Diffusion in a dendritic spine: The role of geometry

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Dendritic spines, the sites where excitatory synapses are made in most neurons, can dynamically regulate diffusing molecules by changing their shape. We present here a combination of theory, simulations, and experiments to quantify the diffusion time course in dendritic spines. We derive analytical formulas and compared them to Brownian simulations for the mean sojourn time a diffusing molecule stays inside a dendritic spine when either the molecule can reenter the spine head or not, once it is located in the spine neck. We show that the spine length is the fundamental regulatory geometrical parameter for the diffusion decay rate in the neck only. By changing the spine length, dendritic spines can be dynamically coupled or uncoupled to their parent dendrites, which regulates diffusion, and this property makes them unique structures, different from static dendrites.

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I. INTRODUCTION

Dendritic spines, the sites where excitatory synapses are made in most neurons of the brain, are considered to be physical microdomains, where crucial chemical reactions, related to synaptic transmission and plasticity, are taking place. Due to their specific geometric shapes, which can be approximated by a head connected through a cylindrical neck to the dendrite, dendritic spines have been considered as a nearly independent calcium compartment [1,2]. Indeed, the spine head contains many key calcium activated molecules involved in the regulation of both the number and the conductance states of ionotropic receptors. Thus spine geometry regulation may provide important clues to synaptic transmission and plasticity. In spite of its intuitive significance, the role of geometry in spine functions is not entirely clear. Recent studies have reported changes in spine geometry in association with functional plasticity [3]. These studies call for a quantitative analysis of the role of spine geometry in the trafficking of ions and proteins or transcription factors. For example, to elucidate the way spine geometry restricts diffusion or regulates the time and the amount of diffusing calcium ions, several groups have attempted to obtain quantitative information about spine dynamics from both experimental methods and theoretical analysis [4–7].

We propose here to analyze the role of spine geometry on diffusing molecules by using a combination of mathematical analysis, experimental data, and numerical simulations. We recently obtained [8–10] an estimation of the mean time a diffusing ion or molecule stays inside a spine and this time can be asymptotically computed from a three-dimensional analysis of the geometry in the limit of a small ratio of the spine neck-to-spine head radii. For a small aspect ratio of neck radius to the spine length (say less than 1/3), the mean sojourn time a diffusing molecule stays inside the total spine or inside the spine neck is exponentially distributed, with a rate that depends on the spine length. We will emphasize here that the mean sojourn time inside the spine neck merely depends on its width, while it influences significantly the sojourn time in the full spine.

II. RESULTS

Intuitive mathematical formulas based on one-dimensional considerations, using electrical analogy, have been used by Svoboda *et al.* [6] to estimate the calcium dynamics [11] and more recently diffusion of inert molecules in spines [12]. Early attempts at the derivation of such formulas were made by Koch and Poggio [13], Wilson [14], and Rall [15]. An explicit formula, derived mathematically from a diffusion model in three dimensions, was first given in Holcman *et al.* [8] and Singer *et al.* [9,10]. It shows significant qualitative differences from previous analysis: the mean sojourn time of a diffusing molecule inside an empty spine of neck length L, longer than the neck radius ε ($L > \varepsilon$) is given by

$$\tau_1 \approx \frac{V}{4D\varepsilon} + \frac{L^2}{2D}, \tag{1}$$

where V is the volume of the spine head and D is the diffusion coefficient. Formula (1) means that the mean escape time of a diffusing particle from a dendritic spine is the sum of the mean first passage time to the entrance of the neck (assuming that a diffusing particle entering the spine neck cannot return into the spine head), plus the mean time to travel through the spine neck. Formula (1) shows that both changes in the volume V of the spine head and neck length L affect the mean diffusion time τ_1. For example, in a spine of length $L=1$ μm, volume $V=1$ μm^3, radius $\varepsilon=0.1$ μm, and diffusion coefficient $D=400$ μm^2/s, we have $\frac{V}{4D\varepsilon}=6.25$ ms while $\frac{L^2}{2D}=1.25$ ms. Thus a calcium ion spends most of its sojourn time in the head. Moreover, when the spine neck is small compared to the radius of the head, it can be shown, using mathematical considerations [9,10], that any transient diffusion process in the entire spine can be fitted by a single exponential of rate $\frac{1}{\tau_1}$. Formula (1) describes the time course of an ion that does not return to the spine head after it enters the spine neck. This situation may represent, for example, a crowded neck entrance occupied by the spine apparatus.

When the neck entrance is not sufficiently crowded to block a Brownian molecule from returning to the spine head, the mean sojourn time inside the total spine $\bar{\tau}_{\text{spine}}$ can be expressed as an infinite sum over all the possibilities to reenter the spine head. Applying Bayes rule, the total sojourn is given by

$$\bar{\tau}_{\text{spine}} = E(\tau|F^0)\Pr(F^0) + E(\tau|F^1)\Pr(F^1) + \cdots,$$

where F^n, $n=0,1,\ldots$, denotes the event that a diffusing molecule starting inside the spine head reenters n times into the spine head before reaching the dendritic shaft where it exits. $E(\tau|F^n)$ represents the mean time the molecule spends inside the spine after exactly n returns into the head. If $p_0=\Pr(F^0)$ denotes the probability of no return, then $\Pr(F^n)=p_0(1-p_0)^n$. We denote by τ_h the mean sojourn time inside the spine head, τ_n is the conditional mean time the diffusing molecule reaches the dendrite for the first time before returning into the head, and τ_r is the mean conditional time a molecule inside the spine neck returns for the first time into the head before reaching the dendrite. Using the relation

$$E(\tau|F^k) = \tau_h + k(\tau_r + \tau_h) + \tau_n,$$

we obtain the expression for the total time

$$\bar{\tau}_{\text{spine}} = (\tau_h + \tau_n)p_0 + \sum_{k=1}^{\infty} [\tau_h + k(\tau_r + \tau_h) + \tau_n](1-p_0)^k p_0$$

$$= \frac{1}{p_0}\tau_h + \tau_n + \frac{1-p_0}{p_0}\tau_r. \quad (2)$$

This computation relies on the following property: when a diffusing molecule reenters the spine head, because the neck radius is small compared to other lengths, after traveling a certain distance away from a boundary layer located at the entrance of the spine head, the diffusing molecules loses its memory of the initial position. To obtain an explicit expression of $\bar{\tau}_{\text{spine}}$, we approximate the spine neck geometry as a one-dimensional interval [H, D], of length L. We define T_x^H and T_x^D as the first time to reach the head and the dendrite, respectively. The probability that a random molecule, starting at position x, enters the head before reaching the dendritic shaft is denoted by $p(x)=\Pr\{T_x^H < T_x^D\}$. It satisfies the equation $p''(x)=0, p(0)=0$, and $p(L)=1$, Karlin *et al.* [16], and the solution is $p(x)=x/L$. Using the probability $q(x)=1-p(x)=\Pr\{T_x^D < T_x^H\}$, the definitions of the conditional mean times $\tau_r(x)=E(T_x^H | T_x^H < T_x^D)$ and $\tau_n(x)=E(T_x^D | T_x^D < T_x^H)$, the functions $v_r(x)=\tau_r(x)q(x)$ and $v_n(x)=\tau_n(x)p(x)$ satisfy, respectively,

$$Dv_r(x) = -q(x),$$

with the boundary conditions $v_r(0)=0, \quad v_r(L)=0,$

$$Dv_n(x) = -p(x),$$

with the boundary conditions $v_n(0)=0, \quad v_n(L)=0.$

The solutions are

$$v_r(x) = \frac{x(x-L)(x-2L)}{6DL}, \quad v_n(x) = \frac{x(L^2-x^2)}{6DL}$$

and thus

$$\tau_r(x) = \frac{x(x-L)(x-2L)}{6DL}\frac{L}{L-x} = \frac{x(2L-x)}{6D},$$

$$\tau_n(x) = \frac{x(L^2-x^2)}{6DL}\frac{L}{x} = \frac{L^2-x^2}{6D}.$$

To approximate the mean sojourn time in a spine head of volume V, we use the results of the narrow escape problem, Holcman *et al.* [8] and Singer *et al.* [9,10], which lead to

$$\tau_h = V/(4D\varepsilon)$$

for a general spine head and to

$$\tau_h = \frac{V}{4\varepsilon D}\left[1 + \frac{\varepsilon}{R}\log\frac{R}{\varepsilon}\right]$$

for a spherical spine head, where we have included in the latter the additional correction term. To derive an explicit expression for formula (2), we now use the previous computations. Due to the presence of a boundary layer of size ε, at the junction between the spine head and the neck, a diffusing molecule will be considered in the neck when it has traveled a distance of the order ε. We approximate the entrance distribution of points at the neck as a Dirac distribution located at position $x=\alpha\varepsilon$, where α should be of order one. We thus obtain the following expressions:

$$p_0 = \frac{\alpha\varepsilon}{L}, \quad \tau_r = \frac{\alpha\varepsilon(2L-\alpha\varepsilon)}{6D} \approx \frac{\alpha\varepsilon L}{3D},$$

and

$$\tau_n = \frac{L^2-(\alpha\varepsilon)^2}{6D} \approx \frac{L^2}{6D}.$$

Finally

$$\bar{\tau}_{\text{spine}} = \begin{cases} \dfrac{VL}{4D\alpha\varepsilon^2} + \dfrac{L^2}{2D} & \text{for a general spine} \\ \dfrac{VL}{4D\alpha\varepsilon^2} + \dfrac{L}{R}\log\dfrac{R}{\alpha\varepsilon} + \dfrac{L^2}{2D} & \text{for a spine with a spherical head.} \end{cases}$$

Estimation of the free parameter α will be discussed below. The addition of binding molecules or a hydrodynamic drift, due to spine twitching, requires another exponential term for the description of the calcium time course (see Holcman *et al.* [17]).

To further analyze the spine neck and the dendrite, we approximate their geometry as thin cylinders. To estimate the effect of spine neck radius, we use an explicit solution of the diffusion equation in a cylindrical geometry. When we use the previous approximation of a cylinder with reflecting lateral boundary, the time course of diffusing molecules, starting uniformly distributed at one side of the cylinder (see Crank [18]) is at first order, the sum of the first two exponential terms in the eigenfunction expansion of the concentration. Indeed, the expansion of the probability density function p is $p(r,z,t) \approx e^{-\lambda_1 t} u_1(z) + e^{-\lambda_2 t} u_2(r,z)$, where the rate constants are given, respectively, by

$$\lambda_1 = \frac{\pi^2 D}{4L^2} \quad \text{and} \quad \lambda_2 = D\left(\frac{\pi^2}{4L^2} + \frac{j_1^2}{\varepsilon^2}\right),$$

where $j_1 = 2.4048$ is the first zero of the Bessel function J_0. This classical result shows that $\lambda_2 > \lambda_1$ and thus the extrusion rate for an empty spine neck depends mainly on the first exponential, which depends, in turn, only on the spine length. Changing the neck radius can affect the diffusion process only through the second exponential, but leaves the first, solely L-dependent exponential term unaffected. For a spine with a neck longer than the neck radius (e.g., by a factor of 3), the spine length is the fundamental regulatory geometrical parameter for the diffusion decay rate.

To confirm these results, we studied diffusion after flash photolysis of a biologically "inert" molecule, fluorescein, at two different dendritic locations. We use here cells, loaded with caged fluorescein, which were not fluorescent initially but became fluorescent under its rapid photolysis. Uncaging methods allowed us to release it from the cage in a local and fast manner. On the other hand, any buffering or extrusion through specific pumps or uptake into stores are not contributing to the dynamics here. Then, the geometry (length, diameter) of the tested object is the only parameter left which may affect the diffusion process. The experiments are designed for fluorescein concentration to be the same in thinner and thicker dendrites due to its loading with a patch pipette. These experiments are in contrast with the ones initiated in Ref. [7] which described the effects of calcium dynamics between the spine head and the parent dendrite. For that purpose NP-EGTA (a caged compound which releases calcium following its photolysis) was used. At that time, we estimated the potential role of the spine neck in calcium extrusion. It was shown there both at an experimental and mathematical level that plasticity of spine neck length is a natural way to isolate longer spines from the dendrite and, on the contrary, to connect shorter ones through calcium removable mechanisms such as calcium exchangers.

Diffusion in a cylindrical dendrite is presented in Fig. 1. Each curve is an averaging result of 20 sequential uncaging trials performed at a rate of 0.1 Hz for each segment. White and gray traces are normalized $\frac{\Delta F}{F}$ conversions related to the thin and the thick dendritic segments. We recall that the ratio $\frac{\Delta F}{F}$ is well used and it represents the "fluorescent" signal well adapted to overcome several limitations of fluorescent microscopy. When an increase in fluorescence is detected, the

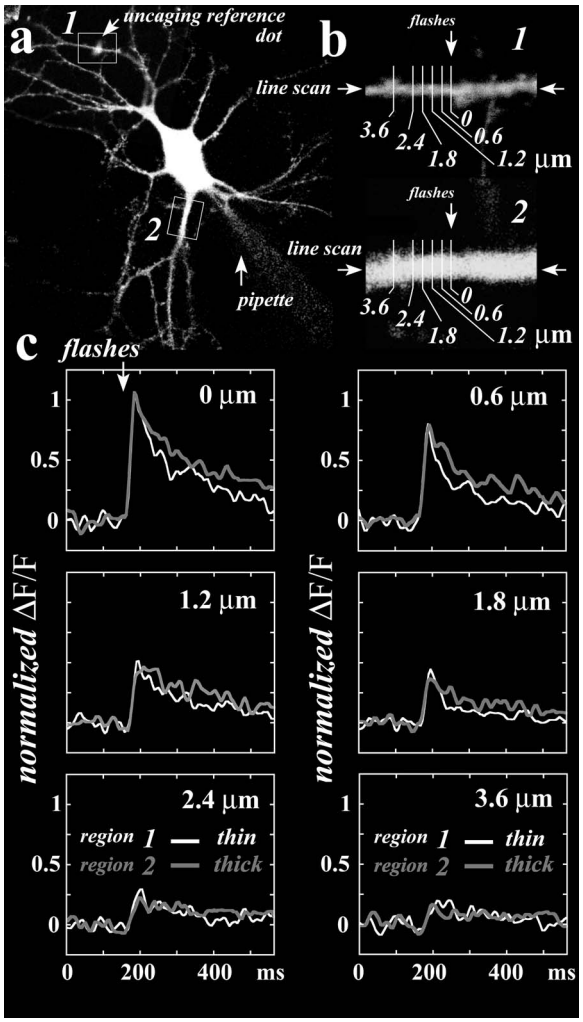

FIG. 1. Comparisons of fluorescence kinetics in thin and thick dendrites following flash photolysis of caged fluorescein, detected with a fast line scan. (a) Cultured hippocampal cell, transfected at 1 week in culture with DsRed plasmid to visualize the dendrites and spines using a lipofectamine 2000™ (Invitrogen) method (for details see Ref. [7]). Image was taken 3 days after transfection using a PASCAL confocal microscope (Zeiss). The helium neon 633 nm laser spot in the middle of box 1 represents the location of the uv (355 nm) flash. White boxes, containing thinner (1) and thicker (2) dendritic segments are shown at higher magnification in (b) 1 and 2. The neuron was patch clamped at the soma with a glass pipette (arrow at the bottom of a) containing 100 μM of caged fluorescein. (b) 1 and 2: thin (1, less than 1 μm in diameter) and thick (2, about 2.5 μm in diameter) dendritic segments, line scanned at a rate of 0.7 ms per line in the middle and along the segment. Measurements were performed at the focus of uncaging (flashes, arrow at 0 μm) and then 0.6, 1.2, 1.8, 2.4, and 3.6 μm apart, as shown in (b). Responses on the left and the right sides of the focus were found symmetric (data not shown). (c) Graphic representations of line scan recordings at the distances, shown in (b).

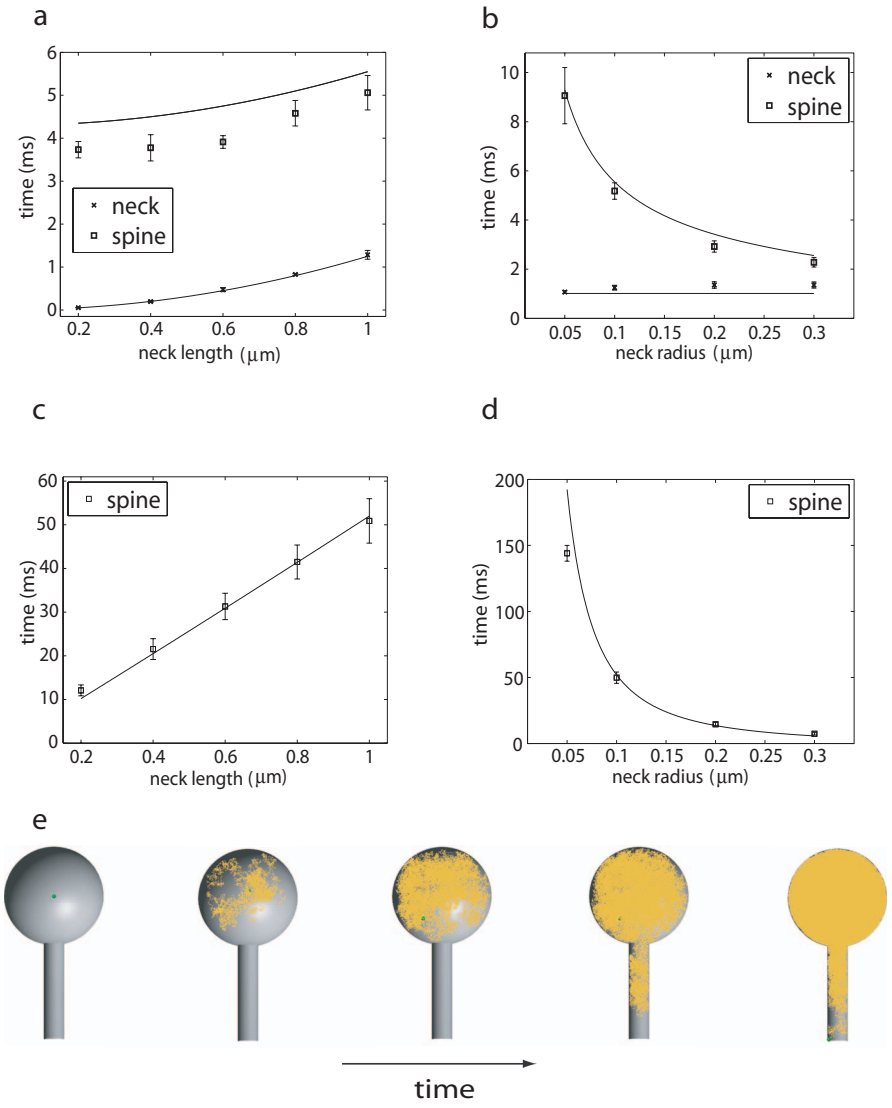

FIG. 2. (Color online) Molecular simulations in a three-dimensional (3D) model of a dendritic spine. The spine geometry is approximated by a spherical head of radius $R=0.5$ μm and a cylindrical neck with radius ε and length L. All particles were initially released from the center of the spine head. (a), (b) Mean sojourn time ($\pm$ standard) of a diffusing molecule in the spine neck and in the spine as a function of the spine neck length (a) and the spine neck radius (b) assuming that no reentries into the spine neck occur after the molecule entered the neck. The theoretical results as predicted by formula (1) are shown as solid lines. (c) and (d) Same settings as in (a) and (b), except that multiple reentries into the spine head were allowed. The theoretical prediction according to formula (2) are shown as solid lines for $\alpha=0.84$ (e) Snapshots of one realization of the Brownian trajectory as traced by one diffusing molecule in a 3D model of a dendritic spine with possible reentries into the spine head ($R=0.5$ μm, $L=1.0$ μm, and $\varepsilon=0.1$ μm). The snapshots are taken at different times $t=0$, 1, 5, 10, and 57.4 ms, where the particle reached the dendritic shaft. Simulation parameters in (a)–(e). Diffusion constant $D=400$ $\mu m^2/s$ (free calcium), time step=1e-7 s, total simulation time: 250 ms.

ratio $\frac{\Delta F}{F}$ allows us to differentiate the signal levels coming from different focal planes. Indeed, signals coming from different focal planes are distorted and this distortion effect can lead to wrong conclusions. The use of confocal microscopy, which takes recordings from relatively thin optical sections (about 1–1.5 μm) partially solves the distortion problem; but when extremely thin biological objects such as dendrites (1 to 2 μm in diameter) or dendritic spines (less than 1 μm) are studied, the confocal imaging seems to be insufficient to compensate for the distortion in thickness, but using $\frac{\Delta F}{F}$ resolves this issue. Using this analysis, we found that the diffusion rates and the spread distances as well as decay times are very similar for both a thin and thick dendrite, with a decay time course being slightly slower in the thicker dendrite. However, this difference was found to be not statistically significant ($n=3$ cells, eight dendritic segment pairs, $p>0.1$). In both dendritic segments, the fluorescence spread was highly restricted. As predicted by the theory above, the diffusion spread is quite insensitive to the neck radius. Similar experiments were performed on dendritic spines where uncaging occurred on the spine head or on the adjacent dendrite (data not shown). We found that the length of the spine neck, but not its neck diameter, affects diffusion between the two compartments. However, due to the limitation of the

optical imaging resolution, the estimation of the spine neck radius is in general problematic, thus we decided not to present these results.

To confirm our results, we use formula (1) to estimate the mean time a diffusing molecule stays both inside the total spine and the spine neck when no reentries into the spine head is possible. For that purpose, we ran 5–10 repetitions (we ran 5 and 10 for large and small radii, respectively) of Langevin simulations (Monte Carlo simulations) of 100 diffusing molecules in a generic dendritic spine for different settings of the spine neck radius and length. Because molecules do not interact, this corresponds to a total of 500–1000 realizations. In each numerical experiment, we plotted the statistics (mean and variance) of the Brownian particles, initially released at the center of the spine head.

The results of the simulations and the theoretical predictions (solid lines) for the mean sojourn time in the neck and spine for different spine neck length L and spine neck radius are shown in Figs. 2(a) and 2(b). According to Fig. 2(b), the mean time to travel across the spine neck does not change significantly with the spine neck radius as predicted by the theory. Thus the neck radius has little influence on the time that a diffusing molecule spends in the spine neck. On the contrary, the mean time in the spine neck depends quadratically on its length, as revealed by the one-dimensional-theoretical formula (1) and as validated by the fit presented in Fig. 2(a).

We next tested in numerical simulations the validity of expression (2). As before, we analyzed the mean sojourn time of a diffusing molecule inside the spine for different values of the spine neck length and the spine neck radius, but now multiple reentries into the spine head were possible [Figs. 2(c) and 2(d)]. The numerical data were analyzed as before. We estimated the parameter α by optimally fitting (least-squares sense) the mean sojourn times in formula (2) resulting in a value of $\alpha=0.84$.

The excellent agreement with the theoretical predictions shows that the formula for $\bar{\tau}_{\text{spine}}$ captures the main geometrical features of the dendritic spine. We conclude that for a diffusing molecule which can return into the spine head after it entered the neck, the mean sojourn time in the spine is drastically changed compared to the situation where no such reentries are possible. Finally, the formula for $\bar{\tau}_{\text{spine}}$ shows that the neck length and the spine radius determine the overall time course of a diffusing molecule inside the dendritic spine.

III. CONCLUSION

We conclude that in the first approximation, changes in the spine neck radius will not affect the diffusion decay of diffusing molecules, contrary to the interpretation proposed in Bloodgood *et al.* [12], which is based on a guessed mathematical formula. This discrepancy suggests that changes in diffusion, induced by physiological conditions, may be due to internal space reorganization, such as to moving organelles. Second, it is surprising to see that the passive decay rate, which is the reciprocal of the mean time a diffusing molecule spends inside a dendritic spine, can be significantly increased (to a factor of 15), when the diffusing molecules may go back inside the spine head (Fig. 2). A diffusing ion may stay up to 150 ms inside a spine if it can go back into the head, while it is less than 10 ms otherwise. These results are compatible with the experimental observations reported in Ref. [6]. It is conceivable that the spine apparatus or any other organelles located at the entrance of the spine neck may regulate this possibility.

In addition to passive geometrical constraints imposed by the spine shape, calcium dynamics, for example, depends on active processes such as calcium pumps, buffers, or stores; but, remarkably, the regulation of spine length may be sufficient to explain the diffusing dynamical coupling between the spine and the dendrite described in Refs. [7] and [12]. Specifically, because the sojourn time of an ion in the neck increases quadratically with the neck length, while keeping the number of calcium exchangers or pumps constant, we propose that for small L, a spine can be completely coupled to the dendrite via diffusion, while for L larger than a critical value, most of the calcium ions are pumped outside or inside the endoplasmic reticulum and thus do not reach the dendrite, as noted in Korkotian *et al.* [7] and Holcman *et al.* [19]. The ratio of pumped ions to those that have reached the dendrite depends on the density and the distribution of exchangers, the diffusion constant, and the extrusion rate. This dynamical role of spines has to be compared to the static role of dendrites, which can also compartmentalize calcium. Indeed, imaging experiments have shown that calcium dynamics in aspiny dendrites can be compartmentalized with the same time scale and over the same space (Goldberg *et al.* [20]) as in spines.

Trafficking of ions or larger molecules in spines is critical for induction and maintenance of plasticity processes. The present analysis quantifies the role of the spine geometry for its function, as a calcium conductor and as a regulatory machine for any diffusing molecules. Specifically, the ability of dendritic spines to regulate their own geometrical shape makes them unique structures different from static dendrites. It is this ability which allows spines to control very efficiently calcium signaling between the spine head and its parent dendrite.

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