Introduction
In this laboratory, you will obtain extracellular recordings of action potentials from an anesthetized earthworm (Lumbricus spp.). You will examine the threshold potential, the all-or-none response, the refractory period, and the conduction velocity of the nerve.

Background
A characteristic of the nervous system is that signals are conducted from place to place by nerve impulses: action potentials that travel along a nerve fiber. All sensation, thought and movement is mediated by coded patterns of nerve impulses. The properties of the action potential in single fibers are therefore of fundamental physiological importance.

Annelids, such as the common earthworms (Lumbricus species), and also arthropods, have a ventral nerve cord that is analogous to the dorsal spinal cord of chordates.

Figure 1: Earthworm ventral nerve cord
As well as a multitude of small nerves that do not run the length of the animal (there are about 500-1000 neurons per segment, or about 100,000 neurons altogether), the earthworm has a medial giant fiber and two lateral giant fibers that lie on either side of the medial one.

In reality, each giant fiber is made up of large individual cells (one per segment) that are electrically coupled to each other through gap junctions. This results in the rapid conduction of action potentials from cell to cell so that each giant fiber behaves as though it were a single axon. In addition, the lateral giant fibers are extensively linked to each other by cross-connections and therefore function as a single unit.

Historically, giant axons played an important part in the discovery of the membrane mechanisms underlying the action potential. The squid giant axon, for example, was used extensively for voltage clamp experiments.

The role of these giant fibers, which conduct action potentials far faster than the small nerves, is to allow the animal to respond rapidly to threatening situations.
They thus take part in a variety of ‘escape’ behaviors. (In chordates, the development of myelination allowed conduction velocities of similar magnitude in nerves of much smaller size.)

A major experimental advantage of the earthworm nervous system is that these giant fibers can be stimulated by electrodes placed on the body of the animal and their responses can be recorded extracellularly from the animal’s surface. Since the small nerves do not run over extended distances, their activity is not recorded.

Whereas, an action potential recorded intracellularly typically has an amplitude of 80–100 mV, the inside of the cell being negative to the extracellular fluid, extracellular electrodes do not have access to this potential. Instead, what is recorded is a small potential difference. Most of the loss of amplitude is due to internal current flow in the worm’s body. The amplitude also depends on the recording conditions: how easily current can flow outside the worm. If the region between the recording electrodes is very wet with solution, the peak deflection may be as small as 20 µV. The recorded response may thus be less than a thousandth of the amplitude of the action potential itself.

Action potentials occur when specialized voltage-sensitive membrane sodium channels are activated. The large increase in sodium permeability results in membrane depolarization. This is followed by repolarization as the sodium permeability returns to its low baseline value and potassium permeability is transiently increased. [Note that the actual numbers of ions moving during each action potential, however, are very small and cell ion concentrations are not altered measurably]. From the beginning of the action potential to the restoration of the resting membrane potential, the neuron is incapable of producing another action potential. This period is referred to as the refractory period, which can be divided into two phases. Initially there is the absolute refractory period, where it is impossible to initiate a second action potential. This is followed by the relative refractory period, where a stimulus of greater than normal intensity can elicit a response.

Action potentials are “all-or-none” events. Once an action potential begins, it propagates down the length of the axon. When a response occurs, it is full-sized. There is no relation between stimulus strength and response amplitude in a single axon.

You are recording extracellularly and measuring the potential difference between two recording electrodes. In the absence of an action potential, there is no potential difference between the electrodes. Once an action potential arises, a wave of surface depolarization passes down the nerve. When it passes under the first recording electrode, this region of the surface becomes negative relative to
the second electrode. This region rapidly recovers but the region under the second electrode becomes negative for a very short time. This propagation therefore results in a biphasic recording. By convention, the negative recording electrode is positioned nearer to the stimulating electrodes. Thus when the surface beneath the first electrode becomes negative, this is recorded as a positive deflection. When the wave reaches the second positive electrode, a negative deflection is recorded.

There can be some variation in the shape of the response, depending mainly on the distance between the recording electrodes. If the two electrodes are close enough together, the deflections overlap in time and appear almost monophasic.

Because you are stimulating externally, a significant voltage is required to obtain any response. Once you have reached a threshold voltage for the median giant fiber, you need to raise the voltage further to stimulate the lateral giant fibers which have higher stimulus thresholds.

In addition to their higher stimulus thresholds, the lateral fibers conduct more slowly than the median fiber. Thus their action potential reaches the recording electrodes after a longer latent period and is seen as a second, delayed peak in the recording.

It seems to be universal in the animal kingdom that nerve fibers conduct impulses in either direction with equal facility. In most, if not all, nerve fibers, there is a preferred natural direction of conduction ("orthodromic"), but this is determined by the fiber's connections and not by the membrane ionic mechanisms responsible for the action potential. Specializations affecting the direction of transmission occur mainly at interconnections, such as synapses, between neurons. In the earthworm, the median and lateral fibers conduct their naturally generated impulses in opposite directions, stimulation of the median giant fiber results in rapid head withdrawal, stimulation of the lateral giant fibers results in rapid tail withdrawal.
What you will do in the laboratory
There are five exercises that you will complete during this Lab.

1. **Evoked action potentials.** In this exercise, you will evoke action potentials from the median giant fiber explore the concept of threshold, and show that the action potential is an all-or-nothing response.

2. **Recruitment of the lateral giant axons.** Here you will show that the worm has two giant fiber systems that conduct action potentials with different thresholds and latent periods.

3. **Axonal conduction velocity.** Here you will calculate the velocity of the median and lateral giant fibers, and compare the absolute and difference methods for calculating conduction velocity.

4. **Refractory period.** Here you will show that an axon can fire a second action potential only after a short period of recovery from a preceding one.

5. **Bidirectionality of nerve impulse.** In this exercise, you will demonstrate that nerve impulses can travel in either direction along an axon.