Exploring new roles for actin upon LTP induction in dendritic spines

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Abstract

Dendritic spines, small protrusions of the dendrites, enlarge upon LTP induction, linking morphological and functional properties. Although the role of actin in spine enlargement has been well studied, little is known about its relationship with mechanical membrane properties, such as membrane tension, which is involved in many cell processes, like exocytosis. Here, we use a 3D model of the dendritic spine to investigate how polymerization of actin filaments can effectively elevate the membrane tension to trigger exocytosis in a domain close to the tip of the spine. Moreover, we show that the same pool of actin promotes full membrane fusion after exocytosis and spine stabilization.

1 Introduction

2 Dendritic spines are small protrusions from the dendrites where the postsynap-
3 tic part of most excitatory synapses are located. At the tip of the spines and
4 opposite to the presynaptic bouton, there is a specialized substructure called

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the postsynaptic density (PSD) where receptors and signalling molecules are localized. The AMPA-type glutamate receptors (AMPA-Rs) in the PSD are activated when the presynaptic neuron releases glutamate. The spine depolarizes when these receptors are strongly enough activated, allowing calcium entry via N-methyl-D-aspartate receptors (NMDARs). This triggers an increase in spine size that is associated with an increase in AMPA-receptor-mediated currents and depends on NMDARs, calmodulin, and actin polymerization [1]. Such an increase in AMPA-receptor-mediated current is due to an increment of AMPARs at the synapse by exocytosis or lateral movement [2, 3]. Moreover, it enhances the signal transmission between two neurons, thereby strengthening their synapse in a process called long-term potentiation (LTP). These activity-dependent structural modifications of dendritic spines are thought to be a cellular basis for learning and memory [1].

Spines experience an increase in their volume of $\approx 200\%$, which happens within five minutes after stimulation [1, 4, 5]. In some spines, volume increments of $\approx 50\%$ have been observed after 100 minutes of initial stimulation [1], indicating that the initial increase of spine volume after stimulation is transient and that spine volume stabilizes at a smaller value, that is still larger than the initial value. This volume change is possible due to actin, the principal cytoskeleton component in the spine [4]. Actin is a globular protein (G-actin) that assembles into filaments (F-actin), which are polar structures that undergo a continuous treadmilling process, where G-actin with bound ATP is polymerized at the barbed (+) end of the filament. Furthermore, bound ATP hydrolyzes to ADP promoting depolymerization of G-actin at the pointed (-) end of the filament. Actin binding proteins (ABPs) promote polymerization and depolymerization, as well as branching and severing of F-actin.

Within spines, there is a dynamic equilibrium between G- and F-actin [6].

Moreover, actin filaments are organized in two different pools: a dynamic pool localized at the tip of the spine with fast treadmilling velocity, and a static pool with slow treadmilling velocity at the base of the spine head [7]. After activity induction, a third pool, called the “enlargement” pool, forms [7]. Importantly, actin serves as the main anchoring site for many postsynaptic proteins including NMDA and AMPA receptors [8]. Interestingly, the PSD size correlates with spine size and the PSD grows in spines that show persistent enlargement [9].

Upon LTP, the structural changes in spines can be divided into three temporal phases [5]. During the first phase (1-7 min), spines rapidly expand due to an increase in the concentration of ABPs, such as Arp2/3 and Aip1, that modify F-actin through severing, branching and capping. At the same time, the pool of ABPs, which stabilizes F-actin, is transiently depleted. Thus, during this phase there is an increase in actin polymerization at the barbed ends that elongates F-actin, promoted by F-actin severing, resulting in a profound remodelling of the spine. During the second phase (7-60 min) ABPs that modify F-actin and ABPs that promote F-actin stabilization return to their basal levels, thereby stabilizing the F-actin cytoskeleton. In the third phase (>60 min), the concentration of some PSD proteins within the spine increases. These proteins are translocated to the PSD, which results in PSD enlargement, thereby, recovering the relation between spine and PSD size. Note, however, that some studies also show that different PSD proteins experience an almost immediate increase in their concentration upon LTP [9].

Actin polymerization at the barbed ends near the membrane generates a force that pushes the membrane forward [10] and raises the membrane tension locally [11], which is involved in many cell processes. For example, it controls cell motility by producing the signal that coordinates membrane trafficking (via exocytosis), actomyosin contraction, and plasma membrane area change [12].

Moreover, the membrane tension generated by F-actin polymerization facilitates shrinking of the fusion-generated Ω -profiles during exocytotic events [13]. A recent theoretical model shows that membrane tension is important for maintenance of different spines shapes [14]. However, to our knowledge, the effects of spine enlargement after induction of LTP on membrane tension and its consequences in cellular processes, such as exocytosis, have been neglected both experimentally and theoretically.

Here, we use a theoretical 3D model to investigate how changes in spine shape affect membrane tension. Assuming that an increase in membrane tension acts as a mechanical signal that triggers exocytosis, we examine how actin polymerization induces such an increase by simulating different scenarios. Moreover, we study how actin facilitates membrane fusion during exocytosis, and size stabilization. We find that a discrete F-actin polymerization focus located near the PSD increases membrane tension most efficiently and aids membrane fusion and spine stabilization, suggesting that these events co-localize and result from the same actin pool.

Results

We used a theoretical model, based on [15], in which asymmetric spine shape changes result from an imbalance between a force generated by actin polymerization, that pushes the membrane forward, and the membrane force that counteracts these deformations. To allow for a better measure of the membrane tension, we used a simplified description of the actin dynamics. Moreover, we only considered the actin that is polymerizing close to the spine membrane and, thus, affects its shape.

83 Distribution of F-actin polymerization affects the force gen- 84 erated by membrane tension

85 In the absence of external forces, our modeled spine membrane reaches a resting
86 shape that minimizes the membrane energy ($\mathcal{E}_{mem}$ in Eq. (4), black shape in
87 Fig. 1A). After obtaining the resting shape for a spine, considering experimental
88 data of spine size and PSD surface area in [9, 16] (see Methods for details), we
89 simulated LTP induction by including a force generated by rapid and persistent
90 F-actin polymerization [6]. This corresponds to the first phase of the structural
91 changes in the spine triggered by LTP induction where actin filaments are con-
92 tinually assembled and disassembled [4]. In our model, this is simulated by a
93 continuous force $\mathbf{F}_{actin}$, given by Eq.(2), that pushes the membrane forward
94 and is inversely proportional to the distance between the starting (nucleation)
95 location of the F-actin polymerization focus $\mathbf{f}^i$ and the spine membrane. Thus,
96 the actin polymerization force is greater near the nucleation location and di-
97 minishes as the spine expands. This way, we considered F-actin that branches
98 laterally and has a fast treadmilling velocity.

99 When the membrane deforms due to the actin polymerization force, its en-
100 ergy increases and generates a response force $\mathbf{F}_{mem}$ (Eq. (3)). Figure 1A shows
101 the resulting spine shape in gray. In our simulations, we tracked $\mathbf{F}_{mem}$ and each
102 of its components at different locations along the spine membrane, in particular
103 the work needed to increase the surface area (dots in Fig. 1C). This way, we
104 captured the membrane force generated by tension (tension force) and studied
105 its spatial distribution and evolution over time and whether it depends on the
106 number of actin foci. This method to approximate the membrane tension force
107 resembles experiments that use optical tweezers to calculate tether force [12] in
108 the sense that both obtain the force generated by the displacement of a reference
109 point. In [12] the membrane tension is proportional to the square of the tether

110 force, but here we did not attempt to calculate this relationship and, instead,
111 reported the tension force derived from Eq. (3). During these simulations, the
112 PSD and neck size remained unchanged (yellow dots in Fig. 1A), as observed
113 in experiments within this temporal interval [4, 5, 9].

114 First, we investigated spine expansion and membrane tension force under
115 different scenarios of F-actin polymerization locations upon LTP induction. To
116 allow comparison between these scenarios, $\mathbf{F}_{actin}$ (Eq. (2)) was multiplied by a
117 constant ϕ , that is inversely proportional to the number of polymerization foci.
118 Thus, the force generated by actin polymerization was distributed among the
119 foci.

120 We found that when actin polymerization accumulates in one location near
121 to the PSD, the membrane tension force increased 2.5 fold at that location
122 at time $t^* = 89.5$ seconds. Thereupon, the spine volume is $v^* = 0.1970 \text{ m}\mu^3$,
123 growing by $\approx 125\%$. Spines with more F-actin polymerization foci also increased
124 their volume to v^* , but at different times (Fig. 1D). Because $\mathbf{F}_{actin}$ distributes
125 among the polymerization foci, the time difference to reach the same volume
126 is related to the foci position within the spine. When there are enough foci to
127 be evenly distributed (11 or 22), the spine volume increases similarly, as shown
128 by the purple and green traces in Figure 1D. If there a few foci, two of them
129 could be close enough to cooperate, resulting in a fast enlargement, as in the
130 case with 3 foci. This highlights the complex interaction between $\mathbf{F}_{mem}$ and
131 $\mathbf{F}_{actin}$ in our model that emerges from the spine morphology.

132 Figure 1C shows spines at t^* for different number of foci. Note that spines
133 with more foci increased their volume and the force generated by the membrane
134 tension more isotropic. The histogram of this force, measured by the tracking
135 points when they reach a volume of v^* (Fig. 1B), reveals that only in the spine
136 with one focus it is greater than 2 pN. Moreover, Figure 1E shows that the

137 evolution of the membrane tension force over time, measured at the tracking
138 point with the maximum tension force value at v^* , is lower for spines with more
139 than one focus.

140 When studying the evolution of the distribution of the tension force among
141 the tracking points, we observed that at the start of the simulation 60.61%
142 of the tracking points measured a force less than 0.6 pN (black bars in Fig.
143 1B). Different from this, after t^* only 42.46% of the points in the spine with
144 one focus measured less than 0.6 pN (blue bars in Fig. 1B). Thus, the tension
145 force increases at most locations and has a total increase from 210.6736 pN, at
146 the start of the simulation, to 280.6355 pN at t^* . Spines with more foci also
147 increase the measured tension force at the tracking locations, but the resulting
148 distribution resembles a normal distribution whilst the distribution of the spine
149 with one focus is skewed. Moreover, the sum of the tension force measured
150 at the spines with more than one focus, when they reached a volume of v^* , is
151 less of that of the spine with one focus (272.71 pN, 275.1510 pN, 274.6277 pN,
152 278.8661 pN for spines with 3, 6, 11 and 22 foci, respectively) .

153 We conclude that after LTP induction, the force generated by membrane
154 tension shows a bigger increase when F-actin polymerization concentrates in a
155 specific location than when it is evenly distributed over the spine. Importantly,
156 this is not an effect of the dependency of the number of foci on the parameter ϕ
157 of Eq. (2), as shown in Supplementary Figure S1, which shows the evolution of
158 a spine with one or 22 foci with the same value of ϕ . Since membrane tension
159 serves as a mechanical signal for exocytosis [12] and the force generated by
160 the membrane tension is proportional to the membrane tension, these results
161 are in line with experimental observations of a defined exocytic domain in the
162 spine [17].

Figure 1: Spine enlargement upon LTP for different number of F-actin polymerization foci. **A**, spine resting shape (black), and spine shape after LTP induction (gray). The membrane deformation results from an imbalance between a force exerted by F-actin polymerization (F_{actin} , magenta arrows) and a membrane force (F_{mem} , cyan arrows). The blue dot signals the location of the F-actin polymerization focus. This plot shows the $y-z$ axis for $x = 0$. **B**, histogram of the distribution of the force generated by membrane tension measured at the tracking points when the spines reach a volume equal to v^* . Colors denote the number of polymerization foci distributed in the spine for each simulation. The distribution of the membrane tension for the resting shape, used at the beginning of the simulations, is shown in black. Blue arrow signals maximum tension. **C**, spines shapes (gray) at time t^* with different number of F-actin polymerization foci. Dots are the tracking points color-coded for the force generated by the membrane tension. **D**, spine volume evolution over time, color-coded by the number of polymerization foci. Note that the traces for 11 and 22 foci are very similar. Dotted black lines denote t^* and v^* . **E**, evolution of the membrane tension for the tracking point with maximum membrane tension when the spine reaches a volume v^* .

163 **Position of the F-actin polymerization focus affects mem-** 164 **brane tension**

165 Next, we investigated whether the location of the polymerization focus affects
166 the force generated by membrane tension. We compared the spine shape result-
167 ing from the polymerization focus near the PSD (Fig. 1A) with those having
168 the focus in the middle of the spine head or near the spine neck. Figure 2A
169 shows that the spine shapes, when they reached a volume of v^* , are different,
170 albeit having the same volume. Spines with the polymerization focus far from
171 the PSD reached this volume faster (Fig. 2C). However, Figure 2B shows that,
172 when the spines reach a volume of v^* , the maximum tension force, measured
173 by the tracking points, is higher for the spine with the focus near to the PSD.
174 Moreover, the sum of these forces along the tracking points is also higher in such
175 a spine (280.6355 pN, compared to 275.1624 pN and 278.7298 pN in the spine
176 with the focus in the middle and near the neck, respectively). Likewise, the
177 evolution of the tension force, measured at the tracking points corresponding to
178 the highest tension force in the spines with volume v^* , shows higher values for
179 the spine with the polymerization focus near to the PSD (Fig. 2D).

180 Note that when the polymerization focus is near the PSD, the membrane
181 stretches more, due to the immobility of the PSD. Although the neck was also
182 fixed, the effect on the force generated by the membrane tension is lower, because
183 the neck surface area is smaller. Taking everything together, the force generated
184 by the membrane tension reaches a higher value when the F-actin polymerization
185 focus is near the PSD, indicating that the exocytic domein locates therein, as
186 observed in [17].

Figure 2: **Spine enlargement upon LTP for different locations of the F-actin polymerization focus.** **A**, spine shapes for different locations of the F-actin polymerization focus (dots) when they reached a volume of v^* , color-coded as in **B**. This plot shows the $y - z$ axis, for $x = 0$. **B**, histogram of the distribution of the force generated by membrane tension measured at the tracking points when the spines reached a volume of v^* (this occur at different times, see **C**). **C**, spine volume evolution over time, color-coded by the number of polymerization foci. Dotted black line denotes v^* . **D**, the evolution of the membrane tension for the tracking point with maximum membrane tension when the spine reaches a volume of v^* .

F-actin polymerization promotes membrane fusion upon exocytosis

We have shown in our model that when F-actin polymerization concentrates at a certain location near the PSD, it rapidly elevates the force generated by the membrane tension, which can serve as a signal for exocytosis [12]. In the spine, exocytosis is important for AMPARs and membrane trafficking [2, 17, 18]. To achieve membrane fusion and cargo release, vesicles dock to the membrane and form an Ω -shaped structure [13] that has to shrink and merge with the membrane. In our model, shape deformations, like the Ω -profile, generate a response force in the membrane $\mathbf{F}_{mem}$ that restores it to the resting shape. However, Wen *et al.* [13] show that F-actin polymerization mediates Ω -profile merging. Therefore, we investigated two scenarios for Ω -profile shrinking, namely, shrinking resulting only from $\mathbf{F}_{mem}$ dynamics and shrinking aided by F-actin polymeriza-

tion.

In the first scenario, we assumed that, when exocytosis starts, myosin contracts and pulls the F-actin away from the membrane, as observed in [12]. Consequently, the force generated by actin polymerization ceases. Experimental data shows that this is possible in spines, since myosin II activates upon LTP induction and is required for stabilization of synaptic plasticity [19]. Therefore, we set $\mathbf{F}_{actin} = \mathbf{0}$ and observed that the Ω -profile depicted in Figure 3A (highlighted in magenta) shrinks fully within 30 seconds (see yellow shapes in Fig. 3D), decreasing spine volume and surface area (Figs. 3B-C). However, exocytosis provides membrane to the spine for enlargement upon LTP [17, 18]. Therefore, there must be an increase in surface area of the spine membrane.

Consequently, we considered a second scenario where actin filaments remain close to the membrane and polymerize. Hence, they generate a polymerization force (i.e. $\mathbf{F}_{actin} \neq \mathbf{0}$) from a focus at the tip of the Ω -profile (blue dot in Fig. 1A). Within 10 seconds, the spine membrane surface area returned to its initial value (Fig. 3C), indicating a possible full merge of the Ω -profile, which supplies membrane. The initial decrease in membrane surface area is transient and could be due the distribution of F-actin. Note that during the simulation, the spine volume increases (Fig. 3B) and the Ω -profile shrinks completely. To test whether the location of the actin focus affects the reduction of the Ω -profile, we ran a simulation with the polymerization focus located next it (orange dot in Fig. 3A). In this case, the volume and surface area of the spine increase (Fig. 3B-C), and the Ω -profile shrinks (Fig. 3D). However, the increase is less than for the previous case and the spine membrane is slightly pushed aside.

These results suggest that actin polymerization is needed for membrane insertion resulting from the full merge of the Ω -profile formed upon exocytosis. Moreover, they indicate that the pool of F-actin that polymerizes and elevates

the membrane tension to trigger exocytosis is involved in promoting Ω -profile shrinkage and the full fusion of the vesicle with the spine membrane. Hence, we speculated that there is a pool of F-actin that translocates to a focus near the PSD upon LTP and pushes the membrane forward, which elevates the force generated by the membrane and triggers exocytosis (see Fig. 4). Then, this same pool promotes the shrinkage of the Ω -profile generated when the vesicle docks to the membrane, and hence, full fusion.

Figure 3: Exocytosis with and without the aid of F-actin polymerization. **A**, dendritic spine membrane after fusion with a recycling endosome. The invagination, highlighted in magenta, is the Ω -profile formed after the fusion event. The blue dot represents the F-actin polymerization focus. **B**, spine volume evolution over time, color-coded depending whether the Ω -profile merging is aided by F_{actin} or not. **C**, spine area surface evolution over time, color-coded as in B. **D**, snapshots taken at different times after initiation of spine membrane fusion with the recycling endosome, color-coded as in B.

Figure 4: Actin re-organization upon LTP. **A**, before LTP induction, actin distributes in a stable and dynamic pool (dark and light blue dots, respectively). Spontaneous shape fluctuations (light blue lines) result from polymerization of the dynamic pool that organizes in distinct foci [15,20,21]. Note that only actin polymerizing close to the membrane can push the membrane forward. **B**, ≈ 1 min after LTP induction, actin rapidly assembles and disassembles. Actin polymerizes at a single location near to the PSD, elevating the force generated by the membrane tension, which triggers exocytosis of the recycling endosome. The initial spine shape is in gray. **C**, actin polymerization promotes full fusion of the Ω -profile formed after the docking of the recycling endosome with the spine membrane. **D**, after completing exocytosis, the spine stabilizes. There is an increase in the AMPARs and spine size. Also, the membrane from the recycling endosome (in green) is merged with the spine membrane. Note that the spine enlargement occurs where the polymerization focus of A was located (highlighted in red).

234 Role of the F-actin polymerization focus for spine stabiliza- 235 tion

236 Next, we studied whether the F-actin pool, responsible for triggering and com-
237 pleting the exocytosis events, also accounts for spine stabilization. We hypoth-
238 esized that after exocytosis, F-actin no longer polymerizes at a fast rate close to
239 the spine membrane. This could be due to the contraction of F-actin promoted
240 by myosin [12] or due to changes in ABPs, namely, an increase in proteins that
241 promote F-actin stabilization and the switch in cofilin function: from severing
242 F-actin to the forming of stable filaments [5].

243 In our simulations, we increased the radius of the PSD at a rate of Δ_{PSD} each
244 time-step, and stabilized the F-actin, corresponding to spine enlargement within
245 this radius, by fixing it at a height of h_{PSD} (see Methods). Figures 5A-B show
246 the increase in the PSD surface area and the evolution of the spine volume from
247 an initially expanded shape (similar to that in gray in Fig. 1A) for different

values of Δ_{PSD} . Note that larger values of Δ_{PSD} reach a higher maximum PSD surface area faster. However, eventually, the PSD surface area settles to a lower value that depends on Δ_{PSD} . Here, the fluctuations in the evolution are an artifact of the mesh approximation to the membrane. We speculate that the decrease on the PSD area is a consequence of spine shrinkage due to the membrane force that moves the vertices, corresponding to the enlarged PSD, away from h_{PSD} .

Increasing the PSD area leads to an increase in spine volume, the higher Δ_{PSD} the bigger the spine (Fig. 5B). The difference between spines with and without the proposed F-actin stabilization is shown in Figures 5C-D. After seven minutes, the spine without F-actin stabilization (in orange) reduces its initial volume by 60.56% whilst the spine with F-actin stabilization (in blue) decreases by 52.99% with a PSD increase of 42.87%. At this point, the proportion between spine head volume and PSD size, proposed by Arellano *et al.* [16], is kept: PSD size ($0.1030 \mu\text{m}$) $\approx 0.88 \times$ volume ($0.88 \times 0.1122 \mu\text{m}^3 = 0.0987 \mu\text{m}^3$).

Therefore, our simulations show that the F-actin promoting full membrane fusion in an exocytotic event, can be stabilized and serve as anchoring place for PSD proteins. Although the stabilization of this pool of F-actin leads to a asymmetrical growth of the PSD, it does not perturb the shrinkage of the spine. We assumed that the F-actin stabilization occurs at a faster rate than published measures on increase in PSD proteins concentrations [5,9]. Further experiments are needed to test whether this is true in the spine.

Discussion

We exploited a mathematical model of dendritic spines to test various hypotheses regarding the role and distribution of F-actin polymerization after induction of LTP. Our model operates in a physiological temporal and spatial regime; how-

Figure 5: **Stabilization of F-actin focus.** **A**, PSD surface area over time for different increments of Δ_{PSD} , color-coded as in **B**. **B**, spine volume evolution over time for different increments of Δ_{PSD} . **C**, top view of a spine after 7 minutes of increasing the PSD size (blue, $\Delta_{PSD} = \delta_s/60$) and without changing the PSD (orange). Inset shows only the mesh corresponding to the PSD. **D**, front view of the spines in **C**.

ever, an exact fitting would require additional experimental data, which are currently not available. We adopted a framework where we considered membranes as a two-dimensional elastic continuum [22] without accounting for lipid dynamics [23]. Moreover, we implemented a continuous force generated by actin polymerization rather than simulating the stochastic dynamics of actin [15, 21, 24]. With this framework, we were able to quantify spine enlargement and local membrane tension force. Unlike other 3D models of dendritic spines [14, 25], our model allows for asymmetries that prove to be crucial for membrane properties, such as the force generated by the membrane tension.

Our results indicate that F-actin polymerization increases the force generated by membrane tension and that such an increase is faster for a single focus of polymerization of actin filaments within the spine than for a spine with multiple foci. Moreover, this force is higher if the focus is located near the PSD.

287 Since membrane tension serves as a signal for exocytosis [12], these results are
 288 in line with experimental data that show a specialized zone near the PSD for
 289 exocytosis of recycling endosomes containing glutamate receptors [17]. These
 290 experiments demonstrate that exocytosis is abrupt and massive, and it occurs
 291 in an all-or-non fashion in a zone defined by t-SNARE syntaxin 4 [17], but they
 292 did not investigate the effects on membrane tension. However, Kliesch *et al.*
 293 showed that membrane fusion efficiency mediated by SNARE proteins increases
 294 with membrane tension [11] and Grafmüller *et al.* suggested that tension has
 295 to increase to start vesicle fusion [26]. Therefore, we propose that t-SNARE
 296 syntaxin 4 and an increase in membrane tension, due to actin polymerization,
 297 have to co-locate to trigger exocytosis in spines.

298 Each step of exocytosis is aided by the actin cytoskeleton in different ways
 299 [27]. Actin also executes different functions depending on the vesicle size, which
 300 dictates the duration of the exocytotic event [27]. Here, instead of modeling all
 301 the exocytotic phases, we investigated whether actin polymerization induces a
 302 full merge of the Ω -profile. Since exocytosis allows the membrane to achieve
 303 spine enlargement [18], this full merge is important. We found that, indeed,
 304 surface area and volume increase when actin polymerization is present. Further
 305 studies could consider a more detailed description of the force performed by
 306 actin filaments, like in previous studies of endocytosis [28].

307 Our model shows that the F-actin pool, responsible for increasing the spine
 308 size, and the force generated by the membrane tension also promotes full merge
 309 of the Ω -profile. Therefore, we investigated whether this pool could account for
 310 spine stabilization, when it is not longer polymerizing at a fast rate. For this, we
 311 assumed that F-actin slowly stabilizes and serves as a scaffold for proteins and
 312 receptors at the PSD. We found that without this mechanism, the spine shrinks
 313 to its original size. Therefore, the here-proposed F-actin pool, that needs to

polymerize at a single location close to the PSD to elevate the membrane tension and trigger exocytosis, acts as the “enlargement pool” proposed by Honkura *et al.* [7]. Furthermore, this actin may act as a synaptic “tag” that captures newly synthesized LTP-related proteins [29, 30].

In conclusion, using our model we were able to investigate in detail the relationship between cell morphology, mechanical properties and LTP-induced events in the dendritic spines. This is a first step towards an in silico structural model of LTP, that can be extended to include a more accurate description of actin filaments and lipid dynamics. Moreover, further work can link to AMPAR dynamics, as described in [31, 32].

Methods

Model

Following our previous work [15], we use a 3D mesh of $n_{vertices}$ vertices at positions $\mathbf{x}^k = (x^k, y^k, z^k) \in \mathbb{R}^3$, $k \in \{1, 2, 3, \dots, n_{vertices}\}$ to represent the spine membrane. At each time-step Δ_t , the vertices move according to

$$\frac{d\mathbf{x}^k}{dt} = \zeta (\mathbf{F}_{mem}(\mathbf{x}^k) + \mathbf{F}_{actin}(\mathbf{x}^k)), \quad (1)$$

where $\mathbf{F}_{actin}$ is the force generated by F-actin polymerization, given by

$$\mathbf{F}_{actin}(\mathbf{f}^i, \mathbf{x}^k) = \frac{\alpha \phi(n_f)}{d(\mathbf{f}^i, \mathbf{x}^k)} \mathbf{V}_{(\mathbf{f}^i, \mathbf{x}^k)}, \quad (2)$$

with $d(\mathbf{f}^i, \mathbf{x}^k)$ representing the distance between the i th actin polymerization focus initial position $\mathbf{f}^i$ and the k th mesh vertex $\mathbf{x}^k$. We assume that F-actin extends and branches from this location. Here, $\mathbf{V}_{(\mathbf{f}^i, \mathbf{x}^k)}$ is the normalized direction vector from the focus to the mesh vertex, α is the strength of F-actin

influence and $\phi(n_f) = \frac{n_{fil}}{n_{vertices_0}n_f}$ is a constant proportional to the number of
 F-actin n_{fil} observed experimentally [25] and inversely proportional to the num-
 ber of actin nucleation locations n_f and the number of vertices of the “resting
 shape” $n_{vertices_0}$ (in black, Fig. 1A).

In Eq. (1), ζ is a proportionality constant and $\mathbf{F}_{mem}$ is the force generated
 by the membrane, given by

$$\mathbf{F}_{mem}(\mathbf{x}^k) = -\frac{\partial \mathcal{E}_{mem}}{\partial \mathbf{x}^k}, \quad (3)$$

where

$$\mathcal{E}_{mem} = P \oint dV + \sigma \oint dA + 2\kappa \oint dH^2 \quad (4)$$

represents the membrane energy. Here, P is the difference between internal and
 external pressure, V the volume, σ the surface tension, A the surface area, κ
 the bending modulus, and H the mean curvature. P , σ and κ are constant
 parameters that depend on the cell type [33]. The last term of Eq. (4) cor-
 responds to the bending energy, described by Helfrich [34–36], which increases
 when the membrane is deformed and induces forces that return the membrane
 to its resting shape. The first and second term in Eq. (4) correspond to the
 volume and surface area constraints, respectively. See Figure 1A for a sketch of
 these forces and [15] for details of the calculations.

Implementation

As in [15], we run the simulations in MATLAB on a desktop computer. At each
 time-step, Eq. (1) is solved using a classical Runge-Kutta algorithm. Import-
 antly, for numerical accuracy, the mesh has to be isotropic and conserve the
 number of neighbors of each vertex. Hence, remeshing is needed. For this we
 use the `remeshing.m` function in [37], that is based on Openmesh [38], with

three iterations and a target length of δ_s . Parameters for the simulation are given in Table 1.

The resting shape (black mesh in Fig. 1A) is found as in [15]. In short, from a sphere with radius r_s , the points corresponding to the neck and PSD are fixed to h_{neck} and h_{PSD} , respectively, and $\mathbf{F}_{actin}$ is set to $\mathbf{0}$. We let the shape evolve until it settles. In this resting shape, the tracking points are selected to be isotropic with edge length $2\delta_s$. At each time-step, each term of $\mathbf{F}_{mem}$ (Eq. (3)) is calculated for these points. Particularly, for each tracking point $\mathbf{x}^j$, the force generated by the membrane tension is given by $-\frac{\partial}{\partial \mathbf{x}^j} \left(\sigma \oint dA \right)$. Therefore, our measure of the force generated by tension is equivalent to the mechanical work needed to increase the surface area of the spine. To compare the force generated at different locations, we take its norm $\|\cdot\|$. The location of the F-actin polymerization foci are selected by either choosing a vertex of the resting shape and scale it 99% or by remeshing a scaled version of the resting shape (99%) with a larger vertex length. This way, the foci are equally distributed within the spine.

For the stabilization of F-actin, at each time-step, the PSD radius increases at a rate of Δ_{PSD} and F-actin corresponding to spine enlargement within this radius is stabilized. This is done by fixing the mesh vertices that are within the increased radius in the $x - y$ coordinates with $\|z - h_{PSD}\| \leq 0.001$ to a height of h_{PSD} . This allows to asymmetrically fix the PSD at the locations where the spine experience growth. Selecting a larger tolerance for the z value leads to a more isotropic enlargement of the PSD.

Code Availability

Custom computer code used to generate the findings of this study will be made available in Github.

Table 1: **Model Parameter Values.**

Symbol	Unit	Definition	Value	Source
Δt	s	length of the time-step	1/8	[15]
r_s	μm	initial spine radius	0.4	To match measures in [16]
δ_s	μm	target length of and edge	0.03	[15]
r_{neck}	μm	neck radius	0.0796	[16]
r_{PSD_0}	μm	initial PSD radius	0.1744	To match measures in [16]
h_{neck}	μm	value for fixing the neck	-0.3920	Calculated as in [15]
h_{PSD}	μm	value for fixing the PSD	0.36	Calculated as in [15]
P	$\text{pN}\mu\text{m}^{-2}$	difference between internal and external pressure	75	Calculated as in [15]
σ	$\text{pN}\mu\text{m}^{-1}$	surface tension	15	[15]
κ	$\text{pN}\mu\text{m}$	bending modulus	0.18	[15]
α	pN	strength of filament influence	3.8	[15]
ζ	$\mu\text{m}^2\text{s}^{-1}\text{pN}^{-1}$	strength of force update	0.004	[15]
n_{fil}	1	number of actin filaments in the spine head	70	[25]
δ_s	μm	target length of an edge	0.03	[15]
Δ_{PSD}	μms^{-1}	PSD increasing rate	$\delta_s/60$	-

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Author Contributions

MB-Q performed research and wrote the first draft. MB-Q and FW contributed to the study concept. FW edited the paper.

Competing Interests Statement

The authors declare no competing interests.

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