

Related topics

Nerve and muscle potentials, electrical stimulation, anaesthetization of muscles, electrical resistance of nerve fibres, double pulse stimulation, refractory period

Principle and task

To work on the following themes by measuring nerve and muscle potentials:

- The action of an anaesthetic
- The different conduction velocities of median and lateral giant fibres
- Refractory period of the median giant fibre

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
Stimuli generator	65962.93	1
Earthworm experiment chamber	65981.20	1
Connecting cord, 32 A, $l = 25$ cm, red	07360.01	1
Connecting cord, 32 A, $l = 25$ cm, blue	07360.04	1
Connecting cord, 32 A, $l = 25$ cm, black	07360.05	2
Connecting cord, 32 A, $l = 50$ cm, red	07361.01	1
Connecting cord, 32 A, $l = 50$ cm, blue	07361.04	1
Crocodile clip, insulated, black	07276.05	1
Petri dish, $d = 100$ mm	64705.00	1
Spoon with spatula end, $l = 150$ mm, steel, wide	33398.00	1
Balance SAS51, 200 g/0.01 g, RS232	45990.93	1
Graduated cylinder, 100 ml	36629.00	1
Aluminium foil		
Earthworms		
Chloretone as anaesthetic (pharmacy or dentist: 1,1,1-trichloro-2-methyl-2-propanol)		
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 stimuli generator **PULSE OUTPUT** to Cobra3 **ANALOG IN 1** (red to yellow, blue to white)
- Connect the stimuli electrodes also to the **PULSE OUTPUT** of the stimuli generator (red to +, blue to -). Fasten the crocodile clip of the positive pole to the pin which is 3 cm away from the rear of the earthworm, the crocodile clip of the negative pole to the pin which is 4 cm away from it
- Set the bio-amplifier to EMG, amplification 1000 times

Fig. 2: Earthworm experiment chamber

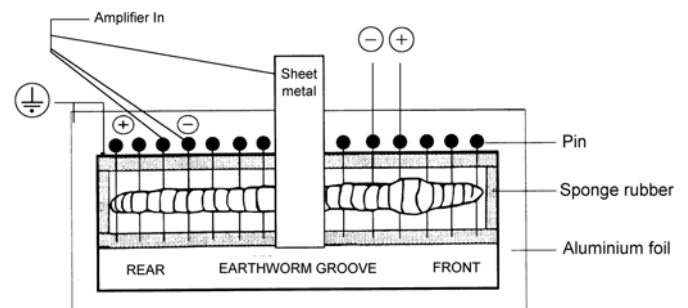


Fig. 1: Experimental set-up



- Set the stimuli generator to single pulse, 0.5 ms pulse width, amplitude at the middle setting

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**
- Switch on the stimuli generator, press the **START** switch and measure the amplitude of the stimulus. Repeat the test measurements to adjust the amplitude to 3.5 V
- 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

Experiment 1:

- Click on the red point and go to measurement with **CONTINUE**
- Stimulate the earthworm (not yet anaesthetized) with a rectangular impulse of 3.5 V and save the result
- Dissolve 0.2 g chlorethone in 100 ml of warm tap water and anaesthetize the earthworm in a Petri dish with this solution for 5 to 10 minutes
- Lay the benumbed animal carefully in the chamber. Caution! Do not hold the earthworm at its ends, as it would then be drawn out extremely long, because of the numbness of the dermal muscular tunic!
- Stimulate the earthworm with a rectangular impulse of 3.5 V as previously and save the result. Should no action potential appear, slightly increase the stimulating voltage or wait a few minutes until the effect of the anaesthetic weakens

Experiment 2:

- Click on the red point and go to measurement with **CONTINUE**

- Stimulate the benumbed earthworm with smaller and smaller rectangular impulses; to do this, decrease the stimulus amplitude in steps of 0.1 V until there is no action potential more to be seen.

Experiment 3:

- Click on the red point and go to measurement with **CONTINUE**
- Set the stimulus amplitude to a value at which only one action potential is evoked (see Experiment 2). Switch from single pulse to double pulse with 10 ms interval
- Stimulate the benumbed earthworm with double pulses at continually smaller intervals; to do this, reduce the double pulse interval in steps of approximately 1 ms until only one action potential appears.

Results and evaluation

- *Experiment 1:* Prior to being anaesthetized, the animal reacts with muscle potentials, which are preceded by action potentials, which overlap each other and can therefore not be properly individually interpreted. With the anaesthetized animal, the anaesthetic paralyzes the dermal muscular tunic, so that muscle potentials no longer appear. Two action potentials can be recognized; first the action potential of the median giant fibre and then that of the lateral giant fibre (Fig. 4). To estimate the conduction velocity v , take the conducting time t as the distance between the maximum and minimum of the action potential (0.45 ms for the median giant fibre in Fig. 4). The stretch covered s , i.e. the distance between the electrodes, is 1 cm. Using the equation $v = s/t$, the conduction velocity is found to be 22.2 m/s.

Fig. 3: Measurement parameters

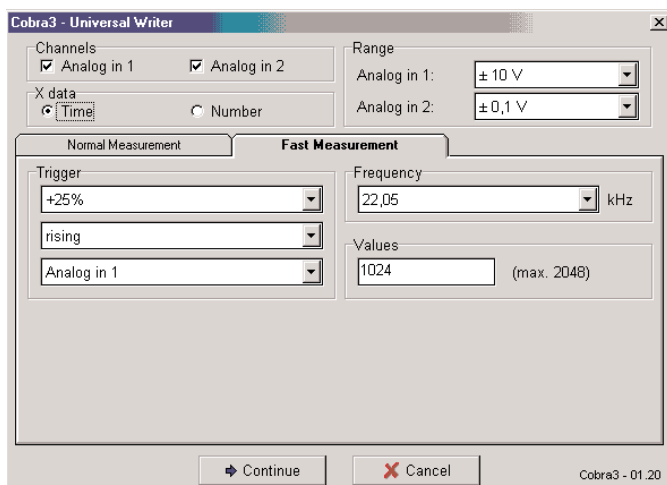
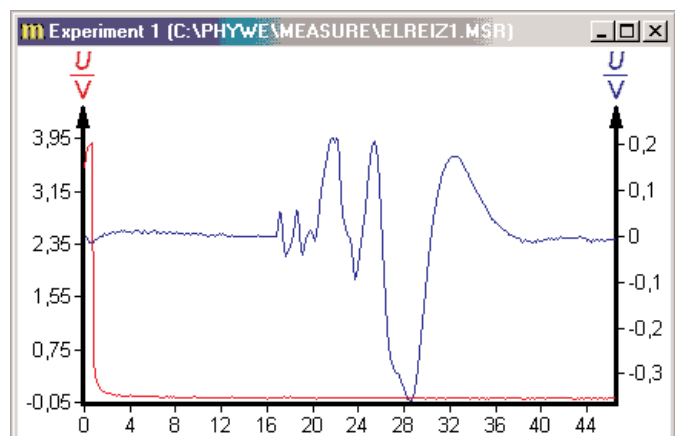


Fig. 4: Result with a strong stimulus



- *Experiment 2:* Because of its lower electrical resistance, the thicker median fibre requires a smaller depolarizing voltage than the thinner lateral giant fibre. On decreasing the exciting voltage to 3.9 V, only the median giant fibre responds (Fig. 5), and on further decreasing it, no action potential at all appears.
- *Experiment 3:* With double pulses at large time intervals, two action potentials appear which are distinctly separate from each other (Fig. 6). On reducing the time interval between the double pulses, the action potentials first approach each other and subsequently the second action potential gets smaller and smaller until it completely disappears (Fig. 7). From the time interval between the double pulses at which only one action potential is triggered, we have as an approximation a refractory period of approx. 2.9 ms.

Notes

- *On Experiment 1:* When the animal lies too long in the anaesthetic solution, the substance diffuses into the ventral cord and neither action potentials nor muscle potentials are recorded. The distinct difference in the conduction velocities of the giant fibres is explained by the difference in the fibre diameters. As the lateral fibres have a smaller conducting cross-section and so a greater longitudinal resistance, the excitation cannot propagate as quickly in them as in the median giant fibre.

- *On Experiment 2:* The excitation threshold of the thicker median giant fibres is lower than that of the thinner lateral giant fibres
- *On Experiment 3:* Longer rest periods should be planned when stimulating with double pulses, as they quickly subject the nerves to "fatigue"

Fig. 6: Result with a double pulse 6 ms

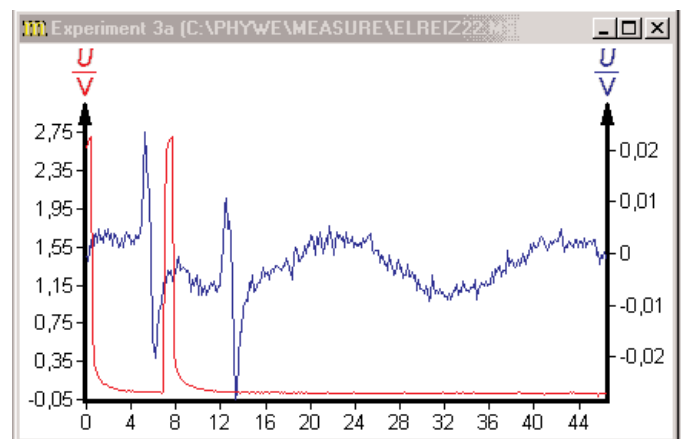


Fig. 5: Result with a weak stimulus

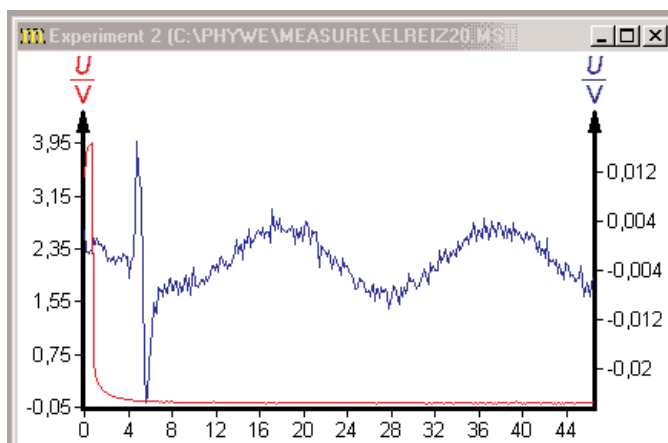
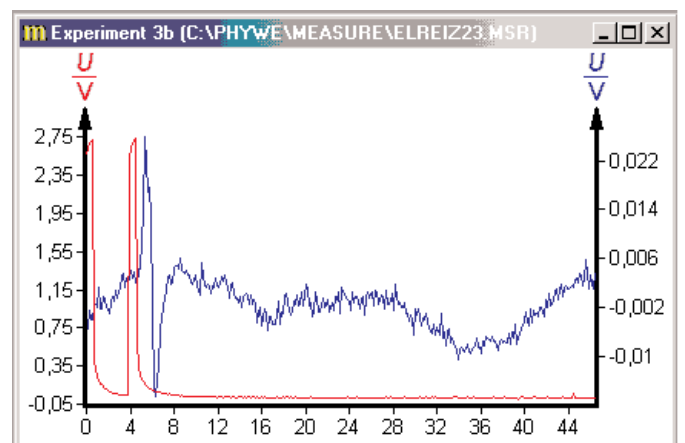


Fig. 7: Result with a double pulse 3 ms



Space for notes