OPEN CHANNEL NOISE.

II. A Test for Coupling Between Current Fluctuations and Conformational Transitions in the Acetylcholine Receptor

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ABSTRACT The first paper of this series demonstrated that the open-channel currents in the acetylcholine receptors in cultured rat muscle show fluctuations on a time scale of ~1 ms. In this paper the hypothesis is tested that these fluctuations are coupled to the gating mechanism that opens and closes the channel. Such a coupling could arise if the channel current and the energy barrier for gating transitions both showed fluctuations having a common origin such as a motion of part of the receptor molecule. A test for coupled fluctuations is made by averaging ~1,000 channel opening or closing transitions to search for the small relaxation in the current that is predicted. At a resolution of ~1% of the single-channel current amplitude, no such relaxation is observed. It is concluded that any coupled fluctuations are small; fluctuations in the energy barrier for the open-closed conformational transition must be smaller than about $0.3kT$.

INTRODUCTION

In the first paper of this series (Sigworth, 1985) fluctuations were analyzed in the current through single open acetylcholine receptor (AChR) channels in cultured rat muscle cells. The power spectrum of the fluctuations was seen to be flat at high frequencies but showed a low frequency excess that could be fitted by a Lorentzian component, as would be predicted by a first-order relaxation process. The apparent corner frequency of this component varied with temperature and voltage in a way suggestive of conformational fluctuations in the channel protein, and corresponded to a characteristic relaxation time ~1 ms. This relaxation time is two orders of magnitude smaller than the channel open time, but could nevertheless be related to the channel's opening and closing (gating) process through an allosteric interaction.

An example of such an interaction is illustrated in Fig. 1. A small motion of part of the receptor molecule affects the gating machinery, transiently changing the probability of transitions between the closed and open conformations of the gate; the motion also has an effect on the flux of ions through the channel (when the channel is open), perhaps by changing slightly the position of ion-coordinating groups in the channel. There is presently no information available about the location or magnitude of such motions, or the degree and polarity of their coupling to the observable channel current or gating transitions. However, since the AChR is a relatively small system one might expect that a motion in one part of the protein could give rise to substantial fluctuations in both of these observable quantities, and the common origin of the fluctuations would cause them to be correlated. The present paper describes a test for such a correlation between the open-channel current fluctuations and the channel gating process. The test consisted of searching for a trend in the open-channel current fluctuations just after the time of chanel opening and just before closing; it yielded a negative result, which, however, places a bound on the magnitude of fluctuations in the channel gating rates.

THEORY

For simplicity we assume that the gating of the channel is a two-state process

$$\text{closed} \underset{\alpha}{\overset{\beta}{\rightleftharpoons}} \text{open},$$

Scheme I

where the transition rates α and β are allowed to fluctuate with time, giving rise to the effects illustrated in Fig. 2. The underlying molecular motions $\mathbf{x}$ are assumed to cause fluctuations in the activation energies ΔG for gating transitions and are assumed also to give rise to part of the open-channel current fluctuations. In the simulation shown in parts *B* and *C* of the figure, a decrease in the activation barrier height was assumed to be correlated with an increase in the channel current. This results in a tendency for the current to overshoot (Fig. 2 *C*) at opening and closing transitions because of the increased tendency for the transitions to occur when the fluctuations are in the proper direction. The overshoot is most clearly seen in the ensemble-averaged time course in Fig. 2 *D*.

The goal of the theory to be presented here is to predict the magnitude and time course of relaxations (such as the overshoot just described) in the average time course of the channel current, from the properties of the fluctuations in

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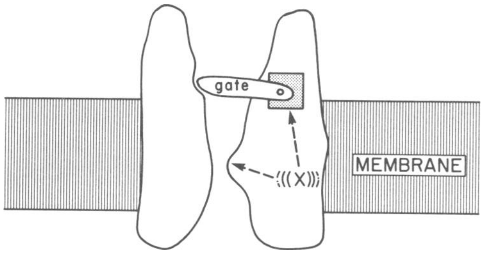

FIGURE 1 Correlated effects of an intramolecular motion. A motion **x** is postulated to affect the channel gating mechanism, changing the opening and closing rates. At the same time, the motion changes the permeation pathway for ions, influencing the ionic current when the channel is open. Because fluctuations in the gating rates and in the currents have a common cause, a correlation between them would be expected to be present.

channel transition rates and in the channel current. Since the latter fluctuations have been characterized, from this theory we will be able to place bounds on the size of fluctuations in the gating transition rates, given bounds on the size of the relaxations. We will derive explicitly here only the effect of fluctuations in the closing rate α, since the derivation for β is essentially the same. The result for α will be fairly general in that it is valid for all kinetic schemes involving a single pathway for the closing of the channel; the result for β would have to be modified if other closed states are added to the two-state scheme used here.

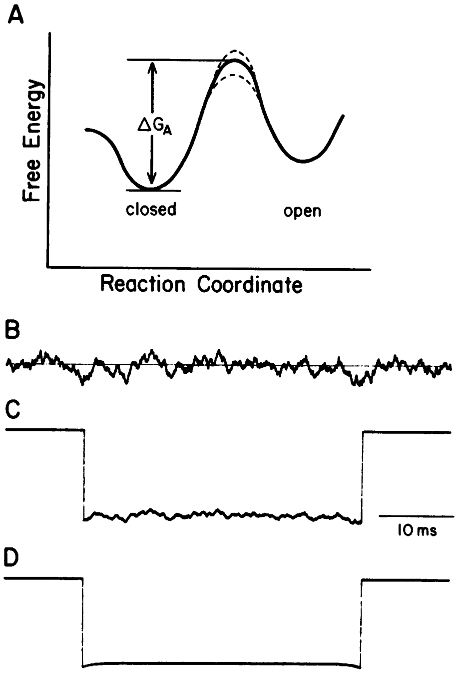

FIGURE 2 Simulation of channel currents in the presence of coupling between current fluctuations and a conformational change. (*A*). Molecular motions are assumed to change the energy barrier for the open-closed conformational change while also changing the open-channel current. (*B*). Simulated time course of a random motion $\mathbf{x}(t)$ having a Lorentzian power spectrum. Downward deflections in *B* correspond to a decreased barrier to conformational change, increasing the probability of transitions at those times. (*C*). Channel current. Current fluctuations due to **x** are present only while the channel is open. The standard electrophysiological convention is used, in which inward currents (such as were recorded through the AChR channels) are plotted downward. (*D*). The expectation of the current time course shows overshoots in the vicinity of times of channel opening and channel closing due to the correlation between current fluctuations and transitions. The open-channel fluctuations in *C* were simulated with the parameter values $\sigma_i = 0.04$ and $f_c = 340$ Hz.

The single-channel current **i** and the rate α are taken to be stochastic processes that fluctuate about their mean values i_0 and α_0 according to

$$\mathbf{i}(t) = i_0[1 + k_{ix}\mathbf{x}(t) + k_{iy}\mathbf{y}(t)] \tag{1a}$$

$$\alpha(t) = \alpha_0[1 + k_{\alpha x}\mathbf{x}(t) + k_{\alpha z}\mathbf{z}(t)], \tag{1b}$$

where the k's are coupling coefficients, and $\mathbf{x}(t)$, $\mathbf{y}(t)$ and $\mathbf{z}(t)$ are zero-mean, stationary random processes representing the molecular motions that give rise to the fluctuations. **x** represents the motions that give rise to fluctuations in both **i** and α, while for generality **y** and **z** are included to represent all other motions which are not correlated with **x**.

The process **x** has variance σ_x^2 and an autocorrelation function

$$R_x(\tau) = \langle \mathbf{x}(0)\,\mathbf{x}(\tau)\rangle. \tag{2}$$

The autocovariance of **i** can be written as the sum of two components

$$\begin{aligned} C_i(\tau) &= \langle [\mathbf{i}(0) - i_0]\,[\mathbf{i}(\tau) - i_0]\rangle \\ &= C_{i(x)}(\tau) + C_{i(y)}(\tau), \end{aligned}$$

where, using R_x and the analogous function R_y for **y**, along with (1A), we can write

$$\begin{aligned} C_{i(x)}(\tau) &= i_0^2 k_{ix}^2 R_x(\tau) \\ C_{i(y)}(\tau) &= i_0^2 k_{iy}^2 R_y(\tau). \end{aligned} \tag{3}$$

The covariance C_i is related, by the Wiener-Khinchin theorem, to the the power spectrum of the current fluctuations, which is obtained experimentally.

To obtain the time course of the relaxation in the current, we calculate the expectation value of **i** near the time of channel closing. First however we must derive various probability functions. The stochastic differential equation for the probability $\mathbf{p}(t)$ of a channel not having closed by time t is

$$\frac{\mathrm{d}}{\mathrm{d}t}\mathbf{p}(t) = -\alpha(t)\,\mathbf{p}(t).$$

We assume that the fluctuations in α are sufficiently small and rapid, so that $\mathbf{p}(t)$ on the right-hand side can be replaced with its ensemble expectation value $\langle \mathbf{p}(t)\rangle$,

$$\frac{\mathrm{d}}{\mathrm{d}t}\mathbf{p}(t) = -\alpha(\mathrm{t})\,\langle \mathbf{p}(t)\rangle. \tag{4}$$

We also introduce the joint probability density function for finding particular values of **i** and α at times t_1 and t_0, respectively

$$f_{i\alpha}(i, t_1; \alpha, t_0)\,\mathrm{d}i\,\mathrm{d}\alpha$$
$$= \mathrm{Prob}\,\{i < \mathbf{i}(t_1) < i + \mathrm{d}i \quad \text{and} \quad \alpha < \boldsymbol{\alpha}(t_0) < \alpha + \mathrm{d}\alpha\}. \quad (5)$$

The joint probability density of channel closing at time t with $\boldsymbol{\alpha}$ taking the value α is, from Eq. 4,

$$f_{\text{close}}(\alpha, t) = \alpha\,\langle \mathbf{p}(t) \rangle \quad (6)$$

which, when averaged over α, gives the simple density of channel closing

$$f_{\text{close}}(t) = \alpha_0\,\langle \mathbf{p}(t) \rangle. \quad (7)$$

Given these quantities we can calculate the desired quantity, the expectation value of $\mathbf{i}$ at t_1 given a channel closing at t_0,

$$E\,\{\mathbf{i}(t_1)|\text{close at } t_0\}$$
$$= \frac{\iint i\, f_{i\alpha}(i, t_1 \,|\, \alpha, t_0)\; f_{\text{close}}(\alpha, t_0)\,\mathrm{d}i\,\mathrm{d}\alpha}{f_{\text{close}}(t_0)}$$
$$= \frac{\iint i\alpha\, f_{i\alpha}(i, t_1; \alpha, t_0)\,\langle \mathbf{p}(t_0) \rangle\,\mathrm{d}i\,\mathrm{d}\alpha}{\alpha_0 \langle \mathbf{p}(t_0) \rangle}. \quad (8)$$

The integral of $i\alpha f_{i\alpha}$ is just the expectation of the product $i\alpha$, so that

$$E\{\mathbf{i}(t_1)\,|\,\text{close at } t_0\} = \frac{1}{\alpha_0}\,\langle \mathbf{i}(t_1)\boldsymbol{\alpha}(t_0) \rangle. \quad (9)$$

Making use of Eqs. 1 and 2 we can rewrite Eq. 9 in terms of the process $\mathbf{x}$,

$$E\{\mathbf{i}(t_1)\,|\,\text{close at } t_0\} = i_0[1 + k_{ix}k_{\alpha x}R_x(t_1 - t_0)], \quad (10)$$

where all terms involving $\mathbf{y}$ and $\mathbf{z}$ vanish because of the lack of correlations. Finally by comparison with the expression (Eq. 3) for $C_{i(x)}(\tau)$, the component of the autocovariance of $\mathbf{i}$ arising from the $\mathbf{x}$ process, we obtain

$$E\{\mathbf{i}(t_1)\,|\,\text{close at } t_0\} = i_0\left[1 + \frac{k_{\alpha x}}{k_{ix}}\,C_{i(x)}(t_1 - t_0)\right]. \quad (11)$$

Case of a Lorentzian Power Spectrum.

As was shown in the previous paper (Sigworth, 1985) the experimental power spectrum of fluctuations in $\mathbf{i}$ can be fitted by the sum of a Lorentzian and a constant of the form

$$G(f) = \frac{4i_0^2\sigma_i^2}{\lambda[1 + (2\pi f/\lambda)^2]} + G_0, \quad (12)$$

where σ_i is the relative, rms amplitude of the Lorentzian fluctuations, and λ is related to the corner frequency f_c by $\lambda = 2\pi f_c$. The hypothesis to be considered in this paper is that the fluctuations $\mathbf{x}$ that are common to both $\mathbf{i}$ and $\boldsymbol{\alpha}$ are those that give rise to the Lorentzian term. The inverse Fourier transform of this term would then give the covariance term

$$C_{i(x)}(\tau) = \sigma_i^2 \exp\,(|-\lambda\tau|). \quad (13)$$

Substituting this into Eq. 11 yields the expected time course of the current relaxation

$$E\{i(t_0 + \tau)\,|\,\text{close at } t_0\} = i_0[1 + A_\alpha \exp\,(-|\lambda\tau|)]. \quad (14)$$

The amplitude of the exponential component is given by

$$A_\alpha = \sigma_i^2 k_{\alpha x}/k_{ix}.$$

We note from Eq. 1 that σ_i, the standard deviation of the fluctuations in $\mathbf{i}$ arising from the process $\mathbf{x}$, is given by

$$\sigma_i = k_{ix}\sigma_x,$$

and the standard deviation of the corresponding fluctuations in α is given by

$$\sigma_\alpha = k_{\alpha x}\sigma_x.$$

We can therefore express the amplitude as

$$A_\alpha = \sigma_i\sigma_\alpha. \quad (15)$$

The result derived here is similar to the fluctuation-dissipation theorem (Kubo, 1956) in that the time course of the relaxation is seen to be the same as that of the autocorrelation of the fluctuations. The amplitude of the relaxation is given by the product of the normalized standard deviations of the fluctuations.

The channel current, and with it the observable current fluctuations, vanishes when the channel closes. For this reason the relaxation in current given by Eq. 14 is observable only for $\tau<0$. On the basis of a derivation essentially identical to the one shown above the relaxation due to fluctuations in the channel opening rate β can be shown to have the same form as Eq. 14 but with the amplitude

$$A_\beta = \sigma_i\sigma_\beta, \quad (16)$$

where $\sigma_\beta = k_{\beta x}\sigma_x$. Since the relaxation will occur on channel opening, it is only observable for $\tau>0$.

EXPERIMENTAL METHODS

The goal of the experimental work was to search for relaxations in the mean time courses of channel opening and closing. Since the magnitude of the fluctuations in $\mathbf{i}$ was typically $\sigma_i = 0.04$ (i.e. 4% of the mean current amplitude) the relaxations are expected to be on the order of that size or smaller. A practical problem in looking for small relaxations in the channel transitions is the accuracy of the step response of the recording system; a residual "creep" of 0.5% in the step response is not uncommon, typically arising from imperfect matching of time constants in the patch-clamp circuit (see Hamill et al., 1981). This difficulty can be avoided by comparing the time courses of channel opening and channel closing with each other, rather than comparing them separately with a step function. In the absence of a relaxation, the linearity of the recording system implies that the opening and closing time courses should be completely symmetrical, such that if they are aligned in time, they should sum to a constant. A relaxation before the channel closing event or

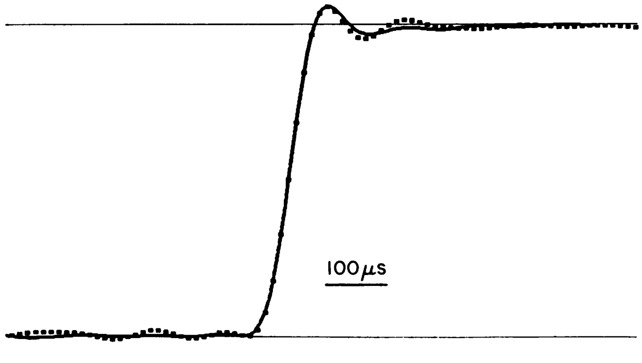

FIGURE 3 Comparison of averaged channel closings (dotted trace) with the step response of the recording system. Approximately 500 channel closing transitions were aligned and averaged as described in the text. The step response was measured by applying a square wave to the test input of the EPC-7 patch clamp. Both the channel currents and the step response signal were recorded on tape, filtered (4 kHz, Butterworth response) and sampled at 50 μs intervals. Note the close correspondence between the two traces.

following the opening, however should be apparent as an asymmetrical component that does not cancel in the summation.

The experimental recordings are the same as were used in the previous study (Sigworth, 1985). They were made from ACh receptors in rat myoballs activated with 0.3–1 μM ACh and recorded in cell-attached or outside-out patch configurations. The currents were recorded using an EPC-5 or EPC-7 patch clamp (List Electronic) and were recorded at 5 or 10 kHz bandwidth on analog tape (RACAL Store-4 or Store-4 DS). The data were digitized using the CATCH program (Sigworth, 1983) at a sample interval of 0.1 or 0.05 ms after Bessel filtering at 2 or 4 kHz, respectively.

To obtain average channel-opening and closing time courses, many hundreds of channel transitions were selected, aligned and summed. The alignment of channel transitions was used by Auerbach and Sachs (1984) to observe the kinetics and amplitude of transitions to sublevels of channel conductance. In contrast to their work, the selection procedure used here was designed to eliminate events that contained any extra transitions (or even brief gaps) within the length of the segments that were averaged (3.2 or 6.4 ms). The procedure used was, first, to accumulate averages of 10–20 transitions that were selected by visual inspection. After this an automatic routine was started which selected and formed separate averages of opening and closing events according to three criteria: the lack of additonal transitions, identified as crossings of the 50% threshold within the interval; the size of the transition; and, after alignment, the squared sum of the deviations from the accumulated mean time course. The allowed deviation in amplitude was 10%, and the threshold for the squared deviations was chosen so that roughly 20% of all "visually acceptable" events were excluded. For alignment, segments were first interpolated to intervals of one fourth the original sample interval using a cubic spline (Colquhoun and Sigworth, 1983), and linear interpolation was then used to provide further fractional shifts of the time axis to completely align the transition midpoints.

The alignment of traces is affected by the presence of relaxations like the ones sought here, and by noise. A relaxation in the vicinity of a transition introduces a small systematic error in the alignment whose effect can be predicted and which has been included in the theoretical curves to be shown in Fig. 4. The effect of noise is to introduce random time errors in the alignment; to first order the standard deviation σ_t of these errors is

$$\sigma_t = \frac{T_r \sigma_n}{i_0},$$

where σ_n is the rms noise amplitude, i_0 is the amplitude of the transition, and T_r is the risetime of the recording system. The result of this error is expected to be only a slight (<5%) reduction in the effective bandwidth of the averaged signal. In fact, the time course of the averaged transitions obtained in the way described matches very closely the step response of the recording system, as is shown in Fig. 3. This also confirms the observation (Hamill et al., 1981) that opening of these channels is instantaneous on the time scale of patch clamp recordings.

RESULTS AND DISCUSSION

Transitions were averaged and analyzed in a total of seven experimental runs, but no relaxations having the expected time course could be detected. Fig. 4 shows the transitions from the three runs whose averages had the best signal-to-noise ratio, and would allow relaxations of ~1% in

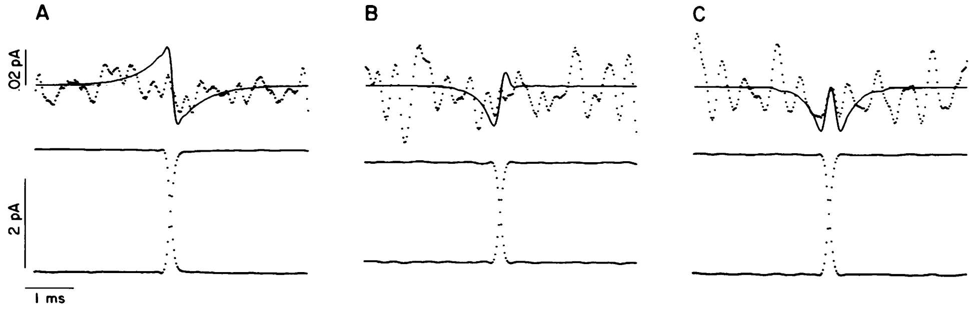

FIGURE 4 Three sets of averaged transitions (*lower* traces) are shown centered on the time of transition. Channel openings are downward-going, and channel closings are superimposed, upward-going traces. The sums of opening and closing traces (*upper*, dotted traces) are plotted with the vertical scale expanded by a factor of 40. Superimposed on the sums are solid curves showing possible time-courses of relaxations, with time constants obtained from the corresponding power spectra and amplitudes of 1% (in *A*) or 2% (in *B* and *C*). Panel *A* is from run 45.1720, an outside-out patch recording at −100 mV and 9°; the corresponding spectrum is shown in Fig. 4 *A* of Sigworth (1985). The sample interval was 0.1 ms, but because of interpolation each dot in the trace corresponds to 25 μs. Totals of $N_o = 860$ opening events and $N_c = 916$ closing events were averaged. The fit to the spectrum gave $\sigma_i = 0.037$ and $f_c = 353$ Hz; the solid curve is calculated with $A_\alpha = -0.01$ and $A_\beta = 0.01$. Panels *B* and *C* are both from a cell-attached patch at 14° and at holding potentials of approximately −80 and −100 mV, respectively (runs 26.1230 and 26.720). The sampling interval was 50 μs. In *B*, the parameter values are $N_o = 786$, $N_c = 742$, $\sigma_i = 0.046$, $f_c = 800$ Hz; the curve was calculated with $A_\alpha = 0.02$ and $A_\beta = 0$. In *C* the values are $N_o = 1{,}294$, $N_c = 1{,}194$; $\sigma_i = 0.042$, $f_c = 1{,}100$ Hz; $A_\alpha = A_\beta = 0.02$. The spectrum corresponding to the traces in *C* is shown in Fig. 4 *C* of Sigworth (1985).

amplitude to be detected. In each case about 1,000 events were averaged, and the resulting opening and closing time courses (shown superimposed as the lower traces in the figure) are essentially the same as the step response of the Bessel filter that was used. The sums of the opening and closing time courses, scaled up by a factor of 40, are shown as the dotted curves in upper traces in the figure. These sum traces are expected to be essentially flat in the absence of any relaxations, because the time courses of the current recorded from channel openings and closings would then be identical but opposite in polarity.

In general, a relaxation could be of either polarity, depending on the signs of the coupling coefficients k_{ix}, $k_{\alpha x}$ and $k_{\beta x}$, and there is in fact no a priori reason to expect that the coefficients for α and β should have the same polarity or value. The solid curves superimposed on the upper traces in Fig. 4 are theoretical curves that were calculated to show various combinations of amplitudes; it can be seen that any trends in the experimental points are smaller in amplitude than these curves.

In part A the amplitudes A_α and A_β were -0.01 and 0.01, respectively. The value of A_α corresponds to an upward relaxation of 1% amplitude just before channel closing as would result if fluctuations that decrease the magnitude of the (negative) channel current resulted in an increase in the closing rate α. The positive value of A_β gives rise to a downward relaxation immediately after channel opening, resulting from the overshoot from an increased opening rate accompanying larger channel currents. In part B, A_α was chosen to be 0.02 while $A_\beta = 0$, i.e. fluctuations increasing the current were assumed to increase α only, with no effect on β. In C both amplitudes were 0.02. The experimental traces appear to show small trends in the vicinity of the channel transition times like those in the theoretical curves, but the trends were not consistent in polarity among these or the other four runs, and are likely to have arisen from random noise. It is concluded, therefore, that no relaxations are visible at the level of resolution afforded by the data.

Sources of Artifacts

Since in this study we are concerned with very small differences between opening and closing time-courses, it is important to consider possible artifacts that could affect the symmetry of the time courses. It turns out that the main sources of artifacts tend to introduce assymetries rather than remove them. One important artifact would arise from a substantial impedance in series with the patch membrane. A series resistance of 400 megohms could give rise to a 1% relaxation since upon channel opening it would be in series with the channel resistance (~40 gigohms), while upon closing the channel resistance would be infinite. The relaxation would not be entirely symmetrical because the changes in patch membrane resistance would alter the charging and discharging time constants (See Hamill et al., 1981 for an illustration and analysis of this situation), yielding a net relaxation in the summing procedure used here. Artifacts due to this mechanism are unlikely because the series resistance was typically in the range of 10–30 megohms, as measured just before isolation of outside-out patches, or in the range of a few megohms for cell-attached patches. The close agreement of the channel transition time course and the step response of the electronics (Fig. 3) also argues against a substantial series resistance artifact.

Another important source of artifacts would be the contribution of brief channel closings to the time course of the average current. Auerbach and Sachs (1984) have demonstrated a relaxation in the average time course of the beginnings and ends of channel-opening bursts due to sojourns in a low-conductance state; a similar phenomenon could occur here if a sufficient number of brief closing events were included in the segments to be averaged, and if the frequency of occurrence of these gaps changed in the vicinity of opening or closing transitions. An analysis of the frequency of resolvable gaps however shows that this is unlikely to be a problem. In the run of Fig. 4 *A* gaps of mean duration $\tau_g = 0.22$ ms occurred at a mean rate $\lambda_g = 40\ \mathrm{s}^{-1}$ while channels were open; the maximum possible amplitude of a relaxation arising from these gaps would be $\tau_g\lambda_g = 0.9\%$. However, at least 70% of these gaps would be detected and eliminated by the automatic selection procedure, since with the 2 kHz filtering used for this run the minimum gap duration for detection is $t_{\min} = 80\ \mu\mathrm{s}$ (Colquhoun and Sigworth, 1983). Any relaxation component due to these gaps would then be smaller than $0.3t_{\min}\lambda_g \sim 0.1\%$, an order of magnitude smaller than the amplitudes shown in Fig. 4.

Bounds on the Fluctuations in α and β

Given experimental values for σ_i from spectral analysis along with bounds for the amplitudes A_α and A_β, we can use Eqs. 15 and 16 to calculate bounds on the fluctuations in the rate constants that are related to the open-channel current fluctuations. We take the magnitudes for A_α and A_β, 0.01, 0.02, and 0.02 in the three panels of Fig. 4, as upper bounds. Then, from the corresponding values for σ_i (given in the legend) one obtains upper bounds for σ_α and σ_β of 0.27, 0.43, and 0.48, respectively. In view of the lack of a consistent pattern in the polarities of the deviations, it is likely that σ_α and σ_β are in fact much smaller. Taking the bound for their magnitude to be 0.3 implies that any fluctuations in the rates that arise from the common process **x** must have a standard deviation of 30%. (Other, uncorrelated fluctuations could be of any magnitude, however.)

Returning to the model in which changes in the rates reflect fluctuations in an energy barrier (Fig. 2 *A*), the corresponding barrier fluctuations would have an upper bound of 0.3 kT rms. This is a small fluctuation compared to the total barrier height, roughly 20 kT, that one calculates from rate theory. It is concluded, therefore, that any fluctuations in the channel transition rates are either small or weakly correlated with the open channel current fluctuations that have been observed in the ACh receptor. This implies that the molecular motions underlying the open-channel current fluctuations are not strongly coupled to the open-closed conformational changes of the channel.

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