

The Cockroach Ventral Nerve Cord: Extracellular Action Potentials

This note discusses how to use PowerLab with Scope to record action potentials and illustrate conduction down the ventral nerve cord of cockroaches.

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Introduction

This simple and popular laboratory exercise uses cockroaches to demonstrate action potentials and illustrate conduction down the ventral nerve cord. Activity is produced by applying gentle puffs of air to the anal cerci, which produces action potentials in a number of “giant” axons in the ventral nerve cord. The large diameter of the axons produces large amplitude spikes in an extracellular recording set-up.

Equipment

Scope or Chart/s software
PowerLab
ML132 Bio Amp

Method

The dissection of the ventral nerve cord is simple and requires care rather than skill. While the laboratory provides a good illustration of action potentials, it can also be used for students to practice fine dissection under the microscope. A decapitated cockroach is pinned to the base of a

dissection dish with its ventral surface uppermost. The preparation is viewed through a dissection microscope while fine scissors and forceps are used to remove the cuticle from the ventral surface of the abdomen. Starting at the rostral end, the cuticle over one segment is cut laterally, peeled away and removed – cutting with the scissors is not required to free the cuticle from the soft underlying body parts. This process is repeated until only the two terminal segments are left.

The gut and trachea are removed by grasping from one side with forceps and pulling to that side. The tissue is cut away, being careful not to cut in the mid line. Flushing the body cavity with insect saline reveals a pair of muscles running parallel with the ventral nerve cord. The muscles are carefully removed together with any extraneous material on the ventral nerve cord - extreme care must be taken not to pinch the nerve. The cleared nerve cord is composed of a series of ganglia (swellings resembling small white grapes) which are linked by a pair of nerves.

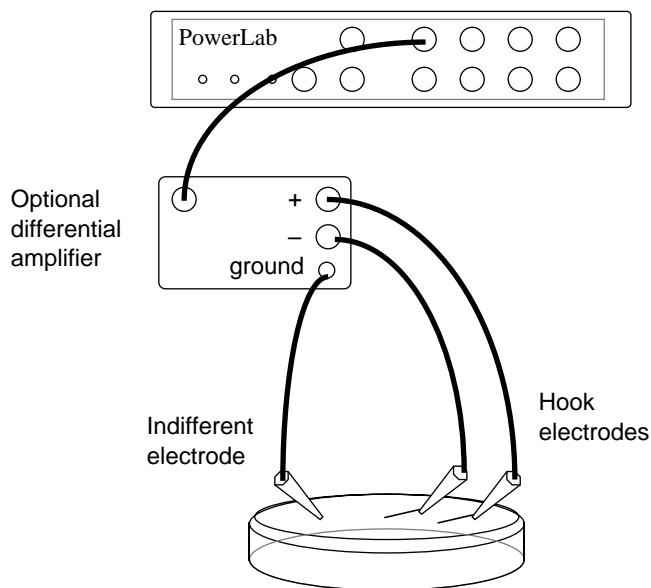


Figure 1. Diagram showing connection of PowerLab and the general set up. The optional differential amplifier can be a ML132 Bio Amp which provides high and low pass filtering. The hook electrodes could alternatively be directly connected to the differential inputs of one of the PowerLab channels.

A pair of metal hook electrodes is used to record spike activity from the ventral nerve cord. The electrodes can be made from twin core screened cable and two short lengths of silver wire. Silver wire is soldered to each wire, and the cable is inserted into a rigid plastic tube and held in place with shrink tubing. The ends of the silver wire are bent to form tiny hooks. The plastic tube is held in the manipulator and the two wires are connected such that:

- The electrodes which can be directly connected to the differential inputs of one of the channels of a PowerLab unit; or
- A differential amplifier with the the screen grounded, such as a ML132 Bio Amp (see Application Note AN325 - Recording Nerve Activity), especially if high and low pass filtering is required. A single BNC cable is used to connect the amplifier output to the PowerLab, Figure 1.

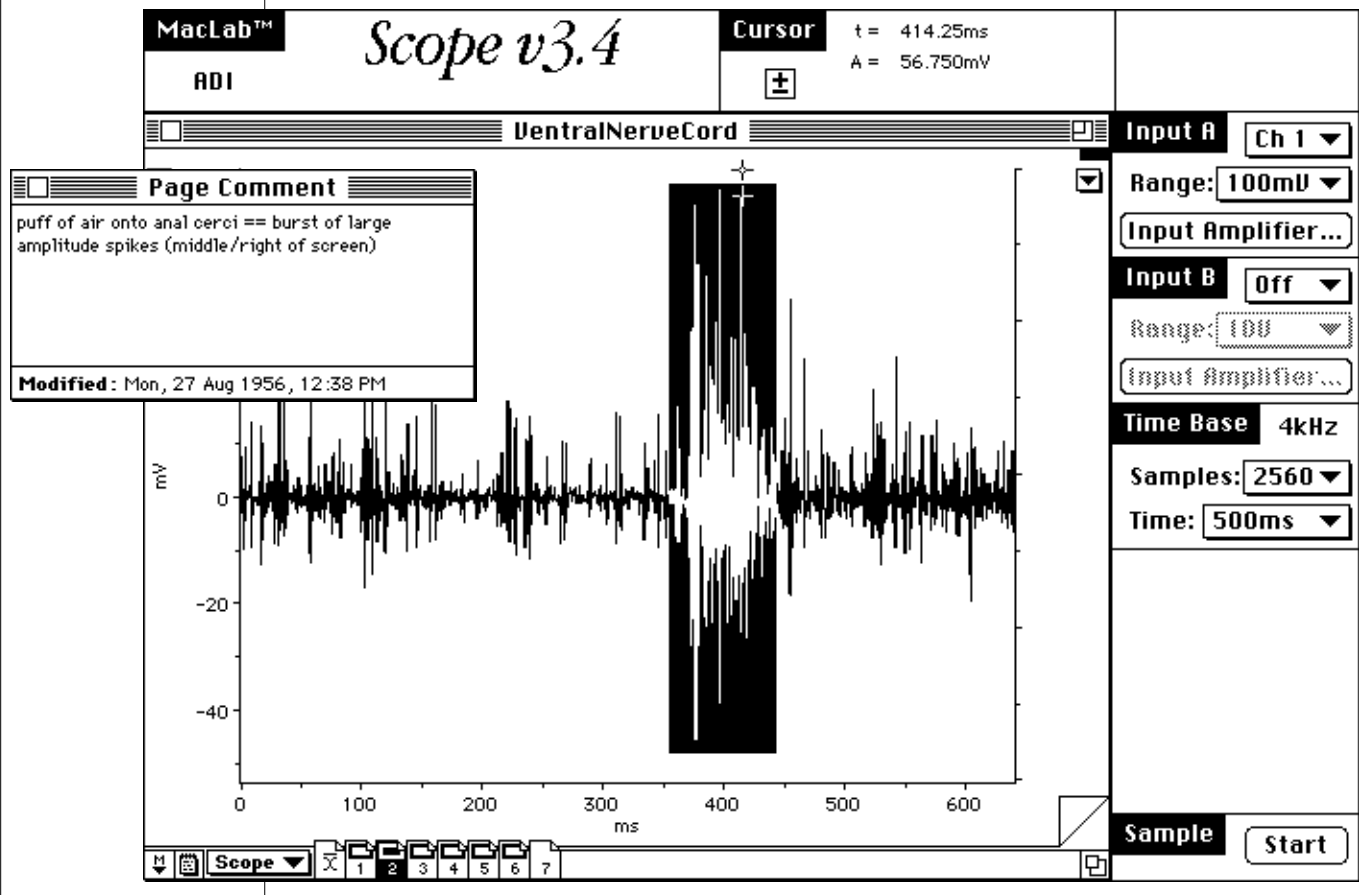
A BNC T-connector can be used to connect the output of the amplifier simultaneously to the PowerLab, a traditional oscilloscope, or to an audio unit. In this way students can monitor nervous activity with Scope software and the PowerLab, the oscilloscope, while listening to the signals on a loud speaker.

The manipulator is used to place the silver hook electrodes under the exposed ventral nerve cord.

The nerve cord is gently lifted away from the body cavity and a small piece of paper wipe is used to remove excess saline from the recording electrodes. It should be noted that, unlike crustacean preparations, this insect preparation should not be immersed in saline for a prolonged period. Adjustment of the PowerLab's gain will allow the student to observe spontaneous nervous activity in the ventral nerve cord. Gently blowing on the anal cerci produces large amplitude spikes; at this stage most students must reduce the gain. This demonstration of action potentials can be made even more dramatic if the output of the amplifier is also fed into a speaker. The roaring of spikes heard on the speaker usually brings the entire laboratory to a halt.

Using Scope software

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: turning off unused channels, renaming the axes and setting the sampling rate, time base and voltage range. The variability of the signal recorded from different preparations may require that the students change some of the settings. This is a simple operation because the PowerLab interface is intuitive and easily



understood.

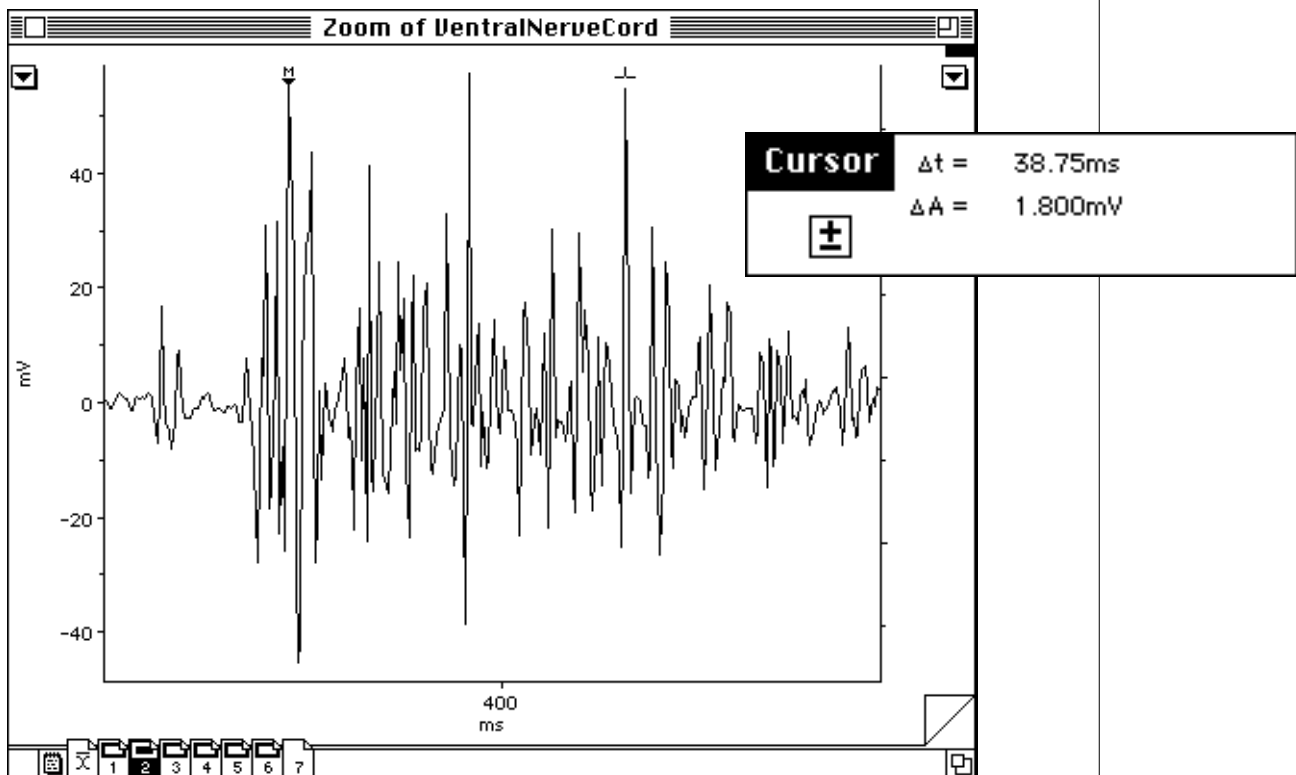
The “Input Amplifier” feature of Scope can be used to monitor the incoming signal without committing it to memory. This facility may be used to perform a “dry run” prior to recording a response. At this stage the student can return to the SCOPE screen and click on <Start> to initiate the trace and stimulate the preparation. The instructor may provide each student group with a marker, which can be connected to a second recording channel. The marker can be as simple as a contact switch connected to a battery; this can be used by the students to indicate when the cerci were being stimulated with a puff of air from a dry Pasteur pipet. Each trial is displayed on a “Page”, which is analogous to a screen shot on a storage oscilloscope. During the recording session students should be encouraged to type annotations into the “Notebook” (for general observations), and into the “Page Comments” (specific to a particular run) which are unique to each page. Scope can be

regarded as an electronic laboratory note book - all observations are stored with the relevant data.

Data Analysis

Analysing data using SCOPE software is easy and does not require a PowerLab unit. Therefore, students can analyse their data on any computer with sufficient RAM and the SCOPE software — student packs of Scope complete with manuals are 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.

Accurate measurements can be made using the “Marker” and the cursor. In this experiment, measurements can be made to determine how many neurons fire based on spike amplitude. Close inspection of selected regions of a trace can be achieved by dragging to “Zoom Window” in the



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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 then paste the window directly into the Scrapbook or into a word processing document as part of the student's laboratory report.

More to do

Additional experiments include determining how many cercal hairs must be stimulated to produce an action potential and whether stimulation from different directions produces different response. This latter study may be facilitated by a recording in which one or two units can be recognised in successive recordings.

If sufficient equipment is available a second set of

hook electrodes can be placed some distance down the nerve cord. Stimulation of the anal cerci will produce a similar recording at both locations. If the same single unit can be identified at both locations, the time delay between the unit in the two traces and the distance between the two electrodes can be measured. These data can be used to provide an estimate of conduction velocity, which can be compared with values obtained from the frog sciatic nerve. This not only correlates the two laboratory exercises, but micrographs of cross sections of the two nerves can be used in a class discussion on the effects of myelin and axon diameter on conduction velocity.

