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Balancing Structure and Function at Hippocampal Dendritic Spines

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Abstract

Dendritic spines are the primary recipients of excitatory input in the central nervous system. They provide biochemical compartments that control locally the mechanisms of signaling at individual synapses. Hippocampal spines show structural plasticity as the basis for physiological changes in synaptic efficacy that underlie learning and memory. Spine structure is regulated by molecular mechanisms that are fine-tuned and adjusted according to developmental age, level and direction of synaptic activity, specific brain region, and exact behavioral or experimental conditions. Reciprocal changes between the structure and function of spines impact both local and global integration of signals within dendrites. Advances in imaging and computing technologies may provide the resources needed to reconstruct entire neural circuits. Key to this endeavor is having sufficient resolution to determine the extrinsic factors (such as perisynaptic astroglia) and the intrinsic factors (such as core subcellular organelles) that are required to build and maintain synapses.

Keywords

serial section transmission electron microscopy; long-term potentiation; long-term depression; development; morphological plasticity

Introduction

Since Golgi and Cajal first revealed the intricate structure of dendrites more than 100 years ago, scientists have wondered: Why are dendritic spines distributed nonuniformly along dendrites? Why do dendrites become grossly distorted among individuals with severe neuropathology and mental retardation? Is the number of spines limited by size? Does the number reach saturation? Do more or less spiny dendrites have a greater capacity for plasticity? What are the intrinsic and extrinsic features that control dendritic plasticity or allow for homeostatic regulation? As protrusions with diverse lengths and shapes, spines allow more connections to form in a compact neuropil. A constricted neck compartmentalizes molecular signals in the spine head and imparts synapse specificity, promotes plasticity, and protects the parent dendrite from excitotoxicity. Spine shape can reflect different inputs in some brain regions such as the lateral nucleus of the amygdala where cortical inputs synapse on thin spines and thalamic inputs synapse on mushroom spines (Humeau et al. 2005). Conversely, both thin and mushroom spines can synapse with the same CA3 inputs in the hippocampus (Harris & Stevens 1989). Furthermore, cerebellar Purkinje cell spines appear club-shaped even without synaptic input (Cesa & Strata 2005). Live imaging with two-photon microscopy has revealed rapid, activity-dependent turnover of spines that is common during development but as an

animal matures more spines stabilize (Alvarez & Sabatini 2007). This form of imaging also reveals dynamic changes in the shapes of individual spines, but is not of sufficient resolution to measure dimensions, count numbers, determine local subcellular or molecular composition, or identify exactly where synapses occur. Electron microscopy is needed to reveal these features (Harris et al. 2006, Rostaing et al. 2006, Masugi-Tokita & Shigemoto 2007). New approaches to combine light and electron microscopy are promising (Zito et al. 1999, Knott et al. 2006, Nagerl et al. 2007), although refinement is needed because the reaction products used to track the dendrites often obscure synapses and subcellular organelles.

This review concentrates on hippocampal dendritic spines. Spatial training (Moser et al. 1997) and exposure to enriched environments (Kozorovitskiy et al. 2005) alters hippocampal spine number. Long-term potentiation (LTP) alters spine number, shape, and subcellular composition in both the immature (Maletic-Savatic et al. 1999, Engert & Bonhoeffer 1999, Ostroff et al. 2002, Lang et al. 2004, Matsuzaki et al. 2004, Kopec et al. 2006, Nagerl et al. 2007) and the mature hippocampus (Van Harrevelde & Fifkova 1975, Trommald et al. 1996, Popov et al. 2004, Stewart et al. 2005, Bourne et al. 2007b). Conversely, long-term depression (LTD) decreases spine number and size (Chen et al. 2004, Nagerl et al. 2004, Zhou et al. 2004). Structural spine plasticity in the hippocampus involves altering the size and composition of the postsynaptic density (PSD); assembly and disassembly of actin filaments; exocytosis and endocytosis of glutamate receptors and ion channels; regulation of local protein synthesis by redistribution of polyribosomes and proteasomes; dynamic repositioning of smooth endoplasmic reticulum (SER) and mitochondria; and metabolic and structural interactions between spines and perisynaptic astroglia. The extent and type of structural change depends partly on experimental methods, developmental age, and regional differences in synaptic organization. This review discusses factors that regulate spine structure and function during hippocampal synaptogenesis and plasticity (Table 1).

Structure and Composition of Dendritic Spines

In the hippocampus, spines vary greatly in size and shape even along short dendritic segments (Figure 1). Most spines have constricted necks and are either mushroom shaped with heads exceeding 0.6 microns in diameter or thin shaped with smaller heads (Harris et al. 1992). Other spines are stubby with head widths equal to neck lengths, branched with two or more heads, or multisynaptic single protrusions. These features provide measurably distinct shape categories (Figure 1F) that might reflect functional histories of the spines. Mushroom spines have larger, more complex PSDs (Harris et al. 1992) with a higher density of glutamate receptors (Matsuzaki et al. 2001, Nicholson et al. 2006). Larger spines are more likely to have SER (Spacek & Harris 1997), polyribosomes (Ostroff et al. 2002, Bourne et al. 2007b), endosomal compartments (Cooney et al. 2002, Park et al. 2006) and perisynaptic astroglia (Witcher et al. 2007). These features suggest that larger spines are functionally stronger in their response to glutamate, local regulation of intracellular calcium, endosomal recycling, protein translation and degradation, and interaction with astroglia. Smaller spines may be more flexible, rapidly enlarging or shrinking in response to subsequent activation (Bourne & Harris 2007).

Postsynaptic density (PSD)

Spine heads provide a local biochemical compartment where ions and signaling molecules become concentrated following synaptic activation. The PSD is an electron dense thickening on spine heads that is apposed to the presynaptic active zone. The PSD contains hundreds of proteins including NMDA, AMPA, and metabotropic glutamate receptors, scaffolding proteins such as PSD-95, and signaling proteins such as calcium/calmodulin-dependent kinase II (CamKII) (Okabe 2007). The surfaces of the PSDs vary from small discs to large irregular shapes that can be perforated by electron lucent regions. Differences in PSD dimensions can

reflect distance-dependent differences in dendritic function (Magee & Johnston 2005). Relatively more of the distal synapses on CA1 pyramidal cells have perforated synapses; however, perforated synapses associated with the distal input of entorhinal cortex host a lower density of AMPA receptors than perforated synapses at proximal CA3 input of the same CA1 cells (Nicholson et al. 2006). PSDs appear larger and are more likely to have perforations shortly after the induction of LTP (Geinisman et al. 1991, Toni et al. 1999, Mezey et al. 2004, Popov et al. 2004, Dhanrajan et al. 2004, Stewart et al. 2005); consistent with the idea that perforations are transient structural perturbations responding to activation (Lisman & Harris 1994, Sorra et al. 1998, Fiala et al. 2002, Spacek & Harris 2004). Larger spines with more AMPA and NMDA receptors in the PSD are more sensitive to glutamate (Takumi et al. 1999a, Takumi et al. 1999b, Matsuzaki et al. 2001). Small “silent” spine synapses only contain NMDA receptors and LTP activates them with exocytic insertion of AMPA receptors (Isaac et al. 1995, Liao et al. 1995, Liao et al. 1999, Petralia et al. 1999, Lu et al. 2001, Park et al. 2004, Kopec et al. 2006). AMPA receptors must be constitutively exchanged to sustain the newly active spines; fortunately lateral diffusion of AMPA receptors out of a spine is limited by the constricted spine neck (Adesnik H et al. 2005, Ashby et al. 2006). AMPA receptors can also be actively removed via endocytosis during LTD (Beattie et al. 2000, Man et al. 2000, Snyder et al. 2001, Xiao et al. 2001, Lee et al. 2002, Brown et al. 2005). Both exo- and endocytic processes alter spine shape. Since the size of the PSD is well correlated with spine head volume and the number of presynaptic vesicles (Harris & Stevens 1989, Harris et al. 1992), there is likely a trans-synaptic mechanism to coordinate them during plasticity (Lisman & Harris 1993, Spacek & Harris 2004).

Actin Cytoskeleton

Spine formation and morphology is regulated by actin filaments (Matus 2000, Zito et al. 2004). Filamentous actin (F-actin) forms organized bundles in spine necks and altered polymerization-depolymerization states accompany changes in head shapes (Star et al. 2002). Induction of LTP briefly depolymerizes actin filaments (Ouyang et al. 2005), while maintenance of LTP and sustained spine enlargement requires polymerization of F-actin (Kim & Lisman 1999, Krucker et al. 2000, Fukazawa et al. 2003, Lin et al. 2005). In contrast, LTD results in the depolymerization of actin and spine elongation or shrinkage of spine heads (Chen et al. 2004, Nagerl et al. 2004, Zhou et al. 2004). The actin cytoskeleton is regulated by actin-binding proteins (Ethell & Pasquale 2005, Tada & Sheng 2006). Profilin is a promoter of actin polymerization that could facilitate LTP-induced actin assembly and spine enlargement (Ackermann & Matus 2003). Cofilin is an actin-binding protein that causes actin depolymerization; induction of LTP or exposure to enriched environments causes a phosphorylation-mediated inhibition of cofilin and promotes spine enlargement (Chen et al. 2007, Fedulov et al. 2007). Rap1 is an actin binding protein that localizes AF-6 to the synaptic membrane where it induces rearrangement of actin filaments and promotes removal of AMPA receptors (Xie et al. 2005) and spine elongation, a morphological correlate of LTD (Zhu et al. 2002). Conversely, inactivation of Rap1 releases AF-6 from the synaptic membrane to regulate a different pool of actin filaments that promote recruitment of AMPA receptors to the synapse and spine enlargement with LTP (Xie et al. 2005). Myosins IIb and VI are motor proteins enriched in the PSD that translocate along, and regulate contractility of actin filaments and spine shape (Osterweil et al. 2005, Ryu et al. 2006). Myosin VI-deficient spines have disrupted clathrin-mediated endocytosis of AMPA receptors, suggesting a role in LTD (Osterweil et al. 2005).

Recycling Endosomes

LTP requires exocytosis-mediated insertion of AMPA receptors (Lu et al. 2001, Park et al. 2004, Kopec et al. 2006) and is accompanied by endocytosis of Kv4.2 subunits of voltage-gated A-type K^+ channels, which enhances local dendritic excitability (Kim et al. 2007).

Patches of preassembled clathrin provide hot spots of endocytosis along spine and dendritic membranes (Blanpied et al. 2002, Racz et al. 2004). Spine shape is regulated by recycling endosomes and blocking this pathway results in significant spine loss (Park et al. 2006). Following the induction of LTP, live imaging and ssTEM revealed translocation into spines of endosomes having sufficient surface area to provide an abundant resource for spine growth. Two pools of membrane were identified: recycling endosomes with tubules, vesicles, and clathrin-coated pits or buds, and large amorphous vesicular clumps (AVC). Quantification suggested that AVCs provided membrane for new or enlarged spines and recycling endosomes maintained them. LTD results in AMPA receptor internalization and reduced spine and synapse size (Man et al. 2000, Zhou et al. 2004, Nagerl et al. 2004, Chen et al. 2004, Brown et al. 2005). Interference with this AMPA receptor internalization leads to excitotoxicity via increased sensitivity to glutamate and eventual spine loss (Halpain et al. 1998, Hasbani et al. 2001). Thus, exo- and endocytosis must maintain an activity-dependent balance to fine-tune the physiological and structural responses of spines to synaptic plasticity.

Polyribosomes and Proteasomes

The response of dendritic spines to synaptic plasticity relies on their ability to regulate protein synthesis and degradation. Treatment with anisomycin prevents spine enlargement during LTP (Fifkova et al. 1982, Kelleher, III et al. 2004). Other findings show that polyribosomes, the machinery necessary to translate proteins, occur at the base of dendritic spines (Steward & Levy 1982) and preferentially redistribute into dendritic spines with enlarged heads and synapses during LTP (Ostroff et al. 2002, Bourne et al. 2007b). What plasticity-related proteins could be translated by these local polyribosomes to increase the size of the PSD? One candidate is CamKII, a cytoplasmic protein highly enriched in the PSD (Kennedy et al. 1983, Kennedy et al. 1990, Otmakhov et al. 2004). CamKII becomes autophosphorylated (Miller & Kennedy 1986) following activation and can regulate glutamate receptors both directly and indirectly long after calcium levels have returned to baseline during LTP (McGlade-McCulloh et al. 1993, Pettit et al. 1994, Liao et al. 1995, Lledo et al. 1995). Furthermore, the mRNA transcripts for CamKII are present in dendrites (Martone et al. 1996, Havik et al. 2003) and translation of CamKII is upregulated (Ouyang et al. 1997, Ouyang et al. 1999, Aakalu et al. 2001) and more CamKII is present in the PSD after LTP (Otmakhov et al. 2004). Induction of LTD through activation of metabotropic glutamate receptors (mGluRs) is dependent on protein synthesis in adolescent but not neonatal rats (Huber et al. 2001, Nosyreva & Huber 2005). Stimulation of mGluRs in synaptoneurosomes triggers the aggregation of polyribosomes and the translation of proteins, including the Fragile X mental retardation protein (Weiler et al. 1997), although the dendritic distribution of polyribosomes following induction of LTD has not yet been examined.

Rough endoplasmic reticulum (RER) and Golgi have been identified in dendrites where they could locally synthesize and regulate integral membrane proteins (Steward & Reeves 1988, Gardiol et al. 1999, Cooney et al. 2002, Horton & Ehlers 2004, Grigston et al. 2005). An intriguing possibility is that the enigmatic spine apparatus which occurs in about 10–15% of mature hippocampal spines (Spacek & Harris 1997) may also be an extension of the Golgi apparatus (Pierce et al. 2000). Localized synthesis of the GluR1 and GluR2 subunits for AMPA glutamate receptors has been demonstrated in hippocampal dendrites (Kacharmina et al. 2000, Ju et al. 2004, Grooms et al. 2006) and the mRNAs for other integral membrane and secretory proteins are found throughout the dendritic arbor (Steward & Schuman 2003).

Maintenance of LTP also relies on proteasomes to degrade proteins (Fonseca et al. 2006, Karpova et al. 2006). Lysosomes and multivesicular bodies also occur in dendritic spines (Spacek & Harris 1997, Cooney et al. 2002). It will be interesting to learn whether the balance

of protein synthesis and degradation is shifted depending on whether a synapse is potentiated or depressed.

SER

Many dendritic spines contain smooth endoplasmic reticulum (SER) which is thought to regulate calcium. SER is present in all dendritic spines of cerebellar Purkinje neurons (Harris & Stevens 1988) but in less than half of cortical or hippocampal spines (Spacek 1985a, Spacek & Harris 1997). Calcium influx can trigger release from SER thereby extending its elevation in stimulated spine heads (Sabatini et al. 2001). The elevated calcium facilitates remodeling of the actin cytoskeleton (Oertner & Matus 2005). Laminae of SER and dense-staining material form a spine apparatus in about 10–20% of hippocampal and cortical spines. Synaptopodin is an actin-associated protein that occurs in the spine apparatus and mice lacking synaptopodin also lack a spine apparatus and display deficits in synaptic plasticity (Deller et al. 2007). SER can shift throughout the dendrite (Toresson & Grant 2005) and it will be interesting to learn whether these dynamics are influenced by synaptic plasticity.

Mitochondria

Mitochondria are abundant in dendritic shafts and the ATP they produce likely diffuses into spines to provide energy for signal transduction. In contrast, mitochondria are rarely found in dendritic spines and are usually restricted to very large and complex spines, such as the branched spines or thorny excrescences located on proximal dendrites of CA3 pyramidal cells (Chicurel & Harris 1992). In cultured neurons from area CA1, it appears that mitochondria occasionally migrate into some dendritic spines during periods of intense synaptic remodeling (Li et al. 2004). The enzymes involved in the glycolic generation and regulation of ATP have been localized to isolated PSDs suggesting a mechanism for direct synthesis of ATP at individual synapses even in the absence of mitochondria in spines (Rogalski-Wilk & Cohen 1997, Wu et al. 1997). Synaptic ATP could provide an energy source for signaling via protein kinases found at the PSD, such as protein kinase A, protein kinase C and CamKII, and for local protein synthesis by polyribosomes. Although enzymes localized to the PSD are a potentially important source of ATP, it would be interesting to know whether the distances between dendritic mitochondria and spines are altered in response to input specific plasticity, such as LTP and LTD, along with the recruitment of polyribosomes to some spines with subsequently enlarged synapses.

The formation and stabilization of new spines

New spines are formed in the hippocampus during development and some forms of plasticity in adults. Filopodia are nonsynaptic or multisynaptic, actin-rich protrusions with pointy tips (Fiala et al. 1998) that tend to be transient, lasting about 10 minutes during development (Ziv & Smith 1996). With maturation, the density of the neuropil increases and additional mechanisms may be required for new spines to find, compete for, and maintain presynaptic partners.

Development

During the first few weeks of postnatal life, hippocampal dendrites have numerous filopodia (Papa & Segal 1996, Ziv & Smith 1996, Fiala et al. 1998). Some filopodia become spines with synapses (Marrs et al. 2001), while others withdraw into the dendrite to form synapses on the dendritic shaft (Fiala et al. 1998, Marrs et al. 2001). These shaft synapses either re-emerge as spines or are preferentially eliminated later in life (Harris 1999, Bourne & Harris 2007).

Stabilization of hippocampal spines requires assembly of pre and postsynaptic elements although the timing of these events may vary (Harris et al. 2003, Ostroff & Harris 2004, Risher

W. C. et al. 2006, Nagerl et al. 2007). Dense core vesicles containing piccolo and bassoon appear in axonal processes within 2 days and cluster along dendritic profiles by 4 days in vitro in cultured hippocampal neurons suggesting presynaptic active zones are prepackaged (Zhai et al. 2001, Shapira et al. 2003). PSD-95 is necessary to stabilize the spine as evidenced by RNAi knockdowns that lead to spine loss (Ehrlich et al. 2007). Assembly of PSD-95 is spatially and temporally correlated with spine morphogenesis (Marrs et al. 2001) and the clustering of presynaptic vesicle proteins (Okabe et al. 2001). Stabilization of dendritic spines also relies on the insertion and activation of glutamate receptors; AMPA receptor activation in particular decreases spine motility and stabilizes spine shape (Fischer et al. 2000). Blocking NMDA receptor signaling does not affect the emergence or density of spines during development (Rao & Craig 1997, Kirov et al. 2004a, Alvarez et al. 2007), but knocking down NMDA receptors through RNA interference (RNAi) results in increased spine motility and eventual elimination (Alvarez et al. 2007).

Synaptogenesis requires that filopodia to be maintained long enough to find appropriate presynaptic partners. Telencephalin is an adhesion molecule of the Ig superfamily and SynGAP is a ras-GTPase activating protein; both of these proteins maintain filopodia in a dynamic state during synaptogenesis and mice deficient in either protein show accelerated spine development and larger spine heads (Vazquez et al. 2004, Matsuno et al. 2006). Once filopodia become spines, telencephalin relocates to the dendritic shaft and is replaced with adhesion molecules N-cadherin and α -catenin that act to stabilize the new spine (Bozdagi et al. 2000, Togashi et al. 2002, Abe et al. 2004). SynGAP remains at the synapse and is bound to PSD-95 through its PDZ domain (Chen et al. 1998, Kim et al. 1998). Activation of NMDA receptors alters the phosphorylation state of different SynGAP isoforms, providing a link between NMDA receptor activation and Ras signaling pathways (Chen et al. 1998, Oh et al. 2004, Krapivinsky et al. 2004).

Spinogenesis is also regulated by microRNAs, small non-coding RNAs that control the translation of messenger RNAs. miR-134 is a brain-specific microRNA localized to dendritic spines that negatively regulates spine size by inhibiting the translation of the protein kinase Limk1 (Schratt et al. 2006). Treatment with brain derived neurotrophic factor (BDNF) relieves miR-134-mediated inhibition of Limk1 translation, suggesting synaptic stimuli and extracellular signals can regulate spine development through local translation mechanisms.

Spinogenesis in the mature hippocampus

Filopodia are rarely observed in the mature hippocampus; however, blocking synaptic transmission in mature hippocampal slices triggers filopodia and new spines in an apparent attempt to compensate for the loss of synaptic input (Kirov & Harris 1999). Chilling hippocampal slices during preparation results in an immediate disappearance of spines but upon re-warming new spines proliferate beyond levels found in vivo (Kirov et al. 1999, Kirov et al. 2004b). Instead, if slices are prepared rapidly at room temperature then spine density matches that found in perfusion fixed hippocampus even several hours later (Bourne et al. 2007a). Hibernating ground squirrels also show substantial spine loss at near-freezing temperatures, but rapid spinogenesis occurs within minutes of awakening and return to warmer body temperatures (Popov et al. 1992, Popov & Bocharova 1992). Telencephalin levels remain high in adulthood suggesting an ongoing involvement in transforming filopodia to new spines in the mature brain (Matsuno et al. 2006).

Adhesion and Trans-synaptic signaling

Cell adhesion molecules such as N-cadherins, catenins, neuexins and neuroligins, and Ephs and ephrins begin to cluster on the pre and postsynaptic sides and help to stabilize the nascent spines and their synapses (Calabrese et al. 2006). N-cadherin is an adhesive molecule that links

pre and postsynaptic elements through calcium dependent homophilic interactions. N-cadherin and β -catenin form a calcium-regulated complex with AMPA receptors and overexpression of N-cadherin increases the surface expression of the AMPA receptor subunit GluR1 (Nuriya & Haganir 2006, Tai et al. 2007). NMDA receptor activation increases the concentration of unphosphorylated β -catenin and inhibits endocytosis of N-cadherin (Tai et al. 2007). N-cadherin also regulates spine morphology via its binding proteins, α - and β -catenin, which interact with the actin cytoskeleton (Kosik et al. 2005). Thus synaptic activity stabilizes synapse structure via N-cadherin which in turn recruits AMPA receptors and maintains synaptic efficacy. Interestingly, prolonged stability of N-cadherin abolishes NMDA receptor-induced LTD, perhaps because N-cadherin prevents the internalization of AMPA receptors associated with synaptic depression (Tai et al. 2007).

Eph receptor-ephrin binding results in multimeric clusters that bridge juxtaposed cell surfaces and mediate cell-cell adhesion and bidirectional signaling. Trans-endocytosis of the eph-ephrin complex loosens the adhesion between the pre and postsynaptic elements which may permit the structural synaptic plasticity. EphB receptors have been shown to associate directly with NMDA receptors at synapses and ephrinB-induced activation of EphB receptors causes NMDA receptor clustering (Dalva et al. 2000). At the mossy fiber synapse in CA3, post-synaptic EphB2 receptors interact with a PDZ-domain protein, glutamate receptor interacting protein (GRIP), to mediate AMPA receptor-dependent LTP (Contractor et al. 2002). EphB2 has also been shown to associate with the GTP exchange factors intersectin and kalirin (Penzes et al. 2003, Irie & Yamaguchi 2004). The intersectin-Cdc42-Wasp-actin and kalirin-Rac-Pak-actin pathways may regulate the EphB-receptor mediated morphogenesis and maturation of dendritic spines in cultured hippocampal and cortical neurons. Perhaps the interaction of presynaptic ephrins with postsynaptic Eph receptors serves to coordinate the establishment of the well-known correlation between presynaptic vesicle number and postsynaptic size during structural synaptic plasticity.

Trans-synaptic signaling may also be mediated by the formation of spinules. Spinules are double-membrane structures that emerge primarily from dendritic spines into presynaptic or neighboring axons or astroglial processes (Spacek & Harris 2004). Spinules appear to be involved in an active trans-endocytosis as evidenced by the presence of clathrin-like coats along the cytoplasmic surface of the engulfing structure, such as the presynaptic axons, across from the tip of the spinule. In particular, this trans-endocytosis could be the morphological correlate of retrograde signaling via cell surface molecules such as Ephs and ephrins, which must remain in the plasma membrane while signaling. Spinules may also be involved in remodeling of the post-synaptic membrane as suggested by their transient increase shortly after the induction of LTP (Applegate & Landfield 1988, Schuster et al. 1990, Geinisman et al. 1993, Toni et al. 1999).

Perisynaptic astroglia

The development and stabilization of synapses also requires astroglia (Allen & Barres 2005). Astroglia form non-overlapping domains in the hippocampus and cortex and a single astrocyte contacts hundreds of dendrites and thousands of synapses, suggesting it coordinates multiple neuronal networks (Bushong et al. 2002, Halassa et al. 2007). Transient interactions between the ephrin-A3 ligand and the EphA4 receptor regulate the structure of excitatory synaptic connections through neuro-glial cross-talk (Murai et al. 2003b, Grunwald et al. 2004). Activation of EphA4 by ephrin-A3 induces spine retraction whereas inhibiting ephrin/EphA4 interactions distorts spine shape and organization (Murai et al. 2003a). Expression of EphA4 decreases during maturation, suggesting a role in synaptic elimination and refinement of connections. Astrocytes also secrete soluble factors such as thrombospondins and cholesterol that influence spine formation and synapse maturation (Ullian et al. 2004, Christopherson et

al. 2005). In the mature neocortex and hippocampus, less than half of the synapses have perisynaptic astroglial processes (Spacek 1985b, Ventura & Harris 1998); however, synapses with astroglial processes at their perimeter are larger and presumably more effective than those without (Witcher et al. 2007). Importantly, synapse size is associated with the presence of an astroglial process juxtaposed to the postsynaptic spine and/or the synaptic cleft; not the degree to which the astroglial process surrounds the synapse. Even the largest hippocampal or neocortical synapses might have only a small fraction of their perimeters surrounded by an astroglial process suggesting that crosstalk via spillover of neurotransmitters between synapses might be functionally significant. Thus, interactions between cell surface molecules and the release of various soluble factors by astroglia may be of crucial importance to the turnover and enlargement of spines observed with synaptic plasticity.

Conclusions

Modern molecular biology, electrophysiology and imaging studies have provided many insights into the mechanisms of the morphological alterations that dendritic spines undergo during development and synaptic plasticity. Nevertheless, fundamental structural questions remain. Presently, only three-dimensional (3D) reconstruction from ssTEM provides sufficient resolution to determine how intrinsic and extrinsic factors might interact to control the structure and function of spines and synapses. Advances in imaging and computing technologies may soon provide us with resources to reconstruct entire neural circuits (e.g. “projectomes” or “connectomes”) (Kasthuri & Lichtman 2007). It is not sufficient however to have just the wiring diagram because we also need to know what controls the switches. Determining the extrinsic factors that regulate connectivity along dendrites and axons and the intrinsic factors that regulate the availability of core subcellular structures required to build and maintain synapses is necessary to formulate a comprehensive understanding of neural circuits that underlie perception, memory, and cognition.

Summary Points

1. Dendritic spines are complex biochemical compartments that integrate individual synaptic inputs into complex neural networks.
2. Dendritic spines in the hippocampus undergo genesis, elimination and structural modification in response to a variety of stimuli.
3. Spines coordinate the activation of glutamate receptors with calcium regulation, cytoskeletal remodeling, membrane trafficking, protein synthesis and degradation, and trans-synaptic signaling.
4. The dynamic balance of the molecular machinery within spines is manifested by morphological changes in spine shape and density and by the translocation of necessary organelles into and out of spines.
5. Although light level microscopy can provide information on real time dynamics of spines and proteins, ssTEM is required to detect small but crucial changes in spine dimensions, inter-spine spacing, and the presence and distribution of subcellular organelles and perisynaptic astroglia.

Future Issues

1. Determining whether the mechanisms underlying the outgrowth and stabilization of new spines during plasticity in the mature hippocampus are the same as those regulating synaptogenesis during development.

2. Refining the methods used to correlate gross morphological changes observed at the light level with subtle ultrastructural changes observed with ssTEM and developing new strategies to label individual cells, dendrites, and spines in an unobtrusive manner.

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Terms/Definition list

Thin spines

spines that have constricted necks and small heads

Mushroom spines

spines with constricted necks and heads exceeding 0.6 microns in diameter

Stubby spines

spines that have head widths equal to the neck length

Perforated synapse

PSD surface is irregularly shaped with electron lucent region(s) dividing it

Macular synapse

PSD surface is disc-shaped and uniformly dense

Filopodia

dynamic protrusions from dendrites that may become spines

Acronyms list

CamKII

calcium/calmodulin-dependent kinase II

PSD

postsynaptic density

LTP

long-term potentiation

LTD

long-term depression

AMPA

α -amino-3-hydroxyl-5-methyl-4-isoxazole-propionate, glutamate receptor

mGluR

metabotropic glutamate receptor

NMDA

N-methyl-D-aspartate, glutamate receptor

RNAi

ribonucleic acid interference

ssTEM

serial section transmission electron microscopy

Literature Cited

1. Aakalu G, Smith WB, Nguyen N, Jiang C, Schuman EM. Dynamic visualization of local protein synthesis in hippocampal neurons. *Neuron* 2001;30(2):489–502. [PubMed: 11395009]
2. Abe K, Chisaka O, Van Roy F, Takeichi M. Stability of dendritic spines and synaptic contacts is controlled by alpha N-catenin. *Nat Neurosci* 2004;7:357–63. [PubMed: 15034585]
3. Ackermann M, Matus A. Activity-induced targeting of profilin and stabilization of dendritic spine morphology. *Nat Neurosci* 2003;6(11):1194–200. [PubMed: 14555951]
4. Adesnik H, Nicoll RA, England PM. Photoinactivation of native AMPA receptors reveals their real-time trafficking. *Neuron* 2005;48:977–985. 977–85. [PubMed: 16364901]
5. Allen NJ, Barres BA. Signaling between glia and neurons: focus on synaptic plasticity. *Curr Opin Neurobiol* 2005;15(5):542–8. [PubMed: 16144764]
6. Alvarez VA, Ridenour DA, Sabatini BL. Distinct structural and ionotropic roles of NMDA receptors in controlling spine and synapse stability. *J Neurosci* 2007;27(28):7365–76. [PubMed: 17626197]
7. Alvarez VA, Sabatini BL. Anatomical and physiological plasticity of dendritic spines. *Ann Rev Neuro* 2007;30:79–97.
8. Applegate MD, Landfield PW. Synaptic vesicle redistribution during hippocampal frequency potentiation and depression in young and aged rats. *J Neurosci* 1988;8(4):1096–111. [PubMed: 3357011]
9. Ashby MC, Maier SR, Nishimune A, Henley JM. Lateral diffusion drives constitutive exchange of AMPA receptors at dendritic spines and is regulated by spine morphology. *J Neurosci* 2006;26:7046–55. [PubMed: 16807334]
10. Beattie EC, Carroll RC, Yu X, Morishita W, Yasuda H, et al. Regulation of AMPA receptor endocytosis by a signaling mechanism shared with LTD. *Nat Neurosci* 2000;3(12):1291–300. [PubMed: 11100150]
11. Blanpied TA, Scott DB, Ehlers MD. Dynamics and regulation of clathrin coats at specialized endocytic zones of dendrites and spines. *Neuron* 2002;36(3):435–49. [PubMed: 12408846]
12. Bourne J, Harris KM. Do thin spines learn to be mushroom spines that remember? *Curr Opin Neurobiol*. 2007
13. Bourne JN, Kirov SA, Sorra KE, Harris KM. Warmer preparation of hippocampal slices prevents synapse proliferation that might obscure LTP-related structural plasticity. *Neuropharmacology* 2007a;52(1):55–9. [PubMed: 16895730]
14. Bourne JN, Sorra KE, Hurlburt J, Harris KM. Polyribosomes are increased in spines of CA1 dendrites 2 h after the induction of LTP in mature rat hippocampal slices. *Hippocampus* 2007b;17(1):1–4. [PubMed: 17094086]
15. Bozdagi O, Shan W, Tanaka H, Benson DL, Huntley GW. Increasing numbers of synaptic puncta during late-phase LTP: N-cadherin is synthesized, recruited to synaptic sites, and required for potentiation. *Neuron* 2000;28(1):245–59. [PubMed: 11086998]
16. Brown TC, Tran IC, Backos DS, Esteban JA. NMDA receptor-dependent activation of the small GTPase Rab5 drives the removal of synaptic AMPA receptors during hippocampal LTD. *Neuron* 2005:81–94.
17. Bushong EA, Martone ME, Jones YZ, Ellisman MH. Protoplasmic astrocytes in CA1 stratum radiatum occupy separate anatomical domains. *J Neurosci* 2002;22(1):183–92. [PubMed: 11756501]
18. Calabrese B, Wilson MS, Halpain S. Development and regulation of dendritic spine synapses. *Physiology* 2006;21:38–47. [PubMed: 16443821]
19. Cesa R, Strata P. Axonal and synaptic remodeling in the mature cerebellar cortex. *Prog Brain Res* 2005;148:45–56. [PubMed: 15661180]
20. Chen HJ, Rojas-Soto M, Oguni A, Kennedy MB. A synaptic Ras-GTPase activating protein (p135 SynGAP) inhibited by CaM kinase II. *Neuron* 1998;20(5):895–904. [PubMed: 9620694]
21. Chen LY, Rex CS, Casale MS, Gall CM, Lynch G. Changes in synaptic morphology accompany actin signaling during LTP. *J Neurosci* 2007;27:5363–72. [PubMed: 17507558]
22. Chen YC, Bourne J, Pieribone VA, Fitzsimonds RM. The role of actin in the regulation of dendritic spine morphology and bidirectional synaptic plasticity. *NeuroReport* 2004;15(5):829–32. [PubMed: 15073524]

23. Chicurel ME, Harris KM. Three-dimensional analysis of the structure and composition of CA3 branched dendritic spines and their synaptic relationships with mossy fiber boutons in the rat hippocampus. *J Comp Neurol* 1992;325:169–82. [PubMed: 1460112]
24. Christopherson KS, Ullian EM, Stokes CC, Mullen CE, Hell JW, et al. Thrombospondins are astrocyte-secreted proteins that promote CNS synaptogenesis. *Cell* 2005;120(3):421–33. [PubMed: 15707899]
25. Contractor A, Rogers C, Maron C, Henkemeyer M, Swanson GT, Heinemann SF. Trans-synaptic Eph receptor-ephrin signaling in hippocampal mossy fiber LTP. *Science* 2002;296(5574):1864–9. [PubMed: 12052960]
26. Cooney JR, Hurlburt JL, Selig DK, Harris KM, Fiala JC. Endosomal compartments serve multiple hippocampal dendritic spines from a widespread rather than a local store of recycling membrane. *J Neurosci* 2002;22(6):2215–24. [PubMed: 11896161]
27. Dalva MB, Takasu MA, Lin MZ, Shamah SM, Hu L, et al. EphB receptors interact with NMDA receptors and regulate excitatory synapse formation. *Cell* 2000;103(6):945–56. [PubMed: 11136979]
28. Deller T, Orth CB, Del TD, Vlachos A, Burbach GJ, et al. A role for synaptopodin and the spine apparatus in hippocampal synaptic plasticity. *Ann Anat* 2007;189(1):5–16. [PubMed: 17319604]
29. Dhanraj TM, Lynch MA, Kelly A, Popov VI, Rusakov DA, Stewart MG. Expression of long-term potentiation in aged rats involves perforated synapses but dendritic spine branching results from high-frequency stimulation alone. *Hippocampus* 2004;14:255–64. [PubMed: 15098730]
30. Ehrlich I, Klein M, Rumpel S, Malinow R. PSD-95 is required for activity-driven synapse stabilization. *Proc Natl Acad Sci U S A* 2007;104(10):4176–81. [PubMed: 17360496]
31. Engert F, Bonhoeffer T. Dendritic spine changes associated with hippocampal long-term synaptic plasticity. *Nature* 1999;399(6731):66–70. [PubMed: 10331391]
32. Ethell IM, Pasquale EB. Molecular mechanisms of dendritic spine development and remodeling. *Prog Neurobiol* 2005;75:161–205. [PubMed: 15882774]
33. Fedulov V, Rex CS, Simmons DA, Palmer L, Gall CM, Lynch G. Evidence that long-term potentiation occurs within individual hippocampal synapses. *J Neurosci* 2007;27:8031–9. [PubMed: 17652593]
34. Fiala JC, Allwardt B, Harris KM. Dendritic spines do not split during hippocampal LTP or maturation. *Nat Neurosci* 2002;5(4):297–8. [PubMed: 11896399]
35. Fiala JC, Feinberg M, Popov V, Harris KM. Synaptogenesis via dendritic filopodia in developing hippocampal area CA1. *J Neurosci* 1998;18(21):8900–11. [PubMed: 9786995]
36. Fifkova E, Anderson CL, Young SJ, Van Harreveld A. Effect of anisomycin on stimulation-induced changes in dendritic spines of the dentate granule cells. *J Neurocytol* 1982;11(2):183–210. [PubMed: 6279784]
37. Fischer M, Kaech S, Wagner U, Brinkhaus H, Matus A. Glutamate receptors regulate actin-based plasticity in dendritic spines. *Nat Neurosci* 2000;3(9):887–94. [PubMed: 10966619]
38. Fonseca R, Vabulas RM, Hartl FU, Bonhoeffer T, Nagerl UV. A balance of protein synthesis and proteasome-dependent degradation determines the maintenance of LTP. *Neuron* 2006;52(2):239–45. [PubMed: 17046687]
39. Fukazawa Y, Saitoh Y, Ozawa F, Ohta Y, Mizuno K, Inokuchi K. Hippocampal LTP is accompanied by enhanced F-actin content within the dendritic spine that is essential for late LTP maintenance in vivo. *Neuron* 2003;38(3):447–60. [PubMed: 12741991]
40. Gardiol A, Racca C, Triller A. Dendritic and postsynaptic protein synthetic machinery. *J Neurosci* 1999;19(1):168–79. [PubMed: 9870948]
41. Geinisman Y, de Toledo-Morrell L, Morrell F, Heller RE, Rossi M, Parshall RF. Structural synaptic correlate of long-term potentiation: formation of axospinous synapses with multiple, completely partitioned transmission zones. *Hippocampus* 1993;3(4):435–45. [PubMed: 8269035]
42. Geinisman Y, Toledo-Morrell L, Morrell F. Induction of long-term potentiation is associated with an increase in the number of axospinous synapses with segmented postsynaptic densities. *Brain Res* 1991;566(1–2):77–88. [PubMed: 1814558]
43. Grigston JC, VanDongen HM, McNamara JO, VanDongen AM. Translation of an integral membrane protein in distal dendrites of hippocampal neurons. *Europ J Neurosci* 2005;21(6):1457–68.

44. Grooms SY, Noh KM, Regis R, Bassell GJ, Bryan MK, et al. Activity bidirectionally regulates AMPA receptor mRNA abundance in dendrites of hippocampal neurons. *J Neurosci* 2006;26(32):8339–51. [PubMed: 16899729]
45. Grunwald IC, Korte M, Adelmann G, Plueck A, Kullander K, et al. Hippocampal plasticity requires postsynaptic ephrinBs. *Nat Neurosci* 2004;7(1):33–40. [PubMed: 14699416]
46. Halassa MM, Fellin T, Takano H, Dong J-H, Haydon PG. Synaptic islands defined by the territory of a single astrocyte. *J Neurosci* 2007;27:6473–7. [PubMed: 17567808]
47. Halpain S, Hipolito A, Saffer L. Regulation of F-actin stability in dendritic spines by glutamate receptors and calcineurin. *J Neurosci* 1998;18(23):9835–44. [PubMed: 9822742]
48. Harris KM. Structure, development, and plasticity of dendritic spines. *Curr Opin Neurobiol* 1999;9:343–8. [PubMed: 10395574]
49. Harris KM, Fiala JC, Ostroff L. Structural changes at dendritic spine synapses during long-term potentiation. *Philos Trans R Soc Lond B Biol Sci* 2003;358(1432):745–8. [PubMed: 12740121]
50. Harris KM, Jensen FE, Tsao B. Three-dimensional structure of dendritic spines and synapses in rat hippocampus (CA1) at postnatal day 15 and adult ages: Implications for the maturation of synaptic physiology and long-term potentiation. *J Neurosci* 1992;12:2685–705. [PubMed: 1613552]
51. Harris KM, Perry E, Bourne J, Feinberg M, Ostroff L, Hurlburt J. Uniform serial sectioning for transmission electron microscopy. *J Neurosci* 2006;26(47):12101–3. [PubMed: 17122034]
52. Harris KM, Stevens JK. Dendritic spines of rat cerebellar Purkinje cells: Serial electron microscopy with reference to their biophysical characteristics. *J Neurosci* 1988;8:4455–69. [PubMed: 3199186]
53. Harris KM, Stevens JK. Dendritic spines of CA1 pyramidal cells in the rat hippocampus: serial electron microscopy with reference to their biophysical characteristics. *J Neurosci* 1989;9:2982–97. [PubMed: 2769375]
54. Hasbani MJ, Schlieff ML, Fisher DA, Goldberg MP. Dendritic spines lost during glutamate receptor activation reemerge at original sites of synaptic contact. *J Neurosci* 2001;21(7):2393–403. [PubMed: 11264313]
55. Havik B, Rokke H, Bardsen K, Davanger S, Bramham CR. Bursts of high-frequency stimulation trigger rapid delivery of pre-existing alpha-CaMKII mRNA to synapses: a mechanism in dendritic protein synthesis during long-term potentiation in adult awake rats. *Eur J Neurosci* 2003;17(12):2679–89. [PubMed: 12823475]
56. Horton AC, Ehlers MD. Secretory trafficking in neuronal dendrites. *Nat Cell Biol* 2004;6(7):585–91. [PubMed: 15232591]
57. Huber KM, Roder JC, Bear MF. Chemical induction of mGluR5- and protein synthesis--dependent long-term depression in hippocampal area CA1. *J Neurophysiol* 2001;86(1):321–5. [PubMed: 11431513]
58. Humeau Y, Herry C, Kemp N, Shaban H, Fourcaudot E, et al. Dendritic spine heterogeneity determines afferent-specific Hebbian plasticity in the amygdala. *Neuron* 2005;45(1):119–31. [PubMed: 15629707]
59. Irie F, Yamaguchi Y. EPHB receptor signaling in dendritic spine development. *Front Biosci* 2004;9:1365–73. [PubMed: 14977552]
60. Isaac JT, Nicoll RA, Malenka RC. Evidence for silent synapses: implications for the expression of LTP. *Neuron* 1995;15(2):427–34. [PubMed: 7646894]
61. Ju W, Morishita W, Tsui J, Gaietta G, Deerinck TJ, et al. Activity-dependent regulation of dendritic synthesis and trafficking of AMPA receptors. *Nat Neurosci* 2004;7(3):244–53. [PubMed: 14770185]
62. Kacharina JE, Job C, Crino P, Eberwine J. Stimulation of glutamate receptor protein synthesis and membrane insertion within isolated neuronal dendrites. *Proc Natl Acad Sci U S A* 2000;97(21):11545–50. [PubMed: 11027353]
63. Karpova A, Mikhaylova M, Thomas U, Knopfel T, Behnisch T. Involvement of protein synthesis and degradation in long-term potentiation of Schaffer collateral CA1 synapses. *J Neurosci* 2006;26(18):4949–55. [PubMed: 16672670]
64. Kelleher RJ III, Govindarajan A, Jung HY, Kang H, Tonegawa S. Translational control by MAPK signaling in long-term synaptic plasticity and memory. *Cell* 2004;116(3):467–79. [PubMed: 15016380]

65. Kennedy MB, Bennett MK, Bulliet RF, Erondy NE, Jennings VR, et al. Structure and regulation of type II calcium/calmodulin-dependent protein kinase in central nervous system neurons. *Cld Spr Harbor Symp Quant Biol* 1990;55:101–10.
66. Kennedy MB, Bennett MK, Erondy NE. Biochemical and immunochemical evidence that the “major postsynaptic density protein” is a subunit of a calmodulin-dependent protein kinase. *Proc Natl Acad Sci U S A* 1983;80:7357–61. [PubMed: 6580651]
67. Kim CH, Lisman JE. A role of actin filament in synaptic transmission and long-term potentiation. *J Neurosci* 1999;19:4314–24. [PubMed: 10341235]
68. Kim J, Jung SC, Clemens AM, Petralia RS, Hoffman DA. Regulation of dendritic excitability by activity-dependent trafficking of the A-type K⁺ channel subunit Kv4.2 in hippocampal neurons. *Neuron* 2007;54(6):933–47. [PubMed: 17582333]
69. Kim JH, Liao D, Lau LF, Haganir RL. SynGAP: a synaptic RasGAP that associates with the PSD-95/SAP90 protein family. *Neuron* 1998;20(4):683–91. [PubMed: 9581761]
70. Kirov SA, Goddard CA, Harris KM. Age-dependence in the homeostatic upregulation of hippocampal dendritic spine number during blocked synaptic transmission. *Neuropharmacology* 2004a;47(5):640–8. [PubMed: 15458835]
71. Kirov SA, Harris KM. Dendrites are more spiny on mature hippocampal neurons when synapses are inactivated. *Nat Neurosci* 1999;2(10):878–83. [PubMed: 10491607]
72. Kirov SA, Petrak LJ, Fiala JC, Harris KM. Dendritic spines disappear with chilling but proliferate excessively upon rewarming of mature hippocampus. *Neurosci* 2004b;127(1):69–80.
73. Kirov SA, Sorra KE, Harris KM. Slices have more synapses than perfusion-fixed hippocampus from both young and mature rats. *J Neurosci* 1999;19(8):2876–86. [PubMed: 10191305]
74. Knott GW, Holtmaat A, Wilbrecht L, Welker E, Svoboda K. Spine growth precedes synapse formation in the adult neocortex in vivo. *Nat Neurosci.* 2006
75. Kopeck CD, Li B, Wei W, Boehm J, Malinow R. Glutamate receptor exocytosis and spine enlargement during chemically induced long-term potentiation. *J Neurosci* 2006;26:2000–9. [PubMed: 16481433]
76. Kosik KS, Donahue CP, Israely I, Liu X, Ochiishi T. Delta-catenin at the synaptic-adherens junction. *Trends Cell Biol* 2005;15(3):172–8. [PubMed: 15752981]
77. Kozorovitskiy Y, Gross CG, Kopil C, Battaglia L, McBreen M, et al. Experience induces structural and biochemical changes in the adult primate brain. *Proc Natl Acad Sci USA* 2005;102:17478–82. [PubMed: 16299105]
78. Krapivinsky G, Medina I, Krapivinsky L, Gapon S, Clapham DE. SynGAP-MUPP1-CaMKII synaptic complexes regulate p38 MAP kinase activity and NMDA receptor-dependent synaptic AMPA receptor potentiation. *Neuron* 2004;43(563):574.
79. Krucker T, Siggins GR, Halpain S. Dynamic actin filaments are required for stable long-term potentiation (LTP) in area CA1 of the hippocampus. *Proc Natl Acad Sci U S A* 2000;97(12):6856–61. [PubMed: 10823894]
80. Lang C, Barco A, Zablow L, Kandel ER, Siegelbaum SA, Zakharenko SS. Transient expansion of synaptically connected dendritic spines upon induction of hippocampal long-term potentiation. *Proc Natl Acad Sci U S A* 2004;101(47):16665–70. [PubMed: 15542587]
81. Lee SH, Liu L, Wang YT, Sheng M. Clathrin adaptor AP2 and NSF interact with overlapping sites of GluR2 and play distinct roles in AMPA receptor trafficking and hippocampal LTD. *Neuron* 2002;36(4):661–74. [PubMed: 12441055]
82. Li Z, Okamoto K, Hayashi Y, Sheng M. The importance of dendritic mitochondria in the morphogenesis and plasticity of spines and synapses. *Cell* 2004;119(6):873–87. [PubMed: 15607982]
83. Liao D, Hessler NA, Malinow R. Activation of postsynaptically silent synapses during pairing-induced LTP in CA1 region of hippocampal slice. *Nature* 1995;375(6530):400–4. [PubMed: 7760933]
84. Liao D, Zhang X, O’Brien R, Ehlers MD, Haganir RL. Regulation of morphological postsynaptic silent synapses in developing hippocampal neurons. *Nat Neurosci* 1999;2(1):37–43. [PubMed: 10195178]
85. Lin B, Kramar EA, Bi X, Brucher FA, Gall CM, Lynch G. Theta stimulation polymerizes actin in dendritic spines of hippocampus. *J Neurosci* 2005;25(8):2062–9. [PubMed: 15728846]

86. Lisman J, Harris KM. Quantal analysis and synaptic anatomy - integrating two views of hippocampal plasticity. *Trends Neurosci* 1993;16:141–7. [PubMed: 7682347]
87. Lisman J, Harris KM. Who's been nibbling on my PSD: Is it LTD? *J Physiol (Paris)* 1994;88:193–5. [PubMed: 7833861]
88. Lledo PM, Hjelmstad GO, Mukherji S, Soderling TR, Malenka RC, Nicoll RA. Calcium calmodulin-dependent kinase-II and long term potentiation enhance synaptic transmission by the same mechanism. *Proc Natl Acad Sci USA* 1995;92:11175–9. [PubMed: 7479960]
89. Lu W, Man H, Ju W, Trimble WS, MacDonald JF, Wang YT. Activation of synaptic NMDA receptors induces membrane insertion of new AMPA receptors and LTP in cultured hippocampal neurons. *Neuron* 2001;29(1):243–54. [PubMed: 11182095]
90. Magee JC, Johnston D. Plasticity of dendritic function. *Curr Opin Neurobiol* 2005;15(3):334–42. [PubMed: 15922583]
91. Maletic-Savatic M, Malinow R, Svoboda K. Rapid dendritic morphogenesis in CA1 hippocampal dendrites induced by synaptic activity. *Science* 1999;283(5409):1923–7. [PubMed: 10082466]
92. Man HY, Lin JW, Ju WH, Ahmadian G, Liu L, et al. Regulation of AMPA receptor-mediated synaptic transmission by clathrin-dependent receptor internalization. *Neuron* 2000;25(3):649–62. [PubMed: 10774732]
93. Marrs GS, Green SH, Dailey ME. Rapid formation and remodeling of postsynaptic densities in developing dendrites. *Nat Neurosci* 2001;4(10):1006–13. [PubMed: 11574832]
94. Martone ME, Pollock JA, Jones YZ, Ellisman MH. Ultrastructural localization of dendritic messenger RNA in adult rat hippocampus. *J Neurosci* 1996;16(23):7437–46. [PubMed: 8922399]
95. Masugi-Tokita M, Shigemoto R. High-resolution quantitative visualization of glutamate and GABA receptors at central synapses. *Curr Opin Neurobiol* 2007;17(3):387–93. [PubMed: 17499496]
96. Matsuno H, Okabe S, Mishina M, Yanagida T, Mori K, Yoshihara Y. Telencephalin slows spine maturation. *J Neurosci* 2006;26(6):1776–86. [PubMed: 16467526]
97. Matsuzaki M, Ellis-Davies GC, Nemoto T, Miyashita Y, Iino M, Kasai H. Dendritic spine geometry is critical for AMPA receptor expression in hippocampal CA1 pyramidal neurons. *Nat Neurosci* 2001;4(11):1086–92. [PubMed: 11687814]
98. Matsuzaki M, Honkura N, Ellis-Davies GC, Kasai H. Structural basis of long-term potentiation in single dendritic spines. *Nature* 2004;429(6993):761–6. [PubMed: 15190253]
99. Matus A. Actin-based plasticity in dendritic spines. *Science* 2000;290(5492):754–8. [PubMed: 11052932]
100. McGlade-McCulloh E, Yamamoto H, Tan SE, Brickey DA, Soderling TR. Phosphorylation and regulation of glutamate receptors by calcium/calmodulin-dependent protein kinase II. *Nature* 1993;362(6421):640–2. [PubMed: 8385275]
101. Mezey S, Doyere V, De S I, Harrison E, Cambon K, et al. Long-term synaptic morphometry changes after induction of long-term potentiation and long-term depression in the dentate gyrus of awake rats are not simply mirror phenomena. *Eur J Neurosci* 2004;19(8):2310–8. [PubMed: 15090057]
102. Miller SG, Kennedy MB. Regulation of brain type II Ca²⁺/calmodulin-dependent protein kinase by autophosphorylation: a Ca²⁺-triggered molecular switch. *Cell* 1986;44(6):861–70. [PubMed: 3006921]
103. Moser MB, Trommald M, Egeland T, Andersen P. Spatial training in a complex environment and isolation alter the spine distribution differently in rat CA1 pyramidal cells. *J Comp Neurol* 1997;380(3):373–81. [PubMed: 9087519]
104. Murai KK, Nguyen LN, Irie F, Yamaguchi Y, Pasquale EB. Control of hippocampal dendritic spine morphology through ephrin-A3/EphA4 signaling. *Nat Neurosci* 2003a;6(2):153–60. [PubMed: 12496762]
105. Murai KK, Nguyen LN, Irie F, Yamaguchi Y, Pasquale EB. Control of hippocampal dendritic spine morphology through ephrin-A3/EphA4 signaling. *Nat Neurosci* 2003b;6(2):153–60. [PubMed: 12496762]
106. Nagerl UV, Eberhorn N, Cambridge SB, Bonhoeffer T. Bidirectional activity-dependent morphological plasticity in hippocampal neurons. *Neuron* 2004;44(5):759–67. [PubMed: 15572108]

107. Nagerl UV, Kostinger G, Anderson JC, Martin KA, Bonhoeffer T. Protracted synaptogenesis after activity-dependent spinogenesis in hippocampal neurons. *J Neurosci* 2007;27(30):8149–56. [PubMed: 17652605]
108. Nicholson DA, Trana R, Katz Y, Kath WL, Spruston N, Geinisman Y. Distance-dependent differences in synapse number and AMPA receptor expression in hippocampal CA1 pyramidal neurons. *Neuron* 2006;50(3):431–42. [PubMed: 16675397]
109. Nosyreva ED, Huber KM. Developmental switch in synaptic mechanisms of hippocampal metabotropic glutamate receptor-dependent long-term depression. *J Neurosci* 2005;25(11):2992–3001. [PubMed: 15772359]
110. Nuriya M, Haganir RL. Regulation of AMPA receptor trafficking by N-cadherin. *J Neurochem* 2006;97(3):652–61. [PubMed: 16515543]
111. Oertner TG, Matus A. Calcium regulation of actin dynamics in dendritic spines. *Cell Ca* 2005;37(5):477–82.
112. Oh JS, Manzerra P, Kennedy MB. Regulation of the neuron-specific Ras GTPase-activating protein, synGAP, by Ca²⁺/calmodulin-dependent protein kinase II. *J Biol Chem* 2004;279(17):17980–8. [PubMed: 14970204]
113. Okabe S. Molecular anatomy of the postsynaptic density. *Mol Cell Neurosci* 2007;34(4):503–18. [PubMed: 17321751]
114. Okabe S, Miwa A, Okado H. Spine formation and correlated assembly of presynaptic and postsynaptic molecules. *J Neurosci* 2001;21(16):6105–14. [PubMed: 11487634]
115. Osterweil E, Wells DG, Mooseker MS. A role for myosin VI in postsynaptic structure and glutamate receptor endocytosis. *J Cell Biol* 2005;168(2):329–38. [PubMed: 15657400]
116. Ostroff LE, Fiala JC, Allwardt B, Harris KM. Polyribosomes redistribute from dendritic shafts into spines with enlarged synapses during LTP in developing rat hippocampal slices. *Neuron* 2002;35(3):535–45. [PubMed: 12165474]
117. Ostroff LE, Harris KM. Dynamic dendrites: More and bigger spines and synapses and proliferation of polyribosomes during early LTP. *Soc Neurosci*. 2004(Abstr.)
118. Otmakhov N, Tao-Cheng JH, Carpenter S, Asrican B, Dosemeci A, et al. Persistent accumulation of calcium/calmodulin-dependent protein kinase II in dendritic spines after induction of NMDA receptor-dependent chemical long-term potentiation. *J Neurosci* 2004;24(42):9324–31. [PubMed: 15496668]
119. Ouyang Y, Kantor D, Harris KM, Schuman EM, Kennedy MB. Visualization of the distribution of autophosphorylated Calcium/Calmodulin-dependent protein kinase II after tetanic stimulation in the CA1 area of the hippocampus. *J Neurosci* 1997;17(14):5416–27. [PubMed: 9204925]
120. Ouyang Y, Rosenstein A, Kreiman G, Schuman EM, Kennedy MB. Tetanic stimulation leads to increased accumulation of Ca²⁺/calmodulin-dependent protein kinase II via dendritic protein synthesis in hippocampal neurons. *J Neurosci* 1999;19(18):7823–33. [PubMed: 10479685]
121. Ouyang Y, Wong M, Capani F, Rensing N, Lee CS, et al. Transient decrease in F-actin may be necessary for translocation of proteins into dendritic spines. *Eur J Neurosci* 2005;22(12):2995–3005. [PubMed: 16367766]
122. Papa M, Segal M. Morphological plasticity in dendritic spines of cultured hippocampal neurons. *Neurosci* 1996;71(4):1005–11.
123. Park M, Penick EC, Edwards JG, Kauer JA, Ehlers MD. Recycling endosomes supply AMPA receptors for LTP. *Science* 2004;305(5692):1972–5. [PubMed: 15448273]
124. Park M, Salgado JM, Ostroff L, Helton TD, Robinson CG, et al. Plasticity-induced growth of dendritic spines by exocytic trafficking from recycling endosomes. *Neuron* 2006;52(5):817–30. [PubMed: 17145503]
125. Penzes P, Beeser A, Chernoff J, Schiller MR, Eipper BA, et al. Rapid induction of dendritic spine morphogenesis by trans-synaptic ephrinB-EphB receptor activation of the Rho-GEF kalirin. *Neuron* 2003;37(2):263–74. [PubMed: 12546821]
126. Petralia RS, Esteban JA, Wang Y-X, Partridge JG, Zhao H-M, et al. Selective acquisition of AMPA receptors over postnatal development suggests a molecular basis for silent synapses. *Nat Neurosci* 1999;2:31–6. [PubMed: 10195177]

127. Pettit DL, Perlman S, Malinow R. Potentiated transmission and prevention of further LTP by increased CaMKII activity in postsynaptic hippocampal slice neurons. *Science* 1994;266(5192):1881–5. [PubMed: 7997883]
128. Pierce JP, van Leyen K, McCarthy JB. Translocation machinery for synthesis of integral membrane and secretory proteins in dendritic spines. *Nat Neurosci* 2000;3(4):311–3. [PubMed: 10725917]
129. Popov VI, Bocharova LS. Hibernation-induced structural changes in synaptic contacts between mossy fibres and hippocampal pyramidal neurons. *Neurosci* 1992;48:53–62.
130. Popov VI, Bocharova LS, Bragin AG. Repeated changes of dendritic morphology in the hippocampus of ground squirrels in the course of hibernation. *Neurosci* 1992;48:45–51.
131. Popov VI, Davies HA, Rogachevsky VV, Patrushev IV, Errington ML, et al. Remodelling of synaptic morphology but unchanged synaptic density during late phase long-term potentiation (LTP): a serial section electron micrograph study in the dentate gyrus in the anaesthetised rat. *Neurosci* 2004;128(2):251–62.
132. Racz B, Blanpied TA, Ehlers MD, Weinberg RJ. Lateral organization of endocytic machinery in dendritic spines. *Nat Neurosci* 2004;7(9):917–8. [PubMed: 15322548]
133. Rao A, Craig AM. Activity regulates the synaptic localization of the NMDA receptor in hippocampal neurons. *Neuron* 1997;19(4):801–12. [PubMed: 9354327]
134. Risher WC, Ostroff LE, Harris KM. What dendritic filopodia induced by LTP encounter along their path through the neuropil of PN15 rat hippocampus. *Soc Neurosci Abs.* 2006(Abstr.)
135. Rogalski-Wilk AA, Cohen RS. Glyceraldehyde-3-phosphate dehydrogenase activity and F-actin associations in synaptosomes and postsynaptic densities of porcine cerebral cortex. *Cell Mol Neurobiol* 1997;17(1):51–70. [PubMed: 9118209]
136. Rostaing P, Real E, Siksou L, Lechère JP, Boudier T, et al. Analysis of synaptic ultrastructure without fixative using high-pressure freezing and tomography. *Eur J Neurosci* 2006;24(12):3463–74. [PubMed: 17229095]
137. Ryu J, Liu L, Wong TP, Wu DC, Burette A, et al. A critical role for myosin IIb in dendritic spine morphology and synaptic function. *Neuron* 2006;49(2):175–82. [PubMed: 16423692]
138. Sabatini BL, Maravall M, Svoboda K. Ca²⁺ signaling in dendritic spines. *Curr Opin Neurobiol* 2001;11(3):349–56. [PubMed: 11399434]
139. Schratt GM, Tuebing F, Nigh EA, Kane CG, Sabatini ME, et al. A brain-specific microRNA regulates dendritic spine development. *Nature* 2006;439(7074):283–9. [PubMed: 16421561]
140. Schuster T, Krug M, Wenzel J. Spinules in axospinous synapses of the rat dentate gyrus: changes in density following long-term potentiation. *Brain Res* 1990;523(1):171–4. [PubMed: 2207688]
141. Shapira M, Zhai RG, Dresbach T, Bresler T, Torres VI, et al. Unitary assembly of presynaptic active zones from Piccolo-Bassoon transport vesicles. *Neuron* 2003;38(2):237–52. [PubMed: 12718858]
142. Snyder EM, Philpot BD, Huber KM, Dong X, Fallon JR, Bear MF. Internalization of ionotropic glutamate receptors in response to mGluR activation. *Nat Neurosci* 2001;4(11):1079–85. [PubMed: 11687813]
143. Sorra KE, Fiala JC, Harris KM. Critical assessment of the involvement of perforations, spinules, and spine branching in hippocampal synapse formation. *J Comp Neurol* 1998;398(2):225–40. [PubMed: 9700568]
144. Spacek J. Three-dimensional analysis of dendritic spines. II Spine apparatus and other cytoplasmic components. *Anat Embryol* 1985a;171:235–43. [PubMed: 3985372]
145. Spacek J. Three-dimensional analysis of dendritic spines. III Glial sheath. *Anat Embryol (Berl)* 1985b;171(2):245–52. [PubMed: 3985373]
146. Spacek J, Harris KM. Three-dimensional organization of smooth endoplasmic reticulum in hippocampal CA1 dendrites and dendritic spines of the immature and mature rat. *J Neurosci* 1997;17(1):190–203. [PubMed: 8987748]
147. Spacek J, Harris KM. Trans-endocytosis via spinules in adult rat hippocampus. *J Neurosci* 2004;24(17):4233–41. [PubMed: 15115819]
148. Star EN, Kwiatkowski DJ, Murthy VN. Rapid turnover of actin in dendritic spines and its regulation by activity. *Nat Neurosci* 2002;5(3):239–46. [PubMed: 11850630]

149. Steward O, Levy WB. Preferential localization of polyribosomes under the base of dendritic spines in granule cells of the dentate gyrus. *J Neurosci* 1982;2:284–91. [PubMed: 7062109]
150. Steward O, Reeves TM. Protein-synthetic machinery beneath postsynaptic sites on CNS neurons: association between polyribosomes and other organelles at the synaptic site. *J Neurosci* 1988;8:176–84. [PubMed: 3339407]
151. Steward O, Schuman EM. Compartmentalized synthesis and degradation of proteins in neurons. *Neuron* 2003;40(2):347–59. [PubMed: 14556713]
152. Stewart MG, Medvedev NI, Popov VI, Schoepfer R, Davies HA, et al. Chemically induced long-term potentiation increases the number of perforated and complex postsynaptic densities but does not alter dendritic spine volume in CA1 of adult mouse hippocampal slices. *Eur J Neurosci* 2005;21(12):3368–78. [PubMed: 16026474]
153. Tada T, Sheng M. Molecular mechanisms of dendritic spine morphogenesis. *Curr Opin Neurobiol* 2006;16(1):95–101. [PubMed: 16361095]
154. Tai CY, Mysore SP, Chiu C, Schuman EM. Activity-regulated N-cadherin endocytosis. *Neuron* 2007;54(5):771–85. [PubMed: 17553425]
155. Takumi Y, Matsubara A, Rinvik E, Ottersen OP. The arrangement of glutamate receptors in excitatory synapses. *Ann N Y Acad Sci* 1999a;868:474–82. [PubMed: 10414324]
156. Takumi Y, Ramirez-Leon V, Laake P, Rinvik E, Ottersen OP. Different modes of expression of AMPA and NMDA receptors in hippocampal synapses. *Nat Neurosci* 1999b;2(7):618–24. [PubMed: 10409387]
157. Togashi H, Abe K, Mizoguchi A, Takaoka K, Chisaka O, Takeichi M. Cadherin regulates dendritic spine morphogenesis. *Neuron* 2002;35(1):77–89. [PubMed: 12123610]
158. Toni N, Buchs PA, Nikonenko I, Bron CR, Muller D. LTP promotes formation of multiple spine synapses between a single axon terminal and a dendrite. *Nature* 1999;402(6760):421–5. [PubMed: 10586883]
159. Toresson H, Grant SG. Dynamic distribution of endoplasmic reticulum in hippocampal neuron dendritic spines. *Eur J Neurosci* 2005;22(7):1793–8. [PubMed: 16197520]
160. Trommald M, Hulleberg G, Andersen P. Long-term potentiation is associated with new excitatory spine synapses on rat dentate granule cells. *Learn Mem* 1996;3(2–3):218–28. [PubMed: 10456092]
161. Ullian EM, Christopherson KS, Barres BA. Role for glia in synaptogenesis. *Glia* 2004;47(3):209–16. [PubMed: 15252809]
162. Van Harrevelde A, Fifkova E. Swelling of dendritic spines in the fascia dentata after stimulation of the perforant fibers as a mechanism of post-tetanic potentiation. *Exp Neurol* 1975;49(3):736–49. [PubMed: 173566]
163. Vazquez LE, Chen HJ, Sokolova I, Knuesel I, Kennedy MB. SynGAP regulates spine formation. *J Neurosci* 2004;24(40):8862–72. [PubMed: 15470153]
164. Ventura R, Harris KM. Many hippocampal synapses have astrocytic processes at their perimeter where glutamate could be detected and spillover limited. 1998Submitted
165. Weiler IJ, Irwin SA, Klintsova AY, Spencer CM, Brazelton AD, et al. Fragile X mental retardation protein is translated near synapses in response to neurotransmitter activation. *Proc Natl Acad Sci U S A* 1997;94(10):5395–400. [PubMed: 9144248]
166. Witcher MR, Kirov SA, Harris KM. Plasticity of perisynaptic astroglia during synaptogenesis in the mature rat hippocampus. *Glia* 2007;55(1):13–23. [PubMed: 17001633]
167. Wu K, Aoki C, Elste A, Rogalski-Wilk AA, Siekevitz P. The synthesis of ATP by glycolytic enzymes in the postsynaptic density and the effect of endogenously generated nitric oxide. *Proc Natl Acad Sci U S A* 1997;94(24):13273–8. [PubMed: 9371836]
168. Xiao MY, Zhou Q, Nicoll RA. Metabotropic glutamate receptor activation causes a rapid redistribution of AMPA receptors. *Neuropharmacology* 2001;41(6):664–71. [PubMed: 11640920]
169. Xie Z, Huganir RL, Penzes P. Activity-dependent dendritic spine structural plasticity is regulated by small GTPase Rap1 and its target AF-6. *Neuron* 2005;48(4):605–18. [PubMed: 16301177]
170. Zhai RG, Vardinon-Friedman H, Cases-Langhoff C, Becker B, Gundelfinger ED, et al. Assembling the presynaptic active zone: a characterization of an active one precursor vesicle. *Neuron* 2001;29(1):131–43. [PubMed: 11182086]

171. Zhou Q, Homma KJ, Poo MM. Shrinkage of dendritic spines associated with long-term depression of hippocampal synapses. *Neuron* 2004;44(5):749–57. [PubMed: 15572107]
172. Zhu JJ, Qin Y, Zhao M, Van AL, Malinow R. Ras and Rap control AMPA receptor trafficking during synaptic plasticity. *Cell* 2002;110(4):443–55. [PubMed: 12202034]
173. Zito K, Knott G, Shepherd GM, Shenolikar S, Svoboda K. Induction of spine growth and synapse formation by regulation of the spine actin cytoskeleton. *Neuron* 2004;44(2):321–34. [PubMed: 15473970]
174. Zito K, Parnas D, Fetter RD, Isacoff EY, Goodman CS. Watching a synapse grow: noninvasive confocal imaging of synaptic growth in *Drosophila*. *Neuron* 1999;22(4):719–29. [PubMed: 10230792]
175. Ziv NE, Smith SJ. Evidence for a role of dendritic filopodia in synaptogenesis and spine formation. *Neuron* 1996;17(1):91–102. [PubMed: 8755481]

Figure 1.

Variability in spine shape and size. **A.** 3-dimensional reconstruction of a hippocampal dendrite (gray) illustrating different spine shapes including mushroom (blue), thin (red), stubby (green), and branched (yellow). PSDs (red) also vary in size and shape. Scale cube = $0.5 \mu\text{m}^3$. **B.** An example of a mushroom spine (blue) with a head diameter exceeding 0.6 microns and a narrow neck. **C.** An example of a thin spine (red) with a small head and narrow neck. **D.** An example of a stubby spine (green) with an equal head and neck diameter and an overall length that equals its width. **E.** An example of a branched spine (yellow) where both branches are thin spines. Scale bar = $0.5 \mu\text{m}$ and arrows indicate where the head and neck diameters were measured for each spine in B–E. **F.** A graph plotting the ratio of head diameters to neck diameters for the spines on the dendrite reconstructed in A. Mushroom spines (blue diamonds), stubby spines

(green diamonds) and thin spines (red diamonds) segregated into distinct groups. Both branches of the branched spine were of a thin shape and are situated among the thin spine dimensions (yellow diamonds).

Table 1**Molecular mediators of spine morphology**

Protein	Function	Citations
PSD-95	Stabilizes nascent spines and anchors receptors and scaffolding proteins at the synapse.	Marrs et al., 2001; Okabe et al., 2001; Ehrlich et al., 2007
CamKII	Increases the thickness of the PSD and phosphorylates signaling molecules involved in plasticity.	Kennedy et al., 1983, 1990; McGlade-McCulloh et al., 1993; Pettit et al., 1994; Liao et al., 1995; Lledo et al., 1995; Martone et al., 1996; Havik et al., 2003; Ouyang et al., 1997, 1999; Aakalu et al., 2001
Actin	Regulates the extension of filopodia and mediates the expansion of spine heads with LTP and the shrinkage of spine heads with LTD.	Matus, 2000; Star et al., 2002; Krucker et al., 1999; Kim and Lisman, 1999; Ouyang et al., 2005; Lin et al., 2005; Fukazawa et al., 2003; Zhou et al., 2004; Chen et al., 2004; Nagerl et al., 2004.
Profilin	Activity-dependent promoter of actin polymerization and actin stabilizer.	Tada and Sheng, 2006; Ethell and Pasquale, 2005; Ackermann and Matus, 2003
Cofilin	Depolymerizes actin filaments but LTP or learning induced phosphorylation decreases its affinity for actin, promoting polymerization and spine enlargement.	Chen et al., 2007; Fedulov et al., 2007.
Rap1/AF-6	Activation elongates spines and removes AMPA receptors while inactivation enlarges spines and recruits AMPA receptors.	Xie et al., 2005; Zhu et al., 2002
Myosin IIb	Stabilizes mushroom spines.	Ryu et al., 2006
Myosin VI	Regulates clathrin-mediated endocytosis of AMPA receptors.	Osterweil et al., 2005
Synaptopodin	Binds to the spine apparatus and may mediate interactions between the actin cytoskeleton and calcium signaling. Synaptopodin-deficient mice have normal spine morphology and density, but all spines lack a spine apparatus.	Deller et al., 2007
Telencephalin	Overexpression slows the development of dendritic spines while deletion accelerates the spine development, suggesting a role in maintaining filopodia during development.	Matsuno et al., 2006
SynGAP	Maintains filopodia during development and localizes to the synapse to negatively regulate Ras signaling pathways, which promote spine formation and growth.	Vazquez et al., 2004; Chen et al., 1998; Kim et al., 1998; Oh et al., 2004; Krapivinsky et al., 2004
miR-134	MicroRNA that negatively regulates spine development by inhibiting translation of Limk1. Overexpression of miR-134 results in a decrease of spine volume.	Schratt et al., 2006
N-Cadherin	Stabilizes mature synapses and regulates spine morphology and synaptic efficacy.	Bozdagi et al., 2000; Togashi et al., 2002; Abe et al., 2004; Kosik et al., 2005; Tai et al., 2007; Nuriya and Huganir, 2006
EphB/EphrinB	Trans-synaptic signaling complex that clusters receptors and mediates spine morphology by recruiting molecules involved in actin polymerization.	Dalva et al., 2002; Contractor et al., 2002; Irie and Yamaguchi, 2004; Penzes et al., 2003; Grunwald et al., 2004.
EphA/EphrinA	Regulates neuro-glial signaling and induces the retraction of spines. Expression decreases during development and is inactive in mature brains suggesting a potential role in synaptic pruning.	Murai et al., 2003; Grunwald et al., 2004; Allen and Barres, 2005