A thorny question: the role of spine morphogenesis in adaptive plasticity

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One of the fundamental questions in neuroscience today is also one of the oldest: how does the brain change in response to sensory stimuli? This ability of the central nervous system to adapt in response to experience, known as experience-dependent plasticity, is essential not only for the fine-tuning of developing circuits, but also for learning and remembering as adults. Over the past fifty years, there has been an intense search for the cellular basis of experience-dependent plasticity. Evidence has been accumulating that changes in neuronal structure underlie experience-dependent plasticity; one structure that has been the focus of intense investigation is the dendritic spine. Recent experiments have demonstrated that dendritic spines are dynamic structures, that changes in spine morphology reflect changes in spine synapse function, and that spine morphological plasticity accompanies circuit plasticity induced by altered sensory experience. These data strongly support the hypothesis that spine synapse plasticity may be responsible for adaptive plasticity of the brain, although definitive evidence remains elusive. In this chapter, we describe some of the most exciting recent discoveries concerning the mechanisms of spine synapse plasticity, and outline what we feel are the most intriguing questions which still loom over these tiny cytoplasmic extrusions.

DO DENDRITIC SPINES TWITCH?

It was hypothesized that dendritic spines might be dynamic structures even at the time of their first discovery in the late 1800s (Cajal, 1893), and again in the early 1980s (Crick, 1982), yet it wasn't until the application of time-lapse imaging of fluorescently labeled living neurons in the mid-1990s that it was definitively demonstrated that spines were continuously emerging, retracting, and changing shape (Dailey and Smith, 1996; Ziv and Smith, 1996). These early live imaging studies examined fluorescently labeled neurons in dissociated cultures (Ziv and Smith, 1996) or cultured brain slices (Dailey and Smith, 1996) in order to characterize the short term (minutes to hours) dynamics of dendritic spines. The authors found that spines grew and retracted, lengthened and shortened, and rapidly changed shape. They proposed that this exuberant dendritic motility reflected an active search for presynaptic partners (Ziv and Smith, 1996) and that the generation of new connections during circuit plasticity in the brain involved the emergence and stabilization of these motile structures. Indeed, the extent of spine dynamics decreased as the slices matured (Dailey and Smith, 1996), a trend similar to the decline of plasticity that is observed in the aging brain.

An extensive actin network is one of the hallmarks of dendritic spines (**FIG 1**), and it is the dynamicity of this network which permits spines to rapidly change shape with time scales on the order of seconds to minutes (Matus, 1999; Star et al., 2002). The spine actin network has recently been shown to contain two distinct pools of actin fibers; a stable, slowly treadmilling pool, which tends to stay constant as long as the spine does not retract entirely, and a quickly cycling pool proposed to be the "enlargement" pool, which allows for fast changes in spine size (Honkura et al., 2008). While actin is the dominant cytoskeletal element within spines, recent work has suggested that microtubule invasion does occur infrequently and in response to elevated synaptic activity (Hu et al., 2008; Jaworski et al., 2009). Although these recent experiments suggest a potential specialized role for microtubules in spine morphogenesis, blocking actin dynamics with cytochalasin D, a drug that inhibits actin polymerization, is sufficient to completely block spine motility (Fischer et al., 1998).

The early studies demonstrating spine dynamics were quite convincing and highly regarded; however, skeptics remained concerned that spine motility was an artifact of *in vitro* preparations and that it would not be observed in the living animal. These criticisms were soon addressed with advanced *in vivo* imaging approaches using two-photon microscopy (Denk and Svoboda, 1997), which allowed high

resolution imaging of these tiny structures deep within brain tissue. The first set of *in vivo* imaging experiments (Lendvai et al., 2000) examined dendrites of neurons in the somatosensory cortex of young rats infected with Sindbis virus expressing green fluorescent protein (GFP). The authors demonstrated that spines were indeed actively undergoing morphological plasticity over minutes to hours in the living animal. In addition, spine motility decreased as the animals grew older, corroborating the decline of motility observed as slices matured, and once again in parallel with the decrease in plasticity observed upon aging.

This remarkable finding that spines are moving acutely in the brain inspired further characterization of spine morphological changes over longer time intervals. Chronic imaging approaches were developed that allowed repeated imaging of the same dendrites over the course of days, weeks, and even months (Grutzendler et al., 2002; Trachtenberg et al., 2002). These experiments utilized transgenic animals expressing GFP in a subset of cortical pyramidal neurons (Feng et al., 2000), avoiding negative side effects associated with long-term viral infection of neurons. Remarkably, both studies observed that spines appeared and disappeared throughout the lifetime of the animal. However, there were dramatic differences between the studies concerning the extent of spine motility *in vivo*. Trachtenberg and colleagues observed that only ~50% of spines persisted for one month in the young adult, whereas the remainder were transient, mostly living less than one day (Trachtenberg et al., 2002). They suggested that persistent spines represent elements of established neural circuitry, whereas transient spines represent substrates for new synaptic connections during brain plasticity. In contrast, Grutzendler and colleagues observed that ~73% of spines in young animals and ~96% of spines in the adult remained stable over a one month interval (Grutzendler et al., 2002), suggesting a much lower level of plasticity.

The large discrepancy in the spine turnover rates observed by the two studies stirred quite a controversy. However, it is important to note that there were a number of major differences in experimental design between the two initial chronic imaging studies from the Gan and Svoboda labs. First, the laboratories employed different techniques to access the brain for *in vivo* imaging – the Gan lab used a "thinned-skull" approach, in which a dental drill is used to thin the skull before imaging; while the Svoboda lab replaced a portion of the skull with a glass coverslip, or a "cranial window". Claims were made that use of a cranial window for *in vivo* imaging is associated with high spine turnover because of activation of microglia after surgery (Xu et al., 2007), although these claims have been disputed (Holtmaat et al., 2009). Second, the Gan lab imaged mainly in the visual cortex, which some studies find to be less plastic than the somatosensory cortex (Holtmaat et al., 2005; Majewska et al., 2006), although other studies find no major differences (Zuo et al., 2005a). Other possible reasons for the discrepancies include different housing environments for the mice, different visibility of spines under the two imaging conditions, and different cell types (layer V versus layer VI pyramidal neurons). Despite all of the controversy, it was an enormous step forward to observe that spines were indeed "twitching" in the adult brain.

DOES SENSORY EXPERIENCE INFLUENCE SPINE MOTILITY?

Changes in dendritic spine densities and morphologies have long been shown to increase or to decrease in response to various environmental stimuli (reviewed by Yuste and Bonhoeffer, 2001). For example, hibernating squirrels lose 40% of their spines, which they regain within a few hours after emerging from hibernation (Popov and Bocharova, 1992). Spine densities are reduced by light deprivation in mice (Valverde, 1967) and increased by visual stimulation (Parnavelas et al., 1973). Most profoundly, animals exposed to enriched environments show altered spine morphologies, and at the same time, these animals are better at solving spatial memory tasks (Greenough and Volkmar, 1973). These studies strongly suggested that sensory experience influences spine motility, yet they were all static snapshots and therefore did not address the acute influence of experience on spine morphogenesis, which requires *in vivo* imaging of living neurons in behaving animals.

The first thrilling glimpses that sensory experience can influence spine motility *in vivo* were from the somatosensory cortex of mice. Using *in vivo* two-photon time-lapse imaging, Lendvai and colleagues demonstrated that spine motility in the somatosensory cortex, but not in neighboring cortical areas, declined in response to sensory deprivation (Lendvai et al., 2000). These studies monitored alterations in spine length over time after clipping all whiskers. Further experiments using a more complex checkerboard whisker clipping pattern (designed to induce maximal plasticity throughout the somatosensory cortex) demonstrated that both growth and retraction, or 'turnover', of spines was increased in response to

checkerboard whisker clipping, which also induced circuit plasticity as assessed electrophysiologically (Trachtenberg et al., 2002). This adaptive circuit plasticity was accompanied both by increases in the occurrence of new persistent spines, thought to represent new circuit connections, and by loss of preexisting persistent spines, presumed to represent the loss of old circuits (Holtmaat et al., 2006). Finally, Zuo and colleagues examined the effects of trimming all whiskers on spine turnover *in vivo* (Zuo et al., 2005b). They found that whisker clipping reduced spine loss without affecting spine growth; the reduction in spine loss was abrogated when whiskers were allowed to regrow. Remarkably, a reduction in the rate of spine loss could also be induced simply by infusing drugs that block NMDA receptors, and reversed after drug withdrawal, suggesting a role for the NMDA receptor in translating sensory experience into changes in rates of spine morphogenesis.

The visual cortex has also provided a great resource for defining the influence of sensory experience on spine motility *in vivo*. In a challenging set of experiments designed to link functional plasticity and specific arrangements of dendritic spines, Hofer and colleagues demonstrated that monocular deprivation, which biases electrophysiological responses in the binocular region of the primary visual cortex toward the open eye, increased the rate of spine formation on apical dendrites of layer V cells, leading to an increase in spine density (Hofer et al., 2009). Restoring binocular vision restored the electrophysiological responses and the rate of spine formation to normal levels, however spine densities remained elevated. Remarkably, spine addition did not increase a second time when the same eye was closed again, even though the electrophysiological responses shifted again and even more rapidly. The authors suggested that those spines added during the first monocular deprivation provide a structural basis for subsequent functional shifts (Hofer et al., 2009); a phenomenon resembling that described for axonal growth during auditory map plasticity in the barn owl (Knudsen, 2002).

Another extraordinary set of experiments in the visual cortex helped to define a role for the extracellular matrix (ECM) in regulating the spine structural changes induced by experience. Mataga and colleagues demonstrated that targeted disruption of the tissue-type plasminogen activator (tPA) prevented spine loss on apical dendrites of layer II/III cells induced by monocular deprivation (Mataga et al., 2004). Because proteolysis by tPA increased with monocular deprivation and declined with age (Mataga et al., 2002), this result was interpreted to mean that proteolysis by tPA is permissive for dendritic spine plasticity and that, in the absence of tPA in older animals, the ECM encapsulates the spine and physically blocks plasticity (Berardi et al., 2004; Mataga et al., 2004). Such a model suggested that removing the ECM might lead to increased plasticity. Indeed, *in vitro* experiments demonstrated that spine motility increased in response to tPA application (Oray et al., 2004). And, in a true tour-de-force set of experiments performed by Maffei and colleagues, *in vivo* application of chondroitinase ABC, an enzyme that digests the ECM, restored robust visual cortical plasticity to adult animals that would normally exhibit very little plasticity (Pizzorusso et al., 2002). The same authors showed that chondroitinase ABC application, when combined with reverse lid-suturing immediately following monocular deprivation, caused a complete recovery of ocular dominance and dendritic spine densities to normal levels (Pizzorusso et al., 2006). These amazing results suggest that targeted degradation of the extracellular matrix might provide a way to restore plasticity in older animals.

WHAT ARE THE PATTERNS OF ACTIVITY THAT INDUCE SPINE MORPHOGENESIS?

What are the specific patterns of activity that lead to spine morphological plasticity in response to experience? Because experience-dependent plasticity in brain circuitry is thought to occur via associative, synapse-specific changes, a remarkable breakthrough came when spine morphogenesis was shown to be evoked by synaptic activity paradigms that induce associative plasticity. The first of these studies came out in the late 1990's (Engert and Bonhoeffer, 1999; Maletic-Savatic et al., 1999). Despite using very different techniques to locally stimulate dendrites with activity patterns known to induce synaptic strengthening (long-term potentiation or LTP), both studies observed increased rates of spine outgrowth in stimulated dendritic regions, but not in unstimulated regions on the same neuron. This outgrowth was inhibited by NMDA receptor blockers, which also inhibit synaptic strengthening. In a complementary study, local stimulation in patterns that induce synaptic weakening (long-term depression or LTD) was shown to decrease synaptic strength and cause spine shrinkage (Zhou et al., 2004), which was inhibited by NMDA receptor blockers. Thus, the NMDA receptor and Hebbian mechanisms of synaptic plasticity appear to play key roles in the induction of spine morphological plasticity.

That spines grew and shrank in response to local stimulation in patterns that induced synaptic strengthening and weakening was indeed remarkable; however, stimulation with local microelectrodes was not restricted to individual spine synapses. Therefore, whether such spine morphological plasticity is input-specific remained unanswered until development of caged glutamate with a suitable two-photon cross section (Matsuzaki et al., 2001). In a series of elegant experiments using two-photon photolysis of caged glutamate at single spines in patterns that induced synaptic strengthening, Matsuzaki and colleagues demonstrated that individual stimulated spines, and not neighboring unstimulated spines, grew in volume as spine synapses increased in strength (Matsuzaki et al., 2004). This growth in spine volume was dependent on NMDA receptors, calmodulin and actin polymerization. In a later study, the same authors demonstrated that long-term spine enlargement also depends on protein synthesis and brain-derived neurotrophic factor (BDNF) action (Tanaka et al., 2008). Thus, spine morphological plasticity can be induced in an input-specific manner and shares many molecular mechanisms with LTP and LTD.

Is spine morphological plasticity always input-specific? A series of very challenging experiments demonstrated that there is cross-talk between spine synapses. The induction of LTP and spine growth at an individual spine using two-photon glutamate uncaging caused nearby spines (within ten microns) along the dendrite to be more receptive to synaptic strengthening using a normally subthreshold LTP inducing protocol (Harvey and Svoboda, 2007). In a subsequent study, Harvey and colleagues showed that induction of LTP at a single spine caused the spread of activated Ras, a small GTPase signaling protein, along the dendrite for up to ten microns, and that blocking Ras signaling prevented the reduction in LTP threshold (Harvey et al., 2008), suggesting a critical role for Ras in cross talk between neighboring synapses. Indeed, in the area surrounding activated spines, the likelihood of new spine appearance is increased (De Roo et al., 2008). These findings suggest that the formation of dendritic spines may be potentiated around sites of high synaptic activity.

Despite all of the success at inducing spine growth and shrinkage in response to glutamate uncaging stimuli, as of yet, *de novo* spine outgrowth or spine retraction in response to glutamate uncaging has not been reported and the repertoire of patterns of activity responsible for the *de novo* gain and complete loss of spines remains somewhat a mystery.

DO CHANGES IN SPINE SHAPE REFLECT CHANGES IN SPINE FUNCTION?

Spines grow and retract in response to experience, but are these changes in spine morphology functionally relevant? Many neurological diseases resulting in mental retardation have been associated with spine loss or spine morphology changes (Fiala et al., 2002); however, whether these changes are causative or a consequence of the disease is not yet clear. Because almost all spines (~96%) in the adult animal serve as the receiving half of excitatory chemical synapses (Arellano et al., 2007), it is easy to assume that the gain or loss of a spine is associated with the gain or loss of a synapse. However, it could also be that those 4% of spines that were not synapse-associated captured in the static ultrastructural study actually are those that are motile in the adult; or, in other words, motile spines don't make synapses, and therefore may not have any physiological relevance.

One very convincing method to address this question is using *in vivo* time-lapse two-photon microscopy to identify new spines followed by retrospective serial section electron microscopy (SSEM) to identify whether new spines are synapse-associated (Knott et al., 2006; Trachtenberg et al., 2002). Trachtenberg and colleagues reconstructed four spines that were each less than one day old and found that two of the four fulfilled the expectations of a spine synapse; they contacted presynaptic boutons and were apposed to active zones containing clusters of synaptic vesicles. Similar results were obtained *in vitro* using retrospective SSEM on new spines that grew in response to local high-frequency stimulation (Nagerl et al., 2007). Thus, new spines in the adult brain can form synapses in less than one day from their time of initial emergence. Further studies provided convincing arguments that these new spines were formed *de novo*, and not from preexisting shaft synapses (Knott et al., 2006), thus suggesting that these new spines are integrated into new circuit connections that represent functional circuit plasticity.

New spines can make anatomically mature synapses as assessed by ultrastructural studies, but are newly formed spines functional? Can they receive signals from a presynaptic terminal? Zito and colleagues combined time-lapse imaging to identify spines of different ages with whole-cell recording to measure the responses of new spines and their neighbors to two-photon glutamate uncaging (Zito et al., 2009). They

found that new spines expressed glutamate sensitive currents that were indistinguishable from mature spines of comparable volumes. Some spines exhibited negligible AMPA receptor-mediated responses, but the occurrence of these 'silent' spines was uncorrelated with spine age. Instead, new spines rapidly accumulated glutamate receptors, within tens of minutes of the time of emergence. In addition, newly emerged spines have been shown to exhibit calcium transients shortly following their appearance (De Roo et al., 2008; Lohmann and Bonhoeffer, 2008). Thus, newly emerged spines rapidly become functional. However, despite these exciting and provocative studies, the time frame with which new spine synapses become functionally relevant for neural circuits remains to be determined.

Experience-dependent spine plasticity can also involve more subtle morphological changes in spine shape. Recent data support that these smaller changes in spine shape could also be functionally relevant. Ultrastructural studies have demonstrated that spine volume, postsynaptic density size, and AMPA receptor content are highly correlated (Harris and Stevens, 1989; Kharazia and Weinberg, 1999; Nusser et al., 1998), suggesting that spine volume should be an accurate indicator of synaptic strength. Indeed, functional studies using two-photon glutamate uncaging demonstrated that the amplitude of excitatory postsynaptic currents at individual spines is proportional to spine volume (Matsuzaki et al., 2001), and that increases in synaptic strength at individual dendritic spines are proportional to spine volume increases (Matsuzaki et al., 2004). Additional studies using local synaptic stimulation found similar results showing that increases and decreases in synaptic strength are associated with increases and decreases in spine volume in the stimulated regions of dendrite, and not in unstimulated regions (Bastrikova et al., 2008; Becker et al., 2008; Wang et al., 2007; Yang et al., 2008; Zhou et al., 2004). Finally, even subtle anatomical changes, like increases in spine neck resistance, are likely to have large effects on spine signaling properties, and have been shown to be associated with long-term potentiation (Bloodgood and Sabatini, 2005). In sum, even small changes in spine shape are functionally relevant.

DO SPINE MORPHOLOGICAL CHANGES UNDERLIE EXPERIENCE-DEPENDENT CHANGES IN NEURONAL CIRCUITS?

The past decade has been a remarkable time for defining the role of dendritic spines in adaptive plasticity in the brain. The experiments described in this chapter demonstrated that spines are motile *in vivo*, that the rate of spine motility can be modulated by experience, that changes in spine morphology represent functional changes in synapses. These studies provide strong support for the hypothesis that spine morphological changes underlie experience-dependent changes in neuronal circuits. However, several key questions remain. Are new spine synapses generated in the adult functionally relevant and integrated into neuronal circuits utilized in the intact organism? Is spine motility necessary for circuit plasticity? And finally, will increasing spine motility in adults reactivate plasticity mechanisms that could enable recovery from damage to the central nervous system? These and other key questions related to molecular mechanisms of spine synapse plasticity will no doubt be the focus of intense investigation in the decade to come.

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FIGURE LEGENDS

Figure 1. Structure of a dendritic spine. A GFP-labeled hippocampal pyramidal neuron in cultured slice imaged with a custom two-photon microscope showing dendrites and dendritic spines (AH and KZ, unpublished) and a schematic of a spine synapse. The archetypal dendritic spine is a bulbous extension of the dendritic cytoplasm, with volumes ranging from 0.001 - 1 μm^3, connected to the dendrite by a thin neck (diameter ~ 0.1 μm), which serves to isolate the spine head from the dendritic shaft. Ultrastructurally, a spine synapse is typically defined by three components: (1) a presynaptic axonal bouton with synaptic vesicles separated by (2) a thin synaptic cleft (~ 20 nm) from (3) a dendritic spine containing an electron dense mass known as the postsynaptic density, or PSD.

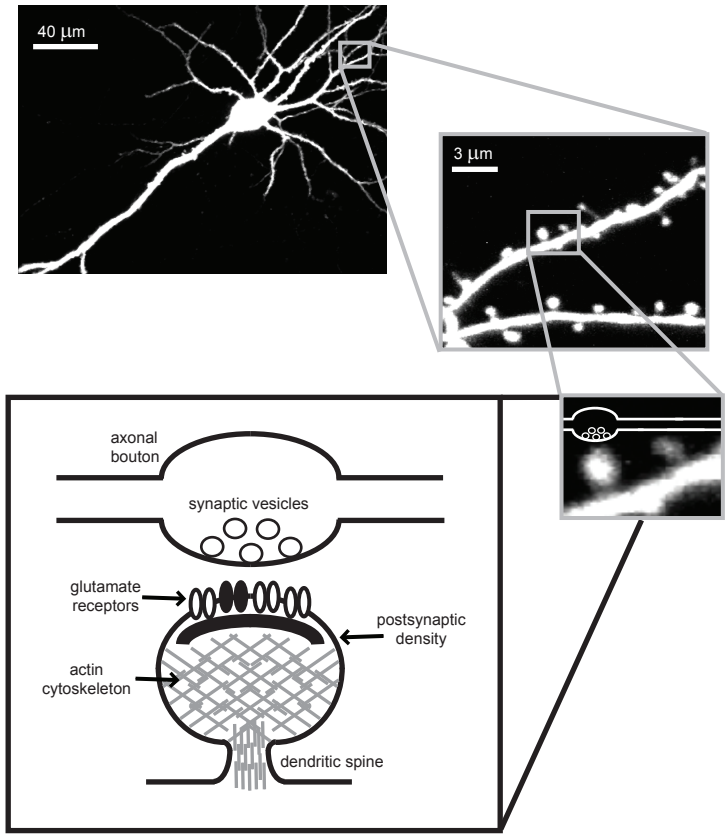
40 µm
3 µm
axonal bouton
synaptic vesicles
glutamate receptors
postsynaptic density
actin cytoskeleton
dendritic spine

FIG 1