A speed limit for conformational change of an allosteric membrane protein

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Neuromuscular acetylcholine receptors are synaptic ion channels that open and close with rate constants of ∼48,000 s⁻¹ and ∼1,700 s⁻¹, respectively (in adult mouse, at 24°C, −100 mV membrane potential). Perturbations of many different sites in the protein can change these rate constants, with those in the extracellular domain mainly affecting channel-opening and many of those in the membrane and intracellular domains mainly affecting channel-closing. We used single-channel recordings to measure the total open time per activation (τₒ) elicited by a low concentration of the natural transmitter, acetylcholine. τₒ increased in constructs with mutations that increased the gating equilibrium constant by either increasing the opening or decreasing the closing rate constant. However, τₒ did not approach the same asymptote in fast-opening and slow-closing constructs. The maximum value for the slow closers was about twice that for the fast openers. One interpretation of this difference is that there is an upper limit to the channel-opening rate constant, which we estimate to be ∼0.86 μs⁻¹. One possibility is that this limit is the rate of conformational change in the absence of an overall activation barrier and thus reflects the kinetic prefactor for the acetylcholine receptor opening isomerization.

Here, we estimate a maximum rate constant for passing through the short-lived, intermediate states that populate the diliganded AChR gating transition-state ensemble.

Materials and Methods

Expression. cDNA clones of α, β, δ, and e subunits of mouse AChR in pRBG4 were transiently expressed in human embryonic kidney cells (HEK 293) by transfection with calcium phosphate. A total of 3.5 μg of DNA per 35-mm culture dish was used with a subunit ratio of 2:1:1:1 (α/β/δ/e). The medium was changed after 24 h, and electrophysiological recording began another 24 h later. All mutations (side-chain substitutions) were made by using the QuikChange (Stratagene) site-directed mutagenesis kit and were confirmed by dideoxy sequencing.

By selecting appropriate combinations of agonist and mutation, we could engineer the gating reaction to study AChRs having a wide range of opening or closing rate constants. The fast-opening constructs had mutations at position D97 [in loop 5, near the transmitter binding site (12)] in both α-subunits. Where noted, a second fast-opening mutation, αS269I, in the extracellular linker (13), was also present. The slow-closing constructs had side-chain substitutions at position L265 or S268 in the membrane domain of the δ-subunit (9).

Electrophysiology and Kinetic Analyses. Single-channel currents were recorded in cell-attached patches (approximately −100-mV membrane potential at 23°C) and sampled at 100 kHz after low-pass filtering at 20 kHz. The acquisition and analyses of the currents were done by using QUB software (www.qub.buffalo.edu). Idealization of the currents was done by using the segmental k-means (SKM) algorithm (14) without additional low-pass filtering. Model-based kinetic analyses of the idealized, noise-free intervals were done by using a maximum interval likelihood method (15) with an imposed dead time of 25 μs.

Single-channel currents were elicited by 1 μM ACh. Usually, the open interval duration distributions were fitted by three exponential components. The time constant of the slowest component (τₒ) corresponds to diliganded AChRs. Our goal was to interpret τₒ by using an equation derived from a kinetic model (Scheme 1 and Eq. 3).

In the fast-opening constructs activated by ACh, our time resolution was less than the lifetime of state A_C (see Scheme 1) and we were therefore unable to measure the opening and closing rate constants directly. Instead, we measured these rate constants (and their ratio, the diliganded gating equilibrium constant, θ) for each construct using a weak agonist, choline, and then extrapolated these values to what we would expect with ACh as the agonist. The core assumption in this extrapolation was that two kinds of perturbation (ligands and mutations) make energetically independent contribu-

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Abbreviations: ACh, acetylcholine; AChR, ACh receptor channels.

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\[ k_{-2} \beta \sigma \leftrightarrow A_2C \rightleftharpoons A_2O \rightarrow A_2 \]

Scheme 1.

To derive these equations, we consider that an ACh receptor is in one of two states: A2C or A2O. The state A2C is the open state, and A2O is the closed state. The rate constants are denoted by \( k_{-2} \), \( \beta \), and \( \sigma \), corresponding to the rate constants of the fast-opening, slow-closing, and activation processes, respectively. The equilibrium constant is denoted by \( \Theta \).

\[ \Theta_{ACh} = 28 \quad \Theta_{choline} = 0.05 \]

Under our experimental conditions, sojourns in A2C are exceedingly brief, and openings that appear to be single events (Fig. 1) are, in fact, "bursts" of individual openings separated by gaps that are too short to be reliably detected or to contribute significantly to the burst duration. In this case (and as shown in ref. 17), the mean duration of a burst (the total open time per activation), \( \tau_b \), is as follows.

\[ \tau_b \approx \left( \frac{\alpha}{1 + \frac{\beta}{\beta_{max} + 1}} \right)^{-1} k_{-2} \]

For WT adult mouse neuromuscular AChRs activated by ACh (−100 mV membrane potential, at 24°C), the approximate parameters (in s⁻¹) are \( \alpha = 1,700, \beta = 48,000, k_{-2} = 50,000, \sigma = 70, \) and \( \beta_{max} = 860,000. \)

**Results and Discussion**

**Kinetic Model.** Scheme 1 encapsulates the kinetic behavior of diliganded closed and open AChRs (A2C and A2O).

\( \beta \) and \( \sigma \) are the opening and closing rate constants and \( k_{-2} \) is the agonist dissociation rate constant from closed AChRs. \( \sigma \) is the sum of the rate constants for agonist dissociation (followed by rapid closure) and desensitization from open AChRs.

Under our experimental conditions, sojourns in A2C are

Table 1. Gating rate constants for AChR mutants

<table>
<thead>
<tr>
<th>Construct</th>
<th>Predicted opening rate constant, ( \beta )</th>
<th>Predicted closing rate constant, ( \alpha )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \delta S268G )</td>
<td>1,700</td>
<td>88</td>
</tr>
<tr>
<td>( \delta S268C )</td>
<td>50,000</td>
<td>131</td>
</tr>
<tr>
<td>( \delta S268T )</td>
<td>70,000</td>
<td>107</td>
</tr>
<tr>
<td>( \delta S268V )</td>
<td>50,000</td>
<td>131</td>
</tr>
<tr>
<td>( \delta S268N )</td>
<td>70,000</td>
<td>107</td>
</tr>
<tr>
<td>( \alpha L265'T )</td>
<td>107</td>
<td>107</td>
</tr>
<tr>
<td>( \alpha L265'T + \alpha D97C )</td>
<td>91</td>
<td>91</td>
</tr>
<tr>
<td>( \alpha L265'T + \alpha D97E )</td>
<td>86</td>
<td>86</td>
</tr>
<tr>
<td>( \alpha L265'T + \alpha D97Q )</td>
<td>85</td>
<td>85</td>
</tr>
<tr>
<td>( \alpha L265'T + \alpha D97A )</td>
<td>72</td>
<td>72</td>
</tr>
</tbody>
</table>

All values are s⁻¹ and pertain to diliganded AChRs activated by ACh. The predicted opening and closing rate constants are calculated from the corresponding values measured for choline and using Eq 1 (see Materials and Methods). The calculated opening and closing rate constants for WT AChRs activated by ACh are 44,349 s⁻¹ and 1,583 s⁻¹, respectively, and are similar to the experimentally determined values of about 48,000 s⁻¹ and 1,700 s⁻¹, respectively. The double-mutant constructs in the slow-closing series also open rapidly, but it is mainly their slow \( \alpha \)-values that cause the \( k^* \) approach zero and, hence, drive \( \tau_b \) towards \( \alpha \)⁻¹ (Eq. 3).

**Estimating the Speed Limit.** Scheme 1 implies that perturbations that specifically slow the closing rate constant \( \alpha \) and those that specifically increase the opening rate constant \( \beta \) should have the same qualitative effect, namely, to increase the probability that bursts terminate from A2O. Eq. 3 predicts that mutations that decrease \( \alpha \) and those that increase \( \beta \) should both shrink \( k^* \) and increase the burst lifetime \( \tau_b \). Quantitatively, Eq. 3 predicts at the extremes (as \( \alpha \rightarrow 0 \) or \( \beta \rightarrow \infty \) that both kinds of mutation should result in the same \( \tau_b \), asymptote, \( \sigma \)⁻¹.

Fig. 1 shows exemplary single-channel currents and burst-
gating rate constants, greater than that of the fast-opening series (duration (Chakrapani and Auerbach PNAS Fig. 2. Slow-closing and fast-opening mutants generate different burst-

either closed or open AChRs) or desensitization (12, 17). The results are summarized in Table 1 and Fig. 2. The specific side-chain substitutions for both classes of perturbation have previously been shown to alter only the gating rate constants and to have little or no effect on agonist dissociation (to either closed or open AChRs) or desensitization (12, 17).

The fast-opening AChRs had mutations of α-subunit residue D97, which is in loop 5 of the extracellular domain (19). With choline as the agonist, mutations of this position increase β but have only a small effect on α (Φ_D97 = 0.93). These constructs reduce k⁺ and increase τ₀ mainly by increasing the denominator of Eq. 3b. The slow-closing AChRs had mutations at one of two positions in the δ-subunit (S268 and L265), both of which are in the membrane domain. These mutations significantly reduce α but have little or no effect on β (Φ_S268 = 0.3 and Φ_L265 = 0.0). These constructs reduce k⁺ and increase τ₀ mainly by decreasing the numerator of Eq. 3b. Although some of these slow-closing AChRs open more rapidly than those of the WT, we chose to classify them as such because it is their slow α-values that are mainly responsible for reducing the magnitude of k⁺ and thus driving τ₀ toward α⁻¹. Eq. 3 predicts that, at the extremes, both kinds of mutation should have the same quantitative effect on τ₀.

To estimate these asymptotic limits for fast-opening and slow-closing mutants, the relationship between the observed values of τ₀ and the calculated values of k⁺ were fitted by Eq. 3 with α and k⁻₂ as free parameters. Fig. 2 shows that the fitted values for the slow-closing series were α = 70.4 ± 5 s⁻¹ and k⁻₂ = 56,405 ± 22,265 s⁻¹. In these mutants, α is small and k⁻₂ is negligible; hence, we take this value of α to be equal to the sum of the rate constants for open-channel dissociation and desensitization (17).

Eq. 3 predicts that the asymptote (α-value) for the fast-opening series should be the same as for the slow-closing series. However, the fitted asymptote for the fast openers was 140 ± 8 s⁻¹, about twice the expected value. The fitted value of k⁻₂ for the fast-opening mutant series was 84,561 ± 17,895 s⁻¹. We do not consider this value to be significantly different from that for the slow-closing series or for WT AChRs estimated by using a variety of methods (~50,000 s⁻¹; ref. 17 and references therein).

We consider three possible explanations for this marked difference in maximum burst duration between fast-opening (i.e., as β →

duration histograms from slow-closing and fast-opening AChR constructs. The asymptote of the slow-closing mutant series is twice the expected value. The fitted value of k⁻₂ for the fast-opening mutant series was 84,561 ± 17,895 s⁻¹. We do not consider this value to be significantly different from that for the slow-closing series or for WT AChRs estimated by using a variety of methods (~50,000 s⁻¹; ref. 17 and references therein).

We consider three possible explanations for this marked difference in maximum burst duration between fast-opening (i.e., as β →
of the gating rate constants, of the perturbation. We have no information about such a constant (brane segment that greatly reduces the channel-closing rate mutations coexist with a background mutation in the transmem-
anism for terminating a burst. However, when these same from the addition of this hypothetical (and unspecified) mech-
slow-closing mutants, 70 to the difference between the limits of the fast-opening and
ymptotes between slow-closing and fast-opening mutants.

If we assume that Φ = 0.93 for all αD97 mutant constructs (see below for an extended discussion on this point), then we estimate from Eq. 4 that Θmax = 675, which is the ratio of gating rate constants θ max = 855,623 s⁻¹ and a = 1,267 s⁻¹. That is, if we attribute the difference in τ asym- we can estimate a maximum gating equilibrium constant, Θmax, and a maximum opening rate constant, βmax. From Eq. 3b, when β > k₁, the limiting value of k* is simply a ratio of the dissociation rate constant k₂ and Θmax (which is equal to βmax/α). With k* = 70 s⁻¹ and k₂ = 50,000 s⁻¹, we calculate Θmax = 714. If we now make the additional approximation that the closing rate constant does not change throughout the fast-opening mutant series (i.e., Φ = 1 for the αD97 constructs), then we calculate (Eq. 1) that the maximum channel-opening rate constant, βmax is 1.17 μs⁻¹. Energy profiles for gating that are consistent with the idea of equilibrium and rate constants having maximum values have been explored (45).

We can refine these estimates by combining Eqs. 1 and 3.

\[
70 \text{ s}^{-1} = \frac{2,000 \text{ s}^{-1} \times \Theta_{\text{max}} \Phi^{-1}}{1 + \frac{2,000 \text{ s}^{-1} \times \Theta_{\text{max}} \Phi^{-1}}{50,000 \text{ s}^{-1}}}
\]

If there are no new exit paths from A₂O (i.e., Scheme 1 and Eq. 3 are valid for all mutants) and if neither k₂ nor σ is altered by the mutations, then the ~2-fold difference in asymptotes for the two mutant series must arise from differences in the values of the gating rate constants, β and α. The smaller asymptote for the fast-opening mutants indicates that k* is reaching a finite, lower limit in the αD97 mutants. This boundary is equal to the difference between the limits of the fast-opening and slow-closing mutants, 70 ± 6 s⁻¹.

According to Eq. 3b, k* will reach a minimum value if α reaches a minimum, if β reaches a maximum, or both. We have no reason to suspect that a rate constant should have a minimum value. Accordingly, we focus our attention on the possibility that the 70 s⁻¹ limit for k* is caused by β reaching a ceiling. That is, we hypothesize that the membrane domain mutations can make channel-closing arbitrarily slow but that the extracellular domain mutations cannot make channel-opening arbitrarily fast. Consequently, we propose (Fig. 2) that the fast-opening series deviates from the slow-closing series because the actual β-values and, hence, the calculated (1/k*) values that are plotted on the x axis are overestimated by using our simple assumptions (see Materials and Methods).

From the limit k* = 70 s⁻¹ we can estimate a maximum gating equilibrium constant, Θmax, and a maximum opening rate constant, βmax. From Eq. 3b, when β > k₂, the limiting value of k* is simply a ratio of the dissociation rate constant k₂ and Θmax (which is equal to βmax/α). With k* = 70 s⁻¹ and k₂ = 50,000 s⁻¹, we calculate Θmax = 714. If we now make the additional approximation that the closing rate constant does not change throughout the fast-opening mutant series (i.e., Φ = 1 for the αD97 constructs), then we calculate (Eq. 1) that the maximum channel-opening rate constant, βmax is 1.17 μs⁻¹. Energy profiles for gating that are consistent with the idea of equilibrium and rate constants having maximum values have been explored (45).

We can refine these estimates by combining Eqs. 1 and 3.
hypothesis that AChRs with ground mutation were similar. This result is consistent with the fitting the is unknown. Therefore, we could not derive an exact expression for fast-opening mutant series, the closing rate constant will diminish. Consequently, throughout the reaction coordinates) will diminish. Thus, the intersection of the equilibrium constant (G0) is linear (1). Any combination of ACh, we were forced to estimate the asymp-
totic limit of AChRs having the mutation in the extracellular linker are somewhat larger than the limiting value of the aD97 series alone because the linker mutation modestly slows \( \alpha \) in addition to increasing \( \beta \).

**\( \Phi \) Values.** In this section we consider the implications of our conjecture that \( \Phi \) is equal to 0.93 for all of the AChR constructs. The Hammond postulate is that the energy of the transition state tends to resemble that of the least-stable end-state (20). Accordingly, as the energies of the transition state and A2C converge progressively throughout the fast-opening mutant series, the intersection point between the two parabolic wells of the end states will shift toward A2C and the \( \Phi \) value for the reaction (i.e., averaged over all reaction coordinates) will diminish. Consequently, throughout the fast-opening mutant series, the closing rate constant \( \alpha \) might shrink more than is expected from our assumption of a constant \( \Phi \) value of 0.93. Because the opening rate constant is too fast to be measured directly, we have no way of specifically probing the \( \Phi \) value of the TBS/aD97 domain for the mutant constructs.

To address the possibility that \( \Phi \) was not 0.93 throughout, we used Eq. 4 to calculate \( \theta_{\text{max}} \) and then Eq. 1 to calculate a channel-opening speed limit (\( \beta_{\text{max}} \)) for different values of \( \Phi \). Reasonable values of these parameters pertain for \( \Phi \) values between 0.5 (\( \theta_{\text{max}} = 289 \) and \( \beta_{\text{max}} = 34,000 \) s\(^{-1}\)) and 1.0 (\( \theta_{\text{max}} = 689 \) and \( \beta_{\text{max}} = 1,378,000 \) s\(^{-1}\)). Any combination of \( \theta_{\text{max}} \) and \( \beta_{\text{max}} \) values between these extremes is consistent with our experimental observations. Our results allow us to conclude only that the minimum AChR channel-opening time is \( >0.72 \) ms.

We next considered the possibility that \( \Phi \) was not constant throughout the aD97 mutant series. In our experiments, a Hammond effect might progressively reduce the \( \Phi \) value as the aD97 mutations progressively increase the gating equilibrium constant. Although we do not know whether, or the precise manner by which, \( \Phi \) might change, we wanted to examine how a sliding \( \Phi \) value in the fast-opening mutant series would bias our \( \beta_{\text{max}} \) estimate.

Because we know of no combination of point mutation and agonist that specifically increases the channel-opening rate constant beyond that of aD97/ACh, we were forced to estimate the asymptotic limit of \( \theta_0 \) by fitting the appropriate analytical function for a constant \( \Phi \), which is a rectangular hyperbola (17). A gradual decrease in \( \Phi \) in the aD97 mutant series (from 1 toward zero) would obviate the use of this exact function. Thus, we wanted to test the possibility that our use of the “wrong” fitting function led us to underestimate the true, asymptotic limit of the burst duration.

The function relating \( \Phi \) to the overall gating equilibrium constant is unknown. Therefore, we could not derive an exact expression for fitting the \( \theta_0 \) vs. \( 1/k^\theta \) relationship. As a first approximation, we assumed that the relationship between \( \Phi \) and the natural logarithm of the equilibrium constant (\( \Delta G_0 \)) is linear (\( \delta \Phi / \delta \Delta G_0 \) is constant), so that \( \Phi \) would progressively decrease from 0.9 to 0.2 during the course of the aD97 mutant series. The results of using this assumption indicate that the \( \theta_0 \) vs. \( 1/k^\theta \) relationship is indeed altered by this progressive reduction in \( \Phi \) but in a manner that is inconsistent with the observed difference between the fast-opening and slow-closing series (data not shown). As \( \Phi \) shrinks, the effect of the change in equilibrium constant is increasingly manifest as a reduction in \( \alpha \), which in turn increases the observed \( \theta_0 \) values more steeply than otherwise. In contrast, our results show a less-steep increase in the observed \( \theta_0 \) values in the fast-opening mutants (Fig. 2). We conclude that a Hammond effect would not impact our estimate of the \( \theta_0 \) asymptote.

**Brief Gaps.** In both WT and mutants AChRs a short-lived (<30 \( \mu \)s) nonconducting state was apparent (Fig. 1). These events are too long-lived to reflect sojourns in A2C and are too frequent to reflect first-order channel-block by the agonist. These events, which have been reported previously in AChRs (21), may represent sojourns in nonconducting diliganded states that are distal to A2O (22) or, perhaps, that populate the A2C \( \rightleftharpoons \) A2O transition-state ensemble (A.A., unpublished work). Because these brief gaps were key determinants of our \( \theta_0 \) measurements, we imposed a dead time of 200 \( \mu \)s to test whether the speed-limit estimate was sensitive to their detection. This change in procedure had a significant effect on the asymptote estimates (70.2 to 15.7 s\(^{-1}\) for the slow-closing series, and 140 to 59 s\(^{-1}\) for the fast-opening series) but only a small effect on the \( \beta_{\text{max}} \) estimate (1.4–2.2 \( \mu \)s\(^{-1}\), assuming a \( \Phi \) of 1.0). Thus, our estimate of the maximum opening rate constant is not highly sensitive to the detection of these short-lived events.

**Interpreting the Speed Limit.** Two-state reactions are often rationalized using absolute rate theories, which codify a rate constant (\( \Lambda \)) as the product of an exponential term (which contains \( E_\text{‡} \), the threshold activation energy) and a preexponential term (\( A^* \)).

\[
k = A^* \exp \left( -\frac{E_\text{‡}}{k_B T} \right)
\]

\( k_B \) is Boltzmann’s constant and \( T \) is the absolute temperature. The prefactor, \( A^* \), is not precisely defined for a chemical reaction as complex as protein conformational change, but it incorporates a term for reaching the transition state once the activation energy has been achieved and a term for actually crossing through the transition state once it has been reached. In Kramers’ theory (23, 24), the reaction is assumed to proceed in one dimension along a double-well potential energy profile, in which case \( A^* \) is a function of the curvature of the reaction-well and barrier top plus a continuum frictional coefficient. These parameters have not been estimated from first principles or from experimental observations for AChR gating. Nonetheless, a rate constant will reach an upper limit (speed limit) if \( E_\text{‡} \) reaches a minimum value, which may be zero, in which case the limit, by definition, reflects only \( A^* \). Note that in Eq. 5, \( A^* \) is an empirical factor that may be different from the theoretical form of the Kramers prefactor (25). We start with this standard formalism to consider several (nonexclusive) mechanisms by which the experimental value of the apparent AChR channel-opening rate constant could reach an upper boundary.

There are several mechanisms by which \( \beta \) could reach a maximum: (i) the binding site and residue perturbations may have not been energetically independent; (ii) there could be a residual energy barrier(s) along the reaction pathway; or (iii) \( E_\text{‡} \) is zero (i.e., the reaction energy profile has no barrier) and, hence, \( \beta_{\text{max}} \) reflects \( A^* \).

The speed-limit estimation was predicated on the assumption that perturbations of residue aD97 (a side-chain substitution) and the transmitter binding sites (a change in the agonist) were independent. For example, we assumed that a 152-fold (5.0 \( k_T \)) increase in the reaction equilibrium constant caused by a D-to-A substitution at \( \theta_7 \) and a 560-fold (6.3 \( k_T \)) increase caused by a choline-to-ACh substitution at the transmitter binding sites together result in an 85,120-fold (11.3 \( k_T \)) increase in the gating equilibrium constant, \( \theta^* \). \( E_\text{‡} \) might appear to reach some non-zero, lower limit if this assumption of energetically independent contributions of perturbations is not valid. Where it has been measured directly, many AChR perturbations do behave independently with respect to changing \( \theta \) (26). Moreover, the two kinds of perturbation that we used influence the diliganded gating equilibrium constants by different mechanisms. aD97 mutations increase \( \theta \) by increasing
The unliganded gating equilibrium constant, whereas the choline-
to-ACh perturbation increases $\Theta$ by increasing the closed/open
equilibrium dissociation constant ratio. However, we have no way
of testing experimentally whether these two perturbations were
indeed independent. In addition, it is possible that these sites
behave independently with small perturbations (which elicit rate
constants that are in a measurable range) but fail to do so with large
excursions in energy.

The second possible explanation for the apparent channel-
opening speed limit is that the perturbations at $\Theta$ and the
transmitter binding sites indeed lower $E^I$ in an independent (i.e.,
energetically additive) manner but that a residual energy barri-
er(s) along the reaction pathway that was previously too small to
contribute significantly to the reaction equilibrium/rate con-
stants now becomes rate-limiting. This mechanism would con-
stitute a change in the transition state, as has been suggested to
occur in unliganded AChR gating (27). Although we cannot
exclude this possibility, our experiments indicate that the net
equilibrium constant for the residual reaction(s) is $\approx 675$ and
that this equilibrium constant does not seem to be affected by the
mutations and agonists that we have examined.

The third possibility is that at the channel-opening speed limit $E^I
\approx 0$ and that the AChR gating reaction has essentially no activa-
tion barrier. In this case, the maximum rate constant is equal to the
prefactor, $A^n$. In these experiments when $\Theta = 1$, which we will call
the intrinsic gating condition, the opening and closing rate con-
stants are each $\approx 2000$ s$^{-1}$. Accordingly, we calculate (Eq. 5, with
$A^n = 0.86 \times 10^9$ s$^{-1}$) that the intrinsic activation energy for
diluted AChR gating is $\approx 6.1$ kT. This estimate is in the range of
that estimated using a completely different method (A. Mitra, T.
Rascione, A.A., and S. Licht, unpublished work). We have no way of
distinguishing between these alternatives.

Natural Selection. Is there a physiological consequence to the gating
speed limit that makes it subject to natural selection? Using rate
constants that pertain to the mouse neuromuscular synapse (see
Materials and Methods), we calculate that synaptic decay time
constants ($\tau_n$) are essentially the same with (0.87 ms) and without
(0.89 ms) the incorporation of a speed limit. However, for a
naturally occurring human AChR mutation that causes a slow-
channel congenital myasthenic syndrome (aS269I (12)), we calcu-
late a decay time constant of $8.4$ ms and $10.2$ ms, with and without
a speed limit. The experimental $\tau_n$ for this construct was $8.3$ ms.
The existence of a speed limit suggests that in AChRs having a WT
dissociation rate constant, the ratio $\beta/k$ cannot exceed $\approx 17$ (Eq.
3). Under extreme conditions, the imposition of a channel-opening
speed limit can serve as a protective mechanism that shortens the
synaptic current decay time constant.

Other Proteins. There have been estimates of speed limits for protein
folding (in a microsecond time scale) and intrachain contact
formation in unfolded polypeptide chains (in a nanosecond time
scale) (28–38). However, there is little known about speed limits for
conformational change in native proteins. Eaton et al. (39) esti-
imated that the time required for communication between subunits
in hemoglobin during the R-to-T allosteric transition is $\approx 1 \mu$s. With
regard to ion channels, a fast component of the Shaker gating
current has a time constant of $\approx 5 \mu$s and has been interpreted as
arising from the diffusion of charges in a potential well (40–42). In
gramicidin, the open-channel noise spectrum has an $\approx 1-\mu$s com-
ponent that has been attributed to structural fluctuations of pore
residues (43). Our estimate for the speed limit for AChR gating,
which we deduced indirectly using a simple kinetic model and low-bandwidth recordings, is similar to these direct measurements
of the fastest rates for native protein conformational change.

It is remarkable that the AChR, a large, multimeric membrane
protein, can undergo a global isomerization in $\approx 1.2 \mu$s given that slow
side-chain motions in native structures (44) would be expected to
slow substantially the dynamics of conformational change. In a companion study (45), the observation that residues
are organized into discrete, rigid-body gating domains (“blocks”) and
the channel-opening speed limit are discussed in terms transition-state energy profiles that are consistent with the
Brownian motion of individual blocks.

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