LETTER

REPLY TO PITT AND LEE:

Occupancies of Ca^{2+} in complexes of calmodulin with voltage-gated sodium channels

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In PNAS, we describe a crystal structure of Ca^{2+}-loaded calmodulin (Ca^{2+}/CaM) in complex with the C-terminal region (CT) of the voltage-gated sodium channel $Na_V1.5$ (1). We contrasted our findings with those of Wang et al. (2) for the following reasons: The title and abstract had suggested a structure of Ca^{2+}/CaM in complex with Na_V CTs, and the corresponding Protein Data Bank (PDB) files (4JPZ and 4JQ) contain fully occupied Ca^{2+} ions in EF hands 3 and 4, which is not supported by the conformation and electron density. A similar comparison was previously made by Hovey et al. (3), who concluded that the structures by Wang et al. (2) did not support Ca^{2+} binding to the C-lobes.

In response to Pitt and Lee (4), we acknowledge that Wang et al. (2) had also stated that the C-lobes in their structures were not fully occupied with Ca^{2+} and that we fail to convey this message in our manuscript. However, in light of this analysis, as well as by Hovey et al. (3), and Gardill et al. (1), it is our opinion that assigning full occupancy for Ca^{2+} in EF-hands 3 and 4 in the corresponding PDB files is erroneous. Together with a misleading title, this has unfortunately brought confusion to the field. We do want to make it clear that there is no scientific disagreement between our studies. We show that the differences are due to the presence or absence of a fibroblast growth factor homologous factor (FHF), which can allosterically obstruct the binding site for the Ca^{2+}/C-lobe we report. Comparing the structures from the 2 studies thus provides mechanistic insights into how FHFs can affect CaM regulation of Na_Vs.

Pitt and Lee (2) claim that we do not acknowledge a previous finding that FHF can change the conformation of the Na_V CT. This statement is wrong, because on several occasions we reference the finding by Gabelli et al. (5), which was the first one to show this effect. For example, in our introduction, we write, "The authors also noted a different relative orientation of the EF-hand domain to the IQ domain depending on the presence of an FHF (33)." We note the efforts by Wang et al. (2), who were the first to describe the presence of a Ca^{2+}/N-lobe binding site immediately downstream of the IQ domain. In our manuscript, our goal was to compare this downstream binding site in $Na_V1.4$ and $Na_V1.5$, and we concluded that the site is present for $Na_V1.5$, but not $Na_V1.4$, a finding also made independently by Yoder et al. (6). We did reference the downstream binding site for the Ca^{2+}/N-lobe reported by Wang et al. (2) in the context of the structure, and analyzed the possibility that this site could be compatible with our reported Ca^{2+}/C-lobe binding site [see figure 4D in Gardill et al. (1) and the corresponding results section]. We apologize for our neglect to also reference the isothermal titration calorimetry results by Wang et al. (2), which are in agreement with the results of Yoder et al. (6) and our study (1).

1 B. R. Gardill, R. E. Rivera-Acevedo, C. C. Tung, F. Van Petegem, Crystal structures of Ca^{2+}–calmodulin bound to Na_V C-terminal regions suggest role for EF-hand domain in binding and inactivation. *Proc. Natl. Acad. Sci. U.S.A.* **116**, 10763–10772 (2019).

2 C. Wang *et al.*, Structural analyses of Ca^{2+}/CaM interaction with Na_V channel C-termini reveal mechanisms of calcium-dependent regulation. *Nat. Commun.* **5**, 4896 (2014).

3 L. Hovey *et al.*, Calcium triggers reversal of calmodulin on nested anti-parallel sites in the IQ motif of the neuronal voltage-dependent sodium channel $Na_V1.2$. *Biophys. Chem.* **224**, 1–19 (2017).

4 G. S. Pitt, S.-Y. Lee, Ca^{2+}/CaM interaction with voltage-gated Na^+ channels. *Proc. Natl. Acad. Sci. U.S.A.* **116**, 26150–26151 (2019).

5 S. B. Gabelli *et al.*, Regulation of the $Na_V1.5$ cytoplasmic domain by calmodulin. *Nat. Commun.* **5**, 5126 (2014).

6 J. B. Yoder *et al.*, Ca^{2+}-dependent regulation of sodium channels $Na_V1.4$ and $Na_V1.5$ is controlled by the post-IQ motif. *Nat. Commun.* **10**, 1514 (2019).

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The authors declare no competing interest.

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