Growth of dendritic spines: a continuing story
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Dendritic spines, which are present at the vast majority of excitatory synapses in the central nervous system, have a specialized cytoskeleton of dynamic actin filaments that makes them capable of rapid morphological plasticity. During development, structural remodeling of nascent spines is an important factor in experience-dependent shaping of neuronal circuits, whereas in the adult brain spines maintain a balance between morphological stability and plasticity.

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Current Opinion in Neurobiology 2005, 15:67–72
This review comes from a themed issue on Development
Edited by Jane Dodd and Alex L Kolodkin
Available online 26th January 2005
0959-4388/$ – see front matter
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DOI 10.1016/j.conb.2005.01.015

Introduction
The construction of neuronal circuits in the developing brain requires the correct assembly of trillions of synaptic connections. How, given the enormous numbers of axons and dendrites seeking contact in the massive tangle of the growing neuropil, do the right partners manage to find one another? The challenge is met by a combination of genetically fixed routines that produce a ‘rough draft’ of the final circuitry, followed by a process of experience-driven plasticity that refines the detailed pattern of connectivity during a critical period of juvenile learning [1,2]. Dendritic spines, minute protrusions scattered along the dendrites of many brain neurons, play a crucial part in this process. During development, immature dendrites first produce motile filopodia that sample the developing neuropil for active presynaptic partners with which to form synaptic contacts [3–6]. These filopodia are later replaced by more stable mature spines, which typically comprise an expanded head joined to the dendrite shaft by a narrow neck (Figure 1e–f). In this morphologically mature state plasticity is restricted to motile ruffling of the spine head [7,8,9].

The motility of both filopodia and spines depends on the turnover of actin filaments in the spine cytoskeleton [10,11,12], suggesting that the transition from filopodium to mature spine involves the down-regulation of actin dynamics. These are the basics, but despite much work the cellular mechanisms involved in this changeover are not well understood and fundamental questions, such the extent of plasticity retained by mature spines, remain unresolved. Here, I focus on recent advances in understanding how the growth and stabilization of spines and synapses are managed.

Filopodia and synapse formation
Filopodia appear well before mature dendritic spines, moreover, they are produced by developing dendrites of both spiny and non-spiny neurons, suggesting that their fundamental role is in dendrite development [4,13]. This interpretation is supported by evidence that neocortical pyramidal cells produce two distinct classes of dendritic filopodia, those on terminal growth cones, which are more numerous early in development when dendrites are growing and branching, and those produced as lateral protrusions from established dendrites, which are associated with spine formation [12].

A recent study of developing dendrites in non-spiny neurons of zebrafish optic tectum [14] provides compelling evidence linking terminal filopodia to dendrite branching and synapse formation. On the growing dendrites of these cells, postsynaptic puncta, which indicate the location of putative nascent synapses, form preferentially in motile terminal filopodia. Significantly, filopodia containing stable synaptic puncta develop into stable dendrite branches, whereas filopodia that didn’t acquire synaptic puncta or that later lost puncta retracted and disappeared [14]. This process shares key features with the development of spine synapses on hippocampal pyramidal cells. Here, contact between a dendritic filopodium and a developing axon triggers delivery of presynaptic proteins, as preformed vesicular ‘transport packets’, to nascent synaptic sites in the axon [15,16]. This is closely followed by the appearance of postsynaptic puncta at adjacent sites in the dendrite, apparently by accretion of molecules from a diffuse cytoplasmic pool ([3,17,18,19,20] but see [21]). About 90% of new postsynaptic puncta first appear in spine-like protrusions that are stable, persisting for hours or even days in time-lapse recordings. However, headless filopodia that lack postsynaptic puncta are unstable and usually disappear within minutes [17,18,19]. The similarities in the patterns of events in zebrafish tectal neurons and rat hippocampal pyramidal cells suggest a fundamental mechanism by which motile filopodia induce the formation of presynaptic sites that are then instrumental in stabilizing a new dendritic branch or spine (Figure 1).
In hippocampal neurons, stimulation protocols associated with long-term potentiation (LTP) can induce the growth of new spines \[22–24\]. An even more extreme example is provided by the medium spiny neurons of the striatum. The dendrites of these cells are richly covered with spines in vivo but are completely smooth in cell culture unless they are grown together with electrically active cortical neurons to provide excitatory input \[25\]. Cerebellar Purkinje cells behave in exactly the opposite fashion, making and maintaining morphologically mature spines even when the granule cells axons that provide their normal presynaptic input are entirely absent \[26\]. These extreme variations are difficult to reconcile with a single universal mechanism of spinogenesis, and instead suggest that spine growth involves different molecular mechanisms in different types of neurons. This possibility is illustrated by espin, an actin binding and bundling protein, which in rat brain is expressed exclusively in cerebellar Purkinje cells, where it is concentrated in dendritic spines \[27\]. Espin binds to actin filaments with an affinity one to two orders of magnitude greater than other actin cross-linking proteins and its binding is not inhibited by Ca\(^{2+}\) \[27\], properties that could contribute...
significantly to the striking stability of Purkinje cell dendritic spines.

Insight into the role of activity also comes from knockout mice lacking either munc13 or munc18, two genes essential for neurotransmitter release [28,29]. Although developing neuronal circuits in these animals are electrically silent, they nevertheless produce abundant synapses with typical morphological features including synaptic junctions and presynaptic boutons [28,29]. These animals die at birth, before spines are generated, but spine formation can be studied using cultures of hippocampal neurons [29]. After 21 days in vitro, dendrites of munc13 null neurons had the same density of synaptophysin-positive synapses as wild type neurons and, although this study did not explicitly examine spine morphology, an electron micrograph shows an apparently normal spine synapse [29]. More data are needed, but these preliminary observations strongly suggest that both synapses and dendritic spines can form normally in the absence of synaptic transmission.

Activity thus seems nonessential for spine formation but, at least in pyramidal neurons, to have a modulatory role. This conclusion is supported by in vivo imaging of synapses and filopodia on pyramidal neurons in developing mouse cortex [30,31**]. Depriving pyramidal cells of their sensory input, either by trimming whiskers for the somatosensory cortex [30] or by suturing the eyelids for visual cortex [31**], produced a ~50% decrease in spine motility without changing either their number or their size. Significantly, the effects of visual deprivation were seen at a time when synapses had already been formed and when activity-dependent remodeling of circuits takes place [31**]. For technical reasons in vivo studies of spine motility in the cortex have been limited to the apical tufts of layer 5 pyramidal cell dendrites, which receive inputs from other pyramidal cells. A recent study used acute brain slices to examine spine plasticity in the basal dendrites of these same cells, which in vivo are recipients of thalamic inputs [32*]. In this case dark rearing, which extends the critical period, produced a small but significant decrease in spine motility; however, it did not delay the developmental switch from motile, filopodia-like protrusions to mature dendritic spines [32*]. Taken together, these data seem to favor two distinct modulatory processes influencing spine plasticity in the developing brain: an activity-independent switch from unstable filopodia to stable spines and activity-modulated effect on spine motility that is particularly prominent during the critical period.

**Plasticity and stability in mature dendritic spines**

In vivo studies also demonstrate that spines are more stable in the adult than in the developing brain [31**,**33,**34,**35*]. Spines on pyramidal cells in area CA1 of hippocampus showed no significant change in morphology during a time period of many hours, even after experimentally induced epileptic seizures [35*]. In the visual cortex ~96% of spines were stable during a month of repeated imaging [33], but even at P42 adult spines remained motile in the form of significant changes in length within minutes [31**]. By contrast, in the somatosensory barrel cortex only ~50% of spines were stable over a month, whereas ~17% lasted for less than one day. Moreover, trimming the whiskers to deprive cells of sensory input significantly increased the fraction of unstable spines with lifetimes of a day or less [34]. The disparity in apparent plasticity of spine stability between visual and somatosensory cortex seems to reflect intrinsic differences, because measurements of spine motility were similar for the two areas [31**].

Despite their increasing stability during the neonatal period, mature spines retain morphological plasticity in the form of rapid changes in head shape driven by turnover of actin filaments [7,**8,**11]. High resolution imaging of the dendrite surface using membrane-targeted green fluorescent protein (GFP) shows that this motility is greatest at the tips of spines where they contact presynaptic boutons, suggesting a close anatomical relationship between morphological plasticity and synaptic transmission [9*]. Indeed, spine motility is blocked by stimulating postsynaptic glutamate receptors, suggesting that activity consolidates synaptic morphology [36,**37**]. Interestingly, two major postsynaptic glutamate receptor subtypes have distinct effects; whereas stimulating α-amino-3-hydroxy-5-methyl-4-isoxazole-propionate (AMPA) receptors blocks spine motility rapidly and reversibly, the effects of NMDA receptor stimulation require 30 min to develop and spines are still immotile many hours after stimulation [37**,**38*].

Evidence suggests that these receptor-induced effects on spine motility reflect the reciprocal influence on one another of synaptic transmission and the actin cytoskeleton. Drugs that inhibit actin dynamics selectively block LTP [39,**40,**41*] and slightly enhance long-term depression (LTD) [41*]. Conversely, inducing LTD leads to a significant decrease in the rate of actin turnover in dendritic spines [11]. In the brain, high-frequency stimulation of axons in the hippocampus increased the level of polymerized actin in spines postsynaptic to the stimulated pathway [42*] and this effect was still detectable days after stimulation, consistent with the long-lasting blockade of actin dynamics in spines following N-methyl-D-aspartate (NMDA) receptor activation [37**,**38*]. Taken together, these data strongly suggest that in mature dendritic spines actin filament dynamics are closely coupled to LTP and LTD.

Recent studies using advanced stimulation and imaging techniques have confirmed that stimulation patterns
associated with LTP increase the level of polymerized actin in spines and, moreover, that this is associated with increases in spine volume of up to twofold \cite{43,44}. These effects are mediated by NMDA-type glutamate receptors, the activation of which also leads to an increase in the levels of both actin and actin binding proteins in the spine cytoskeleton \cite{37,44,45}. Using the spatial precision of 2-photon confocal microscopy to uncease chemically sequestered glutamate, Matsuzaki et al. \cite{43} were able to both stimulate individual dendritic spines and map their associated AMPA receptor currents. They found that repetitive stimulation produced a size increase so that small spines were converted into spines with large heads. Concomitantly, AMPA receptor currents increased by up to 50% and this effect only persisted in spines where the increase in head size was lasting; in spines where the head size increase was transitory, AMPA receptor currents did not remain enhanced \cite{43}. Previous studies have shown that AMPA receptor expression scales with spine size \cite{46,47}, moreover, the increase in synaptic signal strength induced by LTP correlates with increased surface expression of AMPA receptors \cite{48,49}. Taken together, these data suggest a sequence of events in which actin polymerization leads to an increase in spine size that is coupled to increased expression of AMPA receptors on the spine surface. This in turn produces larger postsynaptic currents and hence LTP (Figure 1f–h).

Stimulation induces spine growth in small spines but has no effect on large spines \cite{43}. Nevertheless, large spines are morphologically active showing prominent motile lamellipodia that appear to enclose, to varying degrees, their associated presynaptic bouton \cite{9}. One suggestion for a physiological role for this form of plasticity is that it reflects fine scale 'tuning' of synaptic functions, such as postsynaptic receptor distribution, Ca\(^{2+}\) transients or neurotransmitter spillover \cite{9}. These changes in configuration of the spine head produced by lamellipodial ruffling might significantly alter the time-course of glutamate in the synaptic cleft and hence the parameters of synaptic transmission by way of AMPA receptors. It has also been demonstrated that actin polymerization close to the membrane influences protein diffusion in the plane of membrane \cite{50}, potentially affecting AMPA receptor trafficking that in turn modulates the distribution of glutamate receptors in the postsynaptic membrane \cite{51}.

**Conclusions**

These latest studies confirm the importance of the actin cytoskeleton in regulating dendritic spine function in both developing and mature synaptic circuits. It is also becoming increasingly clear that actin dynamics influence multiple aspects of synaptic function. A core set of events, set in train by activation of NMDA receptors, has been identified that influences the size, motility and level of AMPA receptor expression in spines. Moreover, each of these functions might provide feedback regulation of the others. It is not yet clear whether these processes are causally related or if they are independent mechanisms triggered in parallel. Nor is it known how these actin-based functions, together or separately, influence animal behavior. Similar to the phenomenon itself, our knowledge of spine development seems likely to remain a continuing story for some time to come.

**Acknowledgements**

I thank T. Gertner for critical reading of the manuscript. Figure 1e is based on an original illustration by M. Roelandse.

**References and recommended reading**

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest


2. Hensch TK: Critical period regulation. *Annu Rev Neurosci* 2004, 27:549-579. This review and that by Berardi et al. \cite{1} provide excellent up-to-date overviews of critical period mechanisms from complementary viewpoints.


9. Roelandse M, Welman A, Wagner U, Hagmann J, Matus A: Focal motility determines the geometry of dendritic spines. *Neuroscience* 2003, 121:39-49. This study uses transgenic mice expressing membrane-targeted GFP to image the dendrite surface of hippocampal neurons at high resolution. In contrast to the conventional view, in which contact between pre- and post-synaptic elements is largely restricted to the junctional zone, the results show that large spines produce motile lamellipodia that partially enclose the presynaptic bouton. It is suggested that this actin-based motility in the synaptic contact zone fine-tunes various aspects of synaptic transmission.


12. Portera-Cailliau C, Pan DT, Yuste R: Activity-regulated dynamic behavior of early dendritic protrusions: evidence for different types of dendritic filopodia. *J Neurosci* 2003, 23:7129-7142. Filopodial dynamics were recorded from cortical pyramidal cells injected with fluorescent dye. The data confirm that terminal filopodia, present at the ends of growing dendrites, are distinct from lateral filopodia that function as precursors to synapses and dendritic spines.


19. Ebihara T, Kawabata I, Usui S, Sobue K, Okabe S: Synaptic activity drives and focusing on different selections of proteins, this study examines the relationship between synapse formation and dendrite branching in the living brain of zebrafish embryos. A detailed analysis demonstrates that synapse formation is initiated in motile filopodia at the ends of growing dendrites and that consolidation of new dendrite branches depends on stabilization of synaptic contacts.


31. Majewska A, Sur M: Motility of dendritic spines in visual cortex in vivo: changes during the critical period and effects of visual deprivation. Proc Natl Acad Sci USA 2003, 100:16024-16029. The authors present an in vivo analysis of changing plasticity in dendritic spines as they develop in the mouse visual cortex. The results go a long way towards reconciling apparently contradictory data from earlier studies and suggest a close correlation between the degree of spine motility and the critical period of juvenile learning. A thoughtful discussion makes clear the distinct issues involved in developmental and adult spine plasticity.

32. Konur S, Yuste R: Developmental regulation of spine and filopodial motility in primary visual cortex: reduced effects of activity and sensory deprivation. J Neurosci 2004, 24:236-246. Similar to Majewska and Sur [31], this study examines the relationship of dendritic spine motility and critical period plasticity, but reaches somewhat different conclusions. Both studies suggest that morphological plasticity in transient filopodia of developing dendrites is a distinct process to the subsequent experience-dependent motility of mature dendritic spines.


35. Mizrahi A, Crowley JC, Shtoyerman E, Katz LC: High-resolution in vivo imaging of hippocampal dendrites and spines. J Neurosci 2004, 24:3147-3151. Two-photon confocal imaging was used to make time-lapse recordings of dendritic spines in the dorsal hippocampus. The results show that there was no motility during periods of several hours even after seizures were induced.


In cultured hippocampal neurons stimulation of NMDA receptors triggered targeting of an actin-binding protein, profilin II to dendritic spines, and this was accompanied by a complete blockade of spine motility. Both effects required 30 min to develop fully and were long-lasting, in that neither had reversed 16 h following the stimulus. Profilin accumulation does not occur in cells expressing a peptide that inhibits the binding of profilin to polyproline domain surface proteins. Expressing the peptide inhibitor destabilizes spine morphology, suggesting that contact interaction between the actin cytoskeleton and the spine surface regulates spine plasticity.
38. Brunig I, Kaech S, Brinkhaus H, Oertner TG, Matus A:
   • Influx of extracellular calcium regulates actin-dependent morphological plasticity in dendritic spines.
   The authors demonstrate that two distinct mechanisms can block the motile plasticity of mature dendritic spines. One, depending on AMPA receptors, is rapid and reversible, whereas the other, involving NMDA receptors, develops slowly and does not reverse when the stimulus is reversed. Both are dependent on influx of extracellular Ca$$^{2+}$$ into the spine cytoplasm.


   The authors demonstrate that drugs that inhibit actin turnover block LTP but enhance LTD, suggesting a bidirectional mechanism linking actin assembly to spine plasticity and changes in synaptic signal strength.

   In this study, high-frequency stimuli were delivered to the perforant pathway of the hippocampus using electrodes implanted in the brain. This produced marked increases in levels of polymerized actin in spines as measured by staining with the actin filament-specific marker rhodamine-phalloidin. This upregulation was detectable as long as a week after stimulation, consistent with the long-term accumulation of profilin II following NMDA receptor activation of cultured hippocampal neurons [37].

43. Matsuzaki M, Honkura N, Ellis-Davies GC, Kasai H:
   See annotation [44].

   Using complementary techniques, Matsuzaki et al. [43] and Okamoto et al. [44] show that stimulation protocols associated with the induction of LTP lead to increased actin polymerization in dendritic spines and a substantial increase in the size of spine heads. In Matsuzaki et al. [43] this is shown to be accompanied by an increase in AMPA receptor currents, which are considered to underlie the increase in signal strength associated with LTP.


50. Richards DA, De Paola V, Caroni P, Gahwiler BH, McKinney RA:
   Using membrane-targeted GFP it is shown that diffusion of surface proteins is significantly impeded in dendritic spines relative to the dendrite shaft. Interestingly, protein diffusion is inversely correlated with spine motility and this effect is regulated by glutamate receptors acting through the actin cytoskeleton.

   The authors present a detailed overview of the mechanisms that regulate glutamate receptor organization in the dendrite membrane, including discussion of advanced single-molecule tracking techniques for studying receptor trafficking.