Dendritic Spine Morphogenesis and Plasticity

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ABSTRACT: Dendritic spines are small protrusions off the dendrite that receive excitatory synaptic input. Spines vary in size, likely correlating with the strength of the synapses they form. In the developing brain, spines show highly dynamic behavior thought to facilitate the formation of new synaptic contacts. Recent studies have illuminated the numerous molecules regulating spine development, many of which converge on the regulation of actin filaments. In addition, interactions with glial cells are emerging as important regulators of spine morphology. In many cases, spine morphogenesis, plasticity, and maintenance also depend on synaptic activity, as shown by recent studies demonstrating changes in spine dynamics and maintenance with altered sensory experience.
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Dendritic spines were first described at the end of the 19th century by Ramon y Cajal. Cajal proposed that these small protrusions emerging from the dendrites of many neurons were sites of neuronal contact and suggested that changes in the activity of neurons might affect spine morphology (Ramon y Cajal, 1888, 1891). Studies based on both light and electron microscopy have confirmed that spines are indeed sites of synaptic input, with over 90% of excitatory input ending on dendritic spines (Gray, 1959). Moreover, changes in spine numbers and morphology are associated with changes in neuronal activity and experience (reviewed in Yuste and Bonhoeffer, 2001). Therefore, the density and morphology of dendritic spines at a given locus could be interpreted as a readout of the number and state of potentiation of a population of synapses (Kasai et al., 2003). What was not initially appreciated, due to the methods of visualization available, is that spines can be very dynamic structures (Bonhoeffer and Yuste, 2002). In this review, we will first discuss spine formation during development. Next, we will discuss the cellular and molecular underpinnings of spine morphogenesis and dynamics, featuring newly posed factors and mechanisms. Finally, we will end with a discussion of spine dynamics in the intact mature brain, including the role activity and experience play in modulating these dynamics.

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SPINE STRUCTURE

Dendritic spines generally consist of a head (up to a micron in length) attached to a dendrite via a stalk or a neck. Within this general description, spines span a continuum of shapes from short, stocky spines to long-necked spines tipped by a bulbous head. Traditionally, and based on ultrastructural analysis of the adult cerebral cortex (Peters and Kaiserman-Abramhof, 1970), spines have been divided into several types such as stubby, thin, mushroom-shaped, and cup-shaped (Fig. 1). Because spines are now known to be quite dynamic, changing shape on a time scale of minutes (Dunaevsky et al., 1999; Parnass et al., 2000, see below), the validity of these categories, formulated from static images, is not clear. Indeed, recent measurements of spine dimensions do not provide support for the existence of distinct spine categories (Wallace and Bear, 2004). Although the shape of the spine might be important for its function as an electrical or biochemical compartment (Tsay and

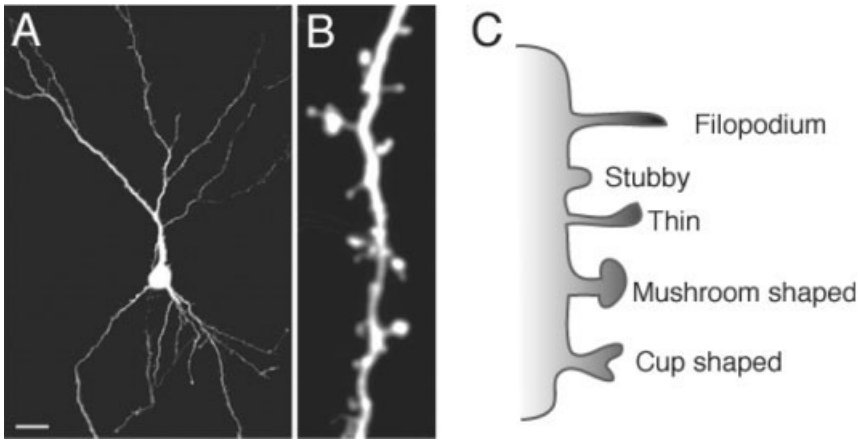

Figure 1 Morphology of dendritic spines. (A) A pyramidal neuron in hippocampal slice culture transfected with green fluorescent protein (GFP) imaged with two-photon microscopy. (B) High magnification image of a hippocampal dendrite demonstrating the diversity in the morphology of dendritic spines. (C) A schematic representation of morphological classifications of dendritic spines. Scale bars: 20 μm in (A) and 2.7 μm in (B).

Yuste, 2004), it is now known that it is the size of the spine head that correlates with synaptic strength (Schikorski and Stevens, 1997; Matsuzaki et al., 2001).

The site of contact between a spine and a presynaptic terminal is marked by the postsynaptic density (PSD), an electron dense thickening of the postsynaptic membrane. The PSD contains the molecular machinery that links synaptic transmission to various signaling cascades and cytoskeletal components (Kennedy, 2000). Unlike the dendritic shaft, the spine head is highly enriched in actin filaments (Fifkova and Delay, 1982; Matus et al., 1982), which mediate spine shape changes and motility (Fischer et al., 1998). Some dendritic spines also contain smooth endoplasmic reticulum, an internal store of calcium. Calcium transients can be restricted to single spines, thus isolating the effect of activation of specific synapses (Sabatini et al., 2001).

SPINE FORMATION

How do spines develop and what regulates spine formation? In most cells, dendritic spines are more prominent in older cells while dendritic filopodia [Fig. 1(C)] are more prominent on younger dendrites (Dailey and Smith, 1996). Although dendritic filopodia have been proposed to be precursors of dendritic spines (Ziv and Smith, 1996; Fiala et al., 1998; Harris, 1999), direct emergence of new spines (with heads) has been observed (Engert and Bonhoeffer, 1999; Marrs et al., 2001), suggesting that spines do not have to transit through a filopodial stage. In addition, on some neurons, dendritic filopodia seen in early stages do not transform into dendritic spines in the adult. Instead, the dendrites become smooth (Wong et al., 2000). In most cases, the development of dendritic spines occurs concurrently with the growth of the presynaptic elements, suggesting that cell-cell interactions and extrinsic cues likely induce the formation of dendritic spines. Although spines and especially filopodia have been thought to actively contact afferents and subsequently induce formation of presynaptic specializations (Ziv and Smith, 1996; Ziv and Garner, 2004), the converse, that ingrowing axons initiate the emergence of dendritic protrusion (Jontes et al., 2000), is also likely. Nevertheless, even in systems such as the cerebellar Purkinje cell, in which the development of dendritic spines and axonal growth occur simultaneously, the formation of dendritic spines can occur in the absence of afferents (Sotelo, 1990), arguing for an intrinsic program for the formation of dendritic spines. Therefore, both extrinsic and intrinsic factors could potentially regulate the formation of dendritic spines, and cell-specific differences in regulation of formation of dendritic spines exist (Yuste and Bonhoeffer, 2004).

SPINE MOTILITY

The ability to image dendritic spines in living preparations has revealed that dendritic protrusions are highly dynamic (Bonhoeffer and Yuste, 2002; Fig. 2). In addition to the protrusive motility of dendritic filopodia on young neurons, which can increase in length at rates of several microns over the course of minutes

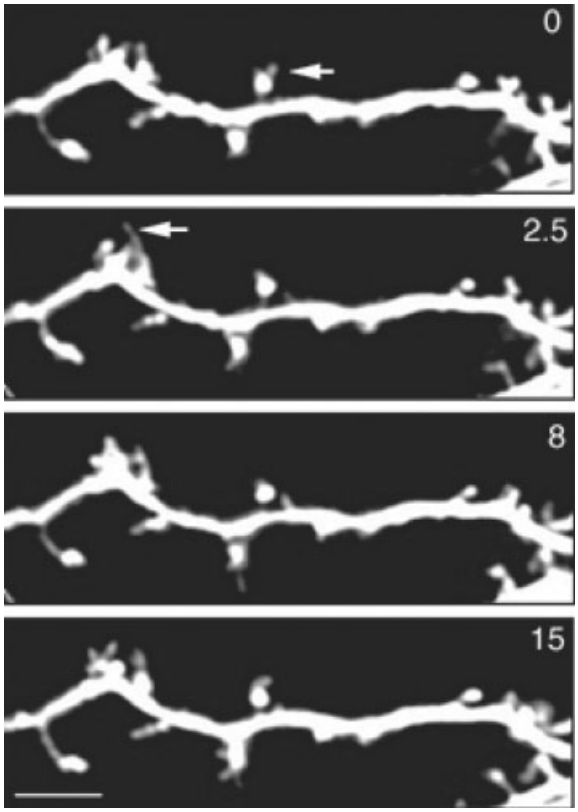

Figure 2 Dendritic spines are dynamic structures. A time-lapse series of a hippocampal dendritic segment imaged at intervals of 30 s. Note how some spines change shape over a time scale of minutes. Time indicated in minutes. Scale bar: 5 μm.

(Dailey and Smith, 1996; Ziv and Smith, 1996), more mature dendritic spines with heads exhibit a more subtle type of motility (Fischer et al., 1998). This motility is powered by actin filament polymerization (Fischer et al., 1998; Dunaevsky et al., 1999) and is developmentally regulated (Dunaevsky et al., 1999). Ultrastructural analysis of previously imaged dendritic spines indicates that spines can continue to be motile even when contacted by a synaptic terminal (Dunaevsky et al., 2001). Moreover, spine motility can continue in the presence of a functional contact (Deng and Dunaevsky, 2004; but see Korkotian and Segal, 2001a). The finding that spines can be motile while bearing synaptic contacts suggests that spine motility might have roles additional to the formation of initial cell-cell contacts, including involvement in synaptic competition (Dunaevsky and Mason, 2003).

In addition, spine motility after synapse formation could serve to alter the signaling at the synapse. Majewska et al. (2000) have shown that alterations in spine shape change calcium dynamics. This type of plasticity could lead to activation of alternative pathways, or even potentiation of certain synapses over others, and may provide a mechanism for activity-dependent learning. In addition, it has been shown that spine motility is caused by rearrangement of actin molecules (Fischer et al., 1998; Dunaevsky et al., 1999), so motility may alter signaling at the synapse by recruiting different, actin-linked molecules to the site of contact. CaMKII, a molecule implicated in LTP, tethers to the actin cytoskeleton and could potentially be brought into the synapse during rearrangement of the actin molecules (Shen et al., 1998). In fact, CaMKII itself has been shown to regulate activity-dependent spine plasticity (Jourdain et al., 2003). It is interesting to note that while dendritic spines are highly dynamic, the presynaptic terminals that contact them are not (Deng and Dunaevsky, 2004).

MECHANISMS REGULATING SPINE FORMATION AND MOTILITY

Several molecular families, including receptors, scaffolding proteins, and regulators of the cytoskeleton have been shown to regulate spine numbers and shape. Not surprisingly, many of those molecules converge on regulation of actin dynamics, which Fischer et al. (1998) identified as the underlying source of spine morphogenesis.

Glutamate Receptors

Many studies indicate that the activation of glutamate receptors on spines stabilizes actin filaments, thus decreasing spine motility. For example, addition of amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) to cultured hippocampal neurons renders the spines and the actin within less dynamic (Fischer et al., 2000). This AMPA-dependent decrease in motility only occurs if the membrane is depolarized and can be blocked through inhibition of low voltage-gated calcium channels (Fischer et al., 2000). In addition to spine stabilization, AMPA receptors may also play a role in spine growth and maintenance (McKinney et al., 1999; Passafaro et al., 2003).

Activation of another type of glutamate receptor, *N*-methyl-D-aspartate (NMDA), causes rapid spine retraction due to loss of f-actin at the spines under some conditions (Halpain et al., 1998), but an increase in spine size under other conditions (Lin et al., 2004). Like AMPA activation, NMDA activation can also lead to stabilization of the dendritic

spines by altering the actin cytoskeleton (Ackermann and Matus, 2003). In a recent study, local activation of glutamate receptors by photo uncaging on single spines of hippocampal pyramidal neurons caused an enlargement of the dendritic spine head (Matsuzaki et al., 2004). Yet in a different study, minute contractions and shortening of dendritic spines were evoked by bursts of action potentials (Korkotian and Segal, 2001b). The response of spines to neuronal activity can therefore be quite diverse and is likely to depend on the exact pattern of activation as well as the size of the spine (possibly reflecting previous activation).

The differing responses caused by activation of glutamate receptors may be due to activation of diverse downstream molecules. For example, spine collapse requires calcineurin activation (Halpain et al., 1998), whereas an increase in size, which is likely due to an increased recruitment of AMPA receptors to the postsynaptic membrane, requires CaMKII (Jourdain et al., 2003; Matsuzaki et al., 2004; Lin et al., 2004). This pattern is reminiscent of LTD and LTP regulation, and is perhaps an endpoint of the same pathway. Strengthening this link, blocking actin dynamics has been shown to impede LTP (Krucker et al., 2000).

PSD Proteins

NMDA receptor activation engages molecules that directly affect actin redistribution. In hippocampal cultures, an actin-binding protein, profilin, moves to the spine head following NMDA receptor activation, suppressing actin dynamics, as seen in time-lapse imaging studies in which profilin and actin were differentially labeled with fluorescent markers (Ackermann and Matus, 2003). This leads to spine stabilization, which could be blocked by inhibiting the movement of profilin to the spine head. Another actin-binding protein, cortactin, shows a similar movement to the dendrite after NMDA activation (Hering and Sheng, 2003). Knocking down cortactin through RNA interference leads to a decrease in spine density, whereas overexpression leads to an elongation of the spines (Hering and Sheng, 2003). In addition to its binding site for actin, the cortactin structure also contains a binding site for Shank, a scaffolding protein found in the PSD that binds NMDA receptors, providing a physical link between the NMDA receptor and the actin that controls spine morphology (Naisbitt et al., 1999). Manipulations of Shank lead to alterations in spine shape; spines heads grow larger following Shank overexpression, especially when coexpressed with Homer1b, while blocking Shank activity leads to a decrease in spine number (Sala et al., 2001, 2003). Inhibition of Shank activity may occur endogenously through Homer1a, which negatively regulates spine growth and synaptic transmission (Sala et al., 2003).

Links to Small GTPases

Another component of the PSD, the protein PSD-95, also links glutamate receptors to the actin cytoskeleton, but through signaling molecules. PSD-95 interacts with the small GTPases Ras, through the GTPase activating protein (GAP) SynGAP (Chen et al., 1998), and Rap, through another GAP, SPAR (Pak et al., 2001). While overexpression of PSD-95 itself will cause maturation of spines (El-Husseini et al., 2000), transfection of SPAR alone is enough to produce not only an enlargement of the spine head but changes that lead to gross abnormalities in spine shape, such as multiple heads that emerge from the same stalk (Pak et al., 2001).

In addition to Rap, other small GTPases, specifically those in the Rho family, play a role in the regulation of spine motility and morphogenesis. Spine density increases as a result of inhibition of RhoA (Tashiro et al., 2000), but decreases in response to Rac1 inhibition (Tashiro and Yuste, 2004; Nakayama et al., 2000). Tashiro and Yuste (2004) have recently shown that blockade of Rho kinase, a downstream effector of RhoA, caused an elongation of spines and an increase in spine motility with no effect on head shape. Overexpression of another Rho family GTPase, Rnd1, resulted in a similar elongation of the spine neck (Ishikawa et al., 2003). While specific suppression of Rnd1 protein expression led to an overall decrease in spine density and width, it increased the number of headless spines (Ishikawa et al., 2003), whereas Rho kinase did not affect spine heads at all (Tashiro and Yuste, 2004). Rnd1 is particularly interesting because unlike the other Rho family GTPases mentioned above, Rnd1 is constitutively active and its expression is highest during the period of synaptogenesis (Ishikawa et al., 2003).

Rho family GTPases are activated by guanine nucleotide exchange factors (GEFs). One of these GEFs, kalirin, can increase spine density (Penzes et al., 2001). Reducing endogenous kalirin in the hippocampus using antisense oligonucleotides for Kal-7, the most common kalirin isoform in the adult rat brain, leads to shortened dendrites and an almost complete reduction in dendritic protrusions (Ma et al., 2003).

These studies demonstrate the involvement of small GTPases, but do not examine the downstream

effects of GTPase activation. Hayashi et al. (2004) addressed this problem by testing the functions of p21-activated kinase (PAK) in this system. When phosphorylated, PAK colocalizes with PSD-95 in the dendritic spines. Transgenic mice expressing a dominant negative form of PAK show decreased spine density when compared to wild-type mice (≈22% lower). The remaining spines have shorter necks with larger heads. Coincident with the notion that larger spines participate in stronger synapses, the neurons of the transgenic mice showed enhanced LTP and reduced LTD while basal synaptic transmission remained constant.

Ephrins

PAK and the Rho GTPases may also be involved in yet another piece of the puzzle of spine formation and dynamics. Recent studies indicate that interfering with the interactions between a class of the membrane-bound ligands ephrins and their tyrosine kinase receptors, Ephs, can both alter spine morphology and incidence. There are two families of Ephs and ephrins, A and B, which both seem to interact with many of the molecules mentioned above. Triple knockout mice lacking EphB1, EphB2, and EphB3 fail to form mature spines by P21 like wild-type mice do, but instead retain immature filopodial processes (Henkemeyer et al., 2003). The triple KO mice show altered clustering of both f-actin and PSD-95 in their long, thin dendritic protrusions. PSD-95 and the presynaptic marker synaptophysin both localize along the shaft, instead of inside spines as in the wild-type mice, indicating abnormal synapse formation in the triple knock-outs. Henkemeyer and colleagues further illustrate that the disruption of synapse formation is specific to glutamatergic synapses. The spine morphology-inducing effects of the EphB receptors may be executed through the binding of these receptors to a common ligand. The likely candidate is ephrin-B2, as application of ephrin-B2-fc fusion protein prematurely induces the shift from filopodia-like to spine-like processes on the dendrite (Henkemeyer et al., 2003), but because Eph receptors have a reputation for promiscuity and can even bind members of the opposite class of ephrins (Himanen et al., 2004), other ligands cannot yet be discounted.

The binding of ephrin-B to EphB triggers a signal transduction pathway beginning with the translocation of kalirin to the active spine. Once at the synapse, kalirin activates Rac1, which in turn activates PAK (Penzes et al., 2003). PAK then interacts with actin, resulting in an increased spine density and size. Blocking any of the molecules in this pathway will block spine morphogenesis. Ephrin-A ligands inhibit PAK and Rac (Wahl et al., 2000), but the effects of ephrin-A on the signal transduction pathway uncovered by Penzes and colleagues remain unstudied. In addition, interactions between ephrin-A3 on the glial membrane and EphA4 on the dendrite may utilize a different pathway, leading to alterations in spine shape (Murai et al., 2003, see below). Ephrin-B binding to EphB also allows EphB to interact directly with NMDA receptors (Dalva et al., 2000), phosphorylating the NMDA receptor and potentiating calcium entry through it (Takasu et al., 2002). Because many forms of spine plasticity and stabilization require calcium-dependent molecules (Halpain et al., 1998; Jourdain et al., 2003; Lin et al., 2004), this Ephrin-B/EphB/NMDA receptor signaling cascade may present a concerted mechanism through which these diverse molecules can modulate the form and function of excitatory synapses.

Adhesion Molecules

Adhesion molecules, such as cadherin-associated protein αN-catenin, can also affect spine stability. Mutations of αN-catenin cause altered spine morphology, but these spines could still form viable synapses (Togashi et al., 2002). Recently, an increase in spine motility in hippocampal cultures from mice lacking αN-catenin was reported, as seen in time-lapse confocal imaging (Abe et al., 2004). Interestingly, one of the most common forms of motility observed in these mice was filopodial protrusion from the spine head. Conversely, transfecting neurons with αN-catenin, causing an overexpression of the molecule, led to a decreased spine turnover and therefore an increased spine density, similar to spine maturation that occurs in normal hippocampal development (Dailey and Smith, 1996). Taken together, these two results illustrate that αN-catenin inhibits spine plasticity and promotes maturation, both by inhibiting changes in motility and by keeping spines from retracting.

Sex Steroids

Yet another class of molecules involved in spine formation and morphogenesis is the sex steroids. Decreased dendritic spine density in the CA1 area of the hippocampus was observed in ovariectomized mice over a decade ago (Gould et al., 1990). This decrease seems to be due to estradiol working through NMDA receptors (Gould et al., 1990; Woolley and McEwen, 1994). Li and colleagues (2004) demonstrated that even without an increase in overall

spine density, *in vivo* treatment with estradiol causes an increase in mushroom shaped spines in the hippocampus of ovariectomized mice. More interestingly, these mice perform the object-placement task, a test of hippocampal-dependent spatial memory, significantly better than vehicle-treated controls. An increase in pre- and postsynaptic markers indicated that synaptogenesis had occurred. This study illustrates that changes in spine morphology can be induced in older mice with immediate behavioral consequences.

Many molecules mentioned above act as modulators that change the density and morphology of spines on spiny neurons. Is there a "spine-forming" molecule that is expressed on spiny neurons but is missing from nonspiny neurons? Interestingly, overexpression of GluR2 or cortactin causes the formation of spines on nonspiny neurons (Passafaro et al., 2003; Hering and Sheng, 2003). It is not clear if other modulating proteins would have a spine-promoting effect on nonspiny neurons as well.

ROLE OF GLIA IN REGULATING SPINE DYNAMICS

Another potential player in the regulation of spine dynamics has recently emerged—glial cells. A close physical relationship between astrocytes and the synapse has long been acknowledged, as seen by electron microscopy studies (Peters et al., 1976). A striking example is the relationship between Bergmann glia and the Purkinje cells of the cerebellar cortex, in which thin lamellar extensions of the glia completely envelop Purkinje cell spines (Spacek, 1985; Grosche et al., 1999). Grosche and colleagues described glial microdomains (Grosche et al., 1999, 2002), thin leaflets of membrane projecting from the appendages of these radial astroglia that ensheathe the synapses between Purkinje cells and parallel fibers. Activation at these ensheathed synapses causes an increase in calcium levels specific to the glial microdomain wrapping that synapse (Grosche et al., 1999), conceivably supporting the synapse-specific enhancement that is thought to occur with learning.

Grosche et al. (1999) hypothesize that these microdomains may function to increase the specificity of synaptic modulation, because microdomains allow for localized compartmentalization of alterations in calcium levels, transmitter uptake, and transmitter release. Another reasonable hypothesis would maintain that the microdomains serve to stabilize the synapse, possibly through two methods—a physical constriction of movement and/or a molecular interaction that would cause the spine to take on a different, possibly more mature, morphology. Ultrastructural studies would support the role of physical constriction of spine motility by the glia that ensheathe it. Because the above mentioned studies have shown that synaptic contact alone is not enough to stabilize the spine (Fischer et al., 1998; Dunaevsky et al., 2001), glial constriction may be a missing factor needed to explain decreased spine motility in the adult (Fig. 3). In fact, by combining time-lapse two-photon imaging with electron microscopy in cerebellar slices, Dunaevsky et al. (2001) demonstrated that spines showing high motility were more likely to be surrounded by extracellular space (fewer neurons

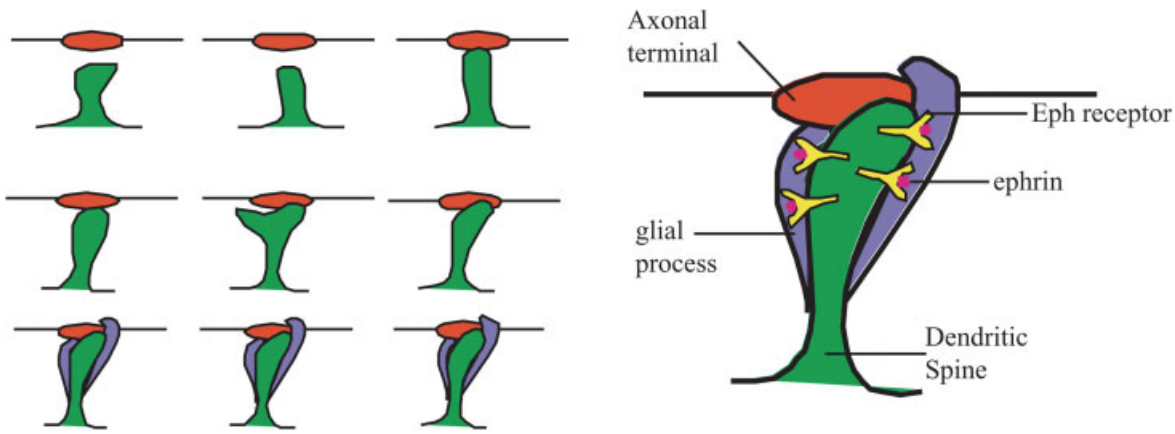

Figure 3 A model for the putative role of glial ensheathement and signaling through the Eph receptor in synaptic stabilization. Motile spines can initiate new contacts with axons. Spine motility can continue in the presence of synaptic contact. Ensheathement of spines by glial processes and signaling through the Eph receptor regulate spine morphogenesis. Reduced motility after glial ensheathement might lead to the stabilization of synaptic contacts.

and/or glia in the surrounding space) than spines showing little motility.

In support of glial-spine molecular interactions influencing spine morphogenesis and maintenance, Murai et al. (2003) demonstrated that disruption of the signaling between the ligand ephrin-A3 on astroglia and its neuronal receptor EphA4 alters spine morphology. In this study, hippocampal slices were treated with either an EphA4-fc protein or a kinase-inactive EphA4 to inhibit ephrin-A3 binding to the endogenous EphA4 receptors or observed dendritic spines in EphA4 knockout mice. All of these manipulations caused disorganization and distortion of spines, particularly with regard to their length. Murai et al. also added an ephrin-A3 fusion protein to promote activation of EphA4. This treatment resulted in a retraction of spines, demonstrating again that communication between these two molecules critically affects proper spine shape and maintenance.

The findings of Murai et al. point to an inhibitory or restrictive role for astrocytes in spine growth, so one might expect to see a reduction in the number of spines with increased numbers of astrocytic processes and vice versa. Supporting this hypothesis, decreased spine density is a common symptom of human diseases associated with glial hypertrophy (Scheibel et al., 1974), and treatments that cause increased numbers of spines often cause astrocytic processes to shrink (Woolley and McEwen, 1992; Klintsova et al., 1995; reviewed in Thompson, 2003). This molecular mechanism of growth inhibition could be in agreement with physical inhibition by ensheathing glia. If the spine is completely covered by an astrocyte that is both physically constricting spine growth and movement while actively stimulating signaling pathways that also inhibit the astrocytic growth, the chances of having a stable spine will increase greatly. If stable spines lead to stable synapses, the system described here has the potential to play an important role in the maintenance of mature connections in the brain. Because synapses are differentially enwrapped, as seen in EM studies (Grosche et al., 2002), the role of the astrocytes may go beyond mere maintenance to actually somehow directing which synapses will be transient and which will become part of the final circuitry of the adult nervous system.

Amateau and McCarthy (2002) suggest another possible pathway in which astrocytes could affect spine plasticity. They found that treating preoptic area neurons with either estradiol or prostaglandin-E2 increases the number of spines on dendrites. The study demonstrated the effect, but not the mechanism by which it occurred. A study by Nicol et al. (1992) showed that one of these molecules, prostaglandin-E2, caused local astrocytes to release glutamate. In addition, other studies show that activation of AMPA/kainate receptors leads to spine induction on the dendrites of the activated receptors (McKinney et al., 1999). Combining these findings with their own, Amateau and McCarthy suggest that glutamate released by glial cells in response to prostaglandin-E2 activates dendritic AMPA/kainate receptors, triggering spine induction. Although multiple studies have replicated the findings of Amateau and McCarthy, that these and similar molecules can alter spine morphology (Gould et al., 1990; Li et al., 2004), evidence implicating astrocytes in this pathway is currently only circumstantial.

Other studies show that astrocytes are necessary for increased synaptogenesis and synaptic efficacy (Ullian et al., 1999, 2001; Mauch et al., 2001; Beattie et al., 2002). Since analysis of synaptic structure was not performed, one can only hypothesize that the spine morphology changes with increased efficacy.

SPINE STABILITY IN THE INTACT ADULT BRAIN

Most of the studies discussed above were performed on either dissociated neurons or slice cultures. Ultimately one would like to know the extent to which dendritic spines are dynamic in the intact adult brain and how spine stability changes with learning and experience. Not surprisingly, considering the technical difficulties in performing such experiments, only a handful of studies have attempted to assess the extent of structural stability of spines, as a mirror of synaptic connections, in the intact mature brain using live imaging approaches. The cortex (Trachtenberg et al., 2002; Grutzendler et al., 2002) and the olfactory bulb (Mizrahi and Katz, 2003), both superficial and therefore accessible, have been imaged. Spine stability has also been recently evaluated *in vivo* in the much less accessible hippocampus (Mizrahi et al., 2004). Two-photon imaging revealed that spines were highly stable in the adult hippocampus over a period of up to 4 h. In order to image the hippocampus in a living mouse, the overlaying neocortex must be removed. Although the ability to view hippocampal spines *in vivo* is extremely important, the current surgically invasive procedure necessary to expose the hippocampus makes this preparation useful only for short-term imaging.

Using two-photon live imaging of the cerebral cortex of mice expressing fluorescent proteins in a subset of neurons (Feng et al., 2000), Grutzendler and colleagues (2002) and Trachtenberg and colleagues

(2002) arrived at different conclusions regarding the baseline structural stability in the adult brain. Both agree that dendritic filopodia and spines are dynamic in young animals and that stabilization occurs with increased age. It is the degree of stabilization that is reached in the adult that is the point of disagreement. Grutzendler et al. viewed spines of layer 5 pyramidal neurons in layer I and II of the primary visual cortex (V1). They observed a high incidence of transient filopodia at 1 month of age. As seen in slices and in younger animals *in vivo* (Lendvai et al., 2000), filopodia grew and retracted on a time scale of minutes with a very high turnover rate. Interestingly, at the same age spines were much more stable, showing both little morphological changes over short imaging periods (4 h) as well as lower turnover rates, with over 70% of spines remaining for a period of 3 months. At the age of 4 months, these authors reported an almost complete absence of filopodia and a remarkable stability of spines with over 90% of the spines persisting for 2 months. Trachtenberg et al. examined the stability of dendritic spines in the barrel cortex and agreed that spines are more dynamic in the young animals, but reported that in "young adult" animals of 6–10 weeks, only 60% of spines persisted for 8 days and only about 50% of spines survived for a month or longer. In addition, using correlative electron microscopy these authors reported that newly formed spines bear a structurally identifiable synapse, thereby demonstrating for the first time that new synapses can be formed in the adult cortex. In summary, Grutzendler et al. report that the vast majority of spines are stable for several months while Trachtenberg et al. state that at least one-half of the spines are dynamic in the adult brain. There are several differences between the studies that could potentially explain the apparently disparate conclusions in these studies. Each group used different mouse strains, potentially revealing different subsets of pyramidal neurons, with different behaviors. An obvious contrast is the cortical area analyzed, visual versus barrel cortex. The imaging approaches were also dissimilar. While Trachtenberg et al. constructed an optical chamber that included the removal of the skull and the implantation of a cover glass, Grutzendler et al. used a less invasive approach of simply thinning the skull. Although thinning the skull might be less damaging, the potentially lower optical resolution obtained with such method might leave smaller, more dynamic spines undetected. Finally, although both studies claimed to image adult cortex, Grutzendler et al. looked at animals of an average age of 4.2 months while the oldest animals in the Trachtenberg et al. study was 2 months younger. Although it is likely that future studies (Zuo et al., 2003) will indicate that spines are much more stable in the adult than has been initially suggested (Trachtenberg et al., 2002), the exact level of stabilization at different regions of the cortex might be different. It is interesting to note that in the olfactory bulb where new neurons are continuously incorporated into the adult circuitry, small dendritic protrusions are in constant flux (Mizrahi and Katz, 2003), while the main dendritic tree is very stable, as it is in the cortex (Trachtenberg et al., 2002). It will be necessary in the future to determine the stability of spines in other nonpyramidal spiny neurons (i.e., Purkinje cells) in the adult animal.

EXPERIENCE-DEPENDENT CHANGES OF SPINE DYNAMICS

Although changes in spine morphology and density with altered experience have been previously reported and extensively reviewed (Yuste and Bonhoeffer, 2001; Nikonenko et al., 2002; Wallace and Bear, 2004; Lamprecht and LeDoux, 2004), the ability to analyze the changes in short and long term stability of spines with experience has only recently become possible. Two groups have examined how sensory deprivation affects spine dynamics in the developing cortex in vivo. Lendvai et al. (2000) have found that sensory deprivation by trimming of whiskers caused a 37% decrease in the motility of spines and filopodia in layer 2/3 barrel cortex. This change in the dynamics of spines was only observed during the critical period P11–13 and was correlated with sensory-deprivation induced abnormal formation of layer 2/3 sensory maps. In the visual cortex, Majewska and Sur (2003) have confirmed the results of Grutzendler et al., that spines become much less dynamic after the critical period for ocular dominance plasticity. They have also examined how sensory deprivation in the visual cortex affects spine short-term dynamics. Similar to sensory deprivation in the barrel cortex, binocular lid suture caused a change in spine dynamics, but only during the peak time of the critical period. Surprisingly, unlike in the somatosensory cortex (Lendvai et al., 2000), sensory deprivation in the visual cortex leads to a 60% increase in spine dynamics (Majewska and Sur, 2003; but see Konur and Yuste, 2004). The authors explain this dissimilarity by a possible difference in the state of the spines in the different cortex regions at the time of sensory deprivation. In the somatosensory cortex the sensory-deprivation induced decrease in spine dynamics occurs at a time of peak synaptogenesis, while in the visual cortex the synapses are already

in place and are undergoing activity–dependent rearrangements. In support of this interpretation, in the somatosensory cortex of young adult mice where synapses are already established, sensory deprivation leads to an increase in transient, thin dendritic spines (Trachtenberg et al., 2002). Thus, alteration in the activity of neurons with sensory deprivation might cause the destabilization and thus increased dynamics of dendritic spines.

These recent studies that allow the visualization of dendritic spines in live mice under normal and sensory-deprived conditions are giant steps towards understanding the cellular and synaptic mechanisms underlying experience-dependent plasticity. What will move the field forward now are studies in which synaptic structural plasticity is correlated with altered experience, not only by sensory deprivation, but with learning and formation of new memories (Rioult-Pedotti et al., 2000).

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