Comments to the Editor

Response to R. Shirokov

This is a response to the comment by R. Shirokov concerning our recent publication entitled "Properties of Deactivation Gating Currents in *Shaker* Channels" (1). His comment deals with the relation between the C-type inactivation of *Shaker* channels and the movement of its voltage-sensor domain (VSD).

The comment first deals with the fact that, because we mention that the slowing of gating currents is independent from C-type inactivation, then "the opposite should also be true: C-type inactivation is not associated with a change in the VS movement." Then Shirokov proceeds to rebut that idea. In fact, we do not believe this assertion is correct simply because our data do not support it. In this regard, we explicitly show in our Supporting Material that the deactivation gating currents (Ig_D) are approximately twofold faster in wild-type *Shaker* than in its nonconducting version W434F (this was also observed previously (2)) and for all prepulse durations tested. Therefore, we are aware that modulations of C-inactivation affect the deactivation gating currents kinetics. The expression "VS movement" is, in fact, too vague to really convey the concept presented in our study, which is the specific changes in the deactivation gating kinetics with prepulse duration, not their absolute magnitude.

Second, Shirokov states that we interpreted the near absence of a depolarization-induced shift in the voltage dependence of gating charge in the W434F mutant as evidence for independence of relaxation from C-inactivation. There are two misunderstandings here. One concerns the "near absence" of Q-V shift. Clearly, our data shows a change in the deactivation kinetics that depends on the duration of the depolarization and when the integration spans the entire time range of the slow decay, the previously found shift is not observed. What this means is that, by doing high-resolution gating current experiments, we can characterize the relaxation by its effect on the deactivation kinetics. In other words, we are saying that the relaxed state is also present in the W434F mutant. The second misunderstanding deals with our interpretation for independence of C-inactivation in the establishment of the relaxation. Indeed, our main evidence for such independence is in fact that the changes in the deactivation kinetics were similar in both fast-inactivating W434F and slow-inactivating WT channels (see below).

We have no comments on the historical note or the kinetic model interpretation because we fully agree that, in a coupled model, the gating current represents a combination of the voltage-dependent steps and voltage-independent steps. Our modeling, starting with the first article on sodium channels (3) to the model presented in Lacroix et al. (1), are all consistent with those basic concepts.

Shirokov states that our results can be explained because in W434F the inactivation rapidly equilibrates, as compared to the wild-type *Shaker*. However, the wild-type still has the same dependence on the deactivation kinetics on pulse duration. This is an important observation because it shows that the time course of entry into the relaxed state is the same in a mutant known to C-inactivate extremely rapidly (see Fig. 1*C* in (1)) as in the wild-type that C-inactivates with a time course of seconds (see Fig. S3 in the Supporting Material in Lacroix et al. (1)). Thus, the rate of slowing of the Ig_D is similar between W434F and wild-type channels. This means that what is causing the VSD to undergo relaxation must be independent of the C-inactivation. This is what we wanted to stress by stating that "C-inactivation does not participate in the biphasic slowing down of Ig_D", although we do agree about the influence of C-inactivation on the overall deactivation kinetics of the VSD movement.

From our point of view, the relaxation process—slowing of Ig_D in *Shaker*—is an intrinsic property of the VSD itself and thus is not triggered by events occurring in the pore. However, events in the pore such as C-inactivation can affect some energetic components of the VSD transitions because the VSD is coupled to the pore. In order to make this point very clear, we usually refer to the relaxation observed as a Q-V shift in the voltage-sensor of Ci-VSP because the latter lacks a pore domain. It is true that in Ci-VSP the presence of the phosphatase domain linked to the S4 segment could be considered as a load acting on the VSD and one could argue that this load (the equivalent of the pore in *Shaker*) is involved in generating the relaxation. However, we have shown that the VSD of Ci-VSP truncated completely after the end of the S4 segment (deletion from 244-576) undergoes an even more pronounced Q-V shift under prolonged polarization (4). To us, this undoubtedly demonstrates that the VSD itself exhibits relaxation.

To summarize, our study and previous observations demonstrate that the relaxation process (slowing of Ig_D or Q-V shift) appears independently of C-inactivation and any load attached to the S4. Therefore the inactivation is not a molecular determinant for the relaxation. However,

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we are not stating that changes in C-inactivation do not perturb the gating charge movement during deactivation as the comment is attributing us stating.

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