Dendritic spine geometry and spine apparatus organization govern the spatiotemporal dynamics of calcium

Miriam Bell[1], Tom Bartol[2], Terrence Sejnowski[2,3], and Padmini Rangamani[1,*]

1 Department of Mechanical and Aerospace Engineering, University of California San Diego, La Jolla, United States
2 Howard Hughes Medical Institute, Salk Institute for Biological Studies, La Jolla, United States
3 Division of Biological Sciences, University of California, San Diego, San Diego, United States

* padmini.rangamani@eng.ucsd.edu.

Abstract

Dendritic spines are small subcompartments protruding from the dendrites of neurons that are important for signaling activity and synaptic communication. These subcompartments have been characterized to have different shapes. While it is known that these shapes are associated with spine function, the nature of this shape-function relationship is not well understood. In this work, we systematically investigated the relationship between the shape and size of both the spine head and spine apparatus in modulating rapid calcium dynamics using mathematical modelling. We developed a spatial multi-compartment reaction-diffusion model of calcium dynamics with various flux sources including N-methyl-D-aspartate receptors (NMDAR), voltage sensitive calcium channels (VSCC), and different ion pumps on the plasma membrane. Using this model, we have made several important predictions – i) size and shape of the spine regulate calcium dynamics, ii) membrane fluxes nonlinearly impact calcium dynamics both temporally and spatially, and iii) the spine apparatus can act as a physical buffer for calcium by acting as a sink and rescaling calcium concentration. These predictions set the stage for future experimental investigations.

Author summary

Dendritic spines, small protrusions from dendrites in neurons, are a hotbed of chemical activity. Synaptic plasticity and signaling in the postsynaptic dendritic spine are closely dependent on the spatiotemporal dynamics of calcium within the spine. This complexity is further enhanced by the distinct shapes and sizes of spines associated with development and physiology. Even so, how spine size and internal organization affects calcium dynamics remains poorly understood. To elucidate the relationship between signaling and geometry, we developed a 3D spatiotemporal model of calcium dynamics in idealized geometries. We used this model to investigate the impact of dendritic spine size, shape, and membrane flux distribution on calcium dynamics. We found that the interaction between spine geometry and the membrane fluxes through various receptors and pumps plays an important role in governing the spatiotemporal dynamics of calcium. Since it is known that dendritic spine size and shape can also change in response to downstream dynamics following calcium influx, our investigation serves as a critical step towards developing a mechanistic framework to interrogate the feedback between signaling and geometry.

Introduction

Dendritic spines, small protein- and actin-rich protrusions located on dendrites of neurons, have emerged as a critical component of learning, memory, and synaptic plasticity in both short term and long term synaptic events [1, 2]. These subcompartments provide valuable surface area for cell-cell interaction at synapses, and important compartmentalization of signaling proteins to control and process incoming signals from the

presynaptic terminal [3,4]. Thus, dendritic spines are hotbeds of electrical and chemical activity. Since calcium is the first incoming signal into the postsynaptic terminal, calcium temporal dynamics have been extensively studied experimentally and more recently computationally [4–7]. In particular, calcium acts as a vital second messenger, triggering various signaling cascades leading to long term potentiation (LTP), long term depression (LTD), actin cytoskeleton rearrangements, and volume expansion amongst other events [1, 2, 8].

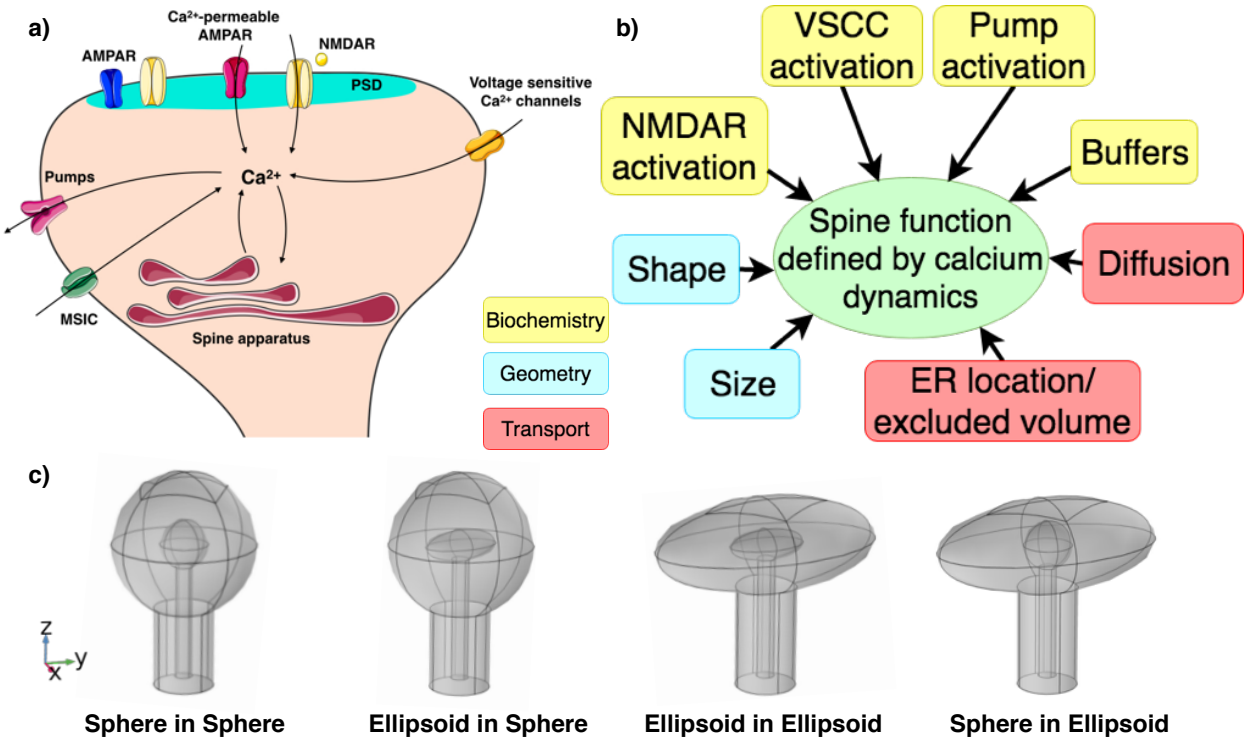

Fig 1. Physical and chemical principles associated with calcium influx in dendritic spines a) Spatio-temporal dynamics of calcium in dendritic spines depend on multiple sources and sinks on both the spine membrane and the spine apparatus membrane. These include receptors (NMDAR), channels (VSCC), and pumps (plasma membrane calcium ATPase (PMCA), sodium/calcium exchanger (NCX)). b) A partial list of factors that can influence these dynamics include biochemical (shown in panel a), geometry, and protein transport components, which are effectively coupled. In this study, we focus on the effects of spine and spine apparatus size, spine and spine apparatus shape, and flux through NMDAR and VSCC distribution on calcium spatiotemporal dynamics. c) Four different combinations of spine head and spine apparatus geometries are used as model geometries – spherical head with spherical apparatus, spherical head with ellipsoidal apparatus, ellipsoidal head with ellipsoidal apparatus, and ellipsoidal head with spherical apparatus – to study how spine geometry affects calcium dynamics. The coordinate axes correspond to 100 nm.

Dendritic spine activity has numerous timescales with signaling pathways operating on the millisecond to the hour timescale following spine activation [1, 9, 10]. Calcium dynamics are on the millisecond timescale, since calcium is the second messenger that floods the spine following the release of neurotransmitter from the presynaptic terminal. The temporal dynamics of calcium have provided valuable insight into the signaling dynamics in dendritic spines and it is quite clear that calcium dynamics are influenced by a large number of factors. Multiple studies have connected the electrical activity of the plasma membrane voltage to calcium dynamics of N-methyl-D-aspartate receptors (NMDAR) [11–13]. The electrophysiology of dendritic spines influence many signaling dynamics through voltage-sensitive (or voltage-dependent) ion channels and thus models of these dynamics can be linked to downstream signaling. Spatial models of calcium dynamics in dendritic spines have also been proposed previously [6, 14, 15]. Spatial-temporal models of calcium dynamics highlighted the role of calcium-induced cytosolic flow and calcium influx regulation by Ca^{2+}-activated K^+

channels (SK channels) [13, 14]. Computational studies using stochastic models of calcium transients have emphasized the role that probes play in altering experimental readouts [6]. Therefore, computational studies have already provided some insight into calcium dynamics.

Dendritic spines have a unique set of shapes and recently the size and shape distribution of spines has been linked to their physiological function [16, 17]. Additionally only about 14% of spines have a specialized endoplasmic reticulum known as the spine apparatus (SA) [18, 19]. Recent advances in imaging and reconstruction techniques have shed light into the complex surface area of a single spine and the ultrastructure of the spine apparatus [6, 20–22]. While the temporal dynamics of calcium have been studied by many groups both experimentally and theoretically [6, 14, 23], the role of spatial and geometrical aspects of dendritic spines and their ultrastructure remains poorly understood. Specifically, we seek to address the following questions: How does the size and shape of both the spine head and spine apparatus affect calcium dynamics? How does the distribution of channels along the synaptic membrane modulate calcium dynamics within the spine? How does the diffusion coefficient of calcium and calcium buffering rates affect the spatiotemporal dynamics of calcium? To answer these questions, we develop a spatial 3D reaction-diffusion model with multiple compartments. We chose a computational approach because it is not yet possible to manipulate spine size, shape, or spine apparatus location with precise control experimentally. However, the insights obtained from computational approaches can lay the groundwork for generating experimentally testable predictions [24].

Model assumptions

In order to interrogate the spatiotemporal dynamics of calcium in dendritic spines, we developed a reaction-diffusion model that accounts for the fluxes through the different sources and sinks shown in Fig. 1. We briefly discuss the assumptions made in developing this model and outline the key equations below.

1. **Time scale**: We model a single dendritic spine of a rat hippocampal area CA1 pyramidal neuron as an isolated system because we are focusing on the 10-100 ms timescale and the ability of the spine neck to act as a diffusion barrier [7, 25–27]. As a result, we do not consider calcium dynamics due to the mitochondria. Even though mitochondria are known to act as calcium stores, their dynamics are on a longer timescale (10 - 100 s) and mitochondria are located in the dendrite outside of the dendritic spine [28].

2. **Spine head**: The average spine head volume is approximately 0.03 μm^3 [6, 29], but a large variation is observed physiologically. Dendritic spines have characteristic shapes such as filopodia-like, stubby, short, and mushroom shaped spines which are the most common [2, 17]. In this work, we consider two idealized geometries – a spherical spine head, which represents a younger spine, and an ellipsoidal spine head, which represents a more mature mushroom spine. The postsynaptic density (PSD) is modelled as a section of the membrane at the top of the spine head. In simulations where the spine size is varied, we assume that the PSD changes surface area approximately proportionally to the spine head volume [30].

3. **Spine apparatus**: Spine apparatus are found primarily in larger mushroom spines [29], which hints at their role in potential regulation of sustained spine volume [31]. We assume that the spine apparatus acts as a calcium sink in the timescale of interest [32]. Another assumption is that the spine apparatus has the same two shapes as the spine head as a generalization of the more complicated and intricate spine apparatus geometry [29].

4. **Plasma membrane fluxes**: To maintain our focus on the short time scale events associated with calcium dynamics, we include the following plasma membrane (PM) sources and sinks of calcium – voltage sensitive calcium channels (VSCC), NMDAR, plasma membrane Ca^{2+}-ATPase (PMCA) pumps, and sodium-calcium exchanger (NCX) pumps. We localized the NMDAR on the postsynaptic membrane adjacent to the postsynaptic density (PSD), designated at the top of the spine head. VSCC, PMCA, and NCX pumps are uniformly distributed along the plasma membrane, including at the spine neck base. Therefore, we model the dendritic spine as an isolated system with the spine neck base modelled in the same manner as the rest of the PM, instead of explicitly modelling the base with an outward flux into the dendrite.

5. **Calcium buffers**: There are a plethora of calcium buffers present in the cytoplasm that act rapidly on any free calcium [6, 9, 27, 33, 34]. We use a time constant, τ, to capture the effect of these buffers on calcium concentration. Specifically, in the cytoplasm, we assume a buffering behavior that acts as exponential decay throughout the whole volume, with τ as the time constant of decay. We explicitly model calcium in the cytoplasm and in the spine apparatus. We assume that the calcium concentration in the extracellular space (ECS) is so large (2 mM) [6, 35] that calcium influx into the spine has an insignificant effect on ECS calcium concentration.

6. **Spine apparatus fluxes**: In this model, the spine apparatus acts as a Ca^{2+} sink in the 10-100 ms timescale [18, 36, 37]. The implications of this assumption are discussed later in the manuscript. The spine apparatus has been observed to have a variety of different calcium influencing receptors and pumps most notably SERCA pumps, Inositol trisphosphate receptors (IP_3Rs), and ryanodine receptors (RyRs). However, for the purpose of this study, we will not focus on calcium induced calcium release (CICR), and therefore do not include RyR or IP_3R dynamics in this study. It should also be noted that not all neurons have been observed to have RyR and IP_3R on the spine apparatus [38]. We assume that SERCA pumps are located uniformly on the spine apparatus membrane.

Based on these observations, we constructed a 3-dimensional spatial model of Ca^{2+} dynamics in dendritic spines. Our control geometry is a medium sized spine with volume of $\sim$0.06 μm^3 including the spine head and neck, with a spine apparatus of volume $\sim$0.003 μm^3. While all simulations are conducted in 3D (see Fig. S6), most of the results will be displayed in 2D for simplicity and ease of interpretation.

Spatial Model of Ca^{2+} influx

The spatiotemporal dynamics of calcium in the spine volume are represented by a single reaction-diffusion equation

$$\frac{\partial Ca_{cyto}}{\partial t} = D\nabla^2 Ca_{cyto} - \frac{Ca_{cyto}}{\tau}. \tag{1}$$

Here, D is the diffusion coefficient of calcium and τ is the time constant associated with buffering. ∇^2 is the Laplacian operator in 3D. The boundary conditions at the PM and the spine apparatus are given by time-dependent fluxes that represent the kinetics of different channels and pumps.

Boundary condition at the PM

We model the calcium influx due to glutamate release in the synaptic cleft as the response of N-methyl-D-aspartate receptors (NMDAR) [11, 12] and due to VSCC permeability [6]. We should note that the majority of existing models for NMDAR and VSCC calcium influx assume well-mixed conditions; in our work, these species are restricted to the boundary of the geometry resulting in a time-dependent flux at the PM. Both the NMDAR and VSCC-mediated calcium influx depend on the membrane voltage; we prescribe this voltage as a set of biexponentials to capture a back propagating action potential (BPAP) and excitatory post-synaptic potential (EPSP), based on [11, 12]. On the PM, we also include PMCA and NCX that activate in response to the change in cytosolic calcium concentration. As a result, the fluxes at the PM are given by

$$-D(n \cdot \nabla Ca^{2+})|_{PM} = J_{NMDAR} + J_{VSCC} - J_{PMCA} - J_{NCX}. \tag{2}$$

The functions that define the flux terms in Eq. 2 are given in Table S1.

Boundary condition at the spine apparatus membrane

In the cases where we included a spine apparatus, we included SERCA pumps along the spine apparatus membrane that respond to the change in cytosolic calcium concentration. The boundary conditions for the flux across the spine apparatus membrane are given by

$$-D(n \cdot \nabla Ca^{2+})|_{SA} = J_{SERCA}. \tag{3}$$

The function that define the flux term in Eq. 3 are given in Table S1.

Geometries used in the model

We modelled the dendritic spines using idealized geometries of spheres and ellipsoids; dendritic spines consist of a spine head attached to a neck, with a similarly structured spine apparatus within the spine, see Fig 1c for the different model geometries. These geometries were inspired by the reconstructions in [6, 23, 39] and were idealized for ease of computation. The geometric parameters including volume and surface area are given in Table S8.

Numerical Methods

Simulations for calcium dynamics were conducted using commercial finite-element software (COMSOL Multiphysics 5.3). Specifically, the general form and boundary partial differential equations (PDEs) interface were used and time-dependent flux boundary conditions were prescribed. A user-defined mesh with a maximum and minimum element size of 0.0574 μm and 0.00717 μm, respectively, was used. Due to the complex boundary conditions used in this model, we also prescribed 4 boundary layers on all membranes. A time-dependent solver was used to solve the system, specifically a MUMPS solver with backward differentiation formula (BDF) time stepping method with a free time stepper. Results were exported to MATLAB for further analysis.

Results

Effect of spine shape and volume on calcium dynamics

We first analyzed how spine shape affects the spatiotemporal dynamics of calcium by simulating calcium dynamics in the spherical spine with spherical apparatus and ellipsoidal spine with ellipsoidal apparatus. We treat these two geometries as model controls and in later cases compare calcium dynamics against these geometries. Calcium dynamics shows a rapid increase in the first 2-3 ms and a decay over approximately 100 ms (Figure 2a). This time course is consistent with experimentally observed calcium dynamics [40]. The spatial profile of calcium in the spherical spine shows a small gradient at 3 ms (Figure 2b) that rapidly dissipates by 10 ms. This is a consequence of localized influx of calcium through the NMDAR in the PSD. Ellipsoid-shaped spines (Figure 2c) also show similar temporal dynamics of calcium as spherical spines. However, the main difference between spherical and ellipsoidal spines is the maximum concentration of calcium at early time (6.9 μM in spherical spines compared to 7.3 μM in ellipsoidal spines). The ellipsoidal spines maintain a higher concentration than the spherical spines until about 90 ms, at which point both geometries have minimal calcium concentrations. Comparing across shapes, the ellipsoid shows a 5% increase in the peak calcium concentration compared to the spherical spine. This difference in shape-dependent effect of spines is because the surface areas of the spherical (young) and the ellipsoidal (mature) spines are different, resulting in more calcium influx at the membrane of the ellipsoidal spines. In addition to the transient response of calcium, the cumulative calcium also carries information with respect to synaptic plasticity [1, 2]. Therefore, we calculated the integrated calcium over the entire spine volume (area under the curve – AUC) over 300 ms. We found that the small transient changes in increased calcium concentration in ellipsoidal spines result in a sustained increase in the cumulative calcium over this short time scale (Figure 2e), resulting in an increase of 3.6% in total calcium in ellipsoidal spines.

In addition to spine shape, spine size (volume) is also known to change during development and plasticity related events [1, 41, 42]. How does changing the volume of the spine, while maintaining the same shape affect calcium dynamics? To answer this question, we conducted simulations in spherical and ellipsoidal spines of different volumes. Recall that the control spine has a cytosolic volume $V_{cyto} = 0.06\mu m^3$ (see Table S8). For each geometry (sphere and ellipsoid), we maintained the spine apparatus size and shape the same as before and only changed the spine cytoplasm volume in the range of $0.5V_{cyto}$ to $1.5V_{cyto}$. We observed that the relationship between spine volume and calcium concentration scales nonlinearly and inversely. For spine volumes smaller than the control, we observed an increase in calcium concentration for both geometries, whereas for larger volumes, calcium concentration decreases (Fig. 3a,b). Furthermore, an increase in spine volume resulted in a transition from shape sensitivity of peak calcium at smaller volumes to no effect of shape on peak calcium at larger volumes (Fig. 3b, for peak calcium times see Fig. S2a). We then calculated

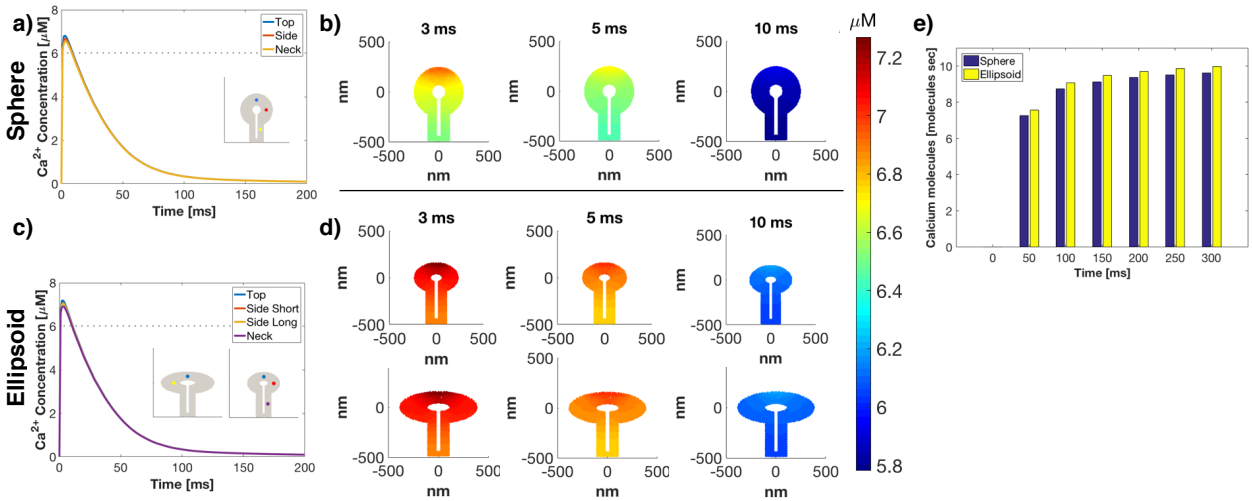

Fig 2. Calcium dynamics during spine activation in spherical and ellipsoidal spines. a) The temporal dynamics of calcium in spherical spines at three locations (top, side, and neck, see inset) in the spherical spine are shown. b) Spatial distribution of calcium in spherical spines at three different time points (3 ms, 5 ms, and 10 ms). c) The temporal dynamics of calcium in ellipsoidal spines at four locations in the ellipsoidal spine (see insets). d) Spatial distribution of calcium in ellipsoidal spines at three different time points (3 ms, 5 ms, and 10 ms). The top panel shows 2D slices along the short axis and the bottom panel shows 2D slides along the long axis. e) Calcium accumulation at different times was calculated using the area under the curve (AUC) of the spatial and temporal dynamics of calcium throughout the volume to obtain the total calcium in the spines.

the cumulative calcium for each of these volumes. First, as expected, we found that for both geometries, an increase in spine volume resulted in an increase in cumulative calcium, see Fig. 3c. Second, we found that the change in cumulative calcium has a direct relationship with the change in spine volume, but this relationship was not linear. More specifically, we see a larger difference in AUC when the spine volume increases as compared to decreases. For peak calcium values however, we see a greater difference between the geometries for smaller volumes. And finally, for each volume, ellipsoidal spines have higher cumulative calcium than spherical spines, suggesting that the shape effect is conserved even in different volumes for cumulative calcium and for peak calcium.

Effect of spine apparatus size and geometry

Although only 14% of dendritic spines have a spine apparatus, the ultrastructural organization of this organelle is not fully understood [18]. Indeed, the complex architecture of the spine apparatus was only recently elucidated in a focused ion beam scanning electron microscopy study by Wu et al. [20]. Since we cannot yet manipulate the shape of the spine apparatus *in vivo*, we varied the geometric features of the spine apparatus *in silico* to see how they affect calcium dynamics. We did this in two ways – *first*, for a given spine shape, we varied the spine apparatus shape while maintaining the same volume-to-volume ratio. In this case, the spine apparatus was varied from a sphere to an ellipsoid with an aspect ratio of its axes b:a:c of 5:3:2 (See Supplementary 2 for more details). *Second*, for a given spine shape, we varied the volume of the spine apparatus to modulate the cytosolic volume to spine apparatus volume ratio. In this case, by varying the spine apparatus volume we changed the spine volume to be 50% and 75% of V_{cyto}, the control spine volume.

We first change the shape of the spine apparatus so we have different spine head and spine apparatus shape combinations (Fig. 4, see Fig. S1 for temporal and AUC results). We first observed that despite the shape of the spine apparatus, the ellipsoidal spines had higher calcium and higher AUC. Next, irrespective of the shape of the spine head, spines with a spherical spine apparatus correspond to a higher calcium concentration when compared to an ellipsoidal spine apparatus (Fig. 4a, c). Since the apparatus acts as a sink, it appears that the reduction in surface area to volume ratio of the spherical apparatus leads to less influx into the apparatus. Therefore, while the ellipsoidal spine heads tend to have higher calcium concentrations and AUC (see Fig. 4b,

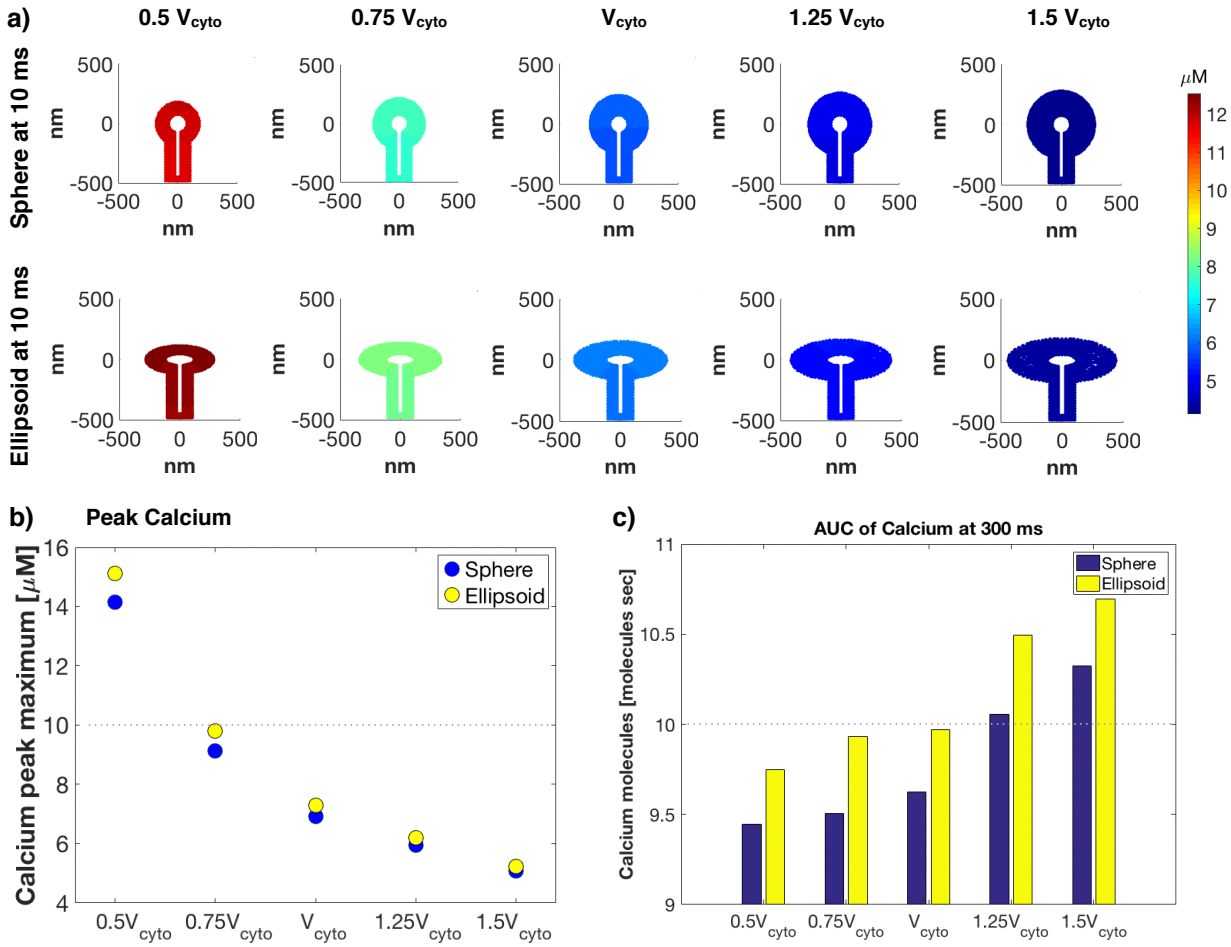

Fig 3. Effect of spine head volume on calcium dynamics. a) Calcium dynamics in spines of different sizes show that calcium concentration in the spine and spine volume are inversely but non-linearly proportional. For smaller spine volumes, we see that the ellipsoidal spine has higher concentrations compared to the spherical spines. The effect of spine size on the temporal dynamics of calcium is seen in the (b) peak values and (c) AUCs of calcium. Increasing spine volume decreases calcium concentration in the spine but increases overall AUC in the spine. For both geometries, the peak calcium concentration increases for decreasing volume, but we see that the difference between the sphere and ellipsoid shapes also increases for decreasing volume. Thus, the effect of shape is more prevalent at smaller volumes.

d) compared to the spherical spine heads due to more PM surface area, the ellipsoidal SA lead to higher influx into the SA compared to the spherical SA due to SA surface area. This result predicts that a combination of large PM area such as that in an ellipsoidal spine combined with a small SA surface area such as that in a spherical SA is conducive to high calcium concentrations at short time scales. Conversely, to closely regulate the calcium concentration, a small PM surface area coupled with a large SA surface area could be effective. Further, this result emphasizes the non-intuitive relationships between PM area and organelle area in cells.

Another key geometric variable that regulates calcium dynamics is the spine volume (Fig. 5). We next asked if changing the spine cytosolic volume by changing the volume of the spine apparatus would impact calcium dynamics. We changed the spine apparatus volume such that the resulting spine volume was either 0.5 V_{cyto} or 0.75 V_{cyto}. We found that a larger spine apparatus leads to an increase in calcium concentrations and peak calcium (Fig. 5a, b; see Fig. S2b for peak calcium times). We also observed that a larger SA leads to a lower AUC (Fig. 5c). It should be noted that the 50% V_{cyto} ellipsoidal spine has a much higher peak calcium compared to the 50% V_{cyto} spherical spine, and that the decrease in AUC for the 50% V_{cyto} spherical spine is much more substantial than the ellipsoidal spine. From these observations, we conclude that spine

volume coupled with spine apparatus volume and surface area is an important regulator of calcium dynamics in dendritic spines.

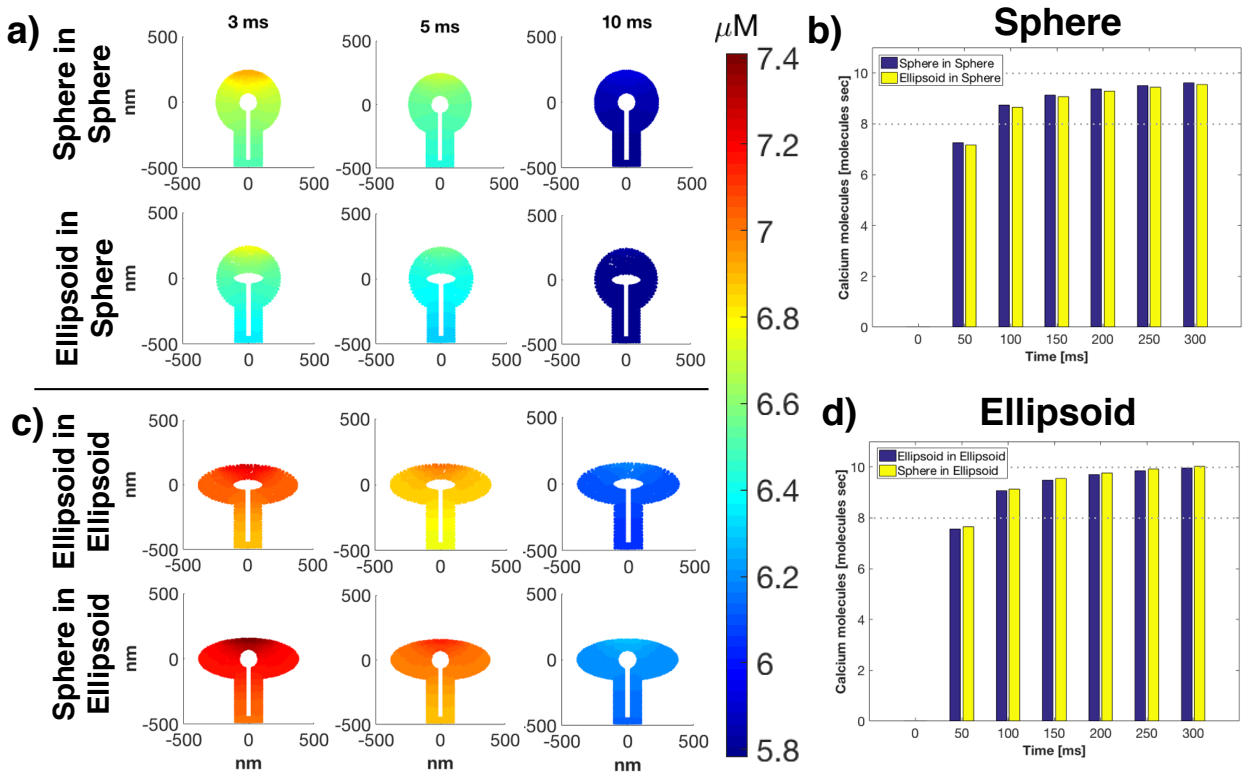

Fig 4. Change in spine shape affects the spatiotemporal dynamics of calcium. a) Calcium spatial dynamics in spherical spines with different geometries of spine apparatus shown in Fig 1C. The effect of the spine apparatus geometry is evident in the calcium concentration at the early time point (3 ms) but not at later times. b) The AUC for calcium shows very little dependence on the spine apparatus geometry. c) Calcium spatial dynamics in ellipsoidal spines with different geometries of spine apparatus shown in Fig 1C. Similar to the spherical spine head, the spherical spine apparatus causes a higher calcium concentration that is still noticeable at 10ms. The maximum calcium concentration difference between the spherical spine with ellipsoidal spine apparatus and ellipsoidal spine with spherical spine apparatus is 9.31% relative to the smaller concentration at 3 ms. d) While the AUC shows very little dependence on the spine apparatus, the ellipsoidal spines have elevated AUC compared to the spherical spines in b).

Spine apparatus can rescale the calcium dynamics by acting as a spatial buffer

Since the spine apparatus is only present in some dendritic spines [18], we next considered how calcium dynamics are affected when the spine apparatus is removed (Fig. 6). We observed that spines without the apparatus have both a higher concentration (Fig. 6 a,b,d,e) and higher AUC (Fig. 6c,f) than the spines with apparatus, regardless of the spine shape. Between the two spine shapes, the spherical spine has a more pronounced difference between the spines with and without the apparatus than the ellipsoidal spine (compare Fig. 6 a-c to Fig. 6 d-f). An intriguing feature of the spine apparatus in particular (Fig. 7a) [20] and the ER in general [43] is the large surface to volume ratio occupied by this organelle. We also considered the effect of the volume to surface area ratio 'n' (given in units of m) of the spine apparatus (Fig. 7). We modelled the boundary flux on the SA membrane such that this flux is now proportional to n_{SA} (n for the SA) so that when we increase the 'volume' of the spine apparatus by increasing n_{SA}, calcium flux into the SA would increase. We noticed that at lower n_{SA} values, both the peak calcium and AUC of calcium (Fig. 7b,c; see Fig. S2c for peak calcium times) plateau, but have a substantial decrease at larger n_{SA} values.

From these observations, we conclude that the spine apparatus acts as a physical and spatial buffer for

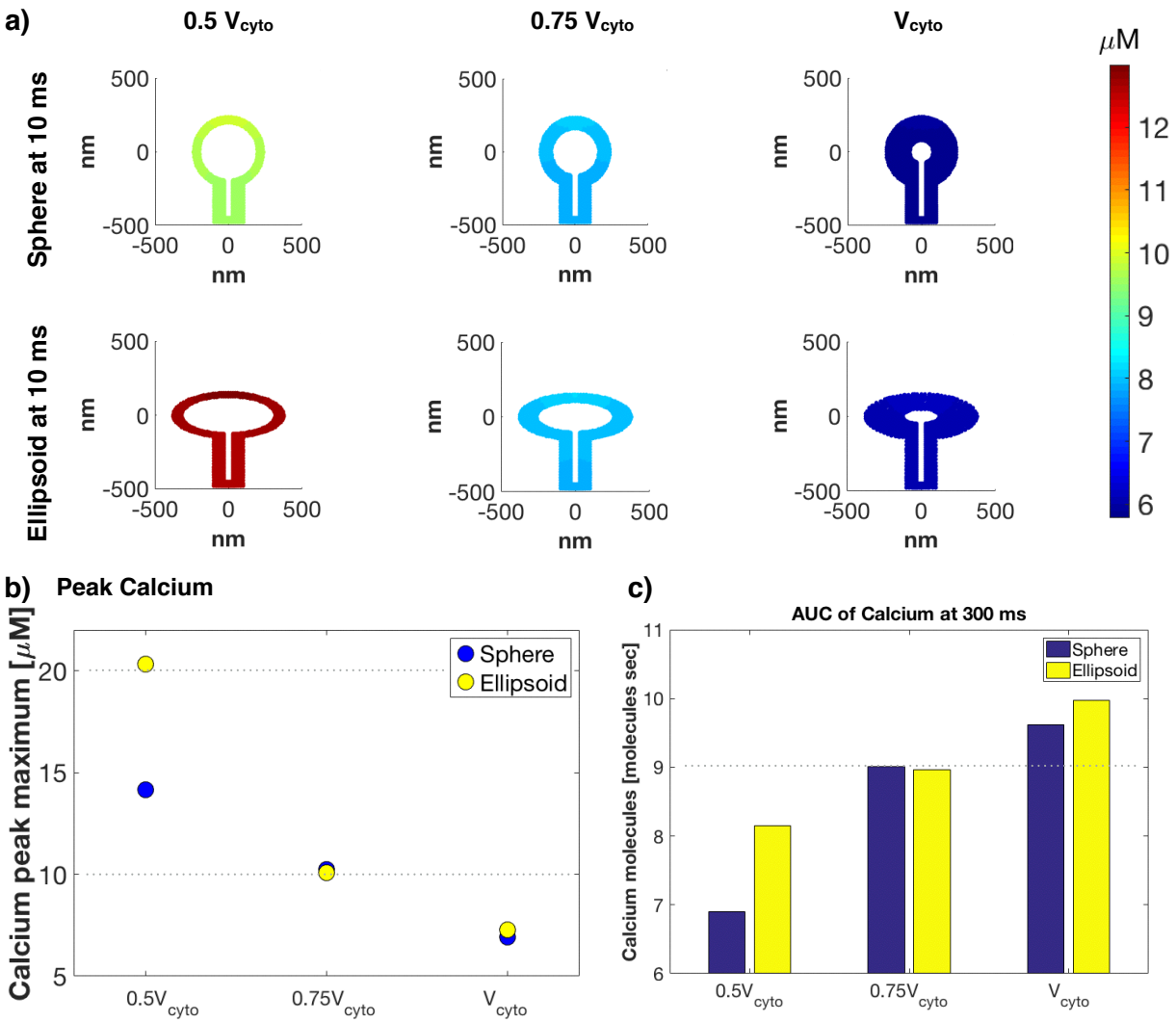

Fig 5. Effect of spine apparatus size on calcium dynamics. a) Calcium dynamics in spines with different spine apparatus sizes show that calcium concentration in the spine and spine apparatus volume are nonlinearly proportional. The effect of spine apparatus size on the temporal dynamics of calcium is seen in the (b) peak values and (c) AUCs of calcium. Decreasing spine volume increases calcium concentration in the spine but decreases overall AUC in the spine. For both geometries the peak calcium concentration increases for decreasing volume, but we see that the difference between the sphere and ellipsoid shapes also increases for decreasing volume. Thus, shape effect differences are most prevalent at smaller volumes.

calcium dynamics by regulating the timescale through surface to volume regulation in the interior of the spine. This is because the spine apparatus acts as a calcium sink in the timescale of interest [18, 36, 37] and in the absence of the spine apparatus the only way to remove calcium from this system is through chemical buffers (see Fig. S5 for the case in which calcium can escape through the spine neck). Furthermore, since the spine apparatus has been known to grow and retract from the dendritic spine in response to stimuli [44], regulation of spine apparatus surface area can also allow for rescaling calcium dynamics in the spine [45].

While the spine apparatus acts as a buffer, there are also molecular buffers in the cytoplasm that play an important role in calcium dynamics [1, 6, 9, 10, 46]. To elucidate the role of these buffers (captured in one time constant, τ) and diffusion, another influential parameter, we varied both parameters in Fig. S3. We see that the combination of buffering and diffusion rates can switch the system between a diffusion-dominated and reaction-dominated regime. For the rates used in this study, the system is in a diffusion-dominated region

(see Supplementary 3 for a more detailed analysis).

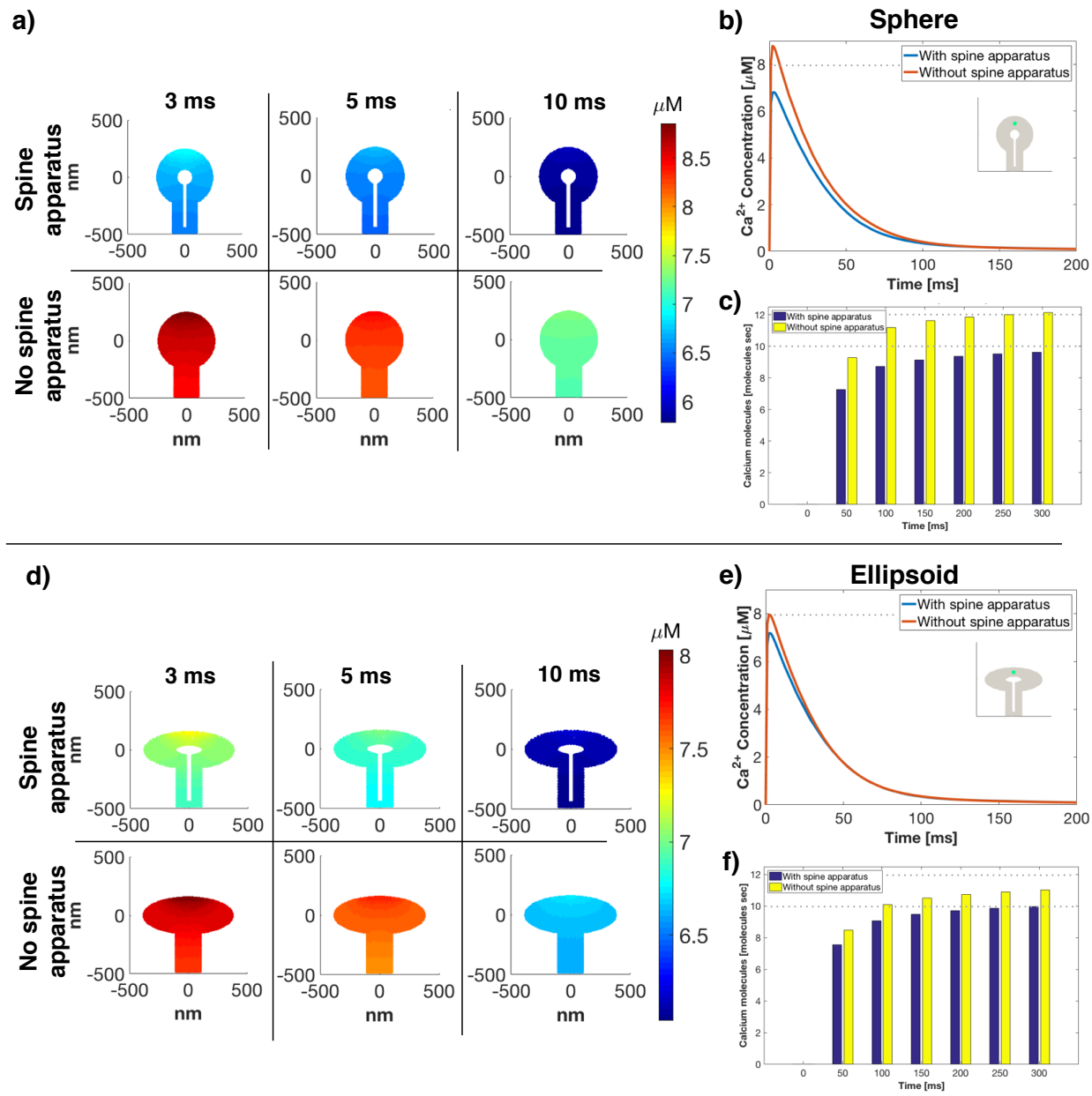

Fig 6. Effect of the presence or absence of spine apparatus. a) Spatial dynamics of spherical spines with and without spine apparatus. Despite having a 5.12% increase in volume, the spherical spine without a spine apparatus has a higher calcium concentration with an increased maximum of 27.81%. The temporal dynamics (b) and AUC (c) for spherical spine highlight this increase in both calcium maximum and total AUC. The time dynamics for the spherical spine show much higher concentrations for the spine without the spine apparatus. The AUC for the spine without an apparatus has an increase of 26.21%, which is sustained throughout the entire time period. d) Spatial dynamics of ellipsoidal spines with and without spine apparatus. Similarly to the spherical results, the ellipsoidal spine without the spine apparatus has higher calcium concentration by 10.48%, while increasing in volume by 4.96%. The temporal dynamics (e) and AUC (f) for the ellipsoidal spine also show this increase in the spine without the apparatus. The spine without a spine apparatus again has a higher total AUC by 10.57%. However, the difference between the two ellipsoidal spines is not nearly as dramatic as the difference between the spherical spines.

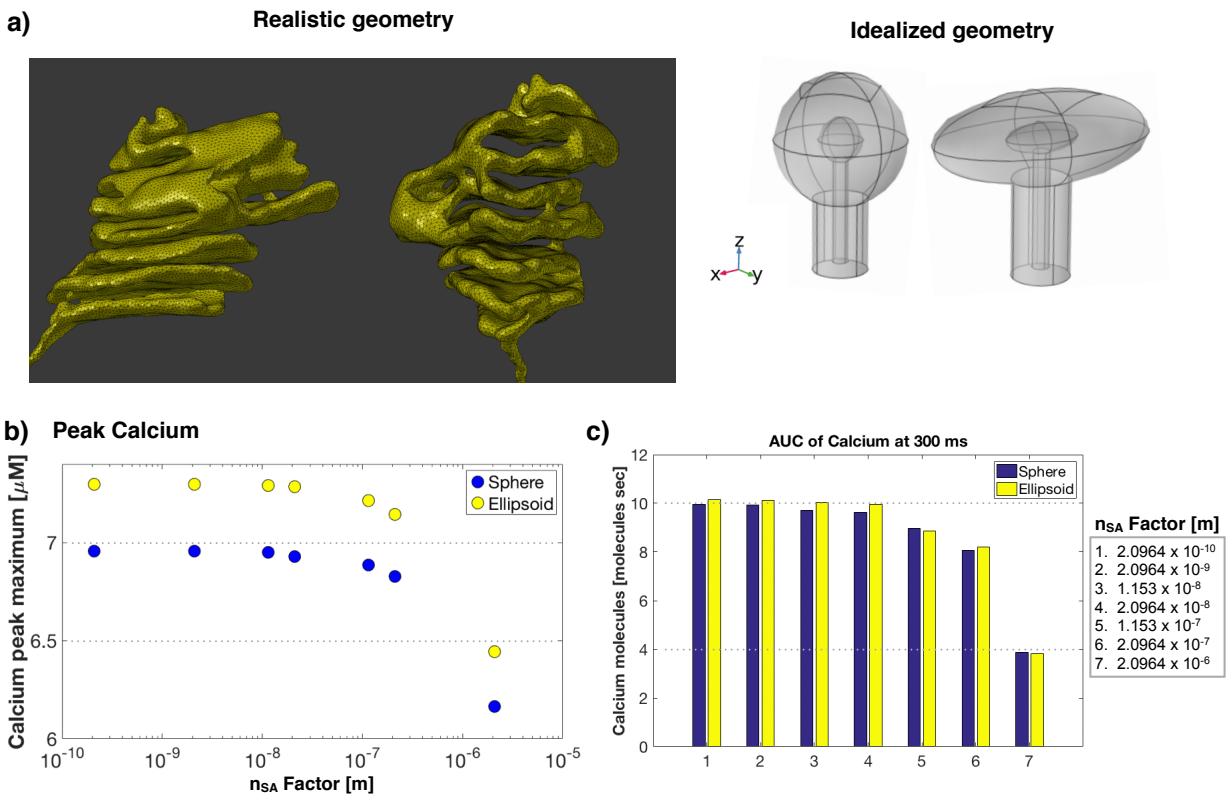

Fig 7. Effect of volume to surface area ratio of the spine apparatus on calcium dynamics. a) Schematic of the realistic geometry of the spine apparatus versus the idealized geometry. The spine apparatus typically is comprised of a series of interconnected stacks which has a very different volume to surface area ratio as compared to the idealized spherical or ellipsoidal spine apparatus of this model. Therefore, we vary the volume to surface area ratio, 'n_{SA},' of the spine apparatus while maintaining the geometry of the model. We take volume and surface area in units of m^3 and m^2 so the n_{SA} factor has units of m. b) Peak calcium concentrations in spherical and ellipsoidal spines for various n_{SA} values. For smaller n_{SA} values, the peak calcium value increases but plateaus. For larger n_{SA} values, we see a dramatic decrease in peak value around 10^{-7} [m]. The ellipsoidal values are consistently higher than the spherical values. c) We see a similar trend for the AUC of calcium at 300 ms for ellipsoidal and spherical spines for various n_{SA} values. The AUC plateaus for lower n_{SA} values but decreases for larger n_{SA} values.

Spatial distribution of membrane fluxes governs calcium dynamics supralinearly in dendritic spines

Dendritic spines are observed to have a range of VSCC densities and a variance in the number of NMDAR that open in response to stimuli [47]. One of the primary determinants of calcium dynamics in the spine is the extent of membrane fluxes because these fluxes serve as a source at the PM and a sink at the spine apparatus membrane. We investigated the effect of the spatial distribution of membrane fluxes on calcium dynamics in the dendritic spine (Fig. 8a). We observed that if only NMDAR activity was present but no VSCC, then calcium concentration was high in the PSD region due to the localization of NMDAR to the PSD but the overall calcium concentration was small regardless of the spine head shape. However, if VSCC were active with no NMDAR activity, then the spatial gradient of calcium is reversed with a higher gradient in the spine neck. We should note that these gradients are short-lived. The temporal dynamics are also affected by the receptor and channel distributions (Fig. 8b). Without NMDAR but with VSCC, there is a larger calcium peak but with faster decay dynamics, whereas with NMDAR but without VSCC results in a lower calcium maximum concentration but a more prolonged transient. The AUC for calcium for the two geometries highlights the complex interactions between the various fluxes (Fig. S4). Therefore, membrane

flux distribution can impact the spatial and temporal dynamics of calcium in a nonlinear manner. This agrees with experimental results stating that the various calcium sources behave supralinearly [27] and a balance between various calcium fluxes is required for tightly regulating calcium concentrations in these small volumes.

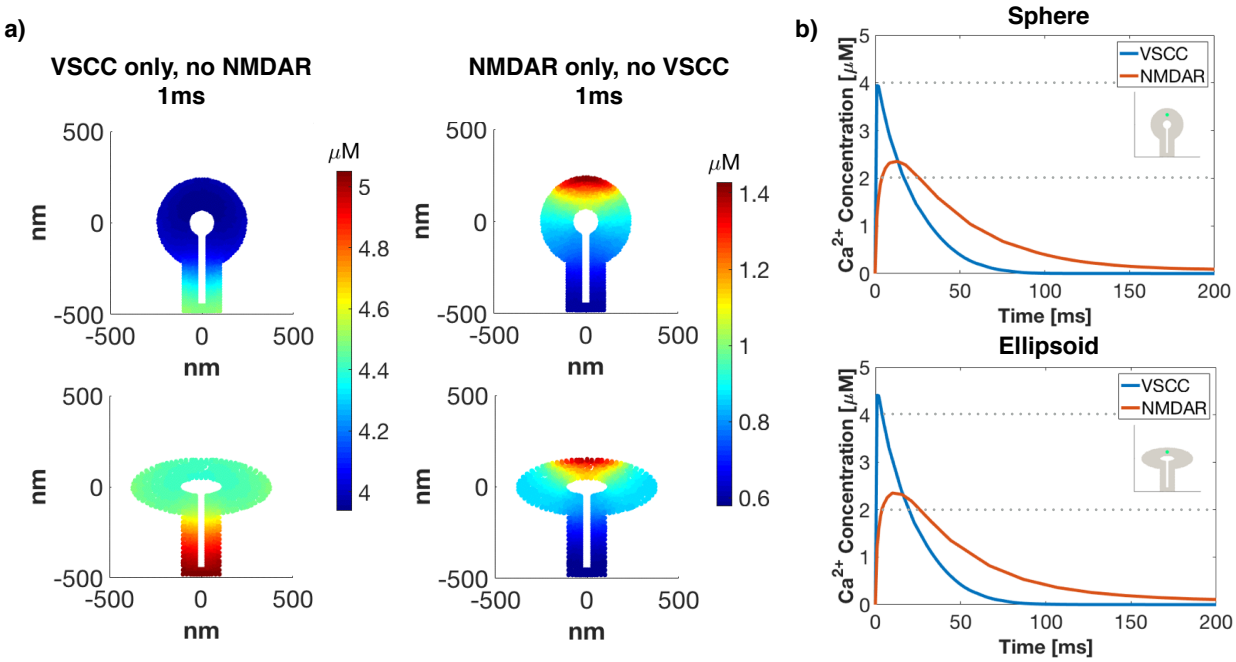

Fig 8. Effect of calcium fluxes through NMDAR and VSCC. a) Spatial dynamics at 1 ms for spherical and ellipsoidal spines with all of the pumps active but with only NMDAR or VSCC active. When the main calcium source is VSCC, we see a clear flip in spatial gradient from the controls, with a higher calcium concentration at the neck. When NMDAR is the main calcium source, we see a large spatial gradient with a higher concentration at the PSD. b) Temporal dynamics for spherical and ellipsoidal spines with either VSCC or NMDAR as the calcium source. Temporal dynamics clearly show that VSCC act on a faster timescale and have a higher max calcium compared to the NMDAR. However, NMDAR leads to a more prolonged calcium transient. Therefore, when considering total AUC for these two cases, NMDAR activation leads to a larger AUC compared to the VSCC (see Fig. S4).

Discussion

Calcium is a fundamental player in both neuronal (cellular) and neural (systems) functionality. Compartmentalized by dendritic spines, calcium has a vital role in triggering signaling pathways for LTP, LTD, synaptic plasticity, and other processes associated with learning and memory formation. However while dendritic spines are known to form functional subcompartments, it is less understood how this specialized compartmentalization impacts calcium dynamics [9]. In this study, we explored the intricate relationship between calcium dynamics and the shape and size of dendritic spine structures. We asked given a size and shape of dendritic spine, and a distribution of membrane flux through various receptors, pumps, and channels, what spatiotemporal dynamics of calcium can we expect. We found a complicated relationship between geometry and signaling that emphasizes the role of shape in cellular functions.

Dendritic spines are known to have characteristic shapes but the majority of mature spines are larger mushroom-shaped spines that are more likely to contain spine apparatus [29]. We found that in similarly sized mushroom-shaped spines, differences in calcium dynamics can result due to differences in shape (spherical verus ellipsoidal spines Fig. 2). Furthermore, we found that differences in spine apparatus shape, while still preserving spine apparatus volume, also affected calcium dynamics within these spines (Fig. 4). Therefore, when keeping a constant surface density of pumps, and channels, the surface area-to-volume ratio of the spine

influences calcium dynamics. Building on the importance of membrane flux, we determined that the various pumps, channels, and receptors had a nonlinear impact on calcium dynamics (Fig. 8). In particular, the spatial distribution of receptors and channels influenced both the spatial and temporal calcium dynamics. Thus, in various disease states that impact the distribution of membrane components, we predict atypical spatial calcium gradients are possible that could impact downstream signaling pathways. For example, NMDAR dysfunction whether leading to increased or decreased functionality can potentially lead to central nervous system diseases such as stroke, Huntington's disease, or schizophrenia [48].

Dendritic spine size is another factor of interest as dendritic spines remain dynamic on the minute timescale. We found an inverse relationship between spine volume and calcium concentration (Fig. 3). In particular, smaller spines had higher calcium concentrations but had lower AUC (total calcium) compared to larger spines. We also investigated spine volume effects by increasing the spine apparatus volume while maintaining spine surface area (Fig. 5). We found similar trends with the smaller volumes having higher calcium peaks and lower AUC, but we observed a larger difference between geometries for the 50% V_{cyto} spines when the spine volume change occurred due to spine apparatus volume increase. Experimental results have already shown different behavior in large versus small dendritic spines [39], this result can help describe how calcium dynamics play into those differences based on spine size.

One of the main findings of this study is the pronounced difference in calcium dynamics for spines with and without a spine apparatus (Fig. 6). We further found that this difference can be enhanced by the shape of the spine head. Studies on dendritic spine geometry show that stable, mature spines tend to be larger mushroom spines that tend towards ellipsoidal shapes as they grow around adjoining axons, and are more likely to have a spine apparatus [29]. In comparison, younger, less stable spines tend to be smaller and more spherical [17, 29]. Therefore, we predict that the mature ellipsoidal spines would be more resilient to any changes in their spine apparatus, as opposed to the smaller spherical spines. This result should be investigated further to elucidate any relationship between spine apparatus and spine shape. Related to this effect, this study highlighted the role of the spine apparatus as a calcium sink (Fig. 6). Building on the effect of the spine apparatus, we investigated the role of volume-to-surface area ratio of the spine apparatus on calcium dynamics (Fig. 7). We found that at smaller values of n_{SA}, the effect of a spine apparatus is minimal since it fails to act as a calcium sink, but larger values of n_{SA} can significantly decrease both peak calcium and AUC of calcium. This highlights the importance of considering the realistic geometry and internal organization of the spine apparatus.

While our model assumes an idealized spine geometry for the dendritic spine and spine apparatus, dendritic spines exist in a wide variety of shape variations and sizes. Therefore, one must be careful when extrapolating our findings to a wider range of natural spines. In particular, the width of the spine neck has been showed as an important determinant of calcium dynamics when comparing larger and smaller spines [30, 49, 50]. Furthermore, while we modeled our dendritic spines as cytoplasm with a spine apparatus, in reality the dendritic spine is a crowded environment with a plethora of cytoskeleton features and signaling proteins. Therefore, it is possible that calcium diffuses through a crowded space, particularly in the PSD, and the exact mechanisms of such transport remain unclear [26, 51, 52]. Within this crowded environment is an abundance of actin, which has previously been shown to have the potential to create cytosolic flow through contraction following spine activation, leading to faster calcium diffusion [14]. We do not address this spine contraction in this model, but this remains the focus of future work. While we touched upon the role of diffusion and buffering in Fig. S3, much work remains to be done on the true impact of the dense actin network within dendritic spines [53].

Another potential limitation of our model also lies in an assumption related to the second Damkohler number, the buffering term. While the assumption of exponential decay allowed for an elegant simplification regarding the surplus of binding partners for calcium and has experimental backing [14], it fails to capture the spatial distribution of buffers. In particular, several calcium buffers are believed to be immobile, which can affect calcium dynamics both spatially and temporally [54, 55]. We would also like to address that due to the small size of the dendritic spine, the system is in a few molecule region that is most commonly addressed with the use of stochastic modelling [6, 24]. However, to elucidate the underlying physics of this system, we choose to address this problem from a deterministic standpoint. The development of a joint stochastic-deterministic model can help combine these two regimes to address the fundamental physics that occurs in these small systems with complex membrane dynamics and few molecule situations.

Despite these shortcomings, we have identified several key features of the relationship between dendritic

spine geometry and calcium dynamics. Our findings can be summarized as calcium dynamics in dendritic spines depend on complex relationships between the PM surface area, cytosolic volume, spine apparatus surface area, and the volume enclosed by the spine apparatus. Therefore, while various spine functions are determined by calcium dynamics, calcium dynamics are influenced by a variety of biochemical, geometric, and transport factors. The spatial aspects of calcium dynamics are fundamental towards understanding the downstream dynamics of critical molecules such as CaMKII, the small RhoGTPases (Cdc42, Rho, and Rac), and subsequently actin dynamics in dendritic spine remodeling [1, 4, 56–59]. Going beyond single spine dynamics, the propagation of the downstream mechanochemical activity to neighboring spines is a key step towards integrating single spine behavior to multiple spines, across the dendrite, and ultimately the whole cell [7, 60]. Thus, accounting for the spatial and physical aspects of calcium dynamics is the first step towards deciphering the complex shape function relationships of dendritic spines.

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