Fractal models, Markov models, and channel kinetics

Dear Sir:

Our findings (McManus et al., 1988) and those of McManus and Magleby (1988), Korn and Horn (1988), and Liebovitch (1989, Fig. 1) indicate that the fractal model (Liebovitch et al., 1987) is unlikely to serve as a general gating mechanism for at least four different ion channels. Liebovitch (1989) does not disagree with this conclusion in his letter. Instead, he argues against Markov models. Although his arguments cannot alter the conclusion that the fractal model is inadequate, as the fractal model can be rejected without comparison to other models (see below; McManus et al., 1988; Korn and Horn, 1988; Horn and Korn, 1989), his letter might be misleading for those unfamiliar with some details of kinetic analysis. Consequently, we find it necessary to address the topics raised in his letter.

Assumptions of the fractal model

Liebovitch suggests in his letter that the fractal model does not describe the data because it is "too simple." Another possibility may be that the underlying assumption of the fractal model, that the rate of leaving any given state is a function of the amount of time spent in that state, is not correct. The fractal assumption of transition rates which vary with time contrasts with the assumption in Markov models of transition rates independent of time. If transition rates remain constant, then the time constants of the exponential components describing dwell time distributions should be independent of previous channel activity (Fredkin et al., 1985). We have tested whether transition rates are independent of time by comparing the distributions of open intervals, which occur next to brief duration shut intervals, with those that occur next to long duration shut intervals. We found that the time constants of the components are independent of the adjacent shut interval durations (Magleby and McManus, 1985; Blatz and Magleby, 1989; Weiss and Magleby, 1989). Such a finding is inconsistent with the fractal assumption that the rate of leaving any given state is a function of the time spent in that state. Further support for Markov gating is the observation by Hahin (1988) that sodium current decays are time-invariant after removal of inactivation.

The fractal model can be rejected without comparison to Markov models

The fractal model does not predict multiple bumps in log-log plots of distributions of interval durations (Liebovitch et al., 1987; McManus et al. 1988). Thus, the consistent observation of such bumps in the experimental data (McManus et al., 1988) is sufficient to reject the fractal model without statistical tests or comparisons to other models. A simple chi-squared goodness of fit test also indicated that the fractal model was inconsistent with the observed data (McManus et al., 1988).

Discrete or a continuum of states

Liebovitch argues that the observations of many or even a continuum of conformational states in studies of (non-channel) proteins dynamics is inconsistent with the limited number of states in discrete Markov models. We disagree. A difference in the numbers of states in these two types of studies might be expected for a number of reasons.

First, many of the experimental observations that Liebovitch interprets as arising from a continuum of states could arise from discrete states. For example, power type distributions are easily generated by discrete Markov models with sums of exponentials, with either a limited number of discrete states (Fig. 1, McManus et al., 1988) or many discrete states (Millhauser et al., 1988; Läuger, 1988).

Second, none of the studies on protein dynamics referred to by Liebovitch are for channel proteins undergoing gating. Gating motions in channel proteins may differ from the studied motions in some non-channel proteins. In fact, agonist-activated channels might well be expected to display discrete states associated with different numbers of bound agonist molecules (see below).

Third, Liebovitch does not make a distinction between kinetic and conformational states. Kinetic analysis detects only those states that affect the gating within the time resolution of the analysis. Kinetic models are minimal models, and do not exclude the possibility that each detected kinetic state could arise from one or more conformational states or conformational substates. In fact, this is likely to be the case (Frauenfelder et al., 1988). Conformational states or substates in rapid equilibrium with one another, when compared to the time resolution of the single channel data, would be detected as a single kinetic state. Discrete Markov models derived from single channel data also do not describe the potentially large numbers of incremental conformations that a channel may pass through when it changes conformation from one detected kinetic state to another, as the transitional states are too brief to detect. Thus, it is to be expected that conformational studies with high time resolution may detect more states than kinetic studies of single channel data.

Obviously, channels may have many conformational states. The implication of discrete Markov models is that the time spent in the major kinetic states exceeds that spent in the transitions among the states. This implication is consistent with the observation that the conformational change that can be most directly measured for single channels, the open-shut transition, occurs on a time course that is rapid compared to the lifetimes of the detected kinetic states. Further evidence for some discrete states is the observation of discrete subconductance states (Hamill et al., 1983).

Finally, numbers of detected conformational and kinetic states would differ if channels enter some conformational states that have little effect on channel gating. Conformational studies might detect these states, whereas kinetic studies might not.

Comparing models with different numbers of free parameters

Liebovitch argues that Markov models describe the data, not necessarily because they have any physical meaning, but because they have enough adjustable parameters. This argu-

ment is unfounded. First, unlike the empirical fractal and polynomial models that Liebovitch considers, the basis for the derivation of equations describing discrete Markov models is well established (Colquhoun and Hawkes, 1981). Second, rather than having too many adjustable parameters, Markov models appear to have about the required number. For example, Hill plots suggest that four or more Ca ions typically bind to the large conductance calcium-activated K channel during activation (Golowasch et al., 1986). Binding four Ca ions would require at least five shut states: one unbound state, and four additional states with one, two, three, or four bound Ca. If the channel can open with different numbers of bound Ca (Magleby and Pallotta, 1983), then there could be at least four open states. Such a model would generate five shut and four open exponential components. This is similar to the number of dominant exponential components detected from kinetic analysis of single channel data (McManus and Magleby, 1988).

Exponential components

Based on a series of horror stories, Liebovitch suggests that exponential components cannot be reliably determined, and asks whether methods of analysis involving exponentials "work" for single channel data. Of course they work. Many fundamental questions about channel kinetics have already been answered using such analysis. In our study of fractal and Markov models (McManus et al., 1988) we show that the parameters describing exponential components can be defined sufficiently well to establish that Markov models are consistent with the experimental observations. With maximum likelihood fitting we have found that the same exponential parameters can be obtained in a repeatable manner with different starting values for the fits. Furthermore, the literature is filled with examples of exponential analyses that work, including estimates of confidence limits (Horn and Vandenberg, 1984; Colquhoun and Sakmann, 1985; Blatz and Magleby, 1986a,b; Kerry et al., 1988).

Are the processes independent?

Since distributions of shut intervals can decay as approximate power functions (Blatz and Magleby, 1986b; Millhauser et al., 1988), Liebovitch suggests that the observed components may not be independent, and questions whether this would affect our fitting procedures.

It is well established that the observed exponential components generated by discrete Markov models are not necessarily independent (Colquhoun and Hawkes, 1981). A change in a single transition rate between only two of any number of connected shut states can affect all of the exponential components generated by the connected shut states. This lack of independence between observed exponential components would not affect our fitting procedures or the statistical conclusion reached in our paper (McManus et al., 1988), that Markov models are tens to thousands of orders of magnitude more likely than fractal models.

Liebovitch further suggests that we (and presumably others) have not proposed a mechanism for the approximate power decay of shut distributions that can be observed. This is not true.

First, a fundamental basis for such decays has been presented in detail by Colquhoun and Hawkes (1981). When shut states are connected in series, a sojourn to any given shut state requires passage through all the preceding shut states; the further an entered shut state is from the open states, the longer the duration of the observed shut interval. The natural consequence of such a mechanism is that for similar rate constants, or for rate constants that decrease as the distance from the open states increases, the frequency of observed intervals decreases with their duration. If one is willing to ignore the observed bumps in the decays, then the decays can appear as approximate power functions. A complete quantitative description of such decays in terms of Markov models with limited numbers of states has already been presented for the fast Cl channel (Blatz and Magleby, 1986b) and large conductance calcium-activated K channel (McManus and Magleby, 1986). Examples of such decays for discrete Markov models with many states are found in Millhauser et al. (1988) and Läuger (1988).

Incidently, an assumption of Markov models is that the transition rates do not change in time, not that they are independent of one another. Constant transition rates that are all the same, all different, or change in some type of progressive manner, depending on the positions of the various states, would all be consistent with a Markov process.

Plotting log k_{eff} versus log t_{eff}

Liebovitch expresses disappointment that we did not present k_{eff} plots in our paper. Such plots were not included, as they only substantiated the findings obtained from analysis of the dwell time distributions. In unpublished results we found that discrete Markov models could account for k_{eff} plots; theoretical k_{eff} curves calculated from Markov models fit to the distributions of dwell times gave excellent descriptions of k_{eff} plots of the observed data. The "instantaneous" decay rate used in the k_{eff} plots was calculated from successive pairs of data points in the dwell time distributions.

It should be mentioned that, contrary to the suggestion of Liebovitch, an absence of plateaus in k_{eff} plots is not sufficient to exclude discrete Markov models. This is the case since k_{eff} plots only generate plateaus for a single exponential decay, whereas the distributions predicted by multistate Markov models are sums of exponentials, and therefore, do not necessarily decay as single exponentials.

Exclusive use of discrete Markov models

Liebovitch suggests that it is not justified to model ion channel kinetics exclusively as the sum of a limited number of exponentials. We agree, but only if the interpretation of such studies allows for the possibility that models inconsistent with the experimental observations are unlikely to be correct.

Additional models

Liebovitch (1989) has proposed some additional models in his letter. The experimental observations that durations of adjacent open and shut times are correlated (McManus et al., 1985;

Kerry et al., 1988) suggest that models of the type he presents in Fig. 2 *B* with one transition pathway between open and shut states, and in Fig. 2 *C* with one open state, are probably too simple. A correlation between adjacent open and shut dwell times suggests two or more open states and two or more independent transition pathways between open and shut states (Fredkin et al., 1985).

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