ELSEVIER

Do thin spines learn to be mushroom spines that remember?

Jennifer Bourne and Kristen M Harris

Dendritic spines are the primary site of excitatory input on most principal neurons. Long-lasting changes in synaptic activity are accompanied by alterations in spine shape, size and number. The responsiveness of thin spines to increases and decreases in synaptic activity has led to the suggestion that they are 'learning spines', whereas the stability of mushroom spines suggests that they are 'memory spines'. Synaptic enhancement leads to an enlargement of thin spines into mushroom spines and the mobilization of subcellular resources to potentiated synapses. Thin spines also concentrate biochemical signals such as Ca^{2+}, providing the synaptic specificity required for learning. Determining the mechanisms that regulate spine morphology is essential for understanding the cellular changes that underlie learning and memory.

Addresses
Center for Learning and Memory, Department of Neurobiology, University of Texas, Austin, TX 78712-0805, USA

Corresponding author: Harris, Kristen M (kharris@mail.clm.utexas.edu)

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Introduction

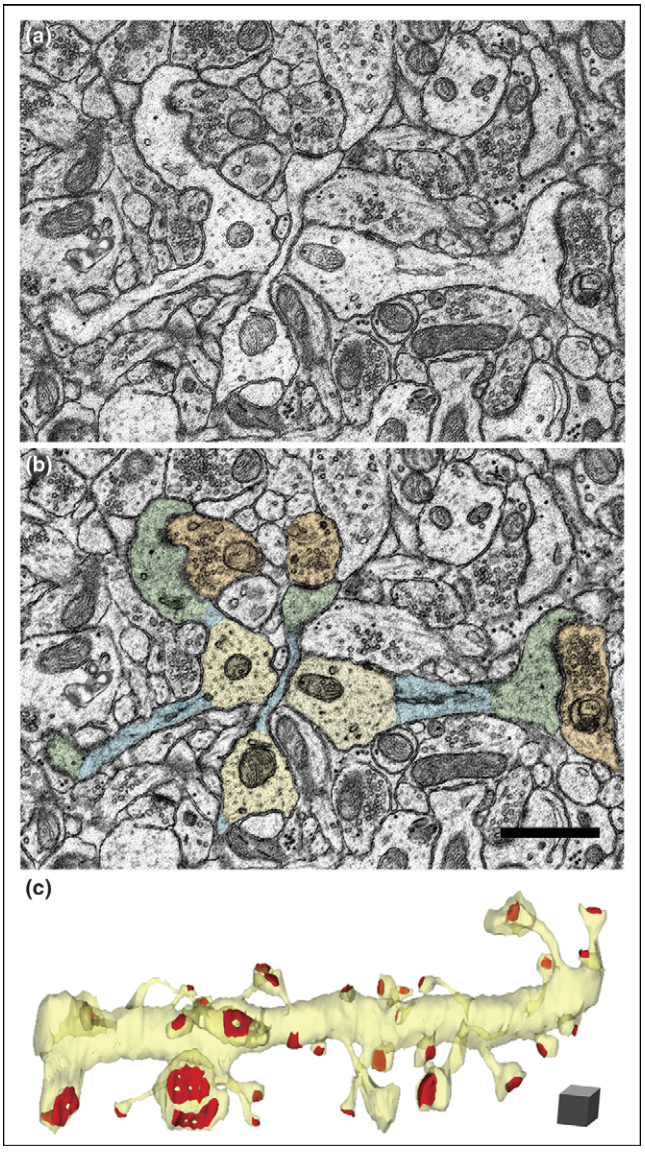
The majority of excitatory synapses in the brain occur on dendritic spines. Mature spines have a bulbous head that forms part of an excitatory synapse and is connected to the dendrite by a constricted neck. Neighboring spines vary dramatically in size and shape (Figure 1). In adult hippocampus and neocortex, spine shapes differ categorically with >65% of spines being 'thin' and ~25% being 'mushroom', having head diameters >0.6 μm [1,2]. Under normal circumstances, ~10% of spines in the mature brain have immature shapes: stubby, multisynaptic, filopodial or branched [1–4]. These shapes can be recognized using light microscopy if the spine is properly oriented, but accurate identification and measurement of spine synapses, dimensions and composition requires reconstruction from serial section transmission electron microscopy (ssTEM). Here we evaluate evidence from the past few years that addresses the question of whether thin and mushroom spines represent distinct categories, or whether they instead switch shapes depending on synaptic plasticity during learning.

Maturation and stabilization of spines

Spines tend to stabilize with maturation [5•]; however, a small proportion continues to turnover in more mature brains [5•–7•]. The transient spines are thin spines that emerge and disappear over a few days, whereas mushroom spines can persist for months [5•,6•]. Mushroom spines have larger postsynaptic densities (PSDs) [1], which anchor more AMPA glutamate receptors and make these synapses functionally stronger [8–12]. Mushroom spines are more likely than thin spines to contain smooth endoplasmic reticulum, which can regulate Ca^{2+} locally [13], and spines that have larger synapses are also more likely to contain polyribosomes for local protein synthesis [14]. Furthermore, large but not small spines have perisynaptic astroglial processes, which can provide synaptic stabilization and regulate levels of glutamate and other substances [15•,16]. These features suggest that mushroom spines are more stable 'memory spines' [17]. By contrast, thin spines form or disappear relatively rapidly in response to different levels of synaptic activity [18,19]. Thin spines have smaller PSDs that contain NMDA receptors but few AMPA receptors, making them ready for strengthening by addition of AMPA receptors [8–12]. Thin spines maintain structural flexibility to enlarge and stabilize, or shrink and dismantle, as they accommodate new, enhanced, or recently weakened inputs, making them candidate 'learning spines' [5•,6•,17].

During the first postnatal week in rats, dendritic filopodia emerge and interact with axons to form nascent synapses. Most of these developmental filopodia contract resulting in shaft synapses or stubby spines. During the second postnatal week, thin and mushroom spines begin to emerge [3]. In more mature brains, filopodia-like protrusions can also emerge and ssTEM shows that they lack synapses [6•,20••,21]; by contrast, spines with bulbous heads that persist four or more days have synapses [20••]. Blocking synaptic transmission in mature, but not immature, hippocampal slices results in a homeostatic spinogenesis that significantly increases numbers of nonsynaptic filopodia, shaft synapses, multisynaptic protrusions and stubby spines, suggesting a recapitulation of early development [21,22]. If the head of the filopodium swells to accommodate a PSD and other subcellular organelles, then it becomes a dendritic spine. The adult neuropil is more compact and might prevent contraction of nonsynaptic filopodia back to the dendritic shaft. In addition, mature dendrites might possess more local resources (e.g. proteins, mRNA and organelles) that can be transported into a

Figure 1



Dendritic spines occur in a variety of shapes and sizes. **(a)** An electron micrograph from area CA1 of the mature rat hippocampus shows three cross-sectioned dendrites with longitudinally sectioned dendritic spines. **(b)** A colorized version of the micrograph in (a), highlighting the dendrite shaft (yellow), spine necks (blue), spine heads (green) and presynaptic boutons (orange). Scale bar, 0.5 μm. **(c)** Three-dimensional reconstruction of an 8.5 μm long dendrite (yellow) reveals how spines and PSDs (red) vary greatly in size and morphology even along short segments of dendrite. Scale cube, 0.5 μm^3.

filopodium to support local maturation of the synapse, and this could explain why some filopodia convert directly into spines with bulbous heads in more mature brains.

Distance-dependent or input-dependent regulation of spine shape

Differences in synapse dimensions might also compensate for distance-dependent differences in dendritic function [23]. Recent studies show that nearly all of the most distal synapses on the apical dendritic tufts of hippocampal CA1 pyramidal cells have large perforated synapses [24]. Perforations in synapses have been seen only on large mushroom spines and they seem to be transient results of intense presynaptic activation [4]. Nevertheless, the perforations categorically identify large mushroom spines. The composition of perforated synapses seems to be input specific. For example, perforated synapses located in striatum radiatum that receive axonal input primarily from area CA3 have a higher density of AMPA receptors than perforated synapses located in the distal apical tuft that receive axonal input primarily from the entorhinal cortex [24]. Thin and mushroom spines also seem to distinguish between different inputs in the amygdala: in the lateral nucleus, large 'mushroom' spines receive input primarily from thalamic afferents and have larger Ca^{2+} transients than do cortical afferents that synapse on neighboring thin spines [25••]. Whether these are strictly input-specific differences in spine shape or reflect different levels of activation remains to be determined.

Spine necks regulate biochemical and electrical signals in large and small spines

Compartmentalization of Ca^{2+} within the spine head is controlled by spine neck dimensions in both mushroom and thin spines of CA1 pyramidal cells [26•]: spines that have narrower or longer necks appear to retain more Ca^{2+} in their heads following synaptic activation than do wider shorter spines. Depending on the absolute concentration achieved, the localized increase in Ca^{2+} levels could modulate signaling cascades that strengthen or weaken spine synapses. The bidirectional diffusion of proteins also seems to be mediated by an activity-dependent barrier in the spine neck [27]. Longer thinner spine necks transiently trap more molecules such as inositol 1,4,5-triphosphate [28] and PSD95 [29], which further regulate Ca^{2+} or synaptic efficacy. The length of time that PSD95 remains within a spine before diffusing into the dendrite is developmentally regulated and experience dependent [29], as is the aforementioned formation of spine necks. The number of isolated spines increases with neuronal activity, suggesting a synapse-specific mechanism to amplify the biochemical signals necessary for synapse growth or removal [27].

The impact of spine neck geometry on electrical signals seems to differ across brain regions. Early ssTEM and modeling studies suggested that most hippocampal, striatal and Purkinje cell spine necks are not constricted enough to attenuate charge transfer to the parent dendrites significantly [1,30,31], and imaging and electrophysiology studies have confirmed these original models for these spines [32]. By contrast, recent studies suggest that thin spines on basilar dendrites of neocortical layer 5 pyramidal cells are long and constricted enough to

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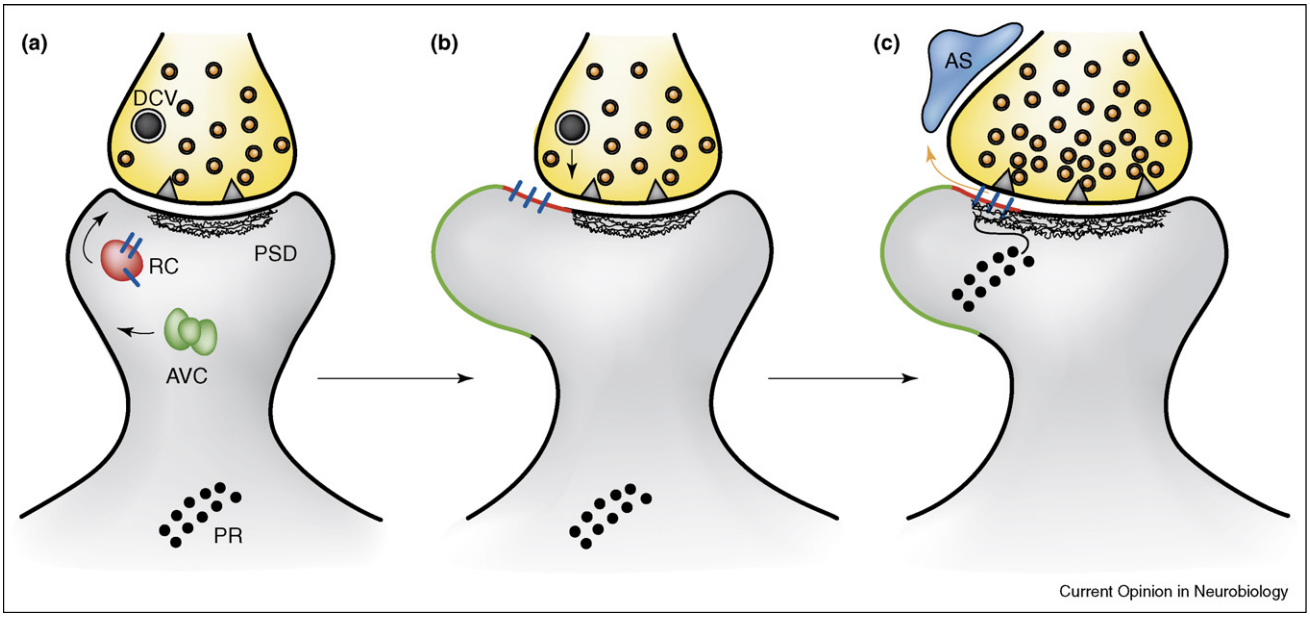
reduce charge transfer [33,34•]. Long-necked spines are essentially electrically silent at the soma, although Ca^{2+} indicators demonstrated that they are activated by uncaging of glutamate at their synapses. It will be interesting to know whether the absolute dimensions of these spine necks are in the special range where slight changes modulate charge transfer [35] and whether these basilar dendrites lack active properties that could boost charge transfer to the soma (in contrast to apical dendrites of hippocampal CA1 cells, where dendritic spikes can amplify synaptic events [23]). ssTEM analysis of the cortical spines would also reveal how the cytoarchitecture and presence or absence of organelles could impact the transfer of charge and the flow of biochemical signals.

Long-term potentiation converts 'learning spines' into 'memory spines'

Long-term potentiation (LTP) is an enduring enhancement of synaptic transmission that is thought to be the cellular correlate of learning and memory. In the immature hippocampus, one effect of LTP is to increase spine head size [36,37,38•,39••], which is followed by an accumulation of AMPA receptors at the synapse (Figure 2) [38•]. Both large and small spines undergo the same absolute increase in head volume and surface area [37,38•]. Recent work reveals a mobilization of recycling endosomes and vesicles (RCs) and amorphous vesicular clumps (AVCs) into spines within minutes after the induction of LTP [39••]. AVCs provide a source of plasma membrane for spine enlargement and RCs probably transport AMPA receptors. By two hours after the induction of LTP, polyribosomes redistribute into the heads of dendritic spines that have enlarged synapses [14]. A transient decrease in levels of F-actin occurs immediately after the induction of LTP and this might enable the transport of polyribosomes and other plasticity-related proteins into the potentiated spines [40]; however, sustained spine enlargement is accompanied by an increase in F-actin levels [41•]. New spines are also formed in response to stimulation paradigms that can induce LTP, and with time their spine heads also enlarge [42,43].

Structural synaptic plasticity also occurs in the more mature hippocampus after the induction of LTP. Polyribosomes are significantly upregulated in dendritic spines two hours after the induction of LTP, and spines that have polyribosomes also have enlarged synapses [44]. The proportion of perforated and complex PSDs is increased one hour after induction of LTP [45]. The volume and area of thin and mushroom spines are increased relative to control stimulation six hours after the induction of LTP in the dentate gyrus *in vivo* [46]. ssTEM has shown that the size of the PSD is perfectly

Figure 2



Model of LTP-related enlargement of dendritic spines and synapses. **(a)** Amorphous vesicular clumps (green, AVC) and recycling vesicles (red, RC) are recruited to potentiated dendritic spines. **(b)** AVCs insert new membrane as the spine head enlarges. RCs that contain AMPA receptors (blue lines) are inserted and then receptors migrate to the vicinity of the synapse; this migration might be facilitated by the fact that the newly inserted membrane is less crowded with other proteins. **(c)** Polyribosomes (black dots, PR) are unmasked and/or recruited to the heads of potentiated spines, where proteins are synthesized locally to stabilize the AMPA receptors and enlarge the postsynaptic density (PSD). At some point the presynaptic axon enlarges, vesicles are recruited and a dense core vesicle (DCV) fuses to enlarge the presynaptic active zone to match the enlarged PSD. Astroglial processes (AS) are attracted to the perimeter of the enlarged synapses, possibly by the spill-out of glutamate from the synaptic cleft (orange arrow).

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correlated with the size of the presynaptic bouton and the number of vesicles it contains [1]; hence, at some point there must be an enlargement of the presynaptic active zone and increase in the number of presynaptic vesicles (Figure 2). Dense core vesicles recently found in mature presynaptic axons seem to be the transport vesicles in active zones [47] that enable rapid formation or enlargement of the active zone in parallel with PSD enlargement (Figure 2) [48]. Blocking synaptic transmission in the more mature hippocampus results in homeostatic upregulation in spines and synapses that recapitulates developmental synaptogenesis [21]; for example, ssTEM reveals a decrease in spine number two hours after induction of LTP relative to control stimulation in relatively mature slices that had been prepared under ice-cold conditions [44]. However, it is possible that this decrease reflects the strengthening of some newly formed synapses by LTP and the elimination of others owing to the ice-cold slicing conditions, so that the control site retained a larger number of the synapses. Recent findings show that chopping slices under mildly hypothermic conditions (room temperature) and transferring them rapidly (in <7 min) to a life-support chamber results in the same density of spines and synapses as in perfusion-fixed hippocampus [49]. These conditions might be more conducive to revealing LTP-related synaptogenesis in more mature hippocampal slices.

Long-term depression converts 'memory spines' into 'learning spines'

Long-term depression (LTD) also has an integral role in the processing and retention of information but, in contrast to LTP, LTD is a long-lasting reduction in synaptic transmission that results from low-frequency stimulation. Induction of LTD results in shrinkage [50] or retraction of dendritic spines [51] associated with a depolymerization of actin [52]. Perhaps the conversion of large 'memory spines' back into smaller 'learning spines' resets the plasticity potential of the dendrite.

Conclusions

Age-related and disease-related declines in cognitive ability are accompanied by decreases in spine density [53,54••–56••,57]. Treatments aimed to counteract age-related cognitive decline result in an increase in numbers of thin spines specifically [56••], suggesting that thin spines are necessary to restore the potential for synaptic plasticity and learning in the aged brain. In addition, the structural stability and abundance of subcellular resources supports the hypothesis that mushroom spines are the more stable 'memory spines'. LTP results in a morphological shift from thin to mushroom spines whereas LTD results in spine shrinkage and retraction. Developmental disorders such as Fragile X syndrome that are accompanied by varying degrees of mental retardation have been characterized by thinner more elongated spines that do not mature into large, mushroom spines [57,58]; these spines also display enhancement of LTD mediated by metabotropic glutamate receptors [59]. Although light-level imaging techniques reveal gross morphological changes, ssTEM is needed to detect and measure changes in dimensions and to provide information about the subcellular events that mediate morphological changes [39••]. Several questions remain. Which structural changes are specific to the different phases of LTP and LTD and other forms of synaptic plasticity? How long does each structural change last? Is the structural synaptic plasticity that is found in the mature brain a recapitulation of development, or fundamentally different? Which structural changes are specific to particular classes of synapse? Answers to these and related questions are needed to understand how distorted spine and synaptic structure affect brain function.

Acknowledgements

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