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LETTER TO THE EDITOR

About hysteresis in Shaker: A note on Cowgill and Chanda

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Introduction

I am writing regarding the recently published article titled "Charge-voltage curves of Shaker potassium channels are not hysteretic at steady state" by John Cowgill and Baron Chanda (Cowgill and Chanda, 2023). The article presents a very interesting take on hysteresis in voltage-gated channels, which has become a topic very close to my heart. I have read the article several times since it was published in *JGP* and found it intriguing but conflicting with my understanding of the phenomenon in question. I don't take this matter lightly because I have great respect and admiration for Dr. Chanda. For that reason, I have very carefully considered sending this letter to point out what I believe is an oversight in the study.

As the authors pointed out, "hysteretic behavior of channel function has a clear physiological significance as it confers 'memory' to channel's gating properties." Thus, identifying the nature of this phenomenon is critical for our understanding of channel physiology. Towards this goal, the article takes aim at a core problem that could be summarized by the following question: *Is the activity-induced shift in a channel's voltage dependence a steady-state property or a transitory feature in the dynamic of these proteins?* The paper by Cowgill and Chanda (2023) makes the case for the second option, showing that recording gating currents for prolonged periods of time—nearing steady state—reveals that the voltage-dependence for gating charge movement seems to converge into a single charge–vs.–potential (Q-V) relationship irrespectively of the initial condition. This is a very interesting idea that goes along with the report by Lacroix, Labro, and Bezanilla from 2011 (Lacroix et al., 2011). Also using Shaker as a model protein, Lacroix and colleagues showed that the net gating charge movement can be underestimated when the slower late phase of the deactivating gating currents is overlooked. In this latter case, they showed that this oversight led to overestimating the depolarization-induced Q-V curve shift. Using a similar argument, Cowgill and Chanda proposed that applying very long-lasting voltage test pulses leads to the observation of no change in voltage dependence.

Discussion

Back to the question of the nature of voltage-gated channel hysteresis: Cowgill and Chanda designed simple but skillfully challenging experiments to address whether the shift in voltage dependence is either a transient process (a kinetic feature) or a *bona fide* steady-state behavior (a thermodynamic property) of Shaker's activity. To that end, they kept the membrane at a holding potential of either −110 or 0 mV to drive the channel's voltage-sensing domain (VSD) to a steady "down" or "up" state, respectively. To evoke gating currents, they applied test pulses to different potentials, driving charge movement. Following this, they applied a third pulse to 0 mV to assess how much net charge was displaced during the test pulse, and therefore determined whether the Q-V curve changed as a function of the initial holding potential. Using this approach, the authors showed that the Q-V curves generated from gating currents measured from an initial holding potential of −110 and 0 mV tended to converge as the test pulses were made longer.

As the authors pointed out, recording gating currents at 0 mV in *Xenopus* oocytes expressing Shaker is a good idea because, at that potential, gating currents are large and usually clean of other currents. However, calculating the charge movement only at 0 mV is also the Achilles' heel of the study. To make my point, I will call your attention to Fig. S2 and Fig. 2 of the paper which, in my opinion, illustrate the core concept of the article (Cowgill and Chanda, 2023).

Fig. S2 A of the paper (Fig. 1 here) shows a simple four-state kinetic scheme with two voltage-dependent transitions (depicted horizontally) and two voltage-independent transitions (depicted vertically). In steady state at −110 mV, the model will mainly reside at the stable closed (deactivated) state (C_S). In contrast, in steady state at 0 mV, the model will reside in the stable open (activated) state (O_S). Following depolarization from a very negative holding potential, the model transitions towards the unstable open (activated) state (O_U) to later reach O_S. When the membrane potential returns to the original negative potential, the model goes back from the O_S state to the initial C_S state, preferentially following the route through the unstable closed

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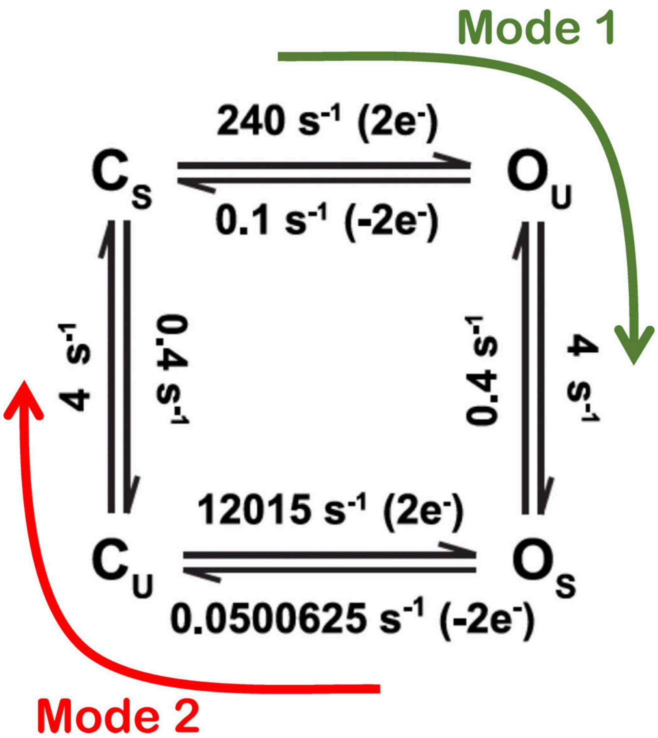

Figure 1. **Four-state model adapted from** Cowgill and Chanda (2023). The model consists of two closed/deactivated states (C_S and C_U) and two open/activated states (O_S and O_U). The subindices "S" and "U" stand for stable and unstable states, respectively. The transitions between C_S and O_U and between C_U and O_S are voltage dependent in both directions. The associated gating charges for each rate are in parentheses. The transitions between C_S and C_U and between O_U and O_S are voltage independent in both directions. The arrows highlight the mode of activity. These modes constitute the path for the movement of gating charges. The total net amount of charge displaced in Mode 1 is the same as that in Mode 2.

(deactivated) state (C_U). This happens because voltage-dependent rates are exponential functions of the membrane potential, and the rate $O_S \rightarrow C_U$ is larger than the rate $O_S \rightarrow O_U$ at voltages below −26 mV. Nonetheless, the bottom line is that the model populates the C_S state in steady state at −110 mV, while switching to the O_S state in steady state at 0 mV.

Fig. 2 A of the paper shows gating-current recordings from the voltage-gated K^+-channel Shaker carrying the mutation W434F. On the left, Fig. 2 A shows gating currents recorded upon the application of test pulses to different voltages from a holding membrane potential of −110 mV. On the right, the figure shows equivalent recordings but from a holding membrane potential of 0 mV. Integration of the gating currents from these recordings yielded the net gating charge moved during each voltage pulse. For 50-ms pulses, the Q-V curves were different for a holding potential of 0 mV with respect to that set at −110 mV (Cowgill and Chanda's Fig. 2 B, left). However, as the pulses were prolonged, the difference between the Q-V curves started to vanish (Cowgill and Chanda's Fig. 2 B, center and right). When the test pulses were as long as 18 s, the two curves were almost identical. This observation led the authors to conclude that the shift in voltage-dependence was transitory.

The elegance of the experiment emerges from its simplicity. However, it has some unintended consequences. To be blunt, the observed convergence of the Q-V curves was not only expected, but it might have been also misinterpreted as proof that hysteresis in Shaker is transitory. Why do I think that? The devil is in the details. The key is "to follow the charge." Let us suppose that the model represents what happened with Shaker. During activation from a holding potential of −110 mV, the most likely path of activation was the sequence $C_S \rightarrow O_U \rightarrow O_S$, where gating currents developed during the first transition; this is Mode 1 of activity (Fig. 1, green arrow). On the other hand, from a depolarized holding potential like 0 mV, the deactivation of the VSD should have followed the path $O_S \rightarrow C_U \rightarrow C_S$, where gating currents were observed during the O_S-to-C_U transition; this is Mode 2 of activity (Fig. 1, red arrow). Let us now consider the recordings in Fig. 2 A from the paper. There, Shaker was expected to be in the C_S state as the holding potential is set to −110 mV. When a pulse to 0 mV is applied, gating currents were produced through Mode 1 of activity. Following that, at the final step, the remaining charge movement was recorded as the 0-mV pulse was applied. These lasting gating currents were still emerging from Shaker being in its Mode 1 of activity.

When a −110-mV test pulse was applied from a holding potential of 0 mV, gating currents were generated by channels in Mode 2 of activity. Then, if the third and final pulse would have been applied to −110 mV, the gating currents would have also emerged from channels in Mode 2—analogous to what happen in the previous case. However, the protocol used in Fig. 2 brought the membrane potential back to 0 mV. In this case, two different sources of gating currents emerged: one source was from those channels that were still in Mode 2 of activity and following the path $C_U \rightarrow O_S$. The second source would have emerged from those channels that already transitioned to the C_S state, so gating currents were produced by the transition $C_S \rightarrow O_U$. If the test pulse was short, most of channels would have been in Mode 2. However, if the pulse was much longer, a larger fraction of the channels would have moved to Mode 1. And, if the pulse length was infinitively long, then, all channels would have moved to Mode 1.

The explanation offered above only considered two extreme cases: (1) when pulsing to 0 mV from a holding potential of −110 mV, and (2) when pulsing to −110 mV from a holding potential of 0 mV. So, what would have happened when applying test pulses of intermediate amplitude? Let us consider the case when applying a test pulse between −110 and 0 mV from a holding potential of −110 mV. In this case, the test pulse would drive the channels to populate all four of the states. Consequently, gating currents recording during the 0-mV pulse would come while in both Mode 1 and 2. Furthermore, if the test pulse is long enough to reach steady state at each test potential, the voltage dependence of the charge movement would become independent of the holding potential. Therefore, the Q-V curve shift would artificially disappear due to the adoption of a new steady state during each infinitively long test pulse. Therefore, I concluded that using the Q-V derived from the gating currents recorded during the third, 0-mV pulse might not be suitable to assess voltage dependence as a function of the holding potential.

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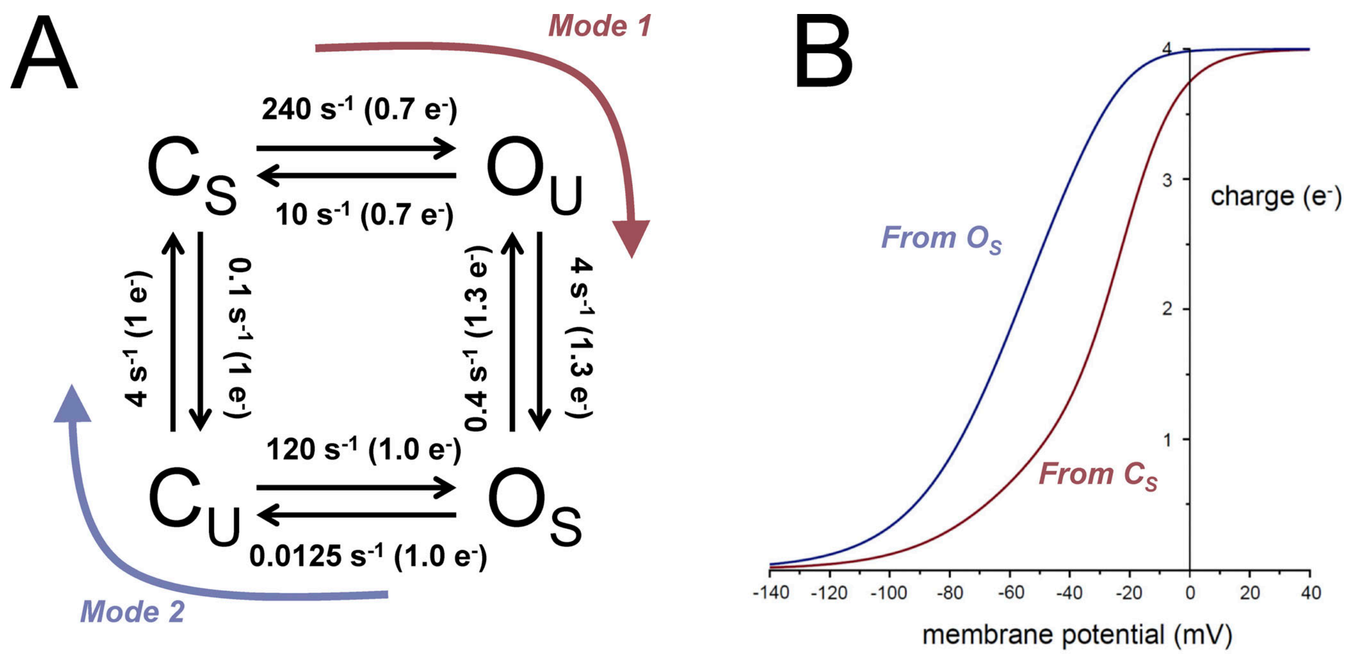

Figure 2. **Four-state model with distinct steady-state voltage dependence for each mode of activity. (A)** In contrast to the model in Fig. 1, the transitions between C_S and C_U and between O_U and O_S are voltage dependent in both directions. As with Fig. 1, the gating charge associated with each transition are in parentheses. **(B)** Q-V curves calculated from the model when the initial holding potential tends to −∞, so charge movement starts from the C_S state (red line), and when the initial holding potential tends to +∞, charge movement starts from the O_S state (blue line).

I truly believe that the results in the paper by Cowgill and Chanda (2023) can be explained by the arguments outlined above. This led me to conclude that the approach employed by the authors seems to be insufficient to rule out hysteresis as a steady-state feature in the activity voltage-gated channels—or at least for the activity of Shaker.

One final thought: The work of Cowgill and Chanda clearly shows how dynamic the voltage dependence of channels can be, and how impactful hysteresis is on their activity. Also, this work has made it clear that a model consisting of a pair of voltage-dependent transitions connected by a pair of voltage-independent transitions seems to be unable to describe hysteresis as a steady-state property. Although activity-dependent changes in kinetics can certainly be described by such models (Villalba-Galea, 2017; Villalba-Galea and Chiem, 2020), Cowgill and Chanda clearly showed in Fig. S2 B that this type of model does not describe activity-dependent changes in voltage dependence—*mea culpa*!

So, what is a more suitable model? It is yet to be clear what the minimum requirements for such a model would be. However, I can only say today that it is likely, but not proven, that the $C_S \rightarrow C_U$ and $O_U \rightarrow O_S$ transitions must be also voltage dependent for the model to show a steady-state shift in voltage dependence (Fig. 2). In summary, much more work remains to be done to understand the underpinnings and thermodynamic nature of hysteresis in voltage-gated channels.

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