

# Postsynaptic Potentials in Crayfish Muscle

This note discusses how to use a PowerLab with Scope software to evoke excitatory and inhibitory postsynaptic potentials from single muscle fibres.

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## Introduction

A simple invertebrate preparation can be used to record evoked excitatory and inhibitory postsynaptic potentials from single muscle fibres. The exercise uses intracellular techniques and introduces students to the stimulator and the concept of triggering PowerLab with Scope software.

## Equipment needed

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

## Method

The dissection of the fast extensor muscles in the crayfish tail is easy, even for students with minimal dissection skills. Each abdominal segment contains a pair of fast extensor muscles, so there are many available for preparations. The macroscopic dissection is followed by removal of excess flexor muscle, connective tissue and the gut from the preparation using the dissection microscope.

The muscle in each hemisegment is innervated by a branch of a segmental nerve. The student must first select a particular hemisegment in which the fast extensor muscle fibres appear to be intact. A manipulator is used to place a pair of metal hook electrodes lateral to the fast extensor muscle in the caudal portion of the hemisegment; it is not necessary to identify the (cut) nerve in this preparation. Brief (1 ms) electrical shocks are applied through the hook electrodes to stimulate the nerve and produce a muscle response. In most fresh preparations, increasing the stimulus voltage above a critical threshold produces rapid muscle twitches, which can be seen through the microscope. At this stage the stimulation should be halted and the voltage reduced to a minimum level.

Conventional glass microelectrode techniques are used to penetrate single fast extensor muscle fibres.

The probe, which holds the microelectrode, and the indifferent electrode are connected to the electrometer, and a BNC cable is used to attach the output of the electrometer to an input channel on the PowerLab.

A laboratory stimulator may be used to stimulate the nerve and trigger the PowerLab. However, the PowerLab unit contains a sophisticated stimulator, the parameters of which are similar to those on a laboratory stimulator. So a student conversant with one will be able to easily use the other. In addition the “Sampling” command permits the triggering parameters to be set as well as options such as single and repetitive stimulation, and averaging. When the stimulator has been invoked the parameters are displayed on the Scope screen, so that students can make changes without navigating through the menus to return to the original window.

The PowerLab output acts like a traditional stimulator except that it is controlled from the Scope software. No special cables are required for triggering the PowerLab — when the Start button is pressed, recording and stimulus are both controlled from the Scope software. Adjustment of the PowerLab voltage output through the stimulator panel allows the delivery of a shock to the nerve to be synchronised with the initiation of the sweep.

The “Input Amplifier” can be used to monitor the signal from the electrometer without committing it to memory. This facility may be used to penetrate single muscle fibres, set the DC offset to an appropriate level, and find the voltage threshold for the production of an excitatory postsynaptic potential. The Stimulator voltage is slowly increased until a postsynaptic response is seen shortly after the stimulus artifact. At this stage the student can return to the main Scope screen and click on “Start” to initiate the trace and stimulate the preparation.

Adjustment of the voltage intensity over several trials introduces the student to the concept of threshold and innervation of a particular muscle fibre by more than one motor neuron. Each trial is displayed on a “Page”, which is analogous to a

screen shot on a storage oscilloscope.

In this preparation, most fibres are innervated by the common excitor and common inhibitor innervating that hemisegment. The location of the stimulating electrodes may be altered and the polarity changed to differentially stimulate the two motor neurons. The common excitor produces an action potential in most fibres in the hemisegment. The evoked contractions can be a problem, since the glass microelectrodes are often dislodged by muscle twitches. On the screen the stimulus artifact is followed by an action potential and a rapid upward deflection of the trace as the electrode comes out of the cell. This movement problem may be minimised by placing the electrode tip in a region showing little movement, or by fatiguing the preparation by repetitive stimulation. In this latter case, the action potential is often replaced by an excitatory postsynaptic potential (EPSP), however there is often a concomitant reduction in the amplitude of EPSP's produced by the other motor neurons.

Six motor neurons are located in the nerve in each hemisegment. The innervation patterns can be examined by recording from muscle fibres in different locations with the hemisegment. Careful adjustment of the shock voltage can be used to determine how many motor neurons innervate a

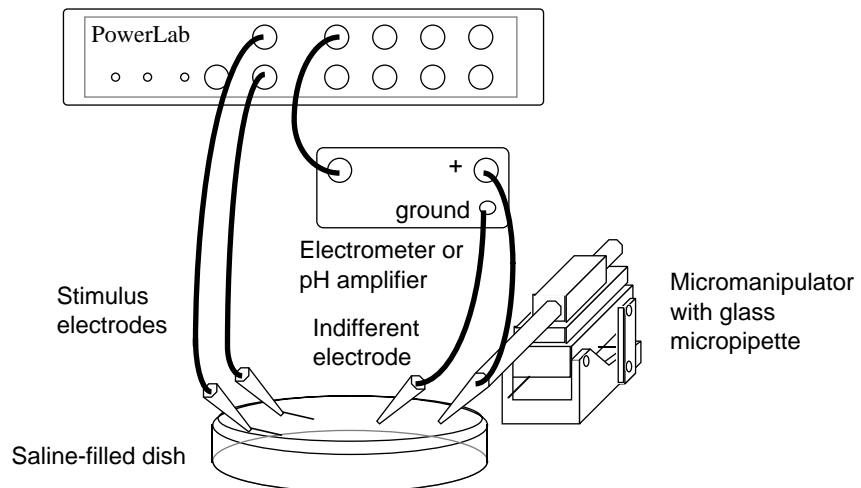
particular muscle fibre. This preparation can also be used to illustrate postsynaptic inhibition as well as facilitation and temporal summation.

### Connecting the PowerLab

A BNC cable from the electrometer can be connected directly to the PowerLab data acquisition unit as in Figure 1. Alternatively, a BNC T-connector can be used to connect the electrometer to the oscilloscope and to the PowerLab unit. In this way students can monitor membrane penetration and evoked postsynaptic potentials on the oscilloscope while making "hard copies" in Scope.

### Data Display

Data analysis in Scope is software driven and does not require the PowerLab unit. Any computer with Scope software can analyse the data. 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. The Show Overlay command superimposes all of the addressed traces — command clicking on the page icon controls which scans will be shown in the overlay. In this way students can observe, for example, several traces in



**Figure 1.** Diagram showing a PowerLab connected to a pair of stimulating electrodes and to an electrometer from which data will be recorded

①

subthreshold voltage

②

increase voltage == small EPSP

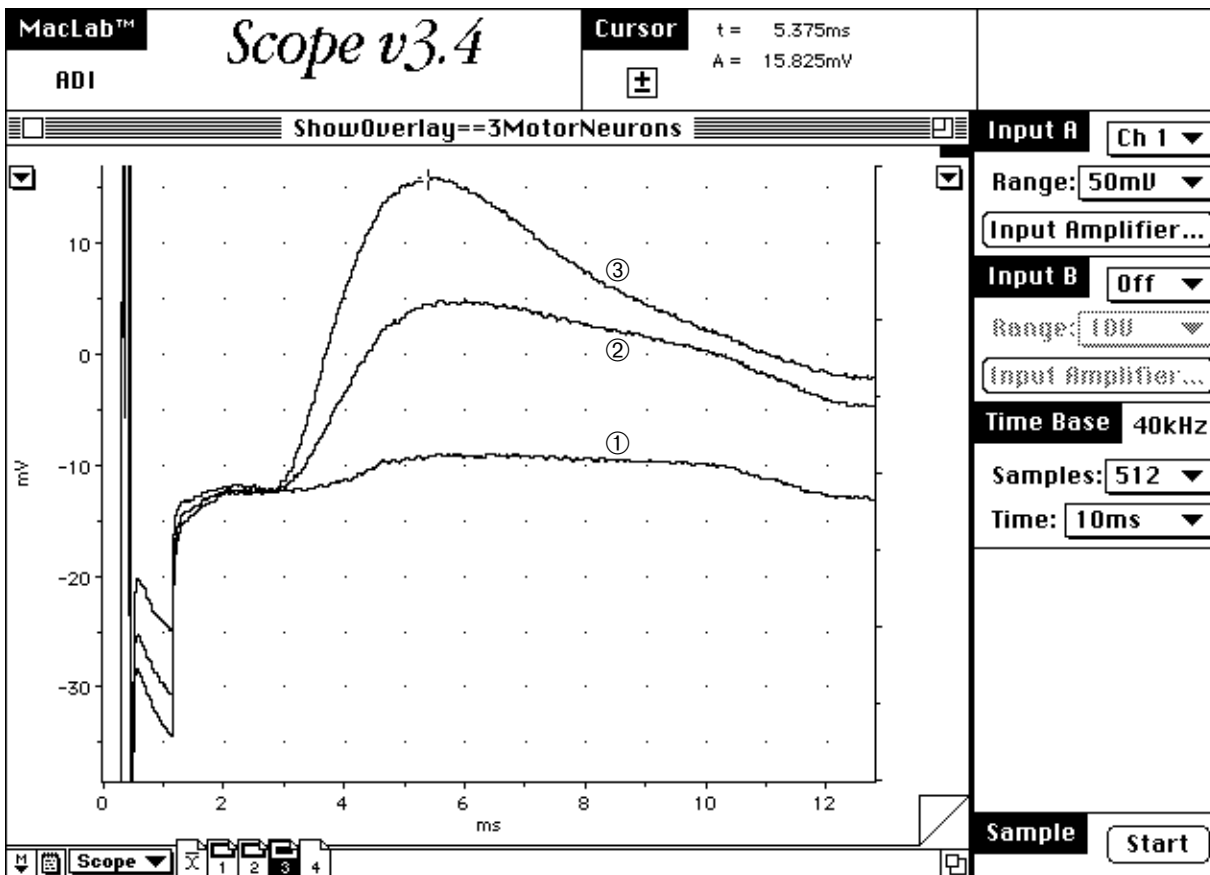
Modifie

③

Increase voltage == larger response  
Thus, two motor neurons innervate this lateral fiber in segment #4.

Modifie

Modified: Mon, 27 Aug 1956, 3:01 PM



**Figure 2.** Three traces collected and displayed with Scope. All three traces are shown overlaid. Notes about each trace can be stored in the Page Comments windows, shown above.

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which the response amplitude changed as the voltage intensity was changed, Figure 2. Accurate measurements can be made using the Marker and the cursor. Close inspection of selected regions of a trace can be made in the Zoom window.

## Annotation

During the recording session students should be encouraged to 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!).

## 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 Vaseline 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 (e.g. 0.5 M cobaltous chloride). After overnight 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.