Related topics
Nerve and muscle potentials, mechanical stimulation, biphasic action potential, frequency modulation, median and lateral giant nerve fibres, conduction velocity

Principle and task
To work on the following themes by measuring nerve and muscle potentials:
- The course of a biphasic action potential over time
- Estimation of the conduction velocity
- Coding of the stimulant intensity as frequency modulation

Equipment
Cobra3 Basic Unit 12150.00 1
Power supply, 12 V 12151.99 1
RS232 data cable 14602.00 1
Cobra3 Universal Recorder software 14504.61 1
Bio-amplifier 65961.93 1
Earthworm experiment chamber 65981.20 1
Stimulus bristle, triggering 65981.21 1
Connecting cord, 32 A, \( l = 25 \text{ cm} \), red 07360.01 2
Connecting cord, 32 A, \( l = 25 \text{ cm} \), blue 07360.04 2
Connecting cord, 32 A, \( l = 25 \text{ cm} \), black 07360.05 2
Crocodile clip, insulated, black 07276.05 1
Adapter BNC-plug/4-mm-sockets 07542.26 1
Aluminium foil
Earthworms
PC, Windows® 95 or higher

Set-up
— Connect the instruments as shown in Fig. 1.

— Place the earthworm experiment chamber on aluminium foil and remove the lid
— Connect the bio-amplifier AMPLIFIER IN to the pins or the sheet metal of the chamber, so that the + electrode is 3 cm and the – electrode 4 cm away from the rear end of the worm. Fix the sheet metal for earthing the worm between the lid of the chamber and the earthworm (Fig. 2)
— Connect the earthed socket of the bio-amplifier to the earthing socket of the chamber, and to the aluminium foil (with the crocodile clip)
— Connect the bio-amplifier AMPLIFIER OUT to Cobra3 ANALOG IN 2 (red to +, blue to –)
— Connect the stimulus bristle to the bio-amplifier TRIGGER IN
— Connect the bio-amplifier TRIGGER OUT to Cobra3 ANALOG IN 1: the red (or most central) jack of the Adapter with the upper yellow jack (+) of Analog In 1; the black (or lateral) jack of the Adapter with the white jack (\( \frac{1}{2} \)) of Analog In 1
— Set the bio-amplifier to EMG, amplification 1000 times

Fig. 1: Experimental set-up

Fig. 2: Earthworm experiment chamber
Procedure
- Call up the COBRA3 MEASURE programme in Windows
- Select the UNIVERSAL WRITER as measuring instrument
- Set the measurement parameters (see Fig. 3) and go to measurement with CONTINUE

Preliminary experiment:
- Form a 12-15 cm long dummy earthworm from a wet paper tissue and lay this in the chamber
- Put the lid (ruler) on, fit the earthing electrode (sheet metal) in and fix the lid with rubber bands
- Start the measurement by using the triggering bristle, save the result of the measurement

Earthworm experiment:
- Wash and dry the earthworm and place it in the groove in the chamber. Prevent the earthworm from crawling out of the chamber by appropriately positioning the lid and rubber bands, and the pieces of sponge rubber at the two ends of the groove
- Click on the red point and go to measurement with CONTINUE
- Insert the stimulus bristle through the hole in the chamber lid and weakly stimulate the rear end of the earthworm, save the result
- Repeat the measurement procedure with a moderate and a strong stimulation. To effect strong stimulation, strongly dent the rear end, or touch the tip of it.

Results and evaluation
- Preliminary experiment: The noise signals from the dummy worm should have an amplitude of less than 10 µV (take the 1000 times pre-amplification into consideration!). With higher amplitudes, check the earthing of the aluminium foil, the chamber and the sheet metal

Experiment with weak stimulation: Fig. 4 shows only a single, biphasic action potential, with an amplitude of 80 µV and of 2 ms duration. To estimate the conduction velocity $v$, take the conducting time $t$ as the distance between the maximum and minimum of the action potential (0.9 ms in Fig. 4). The stretch covered $s$, i.e. the distance between the electrodes, is 1 cm. Using the equation $v = \frac{s}{t}$, the conduction velocity is found to be 11.1 m/s.

Experiment with moderate stimulation: Fig. 5 shows two biphasic action potentials at a distance of 9 ms. The amplitude of 80 µV is the same size as that for weak stimulation. The stimulus intensity is therefore not coded to the height of the amplitude (amplitude modulation) but to the distance of the action potentials (frequency modulation).
— Experiment with strong stimulation: Fig. 6 shows three nerve action potentials in short succession and a completely different form of potential. This potential is a muscle potential with an amplitude of 80 µV and of 20 ms duration.

Notes
— Earthworms have a median giant nerve fibre, which reacts to stimulation at the front end, and two lateral giant fibres, which can be stimulated at the rear end. The median giant fibre has a diameter of 70 µm and conducts the action potential at a high velocity (17–25 m/s). The lateral giant fibres have a slower excitation conduction (7–12 m/s).
— Muscle potentials can always be clearly differentiated from nerve potentials, as they last far longer and usually have a higher amplitude.

Fig. 6: Result with strong stimulation
Derivation of nerve and muscle potentials by mechanical stimulation at the rear end of an earthworm