Investigation of fibre size stimulation selectivity using earthworm model

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Abstract

Fibre type and diameter selective stimulation may allow to restore various motor and sensory functions of human body that have been lost due to disease or injury. Already many stimulation techniques have been proposed for that purpose. They were verified performing computer simulations and in some cases also by in vivo *experiments on mammalian models. Results of computer simulations still need to be confirmed by in vivo experiments, however experiments on mammalian models, due to high number of fibres within stimulated nerve, can be very complex to perform and obtained results difficult to interpret. In this paper, we propose the earthworm (Lumbricus terrestris) as a model for selective stimulation. The earthworm has three giant nerve fibres, with two distinctly different conduction velocities. Therefore it is very easy to distinguish between fibres that are firing at the moment. As a consequence the selectivity of stimulation may be immediately verified without application of sophisticated signal processing and averaging techniques. During performed experiments we have proofed that experimental procedure is simple and the obtained results easy to interpret.*

Keywords*: selective stimulation, fibre size selectivity, anodal block, animal studies, earthworms*

Introduction

Electrical stimulation is commonly used to restore lost body functions. To most spread devices applying electrical stimulation for that purpose belong cardiac pacemakers and cochlear implants for hearing restoration. But there are also devices for many other applications [1]. Use of electrical stimulation for restoration of lost body function would be even more frequent, if there was a reliable method allowing for selective activation of only particular type of fibres within the nerve. Since distinct types of fibres have distinct diameters, fibre type stimulation selectivity may be also understood as fibre size stimulation selectivity.

Various strategies for selective electrical stimulation of nerve fibres with distinct diameters have been proposed [2]. The operation of most of those strategies have been investigated using computer simulations and in some cases also verified during experiments on mammalians [3, 4]. Computational models however are often too simplified to observe the real phenomena that occurs when stimulating neural tissue, therefore it is necessary to perform also *in vivo* experiments. On the other hand, mammalian models are very complex and their results difficult to interpret.

Recently Yoshida et al. [5] have used earthworms to validate nerve conduction velocity selective recording technique. The earthworm (*Lumbricus terrestris*) has three giant nerve fibres, with two distinctly different conduction velocities – the big and fast conducting medial giant fibre (MGF) and two smaller and slower conducting, but coupled together by collateral branches, lateral giant fibres (LGF). We believe that due to these properties, earthworm may be a very useful model not only for investigation of recording selectivity, but also stimulation selectivity. In order to prove this statement, we have developed an experimental set-up and have investigated various selective stimulation strategies using earthworms.

Material and Methods

Experimental set-up

The experimental set-up consists of a programmable stimulator (Stim'nD) with 12 current controlled channels, developed by our team in cooperation with MXM-Neuromedics company [6], connected to a personal computer with a data acquisition card (PCI-6251 from National Instruments) and software for control of stimulation (SENISManager, DEMAR Group, University Montpellier 2/MXM-Neuromedics, France) [7] and recording (MrKick, Aalborg Universtity, Denmark). We also used differential amplifiers with gains 2500x and 3750x developed in our team and a few sets of custom made stimulating and recording electrodes. Two types of electrodes were used: cuff-like and needle electrodes. Cuff-like electrodes consisted of 7-25

mm wide paper tape on which 1,5 or 2 mm wide copper stripes were stuck. Depending on the application 2 to 9 stripes were used. Connecting leads were soldered to ends of stripes and paper tape was rolled around the worm in a way ensuring proper contact between copper stripes and the worm. As needle electrodes a few milimeter long tungsten wires (0,075 mm diameter) were used. Tungsten wires were mechanically connected with connecting leads by pushing end of the tungsten wire between wires of the connecting leads. To the other ends of connecting leads appropriate connectors were soldered.

The earthworms were purchased from the a local supplier and kept at room temperature.

Protocol

The earthworms were anaesthetized for 10 minutes in a 15 % ethanol solution aerated with ambient air [5]. Afterwards stimulating and recording electrodes were placed and connected. In most experiments the configuration of electrodes presented in fig. 1 was used. The cuff-like electrodes were used for stimulation and needle electrodes for recording purpose. The two outermost contacts (cooper stripes) of the cuff-like electrode were however short-circuited and connected to the reference inputs of the differential amplifiers used for recording. Two of the remaining contacts of the cuff-like electrode were used for the stimulation, one of them as a cathode and one as an anode. Various distances between the cathode and the anode were investigated (from 1,5 mm to 13,5 mm). The needle electrodes were always inserted from the side, parallel to the ground, on the ventral side of the worm. The distance between the two needle recording electrodes connected to the same differential amplifier was around 5 mm. Three pairs of the recording electrodes were used, two of them located on one side of the stimulating electrode and the third one on the other side. After insertion of the electrodes various techniques for selective activation of MGF and LGF fibres have been investigated and nerve responses to the stimulation were recorded.

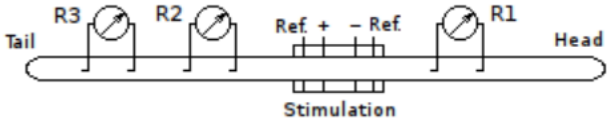

Fig. 1: Electrode connection schema during the performed experiments (R1, R2 and R3 – recording sites, Ref. - reference electrodes for differential recording)

Results

We have performed experiments on 47 earthworms, out of them 20 were excluded because either worm was still moving after anaesthesia or it was not possible to record action potentials (APs) from both MGF and LGF fibres. From remaining 27 earthworms selective activation of LGF fibre (and block of MGF fibre) was achieved in 23 cases. In some cases also selective block of LGF fibres was investigated.

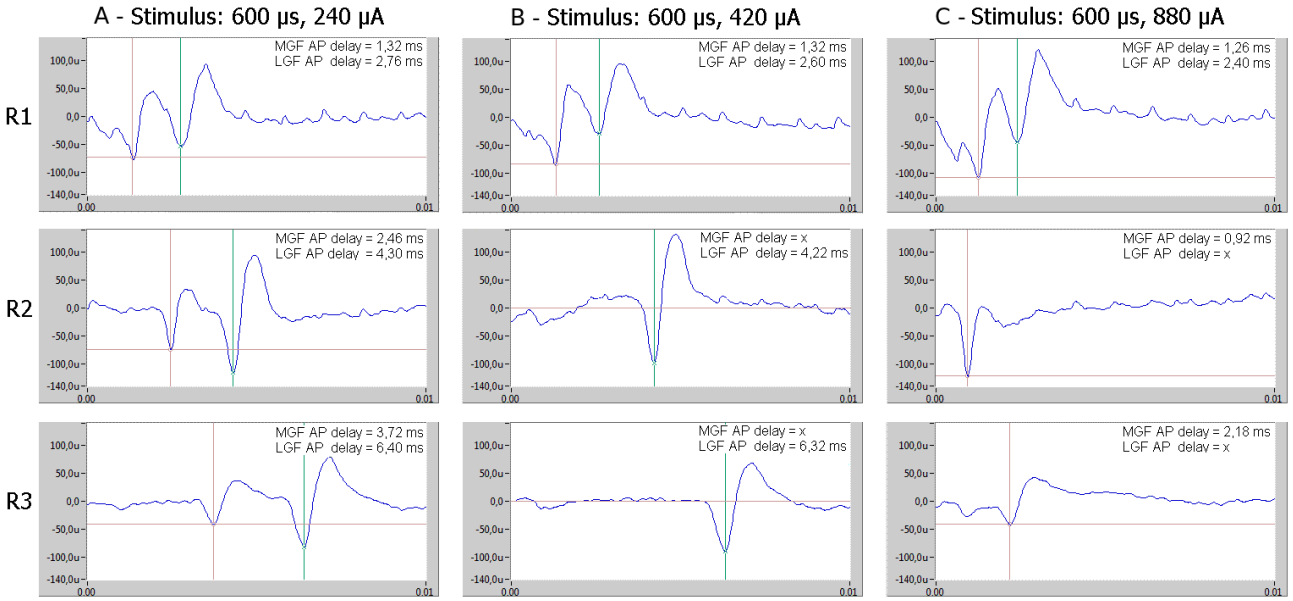

Fig. 2: Example of results recorded at three recording channels for monophasic rectangular stimuli (600 µs long) recorded during first 10 ms from the onset of the stimuli. Single sweeps (without averaging), distance between stimulating electrodes 6 mm, sampling frequency: 50 kHz, high pass filtered: 80 Hz, amplitudes in µV. R1, R2 and R3 – three recording channels. Positions of the stimulating and recording electrodes same as in fig. 1 (R1 located proximally to the stimulating electrode, R2 and R3 located distally). Distance from the cathode to: R1 – 18 mm, R2 – 29 mm, R3 – 46 mm. Column A: threshold to activate both MGF and LGF fibres. Column B: threshold to block distal propagation of MGF Aps. Column C: threshold to block distal propagation of LGF APs, but anodal activation of MGF fibre also visible (APs recorded at the R2 and R3 channels appear earlier than for the lower amplitudes).

The usual signal-to-noise ratio (SNR) was around 10 for cuff-like and 2-4 for needle recording electrodes. Because MGF and LGF fibres have significantly different propagation velocities (in our experiments it was 10-20 m/s for MGF and 5-9 m/s for LGF) and the distances between stimulating and recording electrodes were known, it was very easy to distinguish between APs generated by MGF and LGF fibres. Using the proposed set-up we were able to precisely investigate thresholds for cathodal and anodal activation, as well as anodal block of both types of fibres using various pulse shapes, pulse durations and various distances between stimulating electrodes.

An example of signals recorded during our experiments is presented in fig. 2. In the column B unidirectional block of MGF APs (selective activation of LGF fibres) and in the column Cright column unidirectional block of LGF APs (selective activation of MGF fibre) is shown.

Discussion

The results that we have obtained prove that experiments on earthworms maybe very useful for evaluation of fibre size selective stimulation strategies. Because experiments are performed on real nerve, they may allow to investigate phenomena which could not be observed during computer simulations. In the same time the experimental procedure is much less complex and recorded signals much easier to interpret as in the case of experiments performed on mammalian model. Furthermore, some phenomena (such as for example anodal activation), may be much easier observed on earthworms than on complex mammalian models, because change in AP shape or delay is in this case much easier to observe.

The disadvantage of the proposed method is the inability to verify selectivity of stimulation of high numbers of fibres being located in various distances from the stimulating electrode. Therefore performing experiments on earthworms would be one more important step to perform when investigating techniques for fibre diameter selective stimulation, rather than the substitution of experiments performed on more complex mammalian models.

The other disadvantage of the proposed method is rather low stability of the observed responses. It could be due to many factors. Among them are: stability of anaesthesia, drying up of the worm and nerve damaged due to applied stimulation. Therefore further investigation is necessary in order to determine experimental conditions, which would improve stability of the obtained results.

Conclusions

We have proved that performing experiments on earthworms may allow for non-complex *in vivo* investigation of techniques for fibre diameter and thus also fibre type selective stimulation. Because earthworm has only three giant nerve fibres with two distinctly different conduction velocities, it is possible to distinguish between firing fibres with precision not achievable using much more complex mammalian models.

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