Reply to "On the extraction of kinetic rate constants from experimental data"

Dear Sir:

Dr. Goldman makes the following major points: (*a*) "The Balser et al. analysis is in error because more information is provided ... than they claim"; (*b*) "... more information is required to solve the problem than they have recognized"; (*c*) "in general their ... method cannot in principle provide a unique set of rate constants."; (*d*) "Iterative methods are not required." (*e*) "... the extracted values of the rate constants depended on the initial guesses.".

Dr. Goldman describes pulse conditions needed to extract the rate constants from the three state model that we used. He now demonstrates in analytical form a part of the approach we have taken. Dr. Goldman has apparently misinterpreted the data that we utilized and the method we used to extract the rate constants. We hope we can clarify these issues. We shall address each one, beginning with *d*.

The experimental scientist is always faced with extracting rate constants from a set of real (i.e., noisy) data. Thus, in practice, iterative methods are always required to fit a model to data. This is true whether one fits the analytical solution and then solves it for the rate constants (as Dr. Goldman suggests) or whether one fits the rate equations directly as we have done. We agree with Dr. Goldman that as long as one is faced with fairly simple models, analytical solutions are convenient, but for more complicated models numerical methods are easier to deal with. The key issue is being assured that enough experimental information is available to extract the rate constants.

Points *a–c* of Dr. Goldman are interrelated and none of them are correct. We completely agree that there is potentially an infinite number of rate constants that could give the same fit if insufficient experimental data are utilized to obtain the solutions. This is precisely why we utilized data obtained with different initial conditions and over a wide range of conditions. Whether one uses numerical or analytical solutions is irrelevant. The key question should be "is there sufficient information in the experimental data set to constrain the fits and obtain the correct solution?". Dr. Goldman describes the relevant pulse protocols needed to provide the necessary information in his description leading up to and including Eq. 18. This is the case where $P_2(O)$ is not known and is not zero. In this case, information is needed both from various initial conditions and multiple step potentials. Our data sets did include information obtained at multiple voltages and multiple initial conditions, contrary to what Dr. Goldman states. Thus, he is incorrect in stating (possibly because we did not make clear the pulse protocols used to obtain the data set) that the method cannot work. It does in fact work as we show in our paper, and for the reasons he now outlines in his analytical framework. He provides the analytical reasoning for the data that are required to achieve the correct solution for a three-state model. The improtant point is the requirement for additional information in the data set on the initial rate of change of $P_3(O)$ because it provides the information pertaining to the contribution of P_2 (see Goldman, 1991; Eqs. 7 and 11).

Concerning point *e*, we wish to point out that the extracted rate constants were not functions of the initial guesses as suggested by Dr. Goldman. He has neglected the fact that we were fitting noisy experimental data. To test the ability of the program to converge to the correct solution with these data, we gave randomized initial guesses (see Balser et al., 1990; Table 1). Thus the initial guesses were not biased by our knowledge of the answers. If we used the analytical solutions as a data set and fitted it (i.e., "data" without stochastic gating noise), we always obtained the correct solutions. When using noisy data, the curve-fitting algorithm failed to converge if the initial guesses were sufficiently bad (by several orders of magnitude). The reason for failure of convergence was due to the nature of the search algorithm and not because the system was underdetermined.

We are grateful for the opportunity to clarify these issues. We think we are ultimately in agreement over the experimental conditions necessary to obtain the correct rate constants. Our use of the term "global" was simply intended to emphasize the need to utilize data obtained over a broad range of conditions (including multiple voltages and initial conditions). Ultimately one would like to include single channel data (e.g., open times etc.) to further define the channel gating system. The notion of a *global data* set grew from our need to fit a model to all the relevant experimental data and not just limited subsets.

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REFERENCES

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