



REPLY TO FARRELL:

Experimental evidence is the ultimate judge for model assumptions

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In PNAS (1), we report the full-field interferometric imaging of the dynamics of neuronal deformations during the action potential. The imaging methodology we describe provides a noninvasive approach to observation of the neural signaling and also allows scientists to verify their models with more degrees of freedom provided by the spatial distribution of the deformations, compared to previous single-point measurements. We also show that the mechanical model based on voltage-dependent membrane tension proposed by Zhang et al. (2) fits our observations.

Farrell (3) argues that the membrane tension should change in a parabolic manner with the transmembrane voltage, with its maximum value at 16 mV, as proposed in their model (4). However, all of the experimental measurements of the voltage-dependent cellular deformations we are aware of demonstrate a quasi-linear relationship between the membrane tension and the transmembrane voltage: 1) figure 1c in ref. 2 demonstrates quasi-linear membrane displacement in HEK cells when the membrane potential was controlled from -180 to -60 mV by a patch clamp; 2) figure 4c in ref. 5 shows quasi-linear dependence of the force exerted by PC-12 cells on a piezoelectric nanoribbon when the membrane potential varied from 0 to 120 mV with respect to the resting potential; 3) figure 2A in ref. 6 depicts quasi-linear membrane displacement in HEK cells when the membrane potential varied from -120 to +60 mV; 4) figure 1H in ref. 7 demonstrates a quasi-linear increase in amplitude of

the optical phase change against the stimulus voltage amplitude ranging from 0 to 100 mV in HEK cells; 5) figure 3e in ref. 8 shows a quasi-linear membrane displacement measured at the edge and in the center regions of the HEK cells when the membrane potential was varied by a patch clamp from -100 to +100 mV; and 6) we also have not seen any evidence of an inflection point within the range of action potential: from -70 to +30 mV.

The models suggested by Farrell et al. in ref. 4 depart from these experimental observations, making interpretation of these claims difficult, particularly in the most relevant figure 8a, which includes inconsistencies in its plot of Zhang et al.'s model (2) (shown by the dashed black line). Here, the calculation of the membrane tension change is one order of magnitude larger than our own calculation ($\sim 10 \mu\text{N}/\text{m}$ per 0.1 V) using the same equation, while our result agrees with the experimental measurement in ref. 2. We were unable to find the supplementary material for ref. 4 that expands on that derivation, but in any case the overall outcome is the same: Experimental data support only a quasi-linear voltage-tension dependence in the voltage range relevant to the action potential.

Furthermore, as we mention in the Introduction and Discussion of our paper, many different models have been proposed to explain the mechanism of electromotility, and we encourage anyone working on theoretical modeling in this field to use all of the available experimental data, including ours.

1 T. Ling et al., High-speed interferometric imaging reveals dynamics of neuronal deformation during the action potential. *Proc. Natl. Acad. Sci. U.S.A.* **117**, 10278–10285 (2020).

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