A Bound-Ca^{2+} Model of Synaptic Facilitation Revisited

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Abstract

Synaptic facilitation is a transient stimulation-induced increase in synaptic transmission strength, a ubiquitous form of short-term synaptic plasticity that may play a role in regulating the activity of synaptically coupled neuronal populations on fast time scales. In their pioneering work, Katz and Miledi (1968) and Rahamimoff (1968) demonstrated the dependence of facilitation on presynaptic Ca^{2+} influx, and proposed that facilitation results from the accumulation of residual Ca^{2+} bound to vesicle release triggers. However, in its pure form this bound Ca^{2+} hypothesis contradicts the evidence that facilitation is reduced by exogenous Ca^{2+} buffers, which suggests the importance of free Ca^{2+} for this form of synaptic plasticity. This led to a widely-held view that facilitation must depend solely on the accumulation of Ca^{2+} in free form. Here we consider a more realistic implementation of the bound Ca^{2+} mechanism, taking into account spatial diffusion of Ca^{2+} ions, and show that a model with a slow Ca^{2+} unbinding step can retain sensitivity to free residual Ca^{2+}. We demonstrate that this hybrid free/bound Ca^{2+} model agrees with the facilitation accumulation time course exhibited by the crayfish inhibitor neuromuscular junction (NMJ), and relies on fewer assumptions than the most recent variations of the free residual Ca^{2+} hypothesis. Further, we show that the hybrid mechanism is consistent with the experimental results of Kamiya and Zucker (1994), which revealed that a photolytic liberation of a fast Ca^{2+} buffer decreases the synaptic response within milliseconds. We conclude that a Ca^{2+} binding process with a slow unbinding time step (τ~10s of ms) is a viable mechanism of synaptic facilitation at some synapses, and discuss the experimental evidence for such a mechanism.

INTRODUCTION

Synaptic facilitation is a transient activity-dependent increase in synaptic strength decaying on time scales from tens to hundreds of milliseconds. It is observed in a vast variety of neural systems, from invertebrate junction potentials to neocortical synapses, and along with short-term depression, may play a role in shaping the neural population dynamics on fast time scales. Notwithstanding a few postsynaptic contributions such as temporal summation of successive EPSPs at high stimulation frequencies and the depolarization-dependent relief of Mg^{2+} block of NMDA receptors at central glutamatergic synapses, facilitation is primarily a presynaptic phenomenon, dependent on presynaptic Ca^{2+} entry (see reviews by Magleby, 1987; Fisher et al., 1997; Zucker, 1999; Zucker and Regehr, 2002). Facilitation of the synaptic response may be caused for instance by the facilitation of AP-evoked Ca^{2+} currents, due to a depolarization-dependent relief of G-protein mediated inhibition of Ca^{2+} channels (Brody and Yue, 2000; Bertram et al., 2003). However, in many preparations facilitation can be achieved even under conditions of constant pulse-to-pulse Ca^{2+} influx. This was established in the pioneering studies of amphibian neuromuscular junctions by Katz and Miledi (1968) and Rahamimoff (1968), who proposed that facilitation results from the accumulation of residual Ca^{2+} bound to Ca^{2+}-dependent vesicle release sensors. This so-called bound residual Ca^{2+} hypothesis of synaptic facilitation was later explored in modeling studies (Yamada and Zucker, 1992; Bertram et al., 1996; Bennett et al., 1997; Dittman et al., 2000). However, the interest in the bound Ca^{2+} hypothesis has been limited due to the experimental evidence demonstrating the reduction of facilitation by exogenous Ca^{2+} buffers. Since the main effect of exogenous buffers is to absorb the *free* Ca^{2+} ions, with no effect on Ca^{2+} that is already bound, these results have been used to argue that the free, and not bound residual Ca^{2+} underlies facilitation (see review by Zucker and Regehr, 2002). Notably, experiments of Kamiya and Zucker (1992) on crayfish neuromuscular junctions showed that the synaptic response is reduced within milliseconds of a UV flash dialyzing a fast Ca^{2+}-absorbing buffer diazo-2 in the presynaptic terminal, demonstrating the apparent role of residual *free* Ca^{2+} in facilitated neurotransmitter release.

Thus, it is currently accepted that synaptic facilitation is caused primarily by the gradual accumulation of *free* [Ca^{2+}] during the conditioning train of stimuli. One mechanism for this is the facilitation of AP-evoked Ca^{2+} transients caused by the saturation of endogenous buffers. Proposed on purely theoretical grounds (Klingauf and Neher, 1997; Neher, 1998), this buffer saturation mechanism was recently shown to underlie facilitation at calbindin-positive neocortical and hippocampal synapses (Blatow et al., 2003; see also Maeda et al., 1999, Rozov et al., 2001; Jackson and Redman, 2003). Recent modeling results indicate that this mechanism relies on the saturation of buffers in the entire presynaptic terminal, which requires optimal concentrations of fast mobile buffers similar to calbindin (Matveev et al, 2004).

Alternatively, if endogenous buffers are primarily immobile, and are present in sufficient concentrations, they will mostly saturate *locally*, within a Ca^{2+} channel nanodomain, trapping Ca^{2+} that enters during a stimulus and then slowly releasing it during interstimulus intervals. This would lead to accumulation in *residual* free Ca^{2+}, as opposed to an increase in Ca^{2+} *transients* associated with the buffer saturation mechanism (Sala and Hernández-Cruz, 1990; Nowycky and Pinter, 1993; Neher, 1998; Matveev et al., 2002). Recent modeling studies (Tang et al., 2000; Matveev et al, 2002; see also Bennett et al., 2004) have shown that such a free residual Ca^{2+} mechanism of facilitation incorporating two spatially segregated Ca^{2+} binding sites can explain the magnitude and the time course of facilitation growth observed at the crayfish neuromuscular junction (NMJ). However, this two-site free Ca^{2+} model requires a number of assumptions to match the experimental data, such as high tortuosity of the intracellular space close to the Ca^{2+} channel, the immobilization of exogenous Ca^{2+} buffers by the cytoskeleton, and a significant spatial separation (> 150 nm) between two distinct release-controlling Ca^{2+}-binding sites (both of which must be occupied for release to occur).

In this work, we revisit the alternative *bound* residual Ca^{2+} hypothesis of facilitation, and show that it can also account for the properties of synaptic response recorded in the crayfish inhibitor NMJ, and moreover, requires fewer assumptions than the abovementioned two-site mechanism. The model we present is a more biophysically realistic implementation of the bound Ca^{2+} hypothesis that retains sensitivity to free Ca^{2+} by incorporating Ca^{2+} diffusion, and thereby can be characterized as a hybrid free/bound

residual Ca^{2+} model of synaptic facilitation. We show that it can be easily reconciled with the observed effect of exogenous Ca^{2+} buffers on synaptic response. Importantly, the hybrid model we present can readily account for several additional experimentally observed features of synaptic facilitation. For example, the same experiments that revealed the sensitivity of facilitation to exogenous buffers also uncovered a component of release whose decay lagged behind the decay of free residual [Ca^{2+}] (Kamiya and Zucker, 1994; Atluri and Regehr, 1996), suggesting that residual bound Ca^{2+} is one component of facilitation (see also Regehr et al., 1994; Dittman et al., 2000). Further, the supralinear facilitation time-course observed in a variety of synapses, and at the crayfish NMJ in particular (Tang et al., 2000), may suggest the accumulation of both free Ca^{2+} and bound Ca^{2+}. Therefore, we believe that facilitation may depend on the action of both free and bound residual Ca^{2+} in some synapses, and that current experimental evidence does not rule out the involvement in neurotransmitter facilitation of a slow Ca^{2+}-binding step operating on characteristic time scales of tens of milliseconds and above.

METHODS

We first solve the partial differential equations describing the diffusion and mutual binding of Ca^{2+} ions and buffer molecules (Eqs. 1-5). The resulting Ca^{2+} concentration time course is then used to drive the kinetic scheme modeling the binding of Ca^{2+} to vesicle release triggers (Eqs. 6-7). The simulated synaptic response is given directly in terms of the occupancy of the final "*R*" release state in the scheme described by Eqs. 8-9 (see Fig. 2 *E*). We are interested in comparing our hybrid free/bound residual Ca^{2+} model results with those of the two-site free residual Ca^{2+} model; therefore, we use the same geometry and buffering parameters that were used in Matveev et al (2002) (*modified* parameter set), which in turn is based on the work of Tang et al (2000). These models are adapted to a specific experimental system, the crayfish inhibitor NMJ, which exhibits very pronounced facilitation not occluded by concomitant synaptic depression, making it a perfect system for the study of synaptic facilitation.

Equations describing buffered diffusion of Ca^{2+}. We assume that the binding of Ca^{2+} to both the endogenous and the exogenous buffers is described by simple mass action kinetics with one-to-one stoichiometry:

$$B_j + Ca^{2+} \underset{k_j^{off}}{\overset{k_j^{on}}{\rightleftharpoons}} CaB_j \qquad (1)$$

where k_j^{on} and k_j^{off} are, respectively, the binding and the unbinding rates of the j-th Ca^{2+} buffer species, B_j. This leads to the following reaction-diffusion equations for the Ca^{2+} concentration, and the concentrations of the free (unbound) buffers:

$$\begin{cases} \dfrac{\partial [Ca^{2+}]}{\partial t} = D_{Ca}\nabla^2[Ca^{2+}] + \sum_{j=1}^{buffers} R_j([Ca^{2+}],[B_j]) + \dfrac{1}{2F} I_{Ca}(t) \sum_{i=1}^{channels} \delta(r - r_i) & (2) \\ \dfrac{\partial [B_j]}{\partial t} = D_j \nabla^2[\mathrm{B}] + R_j([Ca^{2+}],[B_j]) & (3) \end{cases}$$

where R_j is the reaction term describing the mass-action kinetics given by scheme (1):

$$R_j([Ca],[B_j]) = -k_j^{on}\,[Ca^{2+}][B_j] + k_j^{off}\,(B_j^{total} - [B_j]) \quad . \tag{4}$$

B_j^{total} denotes the total concentration of the *j*-th buffer; D_j and D_{Ca} are the diffusion coefficients in cytosol of the j-th buffer and Ca^{2+}, respectively. We choose D_{Ca}= 0.2 μm^2 ms^{-1} (Allbritton et al., 1992). Following standard convention, in Eqs. 2 and 3 we have assumed that the initial distribution of the buffer is spatially uniform, and that the diffusion coefficient of the buffer is not affected by the binding of Ca^{2+}. Under these assumptions the sum of the bound and the unbound buffer concentrations is constant in space and time, and is equal to the total buffer concentration, $B_{\text{j}}^{\text{total}}$. Thus, $[CaB_{\text{j}}] = B_{\text{j}}^{\text{total}} - [B_{\text{j}}]$. The last term in Eq. 2 represents the Ca^{2+} influx, where F is Faraday's constant, $I_{\text{Ca}}(t)$ is the (inward) calcium current per channel (see below), and $\delta(r-r_{\text{i}})$ is the Dirac delta function centered at the location of the *i*-th channel. We assume that a single species of endogenous buffer is present, which is immobile and has a resting buffering capacity of about 500, as estimated by Tank et al. (1995). The parameters of this buffer are $K_D = k^{\text{off}} / k^{\text{on}}$ =16 μM, k^{on} =0.5 $\mu M^{-1} ms^{-1}$, B^{total} = 8 mM (Matveev et al., 2002). Exogenous buffers fura-2 and diazo-2 are assumed to be mobile (D_{F2}=118 $\mu m^2 ms^{-1}$, D_{d2}=100 $\mu m^2 ms^{-1}$); their parameters are given in the captions to Figs. 3 and 4.

Equations 2-4 are solved inside a box enclosure representing the volume surrounding a single active zone of a crayfish nerve terminal (Figure 1). Using the symmetry assumption, we only model a quarter of this elementary volume (dashed box in Fig. 1), as a box with dimensions 0.8 x 0.8 x 1 μm^3. We assume 16 Ca^{2+} channels per active zone, or 4 channels in our enclosure representing a quarter of an active zone. Following Tang et al (2000) and Matveev et al (2002), each action potential is modeled as a 1-ms long Ca^{2+} current of $I_{\text{Ca}}(t)$=0.2 pA, followed by a larger 0.2 ms-long tail current of 0.8 pA entering through *each* of the Ca^{2+} channels.

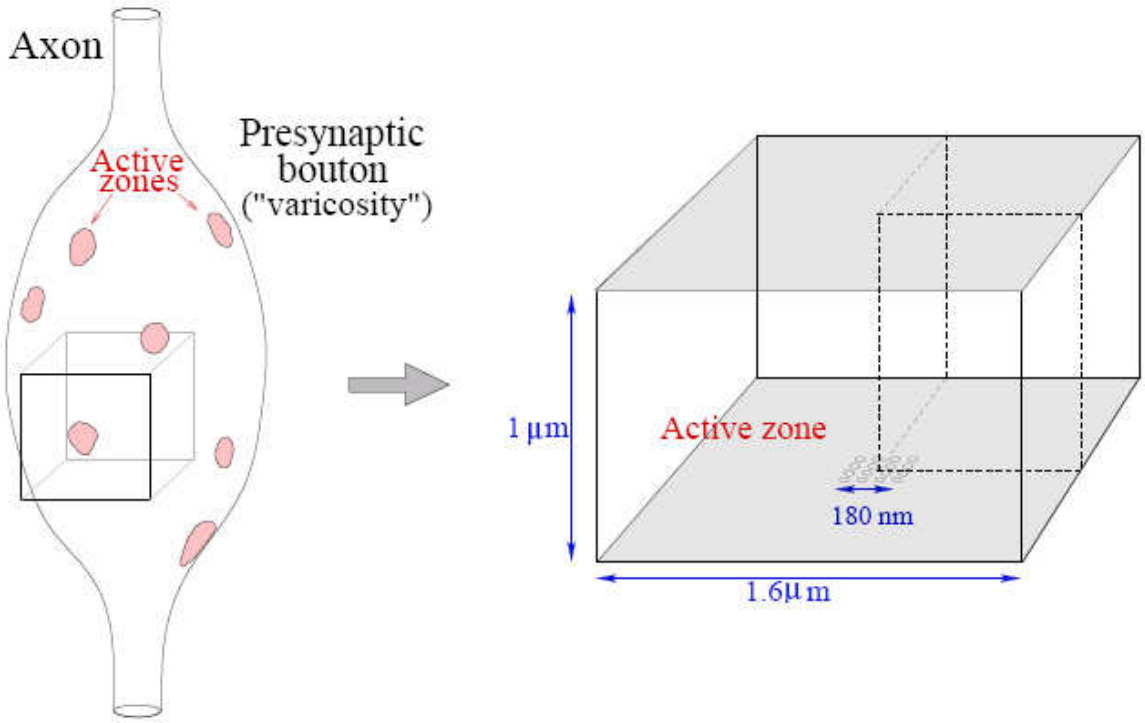


Figure 1. Simulation volume (*right*) approximates the volume surrounding a single active zone of a crayfish presynaptic varicosity (*left*). Assuming the symmetry of the geometry with respect to two mutually perpendicular vertical planes, only one quarter of the total volume is considered (*dashed lines*). Boundary conditions on all side surfaces are zero-flux, while boundary conditions on the bottom and top surfaces take into account outward flux due to Ca^{2+} extrusion by membrane pumps and exchangers.

We impose reflective boundary conditions for Ca^{2+} and buffer(s) on the sides of the box, thereby assuming that the Ca^{2+} and buffer fluxes flowing into the enclosure from the neighboring AZ regions are balanced by the equal fluxes flowing out of the enclosure. The boundary condition for $[Ca^{2+}]$ on the top and bottom surfaces is of Neumann type, simulating Ca^{2+} extrusion by surface pumps:

$$\frac{\partial [Ca^{2+}]}{\partial n} + \frac{M}{D_{Ca}} \frac{[Ca^{2+}] - [Ca^{2+}]_{bgr}}{[Ca^{2+}] + K_P} = 0 \quad . \tag{5}$$

Here M is the maximal pump rate, K_P is the pump dissociation constant, and $[Ca^{2+}]_{bgr}$=0.1μM is the resting background $[Ca^{2+}]$. We use values of K_P=0.2 μM (Dipolo and Beauge, 1983; Carafoli, 1987) and M=0.01 μM μm ms^{-1}. At low $[Ca^{2+}]$, this yields a Ca^{2+} clearance time constant of $\tau \sim (1 + \kappa_0)\, V K_P / (M S) = 5$ sec, where V and S are the volume and the surface area of the bouton (Fig. 1), and κ_0=500 is the resting state endogenous buffering capacity at the crayfish NMJ This agrees with the experimental estimates of Tank et al. (1995).

Ca^{2+} binding and synaptic response. The $[Ca^{2+}]$ time course found by integrating equations 2-5 is used to drive the Ca^{2+}-dependent model of synaptic response. Our Ca^{2+} binding scheme is identical to the one used in Yamada and Zucker (1992), Tang et al. (2000), and Matveev et al. (2002). It assumes the existence of two Ca^{2+} binding sites, X and Y, characterized by fast and slow unbinding rates, respectively:

$$X \underset{k_X^{off}}{\overset{3Ca^{2+}k_X^{on}}{\rightleftharpoons}} CaX \underset{2k_X^{off}}{\overset{2Ca^{2+}k_X^{on}}{\rightleftharpoons}} Ca_2X \underset{3k_X^{off}}{\overset{Ca^{2+}k_X^{on}}{\rightleftharpoons}} Ca_3X \qquad (6)$$

$$Y \underset{k_Y^{off}}{\overset{Ca^{2+}k_Y^{on}}{\rightleftharpoons}} CaY \qquad (7)$$

The binding/unbinding rates are k_X^{on}=0.5 $ms^{-1}\mu M^{-1}$, k_X^{off}=50 ms^{-1}, $K_D^X = k_X^{off} / k_X^{on}$ =100 μM; k_Y^{on}=2.5·10^{-5} $ms^{-1}\mu M^{-1}$, k_Y^{off}=0.01 ms^{-1}; $K_D^Y = k_Y^{off} / k_Y^{on}$ =400 μM. Equations 6 and 7 are converted to ordinary differential equations using the law of mass action. The binding of both sites is necessary to trigger release, schematically described by

$$Ca_3X + CaY \xrightarrow{k_R} R \xrightarrow{k_I} I \qquad (8)$$

where R is the release state, and I is the inactivated state of the release machinery. This leads to the following differential equation for R, which determines the release rate:

$$\frac{dR}{dt} = k_R\, Ca_3X\; CaY - k_I R \quad . \qquad (9)$$

Contrary to the two-site residual free Ca^{2+} model of synaptic facilitation (Tang et al., 2000; Matveev et al., 2002), we assume that both the X and the Y sensor are colocalized

in space, at a distance of about 30 nm from the Ca^{2+} channel, and therefore the same $[Ca^{2+}]$ signal determines the forward rates of binding described by Eqs. 6 and 7.

Numeric simulations. All simulations were performed using the *CalC* ("Calcium Calculator") software developed by one of us (V.M.). *CalC* uses the Alternating-Direction Implicit finite-difference method to solve the buffered diffusion equations (Eqs. 2-4), with second order accuracy in spatial and temporal resolution. To preserve the accuracy of the method in the presence of the non-linear buffering term, equations for $[Ca^{2+}]$ and [B] are solved on separate time grids, shifted with respect to each other by half a time step. *CalC* uses an adaptive time-step method; the spatial grid is non-uniform, with greater density of points close to the Ca^{2+} channel array. Grid size is adjusted to limit the numerical error to about 5% (grid of 34 x 34 x 40 points). *CalC* uses the 4^{th} order adaptive Runge-Kutta method to solve all ordinary differential equations (Eqs. 6-9). *CalC* is freely available from http://web.njit.edu/~matveev/calc.html, and runs on all commonly used computational platforms (UNIX, including Mac OS X, and Windows/Intel). To ensure reproducibility of this work, the commented simulation script files generating the data reported here are available at the *CalC* web site.

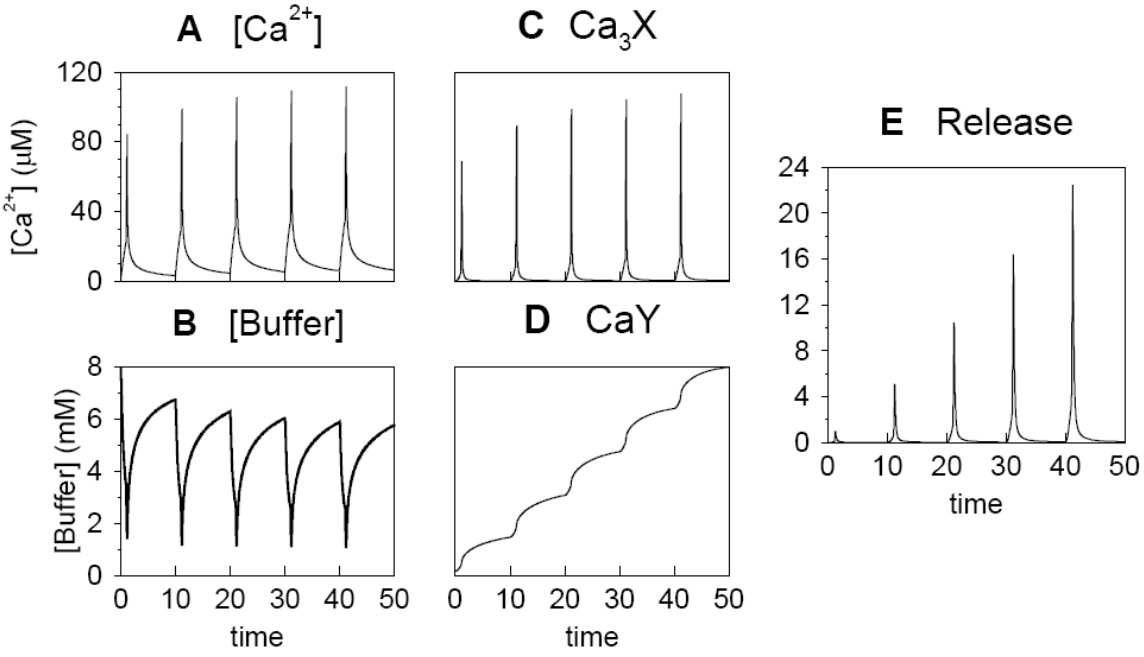
RESULTS

Response to a 5-pulse train of action potentials.

Figure 2 demonstrates the response of the synaptic model when it is driven by a train of five simulated action potentials (Ca^{2+} current pulses), delivered at 100 Hz. Facilitation of release shown in panel *E* results from the increase in the Ca^{2+} binding of the *X* and *Y* sites (Eqs. 6-7) with each action potential (Fig. 2C, D). It is evident that facilitation is caused primarily by the accumulation of CaY, the Ca^{2+}-bound Y site. This accumulation is due to the slow unbinding of Ca^{2+} from this site, so that binding that occurs during a stimulus is only partially removed before the next stimulus. There is also a small contribution coming from the increased occupancy of the *X* site, which is a fast binding site and therefore faithfully follows the $[Ca^{2+}]$ dynamics. Examination of the Ca^{2+} and buffer concentration time courses given in panels *A* and *B* reveals that this small *X*–site contribution arises from the accumulation of residual Ca^{2+} as well as a slight increase in Ca^{2+} transients associated with the local saturation of the Ca^{2+} buffer. Importantly, both

the magnitude and the supralinear (non-saturating) time course of facilitation accumulation are in good agreement with the experimental results of Tang et al. (2000), reproduced in Figure 3 *A* (*control* curve). Although the growth of facilitation in the model is not as rapid as in Fig. 3A, this was achieved with minimal parameter tuning, and no additional assumptions were required, contrary to the two-site mechanism (cf. Fig. 2 of Matveev et al., 2002). In particular, tortuosity (retardation of diffusion close to the membrane) was not included in this model, and the two Ca^{2+} binding sites are *not* spatially segregated, but located at the same distance (30 nm) from the nearest Ca^{2+} channel.

Figure 2. Response of the hybrid free/bound facilitation model to a 100 Hz train of five action potentials. Panels *A* and *B* show, respectively, the Ca^{2+} and buffer concentration time courses at the release site, which is located 30 nm away from the nearest Ca^{2+} channel (see Fig. 1). *C* and *D*: the binding fractions of the fast (*X*) and slow (*Y*) Ca^{2+} sensors. *E*: synaptic response normalized to its magnitude at the first pulse. Facilitation is caused primarily by the increase in the binding of the facilitation site (panel *D*), with some contribution of Ca^{2+} transient growth (panel *A*) due to buffer saturation.



Facilitation is reduced in the presence of exogenous Ca^{2+} buffers.

Our main goal is to explore the sensitivity of a model with a slow Ca^{2+} unbinding step to changes in intracellular buffering capacity. Experimental results of Tang et al. (2000) are shown in Fig. 3 *A* and suggest that facilitation is reduced about two-fold upon injection of 400 μM of the Ca^{2+} indicator dye Fura-2 into the crayfish NMJ. We mimic this experiment by repeating the simulations shown in Fig. 2 after including in our model (Eqs. 2-3) 400 μM of a Fura-2-like buffer. Simulation results presented in Figure 3 *B* show a reduction of facilitation to a degree comparable to that observed experimentally. This reduction is caused by the decrease in the free $[Ca^{2+}]$ in the vicinity of the X and Y binding sites (Fig. 3C), leading to the reduction in the amount of binding achieved during each action potential (Figs. 3D, E). Thus, even though additional buffers cannot accelerate the unbinding of Ca^{2+} from the Y site, the reduction in the free ambient $[Ca^{2+}]$ caused by the added Ca^{2+} buffers is sufficient to reduce the magnitude of synaptic facilitation, demonstrating the sensitivity of the model to free $[Ca^{2+}]$. Note also that adding Fura-2 reduces the local saturation of the immobile buffer, thereby removing the facilitation of Ca^{2+} transients (Fig. 3C), and completely removing the small contribution of the X-site to facilitation (Figs. 3*D*).

Although the variation of the two-site residual free Ca^{2+} model considered by Matveev et al. (2002) was also successful in reproducing the two-fold reduction of facilitation shown in Fig. 3 A, such agreement required an assumption of almost complete immobilization of the indicator dye in the entire presynaptic terminal. This is because a fast mobile buffer is extremely efficient in reducing the residual Ca^{2+} at a remote facilitation site, and would predict a much more dramatic effect of Fura-2 on facilitation than seen experimentally. In contrast, the model we present here does not impose any constraints on the diffusion of the Fura-2, which is assumed to have a diffusion coefficient of D_{F2}=118 $\mu m^2/s$ (Gabso et al., 1997). Further, the two-site model also required an assumption of significant tortuosity retarding Ca^{2+} diffusion in a 200 nm-side layer proximal to the membrane, in order to achieve a non-saturating facilitation accumulation time course. Again, this assumption is not necessary in the present model, where the superlinearilty is caused by the accumulation of both free and bound Ca^{2+}.

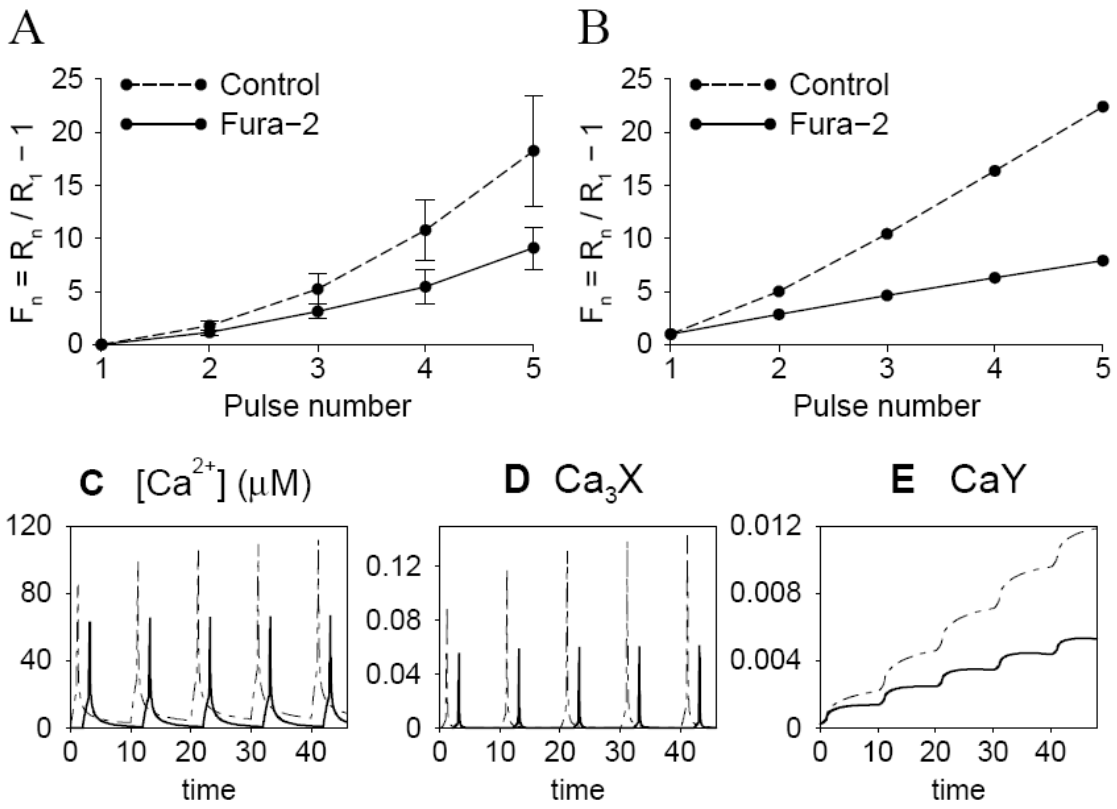


Figure 3. Effect of exogenous buffer application on synaptic response to a five-pulse stimulation train. (*A*) Experimentally measured response time course in the crayfish NMJ, with and without 400 μM Fura-2. Reproduced, with permission, from Tang et al. (2000), (see Fig. 2 *C* therein); (*B*) Simulation results: model response evoked by each of the five action potentials, R_n, normalized to the first response, R_1, with and without the simulated addition of 400μM Fura-2. Bottom panels show (*C*) [Ca^{2+}] time course, (*D*) *X*-binding site, and (*E*) *Y*-binding site occupancy time courses, with (*solid lines*) and without Fura-2 (*dashed lines*; same as in Fig. 2 *A*, *C*, *E*). Fura-2 results in panels *C-E* are shifted to the right by 2 ms for easier comparison with the control curves. The properties of the buffer simulating Fura-2 (K_D=360 nM, D_{F2}=118 μm^2 sec^{-1}, $[\text{Fura-2}]_{total}$ = 400 μM, unbinding rate = 96.7 sec^{-1}) are the same as in Tang et al. (2000).

Ca^{2+} buffering rapidly reduces facilitated response.

A crucial experiment testing the sensitivity of neurotransmitter release to free Ca^{2+} was performed by Kamiya and Zucker (1994), who measured the effect of a rapid

increase in intracellular buffering capacity on facilitated synaptic response, using flash photolysis of the caged Ca^{2+} buffer diazo-2. The UV flash increases the Ca^{2+} affinity of diazo-2, resulting in a rapid sequestering of free Ca^{2+}. When the UV flash was applied after a facilitating train of pulses, the synaptic response to a test pulse administered 10 ms after the flash was reduced dramatically as compared to the control no-flash condition (Fig. 4 *A*). This appears to suggest that the facilitation of neurotransmitter release is rapidly reduced when the free Ca^{2+} is buffered. Figure 4 *B* demonstrates that our hybrid model reproduces these experimental observations. As shown in Figure 4 *C-E*, diazo-2 rapidly buffers the free Ca^{2+}, reducing binding to both the *X* and the *Y* gates. The *X* binding site has rapid Ca^{2+} binding kinetics and is sensitive to the instantaneous concentration of free Ca^{2+}. When Ca^{2+} is buffered (Fig. 4 *C*), binding to the *X* site is reduced during the test pulse (Fig. 4 *D*). Binding of the *Y* site is reduced as well (Fig. 4E), but most of this reduction occurs *after* the first test pulse, due to the slow kinetics of this site (note the difference in time scales between panels *C-D* and *E*). In fact, the binding of the *Y* sensor is reduced by only 4% at the peak of the first test pulse, as compared to a 33% reduction in the binding of the *X* site (Fig. 4 *D*), and the overall reduction of response of 37% (Fig. 4 B).

Thus, in our model diazo-2 has two effects: it reduces the unfacilitated, baseline synaptic response, which is primarily controlled by the *X* site, and it also decreases facilitation by reducing the binding of the *Y* site. Both in the experiment and in our model, diazo-2 does not completely eliminate facilitation during the 10 ms-long interval between the UV flash and the first test pulse. In the model, this is due to the slow unbinding of the *Y* gate. In fact, the decay of facilitation lags significantly behind the decay of residual free Ca^{2+} (cf. Fig. 4 *C* and *E*), in agreement with the results of Kamiya and Zucker (see Fig. 4 therein), and consistent with experiments of Atluri and Regehr (1996) .

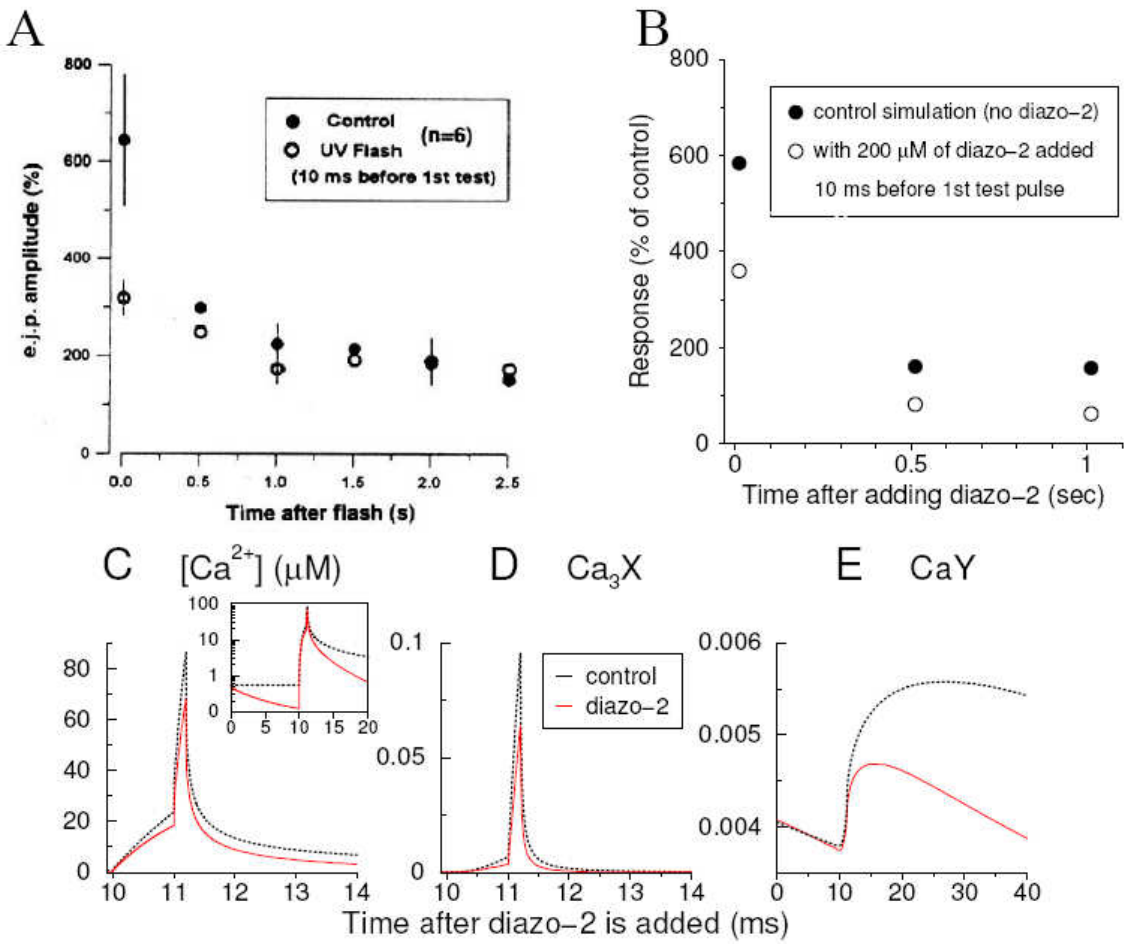


Figure 4. Synaptic response is reduced rapidly upon photolysis of a caged Ca^{2+} buffer diazo-2. (*A*) Experimental results of Kamiya and Zucker (1994), showing the amplitudes of synaptic responses to several test pulses administered at different times following a conditioning train of ten action potentials. The first test pulse is applied 10 ms after a UV flash which increases the Ca^{2+} affinity of the diazo-2 buffer, sequestering Ca^{2+}. (*B*) Simulation of the experiment shown in *A*, showing the model response to test pulses administered 200 ms after a conditioning train of five action potentials applied at 100 Hz. The UV flash is simulated by adding to our model 200 µM of diazo-2-like buffer with affinity of 150 nM, at 10 ms before the 1st test pulse. Lower panels illustrate the effect of adding diazo-2 on the model's response to the first test pulse, and show the time courses of (*C*) Ca^{2+} concentration, (*D*) binding of the secretory *X*-site, and (*E*) binding of the facilitatory *Y*-site. In each of these panels, the black dotted lines indicate the control simulation, while solid red lines correspond to the diazo-2 simulation. The inset in *C* plots $[Ca^{2+}]$ time course using a logarithmic scale, to demonstrate more clearly the buffering effect of diazo-2. Note the difference in time scales among panels.

DISCUSSION

We have shown that a model of synaptic response that relies on a slow Ca^{2+} unbinding step to explain synaptic facilitation is consistent with the properties of facilitation observed at the crayfish neuromuscular junction. Both the magnitude and the accumulation time course are successfully reproduced, and as demonstrated in Figs. 3 and 4, the model agrees with the observed reduction of facilitation upon addition of fast exogenous Ca^{2+} buffers. In particular, the model response is decreased within milliseconds upon adding 200 μM of diazo-2-like buffer, consistent with experiments of Kamiya and Zucker (1992). The model indicates that introducing a fast exogenous buffer into a presynaptic terminal should affect not only the Ca^{2+}-dependent facilitation, but also the baseline, unfacilitated neurotransmitter release. Our model captures both of these effects, predicting the reduction of binding of both the secretory *X* site, as well as the slow-unbinding *Y* gate that is responsible for facilitation. This is because both of these sensors are assumed to be located at a small diffusional distance from the nearest Ca^{2+} channel, so rapid buffering will reduce the number of free Ca^{2+} ions reaching these sites, leading to reduced binding, regardless of their kinetic properties.

Thus, we believe that facilitation due to slow Ca^{2+} unbinding is consistent with available data from the crayfish NMJ, and may apply to other synapses as well. More specifically, the decay time course of facilitation may not be determined solely by the dynamics of intracellular Ca^{2+} diffusion and clearance, but may depend on the intrinsic dynamics of the facilitation mechanism itself. This in fact is suggested by the experimentally observed discrepancy between the time scales of the decay of facilitation and the time scale of the concomitant decrease in intracellular [Ca^{2+}] (Kamiya and Zucker, 1992; Atluri and Regehr, 1996). One experimental approach to test the contribution of a slow Ca^{2+} unbinding step to synaptic facilitation was used by Felmy et al. (2003), who showed that the Ca^{2+}-affinity of neurotransmitter release is not changed during induction of facilitation at the calyx of Held, ruling out the contribution of bound Ca^{2+} to facilitation in that preparation. We note however that a reduction in Ca^{2+} affinity that is correlated with the growth of facilitation was observed in the squid giant synapse

(Stanley, 1986). This suggests that bound Ca^{2+} may contribute to facilitation in some, but not all, synaptic terminals.

It is important to compare the present model and the previously proposed two-site mechanism of synaptic facilitation (Tang et al., 2000, and Matveev et al., 2002). Both models can successfully reproduce the magnitude and the time course of facilitation recorded in the crayfish inhibitor, as well as the reduction of facilitation by fast Ca^{2+} buffers. However, the two-site model assumes that two spatially segregated Ca^{2+}-sensitive release gates control synaptic response, separated by distances of at least 150 nm. Further, in order to explain significant residual facilitation upon application of Ca^{2+} indicator dye Fura-2, the two-site model requires an assumption of strong immobilization of Fura-2 by the cytoskeleton (Matveev et al., 2002). In contrast, the proposed hybrid model assumes that the facilitation gate is colocalized with the Ca^{2+} binding site controlling phasic release, and that mobile exogenous buffers remain mobile upon injection into the terminal.

To conclude, we have demonstrated the viability of the residual bound Ca^{2+} hypothesis originally proposed by Katz and Miledi (1968) and Rahamimoff (1968), and have shown that it may potentially explain facilitation at the crayfish NMJ. We do not believe however that accumulation of bound Ca^{2+} represents the primary mechanism of facilitation at all facilitatory synaptic terminals. Recent studies indicate the existence of several distinct facilitation mechanisms that are differentially expressed in distinct cell types and which may undergo differential regulation during development. For example, saturation of the endogenous buffer calbindin has been implicated in facilitation at calbindin-positive but not calbindin-negative mammalian nerve terminals (Blatow et al., 2003), while the expression of calbindin is known to change during development (Alcantara et al., 1996). Furthermore, experimental evidence suggests that in some synapses facilitation involves a combination of mechanisms. For instance, both buffer saturation and accumulation of residual Ca^{2+} were implicated in facilitation at the calyx of Held (Felmy et al., 2003), and at facilitatory calbindin-positive neocortical and hippocampal synapses (Blatow et al., 2003). We believe that at some synaptic terminals, such as the crayfish NMJ and the squid giant synapse, facilitation is due largely to residual bound Ca^{2+}. The questions of the relative roles of free versus bound residual Ca^{2+}

accumulation requires a study of the dynamics of Ca^{2+} affinity of neurotransmitter release during induction of facilitation (Stanley et al., 1986; Felmy et al, 2003).

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