Experiment AN-10: Crustacean Stretch Receptor

Equipment Required

PC or Mac Computer

IXTA, USB cable, IXTA power supply

NA-100 NeuroAmp

Suction electrode assembly and glass tips (See Appendix for construction instructions and a commercial alternative)

Stand and clamp, or micromanipulator, for electrode.

Plastic crayfish bath chamber

Dissection microscope and light source

Tissue tensioner, or micromanipulator, for flexing tail

Faraday cage and steel base plate (if noise in room)

Cables and alligator clips for grounding equipment

Suture thread

Pasteur pipettes and bulbs

Assorted banana cables and alligator clips

Crayfish

Crayfish salines (see Appendix)

Dissection tools

NA-100 Extracellular Amplifier Setup

1. Locate the NA-100 NeuroAmp extracellular amplifier and its input cable.
2. Plug the DIN-8 cable from the NA-100 extracellular amplifier into Channel A5 of the IXTA.
3. Plug the XLR connector on the input cable into the NA-100.

Figure AN-10-S1: The NA-100 NeuroAmp Extracellular Amplifier and input cable.

Equipment Setup

This experiment uses a suction electrode and the NA-100.

1. Attach the three connectors of the suction electrode assembly to the NA-100 so that:
    - the recording electrode, which is the wire inside the lumen of the suction tubing, is connected to the red (+) connector.
    - the indifferent (reference) electrode, which is the wire wrapped around the suction tubing down to the glass micro- electrode tip, is connected to black (-) connector.
    - the ground electrode, that is in the solution in the bath chamber, is connected to the green connector.
2. Place the barrel of the suction electrode on a micromanipulator placed close to the crayfish preparation dish.

Note: If there is electrical noise in the system, please refer to Appendix IV in the Appendix document. The default filter settings listed are suggested for use in ideal recording conditions. If noise is present in the recording environment, the high and low pass filters can be set at different levels to create a recording with less noise. If noise is caused by AC line voltage used to power the equipment in the lab, the notch filter can be used.

The Dissection

1. Place a crayfish in ice water for 10 minutes. Remove the crayfish from the ice water and quickly cut off its head.

2. Remove the tail (abdomen) from the thorax by cutting around the joint (seam) connecting those two parts.
3. Observe the hinge ridge (Figure AN-10-S2) that runs along each side of the abdomen; only cut on the ventral side of the hinge ridge in order to preserve the hinges that hold the segments of the tail together.
4. Hold the tail and make a longitudinal cut along each side of the abdomen (below the hinge ridge) to loosen the ventral shell, swimmerets, and flexor muscles from the dorsal shell. Leave the tail fins attached to the dorsal exoskeleton.
5. Begin at the anterior end of the abdomen and separate the ventral and dorsal halves of the shell from each other. It may be necessary to cut (use small forceps) the connections that the segmental flexor muscles make to the dorsal shell.

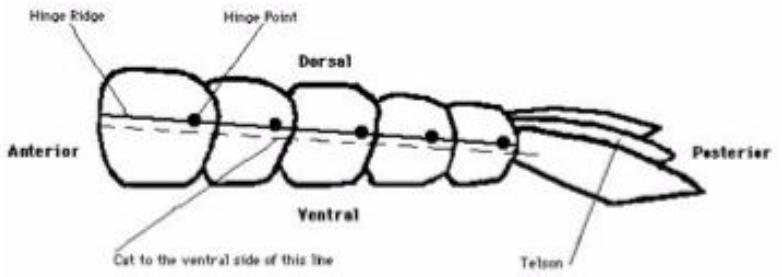

Figure AN-10-S2: The hinge ridge on the crayfish abdomen.

6. Discard the ventral portion of the shell.

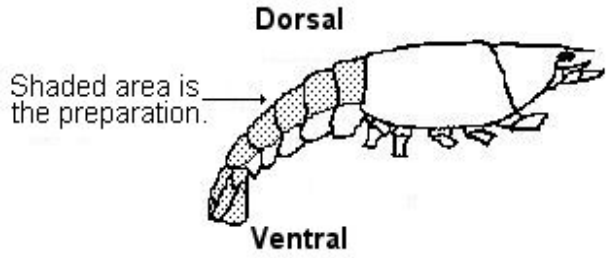

Figure AN-10-S3: Diagram showing the region of the crayfish tail used in the experiment.

7. Place the dorsal shell in the preparation dish and quickly fill the dish with crayfish saline.
8. Push two pins, one on either side, through the shell in the first abdominal segment.

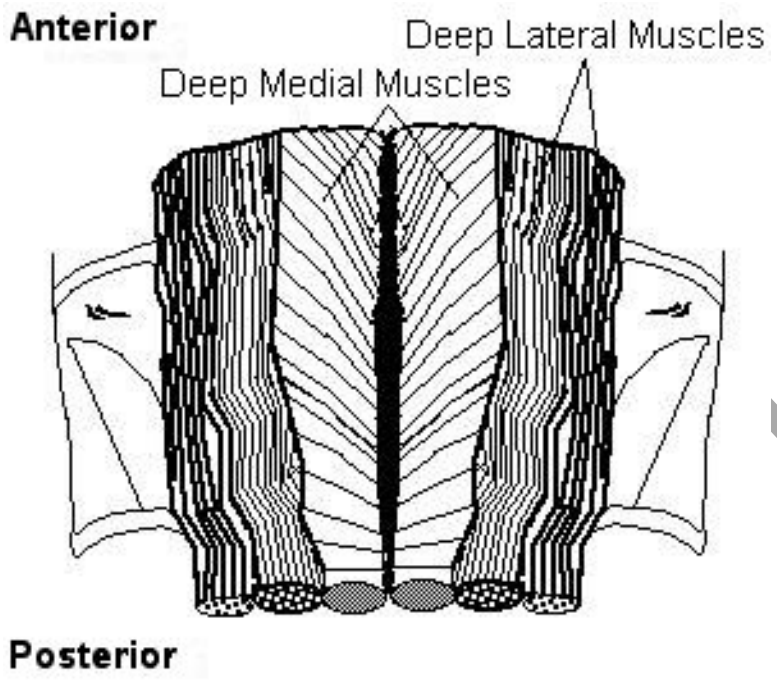

Figure AN-10-S4: Fast extensor muscles inside the dorsal part of second abdominal segment of crayfish.

9. Place the dish under the dissection microscope, position the light for optimal illumination and focus on the preparation. Use small forceps to remove the gut (the green tube in the midline) and any connective tissue from the preparation.
10. Examine the preparation and identify:
    - The six abdominal segments.
    - The paired fast extensor muscles in each segment—one muscle group on either side of the midline.
    - The medial and two lateral bundles in the fast extensor muscle group on each side of a segment.
11. Poke a hole through the most posterior segment (telson) and then thread a suture through this hole. Tie the suture to the tail using a loose loop. The exoskeleton in this area is very thin and tears very easily if knots are tied too tightly.
12. Gently pulling on the string should flex or curl the tail. If the hinge ridge has been damaged, the tail will not flex correctly.
13. Examine the cut edges of the exoskeleton with a dissecting microscope. Locate any cut ends of nerves containing the axons of sensory neurons, extensor motor fibers, and other sensory fibers. Usually the nerve ends float free of other tissue near the posterior and lateral wall of each tergum.

14. With fine forceps, carefully remove any large segments of damaged flexor muscles that obscure the viewing of nerves or interfere with the placement and operation of the suction electrode.

Placement of the Electrode

1. Place the chamber containing the crayfish on the table (or steel grounding plate inside a Faraday cage, if necessary).
2. Position the dissecting microscope over the preparation chamber and focus on the nerve trunks along the edge of the exoskeleton.
3. Place the coiled ground electrode over the side of the specimen chamber, using wax or clay to hold it in place. The copper wire in the lead and the solder joint holding the silver electrodes to the leads must not touch the saline bath.
4. Place two micromanipulators at the end of the chamber nearest the most anterior segment of the tail. Attach the extracellular suction electrode to one of the micromanipulators.
5. Locate a nerve from which to record and grossly position the electrode near the nerve. Pin down the segment in which the nerve is located. Avoid injuring the MROs or the nerves by pinning along the midline or well to the sides.
6. Align the electrode and its manipulator (or stand and clamp) at an angle that still permits the posterior segments of the tail to be flexed without touching the electrode. View the electrode tip through the microscope and move it until it is near or touching the cut end of the nerve. The opening in the tip of the electrode should be the same size or only slightly larger than the diameter of the nerve. Carefully pull back on the plunger of the 3cc syringe (without moving the tubing) and pull the end (or a loop) of the nerve into the electrode.
7. Check the height of the saline in the chamber and inside the glass microelectrode. Both the positive and negative electrodes should be in contact with the saline since the amplifier of the recording channel is used to record differentially between the inner and outer wires.
8. Position another micromanipulator (or tissue tensioner) on the end of the bath with the anterior tail segments. Attach the suture on the telson to the horizontal axis of this device, so the suture does not interfere with the recording electrode when the tail is flexed by movement of the horizontal axis of the micromanipulator or tensioner.

Experiment AN-10: Crayfish Stretch Receptor

Exercise 1: MRO1

Aim: To record action potentials from the slow-adapting MRO1.

Approximate Time: 20 minutes

Procedure

1. Click Record on the LabScribe Main window to begin recording.
2. Type MRO1 in the Mark box. Click the Mark button to attach this notation regarding the type of receptor potentials to be recorded.
3. Move the horizontal axis of the micromanipulator holding the suture on the telson and flex the crayfish tail to a position where the MRO1 begins firing slowly.
4. Click the AutoScale button on the MRO Potential and MRO Firing Frequency channels to adjust the size of the traces displayed on the Main window.

Note: The readings on the scale of the horizontal axis of the manipulator or the number of turns of the tensioner required to create this MRO1 firing frequency. A slow-adapting MRO1 should generate action potentials, like the ones in Figure AN-10-L1.

Figure AN-10-L1: The action potentials from MRO1 are displayed on the upper channel. The frequency of action potentials from MRO1 is displayed on the lower channel.

5. Move the horizontal axis of the micromanipulator or tensioner to flex the tail to a greater degree. The frequency of firing from MRO1 should increase.
6. Move the horizontal axis of the micromanipulator or tensioner to flex the tail to an even greater degree. The frequency of firing from MRO1 should increase, again.
7. Click Stop to halt the recording. Note the readings on the scale of the horizontal axis of the manipulator rack. The tail may then be returned to the original degree of flexion in order to verify the measurements. Relax the tail from its flexed state between recordings to conserve neural activity.
8. Select Save As in the File menu, type a name for the file. Click on the Save button to save the data file.

Exercise 2: MRO2

Aim: To record action potentials from the fast-adapting MRO2.

Approximate Time:

Procedure

1. Click Record. Move the horizontal axis of the micromanipulator holding the suture on the telson and flex the crayfish tail to a position where the MRO1 is firing slowly.
2. Type MRO2 in the Mark box. Click the Mark button to attach this notation regarding the type of receptor potentials to be recorded.
3. Click the AutoScale button on the MRO Potential (CH3) and MRO Firing Frequency channels to adjust the size of the traces displayed on the Main window.
4. While recording action potentials from MRO1, use a pencil to tap the suture holding the tail in the flexed position. This tap should be quick, as if the pencil is bouncing off the suture. If the movement of the pencil depresses and holds the suture too deeply, the electrode could be pulled off the nerve or the nerve could be damaged. With this technique, MRO2 should generate larger action potentials whenever the suture is tapped.
5. Click Stop to halt the recording. Relax the tail from its flexed state between recordings to conserve neural activity.
6. Select Save in the File menu to add this data to the current data file.

Questions

1. Were you able to elicit a response from the MRO2? If you did, what technique did you use?
2. Did you observe any differences between the responses of MRO1 and MRO2? Explain these differences.

Exercise 3: Adaptation of MRO1

Aim: Subject MRO to constant stretch and measure the decline in its rate of firing or adaptation.

Approximate Time: 20 minutes

Procedure

1. Click Record. Move the horizontal axis of the micromanipulator holding the suture and flex the crayfish tail to a position where the MRO1 is firing rapidly.
2. Type MRO1 Adaptation in the Mark box. Click the Mark button to attach this notation regarding adaptation of the MRO1 firing rate.
3. Click the AutoScale button on the MRO Potential and MRO Firing Frequency channels to adjust the size of the traces displayed on the Main window.
4. As you continue to record, press the Mark button every 5 seconds to place a mark on the recording. Continue recording for 2 minutes or until the MRO1 has stopped firing, whichever is shortest.
5. Click Stop to halt the recording. Relax the tail from its flexed state between recordings to conserve neural activity. Wait several minutes between stretches.
6. Select Save in the File menu.
7. Repeat Steps 1 through 6 for a position where the MRO1 is firing less rapidly.
8. Repeat Steps 1 through 6 for a position where the MRO1 is firing even less rapidly.

Data Analysis

1. Scroll through the data file and locate the first recording of constant stretch on the muscle receptor organ.
2. Use the Display Time icons to adjust the Display Time of the Main window to display a 15-second section of recording on the Main window. This section of data can also be selected by:
    - Placing the cursors on either side of the 15-second section of the recording, and
    - Clicking the Zoom between Cursors button on the LabScribe toolbar (Figure AN-10-L2) to expand or contract the fifteen-second recording to the width of the Main window.
3. Data can be collected from the Main window or the Analysis window. If you choose to use the Analysis window, click on the Analysis window icon in the toolbar.
4. The mathematical function, Mean, should appear on screen. The value for Mean can be seen in the table across the top margin of each channel, or to the right of the MRO Potential channel. The value for Mean firing frequency is displayed in the table across the top margin of the MRO Firing Frequency channel.
5. Once the cursors are placed in the correct positions for determining the mean frequency of MRO firing in this section of the recording, the value for Mean can be recorded in the on-line notebook of LabScribe by typing the names and values of the parameters directly into the Journal.

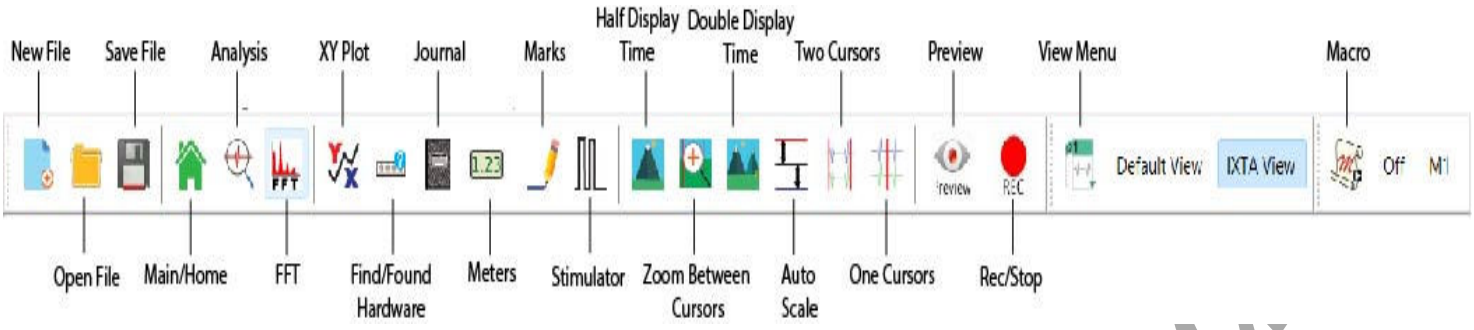

Figure AN-10-L2: The LabScribe toolbar.

6. The functions in the channel menu of the Analysis window can also be used to enter the name and value of the voltage change from the recording to the Journal. To use these functions:
    - Place the cursors at the location used to measure the mean firing frequency.
    - Transfer the names of the parameters to the Journal using the Add Title to Journal function in the MRO Firing Frequency channel menu.
    - Transfer the values for the parameters to the Journal using the Add Ch. Data to Journal function in the MRO Firing Frequency channel menu.
7. On the MRO Firing Frequency channel displayed in the Analysis window, use the mouse to click on and drag the cursors so they are about 5 seconds apart.
8. Record the value for the mean firing frequency (Mean) in the Journal using the one of the techniques described in Steps 5 or 6.
9. Move the left (first) cursor to a point on the recording that is 5 seconds to the right of the position of the second cursor. Determine the frequency of firing in this 5-second block of time. Enter that value in the Journal.
10. Continue to move the cursor to successive 5-second marks, determining the frequency of firing at each mark, and entering those values in the Journal. Continue until the end of the recording is reached.
11. Repeat Steps 1 through 10 for the other two constant stretch recordings that started with lower initial firing frequencies.

Questions

1. Plot the frequency of firing of MRO1 in the first segment as a function of the time after the initiation of the constant stretch. Is the graph linear?
2. What can be concluded about the rate of adaptation of MRO1? How does the duration of the constant stretch affect the response of the MRO1?
3. Plot the frequency of firing of MRO1 in the third (slowest) segment as a function of the time after the initiation of the constant stretch. Is the relationship linear?
4. Can you make any conclusions about the degree of constant flexion and the rate of adaptation?
5. How might an animal benefit from adaptation?

Exercise 4: Flexion & Firing Frequency

Aim: Subject MRO to constant stretch and measure the decline in its rate of firing or adaptation.

Approximate Time: 20 minutes

Procedure

1. Make sure the pinned segment of the crayfish tail remains secure as this exercise is performed.
2. Click Record. Relax the crayfish tail to a position where the MRO1 is scarcely firing. Note the position of the index mark on the scale of the micromanipulator or the position of the tensioner. Click the AutoScale button on the MRO Potential channel to adjust the size of the trace.
3. As the recording continues, quickly rack the manipulator, or tensioner, to increase the flexion of the crayfish tail by one scale unit, or a fraction of a turn. Press the Mark button to mark the recording as the final angle of flexion is reached.
4. Record the firing of the MRO1 with the micromanipulator or tensioner in this position for a period of 5 seconds. Then, relax the crayfish tail to the starting position where the MRO1 was scarcely firing.
5. If you are ready to flex the crayfish tail to a greater degree, continue recording.
6. Quickly rack the micromanipulator or the tensioner with a greater degree of tail flexion (two scale units, or fractions of a turn), mark the recording, and record the firing of the MRO1 in this position for 5 seconds. Note the position of the index on the scale of the micromanipulator or the turns of the tensioner. Relax the crayfish tail to the starting position as done in Step 4.
7. Repeat Steps 3 and 4 three more times with flexions equal to 3, 4, and 5 scale units, or fractions of a turn.
8. Click Stop to halt the recording. Relax the tail from its flexed state to conserve neural activity.
9. Select Save in the File menu.

Data Analysis

Use the same techniques used in Exercise 3 to measure the frequency of MRO1 firing at different degrees of tail flexion.

Questions

1. Plot the initial firing frequency of MRO1 at each degree of tail flexion as a function of the degree of tail flexion (expressed in units of movement for the micromanipulator or tensioner).
2. What is the relationship between the degree of flexion and the initial frequency of firing? Is the relationship linear?
3. Does MRO1 respond to any and all degrees of flexion? Why or why not?

Bibliography

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