Facilitation through Buffer Saturation: Constraints on Endogenous Buffering Properties

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ABSTRACT Synaptic facilitation (SF) is a ubiquitous form of short-term plasticity, regulating synaptic dynamics on fast timescales. Although SF is known to depend on the presynaptic accumulation of Ca²⁺, its precise mechanism is still under debate. Recently it has been shown that at certain central synapses SF results at least in part from the progressive saturation of an endogenous Ca²⁺ buffer (Blatow et al., 2003), as proposed by Klingauf and Neher (1997). Using computer simulations, we study the magnitude of SF that can be achieved by a buffer saturation mechanism (BSM), and explore its dependence on the endogenous buffering properties. We find that a high SF magnitude can be obtained either by a global saturation of a highly mobile buffer in the entire presynaptic terminal, or a local saturation of a completely immobilized buffer. A characteristic feature of BSM in both cases is that SF magnitude depends nonmonotonically on the buffer concentration. In agreement with results of Blatow et al. (2003), we find that SF grows with increasing distance from the Ca²⁺ channel cluster, and increases with increasing external Ca²⁺, [Ca²⁺]₉₉, for small levels of [Ca²⁺]₉₉. We compare our modeling results with the experimental properties of SF at the crayfish neuromuscular junction, and find that the saturation of an endogenous mobile buffer can explain the observed SF magnitude and its supralinear accumulation time course. However, we show that the BSM predicts slowing of the SF decay rate in the presence of exogenous Ca²⁺ buffers, contrary to experimental observations at the crayfish neuromuscular junction. Further modeling and data are required to resolve this aspect of the BSM.

INTRODUCTION

Short-term synaptic facilitation (SF) is the transient increase of synaptic response that can be elicited by a single action potential (AP) or a short train of APs, and decays on timescales from tens to hundreds of milliseconds. It is observed in a variety of systems, from invertebrate neuromuscular junctions to neocortical synapses (reviewed in Magleby, 1987; Fisher et al., 1997; Zucker, 1994 and 1999; Zucker and Regehr, 2002). The pioneering work of Katz and Miledi (1968) and Rahamimoff (1968) showed that SF depends on the extracellular Ca²⁺. However, there is still no agreement on the exact nature of the SF mechanism. Arguably, the simplest explanation is that SF results from the accumulation of free residual Ca²⁺ ([Ca²⁺]₉₉) in the presynaptic terminal. This possibility is supported by the extensive evidence that SF is sensitive to manipulations reducing the free intracellular Ca²⁺ (reviewed in Zucker and Regehr, 2002). However, early modeling work (Chad and Eckert, 1984; Simon and Llinàs, 1985; Fogelson and Zucker, 1985; Roberts, 1994) demonstrated that the Ca²⁺ concentration in the vicinity of an open Ca²⁺ channel ‘domain’ can reach very high values, from tens to hundreds of µM. This is much higher than the residual [Ca²⁺] increase caused by a single AP, believed to range from 10 nM to 1 µM (see reviews by Stanley, 1997; Neher, 1998b; Zucker, 1996 and 1999; and Zucker and Regehr, 2002). If the Ca²⁺-dependent release machinery is located in close proximity to a Ca²⁺ channel, as suggested by the evidence of molecular interactions between the proteins mediating exocytosis and the Ca²⁺ channel proteins (reviewed in Stanley, 1997; Sheng et al., 1998; Catterall, 1999; Fisher and Bourque, 2001; Jarvis and Zamponi, 2001), it is hard to argue that such a small increase in free Ca²⁺ would lead to significant SF. Even taking into account several experimental indications that the Ca²⁺ affinity of the secretory site may be in the 5–20-µM range rather than the 100-µM range (Delaney and Tank, 1994; Ravin et al., 1999; Bollmann et al., 2000; Schneggenburger and Neher, 2000; Ohnuma et al., 2001), it would not be sufficient to explain the high magnitude of SF observed in many systems. One way to resolve this problem is to assume the presence of a separate high-affinity Ca²⁺-sensitive site responsible for SF, distinct from the main secretory trigger, and located far enough from a Ca²⁺ channel, where the residual [Ca²⁺] is comparable to the peak [Ca²⁺] (Tang et al., 2000). We have recently shown that the properties of SF observed at the crayfish neuromuscular junction (NMJ) can be explained by such a model with two Ca²⁺-dependent release-controlling sites, given a sufficient separation between the two sites (~150 nm), and under the additional assumptions of high tortuosity near the active zone and immobilization of Ca²⁺ buffers by the cytoskeleton (Matveev et al., 2002).

An alternative solution is the bound residual Ca²⁺ hypothesis, which postulates that SF results from the buildup of Ca²⁺ bound to the vesicle release sensors (Yamada and Zucker, 1992; Bertram et al., 1996; see also Bennett et al.,
Endogenous Ca\textsuperscript{2+} buffers play a crucial role in both the residual free Ca\textsuperscript{2+} model and the BSM.

Yamada and Zucker (1992), Bertram et al. (1996); see also Regehr et al. (1994), Atluri and Regehr (1996), Bennett et al. (1997), Dittman et al. (2000).

Tang et al. (2000), Matveev et al. (2002).

Klingauf and Neher (1997), Neher (1998a,b), Maeda et al. (1999), Blatow et al. (2003); see also Bennett et al. (2000), Rozov et al. (2001), Felmy et al. (2003), Jackson and Redman (2003), Trommershäuser et al. (2003).
Ultrastructural reconstruction of the crayfish NMJ has shown that the vesicles are located around the edge of the Ca\textsuperscript{2+} channel cluster (Cooper et al., 1996; Atwood et al., 1997). Therefore, we are interested in the Ca\textsuperscript{2+} channels that are not resolved in the current study. Ignoring the point-like nature of Ca\textsuperscript{2+} influx is justified because we are not interested in an accurate description of the Ca\textsuperscript{2+} concentration profile in the microdomain of a single channel. As will be shown in Results, sufficient facilitation magnitude is achieved at distances of at least 40–60 nm away from the edge of the cluster, which is larger than the average separation between neighboring Ca\textsuperscript{2+} channels. We expect the effect of the Ca\textsuperscript{2+} influx localization to be small at these distances, as confirmed by comparing our results with modified simulations in which Ca\textsuperscript{2+} influx was localized, with half entering exactly at the center and the rest entering uniformly along the edge of the AZ (i.e., the circumference of the 160-nm disk). We found that the two simulations yielded nearly identical results. Thus, we are interested in what has been previously termed the “submembrane” Ca\textsuperscript{2+} domain (Klingauf and Neher, 1997; Bennett et al., 2000), or an AZ macrodomain. The uniformity assumption for the current distribution may also be viewed as averaging over varying positions of Ca\textsuperscript{2+} channels at individual AZs.

**Equations describing buffered diffusion of Ca\textsuperscript{2+}**

For simplicity, in most of our simulations we assume the presence of a single dominant Ca\textsuperscript{2+} buffer, with the Ca\textsuperscript{2+} binding reaction described by

\[
B + Ca^{2+} \rightleftharpoons CaB,
\]

where \(k_{on}\) and \(k_{off}\) are, respectively, the binding and the unbinding rates of the Ca\textsuperscript{2+}-buffer compound. This leads to the following reaction-diffusion equations for the Ca\textsuperscript{2+} concentration, \([Ca^{2+}]\), and the concentration of the free (unbound) buffer, \([B]\):

\[
\frac{\partial [Ca^{2+}]}{\partial t} = D_{Ca} \nabla^2 [Ca^{2+}] - k_{on} [Ca^{2+}] [B] + k_{off} (B_{total} - [B]) + \frac{1}{2F} I_{Ca} (t) g(\theta) \delta (r - r_b)
\]

\[
\frac{\partial [B]}{\partial t} = D_B \nabla^2 [B] - k_{on} [Ca^{2+}] [B] + k_{off} (B_{total} - [B]).
\]

Here \(D_{Ca}\) and \(D_B\) are the diffusion coefficients in cytosol of the buffer and Ca\textsuperscript{2+}, respectively. We choose \(D_{Ca} = 0.2 \mu m^2 \text{ms}^{-1}\) (Allbritton et al., 1992). Following the 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 a Ca\textsuperscript{2+} ion. 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, \(B_{total}\). This allows one to eliminate the equation for the evolution of the concentration of the bound buffer, \([CaB]\), from \([B]\) + \([Ca^{2+}][B]\) in Eq. 2. Equations 2 and 3 are extended in a straightforward way when simulations include more than one buffer. In this case, each of the buffers evolves according to an equation identical to Eq. 3, and Eq. 2 is expanded to include the binding and the unbinding terms for each of the buffers present (e.g., see Smith et al., 2001).

The last term in Eq. 2 represents the Ca\textsuperscript{2+} influx, where \(F\) is Faraday’s constant, \(I_{Ca}(t)\) is the (inward) calcium current per active zone, and \(\delta (r - r_b)\) is the Dirac delta function, with \(r_b = 1.5 \mu m\) equal to the bouton radius (this describes a Ca\textsuperscript{2+} current flowing through the surface membrane). The surface density function \(g(\theta)\) determines the distribution of the Ca\textsuperscript{2+} current over the AZ surface; it is assumed to be uniform across disk surface area 160 nm in diameter (\(S_{ca}\)), and is zero everywhere else, as illustrated in Fig. 1 (see justification above). The integral of \(g(\theta)\) over the \(S_{ca}\) area should be equal to one, from which we obtain

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**FIGURE 1** Model of a presynaptic crayfish motor bouton. The crayfish motor terminal is approximately represented as a sphere 3 \(\mu m\) in diameter (bottom), with active zones (AZ) distributed evenly over the spherical surface. Ca\textsuperscript{2+} diffusion and buffering are only simulated in the conical region of the sphere surrounding a single AZ. The ring of vesicles around the model AZ is shown for illustration only, and is not included in the simulation. Current density \(g(\theta)\) (see Eqs. 2 and 4) is assumed to be uniformly distributed over a disk surface area 160 nm in diameter (top). Asterisks mark the locations at which the Ca\textsuperscript{2+} concentration is sampled during the simulations. These sites represent locations of the putative Ca\textsuperscript{2+} “sensors” (possible vesicle locations). We record Ca\textsuperscript{2+} concentration 20 nm below the membrane surface, and at lateral distances of 20, 60, and 100 nm away from the edge of the Ca\textsuperscript{2+} current influx (corresponding sites are marked 1–3).
\[
g(\theta) = 1/S_{Ca} = 1/(2\pi a^2 (1 - \cos \alpha)) \sim 1/(\pi r^2 a^2)
\]
for \(\theta \leq \alpha\)
\[
g(\theta) = 0 \quad \text{for} \quad \theta > \alpha,
\]
where \(\alpha\) is the angle corresponding to the edge of the Ca\textsuperscript{2+} influx area: \(\alpha = 80\ \text{nm} / r_0 = 0.0533\ \text{rad}\) (Fig. 1), and \(r_0\) for reactants with diffusion coefficients of 0.2 m\textsuperscript{2}\text{m}\textsuperscript{-1}\text{s}\text{-1}.

The measurements of Ca\textsuperscript{2+} constant. We use values of a fixed Biophysical Journal 86(5) 2691–2709

Matveev et al., 2000), and is within the range of \(I_{Ca} = 10–50\ \text{pA}\) inferred from the measurements of [Ca\textsuperscript{2+}] rise rate during sustained stimulation (Tank et al., 1995; both values are translated to a 1-ms-long current pulse). In Results, we discuss the behavior of the model for variable magnitude of \(I_{Ca}\).

Reflective boundary conditions hold for both [Ca\textsuperscript{2+}] and \([B]\) at all boundaries, except for the boundary condition for [Ca\textsuperscript{2+}] on the top surface of the cone, which assumes Ca\textsuperscript{2+} extrusion by surface pumps:
\[
\frac{\partial [\text{Ca}^{2+}]}{\partial r} + \frac{M}{D_{Ca}} \frac{[\text{Ca}^{2+}]}{[B]} + K_P = 0.
\]

Here \(M\) is the maximal pump rate, and \(K_P\) is the pump dissociation constant. We use values of \(K_P = 0.2\ \mu\text{M}\) (Dipolo and Beaute, 1983; Carafoli, 1987) and \(M = 0.01\ \mu\text{M}\ \text{ms}^{-1}\) (except for Fig. 12, A and B). At low [Ca\textsuperscript{2+}], this yields a Ca\textsuperscript{2+} clearance time constant of \(\tau \sim (1 + K_0) V_KD (M/S) = 5\ \text{s}\), where \(V\) and \(S\) are the volume and the surface area of the bouton (Fig. 1), and \(K_0 = 500\) is the resting state endogenous buffering capacity at the crayfish NMJ (Tank et al., 1995). This agrees with the experimental value of Ca\textsuperscript{2+} clearance rate obtained by Tank et al. (1995). In Results, we will discuss the possibility of higher Ca\textsuperscript{2+} extrusion rates. The resting buffering capacity \(K_0\) (also known as the endogenous buffering ratio or the binding ratio) is an important characteristic of the buffering strength, defined as (Irving et al., 1990; Neher and Augustine, 1992)
\[
K_0 = \frac{\partial [\text{CaB}]}{\partial [\text{Ca}^{2+}]} \big|_{\text{rest}} = \frac{B_\text{rest}}{K_D + Ca_\text{rest}} = \frac{B_\text{total}K_D}{(K_D + Ca_\text{rest})^2}.
\]

The relevant free parameters in the model are summarized in Table 2. Instead of the pair of values of \(k_{\text{off}}\) and \(k_{\text{on}}\) for each other, so in the fixed \(K_0\) case the value of \(k_{\text{on}}\) (\(k_{\text{off}}\)) characterizes the buffer-Ca\textsuperscript{2+} equilibrium kinetics, not just the binding (unbinding) rate. The upper bound of 1 \(\mu\text{M}^2\text{ms}^{-1}\) imposed on the magnitude of \(k_{\text{on}}\) is close to the theoretical diffusion limit on the maximal achievable forward binding rate for reactants with diffusion coefficients of 0.2 \(\mu\text{m}^2\text{ms}^{-1}\) (Keizer, 1987).

To further simplify the analysis, we assume that the resting Ca\textsuperscript{2+} concentration, \(Ca_\text{rest}\), is zero. In this case the expression for the buffering capacity (Eq. 6) reduces to \(K_0 = B_\text{total}/K_D\). However, all our results can be straightforwardly translated to the case of nonzero \(Ca_\text{rest}\), as described in the Appendix. In effect, we subtract out \(Ca_\text{rest}\) from the total Ca\textsuperscript{2+} concentration and subtract the initial bound buffer concentration from \(B_\text{total}\), because these are constant background values that do not affect the dynamics of Ca\textsuperscript{2+} diffusion. Therefore, everywhere in this paper the value of \(B_\text{total}\) should be understood as the initial (resting) total concentration of free buffer, \(B_\text{rest}\).

**Numerical simulations**

The reaction-diffusion Eqs. 2–33 were solved using the Calcium Calculator (CaC) software developed by one of us (V.M.). CaC uses an alternating-direction implicit finite-difference method, second order accurate in spatial and temporal resolution (Morton and Mayers, 1994). To preserve the accuracy of the method in the presence of the buffering term, the equations for [Ca\textsuperscript{2+}] and \([B]\) are solved on separate time grids, shifted with respect to each other by half a time step (Hines, 1984). CaC uses an adaptive time-step method; the spatial grid size is adjusted to limit the error tolerance to at most 1–2% (grid of 36 \(\times\) 36 points in the mobile buffer case, and 70 \(\times\) 70 in the fixed buffer case). CaC is freely available from http://web.njit.edu/~matveev, and runs on Linux, SGI, and Windows/Intel platforms. To ensure reproducibility of this work, the commented simulation script files generating the data reported here will be made available on the above web site.

**RESULTS**

**Facilitation of Ca\textsuperscript{2+} transients: an example**

Fig. 2 simulates the buffer saturation mechanism of SF, showing the facilitation of Ca\textsuperscript{2+} transients (FCT) in response to a train of five equal Ca\textsuperscript{2+} current steps. This growth in simulated peak [Ca\textsuperscript{2+}] transients is caused by the residual Ca\textsuperscript{2+} accumulating from pulse to pulse and gradually binding and depleting the free buffer, thereby reducing more and more the ability of the buffer to shunt the AP-triggered Ca\textsuperscript{2+} transients. Note that the free [Ca\textsuperscript{2+}]\text{res} is negligibly low (see the inset), because most of the residual Ca\textsuperscript{2+} is in fact bound to the buffer, due to a high buffering ratio in our example (\(K_0 = B_\text{total}/K_D = 1000\)). In this and most of the following figures, concentration is shown at a distance of 60 nm from the edge of the Ca\textsuperscript{2+} influx region (site 2 in Fig. 1); dependence on distance will be analyzed later on (see Fig. 6).

The buffer included in the simulation is very mobile, with the diffusion coefficient of \(D_B = 0.2\ \mu\text{m}^2\text{ms}^{-1}\), which is close to the putative mobility of ATP, for example (Naraghi and Neher, 1997). As will be shown below, strong mobility is a crucial requirement for the BSM. Because of high mobility and fast binding, the Ca\textsuperscript{2+} and the buffer are in equilibrium with each other and evenly distributed in space by the end of each 10-ms-long interpulse interval (data not shown). Therefore, the increase in the fifth Ca\textsuperscript{2+} transient, \(P_5\), over the first Ca\textsuperscript{2+} transient, \(P_1\), is completely determined by the reduction in the amount of free buffer remaining before the last pulse, denoted \(\Delta B = B_\text{total} – B_{R}\). Even at moderate values of the buffering capacity (\(K_0 > 10\)), almost all of the incoming Ca\textsuperscript{2+} becomes buffer-bound, so that \(\Delta B\) is approximately equal to the total (volume-averaged) Ca\textsuperscript{2+} influx, and therefore is solely determined by the magnitude of \(I_{Ca}\).

**TABLE 2 Free model parameters**

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Parameter name</th>
<th>Range of values</th>
</tr>
</thead>
<tbody>
<tr>
<td>(B_{\text{total}})</td>
<td>Total free buffer concentration</td>
<td>0–30 mM</td>
</tr>
<tr>
<td>(k_{\text{on}})</td>
<td>Buffer-Ca\textsuperscript{2+} binding rate</td>
<td>0.001–1 (\mu\text{M}^{-1}\text{ms}^{-1})</td>
</tr>
<tr>
<td>(k_{\text{off}})</td>
<td>Buffer-Ca\textsuperscript{2+} unbinding rate</td>
<td>0.01–50 ms\textsuperscript{-1}</td>
</tr>
<tr>
<td>(D_B)</td>
<td>Buffer diffusion coefficient</td>
<td>0–0.2 (\mu\text{m}^2\text{ms}^{-1})</td>
</tr>
<tr>
<td>(I_{Ca})</td>
<td>Calcium current per active zone</td>
<td>1–50 pA</td>
</tr>
<tr>
<td>(d)</td>
<td>Distance from the channel to the SF site</td>
<td>20–100 nm</td>
</tr>
</tbody>
</table>
the fifth pulse; as discussed above, this requires moderately high values of \( k_0 (k_0 > 10) \), and high \( I_{Ca} \). ii), for this reduced buffer availability to make a difference, the buffer should be close to saturation during the last pulse, as well: the free buffer remaining at the end of the last pulse, \( B_P \), should be small. In other words, initially the buffer should be effective in limiting the rise in \( Ca^{2+} \), but at the end of the train, \( Ca^{2+} \) transients should be able to strongly saturate the buffer. This condition is easier to satisfy for low \( B_{total} \) and fast \( Ca^{2+} \)-buffer binding (high \( k_{on} \)).

Notice that one requirement in the two groups coincides (large \( k_{on} \)), whereas the conditions on \( B_{total} \) and \( I_{Ca} \) are in contradiction. Therefore, one may expect the FCT to grow monotonically with increasing \( k_{on} \), but its behavior with respect to \( B_{total} \) and \( I_{Ca} \) may be nonmonotonic. The results presented below confirm this conclusion.

**FCT peaks at a finite value of \( B_{total} \) and grows monotonically with increasing \( k_{on} \)**

Fig. 3 shows the dependence of the five-pulse FCT, defined according to Eq. 7, as a function of \( K_D \) and \( B_{total} \), the first two model parameters in Table 2. In this figure the magnitude of the unbinding rate \( k_{off} \) is kept fixed (\( k_{off} = 0.4 \text{ ms}^{-1} \) for panel A, \( k_{off} = 0.1 \text{ ms}^{-1} \) for panel B), so the value of \( k_{on} \) varies along with the value of \( K_D \), according to the relation \( k_{on} = k_{off}/K_D \). The similarity between FCT values in Fig. 3, A and B, demonstrates that FCT is only weakly dependent on \( k_{off} \) and is predominantly determined by the values of \( k_{on} \) and \( B_{total} \) (note that the range of values of \( k_{off} \) indicated along the right margin, are equivalent in the two panels). However, the lower the value of \( k_{off} \), the lower should be \( K_D \) to achieve a given FCT magnitude.

In agreement with the conditions formulated in the previous subsection, Fig. 3 shows that FCT grows monotonically with increasing \( k_{on} \) but depends nonmonotonically on the value of \( B_{total} \). For any given value of \( k_{on} \) \((K_D) \), maximal FCT is achieved at a certain finite value of \( B_{total} \) which we will denote by \( B^*_{total} \) (gray curve in Fig. 3). Explaining this behavior, Fig. 4 shows the \( Ca^{2+} \) and buffer concentration time courses for three values of \( B_{total} \) that correspond to the + labels in Fig. 3 A. The middle + marks the optimal value of \( B^*_{total} \) \((450 \mu M) \) for \( K_D = 0.5 \mu M \) (Fig. 4 B), corresponding to the maximal FCT magnitude, whereas the two flanking values of \( B_{total} \) yield smaller values of facilitation. For \( B_{total} < B^*_{total} \) (Fig. 4 A, \( B_{total} = 200 \mu M \)), the buffer is completely saturated during the \( Ca^{2+} \) current pulses; even though \( \Delta p \) is very large in this case, the first \( Ca^{2+} \) transient is large as well, resulting in smaller FCT compared to its value for the optimal \( B^*_{total} \). In contrast, when \( B_{total} > B^*_{total} \) (Fig. 4 C, \( B_{total} = 1 \text{ mM} \)), the buffer does not saturate to a sufficient extent, so even though the value of \( P_1 \) is much reduced compared to Fig. 4, A and B, the magnitude of \( \Delta p \) is quite small, leading again to a smaller value of FCT than in the optimal \((B_{total} = B^*_{total}) \) case.

**The conditions for high FCT**

We will quantify FCT by the ratio of the amplitudes of the fifth and the first peaks in \( Ca^{2+} \) concentration:

\[
FCT = P_5/P_1 = 1 + \Delta P/P_1. \tag{7}
\]

To achieve significant FCT, one needs a large increase in the \( Ca^{2+} \) transient magnitude (\( \Delta P \)), and a comparatively small value of the first \( Ca^{2+} \) transient, \( P_1 \) (see Fig. 2). These two requirements lead to two different sets of constraints on model parameters:

1. The requirements for small \( P_1 \) are straightforward and include all conditions that increase the buffering efficiency: i), large \( B_{total} \); ii), fast binding (large \( k_{on} \)); iii), high buffer mobility, \( D_B \); iv), small \( I_{Ca} \).
2. The conditions for large \( \Delta P \) are twofold: i), significant buffer saturation/depletion \( \Delta B \) should be achieved before

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**FIGURE 2** Demonstration of the facilitation of \( Ca^{2+} \) transients caused by buffer saturation. \( Ca^{2+} \) (middle panel) and buffer (bottom panel) concentration time courses produced in response to a 5-pulse train of 1-ms-long \( Ca^{2+} \) current pulses (top panel), as measured at a distance of 60 nm from the edge of the \( Ca^{2+} \) channel cluster (site 2 in Fig. 1). The growth in the \( Ca^{2+} \) transient between the fifth and the first \( Ca^{2+} \) pulses, \( \Delta P \), depends on the degree of buffer saturation achieved right before (\( \Delta B \)) and during (\( B_r \)) the final pulse. See text for details. Parameters are \( B_{total} = 500 \mu M \), \( K_D = 0.5 \mu M \), \( k_{on} = 0.8 \mu M^{-1} \text{ ms}^{-1} \), \( D_B = 0.2 \mu m^2 \text{ ms}^{-1} \), and \( I_{Ca} = 11.7 \text{ pA} \).
As the above analysis suggests, the optimal buffer concentration $B_{\text{total}}$ should depend on the number of pulses in the stimulation train. One should expect $B_{\text{total}}^*$ to increase with increasing train length, because more buffer is required to produce the same saturation effect for a greater total calcium influx, which is proportional to the number of pulses. Our simulations confirm this conclusion, and show a decrease in $B_{\text{total}}^*$ of ~10% when the number of pulses is decreased from five to three (data not shown).

**FCT for a fixed value of buffering ratio**

Even though the values of most of the model parameters summarized in Table 2 are not readily available from experiment, the rest-state endogenous buffering capacity, $\kappa_0$ (see Eq. 6), can be estimated experimentally using fluorescent dye techniques (Neher and Augustine, 1992). For zero $C_{\text{rest}}$, $\kappa_0$ is given simply by the ratio $B_{\text{total}}/K_D$. We have chosen a value of 500, used in Tang et al. (2000) and Matveev et al. (2002), and close to the estimate of 600 inferred using the fluorescent Ca$^{2+}$ sensitive dye experiments at the crayfish NMJ by Tank et al. (1995). In Fig. 3, the set of points corresponding to $\kappa_0 = 500$ lie along the diagonal lines.

Constraining the value of $\kappa_0$ allows us to eliminate one of the parameters from the pair \{$K_D$, $B_{\text{total}}$\}. That is, having restricted ourselves to a constant value of $\kappa_0$ (the diagonal in Fig. 3), in Fig. 5 we can explore the dependence of FCT on an independent variation of $k_{\text{on}}$ and $B_{\text{total}}$ (the latter now also determines $K_D$, because $B_{\text{total}} = \kappa_0 K_D$). In the new parameter space of Fig. 5, the locus of points corresponding to any given value of $k_{\text{off}}$ is a hyperbolic curve. As an example, Fig. 5 A shows the two curves of points that correspond to $k_{\text{off}} = 0.4$ ms$^{-1}$ and $k_{\text{off}} = 0.1$ ms$^{-1}$. These two hyperbolae are the images of the fixed-$\kappa_0$ diagonal lines in Fig. 3, A and B, respectively. Thus, Fig. 5 A compresses all the fixed-$\kappa_0$ information contained in a whole family of figures like Fig. 3, A and B. Moving vertically from one hyperbolic curve to the other in Fig. 5, we see that faster buffers would give greater FCT. Finally, the FCT peak in Fig. 5 A shows that the maximal magnitude of FCT is still achieved at a certain finite value of $B_{\text{total}} (= 500 K_D)$, even under the fixed $\kappa_0$ constraint.

Interestingly, we find that the dependence of FCT on $k_{\text{on}}$ and $B_{\text{total}}$ is qualitatively as well as quantitatively similar at different values of $\kappa_0$. This follows from our earlier result that FCT is primarily determined by the values of $B_{\text{total}}$ and $k_{\text{on}}$ (Fig. 3). From the relationship $\kappa_0 = B_{\text{total}}/K_D = (B_{\text{total}}/k_{\text{on}})/k_{\text{off}}$ it is clear that increasing $k_{\text{off}}$, which leaves FCT almost unchanged (Fig. 3), is equivalent to a decrease in $\kappa_0$. Therefore, lowering the value of $\kappa_0$, say to $\kappa_0 = 50$ (a 10-fold change), and simultaneously increasing $k_{\text{off}}$ 10-fold (10-fold decrease in $K_D$), results only in a moderate reduction of the FCT magnitude (cf. Fig. 5, A and B).

**Dependence on $I_{\text{Ca}}$ is redundant**

Apart from the value of $K_D$, the value of $I_{\text{Ca}}$ per action potential per AZ can also be estimated using fluorescent Ca$^{2+}$ indicator dye measurements. However, the accuracy of...
the Ca\(^{2+}\) influx measurements is not high, because it depends on the optical calibration of the fluorescent Ca\(^{2+}\)-sensitive dye used in the measurements, on the estimates of its concentration, and on the independent estimate of the endogenous buffering capacity, \(K_D\). Further, one may expect significant variability in \(I_{Ca}\) between different AZs, as well as between different NMJ boutons. Therefore, it is important to consider how the variation in the value of \(I_{Ca}\) would affect FCT.

As it turns out, the dependence of FCT on the magnitude of Ca\(^{2+}\) influx per AP is redundant, because knowing the dependence of FCT on \(K_D\) and \(B_{total}\), for a given value of \(k_{off}\) (Figs. 3 and 5), gives full information about its behavior with respect to \(I_{Ca}\). This is a consequence of the freedom in choosing the units of concentration. The FCT magnitude should not be affected if the three parameters with units of concentration, \(K_D\), \(B_{total}\), and \(I_{Ca}\) (current equals concentration times volume per unit time), are rescaled by the same factor. Thus, if the FCT magnitude is calculated for a given value of the Ca\(^{2+}\) current, \(I_{Ca}\), then at any other value of the Ca\(^{2+}\) current, \(I_{Ca}'\), it is given by

\[
FCT(I_{Ca}, B_{total}, K_D; k_{off}) = FCT(I_{Ca}', a B_{total}, a K_D; k_{off}),
\]

where \(a = I_{Ca}/I_{Ca}'\). It follows then that changing the value of the Ca\(^{2+}\) current is equivalent to sliding along the line of constant \(B_{total}/K_D\) ratio (Figs. 3 and 5).

We have to emphasize that the above redundancy condition only holds for the FCT, and not the facilitation of neurotransmitter release. To obtain an expression similar to Eq. 8 for SF proper, given a particular choice of a Ca\(^{2+}\)-dependent exocytosis scheme, the affinity of the release mechanism to Ca\(^{2+}\) would have to be scaled along with \(I_{Ca}\), \(K_D\), and \(B_{total}\) (i.e., it would have to be included in the list of arguments that are multiplied by factor \(a\) on the right-hand side of Eq. 8). However, we assume a simple power-law relationship between release and [Ca\(^{2+}\)], thus avoiding this complication (see “Relationship between FCT and facilitation of release”). A similar caveat concerns the case of nonzero resting free [Ca\(^{2+}\)] value, \(Ca_{rest}\); in general, \(Ca_{rest}\) would have to be included in the argument list on the left- and right-hand sides of Eq. 8. However, the procedure of subtracting out the \(Ca_{rest}\) value, described in the Appendix, preserves the scaling freedom expressed by Eq. 8 in the case of nonzero values of \(Ca_{rest}\) (the arguments in Eq. 8 are to be understood as the “primed” zero-\(Ca_{rest}\) values: see Appendix).

Finally, we note that the Ca\(^{2+}\) pump dissociation constant \(K_P\) (Eq. 5) constitutes yet another concentration parameter, however it mostly affects the decay time course of SF (to be discussed below), and has only moderate influence on the FCT magnitude for short-duration stimulation trains considered here.

**Relationship between FCT and facilitation of release**

To compare our simulation results with the magnitude of SF available from experiment, we have to convert the FCT values into a measure characterizing facilitation of neurotransmitter release. We prefer not to constrain ourselves to a particular choice of a Ca\(^{2+}\)-dependent release model, because doing so would require introduction of additional parameters into our problem. Instead, we will assume that the synaptic response is given by the fourth power of [Ca\(^{2+}\)]. Then, SF is likewise given by the fourth power of FCT:

\[
SF = Facilitation_{Release} = (FCT)^4.
\]

Note that this equation neglects the inevitable saturation of synaptic response, which would occur at sufficiently high
levels of $[\text{Ca}^{2+}]$. Further, the cooperativity value of 4 lies at the high end of experimental range obtained for the crayfish NMJ (Landô and Zucker, 1994; Wright et al., 1996; Ravin et al., 1999; Vyshedskiy and Lin, 2000). Therefore, all facilitation estimates in this paper are to be understood as upper bounds on physiologically attainable facilitation.

Based on experimental observations, we require a five-pulse SF magnitude of $\sim 18–20$ (see Figs. 1 C and 2 C of Tang et al., 2000). This corresponds to FCT of $P_5/P_1 \sim 2.1$ (because $2.1^4 \sim 19.4$). Therefore, in the contour plots of Figs. 3 and 5, the parameter region yielding physiological magnitude of SF lies within the area bounded by the contour of $FCT = 2.0$.

### FCT grows with increasing distance between Ca$^{2+}$ channel cluster and release site

There is substantial evidence for a close association between the vesicle release machinery and the presynaptic Ca$^{2+}$ channels, suggesting their mutual proximity at many synapses (for reviews, see Stanley, 1997; Sheng et al., 1998; Catterall, 1999; Fisher and Bourque, 2001; Jarvis and Zamponi, 2001). At the crayfish NMJ, morphological studies suggest that vesicles are clustered along the edge of the AZ (Cooper et al., 1996; Atwood et al., 1997; Atwood and Karunanithi, 2002). However, at certain synapses, like the calyx of Held, vesicles may be scattered at various distances from the Ca$^{2+}$ channel cluster (Meinrenken et al., 2002; Sätzler et al., 2002). Further, recent experiments on the sensitivity of phasic response to an application of a fast exogenous buffer (1,2-bis(2-aminophenoxy)ethane-N,N,N’,N’-tetraacetic acid (BAPTA)) suggest that there is a significant separation between Ca$^{2+}$ channels and secretion triggers at several strongly facilitating, but not at depressing, neocortical and hippocampal synapses (Rozov et al., 2001; Blatow et al., 2003). For any synapse type, it is possible that various obstacles to diffusion (such as vesicles themselves) and the tortuosity effects associated with the dense cytoskeletal and protein mesh within the AZ may significantly increase the effective diffusional distance between the Ca$^{2+}$ channel and the release-triggering Ca$^{2+}$ sensor. Therefore, we simulated the Ca$^{2+}$ transients at various distances from the Ca$^{2+}$ influx region. Fig. 6 A demonstrates that the magnitude of FCT is increased by an increase in the channel-sensor separation. This result is easy to understand, because the buffer is able to bind more Ca$^{2+}$ when it is acting over a longer diffusional distance (see review by Neher, 1998b), and thereby is more effective in reducing the first response, $P_1$, leading to higher FCT. Sufficient SF is achieved at distances of at least 40–60 nm from the Ca$^{2+}$ channel cluster.

### Facilitation growth is supralinear

As discussed above, Figs. 5 and 6 A demonstrate that the physiological magnitude of five-pulse SF can be achieved within a certain range of values of $k_{on}$ and $B_{total}$ ($K_D$). However, we also need to establish whether BSM can reproduce the supralinear accumulation time course of SF observed at the crayfish NMJ (Figs. 1 C and 2 C of Tang et al., 2000; Fig. 2 C of Winslow et al., 1994). Traces in Fig. 6 B show that the simulated growth of FCT for a chosen parameter point (marked by an asterisk in Fig. 6 A) is indeed supralinear, at all distances from the Ca$^{2+}$ channel cluster, and therefore facilitation of release (Eq. 9) grows supralinearly as well, in agreement with experiment. Fig. 7 A demonstrates the supralinear time course of SF corresponding to the [Ca$^{2+}$]
trace shown Fig. 6Bb, yielding a physiological SF magnitude. In fact, we find that, for a mobile buffer, the parameter region yielding high facilitation coincides with the region of high supralinearity (data not shown). Therefore, BSM can successfully reproduce the SF magnitude and its accumulation time course observed experimentally at the crayfish NMJ.

Effect of exogenous buffers on facilitation growth

The experimental observations of the reduction of SF by exogenously applied buffers were crucial for establishing the role of residual Ca\(^{2+}\) in SF (see review by Zucker and Regehr, 2002), and a successful SF model should reproduce this property. Fig. 7A shows the simulated effect of application of 200 \(\mu\)M of Fura-2, a fast and high-affinity mobile buffer, on SF magnitude, for a set of parameter values marked by an asterisk in Fig. 6Ab. These simulation results agree very well with the experimental results of Tang et al. (2000) (Fig. 7B). However, due to the nonmonotonic dependence of FCT on \(B_{\text{total}}\), adding a mobile high-affinity buffer may actually increase FCT, and hence SF, under certain conditions. Such paradoxical effect of exogenous buffers on SF has been termed “pseudofacilitation” (Neher, 1998a; Rozov et al., 2001; Blatow et al., 2003; see also Discussion).

FCT at lower buffer mobility

Until now we have assumed that the Ca\(^{2+}\) buffer is highly mobile, with a diffusion coefficient comparable to that of ATP, BAPTA, or EGTA (Naraghi and Neher, 1997). There are several studies that agree with such high mobility of endogenous buffers, for instance the experiments at goldfish retinal bipolar cells (Burron et al., 2002), Purkinje cells (Maeda et al., 1999), and frog saccular hair cells (Roberts, 1993). However, endogenous buffers were found to be immobile or poorly mobile in many other preparations, including the calyx of Held (Helmsen et al., 1997), neocortical, and hippocampal pyramidal cells (Helmsen et al., 1996; Ohana and Sakmann, 1998; Lee et al., 2000), axons of Aplysia neurons (Gabso et al., 1997), and bovine adrenal chromaffin cells (Neher and Augustine, 1992; Zhou and Neher, 1993; Xu et al., 1997). At the crayfish NMJ, the mobility of endogenous Ca\(^{2+}\) buffers is not known. To study the dependence of the BSM on buffer mobility, we repeated our simulations for variable values of \(B_{\text{total}}\) and \(D_B\), the only parameter in Table 2 yet to be varied, for fixed values of \(k_0\) and \(k_{\text{on}}\) (\(k_0 = 500\), \(k_{\text{on}} = 0.8\ \mu\text{M}^{-1}\ \text{ms}^{-1}\)). Fig. 8A shows that FCT declines dramatically as \(D_B\) is decreased, which agrees with the conditions for high FCT that were formulated in the section entitled “Facilitation of Ca\(^{2+}\) transients: an...
Ca$^{2+}$ sensor, strongly decreasing the first few responses, and thereby leading to a high FCT magnitude. This regime is most pronounced when the buffer is completely immobile; as Fig. 8 B shows, FCT rapidly decreases even for small nonzero values of $D_B$. Fig. 9 A illustrates the behavior of FCT for $D_B = 0$, as a function of $k_{on}$ and $B_{total} (= 500 K_D)$, at different distances from the Ca$^{2+}$ channel cluster (compare with the mobile buffer case, Fig. 6). FCT is seen to be relatively weakly dependent on $k_{on}$, for sufficiently high values of $k_{on}$. This is because the effective diffusional distance from the point of influx to the Ca$^{2+}$ sensor is significantly increased in the presence of a fixed buffer, assuring that the Ca$^{2+}$ ions will undergo multiple binding-unbinding steps for a wide range of $k_{on}$ values. For this reason, FCT is sensitive to the buffer affinity, $K_D$, and not to $k_{on}$, as in the mobile buffer case. Therefore, FCT is also sensitive to the value of buffering capacity, $\kappa_0$. We find that large $\kappa_0$ is required to achieve high FCT in the fixed buffer case (data not shown).

The [Ca$^{2+}$] traces shown in Fig. 9 B demonstrate that the free [Ca$^{2+}$]$_{res}$ makes a more significant contribution to Ca$^{2+}$ peaks, as compared to the case of a mobile buffer (cf. Fig. 6 B). In fact, we find that the regions of high FCT and high free [Ca$^{2+}$]$_{res}$ significantly overlap (data not shown). When [Ca$^{2+}$]$_{res}$ is nonnegligible, Eq. 9 should be modified to include its contribution to facilitation:

\[
\text{Facilitation}_\text{Release} = ((P_5 + R_4)/P_1)^4
\]

\[
= (\text{FCT} + R_4/P_1)^4,
\]

where $R_4$ equals [Ca$^{2+}$]$_{res}$ achieved at the end of the fourth interpulse interval (see Fig. 9 Bb). The $R_4/P_1$ ratio should remain much smaller than one, because the delayed asynchronous release 10 ms after the fourth pulse is known to be much smaller than the peak synaptic response achieved during the first pulse. The contribution of [Ca$^{2+}$]$_{res}$ increases with increasing $B_{total} (K_D)$, and with increasing distance from the Ca$^{2+}$ channel cluster. In fact, for sufficiently high $B_{total}$ values and at large distances, the fast Ca$^{2+}$ transient becomes completely shunted by the buffer, and the peak in Ca$^{2+}$ concentration is significantly delayed with respect to the end of the pulse (in Fig. 9 Bc, the delay is 1.5 ms). Under these conditions the BSM would predict a physiologically unrealistic latency between the end of the Ca$^{2+}$ influx and the peak of neurotransmitter release. At a given distance, there is only a narrow range of $B_{total}$ Values where the magnitude of facilitation (Eq. 10) reaches experimentally observed levels, whereas the free [Ca$^{2+}$]$_{res}$ contribution remains small ($R_4/P_1 \leq 0.2$). For a distance of 60 nm (Fig. 9 Ab), the corresponding range is $\sim$10–12 mM, approaching unphysiologically high concentrations.

Fig. 10 demonstrates the spatial distribution of the Ca$^{2+}$ ions and the buffer at different times after the cessation of an AP, for a parameter point marked with a + in Fig. 9 A. Contrary to the mobile buffer case, it takes tens of

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**FIGURE 7** Facilitation grows supralinearly in the mobile buffer case. Comparison of (A) simulation results with (B) experimental data from Fig. 3 C of Tang et al. (2000), showing SF for a five-pulse 100-Hz train, with and without Fura-2. In A, simulations are performed for parameter values corresponding to the point marked by an asterisk in Fig. 6 Ab (distance = 60 nm, $B_{total} = 700 \mu M$, $K_D = 1.4 \mu M$, $k_m = 0.5 \mu M^{-1} ms^{-1}$, $D_B = 0.2 \mu m^2 ms^{-1}$, $IC_{50} = 11.7 pA$). Facilitation is normalized to zero for the first peak of neurotransmitter release. At a given distance, there is a realistic latency between the end of the Ca$^{2+}$ influx before it reaches the sensor, strongly decreasing the first few responses, and thereby leading to a high FCT magnitude. This regime is most pronounced when the buffer is completely immobile; as Fig. 8 B shows, FCT rapidly decreases even for small nonzero values of $D_B$. Fig. 9 A illustrates the behavior of FCT for $D_B = 0$, as a function of $k_{on}$ and $B_{total} (= 500 K_D)$, at different distances from the Ca$^{2+}$ channel cluster (compare with the mobile buffer case, Fig. 6). FCT is seen to be relatively weakly dependent on $k_{on}$, for sufficiently high values of $k_{on}$. This is because the effective diffusional distance from the point of influx to the Ca$^{2+}$ sensor is significantly increased in the presence of a fixed buffer, assuring that the Ca$^{2+}$ ions will undergo multiple binding-unbinding steps for a wide range of $k_{on}$ values. For this reason, FCT is sensitive to the buffer affinity, $K_D$, and not to $k_{on}$, as in the mobile buffer case. Therefore, FCT is also sensitive to the value of buffering capacity, $\kappa_0$. We find that large $\kappa_0$ is required to achieve high FCT in the fixed buffer case (data not shown).

The [Ca$^{2+}$] traces shown in Fig. 9 B demonstrate that the free [Ca$^{2+}$]$_{res}$ makes a more significant contribution to Ca$^{2+}$ peaks, as compared to the case of a mobile buffer (cf. Fig. 6 B). In fact, we find that the regions of high FCT and high free [Ca$^{2+}$]$_{res}$ significantly overlap (data not shown). When [Ca$^{2+}$]$_{res}$ is nonnegligible, Eq. 9 should be modified to include its contribution to facilitation:

\[
\text{Facilitation}_\text{Release} = ((P_5 + R_4)/P_1)^4
\]

\[
= (\text{FCT} + R_4/P_1)^4,
\]

where $R_4$ equals [Ca$^{2+}$]$_{res}$ achieved at the end of the fourth interpulse interval (see Fig. 9 Bb). The $R_4/P_1$ ratio should remain much smaller than one, because the delayed asynchronous release 10 ms after the fourth pulse is known to be much smaller than the peak synaptic response achieved during the first pulse. The contribution of [Ca$^{2+}$]$_{res}$ increases with increasing $B_{total} (K_D)$, and with increasing distance from the Ca$^{2+}$ channel cluster. In fact, for sufficiently high $B_{total}$ values and at large distances, the fast Ca$^{2+}$ transient becomes completely shunted by the buffer, and the peak in Ca$^{2+}$ concentration is significantly delayed with respect to the end of the pulse (in Fig. 9 Bc, the delay is 1.5 ms). Under these conditions the BSM would predict a physiologically unrealistic latency between the end of the Ca$^{2+}$ influx and the peak of neurotransmitter release. At a given distance, there is only a narrow range of $B_{total}$ Values where the magnitude of facilitation (Eq. 10) reaches experimentally observed levels, whereas the free [Ca$^{2+}$]$_{res}$ contribution remains small ($R_4/P_1 \leq 0.2$). For a distance of 60 nm (Fig. 9 Ab), the corresponding range is $\sim$10–12 mM, approaching unphysiologically high concentrations.

Fig. 10 demonstrates the spatial distribution of the Ca$^{2+}$ ions and the buffer at different times after the cessation of an AP, for a parameter point marked with a + in Fig. 9 A. Contrary to the mobile buffer case, it takes tens of
milliseconds rather than a fraction of one millisecond for the concentrations to reequilibrate in space, so the free $[Ca^{2+}]_{res}$ elevation and the buffer saturation region are still significantly localized by the end of a 10-ms-long interpulse interval. This illustrates that it is a local rather than global buffer saturation that underlies FCT in the fixed buffer case.

Further, we find that the accumulation time course of SF is sublinear for a fixed buffer. Fig. 9Bb shows the $[Ca^{2+}]$ trace, and Fig. 11 depicts the corresponding SF growth time course at a distance of 60 nm from the edge of the $Ca^{2+}$ influx region, for a parameter point marked with a + in Fig. 9Ab.

In fact, we find that the SF accumulation time course remains sublinear for any choice of parameter values (data not shown).

### Facilitation decay time course and the effect of Fura-2 on facilitation decay

Above we have shown that high buffer mobility is important for the BSM to explain the experimentally observed supralinear accumulation time course of SF. For a highly mobile buffer, the $Ca^{2+}$ concentration rapidly reequilibrates...
throughout the terminal upon termination of the Ca\(^{2+}\) influx caused by an AP. Therefore, the buffer has to become saturated globally (i.e., in the entire presynaptic bouton) to cause significant FCT. The decay time course of facilitation will therefore be determined by the extrusion of Ca\(^{2+}\) by the plasmalemma transport proteins, with possible contribution of uptake into internal Ca\(^{2+}\) stores. Because we were interested in establishing an upper bound on maximal SF magnitude that can be achieved by the BSM, we made a conservative assumption about the Ca\(^{2+}\) extrusion rate in our simulations, choosing a clearance time estimate of 5 s, based on the crayfish NMJ measurements of Tank et al. (1995). This is much longer than the time scales of tens and hundreds of milliseconds that characterize the two components of SF decay observed at this synapse. The above estimate of Ca\(^{2+}\) clearance rate was obtained by tracking [Ca\(^{2+}\)] for tens of seconds after cessation of stimulation, when Ca\(^{2+}\) is low and the main mode of extrusion is through ATP-driven pumps. It is likely that immediately after stimulation the clearance proceeds at a faster rate, in particular due to activation of a lower-affinity but higher-capacity Na\(^{+}\)/Ca\(^{2+}\) exchanger, or some other low-affinity Ca\(^{2+}\) removal mechanism (e.g., a slow low-affinity buffer). Indeed, the decay of Ca\(^{2+}\) transients induced by AP trains was recently measured at the crayfish NMJ by Vyshedskiy and Lin (2000) on a finer time scale, revealing a biphasic decay with time constants of 84 ± 30 and 390 ± 153 ms. These time constants are comparable to the F1 (18 ± 5 ms) and F2 (530 ± 200 ms) components of facilitation decay at the crayfish NMJ (Zucker, 1974; Vyshedskiy and Lin, 1997; Tang et al., 2000). Note that due to the nonlinear dependence of transmitter release on Ca\(^{2+}\), the SF decay times are expected to be somewhat shorter than the corresponding Ca\(^{2+}\) clearance time constants. Therefore, within the BSM framework, the decay time course of SF could be explained by the fast extrusion of Ca\(^{2+}\) from the terminal.

To allow for a faster decay of Ca transients, we have increased the pump dissociation constant from \(K_p = 0.2 \mu M\) to \(K_p = 5 \mu M\), which is comparable to the affinity of the Na\(^+\)/Ca\(^{2+}\) exchanger (Blaustein and Lederer, 1999; Carafoli, 1987; DiPolo and Beauge, 1983), and increased the maximal extrusion rate from \(M = 0.01 \mu M \mu M \text{ ms}^{-1}\) to \(M = 5 \mu M \mu M \text{ ms}^{-1}\) (see Eq. 5). Fig. 12 A shows the resulting SF decay time course, with and without additional Fura-2. Note that our simulations predict a slowing of SF decay in the presence of an exogenous buffer such as Fura-2, contrary to experimental observations (cf. Fig. 12 C). The slower decay is explained by the fact that the Ca\(^{2+}\) clearance time is directly proportional to the total intraterminal buffering capacity (Neher and Augustine, 1992), which is approximately doubled after addition of 200 μM of Fura-2. We verified that this effect is maintained if we assume that the

\[\frac{\text{Control simulation}}{\text{Fura-2 simulation}}\]

![FIGURE 11](image)

**FIGURE 11** Facilitation grows sublinearly in the fixed buffer case. Shown is the simulated SF in response to a five-pulse 100-Hz AP train, arising from the saturation of a fixed buffer, with and without Fura-2. Note the sublinear accumulation time course, contrary to the mobile buffer case and the experimental data (cf. Fig. 7). Parameter values correspond to a point marked by + in Fig. 9 Ab (\(B_{\text{ex}} = 11 \text{ mM}\), \(K_B = 22 \mu M\), \(k_{\text{in}} = 0.2 \mu M^{-1} \text{ ms}^{-1}\); \(d = 60 \text{ nm}\)). Facilitation is normalized to zero for the first pulse. Fura-2 parameters are the same as in Fig. 7 A. The [Ca\(^{2+}\)] trace corresponding to the control simulation curve is shown in Fig. 9 Bb. The residual free Ca\(^{2+}\) contributes ~12% to the fifth [Ca\(^{2+}\)] peak in the control simulation, and ~7% in the Fura-2 simulation.

Throughout the terminal upon termination of the Ca\(^{2+}\) influx caused by an AP, therefore, the buffer has to become saturated globally (i.e., in the entire presynaptic bouton) to cause significant FCT. The decay time course of facilitation will therefore be determined by the extrusion of Ca\(^{2+}\) by the plasmalemma transport proteins, with possible contribution of uptake into internal Ca\(^{2+}\) stores. Because we were interested in establishing an upper bound on maximal SF magnitude that can be achieved by the BSM, we made a conservative assumption about the Ca\(^{2+}\) extrusion rate in our simulations, choosing a clearance time estimate of 5 s, based on the crayfish NMJ measurements of Tank et al. (1995). This is much longer than the time scales of tens and hundreds of milliseconds that characterize the two components of SF decay observed at this synapse. The above estimate of Ca\(^{2+}\) clearance rate was obtained by tracking [Ca\(^{2+}\)] for tens of seconds after cessation of stimulation, when Ca\(^{2+}\) is low and the main mode of extrusion is through ATP-driven pumps. It is likely that immediately after stimulation the clearance proceeds at a faster rate, in particular due to activation of a lower-affinity but higher-capacity Na\(^+\)/Ca\(^{2+}\) exchanger, or some other low-affinity Ca\(^{2+}\) removal mechanism (e.g., a slow low-affinity buffer). Indeed, the decay of Ca\(^{2+}\) transients induced by AP trains was recently measured at the crayfish NMJ by Vyshedskiy and Lin (2000) on a finer time scale, revealing a biphasic decay with time constants of 84 ± 30 and 390 ± 153 ms. These time constants are comparable to the F1 (18 ± 5 ms) and F2 (530 ± 200 ms) components of facilitation decay at the crayfish NMJ (Zucker, 1974; Vyshedskiy and Lin, 1997; Tang et al., 2000). Note that due to the nonlinear dependence of transmitter release on Ca\(^{2+}\), the SF decay times are expected to be somewhat shorter than the corresponding Ca\(^{2+}\) clearance time constants. Therefore, within the BSM framework, the decay time course of SF could be explained by the fast extrusion of Ca\(^{2+}\) from the terminal.

To allow for a faster decay of Ca transients, we have increased the pump dissociation constant from \(K_p = 0.2 \mu M\) to \(K_p = 5 \mu M\), which is comparable to the affinity of the Na\(^+\)/Ca\(^{2+}\) exchanger (Blaustein and Lederer, 1999; Carafoli, 1987; DiPolo and Beauge, 1983), and increased the maximal extrusion rate from \(M = 0.01 \mu M \mu M \text{ ms}^{-1}\) to \(M = 5 \mu M \mu M \text{ ms}^{-1}\) (see Eq. 5). Fig. 12 A shows the resulting SF decay time course, with and without additional Fura-2. Note that our simulations predict a slowing of SF decay in the presence of an exogenous buffer such as Fura-2, contrary to experimental observations (cf. Fig. 12 C). The slower decay is explained by the fact that the Ca\(^{2+}\) clearance time is directly proportional to the total intraterminal buffering capacity (Neher and Augustine, 1992), which is approximately doubled after addition of 200 μM of Fura-2. We verified that this effect is maintained if we assume that the

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**FIGURE 11** Facilitation grows sublinearly in the fixed buffer case. Shown is the simulated SF in response to a five-pulse 100-Hz AP train, arising from the saturation of a fixed buffer, with and without Fura-2. Note the sublinear accumulation time course, contrary to the mobile buffer case and the experimental data (cf. Fig. 7). Parameter values correspond to a point marked by + in Fig. 9 Ab (\(B_{\text{ex}} = 11 \text{ mM}\), \(K_B = 22 \mu M\), \(k_{\text{in}} = 0.2 \mu M^{-1} \text{ ms}^{-1}\); \(d = 60 \text{ nm}\)). Facilitation is normalized to zero for the first pulse. Fura-2 parameters are the same as in Fig. 7 A. The [Ca\(^{2+}\)] trace corresponding to the control simulation curve is shown in Fig. 9 Bb. The residual free Ca\(^{2+}\) contributes ~12% to the fifth [Ca\(^{2+}\)] peak in the control simulation, and ~7% in the Fura-2 simulation.
Fura-2 is immobilized (Matveev et al., 2002), and in simulations where decay of Ca\(^{2+}\) transients is caused by a slow endogenous buffer (data not shown).

One may suggest therefore that the observed decay properties of SF are more consistent with a model relying on the saturation of a fixed buffer; as discussed above, it is a local rather than global saturation that causes FCT when buffers are fixed. Indeed, Figs. 10 and 9B demonstrate that in the fixed buffer case the Ca\(^{2+}\) transients contain a gradually decaying (\(\tau \sim 10\) ms) component caused by the slow spatial reequilibration of Ca\(^{2+}\), which is absent in the case of a mobile buffer (cf. Fig. 6B). As we have shown previously (Matveev et al., 2002), this local free [Ca\(^{2+}\)] is responsible for SF in the two-site model of Tang et al. (2000). In the BSM framework, it partially contributes to SF by adding to the Ca\(^{2+}\) transients (Fig. 9Bb; Eq. 10).

Therefore, in the presence of fixed buffers the SF decay is expected to contain a fast (F1-like) phase due to the spatial reequilibration of Ca\(^{2+}\), which should be strongly reduced in the presence of a mobile high-affinity Ca\(^{2+}\) buffer such as Fura-2, in agreement with experiment (Fig. 12B). However, the second decay phase is slowed down in our simulations, as in the mobile buffer case (cf. Fig. 12A). Thus, the model in its present form cannot successfully explain the experimentally observed effect of exogenous buffers on the decay time of SF, shown in Fig. 12C (but see the Discussion below).

**FCT in the presence of two buffers**

In this work we have concentrated on the case whereby a single dominant endogenous buffer is shaping the intracellular Ca\(^{2+}\) dynamics. We believe that a thorough investigation and understanding of the single-buffer situation is necessary before considering more complicated scenarios. However, one cannot exclude the possibility that several endogenous buffers make a commensurate contribution to the overall buffering capacity at the crayfish NMJ, as well as at other synapses. In particular, one may propose that the immobile or poorly mobile buffer with low capacity (\(k_0; 40–50\)) found in different cell types (references cited in Results) represents the basal buffer present in all synapses, whereas only a subset of cell types possess an additional mobile high-capacity buffer (e.g., calbindin; Nägerl et al., 2000). Although the data on the properties of the putative immobile low-capacity buffer is lacking, evidence from chromaffin cell studies suggest that it may have low affinity (Xu et al., 1997). Therefore, to analyze this possibility we performed simulations in which a mobile buffer with varying \(B_{\text{total}}\) and \(k_{\text{on}}\) was added to a fixed concentration of a low-affinity immobile buffer with \(B_{\text{total}} = 750\) \(\mu\)M, \(K_D = 15\) \(\mu\)M (\(k_0 = 50\)), and \(k_{\text{on}} = 0.1\) \(\mu\)M\(^{-1}\) ms\(^{-1}\) (Fig. 13). Our results show that the FCT is reduced when the low-affinity buffer is introduced, and that the size of this reduction depends on the relative capacities of the

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**FIGURE 12** Experimental and simulated effect of Fura-2 application on the decay time course of SF. Shown is the SF magnitude for the last pulse in a 100-Hz five-pulse train, for a variable time interval between the fourth and the fifth pulses, \(\Delta t_{4-5}\), for (A) mobile buffer simulation, (B) fixed buffer simulation, and (C) experimental data from Fig. 3B of Tang et al. (2000). SF is computed as in Fig. 7. Parameter values in A are the same as in Fig. 7A, except for the faster Ca\(^{2+}\) extrusion (see text), leading to a reduction of SF at \(\Delta t_{4-5} = 10\) ms from 19-fold to 14-fold. Parameter values in B are the same as in Fig. 11. The biexponential fits in A are given by 9.4 exp\((-\Delta t/57\) ms\) + 5.3 exp\((-\Delta t/202\) ms\) (●), and 3.7 exp\((-\Delta t/111\) ms\) + 3.3 exp\((-\Delta t/416\) ms\) (○). In B, fits are 41 exp\((-\Delta t/7.9\) ms\) + 7.1 exp\((-\Delta t/44\) ms\) + 0.29 exp\((-\Delta t/6330\) ms\) (●), and 21.7 exp\((-\Delta t/7.1\) ms\) + 1.16 (○).
two buffers. Namely, the maximal achievable FCT is more significantly reduced when the immobile and mobile buffers have equal capacities (Fig. 13 B), whereas the FCT reduction is less dramatic when the capacity of the mobile buffer is significantly higher than the capacity of the low-affinity fixed buffer (Fig. 13 A). In general, we could not find conditions under which a two-buffer system would yield a higher FCT magnitude as compared to a single buffer system (data not shown). This puts an additional emphasis on the point that the FCT magnitude that we demonstrate is the maximal bound on the FCT that can be achieved under physiological conditions.

**DISCUSSION**

Using a computer model of a crayfish motor bouton, we have explored in detail the phenomenon of facilitation of Ca\(^{2+}\) transients resulting from the saturation of an endogenous Ca\(^{2+}\) buffer. We have given an exhaustive characterization of the dependence of FCT on the endogenous buffering parameters (Table 2), and have described the conditions necessary to obtain a sufficiently high SF magnitude, as summarized below. The requirement of significant distance between the Ca\(^{2+}\) source and the secretion site; our findings on the nonmonotonic dependence of FCT on buffer concentration; and the increase of FCT with increasing [Ca\(^{2+}\)\(_\text{extr} \)] are all consistent with the experimental results of Blatow et al. (2003).

Further, we compare our simulation results with the properties of SF observed at the crayfish NMJ by Tang et al. (2000). The crayfish NMJ is particularly well suited for the study of SF, because it displays pronounced SF under physiological conditions, and the increase of synaptic response is not occluded by concomitant synaptic depression, which is the case in most other systems (reviewed by Zucker and Regehr, 2002).

**FCT and buffer mobility**

As we have shown, buffer mobility is a crucial factor in the BSM. Curiously, we found that to achieve a physiologically relevant SF magnitude, the buffer has to be either highly mobile or highly immobilized, with much smaller FCT achieved for intermediate values of mobility (Fig. 8 B). This is because the mechanism by which buffer saturation causes facilitation is different in the high- versus low-mobility regimes. A highly mobile buffer efficiently carries Ca\(^{2+}\) ions away from the AZ, strongly shunting the response to the first pulses in a stimulation train, while becoming gradually saturated in the entire bouton (Fig. 2). This results in large FCT. A fixed buffer causes facilitation by saturating locally, rather than globally, which traps Ca\(^{2+}\) ions in an area surrounding the AZ (Fig. 10; Nowycky and Pinter, 1993; Neher, 1998a). To absorb a significant fraction of the Ca\(^{2+}\) influx locally, the buffer has to be completely immobile, and its concentration has to be much higher than in the mobile buffer case (Fig. 5). The magnitude of this reduction is more dramatic when the capacities of the two buffers are comparable (cf. panel B and Fig. 5 B).

**FIGURE 13** Dependence of FCT on the properties of a mobile buffer, in the presence of an immobile low-affinity buffer with fixed characteristics. Five-pulse FCT is shown as a function of \(k_{\text{on}}\) and \(B_{\text{total}}\) of the mobile buffer, for mobile buffering capacity of (A) \(k_0 = 500\) and (B) \(k_0 = 50\). The buffering capacity of the immobile low-affinity buffer is \(k_{0}^{F} = 50\), in both panels. The properties of the immobile buffer are fixed: \(B_{\text{total}} = 750 \mu M, K_D = 15 \mu M,\) and \(k_{\text{on}} = 0.1 \mu M^{-1} ms^{-1}\). Other parameters are the same as in Figs. 2–4 (\(D_0 = 0.2 \mu m^2 ms^{-1}; I_{\text{CA}} = 11.7 pA, d = 60 nm\)). Note that introducing an immobile low-affinity buffer reduces FCT, as compared to the single-buffer case (Fig. 5). The magnitude of this reduction is more dramatic when the capacities of the two buffers are comparable (cf. panel B and Fig. 5 B).
the end of the Ca$_{2}^{+}$ influx (Fig. 9Bc), because diffusion is slowed by the fixed buffer, and the trapped Ca$_{2}^{+}$ ions undergo multiple binding/unbinding steps before they reach their secretory target. Under these conditions, a single release site model is no longer consistent with the transient nature of the postsynaptic response. Therefore, the model would have to be expanded to include a proximal secretory trigger, in addition to the more distal facilitation site, as proposed by Tang et al. (2000) and explored more recently by Matveev et al. (2002). The proximal site would preserve the transient nature of transmitter release, because sufficiently close to the channel cluster the decay of Ca$_{2}^{+}$ peaks is fast.

In the present simulations, in the fixed buffer case the mechanism of facilitation undergoes a gradual qualitative change as the parameters are varied, and at high values of distance and $B_{total}$, the accumulation of free [Ca$_{2}^{+}$]$_{ext}$ becomes the principle source of facilitation (Fig. 9Bc). For intermediate values of parameters, buffer saturation begins to contribute to the overall facilitation magnitude (Fig. 9Bb). In contrast, in the mobile buffer case the FCT through buffer saturation represents the only mechanism of SF for a wide range of buffering conditions. These results further illustrate the opposing effects of fixed and mobile buffers on Ca$_{2}^{+}$ dynamics, elucidated in earlier studies (Junge and McLaughlin, 1987; Sala and Hernández-Cruz, 1990; Zhou and Neher, 1993; Nowycky and Pinter, 1993; Winslow et al., 1994; Gabso et al., 1997; Naraghi and Neher, 1997; Neher, 1998a).

Nonmonotonic dependence on buffer concentration

Most studies show a reduction in SF upon introduction of exogenous mobile Ca$_{2}^{+}$ buffers, pointing to the importance of residual Ca$_{2}^{+}$ as the main source of SF (reviewed in Zucker and Regehr, 2002). Our results suggest that this effect is consistent with the BSM (Figs. 7, 11, and 12). However, depending on whether the endogenous buffering conditions correspond to the “ascending” or the “descending” slope of the FCT peak in Fig. 3, and depending on the mobility of the added buffer relative to the endogenous one, one would predict either an increase or a decrease in the facilitation magnitude. Further, regardless of the source of SF under physiological conditions, adding an alien mobile Ca$_{2}^{+}$ buffer may change the physiological mechanism of facilitation to that of FCT. Such “pseudofacilitation” (Neher, 1998a) may turn out to be greater in magnitude than the native facilitation under control conditions, but as Fig. 3 suggests, it would inevitably decline as the concentration of the added buffer is ramped up sufficiently. This agrees completely with the “pseudofacilitation” effect that has been observed at some neocortical and hippocampal synapses in response to BAPTA application (see Fig. 9B in Rozov et al., 2001, and Fig. 3B of Blatow et al., 2003). Pseudofacilitation may also explain the discrepancy between the results of Winslow et al. (1994), showing the lack of effect of BAPTA-acetoxymethyl-ester on facilitation at the crayfish NMJ, and the results of other studies showing that similar manipulations reduce facilitation in this system. It may be argued that the particular concentration of BAPTA-acetoxymethyl-ester used by Winslow et al. caused a pseudofacilitation effect that balanced the concomitant reduction of facilitation occurring through a different mechanism (see also Discussion in Bennett et al., 2000).

Dependence on external Ca$_{2}^{+}$ concentration

The dependence of FCT on the buffer concentration (and hence, on its affinity $K_{D}$, as well) remains nonmonotonic, even if the value of $\kappa_{0}$ is kept fixed (Figs. 5, 6A, and 9A). It follows then that the dependence of FCT on $I_{Ca}$ is also nonmonotonic, because changing the value of $I_{Ca}$ is equivalent to a simultaneous rescaling of the parameters $B_{total}$ and $K_{D}$ in the opposite direction (Figs. 3A and 5A). A consequence of this fact is that the FCT is an increasing function of the external Ca$_{2}^{+}$, [Ca$_{2}^{+}$]$_{ext}$, for small enough values of [Ca$_{2}^{+}$]$_{ext}$, assuming that $I_{Ca}$ is proportional to [Ca$_{2}^{+}$]$_{ext}$. This agrees with the experimental results of Blatow et al. (2003) and Rozov et al. (2001). However, at sufficiently high values of [Ca$_{2}^{+}$]$_{ext}$, the dependence of SF on [Ca$_{2}^{+}$]$_{ext}$ should reverse. Further, the saturation of the putative secretory site and the potential emergence of short-term synaptic depression at higher release rates, two effects not included in our model, would limit the range of [Ca$_{2}^{+}$]$_{ext}$ values for which SF grows with [Ca$_{2}^{+}$]$_{ext}$.

Dependence on the properties of the release mechanism

As discussed above, we have chosen to quantify facilitation solely in terms of the increase in the Ca$_{2}^{+}$ concentration. Inclusion of a specific Ca$_{2}^{+}$-dependent vesicle release scheme would obscure the relationship between the facilitation magnitude and the Ca$_{2}^{+}$-binding properties of the buffer. Saturation of the release mechanism at high [Ca$_{2}^{+}$] would reduce SF estimates calculated according to Eqs. 9 and 10, especially at low values of $B_{total}$ and $k_{on}$, and for small separations between the Ca$_{2}^{+}$ channel and the release site, because these conditions correspond to high Ca$_{2}^{+}$ concentration. Therefore, saturation effects would move the FCT peak in parameter sweep plots of Figs. 3, 5, 6, 8, and 9 to higher values of $B_{total}$, and the height of the peak may be significantly reduced. However, all our conclusions about the qualitative dependence of FCT on the endogenous buffering parameters would remain unchanged.

Facilitation accumulation time course

We have found that the saturation of either a very mobile or a completely immobilized buffer may explain the magnitude
of SF observed at the crayfish NMJ (Figs. 7 and 11). However, the observed supralinear accumulation time course of SF is only predicted if the endogenous buffer is mobile. In agreement with our results, experiments of Blatow et al. (2003) reveal a supralinear SF growth at a hippocampal mossy fiber synapse that contains a highly mobile calbindin (see Fig. 5 C therein; properties of calbindin have been described by Nagerl et al., 2000). Note also that lowering the assumed Ca\(^{2+}\)-cooperativity of release from four to three, and including saturation at high Ca\(^{2+}\) levels, would make the simulated SF growth less steep, favoring even more the mobile buffer model over the fixed buffer case.

It is important to bear in mind that our results show the upper bound on the physiologically attainable level of SF. Therefore, even in the more favorable mobile buffer case, the parameter region corresponding to the observed magnitude of SF may be more narrow than our results in Figs. 3, 5, 6, and 13 suggest, in particular because we assume very high buffer mobility and a nonsaturating relationship between transmitter release and [Ca\(^{2+}\)].

**Acceleration of facilitation decay by exogenous buffers**

Although saturation of a mobile buffer can successfully explain the observed magnitude and accumulation time course of SF, we showed that it is not consistent with the observed acceleration of decay of SF by fast high-affinity exogenous buffers (Fig. 12, A and C). In the fixed buffer case, the faster phase of decay, corresponding to the spatial reequilibration of the locally elevated residual Ca\(^{2+}\), would be indeed accelerated upon adding a mobile buffer. Still, the slower decay phase, corresponding to Ca\(^{2+}\) clearance, would be slowed down, as in the mobile buffer case (Fig. 12 B). However, one may suggest that a slow leak of Ca\(^{2+}\) from the bouton into the axonal compartment, an effect not included in our model, may make a significant contribution to the decay of SF. If the mobility of the exogenous buffer is greater than or equal to the mobility of the endogenous one, then its addition would accelerate the putative leak of Ca\(^{2+}\) into the axon, thereby accelerating SF decay. It is not clear, however, whether the faster decay of both components of SF could be explained by this leak mechanism. Further, recent crayfish NMJ experiments by J.-Y. Lin, Q. Fu, and T. Allana (in preparation) show that the decay of Ca\(^{2+}\) transients on timescales of tens to hundreds of milliseconds is not sensitive to the mobility of the Ca\(^{2+}\)-sensitive dye used to image [Ca\(^{2+}\)], arguing against the importance of this leak. The above study also suggests that the endogenous Ca buffers at the crayfish opener NMJ are characterized by low mobility. Therefore, it is unlikely that BSM can explain the observed properties of SF at the crayfish NMJ. However, further modeling work that would incorporate this new experimental data is necessary to reach a definite conclusion. Parenthetically, we note that at the frog NMJ, addition of exogenous buffer does slow the decay of SF (Robitaille and Charlton, 1991), as predicted by the present model.

We showed previously (Matveev et al., 2002) that both the growth and the decay properties of SF at the crayfish NMJ can be explained by a model with a distant facilitation site proposed by Tang et al. (2000). However, to explain the supralinear accumulation time course, the two-site model had to incorporate additional assumptions of a very large separation between the SF sensor and the Ca\(^{2+}\) channel array (>250 nm), or a high degree of tortuosity near the active zone. The two-site model is similar to the BSM in that both rely exclusively on the dynamics of Ca\(^{2+}\) diffusion and buffering to explain the observed behavior of SF, in particular its decay time course. An alternative possibility is that at least one of the facilitation decay components may result from the slow unbinding of Ca\(^{2+}\) from a release-controlling Ca\(^{2+}\) sensor (Yamada and Zucker, 1992; Bertram et al., 1996; Bennett et al., 1997; Dittman et al., 2000). In its pure form, such a bound Ca\(^{2+}\) model is inconsistent with experimental data demonstrating the importance of free residual Ca\(^{2+}\) for SF (reviewed in Zucker and Regehr, 2002). Yet it is possible that a hybrid model, sensitive to free Ca\(^{2+}\) but including a slow unbinding process, could also reproduce the facilitation behavior observed at the crayfish NMJ. Although recent experiments have ruled out the existence of a slow unbinding step at the calyx of Held terminal (Felny et al., 2003), it was shown to play a role at a cerebellar granule cell synapse (Atluri and Regehr, 1996).

Our analysis shows that the buffer saturation mechanism (better termed the FCT model) and the two-site residual free Ca\(^{2+}\) model can be viewed as being on the opposite sides of the same spectrum (Fig. 8 B). Whereas the FCT is most pronounced when a highly mobile buffer saturates globally, the accumulation of free [Ca\(^{2+}\)]\(_{res}\) is mediated by the local saturation of (local Ca\(^{2+}\) trapping by) a high-concentration fixed buffer. However, there exists an intermediate parameter regime, where the accumulation of free [Ca\(^{2+}\)]\(_{res}\) and the facilitation of Ca\(^{2+}\) transients can both contribute to synaptic facilitation (Fig. 9 Bb).

**APPENDIX: THE CASE OF NONZERO RESTING CA\(^{2+}\) CONCENTRATION**

Although our simulations assume zero resting Ca\(^{2+}\) concentration, Ca\(^{2+}\)rest, the results can straightforwardly be translated to the case of nonzero Ca\(^{2+}\)rest. The Ca\(^{2+}\)rest is a constant term not affecting the dynamics of diffusion or buffering, so its effect is limited to a transformation of model parameters. Let’s express the total [Ca\(^{2+}\)] as a sum of Ca\(^{2+}\)rest and the contribution due to Ca\(^{2+}\) entry during an action potential ([Ca\(^{2+}\)] = [Ca\(^{2+}\)]\(_{Ap}\): [Ca\(^{2+}\)]\(_{local}\) = Ca\(^{2+}\)rest + [Ca\(^{2+}\)]. Because the derivative of the constant resting term Ca\(^{2+}\)rest on the left- and right-hand sides of Eq. 2 is zero, the only change is in the reaction term on the right-hand side of Eqs. 2 and 3:

\[
R = -k_{off}[B][Ca^{2+}] + k_{off}(B_{total} - [B]).
\]
Facilitation through Buffer Saturation

Let us now introduce the resting concentration of free buffer, $B_{\text{rest}}$, in equilibrium with the resting $Ca^{2+}$:

$$k_{\text{off}}C_{\text{a,rest}}B_{\text{rest}} = k_{\text{eff}}(B_{\text{total}} - B_{\text{rest}}).$$  \hspace{1cm} (12)

$$B_{\text{rest}} = \frac{B_{\text{total}}K_D}{C_{\text{a,rest}} + K_D}, \quad \text{where} \quad K_D = \frac{k_{\text{off}}}{k_{\text{on}}}. \hspace{1cm} (13)$$

We can then express the reaction term as

$$R = -k_{\text{off}}[B](Ca^{2+} + Ca^{2+}) + k_{\text{eff}}(B_{\text{total}} - B_{\text{rest}})$$

Using Eq. 12 to eliminate the second term in above expression, this yields

$$R = -k_{\text{off}}[B](Ca^{2+} + Ca^{2+}) + k_{\text{eff}}[B](B_{\text{rest}} - [B]).$$

Now, notice that the reaction term has the same form as in the $Ca_{\text{a,rest}} = 0$ case (Eq. 11), except that $B_{\text{rest}}$ has to be replaced with $B_{\text{rest}}$ (given by Eq. 13), and $k_{\text{off}}$ has to be replaced with $k_{\text{off}} + k_{\text{on}}$, which in turn implies that $K_D$ has to be replaced with $K_D + C_{\text{a,rest}}$. Note that this parameter transformation allows us to recover the expression for the rest-state buffering ratio $k_0$ in the nonzero $Ca_{\text{a,rest}}$ case (Eq. 6):

$$k_0 = B_{\text{total}}K_D \quad \text{where} \quad K_D = K_D + C_{\text{a,rest}}.$$  \hspace{1cm} (16)

where the primed parameters correspond to the case of zero $Ca_{\text{a,rest}}$. As expected, the value of the buffering ratio is invariant with respect to this parameter change.

Thus, to recover the dependence of facilitation on model parameters for the case of nonzero $Ca_{\text{a,rest}}$, the inverse of the above transformations has to be applied to the parameter values in Figs. 2–13:

$$B_{\text{total}} = \frac{B_{\text{total}}'}{1 - C_{\text{a,rest}}/K_D}, \quad \text{where} \quad K_D > C_{\text{a,rest}}.$$  \hspace{1cm} (17)

where $K_D > C_{\text{a,rest}}$. Note that the value of $k_{\text{off}}$ remains unaltered by this substitution. The condition $K_D > C_{\text{a,rest}}$ implies that the magnitude of $Ca_{\text{a,rest}}$ imposes a constraint on the relevant range of values of $K_D$. Given a certain value of $Ca_{\text{a,rest}}$, one should ignore those parts of Figs. 3, 5, 6, and 13 that lie in the region $K_D < C_{\text{a,rest}}$. The transformations described by Eq. 17 will have no effect on the results for the fixed buffer case (Fig. 9), because the value of $K_D$ is much greater than $Ca_{\text{a,rest}}$ in the relevant parameter range. In the mobile buffer case, for the fixed-$k_{\text{eff}}$ plots showing the dependence of facilitation on $B_{\text{total}}$ ($K_D$) and $k_{\text{on}}$ (Figs. 5 and 6), these transformations involve a simple shift to the left by the amount equal to $Ca_{\text{a,rest}}$, after the absissa values are reinterpreted in terms of $K_D$ instead of $B_{\text{total}}$. Note that the constant-$k_{\text{off}}$ proportionality relationship between $K_D$ and $B_{\text{total}}$ no longer holds in this case, but is determined according to Eq. 6:

$$B_{\text{total}} = k_{\text{off}}K_D(1 + C_{\text{a,rest}}/K_D)^2.$$  \hspace{1cm} (18)

Assuming for example $Ca_{\text{a,rest}} = 0.1 \mu M$, the facilitation peak in Figs. 5 A and 6 A at $(k_{\text{off}} = 1 \mu M^{-1} \text{s}^{-1}, B_{\text{total}} = 400 \mu M, K_D = 0.8 \mu M)$ corresponds to $B_{\text{total}} = 457 \mu M, K_D = 0.7 \mu M$. In Fig. 3, where the values of $B_{\text{total}}$ and $K_D (k_{\text{on}})$ are independently varied, the transformation to the nonzero $Ca_{\text{a,rest}}$ would involve a downward shift along the $K_D$ axis by $Ca_{\text{a,rest}}$ as well as a nonhomogenous stretch to the right, shifting the facilitation peak to higher values of $B_{\text{total}}$. The latter shift in the $B_{\text{total}}$ direction is more significant for lower values of $K_D$. Further, the value of $k_{\text{off}}$ will not remain constant throughout panels $A$ and $B$, due to its dependence on $K_D$ (see Eq. 17). Thus, the independence of facilitation on the value of $k_{\text{off}}$ in the mobile buffer case (Fig. 3) is only approximate, holding for values of $K_D \gg Ca_{\text{a,rest}}$.

references


