

COMPOUND ACTION POTENTIALS: FROG SCIATIC NERVE

[Adapted from Oakley, B. and R. Schafer. 1978. Experimental neurobiology. Univ. Michigan, Ann Arbor.]

Introduction

In 1850, Hermann von Helmholtz first estimated the velocity of nerve impulses transmitted in a frog nerve-muscle preparation with a mechanical kymograph and writing levers. During the latter half of the 19th century, concepts underlying the modern theory of nerve conduction were developed by Sherrington and others, but modern electrophysiological research awaited the development of the cathode ray oscilloscope. Using this apparatus, Erlanger and Gasser in 1921 first measured the ionic currents of compound action potentials. Their studies provided an important foundation for our present understanding of nerve function. The frog sciatic nerve was the classical preparation for study of the action potential until experimental researchers developed intracellular recording methods for studying squid giant fibers.

Action potentials can be elicited simultaneously by electrical stimulation in thousands of axons of a peripheral nerve like the sciatic. The collective response is termed a compound action potential. Indiscriminate as this gross recording technique may seem, some basic aspects of neuronal conduction – maximal firing rates, threshold, conduction velocity, and the role of axon size and myelination – can be demonstrated using the whole nerve approach.

Procedure

a. Preliminary setup:

It is essential to familiarize yourself with the instruments and recording system before beginning the dissection. The instrumentation may seem very complex, but generally viability issues of the biological preparation often end the experiment. Therefore, clear up any practical or conceptual problems regarding the equipment before you begin working with the experimental animal.

b. Surgical procedure:

You will be provided with a doubly-pithed Bullfrog (*Rana catesbiana*). Pick up a fold of skin at midabdomen with forceps and, avoiding cutting into the abdominal cavity with your scissors, cut the skin all the way around the frog. Pull the skin down, everting it as you pull, and strip it off the legs. Lay the frog dorsal side up and gently separate the thigh muscles to reveal the white sciatic nerve and accompanying blood vessels (**Figure 1**). Don't mistake white muscle fascia for the nerve. Ask if you're not sure.

Separate the muscles and free the nerve from surrounding tissue using blunt glass tools whenever you must touch the nerve. Make such a tool by heating a glass rod in a bunsen

burner flame and pulling out a working tip smoothed to about the width of a dulled pencil lead. Apply amphibian perfusion fluid (Frog Ringer's solution) liberally as you work. Do not contaminate the nerve with cut tissues or blood, and do not touch the nerve with metal tools or with your fingers. Avoid stretching, pinching, or drying out the nerve.

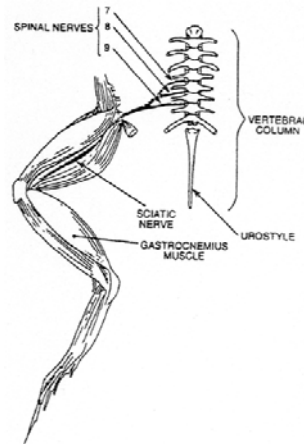


Figure 1: Dorsal view of exposed frog right hind limb and spinal column.

Hold the urostyle up, and carefully cut the muscles on both sides of the bone. Free the caudal end of the urostyle and lift it up to expose the underlying structures. Note the two regions of white fibers that compose the sciatic nerve plexus. Each sciatic nerve originates as three spinal nerve roots. Cut the urostyle at its hinge. Carefully tie the roots together with the end of a 10 cm length of Ringer's-soaked cotton thread. Cut the nerve roots as close to the spinal cord as possible. Now free the nerve from the hip to the knee, lifting with the thread as necessary. [**CAUTION:** Do not stretch the nerve!] When the nerve has been totally freed, cut through the distal end with scissors. Immerse the nerve in a small beaker of perfusion fluid.

While some group members start preparing the nerve for recording, other students should dissect the second sciatic nerve as previously described. Also immerse the nerve in perfusion fluid when done.

c. Setup of equipment (for schematic refer to Appendix I):

- 1) Connect the red and black alligator clips from the stimulator electrodes to two of the metal rungs on opposite sides of the MLT012/B Nerve Bath (Figure 2). The distance between the electrodes should be 0.5 cm. It is not necessary to connect the green (ground) alligator clip.
- 2) Connect the red (positive) BNC connector from the stimulator electrode to the positive (+) analog output connector on the PowerLab. Connect the black (negative) BNC connector from the stimulator electrode to the negative (-) analog output connector.

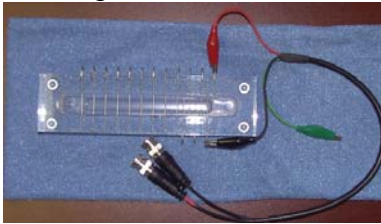


Figure 2. Placement of the stimulus electrodes on the MLT012/B Nerve Bath.

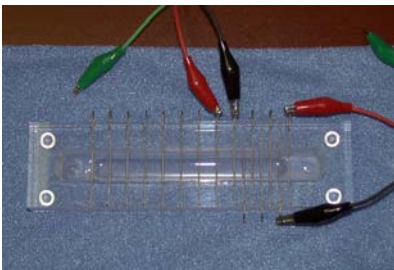


Figure 3. Placement of first recording electrodes on Nerve Bath.

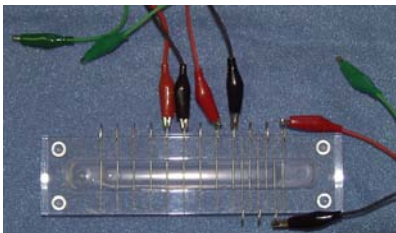


Figure 4. Placement of second recording electrodes on Nerve Bath.

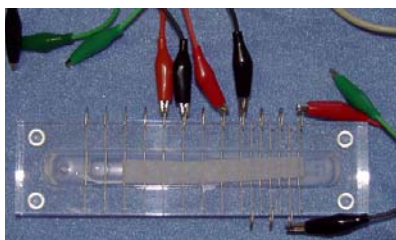


Figure 5. MLT012/B Nerve Bath set up for connection test with filter paper and frog Ringer's.

- 3) Connect the red and black leads from the first recording electrode to two of the metal rungs of the MLT012/B Nerve Bath (Figure 3). Connect the 8-pin DIN connector to the Pod port on Input 1 of the PowerLab.
- 4) Repeat step 3 for the second recording electrode, but place the alligator clips further away from the stimulus electrode (Figure 4). Attach the pod connector to the Pod port on Input 2 on the PowerLab.
- 5) Using an eyedropper or Pasteur pipette, fill the lower reservoir of the Nerve Bath with frog Ringer's solution. Fluid in the lower reservoir must not come in contact with the metal electrode rungs. **Note: OVERFILLING** the Nerve Bath in this manner will cause a short circuit in your experiment.
- 6) Cut a strip of filter paper and lay it over the wires in the nerve bath (Figure 5) so that it touches both stimulating electrodes and both sets of recording electrodes. Moisten the paper strip with frog Ringer's, and place the cover on the nerve bath. This arrangement will be used to test your connections.
- 7) Turn on the PowerLab and make sure it is connected to your computer.

- 8) Open the dialog box for Scope input amplifier (Figure 6) and select the following settings:

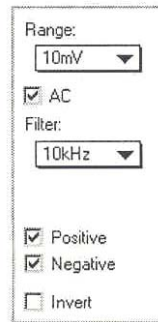


Figure 6: Scope input amplifier settings

Input A

Range: 10 – 20 mV
Low Pass: Off
Positive checkbox: checked
Negative checkbox: checked

Input B

Range: 10 – 20 mV
Low Pass: Off
Positive checkbox: checked
Negative checkbox: checked

Time Base: 10 kHz

- 9) From the Scope Setup menu select the Stimulator dialog box and (Figure 7) and apply the following settings:

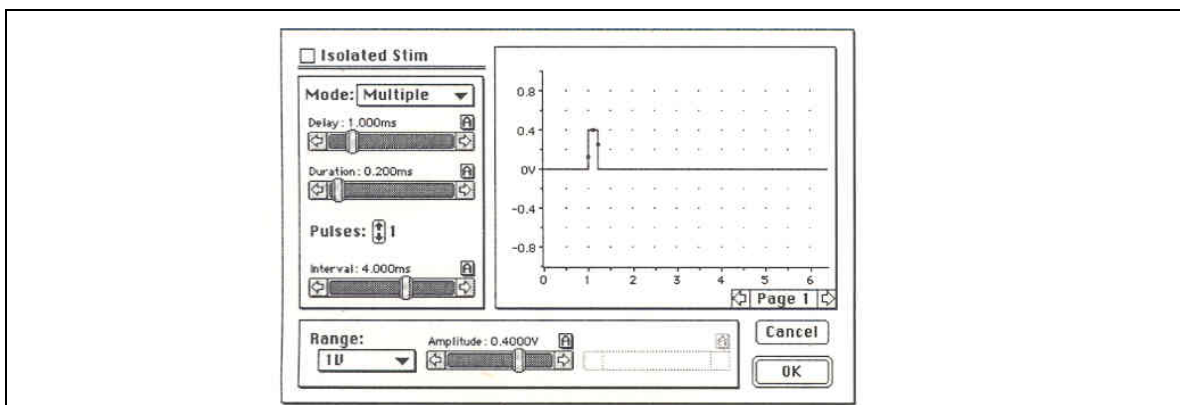


Figure 7: Suggested stimulator settings. This dialog box is displayed when Stimulator... is chosen from the setup menu. Before making these settings insure that the Isolated Stim checkbox is off.

Mode: Multiple
Number of pulses: 5
Isolated checkbox: unchecked
Delay: 0.05 ms
Interval: 10 ms
Duration: 0.15 ms
Range: 500 mV
Amplitude: 150 mV

- 10) Close the Stimulator dialogue box by pressing OK. You will now see a stimulator menu along the top part of the screen. Press Start and record a sweep – Scope will now automatically record data for one second. You should see a series of stimulus pulses recorded, stimuli artifacts (Figure 8). If not, check to make sure that the alligator clips are secure and the filter paper is moist and draped over all the active wires in the Nerve Bath. Once the connections are tested and working, you are ready to begin the lab. Repeat this recording until the stimuli artifacts are readily observable – you may have to change stimulator setting (increase stimulus intensity) or change input amplifier settings (increase sensitivity by manipulating the Range)

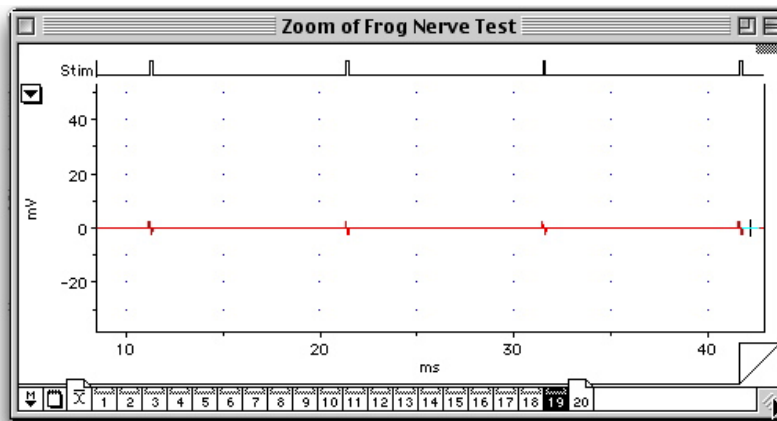


Figure 8: Recording of stimuli artifacts – calibration.

- 11) Remove the filter paper from the Nerve Bath. Lay the nerve across the wire electrodes, making sure it is in contact with each of the active connections (Figure 9). Manipulate the nerve with glass tools as you draw off enough of the fluid so that it comes to rest on the electrode wires. The nerve must be in physical contact with each of the wires, and the level of the fluid must be well below all of the wires to prevent them shorting out. One end of the nerve may remain in the fluid, but not both. If your nerve is too short, adjust the position of the recording electrodes as necessary. Place the cover back on the Nerve Bath.

Note: DO NOT GRASP THE NERVE WITH FORCEPS! Doing so will damage the nerve.

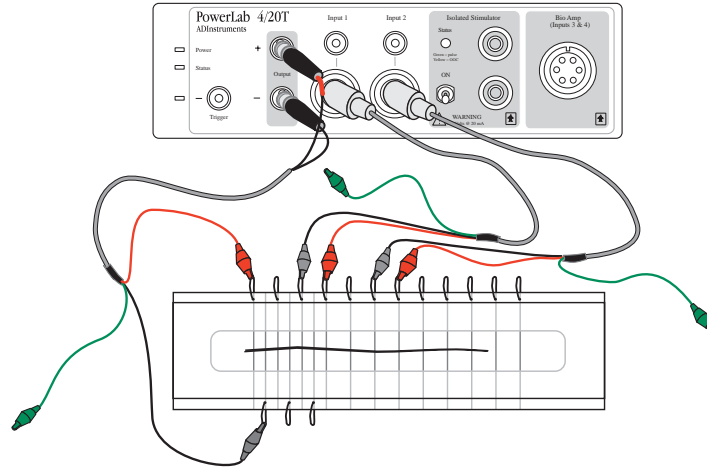


Figure 9: Diagram of the complete setup of the MLT012/B and PowerLab 4/20T for the frog sciatic nerve experiment. Make sure that the nerve is resting on all the electrode pairs.

d. Experimental procedure:

1. Determination of threshold voltage, maximum CAP amplitude, and demonstration of recruitment.

Use the following settings for this procedure:

Input A

Range: 10 – 20 mV
Low Pass: Off
Positive checkbox: checked
Negative checkbox: checked

Input B

Range: 10 – 20 mV
Low Pass: Off
Positive checkbox: checked
Negative checkbox: checked

Time Base: 100 kHz

Stimulator settings:

Mode: Pulse
Isolated checkbox: unchecked
Delay: 1 ms
Duration: 0.15 ms
Range: 500 mV
Amplitude: 0-410 mV in 10 mV steps

In this part of the experiment, you will give the nerve a series of stimuli, each increasing in amplitude. You will then be able to calculate the threshold voltage for the nerve, as well as the voltage required for maximum CAP amplitude.

First, stimulate the nerve with increasing stimuli intensities – start at 10 mV. Gradually increase the stimulus intensity in 10 mV increments. Record a sweep for each stimulus increment and keep track of which sweep used what stimulus intensity – ask your instructor how to insert comments in each sweep for good recordkeeping. You will see the stimulus artifact as the first-appearing wave; this is the stimulating voltage conducted on the outside of the nerve and picked up through the recording electrodes. The artifact can be seen to vary with stimulus duration.

Continue to increase the stimulus voltage until a second wave appears to the right of the artifact. This is the compound action potential. A sample tracing is shown below (**Figure 10**). Note that you will be recording this compound action potential in both Channel 1 and Channel 2. Continue to increase the stimulus until this wave reaches a maximum amplitude.

Reduce the voltage and note the voltage at which the AP first appears. This is the threshold voltage for the most sensitive axons (or those most accessible to the stimulating current). As you progressively increase the stimulus intensity, the amplitude of the CAP increases as the thresholds of more fibers are reached and they, in turn, fire – they are recruited into the response.

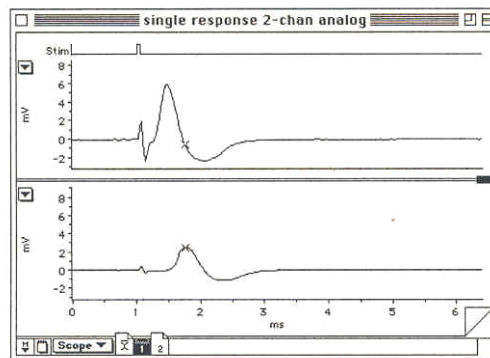


Figure 10: Sample recording of a compound action potential using Scope software.

Increase stimulus intensity until a maximal response is seen. At this point all the nerve fibers are actively conducting AP's (met the threshold for all fibers) and the waveform seen is the sum of all of them. This growth of the AP with increasing stimulus intensity obscures the fact that the action potential of each individual fiber is an all-or-none event. The compound AP has these distinguishing properties: it is not the first deflection observed; its amplitude, though initially increased by raising stimulus intensity, is not a

linear function of stimulus strength; its duration is not a direct function of stimulus duration; it does not have the shape of the stimulus artifact.

2. Conduction velocity:

Use the following settings:

Input A

Range: 10 – 20 mV
Low Pass: Off
Positive checkbox: checked
Negative checkbox: checked

Input B

Range: 10 – 20 mV
Low Pass: Off
Positive checkbox: checked
Negative checkbox: checked

Time Base: 40 kHz

Stimulator settings:

Mode: Pulse
Isolated checkbox: unchecked
Delay: 1 ms
Duration: 0.15 ms
Range: 500 mV or as required
Amplitude: maximal stimulus

In this part of the experiment, you will calculate the velocity of the CAP as it travels down the nerve.

- 1) Using a ruler, measure the distance in centimeters between the black negative leads of each of the two recording electrodes. Record this value.
- 2) Using a maximal stimulus intensity (determined above), stimulate the nerve and record a sweep; repeat three times, and insert appropriate comments.
- 3) You will later use this data to calculate conduction velocity in meters/second.

3. Determination of the refractory period

Input A

Range: 10 – 20 mV
Low Pass: Off
Positive checkbox: checked
Negative checkbox: checked

Input B

Range: 10 – 20 mV
Low Pass: Off
Positive checkbox: checked
Negative checkbox: checked

Time Base: 40 kHz

Stimulator settings:

Mode: Double
Isolated checkbox: unchecked
Delay: 0.25 ms
Duration: 0.15 ms
Range: 500 mV or as required
Amplitude A: supramaximal stimulus from Part 1
Amplitude B: supramaximal stimulus from Part 1
Interval (ms): 4.0, 3.5, 3.0, 2.5, 2.0, 1.9, 1.8, 1.7, 1.6, 1.5, 1.4, 1.3, 1.2, 1.1, 1.0

In this part of the experiment, you will stimulate the nerve with a series of pulses. In each instance/recording, the pulse interval will decrease. You will be able to use this recording to determine the relative and absolute refractory periods of your nerve. Make sure that you can identify specific recordings by inserting comments.

4. Wave form – monophasic and biphasic

Use the following settings for this procedure:

Input A

Range: 10 – 20 mV
Low Pass: Off
Positive checkbox: checked
Negative checkbox: checked

Input B

Range: 10 – 20 mV
Low Pass: Off
Positive checkbox: checked
Negative checkbox: checked

Time Base: 100 kHz

Stimulator settings:

Mode: Pulse
Isolated checkbox: unchecked
Delay: 1 ms
Duration: 0.15 ms
Range: 500 mV or as required
Amplitude: maximal stimulus

The shape of the waveform observed with Scope depends on a number of factors. The distance between stimulating and recording electrodes, time base, sensitivity (range), filter settings, and condition of the nerve all influence the shape of the CAP observed.

Return stimulus to an intensity until the voltage is about 10% above that needed to elicit a maximal response. The compound AP from an undamaged nerve is usually biphasic. As the AP sweeps by the first recording electrode, it drives that electrode negative with respect to the more distant electrode. If you previously arranged the electrodes as above, the initial deflection will be upward. Then, as the wave of depolarization (the AP) arrives at the second recording electrode, making it negative, the oscilloscope trace is deflected downward. Record the biphasic wave in Scope, recording amplification and timebase for reference.

Crush the nerve between the second two (more distant) recording electrodes by pinching it with a pair of fine forceps – if you're not sure where to pinch check with your instructor. This should inactivate the nerve and produce a monophasic recording. You may need to crush and check the nerve several times to ensure that you have a fully monophasic recording. A tiny drop of isotonic KCl (0.16 M) applied to the crushed area will aid in development of a monophasic AP. Record the monophasic wave.

5. Fiber groups

Within the total population of fibers in frog sciatic nerve there are several groups of axons of similar diameter and therefore, similar threshold and conduction velocity.

Connect the electrodes for monophasic recording (see 4 above). Start by using a stimulus intermediate between threshold and maximal intensity. Set up the sweep rate (Timebase) and stimulus frequency (about 5 stimulations/second) so that you can readily observe a CAP recording and attempt to resolve as many peaks of the compound AP as possible. If required slowly increase the stimulus voltage and look for the addition of new peaks – you may also have to play with the Timebase and stimulus frequency. It should be possible to find two of the three major peaks of the compound AP demonstrated by Erlanger and Gasser (1968): A, the largest, corresponding to large myelinated fibers; and C (the slowest wave), corresponding to very fine unmyelinated fibers. Within the A wave you may be able to separate several subpeaks, the A-alpha, A-beta, and A-delta fibers (see **Figure 11** below).

The conduction velocity of the C fibers is only 1/100 that of the A-alpha peak and to see the C peak you must therefore stimulate at a low enough rate for it to appear before the next A wave. The sweep rate should also be low (ca. 50 msec/div) and the stimulus intensity high.

Determine the relative amplitudes, thresholds, and conduction velocities of each group of fibers in your preparation. Fiber diameter is probably the most important determinant of conduction velocity, with large fibers conducting faster.

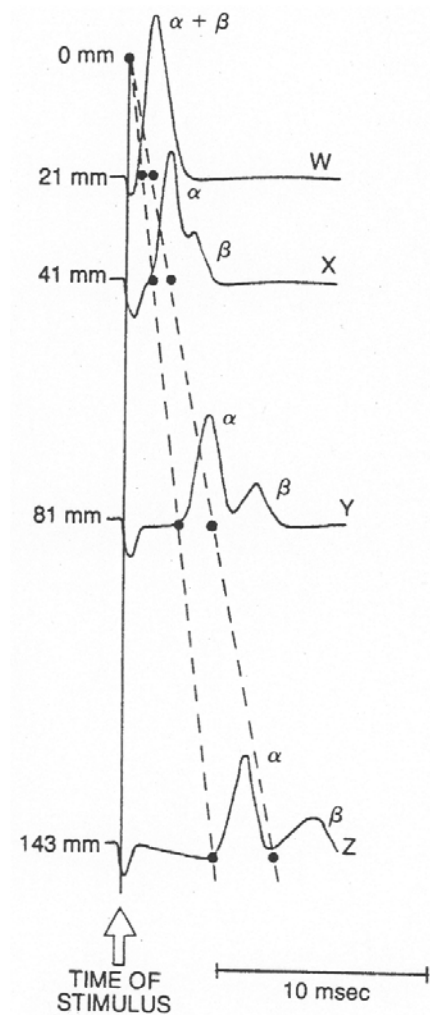


Figure 11: Propagation of the alpha and beta waves of the compound action potential of the bullfrog sciatic nerve. The four recordings (W – Z) were made from four different recording electrodes at distances of 21, 41, 81, and 143 mm from the site of stimulation (0 mm). As the compound action potential passes down the nerve, the alpha and beta components become separated because the velocity of propagation of the alpha and beta fibers is different. At W, near the start, the alpha and beta waves are completely merged into one wave (α and β). In records X through Z, the alpha and beta waves become separated as the running distance along the nerve increases.

The records have been arranged vertically in proportion to the distance of the respective recording electrodes from the point of stimulation (0 mm). The solid line on the left is drawn through the point of initiation of the stimulus artifact. The dashed lines are drawn through the point of initiation of the alpha component and the point of initiation of the beta component. The points of initiation of the alpha and beta components are indicated by dots in each record. The fact that the lines are straight indicates that the velocity of propagation of the alpha and beta components along the nerve is linear. (Adapted from Erlanger and Gasser, 1986)

6. Strength-duration curve:

Use the following settings for this procedure:

Input A

Range: 10 – 20 mV

Low Pass: Off

Positive checkbox: checked

Negative checkbox: checked

Input B

Range: 10 – 20 mV
Low Pass: Off
Positive checkbox: checked
Negative checkbox: checked

Time Base: 100 kHz

Stimulator settings:

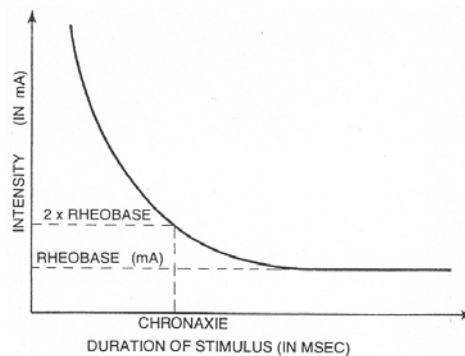
Mode: Pulse
Isolated checkbox: unchecked
Delay: 1 ms
Duration: see below
Range: 500 mV
Amplitude: see below

The ability of the stimulus to elicit a response is dependent on the stimulus duration as well as its intensity. In other words, a response can be obtained using strong current for a short time or a weak current for a long time. The relationship between strength and duration can be determined empirically for your sciatic nerve preparation.

In this part of the experiment you will determine the minimum duration required for a given stimulus intensity to produce a response (for example, a 1 cm deflection – use a constant criterion for recording the response).

Start by setting the stimulus duration to 100 msec and stimulus intensity at 0 mV. Gradually increase the stimulus intensity until a response is noted. Next, decrease the duration to 50 msec and advance the voltage until an identical response is seen. Continue this process for a number of different stimulus durations.

You will later use this to plot a strength-duration curve, with stimulus intensity (V) on the ordinate and duration (msec) on the abscissa. Your curve should look approximately like Figure 7 below.



The minimum stimulus intensity which elicits a response at infinite duration is called the rheobase (the least possible voltage at which it will fire). If the voltage is increased to twice the rheobase voltage, the time required to stimulate the nerve is the chronaxie. Chronaxie is a measure of the excitability of nervous tissue. The smaller its value, the more excitable is the nerve. These concepts have lost some of the importance they once had for understanding nerve function, but chronaxie is still useful to compare excitability of nerve and muscle tissues. Strength-duration curves have also been used experimentally to follow the course of nerve and muscle regeneration.

References [Locate other more recent references by doing searches on your own -- Medline, Medscape, Infotrack (from PhysioLink site)]

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Appendix – Circuitry (note only one recording electrode is shown)

