Structure, development, and plasticity of dendritic spines

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Dendritic spines are distinguished by their shapes, subcellular composition, and synaptic receptor subtypes. Recent studies show that actin-dependent movements take place in spine heads, that spines emerge from stubby and shaft synapses after dendritic filopodia disappear, and that spines can form without synaptic activation, are maintained by optimal activation, and are lost with excessive activation or during degeneration.

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Abbreviations

AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazole-proprionic acid
CNQX	6-cyano-7-nitroquinoxaline-2,3-dione
D-APV	D-2-amino-5-phosphonovaleric acid
EM	electron microscopy
LTP	long-term potentiation
NMDA	*N*-methyl-D-aspartate
PSD	postsynaptic density
SER	smooth endoplasmic reticulum
TTX	tetrodotoxin

Introduction

A diverse assortment of spine-like protrusions emerge from dendrites [1•]. Most spines in the central nervous system have stubby, thin, mushroom or branched shapes [2,3]. Multi-lobed structures called 'thorny excresences' have one or more synapses on each lobe and are present, for example, on proximal dendrites of hippocampal CA3 pyramidal cells [4]. Dendritic spines are present at the squid giant synapse [5], suggesting that they may have developed early in the evolution of the nervous system. We are only just beginning to understand how the structure, formation, and plasticity of relatively simple dendritic spines can influence synaptic function, and it is some of these advances that I will discuss in this review.

Dendritic spine structure

Dendritic spines are the primary postsynaptic targets of excitatory glutamatergic synapses in the mature brain. Even simple spines have remarkably diverse structures. They range in volume from less than 0.01 μm^3 for small thin spines to 0.8 μm^3 for large mushroom spines (Figure 1) [6,7]. Dendritic spines and synapses of different sizes and shapes occur on the same dendrite (Figure 1a). Similarly, a single presynaptic varicosity can form synapses with two or more spines of different dimensions [8,9]. Hence, spine structure is not completely determined by either the presynaptic or the postsynaptic cell.

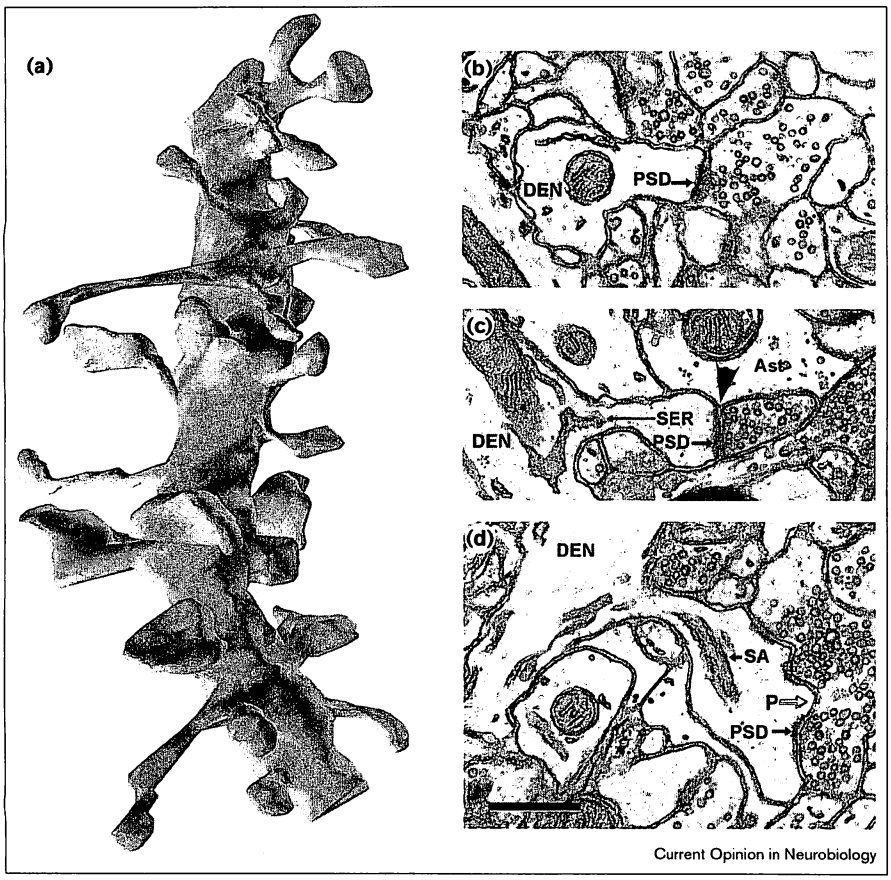
Spine synapses have a thickened postsynaptic density (PSD), which occupies about 10% of the spine surface [6] (see Figure 1b–d). The PSD ranges from a simple disc shape on smaller spines (Figure 1b,c) to a highly irregular shape on larger spines (Figure 1d). Many structural, receptor, and signaling proteins are anchored in the PSD [10•,11•]. The AMPA class of glutamatergic receptors are preferentially located in larger PSDs of hippocampal spines [12••]. Cell–cell adhesion junctions, which contain distinct structural and signaling molecules, are present at the edges of about half of the PSDs and also between spines and neighboring astrocytic processes [13•]. Like the molecules of the PSD, those of the cell adhesion junctions modulate synaptic transmission and plasticity [14,15,16•].

Dendritic spines are further distinguished by their composition of subcellular organelles [17]. For example, about 50% of all hippocampal spines contain smooth endoplasmic reticulum (SER) (Figure 1c), which is specialized to form the 'spine apparatus' in 80% of the large spines (Figure 1d). Some spines contain smooth and/or coated vesicles, multivesicular bodies [17], or polyribosomes [18,19]. Thus, remodeling of synaptic structure via insertion of postsynaptic vesicles [20] or via new protein synthesis could take place in or near spines, *and* degradation could be initiated in spines via the endocytic pathway.

Differences in spine structure can be important for synaptic integration and molecular compartmentalization [21]. Both of these functions are especially sensitive to the length and diameter of the spine neck. Theoretical modeling shows that a thinner and longer neck results in greater depolarization of the spine head for a given synaptic input. Depending on the exact configuration of receptors and voltage-dependent channels, the effects of this property can range from strengthening a particular synapse [22••] to recruiting neighboring synapses in a coordinated depolarization of the dendrite [21]. Similarly, imaging shows that the degree to which calcium is elevated in the spine independently from the dendrite is influenced by spine shape [23,24]. A small calcium signal in a spine can be amplified by an inositol-trisphosphate-dependent release of calcium from the SER. This effect is restricted to neighboring spines along a short dendritic segment [25••,26••]. Limiting the spread of calcium may provide both input specificity for the activated synapses and neuroprotection for the dendritic shaft and soma, where high concentrations of calcium can lead to microtubular breakdown, dendritic swelling, and other degenerative consequences of calcium-induced excitotoxicity [6,27,28].

Rapid fluctuations in spine structure have been visualized by staining their actin-based cytoskeletons in cultured

Figure 1



Dendritic spines in the mature rat hippocampus. **(a)** Three-dimensional reconstruction of a spiny dendritic segment from hippocampal area CA1. This segment is 7 μm long and has 25 spines, three of which are branched (one with three heads and two with two heads), making a total of 29 spine heads. Depicted here are electron micrographs of longitudinally sectioned dendritic spines showing a representative **(b)** stubby spine, **(c)** thin spine, and **(d)** mushroom spine. The small black arrows in (b–d) point to the postsynaptic density (PSD). In (c), smooth endoplasmic reticulum (SER) enters from the parent dendrite into the spine neck, and on adjacent serial sections, the SER continues into the spine head. The arrowhead in (c) indicates where an astrocytic process (Ast) abuts the synapse on the spine head. In (d), the PSD on the mushroom spine is perforated (P) by electron lucent regions where only the plasmalemma is visible. About 80% of mushroom spines contain a spine apparatus (SA), which has stacks of SER with dense-staining fuzz between them. The SER of the SA is also connected to SER in the parent dendrite. DEN, dendrite.

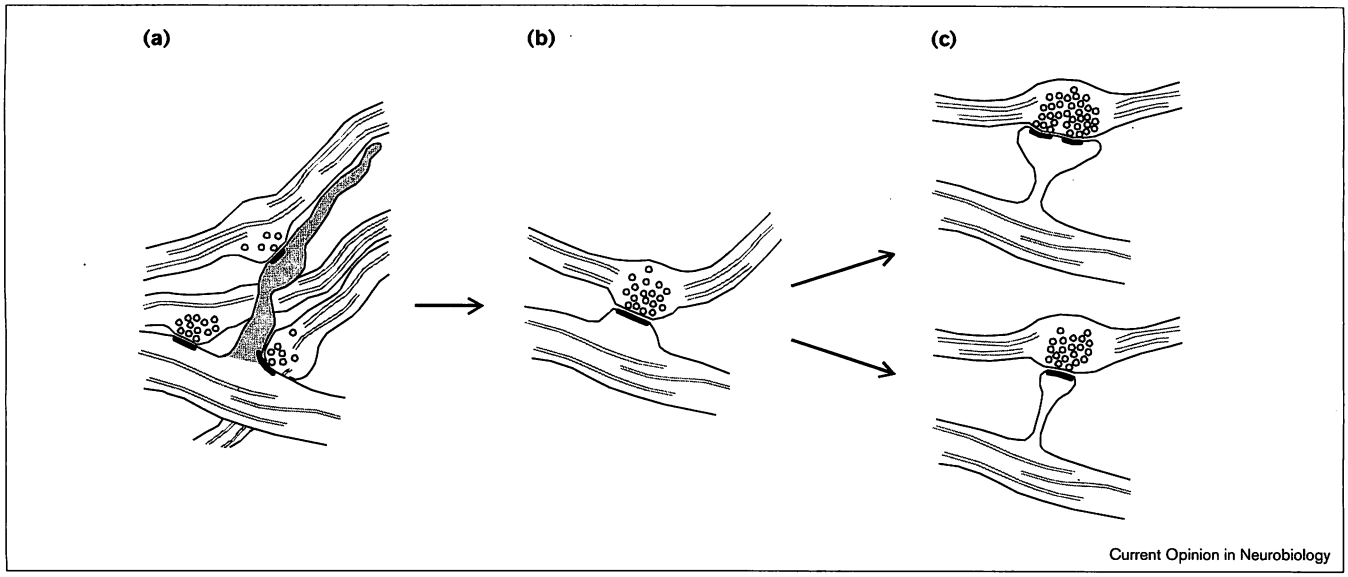
hippocampal neurons [29•]. The movements are mediated by changes in the degree of actin polymerization secondary to changes in the level of internal calcium; and compounds that interfere with actin polymerization prevent these movements [29•]. The images suggest that the spine heads change shape, without much change in the length or volume of the spine. This movement might also reflect an actin-based movement of molecules or organelles within a relatively stable spine membrane.

Dendritic spine formation

Dendritic spines are absent prior to the formation of synapses. Filopodia extend and retract from both dendrites and axons during early stages of synaptogenesis [30,31]. Serial electron microscopy shows that some of the filopodia have synapses at their tips, along their lengths, and at their bases [32••]. Dendritic filopodia recede and are replaced by synapses on dendritic shafts and stubby spines both in hippocampal cultures *in vitro* [33] and after the first postnatal week *in vivo* [32••]. Later, synapses on dendritic shafts and stubby spines decrease, and synapses on thin and mushroom dendritic spines emerge to become the dominant forms in adults [32••,34]. Many of the early synapses are postsynaptically silent, having only NMDA (and no AMPA) glutamatergic receptors [12••,35,36•,37]. Thus, filopodia only appear when a relatively wide extracellular space must be traversed for dendrites and axons to come into apposition, as is the case in dissociated cell culture, organotypic slices and developing neuropil. Filopodia do not provide lasting support for synapses, instead, they appear to guide nascent synapses to dendritic shafts from which spines mature (Figure 2). A lack of spine maturation, specifically the absence of filopodial retraction, is a common feature of conditions leading to severe mental retardation [38•,39].

New dendritic spines are also generated on mature neurons. One hypothesis has been that new spines form through the perforation and splitting of existing synapses [40•]. Such a process would provide new release sites, for example, during hippocampal long-term potentiation (LTP) [41]. However, two lines of evidence argue against this hypothesis. First, branched or 'splitting' spine heads do not share the same presynaptic axon and thus could not arise from a single preexisting spine synapse. Second, perforations in the PSD appear to result from the disassembly or movement of adhesion molecules that span the synaptic cleft [42], in order to accommodate insertion of presynaptic vesicles during synaptic transmission [43•]. Thin evaginations from the spine, called spinules, appear to be engulfed by the presynaptic axon, especially when vesicular release is elevated.

Figure 2



Sequence of synaptogenesis onto hippocampal dendritic spines. Recently, there has been considerable speculation about how dendritic spines are formed and whether filopodia are direct precursors of dendritic spines or whether there is an intermediate stage involving shaft and stubby synapses [32••,52•]. **(a)** Serial EM analyses from hippocampus *in vivo* show that during the first postnatal week (PNW1), more than 70% of the synapses occur directly on the dendritic shafts or at the base of the filopodia (gray shading)[32••]. About 25% of the synapses occur directly on filopodia [32••] and many filopodia have no synapses. **(b)** Serial EM also shows that as synapses double during the second postnatal week (PNW2), more of the synapses occur on stubby protrusions from the dendrite [32••,34,63•]. **(c)** Finally, with maturation, there is another doubling of synapses, most of the shaft and stubby synapses disappear, and dendritic spines emerge as the predominant site of excitatory synapses [34].

Thus, spinules may be involved in removal of postsynaptic membrane in parallel with presynaptic endocytosis. Rather than generating new synapses, this process could coordinate and maintain pre- and postsynaptic structures.

In the mature brain, spines may arise from shaft synapses, such as during development. Alternatively, emerging dendritic filopodia would encounter suitable presynaptic partners at short distances in the compact mature neuropil, and therefore never reach the lengths seen during development, and thus may not be recognized as filopodia. Most of these new spines make synapses with axonal varicosities that already have other synapses on them, giving rise to multiple synapse boutons [44–46]. For example, during the estrous cycle of mature female rats, new spines emerge cyclically, with a concomitant increase in multiple synapse boutons ([45]; see also Woolley, in this issue, pp 349–354). In hippocampal slices from mature male rats, there is a parallel increase in spines and multiple synapse boutons [46]. These findings suggest there are multiple sites on mature presynaptic boutons that can support synapses, and that spines need only to 'discover' these sites for new synapses to form. Such a process would be quite efficient, not requiring a coordinated *de novo* formation of both pre- and postsynaptic sites.

Dendritic spine plasticity

Recent electrophysiological studies suggest that neurons maintain an optimal level of total synaptic input by increasing synaptic strength when activation is low and decreasing synaptic strength when activation is high [47]. One way this could be done is through activity-dependent changes in the receptor composition of existing synapses [48]. Another way is to regulate the total number of active synapses. To define synaptic 'activity' is difficult because different synaptic receptors and channels cause varying degrees of postsynaptic depolarization or induction of signaling cascades. Furthermore, a particular experimental treatment may have different effects depending on the developmental, hormonal, and activation history of a particular neuron (see also the review by Woolley, in this issue, pp 349–354).

Blocking release of neurotransmitter from developing retinal ganglion cell axons with tetrodotoxin (TTX), results in a threefold increase in the number of putative dendritic spines on neurons in the lateral geniculate nucleus [49]. Similarly, inhibiting the NMDA receptor with D-2-amino-5-phosphonovaleric acid (D-APV) results in more than a sixfold increase in putative spines on neurons of the developing lateral geniculate nucleus ([50]; see also [51]). A recent study suggests that localized synaptic activation can induce outgrowth of dendritic filopodia or spines only in the immediate vicinity of the activation, which is blocked by D-APV ([52•]; see also Note added in proof). These results suggest that immature dendrites have more protrusions when the neurons have less global synaptic activation or when there is an

increase in local activation. The global effects may also indicate a developmental arrest in the filopodial state.

There are 40–50% more spines in mature hippocampal slices than in the hippocampus *in vivo*. This increase takes place by the end of a 1 to 2 h recovery period after slicing, a period during which neurons are less responsive [46]. Electron microscopy reveals that the new spines have synapses, and that the effect is specific to an increase in the stubby and mushroom spines. If the mature hippocampal slices are exposed to a combination of TTX, D-APV, CNQX, nimodepine, 0 mM calcium, and 8 mM magnesium, which block synaptic transmission, the dendrites become even more spiny than in control slices, or slices in which synapses are repeatedly activated by electrical stimulation [53]. These findings suggest that mature hippocampal dendrites become spinier when the neurons are less activated.

In organotypic hippocampal slice cultures from the rat, the dendrites have a complement of dendritic spines that are comparable to those present at postnatal day 15 *in vivo*, with approximately equal numbers of stubby, thin, and mushroom spines [34,54••]. As in other systems, if the presynaptic axons are cut and allowed to degenerate for several days, the dendritic spines retract. However, if the lesioned cultures are exposed to AMPA, the spines are retained, suggesting that activation of this glutamatergic receptor is sufficient to maintain dendritic spines in the absence of a presynaptic input [54••]. This hypothesis is supported further by the observation that dendritic spines in cultures are maintained if exposed for 2 days to TTX, which only blocks action potentials, but retract if exposed to botulinum toxin, which prevents all vesicular release [54••]. When the NMDA receptors are blocked with MK801, the dendrites develop more filopodia-like protrusions, suggesting a return to the more immature state [54••].

Long-term potentiation (LTP) is an enduring enhancement of synaptic transmission that could involve a change in synapse number and structure [55]. Recent work shows that overall spine and synapse number, shape, and size are stable 2 to 4 h after inducing LTP, relative to control sites in area CA1 of adult hippocampal slices [56••,57••]. Spine number is also stable after many hours of brief episodes of tetanic stimulation, repeated every 10 min, which also produces sustained synaptic potentiation [53]. These findings suggest that LTP need not be accompanied by a marked or long-lasting change in spine and synapse number or structure. However, unequivocal answers about the role of spine size in synaptic potentiation will require synapse-specific anatomical markers to distinguish silent, active, and previously potentiated synapses from those that were potentiated by the experimentally induced LTP.

Stronger activation of neurons results in spine loss. Exposure of neuronal cultures to NMDA for just 5 min causes a concentration-dependent loss of spines [58••]. Spines in somatosensory cortical slices also retract after just 5 min of exposure to a calcium-free medium [59], which is known to induce epileptiform activity in hippocampal neurons unless magnesium is substantially elevated [60,61]. Chronic epileptic seizures *in vivo* also result in the loss of dendritic spines [62•].

Conclusions

Existing data suggest that more spines form when neurons have less excitatory activation, are maintained by optimal activation, and are lost when activation is too high, or if the presynaptic axons degenerate. This pattern supports the hypothesis that neurons may homeostatically regulate input through spine number. It also suggests a second important fact about dendritic spines. Extra spines that form when excitatory neuronal activation is low can provide a morphological basis to support new synaptic plasticity. Many open questions remain. For example, are spines also formed *in vivo* when excitation is low? How soon after formation are spines activated and incorporated into functional networks? Do the complementary processes of LTP and long-term depression preserve or eliminate spines and synapses? Do spine and synapse number only cycle over days, as during the estrous cycle, or over even shorter times, such as a circadian period, which is accompanied by changes in neuronal activation?

Note added in proof

An interesting new paper [63•] was published, while this review was in preparation, suggesting that highly selective activation may trigger spine formation on developing dendrites.

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- •• of outstanding interest

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56. Sorra KE, Harris KM: **Stability in synapse number and size at 2 hr**
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Interestingly, the authors provide evidence that 30 min after LTP *in vivo*, there are more spines in the dentate gyrus. However, they replicate the findings of Sorra and Harris [56••], showing with confocal microscopy that there are no changes in spine number at 4 h after LTP in area CA1 of adult hippocampal slices (see especially pages 355–356). This paper also reviews the literature on structural correlates of LTP.

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Demonstrates a dramatic loss in dendritic spines in the tetanus-toxin model of epilepsy. It also is a good source of references to earlier literature illustrating a consistent pattern of spine loss when seizure activity is high.

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When synapses along short segments of hippocampal CA1 pyramidal cell dendrites grown in organotypic slice culture are selectively stimulated, potentiation occurs and then 30 minutes later, one or more new dendritic spines appear. No changes occur in total spine density, which is low (0.4 spines/micron) compared to mature hippocampus *in vivo* (2 spines/micron [46]), and stubby spines predominate, hence these are immature dendrites [32••,34]. The findings suggest that synaptic potentiation might initiate developmental processes that induce spines to form, possibly from pre-existing shaft synapses (see Figure 2 of this review).