

Membrane Potentials in Crayfish Muscle

This note discusses how to use a PowerLab with a simple preparation to record membrane potentials from large crayfish muscle fibres.

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Introduction

Membrane potentials from large crayfish muscle fibres can be used to introduce students to intracellular microelectrode techniques. The Nernst and Goldman Equations can be studied by changing extracellular cation concentrations. This exercise can be used in animal and neurophysiology courses.

A PowerLab unit with Chart or Scope software gives students permanent data files. Because data is continuously recorded it also removes the necessity of, and inaccuracy in, making measurements during the experiment. In experiments which vary the level of extracellular cations, the ion levels of the saline can be varied using a simulation, to see the effect on the equilibrium potentials and membrane potential across a squid giant axon membrane, calculated using the Nernst and Goldman Equations. This data can be contrasted with the students' measurements and this comparison has proved invaluable to the students during data analysis.

Equipment needed

PowerLab
Electrometer or ML165 pH amplifier
micro translation stage
electrodes
Chart/s or Scope software

Method

The fast extensor muscle in the tail of a crayfish is a good preparation since the dissection is easy and each of the six abdominal segments contains a pair of fast extensor muscles. Students with rudimentary dissection capabilities are able to dissect the tail with only a pair of curve-blade scissors. The crayfish is cooled on ice, decapitated, and the tail is removed. One blade is inserted into the cut end of the tail and the exoskeleton and flexor muscle are cut along the lateral surface, following the contour line in the cuticle. The cuticle on the dorsal surface is pulled away from the rest of the tail and placed in a petri dish filled with saline and lined with soft

wax. A pin is placed at each end of the tail and a dissection microscope is used to identify a pair of fast extensor muscles in each abdominal segment. Students often comment on the large size of the individual muscle fibres in the two regions of the fast extensor muscle in each hemisegment. At this stage help may be required to remove the gut and any connective tissue over the muscle.

Conventional glass microelectrode techniques are used to penetrate the membrane of a muscle fibre. A glass microelectrode is placed in an adapter and attached to the probe of an electrometer. An electrometer is a device that measures the voltage difference between two electrodes. If an electrometer is unavailable, an ADInstruments ML165 pH amplifier can be used as a substitute. A micro-manipulator firmly holds the probe and is used to place the tip of the microelectrode into the

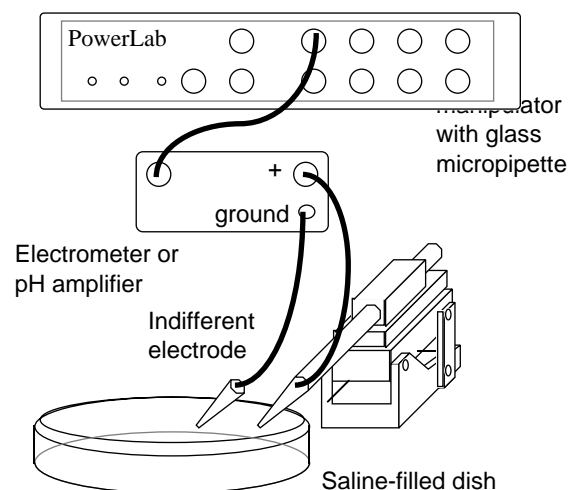
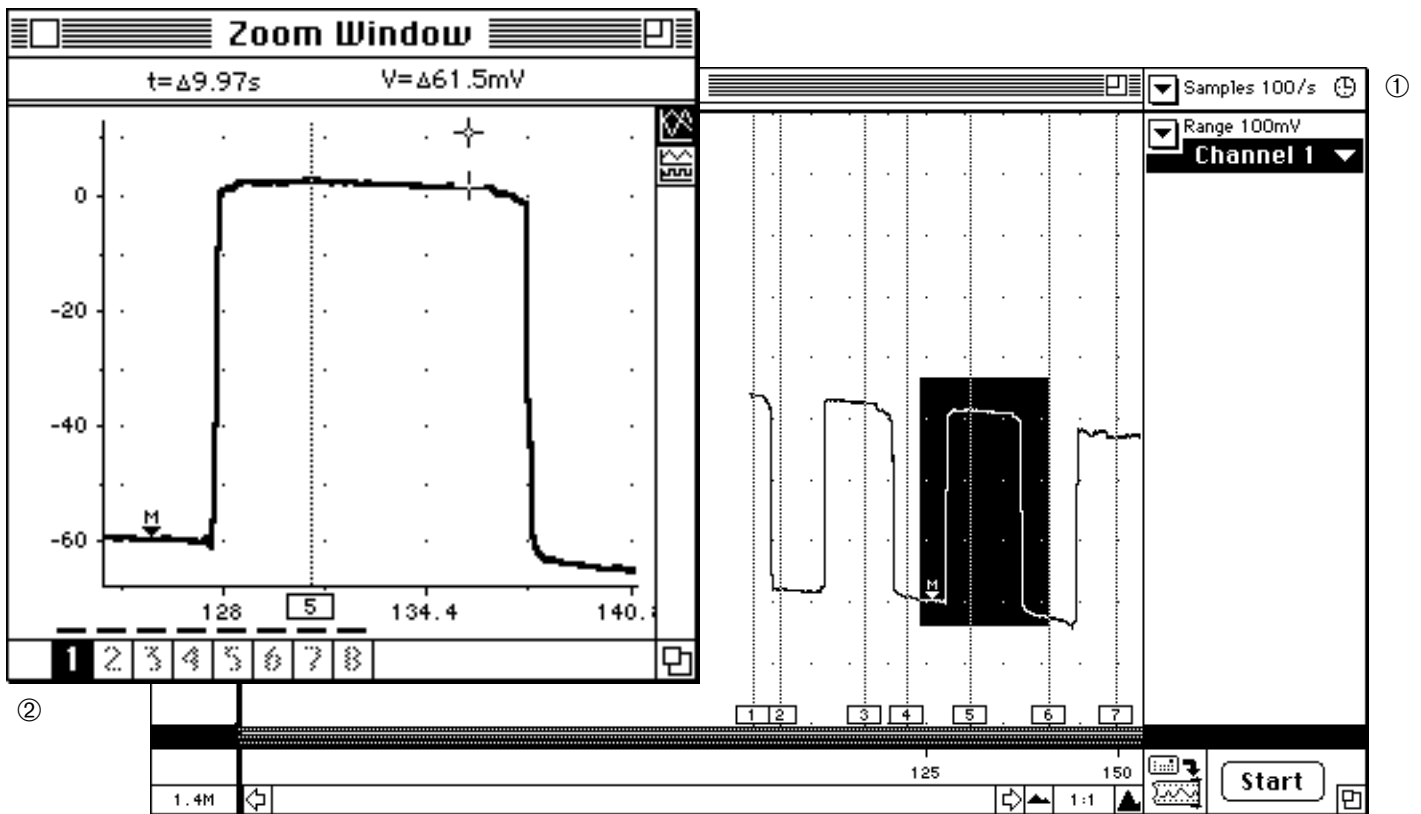


Figure 1. Diagram showing connections between the PowerLab, electrometer, and micropipette electrode (positioned by micromanipulator). The connection from micropipette to electrometer should be shielded.



Comments

Channels: 1 2 3 4 5 6 7 8

	Time	Comment
1	102.5	Out of cell
1	106.12	enter cell - Em about -62 mV
1	117.12	out of cell
1	122.62	Penetrate cell - Em about -65 mV
1	130.75	Out of cell
1	141.12	Penetrate cell - not stable - Em about -68 mV
1	149.87	Out of cell again - end of sequence

Show Times

Delete Go To

Figure 2. A typical trace obtained with PowerLab and Chart software, ①, five point moving average smoothing has been applied to reduce the apparent noise. A selection of the trace has been selected and is shown expanded in the Zoom window, ②. As the electrode penetrates a cell there is a pronounced change in the potential of about 60 – 65 mV. Chart can be left running so that several cycles are completed in the one recording session. Annotations can be made using the comments feature and can be displayed in the Comments window, ③.

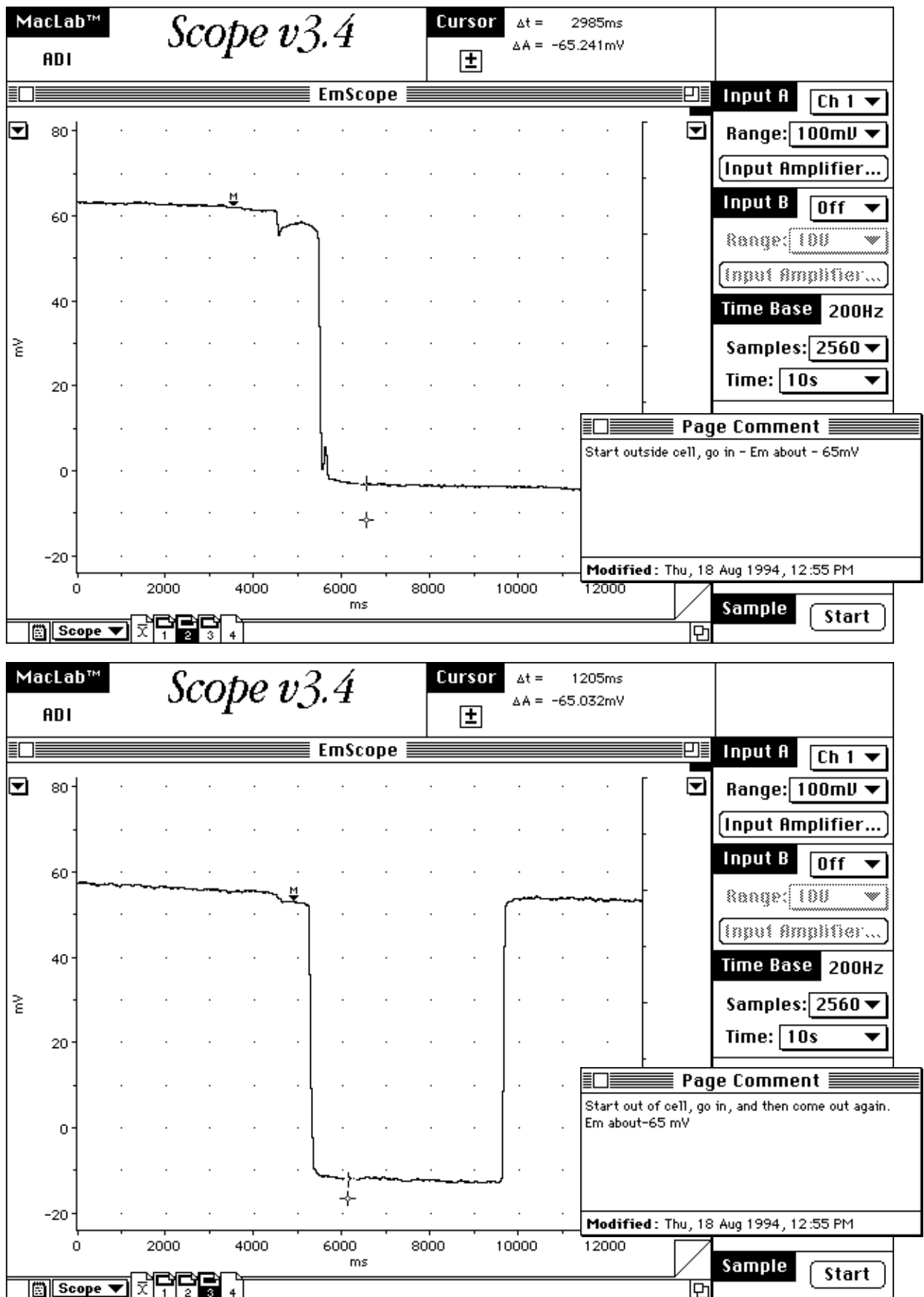


Figure 3. If you are familiar with using an oscilloscope to monitor this type of experiment then you may prefer to use PowerLab with Scope software. Usually you will only have time to record one cycle per Scope 'page', but of course you can record new sweeps on different pages. The marker tool can be used so that the cursor gives a direct reading of ΔV in the Cursor panel (about 65 mV in these figures).

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saline bath. An indifferent electrode is placed in the bath and connected to the electrometer, which in turn is connected to the PowerLab. At this stage the electro-meter is turned on and the trace (as monitored by the PowerLab unit in conjunction with Chart software) is positioned close to the top of the screen using the controls on the two instruments.

The micromanipulator is used to place the tip of the microelectrode over a muscle and then slowly lower the tip. A rapid displacement of the oscilloscope trace indicates that the microelectrode tip has touched the membrane. Membrane penetration is achieved by gently tapping the manipulator or lowering the tip. A fast, maintained downward deflection of the trace indicates that the fibre has been impaled and the membrane potential can be measured. When the equipment has been set up and is working a large number of readings can be taken in a short time period, Figures 2 and 3.

Connecting the PowerLab

The electrometer can be connected directly to the PowerLab data acquisition unit using a simple BNC cable. If the class is composed of students with limited knowledge of intracellular techniques the instructor may decide to first have student use oscilloscopes and then switch to PowerLab units when they feel comfortable with impaling muscle fibres. Alternatively, BNC T-connectors permit the electrometer to be connected to both an oscilloscope and a PowerLab unit. In this way students can monitor membrane penetration on the oscilloscope while making a "hard copy" on the computer. The output of the electrometer can be displayed on the computer screen with either Scope or Chart software.

Data Display

Students can set the recording parameters within Chart or Scope. Alternatively this can be done by the instructor prior to the laboratory session, saving the recording parameters as a separate (locked) "Settings" file. The parameters may include: turning off unused channels, naming and expanding the remaining channel, and setting 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.

Both Chart and Scope software contain the ability to zoom in on a selected region of data for examination and measurement. This can be done to obtain a close-up view of the recording, to insure accurate

measurement with the Marker and the cursor, and the trace in the Zoom Window can be printed. Alternatively the Zoom Window can be copied and then pasted either into the Scrapbook or directly into a word processing document as part of the student's laboratory report.

Annotation

During the recording session, students should be encouraged to type annotations into the Page Comments, which is unique to each page. During the experiment, students use the computer keyboard to annotate specific events on their data file. The student types a descriptive message (in the "Page Comments" in Scope and in "Comments" boxes in Chart) which can be referred to during subsequent data analysis. Types of annotation for this experiment include the region and hemisegment from which the measurement was made and the saline composition.

Data Analysis

Students can save their data as one large file or many smaller files. It may be advisable to provide each student group with a floppy disk, so that computer hard drives do not become cluttered with extraneous files. Furthermore, a floppy disk provides students with the flexibility of performing data analysis on any available computer as the PowerLab unit is not required for data analysis.

Students simply double click on their file to access their data. Data is reviewed by scrolling through the Chart file and clicking on the Comments boxes, or by flipping screens in the Scope programs and reading the Page Comments. In both Chart and Scope the "Marker" is used in conjunction with the cursor to measure the difference in the voltage before and after membrane penetration. Comprehensive instructions on data analysis can be obtained from the manual or Chart Extensions.

More to do

This preparation can be used to measure resting potentials in the two regions of a muscle and compare values from left and right and from different segments. This introduces students to the variability of membrane potential recorded from different fibres and the data can be used to introduce statistical analysis. Additional experiments involve finding a small region of fibres which have similar membrane potentials and replacing the saline with a series of modified salines in which the level of potassium or sodium has been changed.