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

- Supplementary Information -

Tian Wang, Alfredo Gonzalez-Perez, Rima Budvytyte, Andrew D. Jackson, and Thomas Heimburg*
The Niels Bohr Institute, University of Copenhagen,
Blegdamsvej 17, 2100 Copenhagen, Denmark

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FURTHER EXPERIMENTS ON THE COLLISION OF NERVE PULSES IN THE WALKING LEG OF THE LOBSTER

We repeated the experiment shown in Fig. 1 with a slightly different electrode configuration. Instead of switching stimulation electrodes S_2 and recording electrodes R as in Fig. (1), we use two pairs of recording electrodes, R_1 and R_2 as shown in Fig. S1A. Stimulation takes place at S_1 and S_2. Recording at R_1 corresponds to the collision experiment performed by GP2014 [1]. Recording at R_2 corresponds to the collision block experiment by Berg2017 [2]. The data for both experiments are obtained simultaneously at both pairs of recording electrodes. Fig. S1B shows the result of the collision experiment. Upon increasing the stimulation voltage, more and more neurons are activated. Nevertheless, after simultaneous stimulation at S_1 and S_2 the sum of the individual signals (S_1+S_2) is practically identical with the recording after simultaneous stimulation for all voltages. No indication of annihilation can be seen. In contrast, the recording at R_2 shows that at higher voltages the signals from S_1 gradually disappear (Fig. S1C). The signals originating from S_1 have a significantly smaller amplitude than those recorded at R_1. This is a consequence of the larger distance of R_2 from the stimulation site and the different velocities of the pulses in the different neurons. However, none of our experiments show any indication of annihilation at low stimulation voltages in the collision block experiment (as already stated in our reply [3]). This could indicate that we never succeed in stimulating the same neurons at S_1 and S_2 or that the disappearance of signals from S_1 at higher voltages is not due to annihilation. Since, simultaneously, no annihilation was observed at recording electrode R_1, it seems more likely that stimulation with high voltage at site S_2 perturbs the membranes to a degree that renders pulse propagation impossible. In the experiment in Fig. S1B no pulse is required to travel through a highly perturbed region. It is clear from Figs. 1 and S1 that measurements on the same pair of pulses can yield dramatically different results depending on the placement of the recording electrodes. This emphasizes the need for caution in tacitly assuming that differences in experiment design are irrelevant.

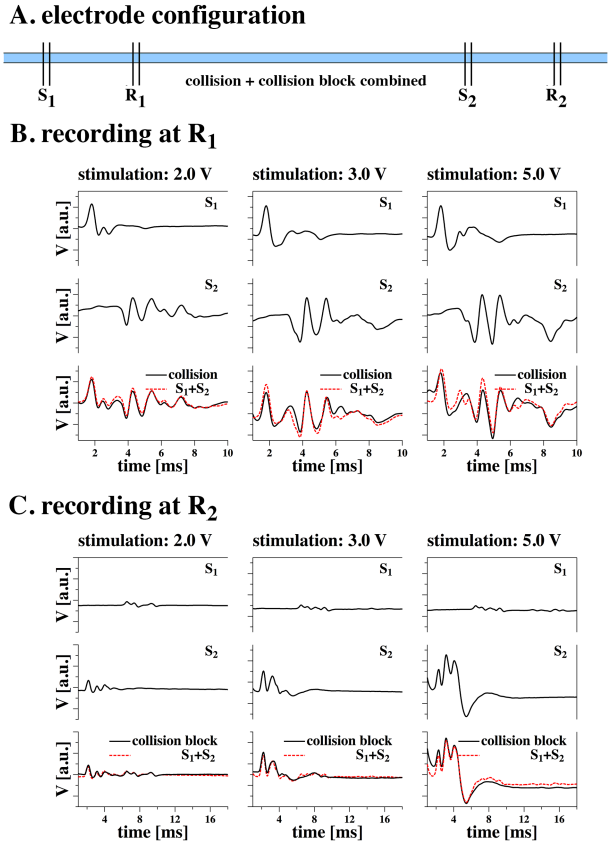

FIG. S1. **A.** Electrode configuration for simultaneous recording of the collision (as in GP2014) and the collision block experiment (as in Berg2017). B: Collision experiment showing penetration of pulses arising from S_1 and S_2 at all voltages. C. Collision block experiment as in Berg2017 showing penetration at low voltage and the absence of some pulses originating from S_1 at high voltage.

We also performed collision experiments with nerves from the ventral cord of lobster tails in a glass capillary where the nerves are always embedded in an electrolyte environment (data will be shown elsewhere). These measurements confirmed our results on the other nerves.

* theimbu@nbi.ku.dk; http://membranes.nbi.ku.dk

This is additional evidence that the criticism made by Berg2017 in their supplement that our results are a consequence of non-physiological conditions is incorrect. For completeness, it should be noted that at high stimulation voltage one can sometimes see single peaks either appearing or disappearing in some collision experiment traces. This might be due to being close to the threshold of single neurons. This does not affect the finding that collision and collision block experiments lead to largely different results. Finally, it is strange that in our experiments we can always stimulate all neurons separately by increasing voltage, both in worm and lobster nerves. This is not seen in the experiments shown by Berg2017.

PULSE VELOCITIES

Berg2017 [2] dedicates considerable space to a discussion of pulse amplitudes and absolute pulse velocities in earthworm axons. These issues are of no relevance for our 2014 article [1]. Nevertheless, we feel that it is useful to comment some of the statements made in Berg2017, which are not generally valid.

1. The authors state that velocities in both directions must be the same. This is incorrect [4]. Both in the HH-model and the soliton theory the velocity depends on the diameter of the axon which changes over the length of the earthworm. Pulses coming from different ends of a neuron travel through regions of different average diameter if the recording electrode is placed between the stimulation sites.
2. The assignment of a velocity to a nerve pulse is ambiguous because it is determined by arbitrarily choosing a reference point in the signal and relating it to the distance between the electrodes. It is difficult to compare the velocities if the shapes of the pulses are not identical.
3. Berg2017 claim that "The results show that the velocity is the same in both directions (within the accuracy of the measurement), and markedly higher (approx. 20m/s) than reported by Gonzalez-Perez et al. (2-8 m/s). A velocity of 20 m/s is in agreement with the literature". It is incorrect that the value of Berg2017 is the general finding in the literature. The velocity of the median giant axon of the earthworm ventral cord can actually vary from 6 to 30 m/s and the velocity of the lateral giant axon of the earthworm from 2 to 10 m/s. In the experiments by GP2014 the velocity of median giant axon was 6-10 m/s in 30 different preparations, and 3-7 m/s in the lateral giant axon. These numbers are consistent with [5].
4. There is no theoretical need for action potentials to maintain their shape, neither in the Hodgkin-Huxley model (where it depends on radius and channel distribution) nor in the Soliton model (where it also depends on the radius due to changing energy densities). The claim that pulses traveling in opposite directions must have the same shape, velocity and amplitude is therefore unjustified. In fact, there are numerous reports showing that orthodromic pulses propagate faster than antidromic pulses, e.g. [4, 6, 7].
5. In addition, both the experimental temperature and the acclimation temperature of the animal influence the velocity in the axons. Lagerspetz et al. [5] found velocities ranging between 6 and 32 m/s in the median giant axon of worms and 3-10 m/s in the lateral giant axon. Kladt et al. (2010) report velocities of 6-14 m/s in the median giant axon and 4-7 m/s depending on temperature (between 2°C and 25°C) in the intact earthworm [8]. In any event, precise determination of the conduction velocity of the median and lateral giant fibers of the intact earthworm (as used by Berg2017) is not possible. The reason is anatomical. These axons are not perfectly straight but are rather bent along the principal axis. Thus, earthworms can stretch two or three times their length without damaging their ventral cords [9]. Any attempt to determine the conduction velocity in the intact earthworm will result only in an apparent velocity. It is shown in [9] that the apparent conduction velocity of earthworm nerves can change from 8 to 20 m/s upon stretching. This is especially relevant when the intact earthworm moves.

Thus, contrary to the claims of the authors of Berg2017, there is clear evidence in the literature that orthodromic and antidromic pulses can have different shapes and velocities. The findings in GP2014 are completely consistent with results reported in the literature.

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