Medical Engineering & Physics 31 (2009) 1261–1270

Contents lists available at ScienceDirect

Medical Engineering & Physics

journal homepage: www.elsevier.com/locate/medengphy

Experimental validation of the nerve conduction velocity selective recording technique using a multi-contact cuff electrode

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ARTICLE INFO

Article history:
Received 31 July 2008
Received in revised form 7 August 2009
Accepted 12 August 2009

Keywords:
Earthworm
Peripheral nerve model
Multi-contact cuff electrode
Velocity sensitive filter
Matched filter
Delay adder filter

ABSTRACT

The earthworm (*Lumbricus terrestris*) is presented as an *in vitro* model of a peripheral nerve containing only two fibers each with distinctly different conduction velocities, the median and lateral giant fibers (MGF and LGF). The worm model is used with a multi-contact cuff electrode to validate the spatial–temporal filtering effect of different electrode contact configurations and the effect of applying a delay adder and matched filter tuned to either the MGF or LGF action potential (AP) to extract conduction direction and velocity from the recording. The results confirmed the known effect of inter-electrode spacing and bipolar and tripolar recording configuration on the AP amplitude. It also demonstrates a crossover point where the amplitude of the tripolar recording is larger than the monopolar recording, an effect of the slower action potential conduction velocities in the worm. The delay adder was found to be an effective velocity sensitive filter, able to discriminate units based on conduction velocity. The matched filter to be an effective means to eliminate artifact and increase signal to noise ratios, however was not found to improve selectivity.

1. Introduction

Different computational models incorporating voltage activated ion channels [1] and the cable equation [2,3] have been developed over the past 50 years [4–7] to understand the biophysics of the electric field captured by an extracellular recording device. Although most models are significantly simplified, they made it possible to simulate a myriad of potential recording configurations and paradigms to give insights into how electrode geometries and processing techniques could improve different types of selectivity of recording. The predictions from these models can next be tested *in vivo* in the acute mammalian model to determine whether they translate to the real world. However, mammalian models can still be complex and their results difficult to interpret. For example, models indicated that fascicle selective recording of nerve activity could be obtained by using a cuff electrode which places multiple electrode contacts at different points along the circumference of the nerve. These predictions were confirmed in [8] to some degree, although the complexity of the model made it difficult to gain insights on what to add to the computational model to further improve the method.

Another type of selectivity, the sensitivity to discriminate neural signals of different nerve fibers of a particular caliber (fiber diameter), or size selectivity, can be optimized by taking advantage of the differences in action potential wavelengths and conduction velocities from different calibers using an appropriate spacing between recording contacts in the direction of nerve conduction [9–11]. By tuning the contact spacing to allow constructive summation of the action potential as a function of wavelength, a spatial–temporal filter can be formed. Multiple, simultaneous observations from multiple locations along the nerve, delayed and summed as suggested by [12] and [13] can be used to create a conduction velocity selective recording paradigm. Such filters are optimized on the conduction velocity making them velocity selective filters (VSF). This class of filter is a Delay and Sum-Beamformer, and has been used in sonar [14] and ultrasound [15] applications. In [13], it was first proposed to use a multi-contact cuff electrode to form multiple tripolar configurations [9] for velocity selective recording (VSR). It described in theory the method of using an array of double differential amplifiers, artificial time delays for each selected velocity, an adder and narrow band filters. Theoretically, selectivity could further be enhanced by passing the raw signal through an optimal match filter [16] before entering the beamforming filter.

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doi:10.1016/j.medengphy.2009.08.005

A major difficulty in the process has been the experimental validation of the theoretical prediction. The first *in vitro* experiments in frog demonstrated evidence of feasibility but managed to identify the conduction velocity of only the dominant fiber group [17]. Recognizing that a larger rejection of stimulus artifacts and a reduction in electrode and amplifier noise were needed, a dedicated low-noise amplifier system was designed [18], which made it then possible to increase the number of fiber populations that could be distinguished [19]. Nevertheless, for use with naturally occurring nerve activity the selectivity needs to be increased further. Enlargement of the delay adder, however, increases the detection threshold as a larger amount of nerve signal power is needed to exceed the increased noise power [20].

Previous experimental validations were limited to electrically evoked compound action potential (CAP) response. Ideally, the optimal and beam forming filters should be trained with data recorded from identified single nerve fibers. However, the process of collecting a set from an amphibian or mammalian nerve bundle that can contain hundreds–thousands of nerve fibers and ensuring that each identified unit is a single unit is a daunting technical challenge. Moreover, because of the relatively high conduction velocities of mammalian nerves, the length of nerves used in previous *in vitro* experiments are short, leading to (1) an overlap in stimulation artifact and CAP response and (2) synchronous contributions from different fiber groups which cannot be individually recognized. Because of these two factors, it is difficult to compare the effect of VSFs of different fiber groups from the CAP.

In the present study, we used a new experimental model, *Lumbricus terrestris*, the earthworm as a representative model of an *in vitro* peripheral nerve preparation. A multi-contact cuff electrode was placed around the entire worm, which modeled a peripheral nerve. This "nerve" contains three myelinated giant fibers, one median giant fiber (MGF) and two lateral giant fibers (LGF) that are located in a ventral nerve cord (VNC) which extends through the entire length of the earthworm [21,22] and have a nerve conduction velocity ranging 15–45 m/s and 7.5–15 m/s respectively [23–25]. These giant fibers mediate rapid withdrawal reflexes of the anterior (MGF) and posterior (LGFs) [26,27]. When action potentials are elicited in the LGFs they are conducted synchronously as the two LGFs electrically coupled by collateral branches and functions as a single unit. The model is representative to the traditional whole nerve cuff electrode recording technique, except that the worm model can be simplified to a nerve containing only two fibers each with distinctly different conduction velocities, which were used to experimentally test two VSF techniques.

2. Methods

2.1. Experimental set-up

The earthworms (*L. terrestris*) used in the present study were purchased from a local supplier (Hvidovre Sport, Hvidovre, Denmark) and kept quiescent at 4 °C. 24 h before the experiments, when they were moved to a cabinet and kept at room temperature (21 °C).

The experimental set consisted of work on twelve adult earthworms. The earthworms were anesthetized in a 10% ethanol solution for 10 min aerated with ambient air. Following the anesthesia the posterior end of the earthworm was threaded through the multi-contact cuff electrode with the aid of a suture. The multi-contact cuff electrode, unlike those used in [28,29], had an inner diameter of 4 mm and contained 11 equally spaced platinum foil ring contacts (0.5 mm wide, 3 mm contact spacing). Subsequently, the earthworms were placed in a Sylgard® bottomed Petri dish which had two additional blocks of Sylgard® (1 cm in height) on which the head and tail of the worm was placed (see Fig. 1). The head and tail of the worm were pinned to the blocks of Sylgard® with two sets of needle electrodes. These two sets of needle electrodes were used for stimulating the anterior and posterior end of the worm, respectively. The multi-contact cuff electrode was placed on the bottom of the Petri dish, which was filled with normal isotonic saline (0.9% NaCl).

Signals from the electrode were differentially amplified and filtered (gain: 1000×, highpass: 0.1 Hz). The two outermost electrode contacts were shorted and served as the reference (see Fig. 1). Adequate stimuli of the MGF and LGF was accomplished using standard 100 μs long rectangular, current controlled pulses (SD9 + PSIU6, Grass-Telefactor, USA). The resulting ENG signals were sampled at 50 kHz. The inter-stimulus interval was 2 s. The experimental acquisition and control was accomplished using a PC with a PCMIO-E1 card (National Instruments, USA) running experimental control and data acquisition software (MrKick, Aalborg University, Denmark).

2.2. Protocol

First, the thresholds intensities (minimum intensity evoking a response from each of the fibers) were established and the supramaximal stimulation intensity, the level that would activate steady both the MGF and LGF fibers, was set to two times the threshold found. Then, the action potentials elicited by at least 50 stimuli to the head of the earthworm were recorded. In six experiments, the tail of the earthworm was subsequently stimulated and the responses to at least 50 stimuli were recorded.

2.3. Signals processing

2.3.1. Electrode spacing

The experimental data were recorded with each channel of the 8 channel amplifier configured to amplify adjacent rings (3 mm electrode spacing) of the multi-contact cuff electrode in a differential bipolar recording configuration. In the data analysis these data were digitally post-processed to obtain all other possible bipolar and tripolar configurations with electrode spacings of 6 mm, 12 mm, 18 mm, and 24 mm. These signals were obtained from the original bipolar signals, where the bipolar channel number n with an electrode spacing of 3 mm ($b_{3\text{mm},n}$) can be written as $m_n - m_{n-1}$ (m denoting a monopolar channel, and n the channel number). Consequently, the bipolar channel $b_{6\text{mm},n}$ can be found as $b_n + b_{n-1} = m_n - m_{n-1} + m_{n-1} - m_{n-2} = m_n - m_{n-2}$. The bipolar signals with 12 mm, 18 mm, 24 mm were obtained by adding 1, 2, and 3 additional 3 mm bipolar channel to this sum, respectively. Once all of the bipolar signals were found, the tripolar signals were calculated from the derived bipolar signals.

2.3.2. True conduction velocities

The "true" conduction velocities of the MGF and LGF units where found from the seven tripolar signals (3 mm inter-electrode spacing). The middle negative peak in each recording indicates when the action potential passed the center electrode of the 3 mm tripolar triple. This instance was identified for each of the seven channels to give the time and position of the unit as the action potential traversed the cuff electrode. A linear least squares regression was made on the time vs position data for each of the two units, and their "true" conduction velocities were estimated as the slope of the regression.

2.3.3. Filters

The VSF tested in the study was implemented by delaying each 3 mm tripolar channel by a multiple of a constant delay (τ) and the channel number (n), and following this with a summing operation of the delayed outputs (see Fig. 2). The delays were transformed to