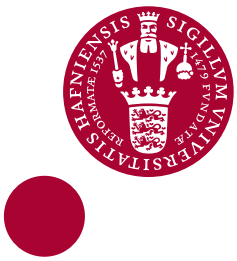

Comment on "Penetration of Action Potentials During Collision in the Median and Lateral Giant Axons of Invertebrates"

Berg, Rune W.; Stauning, Marius Tving; Sorensen, Jakob Balslev; Jahnsen, Henrik

Published in:
Physical Review X

DOI:
10.1103/PhysRevX.7.028001

Publication date:
2017

Document version
Publisher's PDF, also known as Version of record

Comment on "Penetration of Action Potentials During Collision in the Median and Lateral Giant Axons of Invertebrates"

Rune W. Berg,* Marius Tving Stauning, Jakob Balslev Sørensen, and Henrik Jahnsen

Faculty of Health and Medical Sciences, Department of Neuroscience and Pharmacology, University of Copenhagen, Blegdamsvej 3, DK-2200 KBH N, Denmark

(Received 29 June 2015; revised manuscript received 20 November 2015; published 24 April 2017)

The action potential (AP) is an electrical impulse elicited by depolarization of the neuronal membrane from the resting membrane potential (around −70 mV). It propagates along the axon, allowing for rapid and distant communication. Recently, it was claimed that two APs traveling in opposite direction will pass unhindered through each other (penetrate) upon collision [Gonzalez-Perez *et al.* Phys. Rev. X **4**, 031047 (2014)]. We tested this claim under carefully controlled conditions and found that we cannot reproduce penetration. Instead, APs consistently annihilated upon collision. This is consistent with a vast body of literature.

DOI: 10.1103/PhysRevX.7.028001 Subject Areas: Biological Physics

In Ref. [1], Gonzalez-Perez *et al.* reported data on collision of action potentials (APs) in the earthworm medial axon and the lobster ventral cord [1]. They concluded that colliding APs penetrate each other, and they suggested that the AP is essentially a mechanical pressure wave in the membrane, i.e., a soliton. This is a direct challenge of the prevailing view of the AP as an essential electrical phenomenon driven by membrane currents through voltage-gated ion channels [2]. According to this view, two colliding APs annihilate each other because each AP leaves a trace of inactivated Na^+ channels behind, preventing the other APs from passing. Because the results of Ref. [1] are unexpected, we have tested their claim in the earthworm medial axon, in the lobster peripheral nerve, and in the frog sciatic nerve (for experimental procedures, refer to Ref. [3]). We find the following results.

(1) *In the earthworm medial axon, colliding action potentials annihilate*. We stimulated the earthworm at either end and measured using one set of electrodes placed close to the posterior end [Fig. 1(a)]. The AP generated from either end arrives with different delays at measurement electrodes [Figs. 1(b) and 1(c)]. When stimulating simultaneously at both ends with increasing strength, the AP elicited from the anterior end was recorded at lower stimulation voltages [Fig. 1(d)]. At higher stimulation voltages, the AP from the posterior end now appeared, but at the same time, the AP elicited from the anterior end disappeared, indicating that it was annihilated by collision [Fig. 1(d)]. In the case of penetration, both action potentials should have been seen.

(2) *APs in single axons are all-or-none types, and their amplitudes are independent of the direction of propagation*. To establish that an AP in a single axon is being studied, it is necessary to demonstrate the all-or-none characteristic, i.e., that the AP appears abruptly at one stimulation voltage and keeps an unchanged amplitude at higher voltages [Figs. 1(b) and 1(c)]. Unfortunately, Gonzalez-Perez *et al.* did not present the reader with such evidence [1]. Furthermore, we found that the amplitude of an AP measured at the same particular point along the axon is the same in both directions [Figs. 1(e) and 1(f)], which is expected if the velocity and shape of the AP are independent of the direction of propagation (which we establish below). In contrast, the only example shown in Ref. [1] (their Fig. 6) in support of penetration in the medial axon features two signals of different amplitudes, shapes, and durations.

(3) *The action potential propagation velocity in the earthworm medial axon is the same in both directions*. Gonzalez-Perez *et al.* claim that propagation velocity is systematically lower in one direction (antidromic) than in the other (orthodromic) (their Fig. 7). However, they measured the velocity using the time delay from stimulation until AP arrival at a single set of measurement electrodes [mono recording, Figs. 1(h) and 1(i)]. This method underestimates the velocity because the delay between stimulation and initiation of the action potential will corrupt the measurement. Moreover, the method cannot be used for comparing velocities in the two directions because the delays in either end will not be identical, and two different stretches of axon are being measured, which might support different velocities

*Corresponding author.
runeb@sund.ku.dk

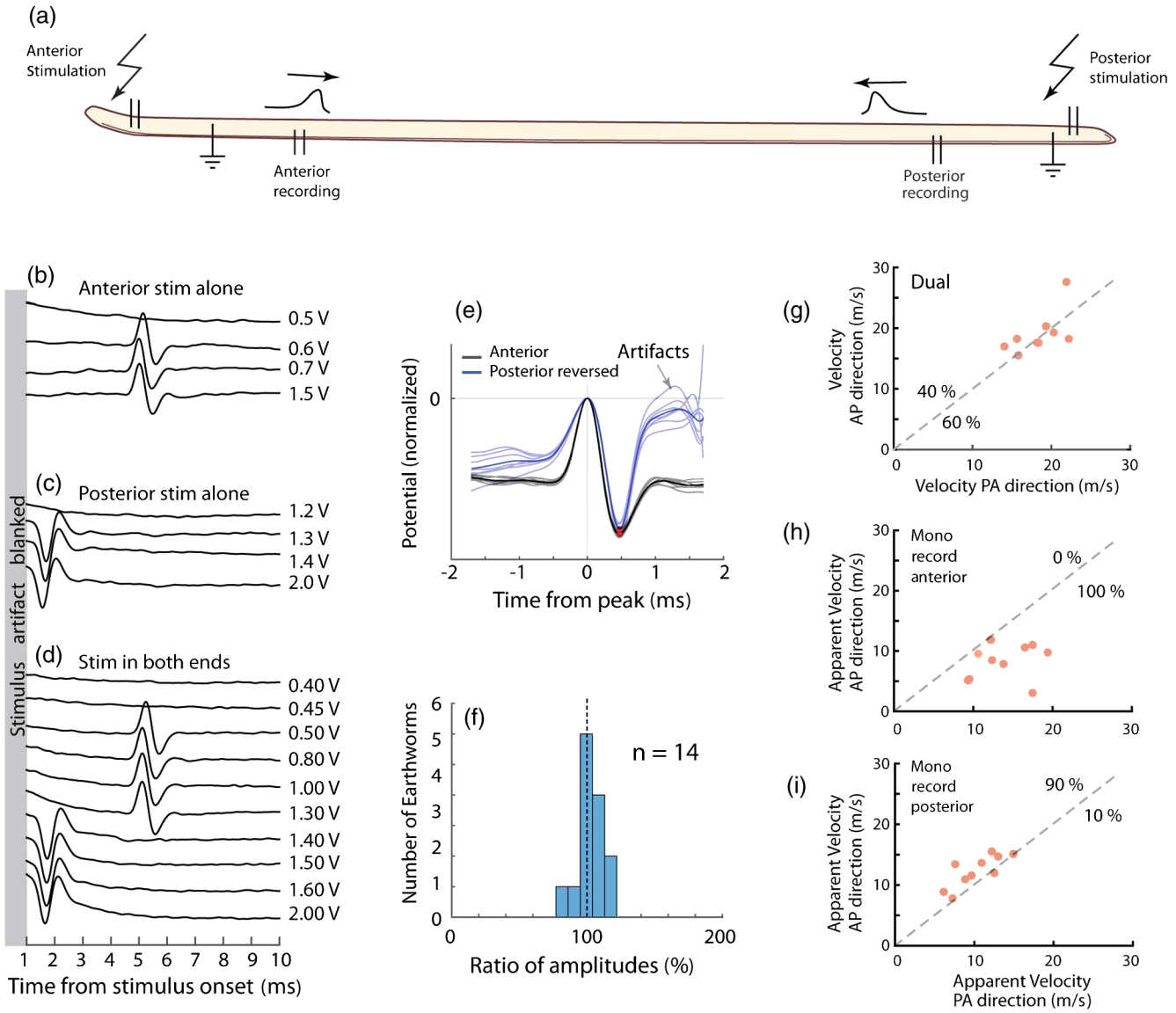

FIG. 1. Action potentials annihilate upon collision. (a) Experimental setup: Pairs of stimulation electrodes for generating APs in both ends, and pairs of recording electrodes are also placed towards both ends. (b) Stimulation of the anterior end of increasing strength and recording at the posterior end (consecutive traces, stimulation strength indicated) evokes APs to illustrate single same-fiber activation by (1) the threshold phenomenon and (2) the amplitude independence of stimulation strength (collectively known as the all-or-none phenomenon). (c) Similar to (b) with stimulation in the posterior end and single APs traveling in opposite directions (posterior recording). Note that the threshold is higher in the posterior than in the anterior end, and the latency is shorter when stimulating posteriorly. (d) Simultaneous stimulation in both ends with increasing strengths first evokes anterior AP until the strength is suprathreshold for generating APs in both ends (1.4 V). Here, the APs propagate from both the anterior and posterior ends and collide in the middle. Since the anterior and posterior APs never appeared together in the same recording, annihilation occurred. This was observed for all earthworms ($n = 14$). (e) Anterior (black lines)/posterior (blue lines, time-reversed) overlaid APs stem from the same nerve fiber as verified by their similarity in peak-to-peak amplitude (ordinate location of red dots, ratio = 96%). (f) Amplitude ratios for the population (PA/AP) are close to 100%. (g) Velocities based on the accurate method (using two sets of electrodes, i.e., dual recording) in the anterior-posterior (A–P) direction plotted against velocities in the reverse direction, i.e., posterioranterior (P–A) ($n = 10$ earthworms). A–P and P–A velocity are the same according to a Kolmogorov-Smirnov test. (h) Apparent velocities using only one set of recording electrodes, as in Ref. [14], in the anterior end (i.e., mono recording). The velocity was estimated as the distance divided by time from stimulation to arrival, thus ignoring the impulse width and AP-generation delay, resulting in a disparity in velocities. (i) Velocities using the reverse configuration (recording in the posterior end), giving opposite disparity in velocities, which illustrates the methodological flaw of using mono recording.

independently of the direction. We measured the propagation velocity using the time difference (and distance) between two sets of measurement electrodes [dual recording, Figs. 1(a) and 1(g)]. The results show that the velocity is the same in both directions (within the accuracy of the measurement), and markedly higher (18.4 ± 0.8 m/s in the anterior-posterior direction; 18.9 ± 1.1 m/s in the posterior-anterior direction) than reported by Gonzalez-Perez *et al.* (2.8–9.7 m/s). A velocity of 18–19 m/s at room temperature is in agreement with the literature [4–8]. When recalculating our data according to Ref. [1], the velocity always appears to be lower in the direction where the stimulation and measurement electrodes are closer together [Figs. 1(h) and 1(i)] because the measurement is dominated by the delay. Thus, the mono recording method of Gonzalez-Perez *et al.* is flawed. We conclude that antidromic and orthodromic pulses are approximately equally fast. While this is not necessarily the case in other preparations (for instance, if measurements are made close to a large electrotonic load such as the cell soma), our experiment implies that, in the preparation used here, action-potential amplitudes should be independent of direction (see also above).

(4) *In uninterrupted axonal bundles, colliding compound action potentials annihilate*. Gonzalez-Perez *et al.* presented collision experiments (their Figs. 9 and 10) carried out in the ventral cord of the lobster. This experiment depends on the ability to identify single action potentials from giant fibers in the very complex recordings (Figs. 9 and 10). However, the data in their Fig. 9 show increased amplitude of the deflections with increasing stimulation strength (compare 1 and 2 V), inconsistent with single action potentials. Instead, these might be compound action potentials from nongiant fibers, which undergo complex synaptic connections within the intervening ganglia, making collision experiments impossible. Nevertheless, collision experiments can be carried out using compound action potentials, provided that uninterrupted axonal bundles are used and all axons are stimulated. We carried out collision experiments using peripheral nerves from the lobster walking legs and frog sciatic nerve, which do not contain ganglia. The results demonstrated annihilation in both species (see Ref. [3]).

(5) *Annihilation has been reported in dozens, probably hundreds, of publications over the course of more than 65 years*. Space does not permit citing all these papers, but annihilation has formed the basis for the so-called collision test, which has been widely used to map out axonal connections [9–12], also in earthworms [13].

We conclude that both our experiments and multiple previous reports show that action potentials annihilate upon collision, in agreement with the view of the action potential as a propagating membrane depolarization driven by membrane currents through voltage-dependent ion channels.

We thank Alex Willumsen for help with data analysis, Morten Bjerre Nielsen for help with the experiments on frog nerves, and William T. Nickell for comments on the manuscript. The work of R. W. B. was funded by The Danish Council for Independent Research Medical Sciences.

[1] A. Gonzalez-Perez, R. Budvytyte, L. D. Mosgaard, S. Nissen, and T. Heimburg, *Penetration of Action Potentials During Collision in the Medial Giant Axon of the Earthworm*, Phys. Rev. X **4**, 031047 (2014).

[2] A. L. Hodgkin and A. F. Huxley, *A Quantitative Description of Membrane Current and Its Applications to Conduction and Excitation in Nerve*, J. Physiol. **117**, 500 (1952).

[3] See Supplemental Material at http://link.aps.org/supplemental/10.1103/PhysRevX.7.028001 for details about, first, the materials and methods for the experiments presented in this study, second, it contains a description of the result of the additional experiments, not described in the main article. These experiments involve the action potential speed in earthworm axons, collision of action potentials in lobster peripheral nerves as well as in the frog sciatic nerve. Furthermore, there is an additional discussion and literature review of the collision experiment in previous scientific reports.

[4] C. D. Drewes, K. B. Landa, and J. L. McFall, *Giant Nerve Fibre Activity in Intact, Freely Moving Earthworms*, J. Exp. Biol. **72**, 217 (1978).

[5] J. Günther, *Impulse Conduction in the Myelinated Giant Fibers of the Earthworm. Structure and Function of the Dorsal Nodes in the Median Giant Fiber*, J. Comp. Neurol. **168**, 505 (1976).

[6] N. Kladt, U. Hanslik, and H.-G. Heinzel, *Teaching Basic Neurophysiology Using Intact Earthworms*, J. Undergrad. Neurosci. Educ. **9**, A20 (2010).

[7] K. Y. H. Lagerspetz and A. Talo, *Tempera Ture Acclimation of the Functional Parameters of the Giant Nerve Fibres in Lumbricus Terrestris L*, J. Exp. Biol. **47**, 471 (1967).

[8] W. A. H. Rushton, *Action Potentials from the Isolated Nerve Cord of the Earthworm*, Proc. R. Soc. B, **132**, 423 (1945).

[9] W. W. Douglas and J. M. Ritchie, *A Technique for Recording Functional Activity in Specific Groups of Medullated and Non-medullated Fibres in Whole Nerve Trunks*, J. Physiol. **138**, 19 (1957).

[10] J. H. Jennings, D. R. Sparta, A. M. Stamatakis, R. L. Ung, K. E. Pleil, T. L. Kash, and G. D. Stuber, *Distinct Extended Amygdala Circuits for Divergent Motivational States*, Nature **496**, 224 (2013).

[11] J. Lipski, *Antidromic Activation of Neurones as an Analytic Tool in the Study of the Central Nervous System*, J. Neurosci. Methods **4**, 1 (1981).

[12] J. Yeomans, *Electrically Evoked Behaviors: Axons and Synapses Mapped with Collision Tests*, Behavioural Brain Research **67**, 121 (1995).

[13] T. H. Bullock, *Functional Organization of the Giant Fiber System of Lumbricus*, J. Neurophysiol. **8**, 55 (1945).