Role of Calmodulin in Synaptic Facilitation

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Abstract

Action potential-dependent release of synaptic vesicles and short-term synaptic facilitation are dynamically controlled by endogenous Ca^{2+} buffers such as adenosine triphosphate, calmodulin, and calbindin found in the presynaptic bouton. Calmodulin is in abundance in the presynaptic bouton and binds with Ca^{2+} faster than any other endogenous Ca^{2+} buffer. The effect of Ca^{2+}-dependent calmodulin dislocation from the neural plasma membrane on the short-term synaptic facilitation has however not been studied in detail. Using a two-compartmental model of Ca^{2+} buffering dynamics and calmodulin dislocation from the neural plasma membrane we show that, at relevant physiological conditions, Ca^{2+} buffering with calmodulin and the subsequent dislocation of calmodulin from the plasma membrane play a significant role in modulating short-term synaptic facilitation.

1 Introduction

The building blocks of the brain are nerve cells called neurons, which are specialised in transmitting information in the form of nerve impulses in the brain. The transmission of information by neurons is done through both electrical and chemical signals. This electrochemical signaling is sustained and propagated by ionic channels in the neural membrane. These transmembrane currents may usually involve ionic species like sodium (Na^+), potassium (K^+), calcium (Ca^{2+}), or chloride (Cl^-) [Dayan and Abbott (2001); and Izhikevich (2007)]. In this work we concentrated on chemical signals, and chemical synapses. Synapses are specialised junctions through which neurons signal to each other. Calcium ions are known to serve as a signal for numerous neuronal functions (Blaustein, 1988).

Increases in Ca^{2+} concentration ($[Ca^{2+}]$) in the presynaptic bouton (see figure 1a) of neurons trigger vesicular release of chemical messengers called neurotransmitters. $[Ca^{2+}]$ changes primarily due to Ca^{2+} influx through voltage-gated calcium channels (VGCCs), which are located at the plasma membrane of the neurons and which become activated during an action potential (AP). More than 95% of Ca^{2+} ions that enter cells under physiological conditions are buffered by endogenous mechanisms [Neher and Augustine (1992); Zhou and Neher (1993); and Blaustein (1988)]. Different endogenous buffers such as calbindin-D$_{28K}$ (CB), calmodulin (CaM), and adenosine triphosphate (ATP), are present in the cytosolic volume of the presynaptic bouton and by interacting with free Ca^{2+} ions they can affect the dynamics of vesicular release and therefore the short-term synaptic plasticity (at the time scale of tens and hundred milliseconds).

One mechanism for synaptic facilitation through Ca^{2+} buffer saturation has been introduced earlier, and CB has been shown to contribute to short-term facilitation through this mechanism in different types of central synapses (Blatow et al., 2003). It has recently been demonstrated by Timofeeva and Volynski (2015), using experimentally constrained three-dimensional diffusion modeling of Ca^{2+} influx-exocytosis coupling at small excitatory synapses, that under relevant physiological conditions CaM can play a more significant role in shaping vesicular release probability than any other buffer. They proposed a novel mechanism for synaptic facilitation through buffer dislocation. CaM molecules are known to be in both mobile and immobile states inside the cytosol, although the precise distribution of CaM in these two states remains unknown. In the work of Timofeeva and Volynski (2015), it was considered that the immobile CaM molecules were attached to the neuronal plasma membrane and they could dissociate from it when cytosolic Ca^{2+} levels increase. It was further assumed that the process of dislocation was instantaneous as well as irreversible.

Computations with the three-dimensional spatial model were however expensive in terms of computational

time, and so in this project we developed a two-compartmental model of Ca^{2+} dynamics and buffering for analysing synaptic facilitation through the mechanism of CaM dislocation. We considered that the dislocation occurred at different rates depending on the number of Ca^{2+} ions and it was as well reversible. In particular, we investigated the role of CaM in short-term synaptic plasticity in the presence of CB and ATP. In section 2 we give a detailed description of the two-compartmental model. Numerical analysis of the results from simulation of the model are given in section 3. Section 4 is devoted to summary of the results obtained, the conclusions drawn from the studies, and suggestions for further studies.

2 Model Description and Implementation

2.1 The Model

Based on the fact that CaM can exist in both mobile and immobile states inside the cytosol, we considered the inside of the presynaptic bouton to be of two compartments. We therefore developed a two-compartmental model for the Ca^{2+} dynamics and buffering with CaM, ATP, and CB. The first compartment is when CaM is attached to the neural plasma membrane, and the second compartment is when CaM is dislocated from the neural plasma membrane. CaM is known to consist of two lobes, the N-lobe and the C-lobe, each containing two Ca^{2+} biding sites (Faas et al., 2011). We considered CaM to be dislocated from the neural plasma membrane when the C-lobe is fully occupied, resulting in the compartmentalisation of the inside of the presynaptic bouton.

In this model we further assumed that CaM dislocation is reversible, that is, CaM can reattach to the neural plasma membrane after some time of its dislocation. However, the rates of possible CaM dislocation from the neural plasma membrane and backward reattachment of CaM to the neural membrane are not known. Thus, CaM molecules could exchange between the two compartments at different rates depending on the number of Ca^{2+} ions at its binding sites. Inside the presynaptic bouton, other calcium binding proteins (or buffers) exist. Typical among them are ATP and CB. The two-compartmental model assumed that the diffusion rates of ATP and CB in the bouton were very small. Therefore, the total concentrations of ATP and CB were conserved and hence uniformly distributed in the presynaptic bouton. Thus, ATP and CB do not exchange across the two compartments.

Concentration of calcium, $[Ca^{2+}]$, outside the presynaptic bouton is higher than the concentration of calcium inside the bouton. During an action potential, concentration of calcium inside the bouton changes due to the influx of Ca^{2+} ions through voltage-gated calcium channels located at the neural plasma membrane. The model thus assumes that a cluster of such VGCCs is located at the membrane of the first compartment. This allows the influx of Ca^{2+} ions during an AP to increase the concentration of calcium in the first compartment. Calcium ions can exchange across the two compartments along a concentration gradient and at some given rate. Excess Ca^{2+} ions are extruded through ionic pumps in each compartment, maintaining the $[Ca^{2+}]$ inside the bouton at relevant physiological levels. Figure 1b gives a pictorial description of the model.

(a) Chemical synapse (Image source: Wikimedia)
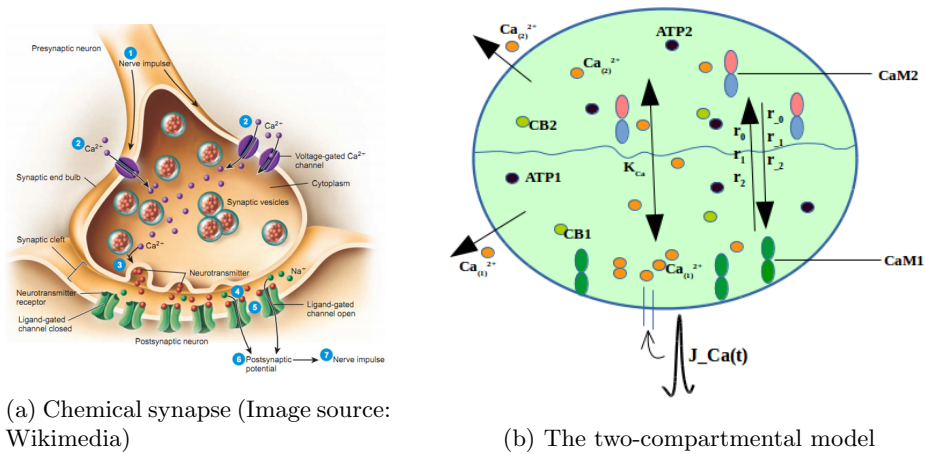

(b) The two-compartmental model

Figure 1: *Left: Inside of the presynaptic bouton. Right: A two-compartmental model description of the reaction dynamics of ATP, CB and CaM with Ca^{2+} ions in the presynaptic bouton. CaM is bound to the neural plasma membrane in the first compartment and it is dislocated from the membrane in the second compartment. ATP and CB are present in both compartments at physiological concentration levels. Changes in $[Ca^{2+}]$ levels is due to influx of Ca^{2+} ions through a cluster of VGCCs located at the plasma membrane of the first compartment. Ca^{2+} ions are extruded from both compartments through ionic pumps.*

The presence of Ca^{2+} ions in the presynaptic bouton results in chemical reactions between Ca^{2+} ions and ATP, CaM, and CB molecules. Ca^{2+} binding to ATP in each compartment was modeled as a second-order reaction given by:
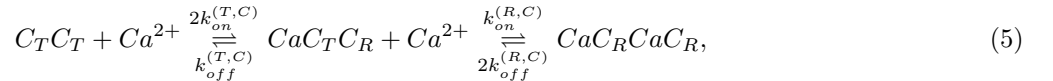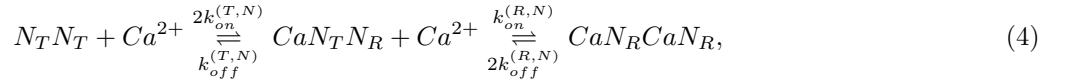
$$ATP + Ca^{2+} \underset{k_{off}^{ATP}}{\overset{k_{on}^{ATP}}{\rightleftharpoons}} CaATP, \tag{1}$$

where $k_{on}^{ATP} = 500\mu M^{-1}s^{-1}; k_{off}^{ATP} = 1.0^5 s^{-1}$ [Ermolyuk et al. (2013); and Timofeeva and Volynski (2015)]. Total ATP concentration is preserved in the bouton and does not exchange between the two compartments due to slow diffusion. Thus, the reaction or binding of Ca^{2+} to ATP molecules occurred independently in each compartment. The total ATP concentration in the presynaptic bouton was $0.9mM$ corresponding to $58\mu M$ of free $[ATP]$ at resting physiological conditions [Faas et al. (2011); Ermolyuk et al. (2013); and Timofeeva and Volynski (2015)].

Similarly, Ca^{2+} binding to CB in each compartment was modeled as a second-order reaction. Each CB molecule contained four independent Ca^{2+} binding sites: two fast, and two slow [Nagerl et al. (2000); Ermolyuk et al. (2013); and Timofeeva and Volynski (2015)]:

$$CB_{fast} + Ca^{2+} \underset{k_{off}^{CB_{fast}}}{\overset{k_{on}^{CB_{fast}}}{\rightleftharpoons}} CaCB_{fast}, \tag{2}$$

$$CB_{slow} + Ca^{2+} \underset{k_{off}^{CB_{slow}}}{\overset{k_{on}^{CB_{slow}}}{\rightleftharpoons}} CaCB_{slow}, \tag{3}$$

where $k_{on}^{CB_{fast}} = 87\mu M^{-1}s^{-1}; k_{off}^{CB_{fast}} = 35.8s^{-1}, k_{on}^{CB_{slow}} = 11\mu M^{-1}s^{-1}; k_{off}^{CB_{slow}} = 2.6s^{-1}$ [Ermolyuk et al. (2013); and Timofeeva and Volynski (2015)]. Like ATP, total CB concentration is also preserved and so CB molecules do not exchange across the two compartments. Thus, the binding of Ca^{2+} to CB molecules occurred independently in each compartment. The total CB concentration was $20\mu M$.

Calcium ions interaction with free CaM was modeled using the two-step binding model of cooperative binding to each N- and C-lobe of CaM [Faas et al. (2011); and Ermolyuk et al. (2013)]:

$$N_T N_T + Ca^{2+} \underset{k_{off}^{(T,N)}}{\overset{2k_{on}^{(T,N)}}{\rightleftharpoons}} CaN_T N_R + Ca^{2+} \underset{2k_{off}^{(R,N)}}{\overset{k_{on}^{(R,N)}}{\rightleftharpoons}} CaN_R CaN_R, \tag{4}$$

$$C_T C_T + Ca^{2+} \underset{k_{off}^{(T,C)}}{\overset{2k_{on}^{(T,C)}}{\rightleftharpoons}} CaC_T C_R + Ca^{2+} \underset{2k_{off}^{(R,C)}}{\overset{k_{on}^{(R,C)}}{\rightleftharpoons}} CaC_R CaC_R, \tag{5}$$

where N_x and C_x represent binding sites on the N- and C-lobes, respectively; after the first binding, the state changes from T to R, giving rise to cooperativity (Faas et al., 2011). The forward and backward reaction rates were given as follows:

$$k_{on}^{T,N} = 770\mu M^{-1}s^{-1}; k_{off}^{T,N} = 1.6 \times 10^5 s^{-1}; k_{on}^{R,N} = 3.2 \times 10^4 \mu M^{-1}s^{-1}; k_{off}^{R,N} = 2.2 \times 10^4 s^{-1};$$

$$k_{on}^{T,C} = 84\mu M^{-1}s^{-1}; k_{off}^{T,C} = 2.6 \times 10^3 s^{-1}; k_{on}^{R,C} = 25\mu M^{-1}s^{-1}; k_{off}^{R,C} = 6.5s^{-1}$$

[Faas et al. (2011); and Timofeeva and Volynski (2015)]. The total CaM concentration was $[CaM]_{total} = 46\mu M$. Depending on the level of $[Ca^{2+}]$ in the first compartment, CaM can dislocate from the cell membrane when the two Ca^{2+} ion binding sites at the C-lobe are fully occupied. The dislocation was assumed to occur at different rates r_0, r_1, and r_2 depending on the state of the N-lobe and it is also reversible. The rates are interpreted as follows: r_0 is the rate of dislocation when all the binding sites at each lobe are fully occupied with Ca^{2+} ions, r_1 is the rate for CaM dislocation when the C-lobe is fully occupied and one binding site at the N-lobe is also filled with calcium, and r_2 is the rate of dislocation when the C-lobe is fully occupied but there is no calcium ion at the N-lobe.

Kinetic schemes (4) and (5), on the other hand, do not show any dynamics of CaM dislocation. The schemes were therefore rewritten to show the various circumstances under which CaM can dislocate from the neuronal membrane. An equivalent scheme for reaction dynamics of calcium with CaM is shown in figure 2. The figure indicates clearly Ca^{2+} binding dynamics at the N- and C-lobes by considering all possible ways in which calcium can bind to CaM. It also shows how CaM can dislocate from the neural plasma membrane.
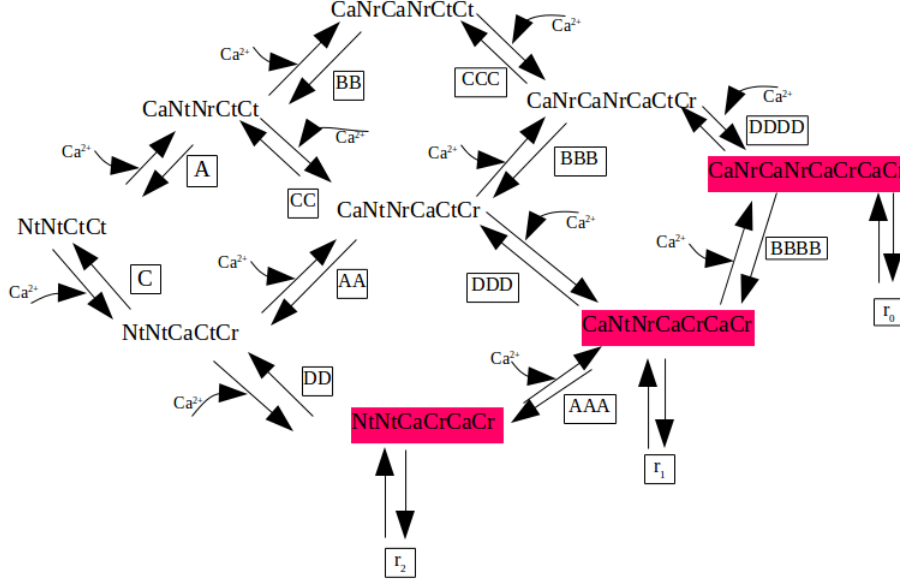
Figure 2: Ca^{2+} *reaction dynamics with CaM molecules in the first compartment. The reactions at each lobe of CaM are illustrated in this figure. CaM is dislocated from the membrane when its C-lobe is fully occupied shown in red. This can occur in three different instances.*

The action potential-evoked Ca^{2+} influx time course $J_{Ca}(t)$ through VGCCs located at the neural plasma membrane (see figure 1b) was modeled by the calcium current given by the function

$$I_{Ca}(t) = \frac{A}{t} exp\left[-B\left[\ln\left(\frac{t}{t_0}\right)\right]^2\right], \tag{6}$$

where $A = 9.2246 \times 10^{-4} pA, B = 15.78, t_0 = 8.036 \times 10^{-4} s$ (Timofeeva and Volynski, 2015). This function, equation (6) (in units pA/s) was then converted to a Ca^{2+} influx (7) (in units $\mu M/s$) as follows:

$$J_{Ca}(t) = \frac{1.0 \times 10^9}{2 \times N_A \times vol \times el} \times I_{Ca}(t), \tag{7}$$

where $N_A = 6.02214 \times 10^{23} mol^{-1}$ is the Avogadro number, $el = 1.6021766^{-19} coulombs$ is the elementary charge (or the charge of one electron), and $vol = 0.11107 \mu m^3$ (see Timofeeva and Volynski (2015)) is the volume of the bouton. The removal of Ca^{2+} from each compartment through the ionic pumps was approximated by a first-order reaction:

$$J_{pump} = -k_{rem}\left([Ca^{2+}] - [Ca^{2+}]_{rest}\right);$$

where $k_{rem} = 3600 s^{-1}$, and $[Ca^{2+}]_{rest} = 0.05 \mu M$ [Ermolyuk et al. (2013); and Timofeeva and Volynski (2015)].

During an AP, Ca^{2+} ions entering the presynaptic terminal triggers synaptic exocytosis and neurotransmitter release (Sudhof, 2013). Three different models have previously been proposed to shed light on the relationship between $[Ca^{2+}]$ in the terminal and paired-pulse facilitation (Blatow et al., 2003). According to the residual calcium hypothesis proposed by Katz and Miledi (1968), facilitation is caused by the increased level of residual Ca^{2+} remaining in the bouton after each AP, which adds to Ca^{2+} influx, increasing the probability of neurotransmitter release (Blatow et al., 2003). Paired-pulse ratios (PPR) for calcium time course after APs were computed for different combinations of buffers as follows:

$$PPR[Ca^{2+}]_{peak} = \frac{[Ca^{2+}]_{peak}^{AP2}}{[Ca^{2+}]_{peak}^{AP1}},$$

where $[Ca^{2+}]_{peak}^{AP1}$ and $[Ca^{2+}]_{peak}^{AP2}$ represent the amplitude of $[Ca^{2+}]$ trace for the first AP and the second AP, respectively.

2.2 Implementation of the Model

Using the law of mass action, the model was implemented as a system of ordinary differential equations (ODEs). The system of ODEs was subsequently solved using MATLAB's ode45 solver (MathWorks Inc.). The ode45 solver implements a Runge-Kutta method with a variable time step for efficient computation of nonstiff system of ODEs.

3 Numerical Analysis of Results

3.1 Stimulation Protocols

The rates of possible CaM dislocation from the neural plasma membrane and backward reattachment remain unknown. However, based on thermodynamic principles it is likely to be comparable to the effective Ca^{2+} dissociation rate [Timofeeva and Volynski (2015)]. We therefore assumed that upon Ca^{2+} binding to the C-lobe, there is a significant chance that CaM will dislocate from the neural plasma membrane (i.e $r0 = r1 = r2 = 650s^{-1}$) (see figure 2). Usually, in real experiments, the cells are stimulated with traces of repeated APs separated by some time, Δt. We therefore considered two stimulation protocols under three different scenarios and three different cases in each scenario. The first stimulation protocol used was the paired-pulse stimulation protocol. In this protocol we used two APs separated by some time, $\Delta t = 20ms$. In the second stimulation protocol, we give a series of APs (burst) with an inter-spike time interval of $20ms$ and wait for a much longer time (here, $300ms$) and give the last AP.

The degree of facilitation of synaptic connection depends on probability of neurotransmitter release and can be quantified by the paired-pulse ratio (PPR). The PPR is defined as the amplitude ratio of the second to the first postsynaptic response after stimulating the connection with two APs (Branco and Staras, 2009). Neural (or paired-pulse) facilitation is thus a form of short-term synaptic plasticity (Zucker and Regehr, 2002). Paired-pulse facilitation arises due to increased presynaptic Ca^{2+} concentration leading to a greater release of neurotransmitter-containing synaptic vesicles (Zucker and Regehr, 2002).

3.2 The Effect of Slow CaM Dislocation on Synaptic Facilitation

The model was simulated for different scenarios and different cases of whether CaM is bound to the neural plasma membrane (the control case) or dislocated from the neural plasma membrane (both reversible and irreversible cases). In the first scenario, we considered that CaM dislocation from the neural plasma membrane was slow. The results for the paired-pulse stimulation protocol for this scenario are shown in figure 3. The

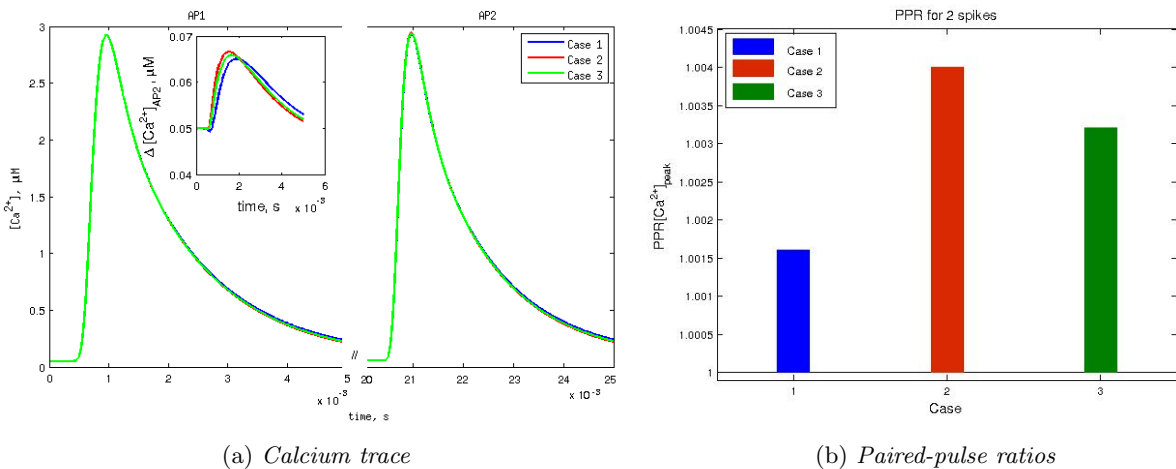(a) *Calcium trace*

(b) *Paired-pulse ratios*

Figure 3: Scenario 1: *Paired-pulse stimulation for slow CaM dislocation. Blue: control case, red: irreversible CaM dislocation, green: reversible CaM dislocation.*

change in calcium concentration ($\Delta[Ca^{2+}]$) at the second AP (inserted in figure 3a) shows that for the slow CaM dislocation from the neural plasma membrane, the change in $[Ca^{2+}]$ was higher for the case when CaM dislocation was irreversible (red), as compared to when CaM is bound to the neural plasma membrane (blue) or when the dislocation from the neural plasma membrane was reversible (green).

The effect of slow CaM dislocation on paired-pulse synaptic facilitation is quantified in terms of the paired-pulse ratios, which are shown in figure 3b. From the PPR values we see that slow-irreversible CaM dislocation from the neural plasma membrane can have an influence on the short-term synaptic facilitation.

In the second stimulation protocol, we used a series of six spikes (burst) given with the inter-spike time interval of $\Delta t = 20ms$ for the first $100ms$. We followed the series of spikes by a last spike after a longer wait of $300ms$. The change or increase in the calcium traces for each spike for all three cases were observed. The first case, where CaM is always bound to the neural plasma membrane, was used as the control case and was compared to the other two cases: reversible, and irreversible CaM dislocation. The results for the calcium traces

for the bursts for all three cases are shown in figure 4. The figure suggests that in the control case, there is no significant changes in the calcium traces for all the spikes (figure 4a). We however observed a slight increase in the calcium traces in the irreversible CaM dislocation case after the first spike and also for the last spike (figure 4b). A similar trend was observed for the reversible CaM dislocation case (figure 4c).

(a) *Control*

(b) *Irreversible CaM dislocation*

(c) *Reversible CaM dislocation*

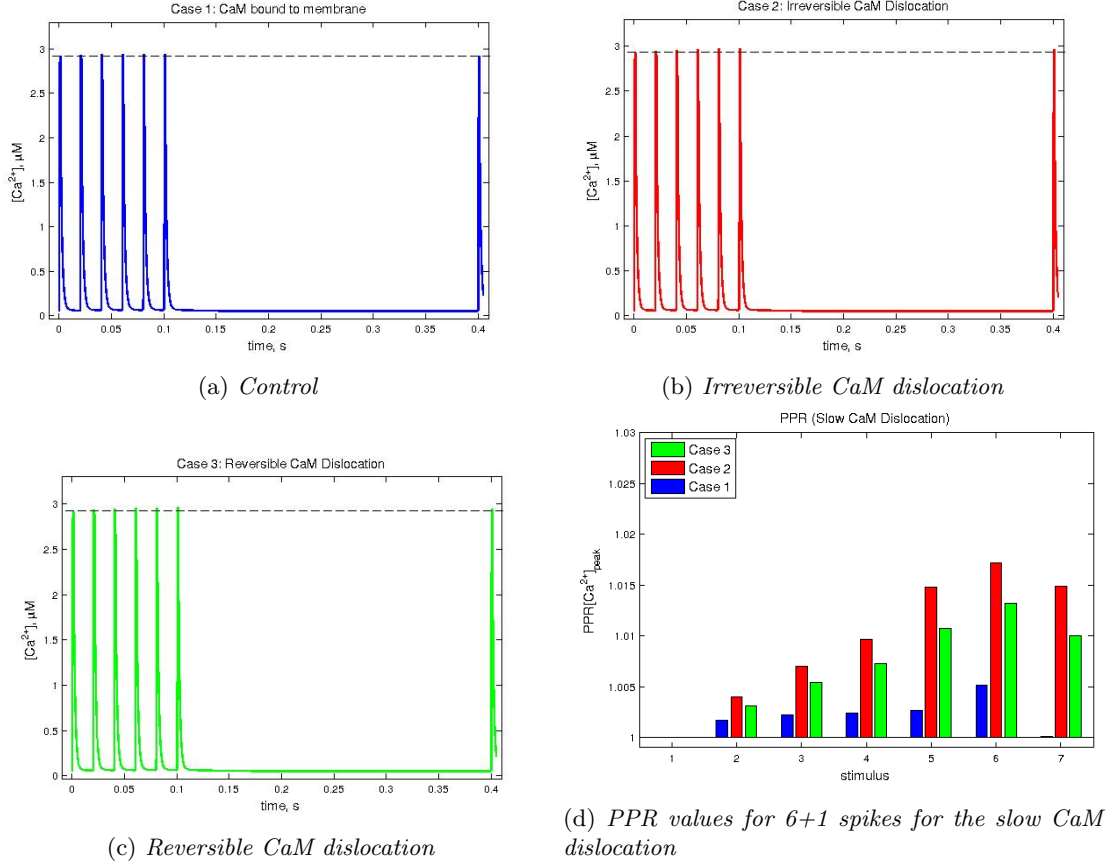(d) *PPR values for 6+1 spikes for the slow CaM dislocation*

Figure 4: Scenario 1: *Slow CaM dislocation from the neural plasma membrane*

These simulation results show that in the first scenario where CaM dislocation from the neural plasma membrane was slow, the case of irreversible CaM dislocation seems to have a significant impact on the short-term synaptic facilitation. The short-term synaptic facilitation are quantified in terms of the PPR values and are shown in figure 4d. The figure shows that the second case of irreversible CaM dislocation from the neural plasma membrane have higher PPR values as compared to the first case (control) and the third case (reversible dislocation). We further explored two other scenarios to confirm what has been observed in this first scenario where we considered only slow CaM dislocation. We thus considered a second scenario where the dislocation of CaM from the neural plasma membrane was instantaneous. The third scenario was where the slow and instantaneous dislocation occurred concurrently.

3.3   The Effect of Instantaneous CaM Dislocation on Synaptic Facilitation

In the next set of simulations, we considered that CaM dislocation from the neural plasma membrane was instantaneous. Due to computational restrictions, the rates of possible CaM dislocation from the neural plasma membrane and backward reattachment were multiplied by one thousand (i.e $r0 = r1 = r2 = 650000s^{-1}$). In this scenario too we considered three different cases just as was done in the first scenario.
The results for the paired-pulse stimulation are shown in figure 5. In figure 5a we see that the change in calcium concentration after the second spike (inserted figure) is much higher in the second case where CaM dislocation is irreversible as compared to the control case and the reversible case. The paired-pulse ratios are shown in figure 5b. The paired-pulse ratios show that the short-term synaptic plasticity is enhanced in the second case of irreversible CaM dislocation from the neural plasma membrane.

Ca^{2+} dynamics during a burst of six APs followed by a single spike $300ms$ after the burst for each case are shown in figure 6. Comparing the irreversible case (figure 6b) and the reversible case (figure 6c) with the control case (figure 6a), we observed a significant increase in calcium concentration during the burst and after
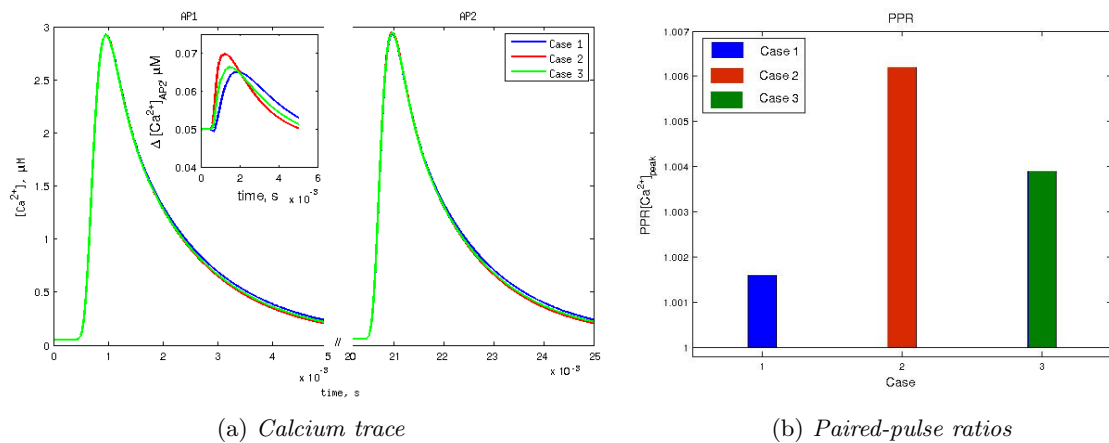
(a) *Calcium trace*

(b) *Paired-pulse ratios*

Figure 5: Scenario 2: *Paired-pulse stimulation for instantaneous CaM dislocation. Blue: control case, red: irreversible CaM dislocation, green: reversible CaM dislocation.*

the burst in the irreversible case than the reversible case. The corresponding PPR values for the burst and the

(a) *Control*

(b) *Irreversible CaM dislocation*

(c) *Reversible CaM dislocation*

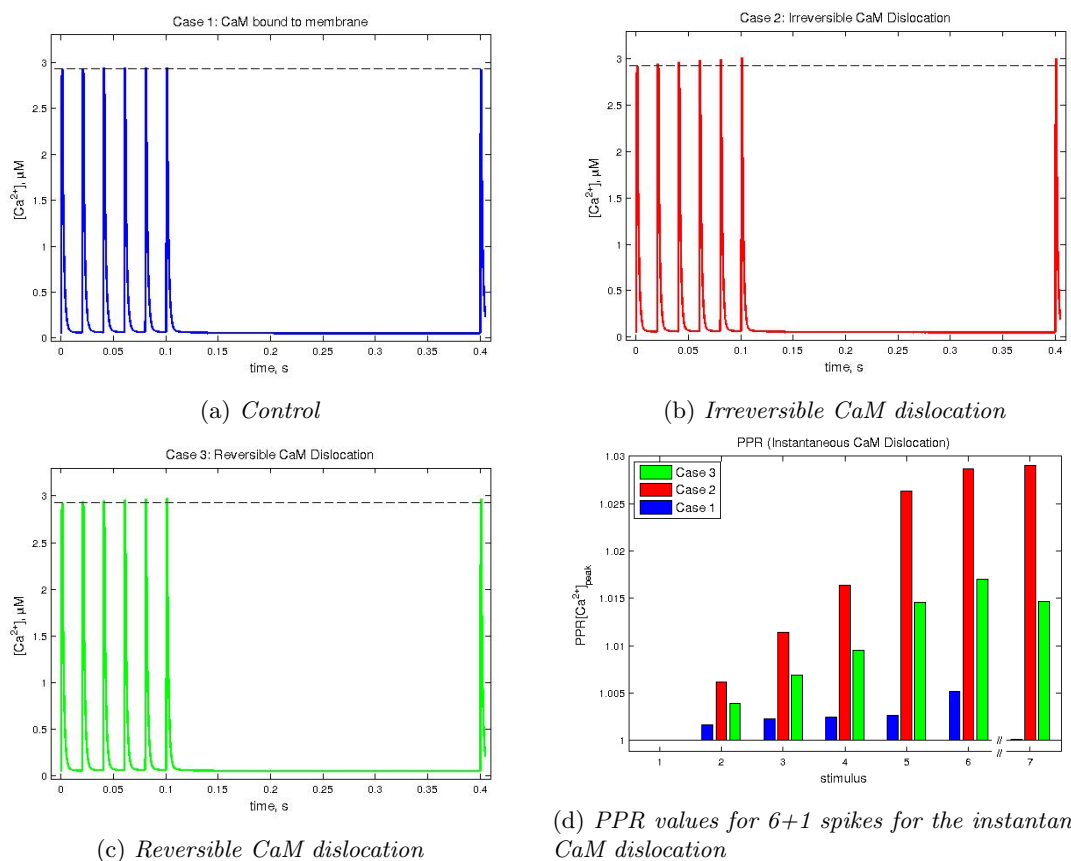(d) *PPR values for 6+1 spikes for the instantaneous CaM dislocation*

Figure 6: Scenario 2: *Instantaneous CaM dislocation from the neural plasma membrane*

single spike at the end of the burst are shown in figure 6d. The PPR values clearly show that short-term synaptic facilitation is influenced by irreversible CaM dislocation from the neural plasma membrane as compared to the control case, and the reversible case. Comparing the results from the first scenario and the second scenario, our simulations consistently showed that irreversible CaM dislocation from the neural plasma membrane has the potential of unraveling the effect of buffer dislocation on short-term synaptic facilitation.

Our model, at this point, is consistent with the results of the three-dimensional spatial model used in the work of Timofeeva and Volynski (2015). Both the two-compartmental model and the three-dimensional spatial model demonstrate that irreversible CaM dislocation from the neural plasma membrane has a significant influence on the short-term synaptic facilitation. Finally, we considered a third scenario where we have a combination of both slow and instantaneous CaM dislocation from the neural plasma membrane. This was done to

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get a broader view of the dislocation dynamics of CaM and the subsequent effect of this dislocation mechanism on the short-term synaptic plasticity of the neurons.

3.4   Mixed Dislocation Dynamics: Slow + Instantaneous CaM Dislocation and its Effect on Synaptic Facilitation

In this set of simulations, we assumed that CaM dislocation from the neural plasma membrane occurred concurrently in a mixture of slow and instantaneous manner (here $r_0 = 650000s^{-1}$, $r_1 = 6500s^{-1}$, and $r_2 = 650s^{-1}$). Like the first two scenarios, we also compared the control case with the second case (irreversible CaM dislocation) and the third case (reversible CaM dislocation). The results from this set of simulations were not different from those obtained in the previous scenarios. Figures 7a and 7b once again show that irreversible CaM dislocation from the neural plasma membrane has an influence on the short-term synaptic plasticity as far as paired-pulse stimulation is concerned.

(a) *Calcium trace*   (b) *Paired-pulse ratios*
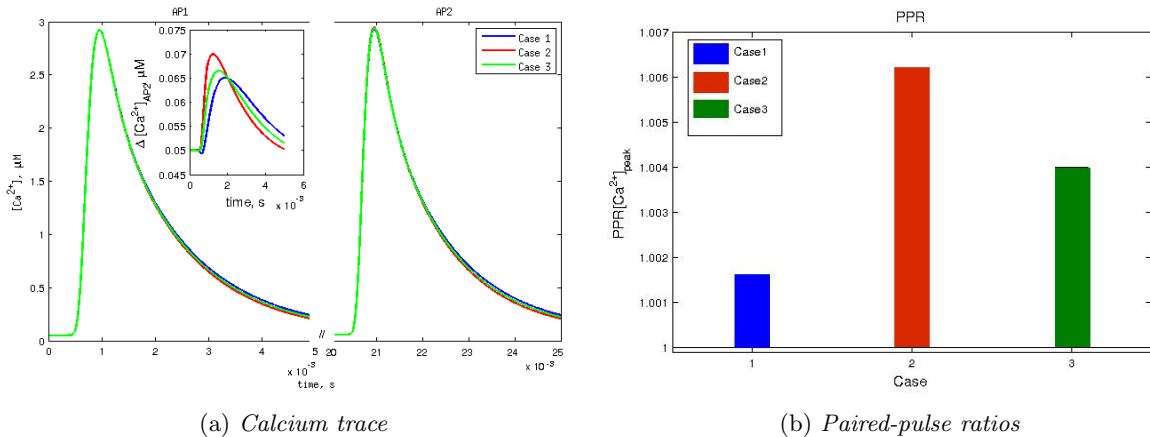
Figure 7: Scenario 3: *Paired-pulse stimulation for a mixture of both slow and instantaneous CaM dislocation. Blue: control case, red: irreversible CaM dislocation, green: reversible CaM dislocation.*

The results for the burst stimulation were also not different from what was observed in the first two scenarios. As seen in figure 8, the second case of irreversible CaM dislocation from the neural plasma membrane in a combination of both slow and instantaneous manner has a significant effect on synaptic facilitation. The PPR values for the burst shown in figure 8d also confirm what we observed in the first two scenarios, that synaptic facilitation is enhanced when there is irreversible CaM dislocation from the neural plasma membrane. Our simulation results so far does not contradict what was observed in the 3-D spatial model (see Timofeeva and Volynski (2015)). All three scenarios considered in our simulation have consistently shown that irreversible CaM dislocation from the neural plasma can have a significant influence on synaptic facilitation.

A comparison of the three scenarios in terms of the paired-pulse ratios is shown in figure 9. The paired-pulse ratios indicate that irreversible (case 2), and reversible (case 3) CaM dislocation from the neural plasma membrane have higher PPR values than the control case (case 1), where CaM is bound to the membrane. We also observed that scenario 2 (instantaneous CaM dislocation), and scenario 3 (slow + instantaneous CaM dislocation) have the same PPR values for case 2. These PPR values were higher than what we observed in scenario 1, where CaM dislocation was slow. This shows that under case 2, there is no difference as to whether the dislocation is instantaneous or a mixture of slow and instantaneous. However, under case 3 we see clear differences between all the three scenarios with the third scenario showing a higher PPR value than the first two scenarios. This shows that in order for reversible CaM dislocation from the neural plasma membrane to enhance synaptic facilitation, the dislocation should happen in a mixture of both slow and instantaneous manner.

Based on these results from our simulations we can argue that irreversible CaM dislocation from the neural plasma membrane has the potential of impacting positively on the short-term synaptic facilitation, especially when the dislocation is instantaneous or a mixture of both slow and instantaneous. Based on the complex nature of biological phenomenon, it is likely for one to speculate based on these results that, the mechanism of irreversible CaM dislocation from the neural plasma membrane that maximizes the synaptic facilitation is the one in which the dislocation can occur in both slow and instantaneous manner concurrently.
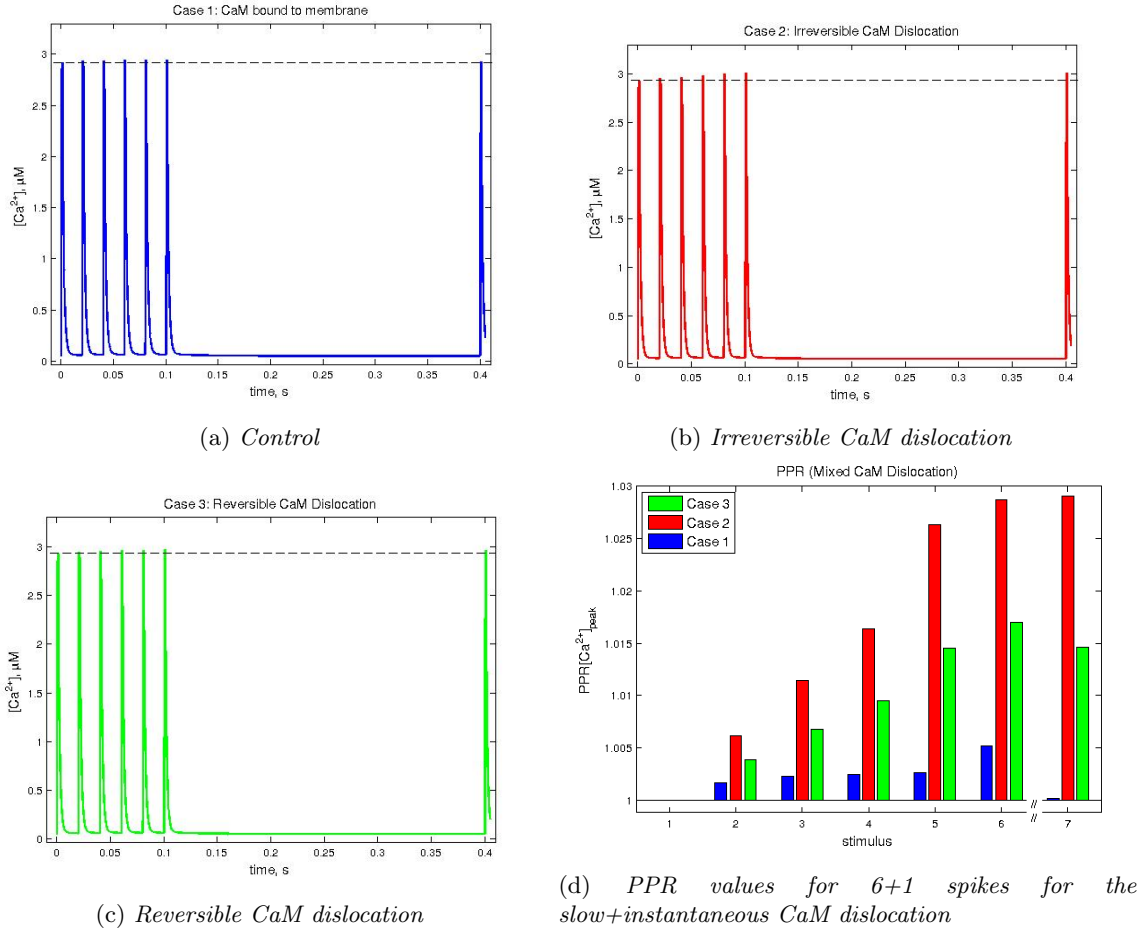
(a) *Control*

(b) *Irreversible CaM dislocation*

(c) *Reversible CaM dislocation*

(d)   *PPR   values   for   6+1   spikes   for   the slow+instantaneous CaM dislocation*

Figure 8: Scenario 3: *Slow+instantaneous CaM dislocation from the neural plasma membrane.*

4   Summary of Results and Conclusion

This study investigates the effects of Ca^{2+} buffering by CaM in the presence of ATP and CB on the short-term synaptic facilitation through buffer dislocation. The multiple roles of CaM in modulating synaptic transmission have been extensively characterised [Xia and Storm (2005); and Ben-Johny and Yue (2014)]. On the other hand, the direct effects of Ca^{2+} buffering by CaM on AP-evoked presynaptic Ca^{2+} dynamics and short-term synaptic facilitation through CaM dislocation have not been systematically explored.

We used a two-compartmental model of AP-evoked presynaptic Ca^{2+} concentration dynamics and buffering with CaM in the presence of ATP and another major Ca^{2+} buffer, CB. We compared the effects of CaM dislocation from the neural plasma membrane on the short-term synaptic facilitation under three main scenarios. The model was simulated for cases where CaM was irreversibly bound to the neural plasma membrane and the result was compared to the cases where dislocation of CaM respectively, occurred reversibly and irreversibly.

Our simulation results demonstrated a noticeable increase in the paired-pulse ratios for the reversible and the irreversible cases when compared to the control case, where CaM is bound to the neural plasma membrane, under each scenario. The effects of Ca^{2+}- dependent CaM dislocation from the neural plasma membrane was even more prominent during the physiological burst-like AP firing, consistent with results from the work of Timofeeva and Volynski (2015). We however observed that when it comes to maximizing synaptic facilitation through irreversible CaM dislocation, it is likely that the dislocation will happen with a mixture of both slow and instantaneous dislocation mechanism.

The rates of possible CaM dislocation from the neural plasma membrane and possible backward reattachment remain unknown. This paved the way for us to consider the various scenarios and cases. That not withstanding, our theoretical modeling study suggests that Ca^{2+}-dependent CaM dislocation from the neural plasma membrane could serve as a potentially powerful mechanism for analysing short-term synaptic facilitation during physiological patterns of activity. This then calls for extensive study of the subject matter and subsequent experimental testing. As plans for future work, we suggest the use of discrete calcium channels
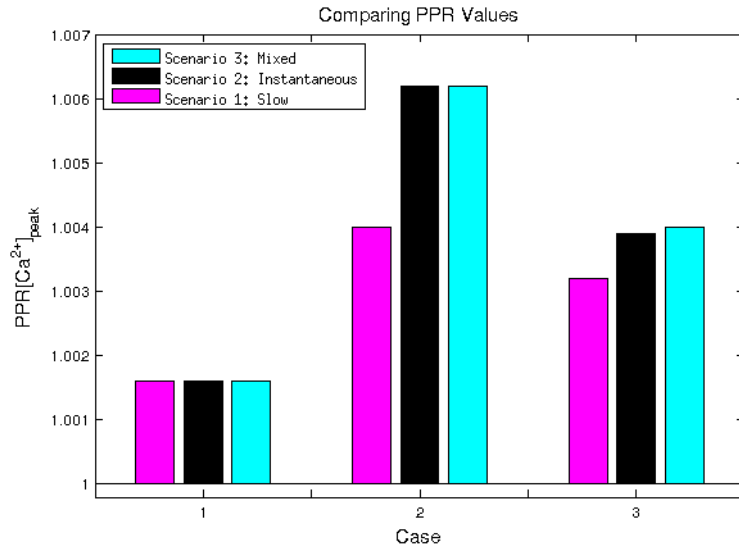
Figure 9: *Comparing PPR for all three scenarios*

instead of the cluster of channels used in this study. We also suggest the inclusion of other buffers in addition to ATP and CB such that all buffers can exchange between the two compartments. Another suggestion for future work is the use of other burst stimulation protocols such as $8 + 1$, $10 + 1$, or $20 + 1$ other than the $6 + 1$ used in this work. Last but not least, one can also consider a generalisation of the two-compartmental model to a three-dimensional spatial model of the presynaptic bouton.

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