Mechanical principles governing the shapes of dendritic spines

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Abstract Dendritic spines are small, bulbous protrusions along the dendrites of neurons and are sites of excitatory postsynaptic activity. The morphology of spines has been implicated in their function in synaptic plasticity and their shapes have been well-characterized, but the potential mechanics underlying their shape development and maintenance have not yet been fully understood. In this work, we explore the mechanical principles that could underlie specific shapes using a minimal biophysical model of membrane-actin interactions. Using this model, we first identify the possible force regimes that give rise to the classic spine shapes – stubby, filopodia, thin, and mushroom-shaped spines. We also use this model to investigate how the spine neck might be stabilized using periodic rings of actin or associated proteins. Finally, we use this model to predict that the cooperation between force generation and ring structures can regulate the energy landscape of spine shapes across a wide range of tensions. Thus, our study provides insights into how mechanical aspects of actin-mediated force generation and tension can play critical roles in spine shape maintenance.

Keyword Lipid bilayer, Dendritic spines, Membrane-actin interactions, Deviatoric curvature.

1 Introduction

Dendritic spines are small, bulbous protrusions along the dendrites of neurons that occur at postsynaptic glutamatergic synapses [1–3]. They respond to a glutamate release by orchestrating a series of biochemical and biophysical events that span multiple spatial and temporal scales [4–6]. Spine morphology is tightly coupled to synaptic function, with larger spines tending to represent stronger synapses [7, 8] due to their greater surface expression of functional glutamate receptors. Synaptic activity regulates spine shape and volume. For example, several forms of physiological synaptic plasticity, such as long-term potentiation (LTP) and long-term depression (LTD) are associated with spine enlargement and spine shrinkage, respectively [9–11]. Although average spine volume is approximately 0.1 femtoliter, the shape and volume of dendritic spines are highly variable, depending both on the developmental stage and a combination of genetic and environmental factors, including the prior history of activity [12–15]. Moreover, spine morphology is highly dynamic on the scale of seconds to minutes, due to a dynamic actin-based cytoskeleton [2, 16].

Despite their broad range of morphological features and highly dynamic nature, dendritic spines can be classified into four broad categories. Spines in the mature nervous system are typically classified as being stubby, thin, or mushroom-shaped [17, 18] (Fig. 1A). These categories of spines can be identified in electron micrographs as postsynaptic structures connected to presynaptic nerve terminals. Stubby spines are short and wide, and lack a discernible neck. Such spines

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appear early during synaptogenesis and may represent an emerging spine, but they also might result from spine shrinkage driven by physiological or pathological conditions (Fig. 1A) [4, 12, 19].

The adult mammalian brain is dominated by either thin or mushroom-shaped spines. Thin spines have a long thin neck that is connected to a small bulbous head (Fig. 1A) [12]. Within the head is the postsynaptic density (PSD), an area just beneath the synaptic plasma membrane containing a high concentration of glutamate receptors, scaffolding molecules, and other proteins essential for postsynaptic function. Thin spines have flexible structures that allow them to adapt their morphology based on different levels of synaptic activity [20, 21]. It has been proposed that thin spines are “learning spines,” because they display a high capacity for expansion and strengthening via insertion of new AMPA-type glutamate receptors into the PSD, which is the key basis for synapse strengthening [20–24]. Compared to thin spines, mushroom-shaped spines have a shorter neck and a greatly expanded head (Fig. 1A) [12]. Mature mushroom-shaped spines are more likely to be stable for months to years [20, 21, 24–26], with slower turnover, and are associated with strong synapse functionality, as they contain on average higher concentrations of AMPA-type glutamate receptors. Such spines have therefore been called “memory spines”, in the sense that their potentiated strength reflects a history of high activity and thus “memory” storage, yet their capacity for further potentiation may be near saturation [22–24, 27–29]. Table 1 provides the reported dimensions for different shape categories of dendritic spines observed in hippocampal neurons [18, 30–33].

In addition to synapse-bearing spines, the fourth category of spine-like protrusions is dendritic filopodia. These are commonly observed during early development, and are thought to facilitate the pairing of presynaptic and postsynaptic glutamatergic sites during synaptogenesis by spatially scanning the neuropil volume for a partner axon [19, 34–36]. Thus, a fraction of these “protospines” become synapse-bearing spines if they come into contact with and are stabilized in partnership with presynaptic nerve terminals [34, 37]. Filopodia are long (>2 µm) and thin (< 0.3 µm diameter) protrusions that lack a bulbous head (Fig. 1A) [30].

Because the size and shape of functional subcellular domains are closely tied to the mechanics of actin-membrane interactions [18, 30, 31], a more complete understanding of dendritic spine dynamics, development, and function would benefit from biophysical models that address the underlying mechanical aspects. We have therefore begun to build a computational model of spines that incorporates both membrane forces and actin-based forces, and their interaction. This model is based on published experimental observations in dendritic spines, non-neuronal cells, and biochemical experiments. The goal of this model is to inform our understanding of the development of spines and the plasticity of their structure under different physiological scenarios.

Currently, there are hundreds of studies that address various aspects of the regulation of dendritic spine size and shape. In building our model, we have chosen to focus on several key observations, as follows.

1. **Actin enrichment in spines:** Dendritic spines are enriched in filamentous actin, which, along with scaffolding molecules, establish spine architecture [38–40]. Membrane-actin interactions associated with spine enlargement and shrinkage during plasticity can be modeled at the single filament level using the elastic Brownian ratchet and the net force acting on the membrane due to actin remodeling can be represented as work done by actin to deform the membrane [41–43].

2. **Different subpopulations of actin:** There appear to be distinct subpopulations of F-actin in dendritic spines, and spine actin can be thought of as an independent network with interconnected nodes [44]. The spine head typically consists of short, cross-linked filaments; branched filaments have been observed in the spine head [39, 45, 46]. The spine neck was initially thought to contain long filaments [47–50], but current evidence has suggested the presence of short, branched filaments [45]. Additionally, recent high resolution imaging techniques have shown that there are likely periodic F-actin structures along the neck region of dendritic spines [51, 52]. These periodic F-actin
structures are very stable and in contrast to long and branched filaments, resist depolymerization [52].

3. **Roles for actin binding proteins:** Actin dynamics in spines are tightly regulated by dozens of various actin binding proteins, some of which must also interact directly or indirectly with the spine plasma membrane [53–55]. First, the turnover of filaments themselves can drive forces against the membrane that regulate the expansion, maintenance, or shrinkage of spine compartments [56]. The key factors that govern this balance are (a) the rate of polymerization, which is regulated by actin nucleating factors such as formins and the Arp2/3 complex [39]; (b) the rate of depolymerization, which is regulated by actin severing factors such as cofilin and gelsolin [57]; (c) the number of free barbed ends, which is regulated via actin severing activity and the activity of barbed end capping proteins [58]; and (d) the concentration of available actin monomer, which is dependent upon the G-actin concentration and also the activities of profilin, which delivers ATP-bound G-actin to the above actin nucleators, and regulators such as N-WASP, which controls Arp2/3 activity [58, 59]. In addition, several proteins that crosslink or stabilize actin filaments, such as cortactin [60], spectrin [61], or drebrin [62] are known to regulate spine shape and separately, myosin motors can affect spine shape either directly by creating contractile forces, or indirectly by regulating the transport of cargo into and out of the spine [58, 63]. Finally, an important role for calcium/calmodulin-dependent protein kinase in structural plasticity of spines has been demonstrated through its ability not only to transduce calcium signals, but also to regulate actin directly through the direct binding of F-actin via its β subunit [64–67].

![Figure 1](image-url) **Figure 1:** Modeling of forces relevant to spine shape. (A) Schematic depiction of different shape categories of dendritic spines (Reprinted with permission from SynapseWeb, Kristen M. Harris, PI, http://synapseweb.clm.utexas.edu/). The inset shows a schematic of a tubular neck with a radius $r$ and a spontaneous deviatoric curvature $D_m$ along the total neck length $l$. (B) The surface parametrization of the membrane geometry in axisymmetric coordinates. $s$ is the arclength, $n$ is the unit normal vector to the membrane surface, and $a_s$ is the unit tangent vector in the direction of arclength. We assume that the actin filaments can apply axial ($F_z$) or normal ($F_n$) forces to the membrane surface. We assume that there is a large membrane reservoir with a fixed area, and we focused on the local region of the membrane under tension $\lambda$, as indicated by the dotted box.

4. **Membrane mechanics:** All cells regulate their shape by coordinating the properties of the cytoskeleton with that of the plasma membrane. Proteins such as MARCKS that interact directly with both F-actin and the lipid bilayer can strongly influence spine shape [68]. Membrane curvature is especially important in spines and represents a specific
mechanical force that is regulated by specific proteins, as well as lipid composition. Bin/Amphiphysin/Rvs (BAR)-
domain containing proteins assemble on the membrane to produce anisotropic curvature and promote tubulation.
Studies have demonstrated critical roles for specific BAR-domain proteins in dendritic spines. Recently, the role
of membrane mechanics has been elucidated in the initiation of dendritic spines [69]. A series of studies showed
that dendritic spines can be initiated by membrane bending due to protein patches containing BAR domains such
as I-BAR and F-BAR proteins [70–73]. These proteins are known to polymerize on the membrane [74–77], induce
anisotropic curvature [78–80], and promote tubulation [76, 81–84].

The above findings suggest that membrane bending and actin-membrane interactions are major determinants of spine
morphology. Recent studies have modeled the role of either membrane mechanics alone [43] or actin dynamics alone in
spines [85], but the interaction between the two has not yet been addressed. Here, we present a general theoretical model
that relates membrane bending and actin-mediated forces to spine morphology. Using this model, we investigate the
mechanical landscape of the different shapes of spines and map the relationships among actin-mediated force generation,
membrane elasticity, and curvature induced by periodic ring structures and proteins such as BAR domains.

Table 1: Dimensions of different spine shapes compiled from the literature.

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<tbody>
<tr>
<td>Total length (L) (µm)</td>
<td>0.44 ± 0.15</td>
<td>2-20</td>
<td>0.98 ± 0.42</td>
<td>1.5 ± 0.25</td>
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<tr>
<td>Length of neck (l) (µm)</td>
<td>–</td>
<td>–</td>
<td>0.51 ± 0.34</td>
<td>0.43 ± 0.21</td>
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<tr>
<td>Neck diameter (2r) (µm)</td>
<td>0.32 ± 0.13</td>
<td>&lt; 0.3</td>
<td>0.1 ± 0.03</td>
<td>0.2 ± 0.07</td>
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<tr>
<td>Total volume (µm³)</td>
<td>0.03 ± 0.01</td>
<td>–</td>
<td>0.04 ± 0.02</td>
<td>0.29 ± 0.13</td>
</tr>
<tr>
<td>Volume of head (V) (µm³)</td>
<td>–</td>
<td>–</td>
<td>0.03 ± 0.15</td>
<td>0.27 ± 0.13</td>
</tr>
<tr>
<td>Total surface area (µm²)</td>
<td>0.45 ± 0.14</td>
<td>–</td>
<td>0.59 ± 0.29</td>
<td>2.7 ± 0.93</td>
</tr>
<tr>
<td>Surface area of head (µm²)</td>
<td>–</td>
<td>–</td>
<td>0.4 ± 0.15</td>
<td>2.4 ± 0.92</td>
</tr>
<tr>
<td>Surface area of PSD (µm²)</td>
<td>0.07 ± 0.02</td>
<td>–</td>
<td>0.05 ± 0.02</td>
<td>0.3 ± 0.1</td>
</tr>
<tr>
<td>Surface area of PSD/head</td>
<td>–</td>
<td>–</td>
<td>0.1 ± 0.06</td>
<td>0.18 ± 0.15</td>
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2 Model development

2.1 Assumptions

- We treat the lipid bilayer as a continuous thin elastic shell, assuming that the membrane thickness is negligible
  compared to the radii of membrane curvature [86, 87]. This allows us to model the bending energy of the membrane
  using the modified version of the Helfrich–Canham energy, including the effect of spatially varying deviatoric
curvature to represent the induced anisotropic curvatures by periodic F-actin rings and other ring-shaped structures
  [81, 82, 87–90].

- We assume that the membrane is locally inextensible, since the stretching modulus of the lipid bilayer is an order
  of magnitude larger than the membrane bending modulus [91]. We implemented this constraint using a Lagrange
  multiplier, which can be interpreted as the membrane tension [92–94]. We note that this membrane tension, in this
study, is better interpreted as the cortical tension including the effective contribution of both the membrane in-plane stresses and membrane-cytoskeleton interactions [95, 96].

- We assume that the time scales of mechanical forces are much faster than other events in dendritic spines, allowing us to assume mechanical equilibrium and neglect inertia [43, 92]. This assumption is justified by the fact that the timescale of the equilibration of the mechanical forces is much smaller than the timescale of actin polymerization in dendritic spines [97].

- We assume that the force exerted by the actin cytoskeleton can be represented as work done on the membrane and do not include the molecular details of the actin network [43, 96, 98–100]. Additionally, we assume that the periodic ring shaped structures of actin and related proteins such as βII spectrin and BAR-domain proteins can be represented using an anisotropic spontaneous curvature [80–82, 89].

- For ease of computation we assume that the geometry of a dendritic spine is rotationally symmetric (see Fig. 1B) [43]. This assumption allows us to parametrize the whole surface by a single parameter, arclength.

### 2.2 Mechanical force balance

In this section we present a concise derivation of the governing mathematical shape equations for the shape of dendritic spines at mechanical equilibrium. The complete derivation with details is given in [92, 98, 101, 102]. The total free energy of the system ($E$) includes the elastic storage energy of the membrane ($E_{\text{elastic}}$), and the work done by the applied forces due to actin filaments ($W_{\text{force}}$) [90, 98, 103, 104] is given by

$$E = E_{\text{elastic}} - W_{\text{force}},$$

(1)

where

$$E_{\text{elastic}} = \int_{\omega} \left( \sigma(H, D, K; \theta^\alpha) + \lambda(\theta^\alpha) \right) da - pV,$$

(2a)

and

$$W_{\text{force}} = \int_{\omega} f(\theta^\alpha) \cdot (r - r_0) da.$$

(2b)

Here, $\omega$ is the total membrane surface area, $\sigma$ is the bending energy density per unit area, $\theta^\alpha$ denotes the surface coordinate where $\alpha \in \{1, 2\}$, $H$ is the mean curvature of the surface, $D$ is the curvature deviator, $K$ is the Gaussian curvature of the surface, $\lambda$ is the tension field and represents the Lagrange multiplier associated with the local area constraint, $p$ is the transmembrane pressure and represents the Lagrange multiplier associated with the volume constraint, $V$ is the enclosed volume, $f$ is the applied force per unit area, $r$ is the position vector in the current configuration, and $r_0$ is the position vector in the reference frame. To model the energy density $\sigma$ in Eq. 2a, we used the modified version of Helfrich energy including the effects of induced anisotropic curvature by periodic F-actin structures and BAR domain proteins [78, 81, 82, 87, 88, 90, 105], given as

$$\sigma(H, D, K; \theta^\alpha) = \kappa H^2 + \kappa(D - D_m(\theta^\alpha))^2,$$

(3)

where $\kappa$ is a constant representing the bending moduli and $D_m$ is the spontaneous (intrinsic) deviatoric curvature which can be spatially heterogeneous along the membrane surface [78, 81, 82]. It should be mentioned that in Eq. 3, we assumed
that periodic rings can only induce anisotropic curvature and we set the isotropic curvature (spontaneous curvature) to be zero throughout this study. Substituting Eqs. 2a, 2b, and 3 into Eq. 1 gives

\[
E = \int_\omega \kappa H^2 da + \int_\omega \kappa (D - D_m)^2 da + \int_\omega \lambda da - pV - \int_\omega f(\theta^a) \cdot (r - r_0) da
\]  

Minimization of the energy (Eq. 4) using the variational approach results in the governing shape equation (Eq. S6) and the incompressibility condition (Eq.S7) for a heterogeneous membrane. The complete equations are presented in the supplementary material along with the complete notation in Table S1.

2.3 Numerical implementation

In axisymmetric coordinates, the membrane shape equation (Eq. S6) and the incompressibility condition (Eq.S7) simplify to a coupled system of first order differential equations (Eq. S24). In order to solve this system of equations along with the prescribed boundary conditions (Eq. S25), we used ‘bvp4c,’ a boundary value problem solver in MATLAB. In all our simulations, we assume that the total area of the membrane is conserved and we also fixed the bending modulus to be \( \kappa = 0.18 \text{ pN} \cdot \mu \text{m} \) based on previous models for spines [106, 107]. We also set the transmembrane pressure to zero \( (p = 0) \) to focus only on the mechanism of membrane-actin interactions in governing the shapes of dendritic spines.

3 Results

Using the model described above, we conducted simulations for different mechanical parameters with the goal of identifying the range of forces, the associated heterogeneities, and the protein-induced and cytoskeleton-induced anisotropic curvatures that could result in shapes and sizes of spines corresponding to those observed experimentally (Table 1). Specifically, we sought to recreate the filopodial, stubby, thin, and mushroom-shaped spines as shown in Fig. 1. We must emphasize that all the shapes are equilibrium shapes, and our model does not provide insight into dynamic transitions from one shape to another. Our simulation results are described below. In these data, we emphasize the relationships among different mechanical parameters to obtain the desired shapes, and give specific values for mechanical parameters that result in sizes as listed in Table 1. These provide some realistic magnitudes for forces present at various locations within the compact spine volume.

3.1 Localized axial forces along the membrane are sufficient for the formation of stubby and filopodial shaped spines

We begin with an analysis of the force-shape relationship of stubby spines. We assumed that actin filaments exert axial forces in the nascent PSD area, which is a small fraction of the membrane surface area (Table 1). This heterogeneous force distribution along the membrane was implemented using a hyperbolic tangent function (Eq. S26). We observed that the relationship between the magnitude of the forces and the length of the stubby spines depends on the value of tension. To map this relationship, we performed the simulation for (i) a fixed height \( (L = 0.44 \mu \text{m}) \) and a wide range of tensions (Fig. 2A) and (ii) a fixed tension \( (e.g., \lambda = 10 \text{ pN/}\mu\text{m}) \) and different heights of the stubby spine (Fig. 2B). As shown in previous studies [108, 109], for a small membrane deformation, such as a stubby spine, the axial force is linearly proportional to both tension and the height of the stubby spine (Figs. 2A & B) [108, 109]. Thus, from a mechanical
standpoint, the stubby spine shape is accessible for a wide range of forces and tensions in the physiological range. If we seek to match a specific height of the stubby spine as noted from experimental observations, our simulations showed that an axial force of $F_z = 7.5 \text{ pN}$ is required to form a stubby spine of the length of $L = 0.44 \mu\text{m}$ (Table 1) when the tension is $\lambda = 10 \text{ pN}/\mu\text{m}$ (Fig. 2C).

Next, we investigated the role of forces in the formation of long spines that resembled filopodia. As expected, we found that the formation of a long filopodium follows well-established results for tube formation in membranes [108]. Ignoring the spherical cap, a filopodium is a tubular membrane and its equilibrium radius ($r$) depends on the tension and bending rigidity of the membrane as $r = \sqrt{\kappa/(2\lambda)}$ (Fig. 2D) [108]. The axial force $F_z$ required to maintain the tubule with radius $r$ and height $L$ is given as $F_z = 2\pi\sqrt{2\kappa\lambda}$ (Fig. 2E) [108]. Results from our simulations are consistent with these theoretical relationships. Specifically, for a filopodial-shaped protrusion of $L = 5 \mu\text{m}$ with a neck radius of $r = 0.1 \mu\text{m}$, the required force was $F_z = 11.12 \text{ pN}$ for a tension of $\lambda = \kappa/(2r^2) = 9 \text{ pN}/\mu\text{m}$ (Fig. 2F) and the magnitude of this axial force is independent of the length of the protrusion (Fig. S3A).

Figure 2: Formation of stubby and filopodia shaped spines with a localized axial force. (A) Linear relationship between the magnitude of axial force and tension in a small stubby-shaped membrane deformation [108, 109]. The dashed line is the fitted curve ($F_z = 0.688\lambda$) with $R^2 = 0.9967$. (B) Linear relationship between the magnitude of axial force and the height of the stubby spine for a fixed tension [108, 109]. The dashed line is the fitted curve ($F_z = 16.82L$) with $R^2 = 0.9896$. (C) A stubby-shaped spine with a total length $L = 0.44 \mu\text{m}$ is formed with $F_z = 7.5 \text{ pN}$ applied along the blue area ($\lambda = 10 \text{ pN}/\mu\text{m}$). (D) Neck radius of a filopodium as a function of tension ($r = \sqrt{\kappa/(2\lambda)}$) [108]. (E) The magnitude of axial force needed to form a filopodium as a function of tension ($F_z = 2\pi\sqrt{2\kappa\lambda}$) [108]. (F) A filopodium-shaped protrusion with a total length $L = 5 \mu\text{m}$ and neck radius $r = 0.2 \mu\text{m}$ is formed with $F_z = 11.2 \text{ pN}$ applied along the spherical cap of the filopodium, which is shown in blue ($\lambda = 9 \text{ pN}/\mu\text{m}$).
3.2 Normal forces along the membrane support the formation of thin shaped spines

We next investigated the nature of forces that could be associated with the formation of thin-shaped spines. Because thin-shaped spines have a bulbous head, axial forces such as those used in Fig. 2 are insufficient to generate the spherical shape of the head. Since spherical shapes can be obtained by a normal force acting locally on the head region, we repeated the simulation in Fig. 2 but now included a localized uniform normal force density along the area of the spine head \( A_{\text{force}} = A_{\text{spine head}} \). It is possible that such normal forces result from the dense actin meshwork in the spine heads [43, 110]. We estimated the forces required to generate a spherical head by assuming that a thin spine is ideally a sphere with radius \( R \) which is connected to a cylinder with radius \( r \) and height \( l \) (Fig. S1B). If a uniform normal force density, \( f_n \), is applied all along the sphere, then, ignoring the interface between the sphere and the cylinder, the total energy of the system can be written as

\[
E = E_{\text{sphere}} + E_{\text{cylinder}},
\]

where \( E_{\text{sphere}} = (\kappa/R^2 + \lambda)4\pi R^2 - (4\pi/3)R^3 f_n \) and \( E_{\text{cylinder}} = 2\pi \sqrt{2\lambda \kappa l} \) (see section 1.5.2 in the supplementary material). Minimizing the total energy of the system with respect to \( R \) by taking \( \partial E/\partial R = 0 \), we obtain the equilibrium normal force density as \( f_n = 2\lambda/R \). This resembles the Young-Laplace equation where normally pressure (normal force density) is a global parameter; in this case, \( f_n \) is a local normal force density. To find the total magnitude of normal force, we need to multiply the force density by the area of the spine, which produces \( F_n = 8\pi R\lambda \). In our simulation, we prescribe the area of the applied force and can rewrite the force as

\[
F_n = 4\lambda \sqrt{\pi A_{\text{force}}},
\]

In order to generate thin-shaped spines, we first fixed the neck diameter based on the magnitude of tension \( (r = \sqrt{\kappa/(2\lambda)}) \) as shown in Fig. 2D. Similar to filopodia, in thin spines, the radius of the neck is related to the tension and the bending rigidity, given by \( r = \sqrt{\kappa/(2\lambda)} \) [108] (Fig. 3A). This relationship suggests that in order to have a thin spine with a neck radius between 0.035 \( \mu \text{m} < r < 0.065 \mu \text{m} \) (given range in Table 1), the tension can vary between 20 \( \text{pN/\mu m} \) \( \leq \lambda \leq 80 \text{ pN/\mu m} \). Based on Eq. 6, the magnitude of the normal force linearly depends on the tension, while it varies as the square root of the area of applied force.

In Fig. 3B, we plotted the magnitude of the normal force as a function of tension obtained from numerical solutions (red squares) versus the analytical expression given in Eq. 6 (dotted line) for fixed \( A_{\text{force}} = 0.44 \mu \text{m}^2 \). We found a good agreement between the analytical solution and the results obtained from simulation such that by changing tension between 20 \( \text{pN/\mu m} \) \( < \lambda < 80 \text{ pN/\mu m} \), the magnitude of the normal force required to form a thin-shaped spine varies in a large range between 150 pN \( < F_n < 400 \text{ pN} \) (Fig. 3B). To further validate our numerical results, we plotted the magnitude of the normal force as a function of the area of the applied force \( A_{\text{force}} \) obtained from numerical solution (red squares) versus the analytical expression given in Eq. 6 (dotted line) for a fixed tension, \( \lambda = 36 \text{ pN/\mu m} \) (Fig. 3C). We observed a good agreement between the analytical solution and the numerical results where by increasing the area of the applied force from \( A_{\text{force}} = 0.25 \mu \text{m}^2 \) to \( A_{\text{force}} = 0.55 \mu \text{m}^2 \), the magnitude of the normal applied force needed to form a thin spine increases from \( F_n \sim 120 \text{ pN} \) to \( F_n \sim 200 \text{ pN} \) (Fig. 3C).

Thus, to form a thin spine with an average neck diameter of \( r = 0.05 \mu \text{m} \) (see Table 1), we set our tension to be \( \lambda = 36 \text{ pN/\mu m} \) \( (r = \sqrt{\kappa/(2\lambda)}) \). Based on our calculation for \( \lambda = 36 \text{ pN/\mu m} \) and \( A_{\text{force}} = 0.44 \mu \text{m}^2 \) (average area of the spine head in Table 1), a total normal force of \( F_n = 168.18 \text{ pN} \) (applied along the red area) is required to form a thin spine with
a total length $L = 0.98 \, \mu m$, a neck radius $r = 0.05 \, \mu m$, and a head volume $V = 0.033 \, \mu m^3$ (Fig. 3D). Also, in Fig. S3B, we show that the magnitude of the normal force needed to form a thin spine is independent of the height of the spine.

Figure 3: Formation of thin-shaped spines with localized normal force along the spine head. (A) Neck radius of a thin-shaped spine as a function of tension ($r = \sqrt{\kappa/(2\lambda)}$) [108]. (B) Linear relationship between the magnitude of normal force needed to form a thin-shaped spine and the tension. Here the area of the applied force is set at $A_{\text{force}} = 0.44 \, \mu m^2$. The red squares represent the results obtained from simulation and the dashed line is the derived analytical solution ($F_n = 4\lambda \sqrt{\pi A_{\text{force}}}$, Eq. 6). (C) The magnitude of a normal force needed to form a thin-shaped spine as a function of the area of the spine head. The tension is set at $\lambda = 36 \, pN/\mu m$. The red squares represent the results obtained from our simulations and the dashed line is the derived analytical solution ($F_n = 4\lambda \sqrt{\pi A_{\text{force}}}$), Eq. 6. (D) A thin-shaped spine with a total length $L = 0.98 \, \mu m$, neck radius $r = 0.05 \, \mu m$, and head volume $V = 0.033 \, \mu m^3$ is formed with $F_n = 168.18 \, pN$ applied along the head of spine which is shown in red ($\lambda = 36 \, pN/\mu m$ and $A_{\text{force}} = 0.44 \, \mu m^2$).

3.3 Non-uniform normal force distributions can result in mushroom-shaped spines

We next asked if changes to the force distributions could result in mushroom-shaped spines. We hypothesized that one possible way is to have a heterogeneous force distribution along the spine head and the PSD area. To understand how non-uniform distributions of normal forces can characterize the morphology of mushroom spines, we performed simulations assuming that the normal force applied along the PSD area is different from the normal force applied along the rest of the spine head (Fig. 4A).

In the case of mushroom-shaped spines, we have multiple geometric parameters to consider – (a) head volume, (b) area fraction of the PSD, and (c) tension. For example, to form a mushroom-shaped spine with a total length $L = 1.51 \, \mu m$, head volume $V = 0.25 \, \mu m^3$, and area of PSD/area of head ratio = 0.2 (see Table 1), normal forces of $F_n = 117.65 \, pN$ and $F_{n,\text{PSD}} = 83.72 \, pN$ are required along the spine head (red region) and the PSD area (gray region), respectively (Fig. 4A). The value of tension was set to $\lambda = 9 \, pN/\mu m$ to obtain a neck radius of about $r \approx 0.1 \, \mu m$ (see Table 1 and Fig. S4). The magnitude of these forces is independent of the height of the spine (Fig. S3C).

We observed that the morphology of the spine head changes with varying magnitude of tension; the spine head flattens for large tensions (Fig. 4B). This is consistent with previous studies that have investigated membrane shape at high tensions, e.g., the membrane remains almost flat during vesicle budding [111, 100], or in the case of a red blood cell, the biconcave cell flattens to a pancake shape [112, 96]. To further investigate how a change in the morphology of the spine head can affect the volume of the head, we plotted the volume of the head ($V$) as a function of tension (Fig. 4B). We found that the head volume is a non-monotonic function of tension; as tension increases, the volume of the spine head increases and then decreases (Fig. 4B).
This is because initially when increasing tension from low to intermediate values the head flattens and the volume of the head increases. However, for high tensions, the shrinkage of the head becomes dominant and as a result the volume decreases (Fig. 4B). Consistent with these observations, a larger normal force is required to bend a stiffer membrane and form a mushroom-shaped spine (Fig. 4C). For example, based on our calculation, when increasing tension from $\lambda = 5 \text{ pN/\mu m}$ to $\lambda = 20 \text{ pN/\mu m}$, the normal forces in the spine head and PSD area increase by almost 150 pN (Fig. 4C).

To study how the ratio of PSD area to the total area of the spine head affects the magnitude of normal forces, we performed simulations for a range of area of PSD/area of head ratios (Fig. S5). Our results show that with increasing area of PSD/area of head ratio, larger normal forces both in the spine head and the PSD region are required (Fig. S5A). Additionally, increasing the ratio of the PSD area to the total area of the head results in the flattening of the spine head with a larger volume (Fig. S5B). Thus, mushroom-shaped spines can be formed from a multitude of mechanical pathways – heterogeneous forces in the spine head, balancing tension and force distributions, and using different area localizations of the forces.

Figure 4: Formation of mushroom-shaped spines with localized normal forces along the spine head and PSD. (A) A mushroom-shaped spine with a total length $L = 1.51 \mu\text{m}$, neck radius $r = 0.1 \mu\text{m}$, head volume $V = 0.25 \mu\text{m}^3$, and area of PSD/area of head $= 0.2$ is formed with $F_n = 117.65 \text{ pN}$ applied along the head of spine (red domain) and $F_n,_{\text{PSD}} = 83.72 \text{ pN}$ applied along the PSD (gray domain) ($\lambda = 9 \text{ pN/\mu m}$). (B) The nonmonotonic behavior of the volume of a mushroom-shaped spine head when increasing tension. Three different shapes of mushroom-shaped spines are shown for low, intermediate, and high tensions. With increasing magnitude of tension, the mushroom-shaped spine head flattens. (C) The magnitude of normal forces in the spine head (red squares) and in PSD (gray squares) increases with increasing tension.

### 3.4 Induced spontaneous deviatoric curvature by periodic F-actins structures and BAR domain proteins can generate characteristic dendritic spine necks

Recently, super-resolution microscopy methods have revealed the presence of ubiquitous actin ring structures along spine necks [51, 52]. It has been suggested that these ring-like structures and BAR-domain proteins can together support the tubular shape of dendritic spines [38, 113]. To understand how periodic F-actin structures and BAR domain proteins can regulate the tubular shape of spine necks, we implemented their net effect in our model by including spontaneous deviatoric curvature in the energy density of the system (Eq. 3) [78, 81–84].

Consider a tubular membrane with radius $r$ and a spontaneous deviatoric curvature $D_m$ along the neck with total length...
l (Fig. 1A), the equilibrium radius in the presence of spontaneous deviatoric curvature is given by \( r = \sqrt{\kappa/(2(\lambda + \kappa D_m^2))} \) (Eq. S41). Since this radius depends on both the value of tension and the spontaneous deviatoric curvature (Fig. 5A), we define an effective tension \( \lambda + \kappa D_m^2 \). As a result, the relationship between neck radius, spontaneous deviatoric curvature, and tension in Fig. 5A collapses onto a single curve (Fig. S6B) as a function of this effective tension. Simulations confirm that the radii of tubular necks obtained from numerical solutions collapse onto a single curve as a function of effective tension (Fig. 5B).

Similarly, the axial force required to maintain a tubular membrane with radius \( r \) and spontaneous deviatoric curvature \( D_m \) along the total length \( L \), is given by \( F_z = 2\pi(\sqrt{2\kappa(\lambda + \kappa D_m^2)} - \kappa D_m) \) (Eq. S41). In Fig. 5C, we plotted the axial force as a function of tension and spontaneous deviatoric curvature. We found that the axial force has a local minimum along the red line (Fig. 5C) where \( \lambda = \kappa D_m^2 \) (Eq. S42) and \( F_{z,\text{min}} = 2\pi\kappa D_m \) (Eq. S42). The 3D surface in Fig. 5C can be reduced to a single curve by defining the effective axial force as \( F_z + 2\pi\kappa D_m \) and plotting it as a function of effective tension (Fig. S6D). We also plotted the effective axial force obtained from numerical solutions as a function of effective tension (Fig. 5D). We observed that consistent with the analytical prediction, for different tensions, the effective axial forces collapse onto a single curve as a function of effective tension (Fig. 5D). These results suggest that effective tension \( \lambda + \kappa D_m^2 \) regulates the radius of dendritic spine necks.

Figure 5: Effective tension including spontaneous deviatoric curvature regulates the neck radius and the magnitude of axial force in a tubular membrane. (A) Analytical solution for the neck radius of a tubular membrane as a function of spontaneous deviatoric curvature and tension \( r = \sqrt{\kappa/(2(\lambda + \kappa D_m^2))} \), Eq. S41). (B) The neck radius obtained from numerical solutions as a function of effective tension \( \lambda + \kappa D_m^2 \). Here, for fixed three different tensions, we varied the effective tension by changing the spontaneous deviatoric curvature between \( 0 < D_m < 30 \mu\text{m}^{-1} \). The radii of the membrane necks collapse onto a single curve for different tensions. (C) Analytical solution for the magnitude of an axial force needed to maintain a tubular protrusion as a function of spontaneous deviatoric curvature and tension \( F_z = 2\pi(\sqrt{2\kappa(\lambda + \kappa D_m^2)} - \kappa D_m) \), Eq. S41). The axial force needed to maintain a tubular protrusion has a local minimum along the red line where \( \lambda = \kappa D_m^2 \) (Eq. S43). (D) The effective axial force \( F_z + 2\pi\kappa D_m \) obtained from numerical solutions as a function of effective tension \( \lambda + \kappa D_m^2 \). Here, for fixed three different tensions, we varied the effective tension by changing the spontaneous deviatoric curvature between \( 0 < D_m < 30 \mu\text{m}^{-1} \). Effective axial forces collapse onto a single curve for different tensions.
3.5 Cooperation of forces and induced spontaneous deviatoric curvature offers multiple pathways for spine shape maintenance

Thus far, we have focused on the role of forces (axial and normal) on spine head shape and the role of spontaneous deviatoric curvature representing periodic rings on the spine neck radius. Next, we asked if the cooperation of these two different mechanisms could further influence the spine geometries and the energy landscape associated with these features. In other words, we asked if the combination of spontaneous deviatoric curvature and applied forces could result in lower energy states for the same spine geometry. To answer this question, we sought to identify the parameters that give rise to thin spines with the same geometric parameters. We explain this approach with a specific example below.

As noted before, when only normal forces are used, a normal force of $F_n = 168.18$ pN under a tension of $\lambda = 36$ pN/$\mu m$ is required to form a thin spine with a neck radius of $r = 0.05$ $\mu m$ and head volume of $V = 0.033$ $\mu m^3$ (Fig. 6A, left). We can also obtain a thin spine with the same dimensions, by using a prescribed spontaneous deviatoric curvature $D_m = 10$ $\mu m^{-1}$ along the neck and an applied force of $F_n = 43$ pN along the head for $\lambda = 10$ pN/$\mu m$ (Fig. 6A, right). Thus, for the same shape parameters, in the presence of spontaneous deviatoric curvature, the value of force required is roughly a quarter of the force required in the absence of spontaneous deviatoric curvature (Fig. 6A). Similarly, when a combination of axial force along the spine head and spontaneous deviatoric curvature along the neck is used, a thin spine with $r \sim 0.05$ $\mu m$ and head volume $V \sim 0.033$ $\mu m^3$ can be formed with $F_z = 7.71$ pN and spontaneous deviatoric curvature $D_m = 10$ $\mu m^{-1}$ when $\lambda = 10$ pN/$\mu m$ (Fig. 6B). Thus, in both these cases (axial and normal forces) for the formation of thin spines, we note that access to spontaneous deviatoric curvature significantly reduces the forces required to form and maintain thin spines.

Not surprisingly, these same results hold for mushroom-shaped spines too. As we have shown before, to form a mushroom spine with a neck radius of $r = 0.1$ $\mu m$ and head volume of $V \sim 0.25$ $\mu m^3$, normal forces of $F_n = 117.65$ pN along the spine head and $F_{n,PSD} = 83.72$ pN along the PSD are required under a tension of $\lambda = 9$ pN/$\mu m$ (Fig. 6C, left). We can also form a mushroom spine with the same dimensions and lower tension ($\lambda = 5.5$ pN/$\mu m$) by prescribing a spontaneous deviatoric curvature $D_m = 1.8$ $\mu m^{-1}$ along the spine neck and normal forces of $F_n = 80.1$ pN and $F_{n,PSD} = 45.17$ pN along the spine head and PSD, respectively (Fig. 6C, right).

In Figs. 6D-F, we plotted the magnitude of forces that are required to form thin and mushroom-shaped spines with or without spontaneous deviatoric curvature as a function of tension alone (with no spontaneous deviatoric curvature) or effective tension (with spontaneous deviatoric curvature). We observed that with increasing effective tension, the magnitude of the normal force that is required to form a thin spine with spontaneous deviatoric curvature (red squares) is almost constant (Fig. 6D). However, the magnitude of the normal force that is needed to form a thin spine without spontaneous deviatoric curvature (dashed line) increases linearly with increasing tension (Eq.6 and Fig. 6D). In the case of the formation of a thin spine with an axial force, we found that in the presence of spontaneous deviatoric curvature, the magnitude of axial force (blue squares) decreases slightly and then becomes constant with increasing effective tension (Fig. 6E). In contrast, without spontaneous deviatoric curvature, the magnitude of axial force (dashed line) increases with increasing tension (Fig. 6E). Similar to the thin-shaped spine, with spontaneous deviatoric curvature along the spine neck, the magnitude of normal forces in the head (red square) and PSD (gray square) region that are required to form a mushroom spine is almost constant with increasing effective tension (Fig. 6F). However, without spontaneous deviatoric curvature, the magnitude of forces in both regions increases with increasing tension (Fig. 6F).

To further compare thin and mushroom spines shown in Fig. 6, we computed the components of energy (Eq. 1) and the total energy of the system for each shape (Tables S2 & S3 and Figs. 7C & D). Based on our results, by prescribing spontaneous deviatoric curvature $D_m$ along the spine neck, the bending energy due to deviatoric curvature decreases...
This is because the deviatoric curvature $D$ along the neck tends to $D_m$ and minimizes the bending energy (Tables S2 & S3). Additionally, in the presence of spontaneous deviatoric curvature, in our simulation, we set the tension to lower values compared to the condition that $D_m = 0$. Therefore, the work that is done by tension and forces to bend the membrane reduces for the case that the spines obtained with a combination of force and spontaneous deviatoric curvature (Tables S2 & S3). For example, to form a thin spine shown in Fig. 6, the work that is done by an axial force with a spontaneous deviatoric curvature (Fig. 6B) is almost one third of the work that is done by a normal force without spontaneous deviatoric curvature (Fig. 6A and Tables S2 & S3).

Figure 6: Formation of thin and mushroom shaped spines with a combination of forces and spontaneous deviatoric curvature. (A) Formation of a thin-shaped spine by applying a uniform normal force density along the spine head (left) versus applying a uniform normal force density along the head and spontaneous deviatoric curvature (purple region) along the spine neck (right) ($\lambda = 10 \text{ pN/\mu m}$). (B) Formation of a thin-shaped spine by applying an axial force along the spherical cap (blue region) and spontaneous deviatoric curvature along the spine neck (purple region), $\lambda = 10 \text{ pN/\mu m}$. All thin spines in panels A and B have a neck radius $r \sim 0.05 \mu m$ and head volume $V \sim 0.033 \mu m^3$. (C) Formation of a mushroom-shaped spine by applying a non-uniform normal force density along the spine head (left) versus applying a non-uniform normal force density along the head and spontaneous deviatoric curvature along the spine neck (purple region), (right), $\lambda = 5.5 \text{ pN/\mu m}$. The formed mushroom spine with normal forces $F_n = 80.1 \text{ pN}$ and $F_{n,PSD} = 46.2 \text{ pN}$ and deviatoric curvature $D_m = 1.8 \mu m^{-1}$ has a neck radius $r \sim 0.1 \mu m$ and head volume $V \sim 0.27 \mu m^3$. (D) The magnitude of a normal force that is required to form a thin-shaped spine with and without spontaneous deviatoric curvature as a function of effective tension and tension, respectively. (E) The magnitude of an axial force that is required to form a thin-shaped spine with and without spontaneous deviatoric curvature as a function of effective tension and tension, respectively. (F) The magnitude of normal forces in the spine head and in PSD that is required to form a mushroom spine with and without spontaneous deviatoric curvature as a function of effective tension and tension, respectively.
In the bar plots of Figs. 7C &D, we compared the total energy of thin and mushroom spines formed with different mechanisms. We observed that in both thin and mushroom spines, the total energy of the system dramatically decreases when the spines form with a combination of forces and spontaneous deviatoric curvature (Figs. 7C &D). This result suggests that spontaneous deviatoric curvature can alter the energy landscape of thin and mushroom dendritic spines to a lower energy state.

4 Discussion

Previously, we showed that the coupled dynamics of signaling and actin remodeling can alter spine volume in a bio-physical model [67] without considering the geometry of the spines or considering the role of spine shape in regulating different signaling pathways [114–118]. In this work, we present a simplified mechanical model for studying the role of different force distributions and energy contributions that are associated with the different spine shapes noted in the literature. Our results show that different spine shapes can be associated with different forces and spontaneous deviatoric curvature distributions, giving us insight into the mechanical design principles of spine formation and maintenance (Fig. 7).

We show that stubby spines can be formed for a wide range of tensions and low forces (Fig. 2). From a spine formation viewpoint, this makes sense, since during development the stubby spines can be the initial protrusions that form out of the dendrites. Given the ubiquitous nature of stubby spines [4, 12, 19], our results suggest that the prevalence of stubby spines could be due to the mechanical ease which they can be formed. They may also represent a temporarily stable state adopted by shrinking spines during synapse removal. Filopodia have the same force-length and force-radius relationships as membrane tubules that can be formed with micropipettes [119], optical tweezers [120], or by kinesin motor proteins [121] (Fig. 2). Based on our results, dendritic filopodia can be formed with a relatively small axial force, which make them good candidates as initial protrusions for the formation of mature thin and mushroom spines. Thin and mushroom spines, which have defined head shapes, require more mechanical features – heterogeneous force distributions, normal or axial forces, and an induced spontaneous deviatoric curvature representing the periodic protein rings or other deviatoric curvature inducing mechanics along the neck.

In the case of thin spines, we find that the mechanical design principles that support the formation of a spherical head are (1) large normal force along the head (Fig. 3), (2) normal force along the head with a spontaneous deviatoric curvature along the neck (Fig. 6A), and (3) an axial force along the head with a spontaneous deviatoric curvature along the neck (Fig. 6B). Within these mechanisms, the presence of spontaneous deviatoric curvature significantly reduces the total energy of the spine (Fig. 7C). Similarly, for mushroom spines, in addition to non-uniform forces along the head and the PSD (Fig. 4), the spine can be formed with a combination of forces in the head and spontaneous deviatoric curvature along the neck (Fig. 6C) while the spontaneous deviatoric curvature results in a lower energy state (Fig. 7D).

These findings have implications for our understanding of how mechanical aspects of membrane dynamics such as bending, tension, membrane-protein interactions, and interactions of the membrane with the cytoskeleton play critical roles in spine geometry maintenance, particularly in structural plasticity. Many of the events associated with synaptic plasticity alter spine size and shape through changes in F-actin dynamics and the dynamics of the actin related proteins [38–40]. The net impact of changes in actin remodeling would likely result in changes in force distribution. Another important and, as yet, under explored aspect of synaptic plasticity is the role of cortical membrane tension, including the effect of the membrane in-plane stresses and membrane-cytoskeleton interactions. We know that spines are sites of active vesicle trafficking events, such as endo- and exocytosis, and that these processes alter the membrane surface area and thereby alter the membrane tension [122, 123]. Here, we show that the effective membrane tension can play an
important role in altering the energy required for the maintenance of different spine shapes. One of the main impacts of such effective tension is that because of the cooperative effects of spontaneous deviatoric curvature and the applied forces, the energy required to maintain certain spine shapes may be lower. Thus, we show that there are different mechanical pathways that are likely associated with the different spine shapes and that some mechanisms may be energetically more favorable than others.

Despite these insights, our model has certain limitations. We do not explicitly consider the remodeling of the actin network or the dynamics of the associated proteins, but use force as a lumped parameter. Additionally, the use of axi-symmetric coordinates restricts our ability to obtain realistic spine shapes [124]. We note that the technology required to simulate these processes is quite challenging [125] and is ongoing research in our group.

Figure 7: Characterizing different shapes of dendritic spines based on the mechanical model. (A) Stubby spines can be formed with an axial force and in a wide range of tensions. (B) An axial force is sufficient to form a long filopodial spine. (C) A thin-shaped spine can be formed with three different mechanisms; (1) a uniform normal force density along the spine head, (2) a uniform normal force density along the spine head and spontaneous deviatoric curvature along the neck, and (3) a uniform axial force density along the spine head and spontaneous deviatoric curvature along the neck. In the bar plot, the total energy of the system is shown for three different mechanisms. The total energy of the system for the second and third mechanisms with spontaneous deviatoric curvature is much less than the energy for the first mechanism with just a normal force. (D) A mushroom-shaped spine can be formed with two different mechanisms; (1) a non-uniform normal force density along the spine head and PSD region and (2) a non-uniform normal force density along the spine head and PSD region plus a spontaneous deviatoric curvature along the spine neck. The resulting mushroom spine with a combination of normal forces and spontaneous deviatoric curvature has lower energy compared to the spine that is formed with just normal forces (bar graph).

The impact of mechanical aspects of actin remodeling and membrane mechanics on structural plasticity is highly intriguing and we are only beginning to understand their effects on spine functionality. This complexity is immediately
apparent in dendritic spines, which undergo dynamic changes, both mechanical and biochemical during structural plasticity spatiotemporal scales. Our minimal model provides insights into the possible mechanical aspects underlying the characteristic geometries associated with dendritic spines. This is an important step towards deciphering the intricate mechanochemistry of structural plasticity and dendritic spine development.

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