Simple and low-cost amplifier system for the study of a ventral nerve action potential in earthworm

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Abstract

Basic Biophysics or Neurobiology courses encounter problems when starting their practices due to the complexity and high cost of the equipment. As a consequence, these experiences are replaced by simulation software, leading students to boredom and disinterest, and finally to the lack of understanding of the basic principles of the phenomenon observed. Classical nerve conduction studies for the visualization of action potentials are an example of this, being able to be developed with simple and low-cost amplification circuits, as described in this article. The system showed stability, expected amplification and high signal-to-noise ratio. The action potential of the medial giant fiber (MGF) as lateral giant fiber (LGF) in the ventral nerve cord of the earthworms *Lumbricus terrestris* was recorded. The experimental system has met expectations and regains practical, effective and stimulating learning for students.

Keywords: Action potential, giant ventral nerve cord, earthworm, low-cost amplifier circuit.

1. Introduction

In the study in classical neurophysiology of neural conduction mechanisms, must pass by the reading of the works carried out by Hodgkin-Huxley.

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26 They describe an electrical change recorded in nerve fibers known as action
27 potentials (AP) [1]. Because most of the nerve fibers are extremely small, AP
28 records were initially performed on giant squid axons (*Loligo forbesi*), but ob-
29 taining them is difficult because of their geographic and seasonal availability,
30 leading to the search for other animals with the same feature.

31 In general, invertebrates, such as earthworms, have larger nerve cords
32 than other taxonomic groups, facilitating conduction studies in the nervous
33 system,[2],[3]. These one have a ventral nerve cord large enough to be used
34 in classical electrophysiology studies [2],[4],[5],[6],[7],[8],[9].

35 On the other hand, most AP records published are made with high-cost
36 amplification equipment, making it difficult to access these types of practical
37 studies by replacing them with virtual experiments through software, causing
38 students to feel bored and disinterested in difficult to learn. Nowadays it is
39 possible to find electronic components of low cost for the implementation of
40 this type of amplifier. Traditional action potential recording systems require
41 basic electronic equipment such as an oscilloscope, a stimulator (eg grass
42 stimulator), an amplifier, a low-pass filter, and a data acquisition system.
43 Also, we need a suction electrode and a micro-manipulator. All of this high-
44 cost equipment except the suction electrode.

45 Recent efforts by some authors [8],[7], in the earthworm AP record, used
46 relatively inexpensive equipment with invasive or non-invasive systems and
47 with or without electrical stimulation, obtaining records without good res-
48 olution and with associated noise. The complete setup configuration is im-
49 portant for good records and it is difficult to dispense equipment such as
50 oscilloscope or acquisition systems with an adequate sampling rate.

51 In an attempt to solve some of these problems the objective of this work
52 was to describe a simple and low-cost amplification circuit for the repro-
53 duction of classical studies of action potentials in the ventral nerve cord in
54 earthworms.

55 2. Materials and Methods

56 2.1 Biological material, ventral nerve dissection and experimental system.

57 The procedure was an adaptation as described by Roberts (1962). Earth-
58 worms of the species *Lombricus terrestris* were obtained around the Sosígenes
59 Costa Campus of the Federal University of Southern Bahia, Porto Seguro,
60 Bahia, Brazil. For maintenance, they were placed in a container containing

61 soil and minced banana. To dissect the ventral nerve cord the earthworm
62 was anesthetized in 10% alcohol for 6 minutes, washed with water to remove
63 alcohol residue and placed in a petri dish filled with hardened silicone. The
64 worm was placed on the petri dish with the dorsal side up and pinned to 2/3
65 of the cephalic part. Using a low-cost common magnifying glass to amplify
66 the tissue, the dorsal part was cut with scissors being careful not to damage
67 the intestine and nerves and it was fixed with pins, exposing the digestive
68 system which was removed with tweezers to expose the ventral nerve cord.
69 Saline solution for earthworm [11] consisting of NaCl (6 g/L), KCl (0.12 g/L)
70 CaCl₂ (0.2 g/L) and NaHCO₃ (0.1 g/L) was added to wipe scraps of tissue
71 and keep saline medium. The nerve was cut at the bottom with scissors and
72 separated from the tissues by the tip of a suction electrode.

73 The loose nerve was suctioned with a glass suction electrode consisting
74 of an internal Ag/AgCl electrode and an Ag/AgCl outer coiled to the 1 ml
75 pipette tip, attached to a 30 cm tube and a 5 mL syringe similar to described
76 by Johnson et al., (2007). To chloridizing the silver wire it was placed in
77 a 5% solution of sodium hypochlorite overnight. This suction electrode is
78 attached to a low-cost manipulation system consisting of articulated metal
79 bars and not to a micro-manipulator as commonly used and connected to
80 the amplifier as described in the following section. The stimulus electrodes
81 were connected in the cephalic part of the earthworm with two electrodes the
82 anode (+) closest to the head and the cathode (-) following it (see Fig.1).
83 The preparation was earthed between the first pair of stimulus electrodes and
84 the nerve cord. The action potentials were amplified and visualized using a
85 Tektronix TBS 1072B digital oscilloscope. The computer was running on
86 battery power to reduce electrical noise. The experiments were carried out
87 at an ambient temperature of 28°C. To determine the conduction velocity
88 (CV) the following equation was used:

$$CV = \frac{d}{latency} \quad (1)$$

89 Where d is the distance between the stimulation electrodes and the suc-
90 tion electrode (5 cm approx.) and latency, which is the time between the
91 stimulus and the first deflection from baseline.

92 Fig. 1

93 2.2 Electronic circuit, stimulus system and data acquisition.

94 To amplify the voltage of action potential was made a circuit consisting
 95 of two followers amplifier connected at the input of a differential amplifier
 96 (INA128P) and an offset regulator made with an amplifier type TL071CN
 97 and two 9 volt batteries that also serve to power the circuit (Fig.2). This off-
 98 set regulator is for adjusting the baseline close to 0 mV. The circuit simulation
 99 can be seen in the following URL, <http://tinyurl.com/yy8vuz49>. According
 100 to the configuration of the suction electrode connections, the internal elec-
 101 trode was connected to the non-inverting input and the external electrode
 102 (screwed into the suction electrode) was connected to the inverting input
 103 of the differential amplifier INA128. The output of the differential ampli-
 104 fier was connected to an inverted offset regulator amplifier TL071CN which
 105 gives finally a non-inverted signal for inverting input and inverted signal
 106 for non-inverting input. In the case of the experimental system assembled,
 107 first detects the potential variation in the non-inverting input (internal suc-
 108 tion electrode) compared to the inverting input (external suction electrode)
 109 which gives the expected response to an extracellular register.

110 Fig. 2

111 Table 1 shows the components used to mount the amplifier and the ap-
 112 proximate cost for each of them.

113 Table 1

114 The gain of the amplifier used was 99 and 1065 times calculated as:

$$G = 1 + \frac{50k\Omega}{Rg} \quad (2)$$

115 Where Rg is the external resistance to be modified with 511 ohms and 47
 116 ohms to have the gains described.

117 The signal-to-noise ratio was calculated as:

$$SNR = 20 \log_{10} \frac{A_{signal}}{A_{noise}} \quad (3)$$

118 Where A_{signal} and A_{noise} are the RMS (root mean square) for each re-
 119 spectively.

120 The amplification system and the data acquisition hardware were placed
 121 inside a Faraday cage connected to the ground to avoid noise.

For the data acquisition and electrical stimulation, USB-6218 digital-analog converter with 250 KS/s (kilo-samples by seconds) and Labview software from National Instruments was used. The Labview program is based on graphical programming and consisting of a viewing window (front panel) and a programming window (block diagram). A pulse generation program was made, and this consisted of a pulse with 100 μ s of duration with an amplitude ranging from 0-10 Volts at 1s intervals as described in Fig.3. A data acquisition program was made as described in a tutorial of the national instrument [13]. The rate acquisition was at 50kHz and stored in .lvb format (Labview measurement) and finally imported and analyzed using the Excel program.

Fig.3

3. Results and Discussion

3.1 Assembly of the experimental system.

The amplification circuit was assembled as described in materials and methods. This showed stability in response and high signal to noise ratio of 25 dB avoiding the use of a filter for the purpose under study. The total cost of the amplification system was approximately US\$ 25 (see Table 1), having a lower value compared to other commercial systems for the same purpose. The most costly was the oscilloscope (US\$ 800), the LabView program (US\$ 2990) and the digital-analog converter USB-6218 (US\$ 1498).

Over the past decade, several authors[6],[7],[8],[9],[14] have provided relatively low cost solutions using commercial equipment to amplify, filter and acquire data as well as the stimulation systems. Some of them use pin approach without having an invasive system in the AP registry but even anesthetized worms move slightly increasing the noise and varying the baseline making it very difficult to adjust the offset of the amplified signal. Low-cost systems are usually related to low acquisition resolution and noise issues. The proposed system considerably reduced the cost of amplification didn't use filters but didn't escape the use of high-cost systems such as oscilloscope and data acquisition system. The stimulation system has been simplified using the same acquisition system but this stimulus can also be generated using other platforms such as Arduino with limitation of the output voltage of the equipment to 5 volt.

3.2 Recording action potentials.

After suctioning the nerve with the electrode suction and turning on the amplifier, it is possible to observe spontaneous fluctuations of potential (Fig. 4a), being these records were reproducible when the earthworm is slightly anesthetized (<6 min) and the suction electrode has suctioned the nerve cord properly. This means that the inner diameter of the pipette must be similar to the nerve diameter to increase the resistance between the internal and external Ag/AgCl electrodes to observe a potential variation.

Under electrical stimulation conditions, there are characteristic fluctuations described as a development of action potential, with a pronounced depolarizing event followed a repolarizing face. It was possible to record action potential from lateral giant fiber (LGF) as medial giant fiber (MGF), featured for its conduction time with approximately 4.5 ms (Fig.4b) and 1.5 ms respectively (Fig.4c). This difference can be explained as described by Gunther et al., 1961 and Shannon et al. (2014), increasing the diameter of the axon, increase their conduction velocity in a relationship that is the square root of the axon diameter. The diameter of the giant fiber is 0.05 mm for the LGF and 0.07 mm for the MGF approximately.

The peak-to-peak amplitude was approximately 100 mV (Gain = 100) (Fig.4b) and 60 mV (Fig.4c) but these values depend on the resistance between the reference electrode and recording electrode in the suction pipette because the measured value is the potential drop in external resistance. The conduction velocity was of 11 m/s for LGF and 33 m/s for MGF. The value found is similar to found by Rushton et al., (1944), Gunther et al., (1961), kadt et al., (2010) and Shannon et al. (2014) and far from reported by Castelfranco and Hartline, 2016. According to Shannon et al. (2014), an increase of 1.4 times the conduction velocity between LGF and MGF is expected but it depends on the fiber size values which may vary depending on the earthworm analyzed.

Doubles AP was also observed when the ventral nerve cord was stimulated with a greater potential (from 1 to 9 V) or when the duration of the pulse increased from 100 μ s to 1 ms (Fig.4d). This is because the stimulation to voltage or pulse duration is high enough to stimulate both fibers in the nerve cord (MGF and LGF).

Fig.4

191 3.3 Troubleshooting.

192 Some problems were encountered while registering AP in the neural cord.
193 If the earthworm is anesthetized for a time larger than 6 minutes we don't
194 observe elicit spike, a situation similar to that described by Shannon et al.,
195 2014. We recommend not to exceed 5 min.

196 AP logging is most often out of the baseline which can be offset by the
197 offset regulator implemented in the amplifier.

198 If the pipette tip on the suction electrode has a diameter larger than the
199 neural cord, AP may not be observed. The pipette tip must have a diameter
200 similar to the neural cord. Failure to adhere properly greatly decreases the
201 resistance between the electrodes and AP cannot be observed.

202 A system of articulated metal bars was used as a guide to the suction
203 electrode, but a good approximation of the electrode to the neural cord is
204 difficult. The use of a micro-manipulator system is necessary but it has a
205 high cost.

206 3.4 Educational considerations.

207 The work in the educational context resumes an experimental perspec-
208 tive that is losing itself every day with access to information internet way.
209 The student wants quick answers through the use of sources and programs
210 of simulation generating cognitive situations with prejudices without an ad-
211 equate theoretical foundation. The described experimental system allowed
212 the student to learn concepts of basic neurobiology in a ludic manner and
213 stimulates him to the implementation of a new low-cost methodology for
214 study pharmacological effects in AP and implementation of other techniques
215 such as voltage-clamp current analysis, among others.

216 Therefore, practices such as these help in teaching-learning, leading to not
217 only undergraduate courses but primary and secondary schools, stimulating
218 students curiosity about basic processes in neurophysiology.

219 4. Conclusions

220 The present work allows recovering in a simple form the classic study
221 of the propagation of nerve impulse by recording action potentials using
222 components of low cost and simple to assemble and experience one of the
223 basic foundations of neuroscience.

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270 Figure caption

271 **Fig.1** Experimental set up for recording action potentials from a ventral
272 neural cord in the earthworm. a) Suction electrode coupled to the manipu-
273 lator; b) ventral nerve after dissection (segmented arrow); c) positioning of
274 the stimulation electrodes on the earthworm head, the recording electrode
275 (suction pipette) and the reference electrode near the latter; d) experimental
276 system diagram indicating the animal used the stimulation, amplification, vi-
277 sualization (oscilloscope) and acquisition and recording system (USB-6218,
278 computer).

279 **Fig.2** Low-cost amplifier for recording action potentials. a) Photograph
280 of the assembled system; b) circuit diagram used for mounting the amplifier
281 consisting of two operational amplifiers, one INA128p, and one TL071CN
282 plus one offset regulator.

283 **Fig.3** Front panel (a) and Block diagram (b) from Labview software for
284 generating the stimulus pulse using the USB-6218.

285 **Fig.4** Action potential record from a ventral neural cord in the earth-
 286 worm. a) spontaneous fluctuation of AP (indicated by continuous arrow)
 287 without electrical stimulus; b) characteristic AP lateral giant fiber with a
 288 latency of 5 ms; b) characteristic AP medium giant fiber with latency of 2
 289 ms ; d) Double AP by increased the stimulus duration from 100 us to 1 ms
 290 indicating the activation of both fibers in the nerve cord.

Fig. 1

Fig 2

a

b

Fig. 3

Fig. 4

Table 1: Material needed to build the amplifier circuit and associated cost. Value quoted in Digi-key electronics (www.digikey.com).

Component	Quantity	Cost US\$	Total (US\$)
INA128p	1	11.16	11.06
TL071	1	0.4	0.4
Resistor 511 ohm	1	0.11	0.11
Resistor 47 ohm	1	0.1	0.1
Resistor 1kohm	1	0.12	0.12
Resistor 715 ohm	3	0.18	0.54
Capacitor 0.1 uF	2	0.23	0.46
Aluminum case 5x10x4 cm	1	8.45	8.45
switch	2	0.87	1.74
potentiometer 10K	1	1.83	1.83
Total (US\$)			24.91
Oscilloscope Tektronix TBS1072B	1		800
USB6218	1	1498	1498
Labview	1	2999	2999