Experiment AN-13: Crayfish Motor Nerve

Equipment Required

PC or Macintosh computer

IXTA, USB cable, IXTA power supply

IX-B3G recording cable

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

Micromanipulator with base

Faraday cage (if necessary)

Preparation dish

Cold crayfish saline (See Appendix)

Dissection microscope and light source

Dissection tools

Bath ground electrode with cable

Disposable plastic transfer pipets

Connect the iWire-B3G prior to turning on the IXTA.

Equipment Setup

1. Locate the iWire-B3G recording cable and insert the connector on the end of the recording cable into the isolated inputs of the iWire 1 channel on the front of the IXTA.

Figure AN-13-S1: The iWire-B3G recording cable.

This experiment uses a suction electrode and the B3G.

1. Attach the three connectors of the suction electrode assembly to the B3G 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 microelectrode tip, is connected to the 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: *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, a digital notch filter (as described in the Electrical Noise section)can be used.*

Note: If electrical noise is an issue, please see Appendix.

The Dissection

1. To anesthetize it, bury a crayfish in ice for 10 minutes.
2. Separate the abdomen (the "tail") from the thorax.
3. Securely pin the abdomen dorsal side down in the dish's wax or Sylgard. Cover the preparation with cold crayfish saline. Cut off the swimmerets which are the paired appendages on the ventral surface of each segment. The movements of the swimmerets interfere with the recording process if they are not removed.
4. Choose one of the middle segments to dissect. Use a scalpel to make a shallow incision along the anterior sternite (the ribs that separate the segments). Cut all the way through the membrane but avoid cutting too deep and damaging the underlying nerve cord and muscles.
5. Use fine forceps to lift up the membrane and make a midline incision extending for the anterior half of the segment. Lift the corner on one side and use small scissors to cut a small piece of the membrane off. This will expose the ventral nerve cord, including the ganglion and the three nerves (I, II, and III) coming out of each side. Identify the superficial branch of the third nerve (SBIII) which extends to the superficial flexor.
6. In this experiment, you will be attaching a suction electrode to the superficial branch of Nerve 3 and recording the spontaneous and stimulated extracellular action potentials from the six motor neurons contained in the nerve.

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Exercise 1: Action Potentials in Motor Neurons

Aim: To record motor nerve extracellular action potentials from the SBIII nerve and sort them by size.

Approximate Time: 30 minutes

Procedure

1. Use the manipulator to position the tip of the suction electrode near the exposed section of the SBIII nerve.
2. Check the size of the tip of the suction electrode. It should be the same size or only slightly larger than the diameter of the nerve.
3. Without moving the tubing, pull back on the plunger of the syringe that is connected to the tubing of the suction electrode, to pull a loop of the nerve into the suction electrode.
4. Type Motor Neurons – Spontaneous Activity in the Mark box.
5. Click Record to begin recording and click the mark button to mark the recording. A sample of this recording can be seen in Figure AN-13-L1.
6. To improve the display of the action potentials in the SBIII nerve on the Main window, Autoscale the recording.
7. If only two or three different types of action potentials are detected, use a suction electrode with a tighter fit or record from a different SBIII nerve.
8. Continue to record for two minutes.
9. Click Stop to halt recording.
10. Type Motor Neurons – Posterior Hairs Stimulated in the Mark box.
11. Click Record to begin recording and click the mark button to mark the recording.
12. Brush the sensory hairs on the posterior end of the tail fins.
13. Continue to record for two minutes.
14. Click Stop to halt recording.
15. Stimulating one of the swimmeret stumps or other sensory hairs visible at the edges of the abdomen may also alter the activity. Mark the recording appropriately and repeat the recording process for as many specific manual stimulations as desired.
16. Select Save in the File menu.

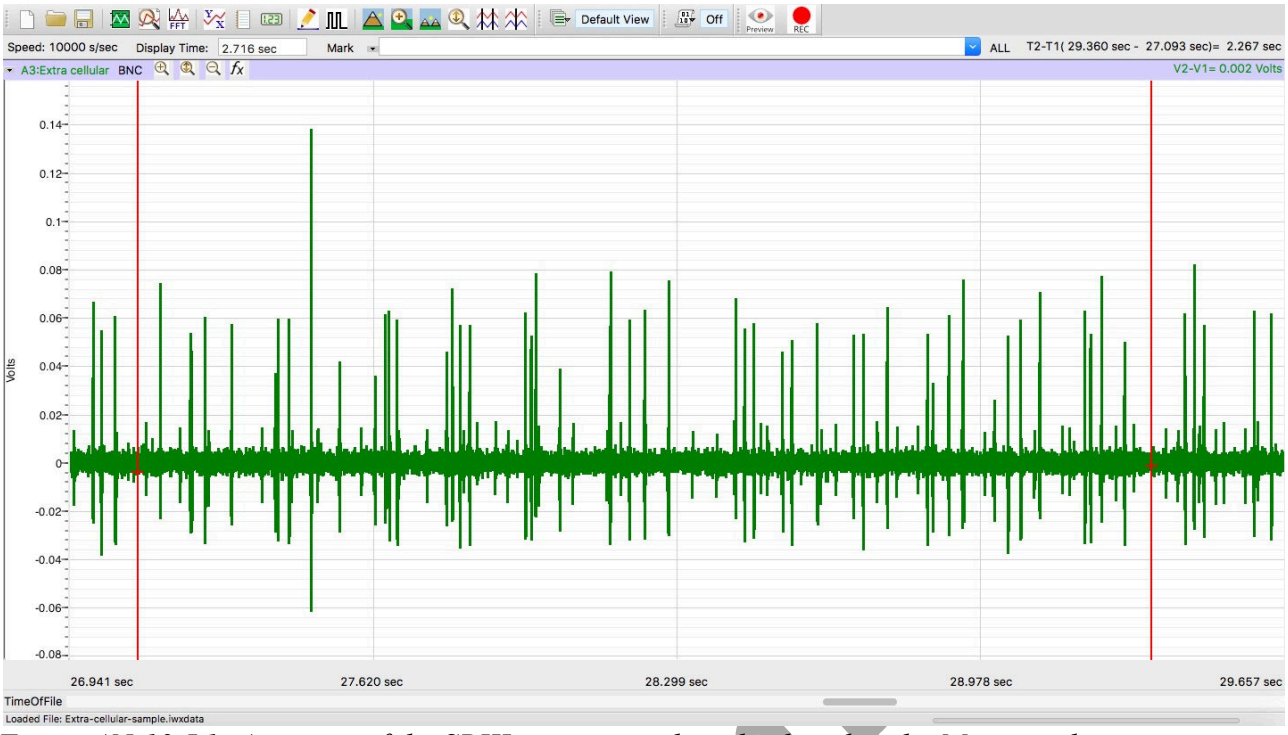

Figure AN-13-L1: A section of the SBIII nerve recording displayed in the Main window.

Data Analysis

1. An inspection of the SBIII recording will discover a number of different amplitude classes of extracellular spikes. The LabScribe Template Match function will help to sort these spikes (Figure AN-13-L2).
2. Choose one of the size classes (e.g. the largest spikes) and isolate a single spike between the cursors. Use the Horizontal Display controls to spread out the data enough that it is possible to isolate just the peak of the spike between the cursors.
3. In the channel's add function menu, choose Template Match. In the resulting dialog, click on Create a Template from data between cursors. With Tolerance set to fixed, click on the up arrow next to the zero until the number reaches .05. Click OK.
4. LabScribe will find all the spikes within the tolerance limits and isolate them in the Template Match channel. Compare the newly created spikes on the Template Match channel to the recording in the SBIII channel. If it seems to have caught most of the spikes of that size class, proceed to the next step. If it caught too few, click on the word Template in the channel menu and choose Setup Function. Increase the Tolerance number and check again. Repeat until most of the similar amplitude spikes are matched. If theTemplate Match caught too many, reduce the Tolerance number until only the correct spikes are matched.
5. Once you have a good match, you can perform steps 2-4 for each of the different size spikes. This will add a channel for each size spike.

6. It is now possible to determine the frequency of the spikes in each of the size classes. To do this, choose the first Template channel and click its add function button. Choose Periodic > Frequency. Adjust the Threshold lines to capture the spikes. Click OK, and you will be able to use the Frequency channel to determine the frequency of that size spike in any section of the data.

Figure AN-13-L2: A section of the SBIII nerve recording with two Template Match channels displaying two sorted spike sizes and the two corresponding spike frequency channels.

7. Click on the Analysis button in the Main Toolbar. Click Add Function > General > Mean. Choose any section of data by placing the cursors around it. The function window on the frequency channel will read the mean frequency of the spikes of that size in the selected section.
8. Repeat Steps 6 and 7 for each of the Template channels.
9. Enter the values in the Frequency column of Table AN-13-L1.
10. Other parameters of the spikes can also be determined. Use these parameters to distinguish the different classes of action potentials recorded in the spontaneous activity from the SBIII nerve:
    - Amplitude (Amp): Measure the amplitude of an action potential by placing one cursor on the baseline before the action potential, and the second cursor on the peak of the potential. The value for the V2-V1 function on the Nerve Action Potential channel is the amplitude.

- Duration (Dur): Measure the duration of an action potential by placing one cursor at the onset of the action potential, and the second cursor on the point where the potential returns to the baseline. The value for the T2-T1 function on the Nerve Action Potential channel is the duration.
- Polarity (Pol.): note whether the waveform of the action potentials goes up (positive) or down (negative) on the screen.

11. Enter information about the types of action potentials recorded in the Journal and Table AN-12-L1.
12. Determine the overall frequency of spikes and the frequencies of each size spike in the different manual stimulation conditions and enter those values into Table AN-13-L2.

Table AN-13-L1: Properties of Action Potentials Recorded from SBIII Nerve.

AP Type	Amp. (mV)	Dur. (msec)	Freq. (Hz)	Pol.(+, -)
1				
2				
3				
4				
5				
6				

Table AN-13-L2: Properties of Action Potentials Recorded from SBIII Nerve.

AP Type	Spontaneous Freq. (Hz)	Stim Cond 1 Freq. (Hz)	Stim Cond 2 Freq. (Hz)	Stim Cond 3 Freq. (Hz)	Stim Cond 4 Freq. (Hz)
1					
2					
3					
4					
5					
6					
Overall					

Questions

1. How many different types of extracellular action potentials did you detect?
2. What membrane properties affect the shape and size of action potentials?
3. If recorded intracellularly, all action potentials would be of a very similar amplitude. Why do extracellularly recorded action potentials vary in amplitude?
4. There are six neurons present in the nerve. You may have seen less than six classes of spikes. Why might this be the case?
5. Did the different types of spikes differ in their frequency response to manual stimulation of sensory hairs or swimmeret stumps?