Calcium dynamics in dendritic spines, modeling and experiments

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Abstract

Dendritic spines are microstructures, about 1 femtoliter in volume, where excitatory synapses are made with incoming afferents, in most neurons of the vertebrate brain. The spine contains all the molecular constituents of the postsynaptic side of the synapse, as well as a contractile element that can cause its movement in space. It also contains calcium handling machineries to allow fast buffering of excess calcium that influx through voltage and NMDA gated channels. The spine is connected to the dendrite through a thin neck that serves as a variable barrier between the spine head and the parent dendrite. We present a novel modeling approach that is more suitable for the accurate description of the stochastic behavior of individual molecules in microstructures. Using this approach, we predict the calcium handling ability of the spine in complex situations associated with synaptic activity, spine motility and plasticity.

1. Introduction

Dendritic spines consist of two compartments, a spine head where an incoming fiber makes an excitatory synapse, and a cylindrical neck which connects the head to the parent dendrite. The spine volume is extremely small, up to 1×10^{-15} L, meaning that movement of very few ions into the spine head will make a large change in concentration of this ion. Their large number, estimated to be between 0.1 - 3×10^5 spines per neuron, (about 1-3 spines per $1 \mu\text{m}$ of dendrite), drastically increase its surface area [1, 2]. The spine contains all the machinery used for the synapse, including receptors, postsynaptic density (PSD)-95, scaffolding molecules, as well as the contractile molecule actin. In addition, it contains calcium buffers, calcium channels and calcium removal mechanisms. Notably, the spine does not contain mitochondria, the largest calcium buffer in the cell. We review here experimental results on dendritic spine motility, intracellular calcium variations and recent relevant models on calcium dynamics in microstructures. The combination of modeling and experimental approaches reveals the complexity of calcium dynamics in dendritic spines.

Contrary to previous belief, which considered the spines a stable locus of long term synaptic memory, recent studies demonstrated that spines are motile structures, which change their shape continuously [3,4], irrespective of the presence of an attached presynaptic terminal. In fact, it has been observed that spines can shrink momentarily in response to influx of calcium ions [5]. This fast twitch causes a decrease in the spine head volume and may cause an instantaneous increase in intra-spine pressure. The significance of this spine twitch is still not known, but it may affect the functioning of the NMDA receptor, shown to be regulated by hydrostatic pressure [6]. The significance of the larger, spontaneous fluctuations in volume is also not known, but they appear to be more pronounced in idle spines, ie, those that do not receive ongoing afferent activity, whereas when the spine is exposed to a glutamate agonist, AMPA, spine motility ceases [7]. On the other hand, a quiet spine can start to move when the culture is treated with tetrodotoxin, which reduces drastically ongoing network activity [4]. Thus, when a spine receives a synaptic input, it may shrink momentarily, and if this activity continues,

the spine will freeze. Further, enhanced activation of the spine, and an even larger influx of calcium will eventually cause spine collapse into the parent dendrite [8].

Due to the small size of the spine, and the small number of ions that flux into this microstructure, the nature of the calcium interactions with the ambient molecules is stochastic in nature. Coupling non-equilibrium chemical reactions with structural changes of a spine was described recently in [9], where the fast contraction not only modifies the time course of calcium, but changes the final calcium distribution between the stores, the dendritic shaft and the calcium that can be pumped out. Moreover through a modeling approach it has been possible to address questions concerning the rules that govern the change in shape of the spine and, in particular, the neck of the spine, and also on how these rules are implemented in molecular terms.

2. Modeling Microstructures

More than ten years ago, the need to understand at a biophysical level microstructures such as dendritic spines, [1, 10-14], pushed the development of phenomenological models, to study chemical reactions with diffusion in confined geometry. This goal was achieved by coupling the diffusion equation for calcium with the ambient chemical reactions. The modeling techniques were based on compartmentalizing the spine into several subunits, where the calcium diffusion process was presented in a discrete manner and ordinary differential equations described the chemical bonding of calcium to buffer molecules.

The first conclusion from the model proposed by Zador et.al [15] was that dendritic spines influence calcium dynamic: it compartmentalizes calcium and thus can regulate the induction of long term potentiation (LTP), which requires a transient and high local increase in $[Ca^{2+}]_i$. In [3], a Monte Carlo simulation was presented, avoiding the solution of the partial differential equation in complex geometry. More recently, two large computational efforts, namely the Virtual Cell [16] and the MCell [13] provide a computational package where stereotype micro-structures such as a spine can be simulated. A different type of effort has lead to models where the magnitude of

synaptic facilitation can be analyzed as a consequence of buffer saturation mechanism. Indeed, it was found by Matveev et.al [17] that synaptic facilitation can either be obtained as a consequence of saturation of mobile buffers in the entire presynaptic domain or as a local saturation of immobilized buffer. The effect of the geometrical distribution of buffers was analyzed, using a reaction-diffusion equations, representing binding and unbinding of calcium ions entering through some specific domain. In a similar approach, Smith et al.[18] studied, near the entrance of an open channel, the extension of calcium for various regimes when buffers are in excess, diffuse slowly or when buffers and calcium have similar time scales. Finally, using a partial differential equation approach, Volfovsky et.al [19], studied the effect of varying the geometrical spine neck length on calcium dynamics, demonstrating that the magnitude of calcium transient is higher for longer spines than shorter ones.

Modeling fast motility in spines at a molecular level, requires to follow ions and contractile molecules at any moment in time in order to compute the total drift that will add to the random motion of the ions (Holcman et. al. [9]). When a dendritic spine twitches, the volume decreases by up to 10% of its initial value, as indicated by Korkotian and Segal [5]. A stochastic approach is required and only from there, the nonlinear nature of the process can be derived in generalized fluid-reaction-diffusion equations (Holcman et. al. [20]).

One consequence of building models at a molecular level by tracking individual particles, is that the notion of concentration has to be abandoned and a more adequate variable is the number of molecules inside a specific volume, especially if the volume can vary.

3. Modeling calcium dynamics: a molecular approach

Models that follow individual molecules became popular in recent years. The new theoretical approach has been motivated by three main factors:

1. The instrumental resolution imposes a limitation on calcium data. More dramatically, calcium dyes perturb significantly calcium dynamics in spine, by binding directly to the calcium ions,

interfering with the normal calcium dynamic, making the interpretation of the experimental data difficult.

2. Due the low number of ions and molecules involved during calcium dynamics in spines— involving from a few to a few hundreds of ions, the number of calcium bound to relevant molecules becomes a stochastic variables, where fluctuations cannot be neglected. This forces us to keep track of any relevant molecules.

3. Using an approach based at a molecular level, it is possible to derive coarse grained equations which capture some of the dendritic spine functions. A similar approach has been used successfully fifty years ago to derive the transistor equations in electronics.

The last step is relevant if one wants to integrate many spines together on a dendrite. It is still unrealistic to simulate millions of ions together in a dendrite. Furthermore, modeling the change of scale from few particles to many, reveals how a cell integrates molecular information into a physiological behavior. A dendritic spine appears to be an ideal microstructure for calcium modeling, not too small compared to a ionic channel, so that electrostatic interaction can be neglected, reducing a lot the complexity of the interaction, and not too big compared to a dendrite so that the number of molecules to track is of the order of hundreds. A dendritic spine is thus a generic microstructure at an intermediate stage between the discrete and the continuum description.

4. Diffusion in microstructures

Although calcium is a charged ion, its dynamics in the spine is well approximated by a pure diffusion process, ignoring any electrostatical effect. Indeed, calcium ions are shielded by water molecules. Diffusion of ions in a microstructure is modeled at the molecular level by a random walk. When an ion meets the membrane of the cell, it is reflected and can only be absorbed in specific regions of the boundary, like the site of a free pump or at the dendritic shaft. The probability $p(x,t)$ to find an ions at time t at a position x satisfies the standard Fokker-Planck equation [21] and when the ions are considered to be independent, the concentration is defined

using the probability $p(x, t)$, by

$$c(x, t) = Np(x, t), \quad (1)$$

where N is the initial number of ions. Ignoring at this stage the effect of any chemical reaction, the effect of the geometry on the characteristic time scale of diffusion is identify as follow: if Ω denotes the domain of the spine (neck+head) and the boundary decomposes into two parts, $\partial\Omega_a$ the absorbing part, where the pumps and the dendritic are located and $\partial\Omega_r$ the reflective part, then the concentration $c(x, t)$ satisfies the diffusion equation

$$\begin{aligned} \frac{\partial c(x, t)}{\partial t} &= D\Delta c(x, t), \\ \frac{\partial c(x, t)}{\partial n} &= 0 \text{ on } \partial\Omega_r, \\ c(x, t) &= 0 \text{ on } \partial\Omega_a, \end{aligned} \quad (2)$$

where the ratio $|\partial\Omega_a| / |\partial\Omega_r|$ is small. If at time 0, the spine is loaded with N calcium ions, equation 2 describes the calcium dynamics due to diffusion only. The solution of such equation is of the form

$$c(x, t) = \sum_{k=1}^{\infty} e^{-\lambda_k t} a_k u_k(x), \quad (3)$$

where λ_k are the eigenvalues and $u_k, k = 1..$ the eigenfunctions. Due to the specific geometry, where the surface of the absorbing boundary is small compared to the total boundary, the characteristic time scale of the diffusion process is captured by the first eigenvalue λ_1 and the contribution coming from the rest of the spectrum can be ignored [22,23]. By using asymptotic analysis, an estimation of the diffusion time scale is obtained by decomposing the time it takes to exit a spine through the dendrite. It is the sum of duration. First the time τ_1 it takes for an ion

inside the head to find the entrance of the neck and second the time τ_2 it takes for an ion to reach the dendrite. The computation of the first time τ_1 is based on the time it takes for a particle to find a small opening in a domain [22,23] and the computation of τ_2 assumes that the spine neck can be modeled as a one dimensional interval. The mean time it takes for an ion or a group of ions to exit a spine is given by

$$\frac{1}{\lambda_1} = \tau = \tau_1 + \tau_2 = \frac{V}{4D\varepsilon} + \frac{L^2}{2D}, \quad (4)$$

where D is the diffusion constant, V is the volume of the spine head, ε is the radius of the spine neck and L is the length of the neck. For $V = 0.7 \mu m^3$, $D = 400 \mu^2 s^{-1}$, $\varepsilon = .2$, $L = 1 \mu m$,

$$\tau \sim 8 ms. \quad (5)$$

The mean time that a brownian particle exits a typical spine is in the order of 10 ms.

5. Modeling the interaction with molecules

Chemical reactions are described by the backward and the forward binding rate, which are usually obtained in aqueous solution, where diffusion is not limited by space. In transient state, in confined micro-domains where the number of ions involved is small, the binding rates have to be converted into local quantity so that the stochastic nature of the binding and unbinding dynamics is restored and thus can be introduced into the models. Let us recall how the rates are converted:

1. Backward binding rate

The mean time that two molecules react chemically is modeled as the mean time the first molecule stays imprisoned in the potential well of the second. The random time interval between the binding and the reappearance of the binding molecule into the Free State is exponentially distributed with a rate constant equal to the backward binding reaction. The exponentially

distributed waiting time for the backward reaction is based on Kramers' theory of activated barrier crossing, as described by Schuss [21]. Thus for transient chemical reaction, each molecule reacting with a calcium ion has two consequences: one the molecule can become activated and second the time course of calcium is delayed.

2. Forward binding rate

The forward binding rate K_{for} corresponds to the flux of particles to the binding sites. Contrary to the backward binding rate, this rate does not contain local properties only, but includes the effect of the global geometry of the domain, where the chemical reaction occurs. Such a rate has been computed at equilibrium by Smoluchowski and can be converted as the effective radius R_a of a ball that mimic the binding site and so that the average probability that an ion meets such ball is equal to the forward rate. The radius is calibrated according to the formula

$$K_{\text{for}} = 2\pi R_a D [Ca^{2+}], \quad (6)$$

where $[Ca^{2+}]$ is the initial calcium concentration, D diffusion constant. This calibration can also be used to estimate the effective radius of a binding molecule in transient state, calibrated for the initial concentration condition.

3. Effect of buffers stores

Calcium binding molecules can be modeled using the previous forward and binding rate. The distribution of calcium-dependent buffers or actin-myosin influences the time course of transient calcium dynamics, (see Holcman et. al. [9]). Calcium binding molecules such as calmodulin, and calcineurin, contributes significantly to the time scale of calcium dynamics, due the forward binding rate. Moreover, for a diffusion time constant of $\tau = 500ms$, (see Majewska et.a l.[12]), the number of bound N_b made by a calcium ion to free binding molecule (with forward rate k_1) is $\tau = \tau_d + N_b k_{-1}$, where τ_d is the diffusion rate. Thus a simple computation leads that the number of bound particles is of the order of hundreds.

Internal calcium stores, which can release or take-up calcium, as a function of the calcium concentration affect also significantly calcium dynamic in a nonlinear manner, since usually calcium are stored below a certain threshold and release above. The effect of calcium stores has been included in Volfovsky et. al. [19] and Majewska et. al.[12] and contribute to calcium dynamics. However no models in dendritic spine, have included yet the effect of calcium stores at a molecular level. Newer models should include stores but they will have to face the first difficulty, which is to build a realistic model of stores at the molecular level in such a way that the macroscopic behavior can be recovered (Friel [24]).

6. Modeling a general calcium ion trajectory

A calcium ion trajectory is described by the Smoluchowski limit in the large damping approximation [21] of the Langevin equation. If $x(t)$ denotes the position at time t then

$$\gamma \left[\dot{x} - V(x, t) \right] + F(x) = \sqrt{2\varepsilon\gamma} w, \quad (7)$$

Here $\varepsilon = k_B T / m$, T is the temperature, k_B the Boltzmann constant, $\gamma = 6\pi a \eta$ is the dynamical viscosity, where η is the viscosity coefficient per unit mass, a is the radius of the ion. w is random white noise modeling the thermal fluctuation and the electrostatic force is

$$F(x) = -ze \nabla_x U_0(x),$$

where U_0 is the potential created by the site where the proteins are located. In a first approximation, each protein creates a parabolic potential, where the depth can be calibrated by using the backward binding rate and the radius by using the forward binding rate [20]. The frictional drag force, $-\gamma \left[\dot{x} - V(x, t) \right]$, is proportional to the relative velocity of the ion and the cytoplasmic fluid. When $V(x, t)$ is induced by calcium ion, we will see below how to compute such term.

7. Fast Spine contraction

Spine fast contraction is attributed to actin, which is found inside the spine head. As in muscle cells, high concentrations of actin molecules indicate that rapid movement can follow the arrival of calcium ions. It has proposed in [9], that calcium ions set the spine in motion by initiating the contraction of actin as they bind at active sites. Each molecule is assumed to give rise to a local contraction. In a simplified model, all contractions add up to achieve a global contraction. We have neglected the anisotropy contraction due to delay interval between each molecule contraction. Also, the possible effect of the change in pressure on the functioning of NMDA receptors have not been dealt with in the current model.

The details of the mechanism goes as follow: once calcium ions enter the spine, they arrive to the binding sites by diffusion and can bind there. When four calcium ions bind to a single protein, a local contraction of the protein occurs. Adding all local contractions at a given time produce a global contraction and induce a hydrodynamic movement of the cytoplasmic fluid. Calcium trajectory are not any more pure Brownian, but contain a drift and thus the probability to reach the dendritic shaft through the spine neck is increased [9]. The flow field $v(x,t)$ was computed in [9] and result of the contraction of actin/myosin proteins that bind enough calcium (4 calcium ions per protein). In [9], a simulation of calcium dynamics in a spine was presented starting when the ions are already inside the spine head (see figure 2), neglecting the entrance processes through the channels. This simulation excluded the cases when the ions enter through voltage sensitive calcium channels, distributed on the surface of the spine head.

8. What can we learn from a stochastic simulation of calcium in a dendritic spine?

The major benefit of a stochastic simulation is to get access to the total number of biochemical bound induced by calcium on specific molecule (see figure 3b, graph 5) and to quantify the amount of structural changes occurring at the spine level. There are many other consequences: first the hydrodynamics component changes the nature of the ion trajectories, second two periods of calcium dynamics can be identified and finally, in the modeling approach, novel

coarse-grained equations can be derived [20].

Occupation of the space

The geometric characteristics of ionic trajectories with the hydrodynamic are distributed differently from pure diffusion (see figure 2). Not only the nature of the movement is different but the hydrodynamic flow causes the ions to drift in the direction of the neck and consequently the time they spend in the spine head is reduced. As a consequence, the probability of a trajectory to leave through a pump located in the head decreased. Similarly, the probability to return to the head from the spine neck is reduced if it has to diffuse upstream, against the hydrodynamic drag force. Thus the ionic trajectory stays inside the spine a shorter time in the presence of the hydrodynamic flow, as compared to the time without it. As discussed in [9], the total number of bound molecules, to calcium can change to 30 percent with and with out the flow.

Two stages of calcium concentration decay

Two very distinct decay rates of the fast extrusion periods have been reported in [12, 25]. The second decay time constant is identified as purely driven by random movement and the time constant equals the first eigenvalue of the Laplacian on the spine domain, with the adequate boundary conditions.

For the first decay time constant, two theories prevail: the first period corresponds to a fast calcium extrusion, measured by Majewska, et al, [12] with a exponential decay rate $\lambda = 0.14s^{-1}$ and is due to the consequence of the diffusion of saturated buffers, binding kinetics of endogenous buffers, diffusion of buffers, buffer calcium diffusion across the spine neck, and the effect of the pumps. The first decay dynamics was ascribed in [12] to the fast binding to calcium stores.

On the other hand, in the simulation resulting of the model [9] (see figure 3), based on fast spine motility, the first time period has a exponential decay rate, constant $\lambda_t = 0.16s^{-1}$ derived in [20].

The decay seems to be a consequence of the dynamics created by the push effect, since stores were neglected. Further studies that includes large number of buffers, should reveal the precise contribution of buffers to the calcium fast decay rate, as compared to the rate imposed by the spine contraction.

9. Calcium dynamics, fast motility and induction of synaptic plasticity

There are two main consequences of the fast spine contraction that induces a hydrodynamic flow: first, calcium ions are directed where molecule like calmodulin are clustered and this it reduces the fluctuation in the number of bound and increases the probability that a given calmodulin molecule will be bound to a calcium ion. Second the coincidence time between bonds with calmodulin is higher, increasing the probability that the number of bound calmodulin exceed a threshold, which can then activate CaM-KII. This is the mechanism by which calcium concentration controls the induction of synaptic plasticity: small concentration induces long term depression (LTD), while large concentration induces LTP. The induction process starts when a certain number of CaM-KII are activated by calmodulin [25].

Having a direct access to the number of bound proteins, (see figure 3 b5), the magnitude of fluctuation explains why the induction process requires long lasting stimulation. The fluctuation in the number of bound calmodulin is estimated to be around 20 percent around the mean without the effect of the drift but decay to 10 percent when the drift is taken into account[9]. Any fluctuation in the number of activated calmodulin is amplified at the level of CaM-KII, due the low number, from 5 to 10.

10. How calcium gets distributed in spines

According to Nimchinsky et.al. [26] and Sabatini et.al [27], after calcium ions flow inside a dendritic spine, the percentage of ions arriving at the dendritic is negligible, of the order of 1 percent, whereas most of the calcium (70 percent) is extruded in the head, and the rest is stored in calcium stores. The results of the simulation [16], suggest that when the hydrodynamics flow is

ignored, indeed most of the ions (70 percent) are pumped outside the spine head. In contrast, when the hydrodynamics flow is taken into account, the fraction of ions arriving at the dendritic shaft increases significantly (more than 50 percent). It has been suggested that calcium entering through VSCC, distributed inside the spine head and stabilizes the spine. Thus calcium entering through specific channels like VSCC might have a specific regulatory control, different from the calcium entering from the top of the spine head through NMDAr. It is thus conceivable that the organization of buffers, calcium binding molecules and stores lead to various calcium dynamics, depending on the origin of the calcium ions.

Pairing APs with EPSCs in the correct temporal order lead to Ca accumulation, suggesting that various factors modulate calcium dynamics and it is conceivable that calcium ions reaching the dendrite are not the same as calcium ions relayed by calcium stores. There are various ways to produce calcium entry at the dendritic shaft. One way is to conduct calcium from the spine directly and the second is to open VSCC at the dendritic shaft, which can occur faster than the diffusion of calcium, and which is induced by a depolarization inside the spine head.

Finally, the redistribution of calcium depends also on the neck length. By modulating its length, a spine can switch between an isolated to a conducting state with the dendrite. In a conducting state, a significant proportion of calcium ion inside the spine head reaches the dendrite, but in the in isolated state. It is unclear if the calcium arriving to the dendrite has a functional role in that it can activate protein synthesis. Further studies are needed to explore this possibility.

11. Derived coarse grained equations and dendritic spine integration

How is the calcium signal from a single spine integrated into a large population of spines? To identify the collective property of many spines, it is not possible to use only the calcium dynamics in a single one, and thus a coarse-grained continuum description is necessary. This requires the development of new equations to describe calcium in a complete dendrite. The first step of this

program is obtained by a simplified description from the coupled equations described in [20], which give estimates on the decay rates of calcium dynamics in a spine.

The main goal of the coarse-graining is to capture the features of calcium dynamics with a limited number of equations, ideally with a single ordinary differential equation, so that a comprehensive model of calcium dynamics in a spiny dendrite can be derived and the effect of thousands of spines can be integrated and coupled to an action potential.

12. Conclusions

The convergence of theory and experiments allows us to study the function of a microstructure, like a dendritic spine, at a molecular level. Such approach reveals that structural changes are regulated in molecular terms and can be quantified by models. In particular it seems likely that calcium ions induce contraction of actin-myosin-type proteins and produce a flow of the cytoplasmic fluid in the direction of the dendritic shaft, thus speeding up the time course of calcium kinetics in the spine, relative to pure diffusion. Experimental and simulation results reveal two time periods in spine calcium dynamics. Simulations show that in the first period, calcium motion is mainly driven by the hydrodynamics, while in the second period it is diffusion. The ultimate goal of the modeling approach is to be predict the consequence of perturbing the spine, for example by knocking out a gene, and thus to predict the physiologically non-functional spine. Only a statistical model approach capturing the effect of proteins together is relevant, since in vivo, compensatory mechanisms can restore partially the impaired specific function.

A dendritic spine is analogous to a transistor device, which can switch from an isolated to conductor state, as a function of the neck length for the spine. In the case of the spine, the neck length may play the role of control parameter. For that reason, a dendritic spine can be viewed as an active filter.

The way a dendrite integrates depolarization depends on the organization of the synaptic inputs and on the dendritic spines distribution. In particular, how dendritic spines influence the propagation of a calcium wave cannot be addressed, without a model of calcium dynamics in spines. For that purpose the derivation of simplified coarse-grained equations is crucial. In

another direction, diffusion coupled to electrical activity has been neglected in all models. Introducing electrical activity at a molecular level appears to be a challenge of modeling approaches, but can have important implications for addressing questions like how long it takes for voltage gated calcium channels to open, following a depolarization and more generally, how the electrical activity carried by charged ions is coupled to calcium dynamics.

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FIGURE CAPTIONS

Figure 1. Kinetics of calcium decay in spines and adjacent dendrites after a flash photolysis of caged calcium inside the dendritic spine. Cultured hippocampal neurons were incubated with the caged EGTA, together with Fluo-4Am for 1 hour. Red-fluorescent cells were identified and imaged in a confocal laser scanning microscope. (a). Low power image of the red fluorescent cell. (b) High power image of the spine and the parent dendrite, with arrows indicating the scanned line. c&d, fluorescence obtained in the scanned line before and immediately after flash photolysis of caged calcium in the spine head. (c) summary diagram of changes obtained in the spine, the neck and the parent dendrite (color code as in (b), right). (d) the raw fluorescence in the different compartments before and after the flash, shown at time=0. Note that the decay of calcium rise can be fit best with two exponential functions, with time constants of 8.6 and 93.7 in the spine head, and a single exponent with a time constant of 39 msec in the parent dendrite (unpublished observations, Korkotian et al).

Figure 2. The filling of space by 5 random trajectories in the spine with no drift (a), and with drift (b). Each color corresponds to a trajectory. Proteins are uniformly distributed in the spine head, and are represented by circles and crossed circles, respectively. A trajectory starts at the top of the spine head where channels are located and continues until it is terminated at the dendritic shaft or at an active pump.

Figure 3. Dynamics of 100 calcium ions in a dendritic spine. (a) Time evolution of the concentration and binding. First row: concentration vs time (in μ sec). From left to right: 1. $[Ca^{2+}]$ in the total spine. 2. $[Ca^{2+}]$ in spine head. 3. Number of ions in the neck. Note that the neck contains only one ion at a time. 4. Number of bound proteins (type 1 – blue, type 2 – green). Note the stochastic nature of those curves. Second row: from left to right: 5. Number of saturated proteins of type 1 vs time. 6. Arrival times of ions at active pumps: the ions leave one at a time. 7. Number of bound ions vs time. 8. Number of active pumps vs time. (b) statistical analysis after 100 ions have crossed to the dendrite. From left to right, first row: 1. Calcium efflux from the spine vs time (in μ sec). 2. Calcium efflux through pumps vs time. 3. Calcium influx into the dendrite vs time. Second row from left to right: 4. Number of proteins that have bound a given number of ions: only 5 proteins bound 400 ions during the entire time course of the simulation. 5. The abscissa is the numbered proteins: 1 to 50 are the proteins of type 1, 51 to 80 are of the type 2. The ordinate is the number of calcium ions that each protein bound in the simulation.

1 μm