a 5V maximum.
2. Draw pictures of CAP at each of 5-10 amplitudes in this range.
3. Plot the amplitude of the peak of the CAP as a function of stimulation voltage. Is this function linear? Why or why not?
 - This peak can be positive or negative, depending on the arrangement of your recording leads.
4. Plot the latency (time from stimulation pulse onset) of the CAP peak as a function of stimulation voltage. Use channel 2 to see the start of the stimulation pulse. What does this function look like? Why?

Determine the conduction velocity of the CAP

1. Make a ballpark estimate of the conduction velocity using the latency observed above and determining the distance between the stimulation and recording leads.
2. To make a more accurate estimate, move your recording leads to at least two different positions along the nerve. Keep the separation of the recording leads constant as you change their position.
 - Is the shape of the CAP the same at all recording positions? Why or why not?
 - You can set your scope display persistence to infinite to help you see this.
3. Make a plot of CAP latency on the X-axis and the distance between stimulation and recording leads on the Y-axis.
 - Each wire is separated by 0.5 cm.
 - What does the slope of this plot tell you?
4. Convert units on the slope of your plot to m/s.
 - Is the value what you would expect? Why or why not?

Examine the refractory period of the CAP

1. Set the stimulation voltage to just above CAP threshold. Set the stimulator switch to “paired pulses”.
 - This will cause the stimulator to deliver two pulses at a fixed separation. Each pulse will have the same amplitude (voltage) and width (duration).
2. Vary the separation of the pulses using the DELAY dial on the stimulator. Start with a separation of 10 ms, and gradually reduce the separation to 1 ms.
 - Be sure that you can see two stimulation pulses on your scope screen.
3. Make a plot of amplitude of second pulse relative to first as you decrease the pulse separation time (also called the inter-pulse interval).
4. Determine the absolute and relative refractory period.
 - What neuronal mechanism underlies this property?

Optional experiments

**You are not limited to the experiments listed below. You may test anything that you find interesting in the remaining lab time.

1. What is the effect of recording electrode separation on the CAP shape? Can you explain these effects?
2. What is the effect of exchanging the anode and cathode?
 - Try looking carefully at the latency of the CAP in both conditions.
 - Using infinite persistence on your scope may help.
3. Try to distinguish two populations of fiber classes in the CAP.
 - You will need a nice long nerve to do this, and your stimulation and recording electrodes should be maximally separated.
4. Determine the effect of the anesthetic Tricaine on the CAP. Drop a small amount of Tricaine along the length of the nerve. Be careful not to touch the nerve or form fluid bridges between the electrodes. Observe the nerve over the next 10 minutes.
 - What is the effect of Tricaine on the CAP shape and amplitude? What is the effect on conduction velocity? **Note: Due to the nature of this experiment, it will become your last one with the nerve, so be sure that you are finished with your other experiments before you proceed with this one.

Frog skeleton dorsal view removed due to copyright restrictions.
See http://www.k-state.edu/organismic/images/frog_skeleton.jpg.

Dorsal view of frog skeleton 1¹

Figure removed due to copyright restrictions. See <http://shs2.westport.k12.ct.us/mjvl/biology/dissect/frog.htm>.

Ventral view of frog skeleton III

Frog nervous system diagram removed due to copyright restrictions. See Figure 49. Bohensky, F. *Photo Manual and Dissection Guide of the Frog*, Avery Publishing Group Inc., 1982.

Ventral view of frog nervous system [1]

[1] Bohensky, F. *Photo Manual and Dissection Guide of the Frog*, Avery Publishing Group Inc., Wayne, NJ, 1982

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