SUCCESSIVE OPENINGS OF THE SAME ACETYLCHOLINE RECEPTOR CHANNEL ARE CORRELATED IN OPEN TIME

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ABSTRACT Previous analysis of single-channel current records has shown that both the opening and closing transitions of chemically activated ion channels are operated by fast and slow kinetic processes. The fast component in the kinetics of channel opening has been interpreted as the reopening of a channel that has just closed. The fast component in the kinetics of channel closure has many possible explanations and is therefore more difficult to interpret. We can gain insight into the closing process by asking whether the lifetimes of successive openings of an acetylcholine receptor channel are correlated in open-state lifetime. Five kinetic models of channel closure are considered. Two of these models predict uncorrelated open-state lifetimes, one predicts correlated open-state lifetimes, and for two others a range of behavior is possible. Acetylcholine receptor channel data from cultured rat muscle are analyzed to show that open-state lifetimes are correlated, eliminating two models of channel gating.

The resolution of fast kinetic processes in the gating of the acetylcholine receptor channel complex opens a new avenue by which channel gating mechanisms can be studied (Colquhoun and Sakmann, 1981; Cull-Candy and Parker, 1982; Jackson and Lecar, 1982; Gration et al., 1982). Distributions of both open times and closed times obtained from single-channel current records reveal large numbers of fast events, in excess of the number expected from a single exponential distribution. The kinetic model of a single population of channels opening and closing with first-order kinetics is eliminated by this observation, but a multitude of more complex models can be considered as alternatives. Such models account for the nonexponential shapes of state lifetime distributions by introducing additional kinetic processes. Because several different models of channel gating predict similar distributions of open-state lifetimes, further analysis of single-channel current data requires new approaches to elucidate the mechanism of gating. The method employed here consists of a concerted scrutiny of both the opening and closing processes. Closely spaced successive reopenings of a channel are identified and tested to see if open-state lifetimes are correlated.

Single-channel currents were recorded with patch electrodes (Neher and Sakmann, 1976) using the gigohm seal techniques (Hamill et al., 1981). Dilute solutions of the agonist suberyldicholine were used to activate the acetylcholine receptor channel. In cultured rat skeletal muscle (Jackson and Lecar, 1979; Nelson et al., 1976), the preparation used in the present work, channel gating proceeds with relatively slower rates than in the frog cutaneous pectoralis (Colquhoun and Sakmann, 1981), so that the fast processes can be studied using our instrumentation, which has a bandwidth of 1 kHz. The cumulative distributions of open-state and closed-state lifetimes displayed in Fig. 1 reveal two exponential components. Data with the same qualitative features have been observed in other preparations (Colquhoun and Sakmann, 1981; Cull-Candy and Parker, 1982; Jackson and Lecar, 1982; Jackson et al., 1982 *a, b*). Open-state lifetime distributions with these features were seen in more than 20 experiments on different cultures of rat muscle obtained from separate dissections. A sum of two exponentials produces an excellent curve fit to these data (using the MLAB curve fitting routine [Knott and Reece, 1972]); single exponential func-

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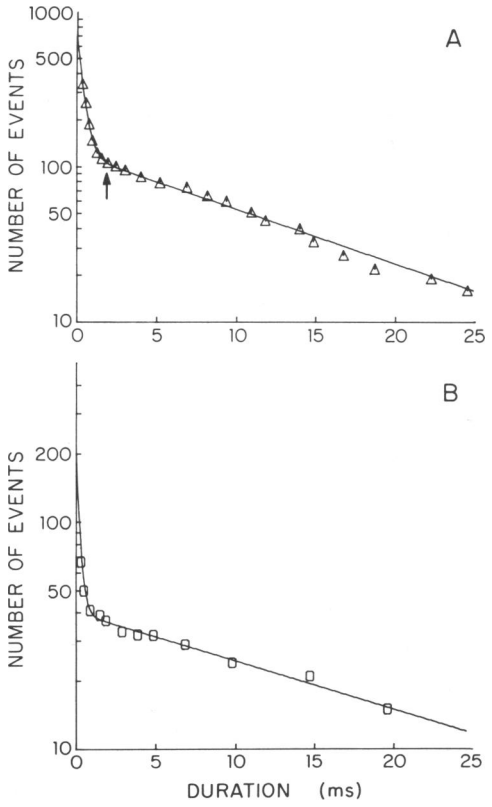

FIGURE 1 (*A*) A cumulative distribution of open-state lifetimes of suberyldicholine activated channels is plotted semilogarithmically (suberyldicholine concentration, 5×10^{-7} M). The curve was drawn using Eq. 1 with parameters determined from a curve fit. The parameter values in this case were $N_s = 120$, $N_f = 691$, $\tau_s = 12.52$ ms, and $\tau_f = 0.36$ ms. The arrow denotes t', the value of t for which the probability densities of the two exponential components are equal. The membrane potential was held at -100 mV during this experiment. (*B*) The distribution of closed-state intervals from the same experiment is plotted semilogarithmically for closed times <50 ms. Of the 342 openings seen in this experiment, 196 were at the beginning of a record (records consisted of 4,096 points sampled at 100-μs intervals) so that it was impossible to determine the elapsed time since the last event. In addition, there were 79 recorded closed times that were longer than 50 ms. These times were not included in this plot to enhance the visibility of the fast component. The low frequency of events (less than two per record) provides optimal conditions for identifying successive openings of one channel.

tions produce inadequate fits. Functions other than double or single exponentials were not tested.

The observation of two exponential components in the kinetics of channel closure raises interesting questions about the mechanism of this process and provides the incentive to consider various kinetic models. Two distinct receptor-channel populations, each of which close with first-order kinetics, will operate simultaneously to produce open-state lifetime distributions that are a sum of two exponentials. Alternatively, a single population of receptor-channel complexes can also have two different open states distinguished by their macromolecular configurations. Whether these two open states close independently or interconvert sequentially (Colquhoun and Hawkes, 1981), a lifetime distribution that is a sum of two exponentials is predicted. The range of models is further enriched by considering the possibility of multiple-receptor binding sites (Adams, 1975; Dreyer and Peper, 1975; Dionne et al., 1978), where receptor-channel complexes with one or two bound agonists produce open states with different rates of closing (Colquhoun and Sakmann, 1981; Cull-Candy and Parker, 1982).

The simplest and most widely accepted interpretation of the fast component of the closed-time distribution is that many channels within a given patch of membrane operate independently, but a channel that has just closed resides for a finite period of time in an activated state with a high probability of opening. The reopening of such recently closed channels accounts for the fast component in the distribution of closed times (Nelson and Sachs, 1979; Colquhoun and Sakmann, 1981; Cull-Candy and Parker 1982; Gration et al., 1982; Dionne and Liebowitz, 1982).

We can exploit the conclusion that closely spaced events are almost always openings of the same channel to explore the significance of the two components in the distribution of open-state lifetimes. This is done by asking the question, are successive openings of the same channel correlated in their open-state lifetime? Two extremes can be visualized: open-state lifetimes could be uncorrelated with short and long lifetime events following one another with independent probabilities, or open-state lifetimes could be correlated, so that one sees successions of fast events or successions of slow events. Varying degrees of correlation, where bursts of openings are partially dominated by one rate of closing, are also possible.

Table I shows five kinetic schemes that can explain the two component lifetime distributions of Fig. 1. In all of these models, the agonist binding step will give rise to an activated closed state and cause closed-state lifetime distributions to have two or more exponential components. Three exponential components would arise from models such as the two population model (Model II), the two agonist model (Model IV), or the two activated closed-state model (Model V), if each closed state reopened with a different rate. Colquhoun and Sakmann (1981) note a small third exponential component in their closed-time distributions that may be relevant to the questions discussed in this communication. The poorer instrumental bandwidth in our recordings prevents us from resolving additional kinetic processes.

Open-state lifetime distributions for all of the models of Table I would be sums of two exponentials with terms of the form $N_1 e^{-t/\tau_1}$ and $N_2 e^{-t/\tau_2}$. The exact values of the factors N_1 and N_2 that multiply the exponentials, as well as the time constants, would depend on which model was being considered. The precise form of the open-time distribution function is not important to the method of analysis developed here, as long as it is a sum of two exponentials.

Although these models make qualitatively similar predictions about open-time and closed-time distributions,

TABLE I
KINETIC SCHEMES OF CHANNEL GATING

Model	Scheme
I	$CR \overset{nA}{\rightleftharpoons} CRA_n \rightleftharpoons O_1RA_n \rightleftharpoons O_2RA_n$
II	$C_1R \overset{nA}{\rightleftharpoons} C_1RA_n \rightleftharpoons O_1RA_n$ $C_2R \overset{nA}{\rightleftharpoons} C_2RA_n \rightleftharpoons O_2RA_n$
III	$CR \overset{nA}{\rightleftharpoons} CRA_n \begin{matrix} \rightarrow O_1RA_n \\ \rightarrow O_2RA_n \end{matrix}$
IV	$CR \overset{A}{\rightleftharpoons} CRA \overset{A}{\rightleftharpoons} CRA_2$ $CRA \rightleftharpoons ORA$ $CRA_2 \rightleftharpoons ORA_2$
V	$CR \overset{nA}{\rightleftharpoons} C_1RA_n \rightleftharpoons C_2RA_n$ $C_1RA_n \rightleftharpoons O_1RA_n$ $C_2RA_n \rightleftharpoons O_2RA_n$

R denotes the receptor; C, a closed channel; O, an open channel; and A, an agonist molecule. As noted in the text, all of these models predict that open-time distributions are a sum of two exponential functions. The closed-time distributions are a sum of two or three exponential functions. For a treatment of the mathematical methods for determining the predicted distribution of dwell times, the reader is referred to the work of Colquhoun and Hawkes (1981).

there are important differences between these models with respect to correlations in open-state lifetime. This is best understood by directing attention towards the activated closed states, and by examining Models I and II. There is only one closed state capable of opening in Model I, whereas in Model II there are two activated closed states that produce two different noninterconverting open states. In Model I the activated closed state entered by a closing channel is independent of the lifetime of the just completed opening. If the channel reopens, it will have no memory of the lifetime of its previous open state, and open-state lifetimes will be uncorrelated. The two distinct closed states of Model II gate independently, opening, closing, and reopening with specified rates. Channels will therefore reopen to the same state they were in during their previous opening, which is characterized by the same rate of closing. Model II therefore predicts successions of channel current pulses with either mostly short duration openings or mostly long duration openings. Open-state lifetimes of successive openings of the same channel would then be correlated.

Considering Models III, IV, and V with regard to correlations in open-state lifetime, Model III, like Model I, has only one activated closed state and would therefore produce successive openings uncorrelated in open-state lifetime. Models IV and V each have two activated closed states that are capable of interconverting. If interconversion is slower than reopening, then these closed states will reopen independently, and lifetimes of successive openings will be correlated. If the two closed states equilibrate before a reopening is likely to occur, then there will be little evidence of correlated open-state lifetimes. When reopening proceeds with a rate that is competitive with the rate of closed-state interconversion, one may expect the lifetimes successive openings to be weakly correlated.

It is important to mention that channels obeying any of the schemes in Table I can produce successions of openings with very similar or very different lifetimes. However, the models make different predictions for the probability that such successions occur. When lifetimes are uncorrelated, the probability of a specific sequence of openings is simply the product of the probabilities of each individual event. Correlation implies that the probability of observing a particular sequence has a more complex functional dependence on the lifetimes of all of the individual events.

Examples of successive openings of a channel with open times that are similar or different are shown in Fig. 2, where two successive long events (Fig. 2 *A*), two successive short events (Fig. 2 *B*), and mixed pairs of events (Fig. 2 *C*) can be seen. Fig. 2 *D* shows one pair of long events and one pair of short events. In fact, sequences of events of the

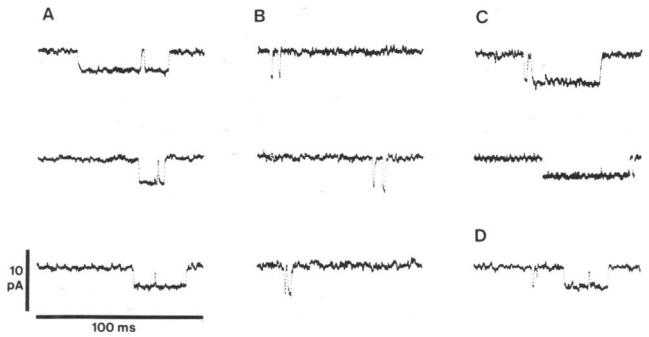

FIGURE 2 Records of channel currents display successive openings that are very probably reopenings of one channel. The classification procedure for identifying fast and slow events is described in the text. (*A*) Successive slow events. (*B*) Successive fast events. (*C*) Pairs consisting of one fast and one slow event. (*D*) A single record with one fast pair and one slow pair. The time interval between the two pairs in *D* is long enough to consider the two pairs as independent instances of channel activation. The second event in the first pair was of such short duration (~0.4 ms) that it is very attenuated by the instrumental bandwidth. Because analysis of such fast events is unreliable, they were generally ignored. The open-state lifetime distributions (Figs. 1 *A* and 3 *A*) include open states with lifetimes as low as 0.7 ms. In all of these experiments, the membrane was held at −90 to −100 mV.

type shown in Fig. 2 *C*, where successive open-state lifetimes appear to be uncorrelated, are rare as the following more quantitative discussion demonstrates.

Analysis begins with a curve fit of an open-state lifetime distribution to a sum of two exponentials

$$N(t) = N_f e^{-t/\tau_f} + N_s e^{-t/\tau_s}. \quad (1)$$

In this way we identify the two different exponential terms by the subscripts s and f, to denote slow or fast, without committing ourselves to any of the above models. Determining these parameters provides us with a means of estimating the probability that a particular event belongs to either the fast component or the slow component.

The next step is to classify events as fast or slow depending on whether the fast or slow exponential term of the best fitting density function is larger for the observed lifetime of an event. This classification can be performed by noting that for a time, t', defined by the relation

$$\frac{N_f e^{-t'/\tau_f}}{\tau_f} = \frac{N_s e^{-t'/\tau_s}}{\tau_s}, \quad (2)$$

the probability densities of the fast and slow events are equal. Thus, if an event has a lifetime $<t'$, it has a higher probability of being a member of the fast kinetic component, whereas if an event has a duration $>t'$, it is more likely to belong to the slow kinetic component.

This method of classifying channels can be shown to satisfy an objective criterion for minimizing the total number of incorrect assignments. Using an arbitrary time, t_o, to divide events into two categories, the number of fast events incorrectly classified as slow can be computed from the first term of Eq. 1 as $N_f e^{-t_o/\tau_f}$; the number of slow events incorrectly taken as fast is $N_s(1 - e^{-t_o/\tau_s})$. The total number of incorrect assignments for any choice of t_c is the sum of these two quantities, and differentiation reveals a minimum in the number of incorrect assignments for $t_o = t'$ as defined by Eq. 2. Even when instrumental bandwidth is taken into account by including a cutoff time limiting our capacity to detect very short-lived events, the number of incorrect assignments is still minimized by using t' determined by solving Eq. 2.

Calculation of the second term on the right side of Eq. 1 indicates that 97% of the 106 channel currents making up the portion of Fig. 1 *A* to the right of t' are part of the slow component. Of the 236 channel currents with durations $<t'$, it can be estimated that <17 are of the slow component. Thus, for our data on suberyldicholine activated channels, this classification procedure produces an incorrect assignment $<10\%$ of the time. This procedure is a useful device in the analysis of correlations of open-state lifetimes subject to the conditions (*a*) that $N_f/\tau_f > N_s/\tau_s$, and (*b*) that there is a large disparity in the two time constants. In Fig. 1 *A*, t' is indicated by an arrow.

A similar analysis of closed-time intervals shows that, in the experiment of Fig. 1 *B*, of the 33 successive pairs of channel events separated by a closed time of <5 ms, 89% are reopenings of the same channel, while 11% are uncorrelated openings that are coincidentally <5 ms apart. In rare instances when three closely spaced channel currents occurred, the first and second events were treated as one successive pair and the second and third events were treated as another successive pair. In two experiments, 33 and 24 pairs of channel currents were separated by 5 ms or less. Each channel current was classified as fast or slow with t' (defined by Eq. 2) determined separately for each experiment from the parameters of a curve-fit of Eq. 1 to the open-state lifetime distribution. Pairs were then classified as fast-fast, slow-slow, or mixed. The numbers of pairs of different types are presented in columns 1 and 5 of Table II. If successive events were uncorrelated in their open-state lifetimes, we would expect many more mixed pairs compared with the number of pairs that are composed of two events of the same classification. The two or three mixed pairs observed in these two examples can easily be accounted for by estimating the number of events incorrectly assigned by our procedures either of classifying successive pairs as reopenings or of classifying events as slow or fast. Subsequent discussion takes up the question of the quantitative significance of this observation.

TABLE II
OBSERVED DISTRIBUTIONS OF SLOW-SLOW (SS), FAST-FAST (FF), AND MIXED PAIRS COMPARED WITH BINOMIAL EXPECTATIONS FOR TWO EXPERIMENTS

	Experiment 1				Experiment 2			
	$t_c < 5$	Binomial Expectation	$10 < t_c < 30$	Binomial Expectation	$t_c < 5$	Binomial Expectation	$10 < t_c < 30$	Binomial Expectation
SS	10	2	3	2	26	3	3	2
FF	11	12	11	14	5	16	10	11
Mixed	3	10	12	10	2	14	9	9
Total	24	—	26	—	33	—	22	—
χ^2	13.6 ($p < 0.001$)		0.0045 ($p = 0.95$)		21.9 ($p < 0.001$)		0.28 ($p = 0.6$)	

Channel currents are classified as fast, or slow as described in the text. Pairs separated by closed times (t_c) of <5 ms or >10 ms are compared. The observed frequencies with which fast and slow events occur are used to compute binomial expectations. For fast and slow frequencies, f_f and f_s, the expected proportions of SS, FF, and mixed pairs are f_f^2, f_s^2, and $2f_f f_s$, respectively. χ^2 are computed using the 2×2 contingency test.

If open-state lifetimes were uncorrelated, we would expect the different categories of pairs of events to occur in proportions different from those presented above. The most likely distributions of different types of pairs can be estimated from the actual numbers of fast and slow events of the appropriate experiment by using the binomial distribution. These values are presented in columns 2 and 6 of Table II along side the observed frequencies for comparison.

To contrast correlated and uncorrelated results we note that whereas the pairs of events separated by short closed-time intervals were examined because they were believed to be reopenings of a channel, pairs of events separated by longer closed-time intervals should be essentially all independent openings of different channels. In the first of the two experiments, 26 pairs were found to be separated by 10–30 ms; in the latter of the two experiments, 22 pairs of events were separated by closed-time intervals of between 10 and 40 ms. The frequencies with which different types of pairs separated by longer closed times were observed is presented in columns three and seven of Table II and the binomial predictions are listed in columns 4 and 8 for comparison. In these examples the observed proportion is very similar to the expected values for uncorrelated open-state lifetimes. Likelihood estimates for the two distributions of pairs, computed from the multinomial distribution, indicate that the observed distributions of pairs separated by long closed-time intervals have approximately half the likelihood of the ideal computed distributions presented in columns 4 and 8 of Table II. The distributions of pairs separated by short closed-time intervals (columns 1 and 5) are 10^6 times less likely than the computed binomial distributions (columns 2 and 6).

The hypothesis of independence in the lifetimes of successive openings can be tested directly by constructing 2×2 contingency tables and computing a value for χ^2. These values are presented at the bottom of each column of observed distributions in Table II. The χ^2 values are high for distributions of pairs separated by short closed-time intervals; they are low for distributions of pairs separated by long closed-time intervals. Thus, the probability that the hypothesis of independence holds for open-state lifetimes of closely spaced pairs is <0.001, suggesting that there is a significant correlation in open-state lifetimes.

Instrumental bandwidth, which cuts off a relatively higher proportion of pairs composed of two fast events, together with the small number of correlated pairs per experiment, prevents us from estimating whether members of the fast and slow components have different probabilities of reopening. As a result quantitative predictions of the frequencies of various combinations of events are not possible for models where open-state lifetimes are correlated. It is possible, however, that slow-slow pairs occur more frequently than fast-fast pairs, and this may reflect a higher opening rate of the activated closed state that gives rise to long duration openings.

The observation of successive events with correlated open-state lifetimes contradicts a prediction made by models with only one activated closed state (Models I and III). Model II, with two independent receptor-channel populations, is consistent with the data. Model IV, where the receptor has two activated closed states distinguished by the occupancy of one or two binding sites, and Model V, where two activated closed states interconvert without gaining or losing agonist, will also produce successions of openings similar to those reported here if the reopening rates are sufficiently faster than the interconversion rates between activated closed states.

Two populations of receptor channels closing with two different characteristic rates pass all of the tests considered so far (Model II). However, the two populations of nicotinic receptor channel so far studied in rat skeletal muscle, termed junctional and extrajunctional, have different conductances as well as different mean open times (Sakmann, 1978). The more rapidly closing junctional channels have a conductance that is ~1.4 times greater than the conductance of the more slowly closing extrajunctional channels. The acetylcholine receptor channel in cultured rat muscle is found to have two discrete conductance levels that are identical to the junctional and extrajunctional conductance levels (Hamill and Sakmann, 1981). For reasons that are not apparent at the present time, we found that cultures from this laboratory have channels with only one conductance level or the other, but rarely both. There are no qualitative differences in the closing kinetics of the two conducting species. Fig. 3 *A* shows a distribution of open-state lifetimes taken from an experiment where the con-

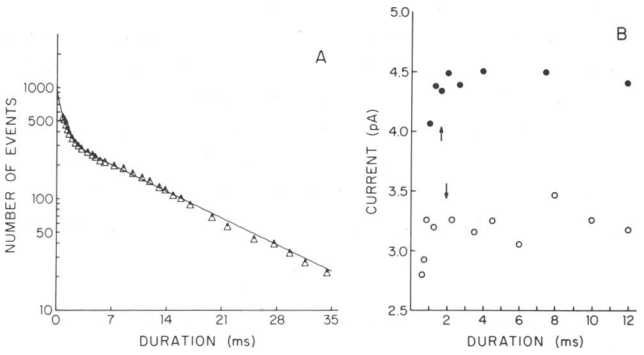

FIGURE 3 (*A*) A distribution of open-state lifetimes is plotted as in Fig. 1 *A*. The parameters were $N_s = 36$, $N_f = 622$, $\tau_s = 7.4$ ms, and $\tau_f = 0.46$ ms. (*B*) Plots of averaged currents for channels with open-state lifetimes within an interval of time vs the average time of the interval. Because channel currents with longer open-state lifetimes occur less frequently, larger intervals were used for averaging amplitudes. The upper plot (filled circles) is derived from the data used to create the distribution of open times of Fig. 3 *A*. These channels were uniform in amplitude and had a conductance of 47 pS. The lower plot (open circles) is derived from data used to create the distribution of open times of Fig. 1 *A*. These channels were also uniform in amplitude and had a conductance of 34 pS. The membrane potential was −100 mV in both experiments. t', derived from parameters determined from curve fits to the lifetime distributions, is denoted by arrows.

ductance was determined to be 47 pS. A conductance of 34 pS was determined in the experiment from which the plot of Fig. 1 *A* was derived. Plots of channel current as a function of open-state lifetime for an experiment with 34-pS channels and an experiment with 47-pS channels are virtually flat over a range of open-state lifetimes including t' (Fig. 3 *B*). The finite bandwidth of the current amplifier attenuates the amplitudes of channel currents that have lifetimes of <1 ms. This plot indicates that if there are two populations of channels underlying the two exponential components in the kinetics of closure, then their conductances are almost identical.

Other reports, in which two exponential components in the kinetics of channel closure have been revealed by patch-clamp experiments, also discuss the question of what is the appropriate kinetic scheme. The two agonist model has been invoked (Colquhoun and Sakmann, 1981; Cull-Candy and Parker, 1982) as well as the two population model (Jackson et al., 1982*a*). Model II of Table I has been eliminated for the glutamate receptor channel of locust muscle (Cull-Candy and Parker, 1982).

Correlations in the open-state lifetimes of successive single-channel events have not been sought in previous single-channel current studies. A simple classification scheme of fast and slow was successful in this study because of the great difference in relaxation times of the two processes that operate the closure of an acetylcholine receptor channel. If the situation were more equivocal, testing for such correlations would require a more elaborate theoretical framework. The maximum likelihood method has been applied to the acetylcholine receptor channel reopening problem (R. Horn and K. Lang, 1983) and may be useful in estimating kinetic rate constants.

The method introduced in this report is uniquely suited to the analysis of single-channel data, providing insights that cannot be obtained from relaxation studies or noise analysis. We have used this procedure to eliminate two models of channel gating that, prior to performing this analysis, we had taken seriously as possible explanations for the double-exponential open-state lifetime distributions.

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Note Added in Proof: A complementary approach to the question of correlations in open time has been developed by P. Labarca, J. Lindstrom, and M. Montal, manuscript submitted for publication. They used conditional probabilities and focused on correlations of a longer time scale than that dealt with here. They interpret their results in terms of multiple states of agonist occupancy similar to our model IV, a model that was not eliminated by our test.

REFERENCES

Adams, P. R. 1975. An analysis of the dose-response curve at voltage-clamped frog endplate. *Pfluegers Arch. Eur. J. Physiol.* 360:145–155.

Colquhoun, D., and A. G. Hawkes. 1981. On the stochastic properties of single ion channels. *Proc. R. Soc. Lond. B. Biol. Sci.* 211:205–235.

Colquhoun, D., and B. Sakmann. 1981. Fluctuations in microsecond time range of the current through single acetylcholine receptor ion channels. *Nature (Lond.).* 294:464–466.

Cull-Candy, S. G., and I. Parker. 1982. Rapid kinetics of single glutamate receptor channels. *Nature (Lond.)* 295:410–412.

Dionne, V. E., and M. D. Liebowitz. 1982. Acetylcholine receptor kinetics. A description from single-channel currents at snake neuromuscular junctions. *Biophys. J.* 39:253–261.

Dionne, V. E., J. H. Steinbach, and C. F. Stevens. 1978. An analysis of the dose-response relationship at voltage-clamped frog neuromuscular junctions. *J. Physiol. (Lond.).* 281:421–444.

Dreyer, F., and K. Peper. 1975. Density and dose-response curve of acetylcholine receptors in frog neuromuscular junction. *Nature (Lond.).* 253:641–643.

Gration, K. A. F., J. J. Lambert, R. L. Ramsey, R. P. Rand, and P. N. R. Usherwood. 1982. Closure of membrane channels gated by glutamate receptors may be a two step process. *Nature (Lond.)* 295:599–601.

Hamill, O. P., and B. Sakmann. 1981. Multiple conductance states of single acteylcholine receptor channels ion cultured embryonic muscle cells. *Nature (Lond.).* 294:462–464.

Hamill, O. P., A. Marty, E. Neher, B. Sakmann, and F. J. Sigworth. 1981. Improved patch-clamp techniques for high resolution current recording from cells and cell-free membrane patches. *Pfluegers Arch. Eur. J. Physiol.* 391:85–100.

Horn, R., and K. Lange. 1983. Estimating kinetic constants from single-channel data. *Biophys. J.* In press.

Jackson, M. B., and H. Lecar. 1979. Single post-synaptic channel currents in tissue-cultured muscle. *Nature (Lond.).* 282:864–865.

Jackson, M. B., and H. Lecar. 1982. Double-exponential channel current lifetime distributions in cultured nerve and muscle. *Biophys. J.* 37(2, Pt. 2):310*a*. (Abstr.)

Jackson, M. B., H. Lecar, V. Askanas, and W. K. Engel. 1982*a*. Single cholinergic receptor channel currents in cultured human muscle. *J. Neurosci.* 2:1465–1473.

Jackson, M. B., H. Lecar, D. A. Mathers, and J. L. Barker. 1982*b*. Single channel currents activated by GABA, muscimol, and (−) pentobarbital in cultured mouse spinal neurons. *J. Neurosci.* 2:889–894.

Knott, G. D., and D. K. Reece. 1972. MLAB: a civilized curve-fitting system. *In* Proceedings of the Online 1972 International Conference. Brunel University, England. 1:497–526.

Neher, E., and B. Sakmann. 1976. Single channel currents recorded from membrane of denervated frog muscle fibers. *Nature (Lond.)* 260:799–802.

Nelson, D. J., and F. Sachs. 1979. Single ion channels observed in tissue-cultured muscle. *Nature (Lond.).* 282:861–863.

Nelson, P. G., C. N. Christian, and M. Nirenberg. 1976. Synapse formation between clonal neuroblastoma × glioma hybrid cells and striated muscle cells. *Proc. Natl. Acad. Sci. USA.* 73:123–127.

Sakmann, B. 1978. Acetylcholine-induced ionic channels in rat skeletal muscle. *Fed. Proc.* 37:2654–2659.