

# Chapter 3

## **Nerve Conduction in Frogs and Humans**

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# Nerve Action Potential and Conduction Velocity

## INTRODUCTION

A transmembrane potential is one of the characteristic features of the living cell. Its source resides in a differential distribution of ions across the membrane with high  $K^+$  inside and high  $Na^+$  and  $Cl^-$  outside. This distribution depends on the permeability properties of the cell membrane and the presence of ion pumps. In "resting cells", this potential is about 75 to 90 millivolts (mv) with the outside of the nerve fiber positive with respect to the inside. The potential can be readily recorded by inserting a fine electrode into the interior of a cell while another electrode touches its outer surface; the constant potential thus recorded is called the **resting potential**.

When a cell is excited (muscle cell contracts, nerve cell transmits, gland cell secretes), there is a characteristic change in this potential (electrogenesis) known as the **action potential**. One of the most useful methods of monitoring activity in cells is the measurement of these action potentials. The action potential in the nerve is the **nerve impulse**.

In this exercise action potentials will be recorded during the stimulation of the frog sciatic nerve. It must be remembered that the sciatic nerve as a nerve trunk, contains the axons of many neurons; consequently the record obtained in your experiment will show a composite action potential. In addition, placing the electrodes on the outside of the nerve trunk will result in a diphasic recording, as opposed to the monophasic action potentials recorded by transmembrane electrodes. For further reference consult Ruch & Patton.

## EQUIPMENT

Your equipment consists of a nerve chamber, stimulator, amplifier and an oscilloscope (Figure 1). Before you dissect the nerve, you should set up your apparatus, ready to record.

1. Connect stimulator output to the stimulating electrodes on nerve chamber (A and B on diagram.)
2. Connect the first recording electrode (C) to the reference terminal of amplifier, and the second and third recording electrode (D to H) to the input of the amplifier.
3. Connect amplifier output to "y" input of oscilloscope and "sync out" of stimulator to "x" input of oscilloscope.

Dissect a long sciatic nerve preparation from a double pithed frog (diagrams provided). Use only glass probes in handling the nerve and keep the nerve wet with amphibian Ringer's throughout the dissection. Tie a piece of thread around one of the spinal nerves which leads into the sciatic nerve and cut the nerve free just beyond the thread. Tie another thread onto the distal end of the sciatic nerve and sever the nerve beyond the thread. Lay the free nerve on a piece of filter paper and blot gently free of water. Now mount the nerve in the nerve chamber, passing the nerve over and under successive electrodes as shown in the diagram. The proximal (spinal) end of the nerve should be in contact with the two stimulating electrodes. Without overstretching the nerve, extend it sufficiently to ensure good contact with all of the electrodes. Pass the thread through the holes in the plexiglass at the two ends of the chamber and secure with plasticine. Moisten the nerve with Ringer's solution, and cover chamber with microscope slide.

If the other sciatic nerve of your frog is not being used by another pair of students, dissect it and store in cold aerated Ringer's.

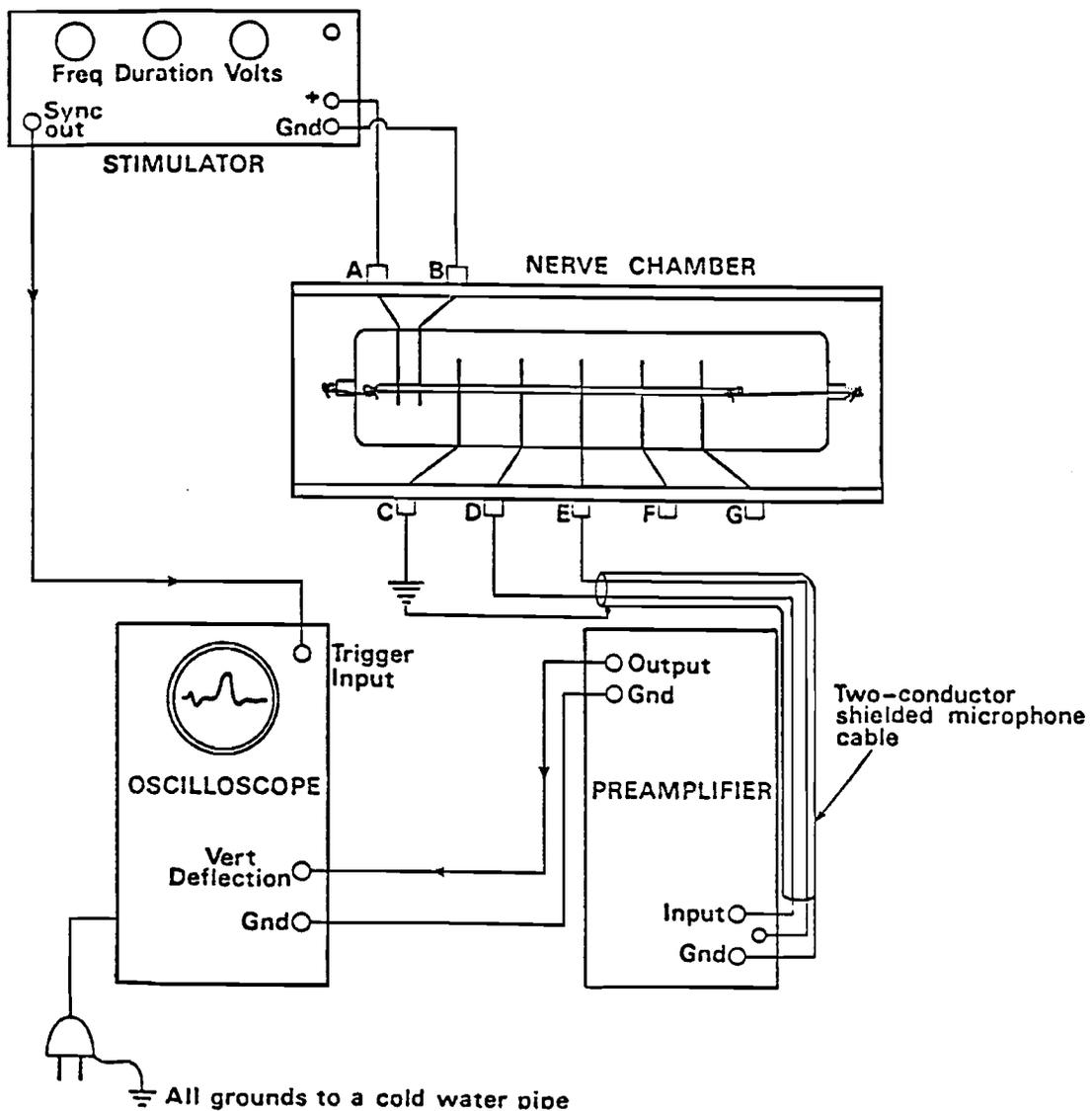


Figure 1. Nerve chamber, stimulator, amplifier and oscilloscope.

## EXPERIMENTAL PROCEDURE

Slowly turn up the stimulus voltage, until you see a "stimulus artifact" (or "shock artifact") appear as a small vertical deflection at the beginning of the sweep. Do not exceed 0.1 volts of intensity at this stage. If there is no trace on the screen, you have to adjust the position of the beam in the following manner:

Turn the intensity dial fully clockwise, depress beam finder button, centre trace on screen using the vertical and horizontal position knobs. Release beam finder knob and make fine adjustments to position until the complete trace is visible on the screen.

After the trace is properly centered, start again with a voltage of 0.0, and increase the intensity of stimulus until you see the stimulus artifact. The stimulus artifact is really an electrical leakage from the stimulating electrodes, which is conducted around the outside of the nerve and picked up by the recording electrodes. This artifact marks the exact time that the nerve is shocked. Continue to gradually increase the stimulus voltage; note that the size of the artifact also increases. Then, a new, small, delayed deflection will appear - the nerve action potential. Increase the voltage until the nerve response reaches maximum height. What is the voltage applied when the response is maximal? Do not exceed this voltage by more than 10-20 mV, to avoid injuring the nerve. Note that the nerve impulse appears as a diphasic wave. Why? Record the voltages for threshold and maximal responses of the nerve trunk. Why should the threshold voltage differ from that which causes maximal response.

To calculate conduction velocity, you should store the trace of the action potential. Connect recording electrode D & E and obtain a tracing of maximal response. Now, set oscilloscope triggering mode to single sweep, depress store button, push erase, and press triggering mode to reset. Allow mode to return to single sweep position, and you should now have a single trace on the screen. To obtain recording from the second point along the nerve, disconnect recording electrode D & E and connect electrode G & H. Repeat procedure for storing trace. You should now have two traces of action potentials on the screen, at two different distances from the stimulus artifact. Measure the distance along the nerve for each trace between the stimulating and the recording electrodes. Read the time between stimulus artifact and action potential for both traces from oscilloscope, using the setting of your time base dial. ( $d_1$  &  $d_2$  on diagram). Subtract time for shorter distance from that of the greater distance and divide this value into the distance between the two recording electrodes on the nerve chamber. The value obtained from this calculation is the velocity of the impulse conduction.

Calculate nerve conduction velocity in meters/sec.

## REFERENCES

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## Refractory Periods

Set up a nerve preparation and equipment as described in the previous exercise, but in place of the regular stimulator, connect the GRASS S 48 twin pulse stimulator. The twin pulse stimulator allows the delivery of two shocks at pre-set intervals, where the time lapse between the two stimuli is regulated by a time delay switch. To set the stimulator; place Stimulus switch to ON position, Pulses-DC to pulses, Function switch to Single position and Duration to 0.15 msec. To assure a square wave stimulus, a Stimulus Isolation Unit (SIU) is connected to your stimulator. Set the voltage multiplication dial on the S 48 to X10 (SIU), and leave it in this position throughout the experiment. On the SIU, set Voltage Input multiply dial to required range, Coupling switch to capacity and Polarity to normal. Regulate voltage range during the experiment using the dial on the SIU unit.

To determine Refractory Period, turn stimulus mode to repeat at 30 pulses/sec., voltage range from SIU to 0.1, and pulses to single. Turn voltage up slowly, using the control on your stimulator, until you just obtain maximal response. Switch to twin pulses and increase delay time gently until you record a second Action Potential. The delay in msec.s represents the total Refractory Period of the nerve trunk. Now turn the voltage to 4-5 times threshold strength and obtain delay time as before. The time obtained using suprathreshold stimulus is the end of the Absolute Refractory period. To determine Relative Refractory Period subtract Absolute Refractory Period from total Refractory Period.

# Nerve Conduction Velocity Measurements in Human

## INTRODUCTION

Nerve conduction velocity in human can be easily determined by stimulating a motor nerve and monitoring the response of its associated muscles. The time interval between the stimulation and the response - latent period - is recorded from two different points along the nerve. The difference between the two latent periods is the time required for the nervous impulse to travel from point A to point B.

In your exercise you will stimulate the ulnar nerve and record muscle contractions in the fifth finger. Before coming to the laboratory, you should consult an anatomy book to familiarize yourself with the location of the ulnar nerve. For further reference, make use of the diagrams on your laboratory benches.

## EXPERIMENTAL PROCEDURE

### Location of the Ulnar Nerve

1. Set Grass stimulator to a frequency of 1/sec.; duration of 20 msec.; voltage 50 V; monophasic; + polarity; and the mode switch to repeat.
2. Connect "sync out" of stimulator to X input of oscilloscope.
3. Connect Grass stim. output to pulse generator input.
4. Plug recording electrodes into amplifier outlets. The first one, which will be the active electrode into RA outlet, the second one (indifferent electrode) into LA and the ground into RL outlet.
5. Cover surface of recording electrodes with contact media and secure them to your hand. The active electrode should be midway along the lateral border of the large muscle above the fifth finger (hypothener eminence); the indifferent electrode is placed at the base of the proximal phalanx of the fifth finger, and the ground electrode to the palmar surface of the hand. (see diagrams in laboratory)
6. Turn the output level of the pulse generator all the way down, and touch your arm with the stimulating electrode. Now increase the output level of the pulse generator until you feel a strong muscle contraction.
7. Move the stimulating electrode to the approximate location of the ulnar nerve. **ALWAYS PLACE THE CATHODE OF THE STIMULATING ELECTRODE NEAREST TO THE MUSCLE BEING STIMULATED.** Locate the ulnar nerve at the wrist, elbow and upper arm, by watching for contraction of the little finger. Mark the location of the nerve at these points with a ball point pen. Now you are ready to record.

### Recording

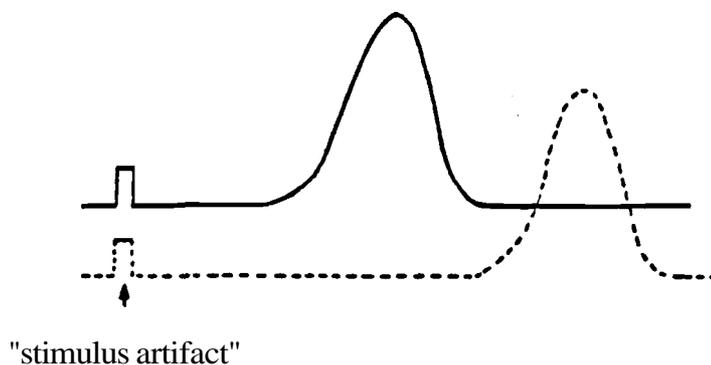
1. Turn oscilloscope ON, Store "off" (button in an out position). Set triggering to ext., mode to norm., and turn CHANNEL 1 on. (Left hand side control panel, and button to be pushed in.)

Use an 0.2 volts/div., a time base of 1 msec/div.

The oscilloscope should now produce a trace only when the Grass stimulator produces a pulse. If this is not happening, it may be necessary to adjust the trigger level on the oscilloscope. If no trace at all is visible on your screen, the position of the beam will have to be adjusted in the following manner:

Turn intensity dial fully clockwise, depress beam finder button, centre trace on screen using the vertical and horizontal position knobs. Release beam finder knob and make fine adjustments to position until the complete trace is visible on the screen.

2. Change oscilloscope triggering to "line". A continuous trace should now be seen. (It may be necessary to readjust trigger level.)
3. Flex the little finger and adjust the gain of the amplifier until substantial trace deflection is seen. Do not proceed until an EMG is observed. If no deflection can be seen on the oscilloscope, check all the connections, particularly the recording electrodes. Return triggering to "EXT".
4. Stimulate the nerve at the points marked earlier. The resulting waveform should appear as follows:



The first deflection is the stimulus artifact. The stimulus artifact is an electrical leakage from the stimulating electrodes, which is conducted along the surface of the arm and picked up by the recording electrodes. It may be necessary to adjust the horizontal position for the stimulus artifact to appear on the display. If the artifact trace disappears off scale, and adjusting the vertical position does not bring it back, reduce the sensitivity (Volts/div.).

The second deflection represents the contraction of the muscle. The time delay between the stimulus and the response represents the latent period. To produce a suitable trace it may be necessary again to make some adjustment to the time base or sensitivity.

### Velocity Measurements

1. Set oscilloscope triggering mode to single swp, STORE-on and press erase.
2. Hold probe on the first mark on your arm and momentarily depress mode switch to reset. When a trace is obtained, repeat procedure on the next point marked on your arm.

3. You should have a composite display of several delay times remaining on the screen. To calculate conduction velocity, record delay times from oscilloscope, and measure the distance between the point of stimulus and the first recording electrode on the arm. Plot the distance along the nerve vs. time delay. The slope of the line will give the conduction velocity of the nerve impulse in the ulnar nerve. Express this velocity in meters/sec., and compare your results to those in the literature.

OR:

Read time delay between peaks from oscilloscope, and record distance between points of stimulus on the ulnar nerve. To calculate conduction velocity, use the following formula:

Distance between points of stimulus/time delay = conduction velocity in cm/msec.

Convert the obtained value to meters/sec.

#### REFERENCES

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