

Spontaneous Nerve and Muscle Activity in Crayfish

This note discusses how to use a PowerLab with Scope to record crayfish nerve and muscle activity.

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Introduction

The superficial flexor is a paired muscle located in each segment of the crayfish tail. The muscle in each hemisegment is innervated by six motor neurons whose axons leave the ventral nerve cord in the third root. The motor neurons exhibit tonic activity and this property makes the superficial flexor preparation ideal to record spontaneous activity from motor neurons and from the target muscle fibers.

Equipment needed

PowerLab
Electrometer or ML165 pH amplifier
micro translation stage
electrodes
ML110 Bridge Amp (optional)
Chart/s or Scope software

Method

In theory the dissection is simple. The isolated tail is pinned to the base of a saline-filled dish with its ventral surface uppermost. The student uses a dissection microscope to identify segments, which are bound by sternites ('bars' of cuticle) at the rostral and caudal ends. Forceps and the tip of a sharp scalpel blade are used to remove the arthroial membrane over the central portion of the segment, to expose the ventral nerve cord and the superficial flexor muscles.

In practice this can be difficult for students with minimal dissection skills. However, since the muscle is segmental up to four potential preparations can be found in a single tail (the first and last segments are usually damaged when the preparation is pinned out). The superficial flexor muscle is located immediately below the arthroial membrane and is easily damaged during dissection. It is critical that a good light source is used and that the student identifies the muscle prior to dissection. It may be helpful to remove the dorsal surface of the tail and much of the fast flexor muscle (the 'meat' of the tail) prior to pinning to the dissection dish. In such a preparation identification of the

muscle and the third root may be facilitated by using a mirror to reflect light from below. Finally, in some preparations connective tissue may be located between the muscle and the ventral nerve cord. While it does not obscure the muscle, the connective tissue must be removed to expose the third root

The superficial flexor muscle has large fibers which can be easily impaled with glass microelectrodes. Conventional microelectrode techniques are used to impale single muscle fibers, but care must be taken since the fibers form a thin sheet and it is easy to push the electrode tip through the muscle. Students who are accustomed to measuring resting membrane potentials are usually amazed at the spontaneous excitatory postsynaptic potentials (EPSPs) they see on the oscilloscope screen. In many fibers they immediately notice that the EPSPs are not the same amplitude and that their frequency is not constant.

Suction electrodes can be made from a pressure half cell and a broken glass microelectrode. The appropriate plug is connected to one wire in the screened cable while the second wire is soldered to silver wire which is wrapped around the glass electrode. The screened wire and plug is bonded into a rigid plastic tube, which is held in a manipulator. Saline is sucked into the electrode and the half-cell and the tip of the electrode is then carefully placed onto the third root. Gentle suction is applied to draw the nerve into the electrode tip.

Connecting the PowerLab

The BNC cable from the electrometer can be connected directly to the PowerLab data acquisition unit as in Figure 1 (an electrometer is a high impedance voltmeter. If an electrometer is unavailable an ADInstruments ML165 pH amplifier can be used as a substitute). Alternatively, a BNC T-connector can be used to connect the electrometer to an oscilloscope and to the PowerLab unit. The output from the suction electrode may be connected directly to the PowerLab unit, or the instructor may prefer to use a differential amplifier (so that high and low pass filters may be used) and then connect this amplifier to the PowerLab unit. In this case the two leads

from the suction electrode are connected to the differential amplifier and the screen is grounded. A single BNC cable is used to connect the amplifier output to a second input channel on the PowerLab.

Data Display

Adjustment of the gain on the second channel allows the student to correlate spontaneous EPSPs with extracellularly recorded motor neuron activity. It should be appreciated that selection of an electrode with a small tip, or suction onto a branch, may permit selective recordings to be made from only a fraction of the six motor neurons. Under these conditions it may be possible to correlate motor neuron spikes with only some EPSPs, Figure 2.

Students can set the recording parameters within Scope, or this can be done by the instructor prior to the laboratory, saving the recording parameters as a separate (locked) "Settings" file. The parameters may include renaming the axes and setting the sampling rate, time base and voltage range. Menu items that are not required for the experiment can be hidden from view or locked. The variability of the signal recorded from different preparations may require that the students change some of the settings.

The "Input Amplifier" control of Scope can be used to monitor the signal from the electrometer while not actually recording it. This facility may be used

while penetrating single muscle fibers to help set the DC offset to an appropriate level. At this stage the student can return to the main Scope window and click on the start button. Recordings are made on a series of "Pages", each of which is analogous to a screen shot on a storage oscilloscope.

Close inspection of selected regions of the traces can be achieved by using the Zoom Window in the Windows menu. In this way the Marker and cursor can be placed in exactly the required location. This option also allows the user to copy and paste the magnified trace directly into the Scrapbook or into a word processing document as part of the student's laboratory report.

Annotation

During the recording session students can type annotations into the Page Comments, which is unique to each page. The Notebook facility can also be used to writeup overall experimental procedures. In this way all observations and comments are recorded along with the data rather using a separate notebook (or scrap of paper!).

Data Analysis

Data analysis in Scope does not require connection to the PowerLab unit. Therefore, students can analyze their data on any computer with sufficient RAM for the Scope software (Student Packs of Scope software complete with documentation are

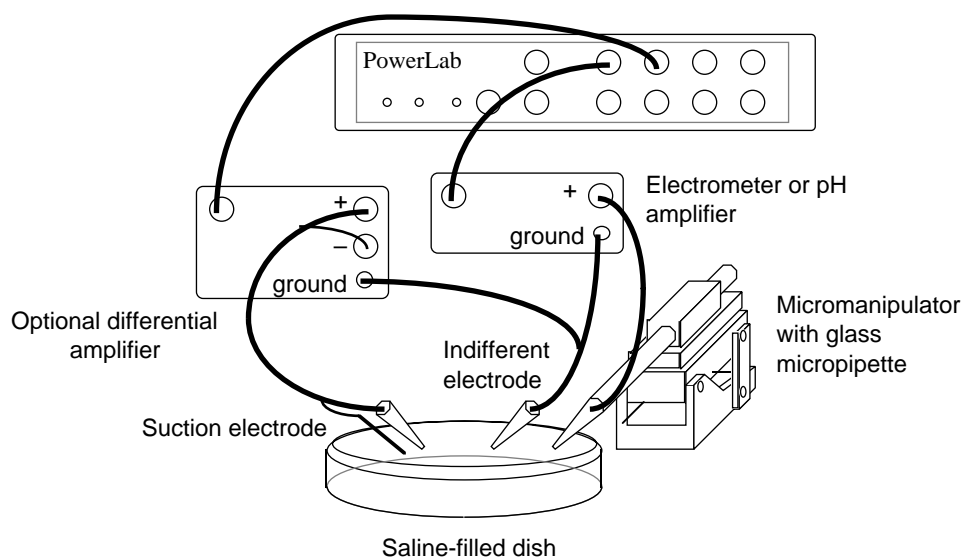


Figure 1. Diagram showing connection of PowerLab to the apparatus. Note that the differential amplifier can be dispensed with and the suction electrode connected to the + and - terminals of Channel 1 of a PowerLab with differential inputs. The connection from micropipette to electrometer should be shielded.

available). It may be advisable to provide each student with a floppy disk so that they can perform data analysis on any available computer. Students simply double-click on their icon to display their data on the computer screen. Pages can be addressed in order by clicking in the icon in the lower right corner, or by clicking in the lower margin on the icon containing the appropriate page number. In this way, data analysis can be performed after the experiment, rather than waste precious laboratory time.

Accurate measurements can be made using the Marker and the cursor. The amplitude of the spikes can be measured and a histogram constructed on the number of each class of spike recorded with a given time frame. This allows students to identify up to six motor neurons on the basis of spike amplitude and obtain an estimate of the rate of firing for each neuron. While this may be repeated using EPSP amplitude it must be appreciated that not all muscle fibers are innervated by all motor neurons. This may be demonstrated elegantly by correlating a nerve spike and the resulting EPSP in the muscle and noting one or more class of nerve spike with no concomitant EPSP in the impaled muscle. Similarly there may be one or more class of EPSP with no

matching nerve spike, Figure 3. This may be observed if the suction electrode tip is small or recordings are made from a branch of the third root containing only a fraction of the motor axons.

More to do

A simple addition to this laboratory exercise involves backfilling of the motor axons in the third root. A dissected ventral nerve cord is pinned to the base of a small saline-filled dish and one or more third roots are identified. A syringe filled with petroleum jelly is used to construct a 'well' with a third root passing through one wall. The end of the root is cut and the saline in the well is filled with a dye (for example 0.5 M cobaltous chloride). After over-night incubation in a refrigerator the preparation is moved to a dish with fresh saline and the cobalt ions are precipitated by adding ammonium sulfide. After a few minutes the filled motor neurons and their axons can be seen through a dissection microscope. The preparations may be subsequently fixed, dehydrated and cleared so that the location of the cell body and the branching patterns of each neuron within the ganglion can be determined.

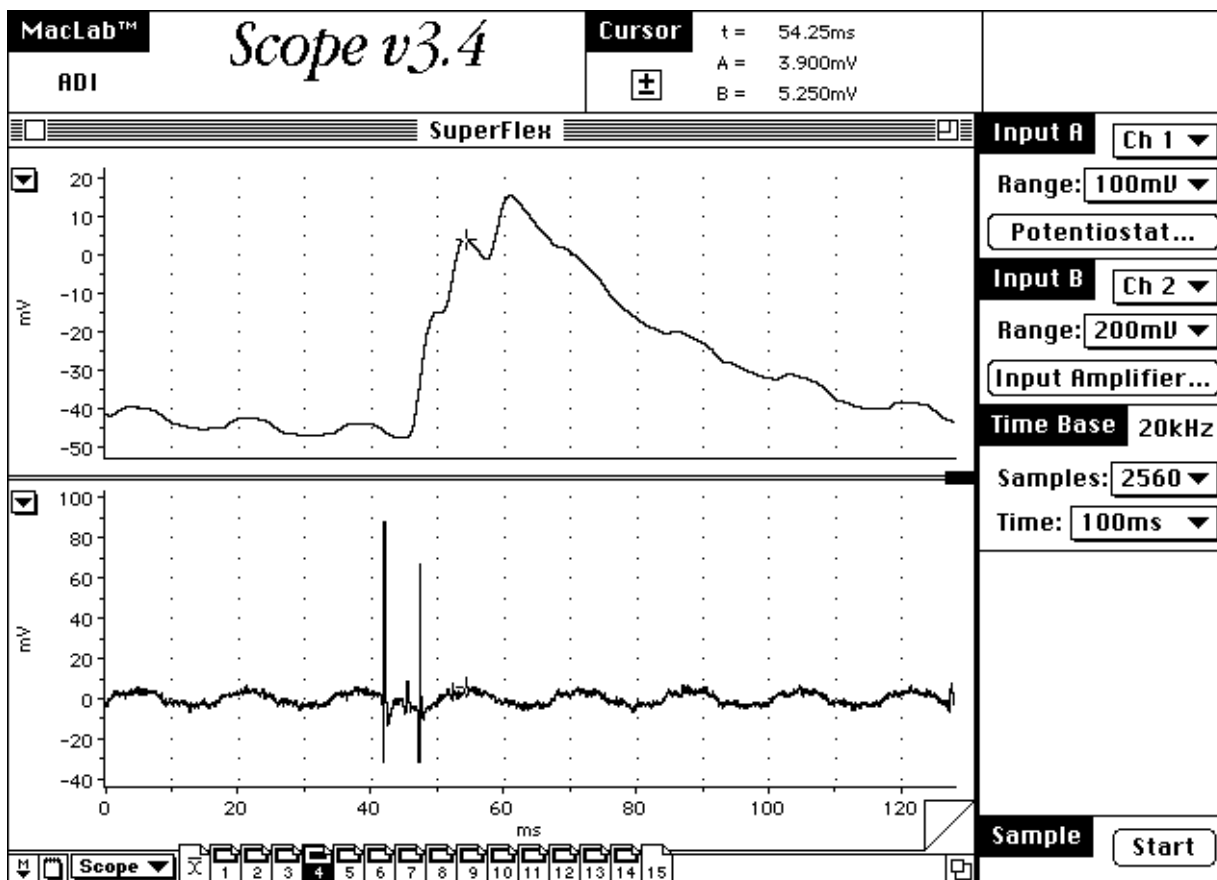


Figure 2. A typical trace obtained using Scope software. Many traces can be stored in the one file and are viewed by selecting the appropriate 'page' at the bottom of the screen. In this case page 4 has been selected for display from the 14 scans in this file. Scan 15 is blank and will be used for the next recording.

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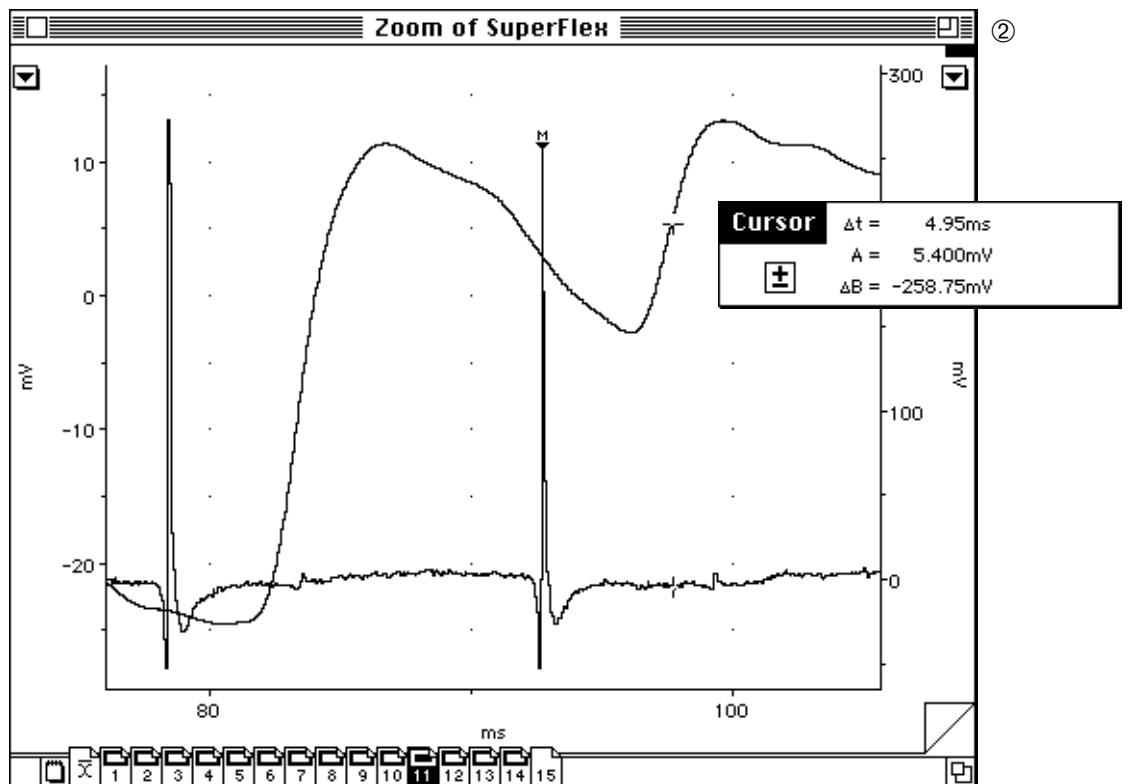
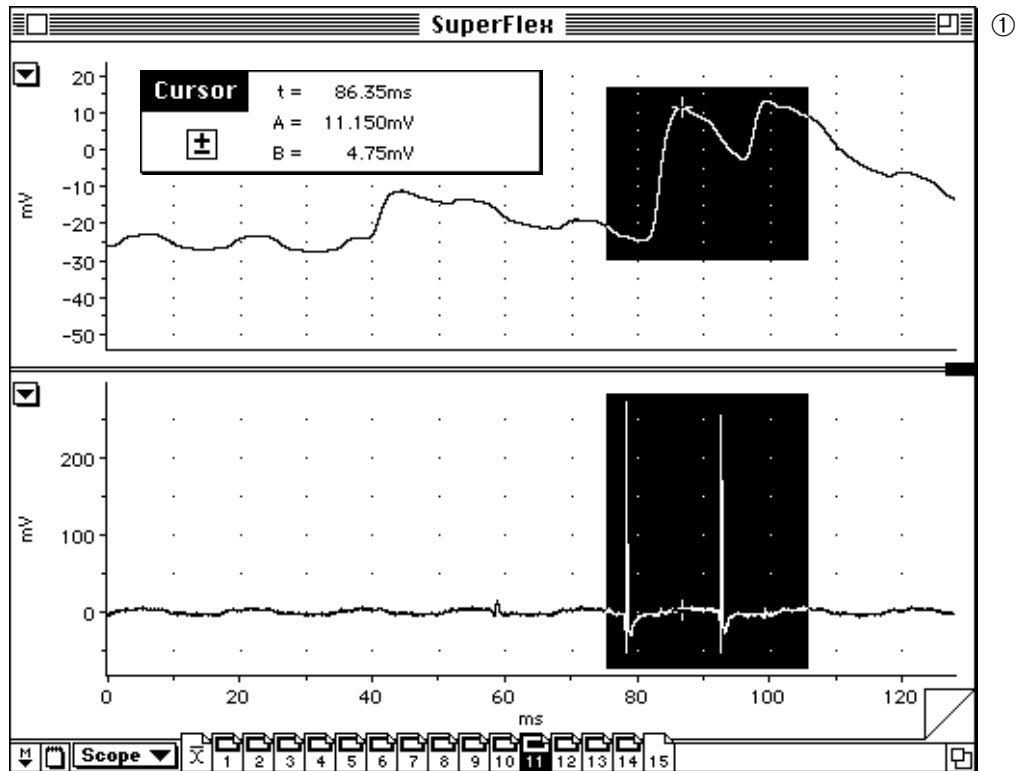


Figure 3. A portion of the signal has been selected on both channels, ①, and the Zoom Window, ②, used to both enlarge and overlay the traces. The marker tool has been placed on a spontaneous EPSP and the cursor on an apparently related increase in motor neuron activity about 5 ms later.