Neuron
Review

Biochemical Computation for Spine Structural Plasticity

Jun Nishiyama[1] and Ryohei Yasuda[1,*]
[1]Max Planck Florida Institute for Neuroscience, One Max Planck Way, Jupiter, FL 33458, USA
*Correspondence: ryohei.yasuda@mpfi.org
http://dx.doi.org/10.1016/j.neuron.2015.05.043

The structural plasticity of dendritic spines is considered to be essential for various forms of synaptic plasticity, learning, and memory. The process is mediated by a complex signaling network consisting of numerous species of molecules. Furthermore, the spatiotemporal dynamics of the biochemical signaling are regulated in a complicated manner because of geometrical restrictions from the unique morphology of the dendritic branches and spines. Recent advances in optical techniques have enabled the exploration of the spatiotemporal aspects of the signal regulations in spines and dendrites and have provided many insights into the principle of the biochemical computation that underlies spine structural plasticity.

Dendritic spines are tiny postsynaptic protrusions covering the dendrites of most of the principal neurons in the CNS. The plasticity of the structure and the function of dendritic spines are considered to be important for synaptic plasticity and memory. Each dendritic spine consists of a small bulbous head (~0.1 fl) connected to its parent dendrite through a narrow neck (~0.1 μm in diameter and ~0.5 μm in length). The neck acts as a diffusional barrier and an electrical resistance, isolating the spine head biochemically (Bloodgood and Sabatini, 2005; Svoboda et al., 1996) and electrically (Grunditz et al., 2008; Harnett et al., 2012; Tønnesen et al., 2014) from its parent dendrite. The structure and function of spines are regulated by biochemical reactions mediated by calcium (Ca^{2+}) and numerous signaling molecules. The spatiotemporal dynamics of the biochemical reaction are restricted in a complicated manner because of the unique morphology of the spines and dendritic shafts. Imaging studies have demonstrated that some signaling activities are restricted to the spine to maintain the synaptic specificity of long-term potentiation (LTP) (Lee et al., 2009; Sabatini et al., 2002; Yuste and Denk, 1995), whereas the other signals spread locally along the dendritic shaft and nearby spines (Harvey et al., 2008; Murakoshi et al., 2011; Yasuda et al., 2006) and distantly even into the nucleus, located a few hundred micrometers away from the stimulated spines (Zhai et al., 2013). Therefore, the distinct spatiotemporal dynamics of biochemical signaling could have a large impact on the length and timescales of various forms of synaptic plasticity. Here we review recent findings demonstrating how the biochemical signals are initiated at single spines and how they are transmitted, computed, and integrated at the distinct neuronal compartments to regulate the functions of the spines and dendrites as well as the nucleus during structural plasticity of the dendritic spines.

Structural Plasticity of Dendritic Spines

Remodeling of neuronal networks through activity-dependent functional modification of synaptic connections and associated structural changes of synapses is hypothesized to be a cellular substrate of learning and memory. Recent studies have revealed that the morphology of the spine head, neck, and its substructures are modified dynamically during various forms of synaptic plasticity.

Plasticity of Spine Heads

The volume of a spine head is proportional to the area of postsynaptic density (PSD) in the spine, the presynaptic area of its synaptic partner, the number of synaptic AMPA receptors (AMPARs), and the amplitude of the AMPAR-mediated currents (Harris and Stevens, 1989; Matsuzaki et al., 2001; Schikorski and Stevens, 1997; Takumi et al., 1999). Therefore, the morphology of the spine is tightly coupled with synaptic function, and a change in spine volume has been considered to be an important substrate of synaptic plasticity. Indeed, many studies have demonstrated that LTP and long-term depression (LTD) are associated with spine enlargement and shrinkage, respectively (Desmond and Levy, 1983; Hayama et al., 2013; Matsuzaki et al., 2004; Nägerl et al., 2004; Oh et al., 2013; Okamoto et al., 2004; Van Harreveld and Fifkova, 1975; Zhou et al., 2004). Studies of spine structural plasticity have been promoted by development of the two-photon glutamate uncaging technique. This technique allows one to selectively stimulate a single spine while simultaneously imaging the morphology of the stimulated spine with two-photon microscopy (Matsuzaki et al., 2001). It has been found that repetitive glutamate uncaging under low-Mg^{2+} (nominally zero) conditions induces a rapid and transient enlargement of the spine head in the first few minutes in hippocampal CA1 pyramidal neurons. This is followed by a volume change sustained for hours (Lee et al., 2009; Matsuzaki et al., 2004). Spine enlargements with similar time course have also been observed in response to the standard LTP induction protocol—high-frequency electrical stimulation of Schaffer collateral axons in the presence of Mg^{2+} (Matsuzaki et al., 2004). The morphological change of the stimulated spine is associated with an increase in postsynaptic glutamate sensitivity. These morphological and functional changes are observed only in the stimulated spine but not in the neighboring spines, indicating that LTP can be induced in an input-specific manner at the single spine level (Figure 1A). In this Review, we refer to this form of spine morphological plasticity as structural LTP.

Similar to functional LTP, there are two distinct temporal stages in structural LTP: protein synthesis-independent, early

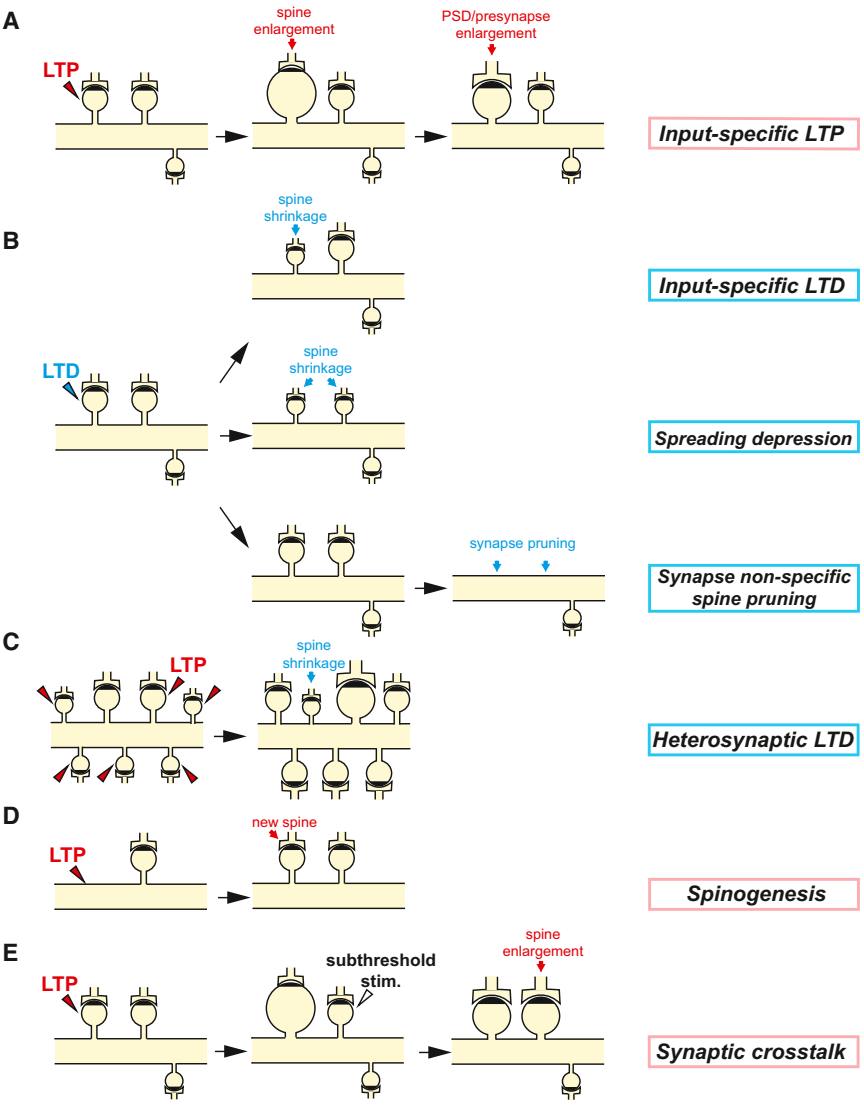

Figure 1. Spine Structural Plasticity

(A) Structural plasticity during LTP. Repetitive two-photon glutamate uncaging (0.5–2 Hz for 1 min) at a single spine under low-Mg^{2+} (nominally zero) conditions or paired with postsynaptic depolarization induces a rapid enlargement of the spine head in a few minutes. The volume of the enlarged spine gradually decreases over ~5 min to a plateau and sustains for more than an hour (Lee et al., 2009; Matsuzaki et al., 2004). The PSD and presynapse increase with a delay of 0.5–3 hr (Bosch et al., 2014; Meyer et al., 2014). Note that the enlargement of the spine volume is restricted to the stimulated spine (input-specific LTP).

(B) Structural plasticity during LTD. Different protocols for LTD induction have been reported to result in distinct structural plasticity. Low-frequency glutamate uncaging (90 pulses at 0.1 Hz) in low extracellular Ca^{2+} (0.3 mM) and Mg^{2+} (nominally zero) concentrations or paired with postsynaptic depolarization induces a spine shrinkage restricted to the stimulated spine (input-specific LTD) (Oh et al., 2013) (top). b-AP paired with subsequent two-photon glutamate uncaging pulses (~10 ms) at a single spine (80 pulses at 1 Hz) shortly after (<50 ms) GABA uncaging at the adjacent dendritic shaft induces a reduction in the volume of the stimulated spine as well as of neighboring non-stimulated spines (spreading depression) (Hayama et al., 2013) (center). The optogenetic stimulation of presynaptic CA3 pyramidal neurons expressing channelrodopsin-2 (1 Hz for 900 light pulses) induces functional LTD but not spine shrinkage in postsynaptic CA1 neurons. However, a few days later, the stimulated spine and many neighboring synapses are removed (synapse-nonspecific spine pruning) (Wiegert and Oertner, 2013) (bottom).

(C) Heterosynaptic LTD. LTP stimulation at multiple spines on a single dendritic segment by glutamate uncaging induces shrinkage of nearby unstimulated spines (Oh et al., 2015).

(D) Spinogenesis induced by glutamate uncaging. Two-photon glutamate uncaging (40 pulses at 2 Hz) at dendritic shafts triggers rapid de novo spinogenesis in young neurons (Kwon and Sabatini, 2011).

(E) Synaptic crosstalk associated with structural plasticity. Repetitive glutamate uncaging (30 pulses at 0.5 Hz, 4-ms pulse duration) is applied to a single spine to induce LTP. A subthreshold stimulus (30 pulses at 0.5 Hz, 1-ms pulse duration), which by itself does not trigger LTP, is then applied to a nearby spine. This induces a sustained structural and functional LTP in the weakly stimulated spine (Harvey and Svoboda, 2007; Harvey et al., 2008).

phase of LTP (E-LTP) and protein synthesis-dependent, late phase of LTP (L-LTP) (Bosch et al., 2014; Govindarajan et al., 2011). L-LTP can be induced in single spines by glutamate uncaging paired with postsynaptic depolarization or the bath application of BDNF or forskolin (an activator of cyclic AMP [cAMP] signaling) (Govindarajan et al., 2011; Tanaka et al., 2008a). Notably, structural and functional plasticity share at least part of their signaling pathways. They both require Ca^{2+} influx through postsynaptic NMDA receptors (NMDARs), activation of Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) and small GTPases, and actin polymerization (Harvey et al., 2008; Kim et al., 2014; Lee et al., 2009; Matsuzaki et al., 2004; Murakoshi et al., 2011). Although structural and functional plasticity can be dissociated under some conditions (Kopec et al., 2007; Sdrulla and Linden, 2007; Wang et al., 2007), these results suggest a substantial overlap between the mechanisms underlying LTP and spine enlargement.

In addition to LTP, two protocols to induce LTD and spine shrinkage using two-photon glutamate uncaging have also been found (Hayama et al., 2013; Oh et al., 2013). In the first protocol, low-frequency glutamate uncaging (0.1 Hz) in low-Ca^{2+} (0.3 mM) extracellular solution under postsynaptic depolarization or nominally zero Mg^{2+} can induce spine-specific LTD and spine shrinkage (Figure 1B). The second protocol is much more complicated. γ-aminobutyric acid (GABA) uncaging at the dendritic shaft ~10 ms prior to back-propagating action potentials (b-APs) followed by glutamate uncaging at single spines has been found to induce LTD and spine shrinkage. Interestingly, this protocol induces volume shrinkages in the surrounding, non-stimulated spines located within ~15 μm of the stimulated spines

as well as in the stimulated spines (Figure 1B). This spreading depression requires the suppression of bAP-evoked Ca^{2+} transients by GABA uncaging. GABA signaling does not appear to encode precise timing information because simple pharmacological activation of GABA receptors can replace GABA uncaging.

Plasticity of Spine Necks

It has been speculated that spines serve as electrical compartments because of the resistance at the necks (Segev and Rall, 1988). The electrical compartmentalization amplifies local excitatory postsynaptic potentials (EPSPs) within the spine and produces a voltage gradient between the spine and the dendritic shaft, reducing dendritic and somatic EPSPs compared with those in the spine. The voltage may be amplified further in spines with voltage-dependent conductance (Bywalez et al., 2015; Grunditz et al., 2008; Yuste, 2013). Indirect estimates of spine neck resistance, based on the cable theory or calculations from the measured diffusional fluxes, vary greatly (Bloodgood and Sabatini, 2005; Harris and Stevens, 1989; Svoboda et al., 1996; Yuste, 2011). However, recent evidence has supported the idea of electrical compartmentalization by the spine neck. A study using whole-cell recordings with glutamate uncaging at individual spines revealed that stimulation of spines with longer necks produces smaller EPSPs at the soma in layer 5 pyramidal neurons (Araya et al., 2006, 2014), although no such correlation was observed in olfactory bulb granule neurons (Bywalez et al., 2015). In addition, Ca^{2+} transients within spines through NMDARs and voltage-sensitive calcium channels (VSCCs) are evoked by subthreshold synaptic stimulation to a degree consistent with the voltage amplification by spine necks (Bloodgood et al., 2009; Grunditz et al., 2008; Kovalchuk et al., 2000; Yuste and Denk, 1995). Voltage-gated sodium channels have also been shown to be activated locally within spines stimulated with glutamate uncaging, leading to the opening of high voltage-activated Ca^{2+} channels in olfactory bulb granule neurons (Bywalez et al., 2015). Furthermore, the ratio of voltage changes in a spine head to that in its parent dendrite has been quantified in CA1 pyramidal neurons using two-photon glutamate uncaging in combination with Ca^{2+} imaging and dendritic patch clamping (Harnett et al., 2012). In this study, the authors measured dendritic EPSPs and associated Ca^{2+} elevations in spines mediated exclusively by VSCCs in response to two-photon glutamate uncaging (in the presence of inhibitors of NMDARs and voltage-gated sodium channels). These dendritic EPSPs were compared with the voltage changes induced by dendritic current injections to depolarize the spine to a level where the associated spine Ca^{2+} signals match those produced by the glutamate uncaging. The experiments revealed that spine necks exhibit a high resistance of $\sim$500 MΩ and amplify the depolarization in the spine heads associated with synaptic activation by 1.5- to 45-fold (Harnett et al., 2012). Although the exact neck resistance is still unknown, these results suggest that spines can act as electrical compartments.

Importantly, the function and morphology of the spine neck is regulated dynamically by neuronal activities (Bloodgood and Sabatini, 2005; Grunditz et al., 2008; Tønnesen et al., 2014). Diffusional coupling between spines and dendrites has been measured with fluorescence recovery after photobleaching (FRAP) of fluorescent proteins or fluorescence decay after photoactivation of photoactivatable GFP (PA-GFP). These studies revealed that the coupling time increases in response to two-photon glutamate uncaging at spines paired with b-APs or a postsynaptic depolarization for a few minutes (Bloodgood and Sabatini, 2005; Grunditz et al., 2008), suggesting that high neuronal activity can cause higher neck resistance. In contrast, a study found that protein synthesis-dependent LTP, induced by two-photon glutamate uncaging paired with b-APs, is coupled with widening of the spine neck (Tanaka et al., 2008a). Furthermore, a recent study using superresolution imaging based on stimulated emission depletion (STED) demonstrated that the spine necks become wider and shorter after LTP induced by two-photon glutamate uncaging (Tønnesen et al., 2014; Figure 1A). Therefore, it appears that LTP induction leads to the lowering of spine neck resistance. However, because the neck widening counteracts the increased biochemical compartmentalization by head enlargement, the degree of the diffusional coupling between spine and dendrite appears not to be altered during structural LTP (Tønnesen et al., 2014). The reduction of neck resistance should decrease the voltage amplification in spines and, therefore, may reduce the probability of further induction of LTP. On the other hand, a shorter and wider neck may facilitate the transport of resources from the dendrites into the spines undergoing LTP (Tønnesen et al., 2014).

Because spine neck plasticity can change electrical filtering by the neck, it could be one mechanism to change EPSPs at the soma during synaptic plasticity (Araya et al., 2014). However, according to a mathematical simulation using measured spine morphology with superresolution microscopy, spine neck plasticity has relatively minor effects on the amplitude of somatic EPSPs in the passive regime (Tønnesen et al., 2014). Therefore, the roles of spine neck plasticity appear to be mainly regulations of local voltage amplification in spines and biochemical compartmentalization.

The molecular mechanisms underlying spine neck plasticity are unknown, but several proteins have been identified to be localized at spine necks. In particular, septins, a highly conserved family of GTPases, are known to assemble into a hetero-oligomeric complex and higher-order structures such as filaments, rings, and gauzes. Interestingly, it has been reported that septin 7 forms a complex with septin 5/11, localizes at the base of spine necks (Tada et al., 2007; Xie et al., 2007), and serves as a diffusion barrier of membrane proteins, including GluA2 (Ewers et al., 2014). Because septins regulate the compartmentalization of the yeast plasma membrane during mitosis by forming rings at the bud necks (Barral et al., 2000; Takizawa et al., 2000), they may also play an important role in regulating the morphology of the spine neck. Further, a recent study has demonstrated that Ankyrin-G, a protein that acts as an adaptor to connect transmembrane proteins to the underlying spectrin-actin cytoskeleton, forms distinct nanodomains within spine heads and necks (Smith et al., 2014). Interestingly, the nanodomain confines AMPARs in spines, possibly acting as a diffusion barrier. In addition, the presence of Ankyrin-G at the spine neck is tightly associated with the larger head volume. When the 190-kDa isoform of Ankyrin-G, a major isoform in spines, is overexpressed, the neck width as well as the head volume are

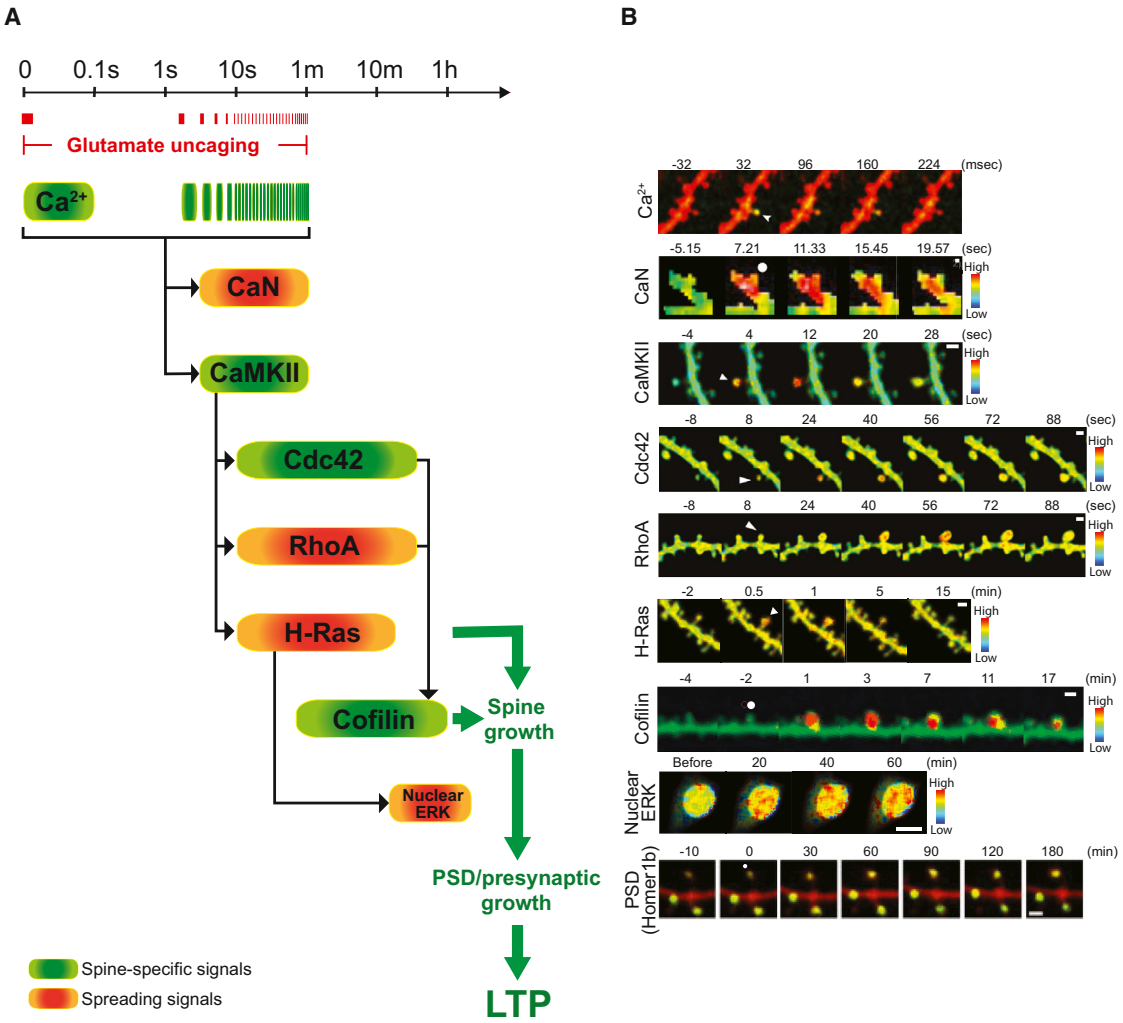

Figure 2. The Spatiotemporal Dynamics of Signaling Activities during Structural LTP

(A) The timescale of signaling activities during structural LTP induced by two-photon glutamate uncaging (0.5–20 Hz). Spine-specific signals and spreading signals are indicated in green and orange, respectively. The timing of glutamate uncaging of a typical LTP induction protocol (0.5 Hz) is shown by red bars. (B) Ca^{2+} elevation; activities of CaN, CaMKII, Cdc42, RhoA, H-Ras, cofilin, and nuclear ERK; and the accumulation of Homer1b during structural LTP induced at a single spine or seven spines (ERK). The arrows and circles show the spines stimulated with glutamate uncaging. Scale bars, 10 μm for ERK and 1 μm for others. The images were adopted and modified from Zhai et al. (2013) for Ca^{2+} and ERK, Fujii et al. (2013) for CaN, Lee et al. (2009) for CaMKII, Murakoshi et al. (2011) for RhoA and Cdc42, Harvey et al. (2008) for H-Ras, and Bosch et al. (2014) for cofilin and Homer1b, with permission. Ca^{2+} elevation is visualized with a Ca^{2+} indicator, Fluo-4FF (green) and Alexa 594 (red). Ca^{2+} elevation in response to the first uncaging pulse during the LTP induction protocol (1 Hz, 60 pulses) is displayed. CaMKII, Cdc42, RhoA, H-Ras, cofilin, and nuclear ERK activities are imaged with 2pFLIM combined with FRET sensors. CaN activities are visualized with dual FRET with optical manipulation (dFOMA). The accumulation of Homer1b in spines is visualized with GFP-tagged Homer1b and RFP (cell fill). Note that Ca^{2+} elevation and activation of CaMKII, Cdc42, and cofilin are restricted to the stimulated spines, whereas activation of CaN, RhoA, and H-Ras spread into the dendritic shafts and nearby spines.

increased significantly. These results suggest that septin and Ankyrin-G may regulate the morphology and function of spine necks during spine structural plasticity.

Plasticity of PSDs and Presynapses

Spine head volume is tightly correlated with the size of the presynaptic active zone and PSD (Harris and Stevens, 1989; Schikorski and Stevens, 1997; Takumi et al., 1999). Therefore, spine growth associated with LTP should be accompanied by a growth in the active zone, PSD, and, potentially, other spine substructures. Indeed, recent studies have revealed that this is the case at the single-spine level. Within the initial few minutes after LTP induction, the actin cytoskeleton grows, and actin and actin binding proteins such as profilin and cofilin are accumulated rapidly in the stimulated spine (Bosch et al., 2014). In contrast, the amount of PSD proteins and PSD size do not increase at this temporal stage (Bosch et al., 2014; Steiner et al., 2008). However, with a delay of a few hours, PSD scaffold proteins such as Homer1b, PSD-95, and shank1b slowly accumulate, and the size of the PSD increases (Bosch et al., 2014; Meyer et al., 2014; Figure 2). These changes have been found to be followed by the slow growth of the presynaptic terminals, suggesting the existence of dynamic retrograde

signaling during glutamate uncaging-evoked LTP (Meyer et al., 2014). These results suggest that, overall, synaptic structures are gradually rescaled over a few hours following LTP induction (Figure 1A).

Interestingly, SynGAP, one of the most abundant proteins in the PSD (Cheng et al., 2006), is dissociated from the PSD within a few minutes of chemical LTP induction (Araki et al., 2015). This dispersion state of SynGAP is sustained for more than half an hour. Because SynGAP is an inactivator of Ras, a signaling protein required for the maintenance of LTP (Harvey et al., 2008; Zhu et al., 2002), de-localization of SynGAP may help to increase Ras activity in stimulated spines, thereby stabilizing LTP. Although it is not clear whether the amount of SynGAP in the PSD eventually follows the size of the PSD or not, the protein content of the PSD and spines appears to be changed dramatically during spine structural plasticity.

Spine Formation and Elimination

In addition to changes in the structure of preexisting spines, LTP induction has also been found to be associated with the formation of new spines and filopodia (Engert and Bonhoeffer, 1999; Maletic-Savatic et al., 1999; Nägerl et al., 2004, Nagerl et al., 2007; Toni et al., 1999). A study has demonstrated that two-photon glutamate uncaging at dendritic shafts in young layer 2/3 pyramidal neurons in the cortex is sufficient to induce rapid de novo spinogenesis within a few micrometers of the stimulated spot and within several tens of seconds during the glutamate uncaging stimuli (Kwon and Sabatini, 2011; Figure 1D). The newly formed spines are functional because they produce Ca^{2+} transients in response to synaptic stimulation. This study also demonstrated that spine formation does not necessarily require an intermediate filopodium stage and that glutamate is sufficient to assemble the machinery required for nucleating spine formation. The long-term stabilization of newly formed spines appears to require further potentiation (Hill and Zito, 2013).

On the other hand, it has been known that LTD induction can cause eliminations of preexisting spines in an NMDAR-dependent manner (Figure 1B; Bastrikova et al., 2008; Nägerl et al., 2004; Okamoto et al., 2004; Zhou et al., 2004). A recent study followed the fate of spines up to 7 days after LTD induction in organotypic hippocampal slices (Wiegert and Oertner, 2013). By combining the optogenetic stimulation of presynaptic CA3 pyramidal neurons expressing channelrodopsin-2 with calcium imaging of spines in postsynaptic CA1 neurons expressing GCaMP3, synapses were stimulated optically, and the activities of individual synapses were monitored with Ca^{2+} responses. In this paradigm, they observed reductions of the success rate and the amplitude of postsynaptic Ca^{2+} transients after LTD induction, suggesting that this form of LTD is induced by both postsynaptic and presynaptic mechanisms. Interestingly, after a few days of LTD induction, these depressed synapses and their neighbors were eliminated (Figure 1B). The delayed elimination of depressed synapses seems not to be correlated with the degree of the initial LTD, but, rather, the synapses with an initially low probability of neurotransmitter release (measured before LTD induction) tend to be eliminated more selectively. Therefore, over days after LTD, the stimulated neurons change the way in which they adjust synaptic strength from an "analog" regulation in the potency of each synapse to a "digital" regulation in the number of synapses.

Heterosynaptic Plasticity in Dendritic Segments

Although individual synapses can serve as independent computational units, it has been reported that there is heterosynaptic spreading of functional plasticity (Abraham, 2008). Recent studies, by utilizing two-photon glutamate uncaging, have shown that several forms of heterosynaptic plasticity can occur even at the single-spine level. For example, LTP induction at a single spine with glutamate uncaging lowers the threshold for LTP induction at surrounding spines (Harvey and Svoboda, 2007; Harvey et al., 2008; Figure 1E). The reduction in the threshold for LTP induction, or "cross-talk" of synaptic plasticity, lasts ~10 min and spreads over ~10 μm along the dendritic shaft. In addition, the induction of protein synthesis-dependent LTP induced by glutamate uncaging combined with a bath application of forskolin can reduce the threshold for LTP induction at surrounding spines (Govindarajan et al., 2011). This heterosynaptic facilitation occurs within ~70 μm of the stimulated spine and lasts ~90 min. Moreover, in young neurons, repetitive glutamate uncaging at single spines reduces the induction threshold for glutamate-induced spinogenesis in the surrounding area for at least a few minutes (Kwon and Sabatini, 2011). In addition to the facilitation of LTP on surrounding synapses, it has been known that LTP induction in one set of synapses causes LTD in the other set of synapses on the same cell (Abraham et al., 1994; Doyère et al., 1997). Similar to this so-called "heterosynaptic LTD," it has been revealed recently that the LTP induction of multiple spines on a single dendritic segment can cause spine shrinkage and synaptic weakening of nearby unstimulated spines located within a few micrometers (Figure 1C; Oh et al., 2015). These results strongly suggest that intracellular signaling factors can spread from the stimulated spines and have a large impact on the surrounding dendritic spines.

Biochemical Computation in Dendritic Branches for Structural Plasticity

In the past decades, signaling pathways leading to LTP and LTD have been studied extensively with pharmacological, genetic, and biochemical tools. These studies have revealed that Ca^{2+} influx through synaptic NMDARs triggers a variety of signaling pathways, which, in turn, induces long-lasting changes in postsynaptic sensitivity to glutamate and/or the probability of glutamate release from presynaptic terminals (Bliss and Collingridge, 2013; Bredt and Nicoll, 2003; Enoki et al., 2009; Huganir and Nicoll, 2013; Zakharenko et al., 2001). Imaging techniques based on Förster resonance energy transfer (FRET) enable the measurement of spatiotemporal dynamics of biochemical signaling activity in living cells. However, these techniques have been difficult to implement because of small fluorescence from the tiny volume of spines and strong light scattering by brain tissue. The development of two-photon fluorescence lifetime imaging microscopy (2pFLIM) in combination with highly optimized FRET-based biosensors has overcome these limitations and allowed researchers to directly monitor biochemical signal transduction at single-spine resolution (Yasuda, 2006, 2012; Yasuda et al., 2006). Using this and other imaging techniques, the detailed

spatiotemporal dynamics of signal transduction during synaptic plasticity have been revealed.

Calcium Sensing

Synaptic stimulation produces a short Ca^{2+} transient largely restricted to the stimulated spines (Mainen et al., 1999; Noguchi et al., 2005; Sobczyk and Svoboda, 2007; Yuste and Denk, 1995). The Ca^{2+} transient lasts only ~0.1 s and, when repeated, initiates biochemical signal transduction crucial for LTP and LTD (Figure 2). When Ca^{2+} flows into the spine through NMDARs, it binds to calmodulin (CaM) and activates CaMKII. It has been well established that this Ca^{2+}-CaM-CaMKII signaling cascade is the first reaction necessary for LTP induction (Lisman et al., 2012; Figure 2A). The kinetics of CaMKII activation during structural LTP were determined by imaging of CaMKII activities using 2pFLIM in combination with a FRET-based CaMKII sensor (Lee et al., 2009; Takao et al., 2005). It has been revealed that the induction of LTP with glutamate uncaging in CA1 pyramidal neurons triggers rapid CaMKII activation restricted to the stimulated spine. This activity decays with a time constant of ~10 s (Figure 2B). These results suggest that CaMKII serves as a relay to extend the short Ca^{2+} transient at a timescale of milliseconds to the signal at a timescale of seconds (Figure 2). Therefore, downstream signaling molecules are required to further extend signals for the persistence of LTP over the course of minutes or hours.

In addition to CaMKII, calcineurin (CaN), a calcium-dependent phosphatase, has been found to be activated during spine enlargement in dissociated neurons (Fujii et al., 2013). In this study, the authors developed a dual FRET system and simultaneously imaged activities of CaMKII and CaN in response to glutamate uncaging at single spines. They reported that strong uncaging stimuli can activate both CaMKII and CaN with similar temporal dynamics with an activation time window of 1 min (Figure 2A). However, the spatial profiles of their activations are distinct. Although CaMKII activation is compartmentalized within the stimulated spines, CaN activity spreads over several micrometers and invades adjacent spines (Figure 2B). When stimulation is weak, only CaN is activated, and the activation is restricted to the stimulated spines. The spreading of CaN from stimulated spines may be important for heterosynaptic LTD because this form of LTD depends on CaN (Oh et al., 2015).

Regulation of the Actin Cytoskeleton

Actin filaments constitute the major cytoskeleton of dendritic spines and, therefore, are an important determinant of spine morphology. Actin monomers in the spine cytoskeleton undergo continuous and rapid turnover because of their dynamic cycles between monomeric G-actin and filamentous F-actin, called treadmilling (Chazeau et al., 2014; Frost et al., 2010; Honkura et al., 2008; Star et al., 2002). Because the equilibration is more shifted toward F-actin at one end (barbed end) than at the other end (pointed end), each actin monomer undergoes a cycle of binding to the barbed end, moving toward the pointed end, and unbinding at the pointed end. Therefore, the flow of actin monomers caused by treadmilling indicates the direction of the filaments. The dynamics of treadmilling within a spine have been observed using photoactivation of PA-GFP tagged actin, and these studies revealed a retrograde flow of actin monomers from the tip to the base of spines (Frost et al., 2010; Honkura et al., 2008). However, recent superresolution imaging studies based on single-particle tracking combined with photoactivated localization microscopy (PALM) have demonstrated that the direction of actin flow is highly inhomogeneous and unoriented in spine heads (Chazeau et al., 2014; Frost et al., 2010). In contrast, the flow is more oriented, directed toward the dendritic shaft in the spine neck (Frost et al., 2010). The unoriented flow of actin is consistent with the relatively unorganized structure of the actin cytoskeleton in spines observed in electron microscopy (Korobova and Svitkina, 2010).

Because functional and structural LTP require actin reorganization (Kim and Lisman, 1999; Krucker et al., 2000; Lang et al., 2004; Matsuzaki et al., 2004; Okamoto et al., 2004), signaling pathways associated with actin polymerization and depolymerization have been studied intensively. Among them, small GTPases, including Ras, Rho, Cdc42, and Rac, and their downstream molecules are known to play critical roles in actin reorganization, spine morphogenesis, and LTP. 2pFLIM imaging of small GTPase activities, including H-Ras, Cdc42, and RhoA, have shown that the induction of LTP at single spines similarly activates these small GTPases within ~1 min of LTP induction (Harvey et al., 2008; Murakoshi et al., 2011; Oliveira and Yasuda, 2014). However, interestingly, their activation profiles are very different. The activities for Cdc42 and RhoA, but not H-Ras, are sustained for more than 30 min. Notably, Cdc42 activity is restricted to the stimulated spine, whereas H-Ras and RhoA activities are not compartmentalized and spread over ~5–10 μm of the dendrite and invade nearby spines (Figure 2B). Inhibition of CaMKII using KN62 or autocamtide CaMKII inhibitor peptide (AIP2) inhibited the activations of H-Ras, Cdc42, and RhoA, indicating that these molecules are downstream of CaMKII (Harvey et al., 2008; Murakoshi et al., 2011). Furthermore, pharmacological inhibition of p21-activated kinase (PAK) and Rho kinase (ROCK), which are the effectors for Cdc42 and RhoA, respectively, inhibited structural LTP (Murakoshi et al., 2011). Therefore, the Ca^{2+}-CaMKII-Cdc42 pathway constitutes spine-specific signal transduction, spanning a timescale of milliseconds to more than half an hour to cause synapse-specific plasticity (Figure 2A).

Activation of small GTPases is known to lead to the activation of actin binding proteins, including cofilin and Arp2/3 (Figure 3). A recent study has shown that cofilin is accumulated rapidly and persistently at the stimulated spine after LTP induction with two-photon glutamate uncaging (Bosch et al., 2014). Imaging the cofilin-actin and cofilin-cofilin interactions with 2pFLIM showed sustained increases of these interactions in the stimulated spines (Figure 2B). These results suggest that LTP induces the formation of a stable actin-cofilin complex restricted to the potentiated spine. Pharmacological analysis suggests that cofilin activation requires several kinases, including LIM kinase (LIMK), PAK, and ROCK (Figure 3). Therefore, overall, cofilin seems to be one of the most important factors that link small GTPase signaling and structural LTP. Interestingly, cofilin also plays an important role in AMPAR trafficking (Gu et al., 2010), further supporting the important role of cofilin in LTP and spine enlargement. In addition to cofilin, Arp2/3 is highly enriched in dendritic spines and generates de novo actin filaments of a branched architecture found in the spine head (Korobova and Svitkina, 2010; Racz and Weinberg, 2008). Arp2/3 is activated

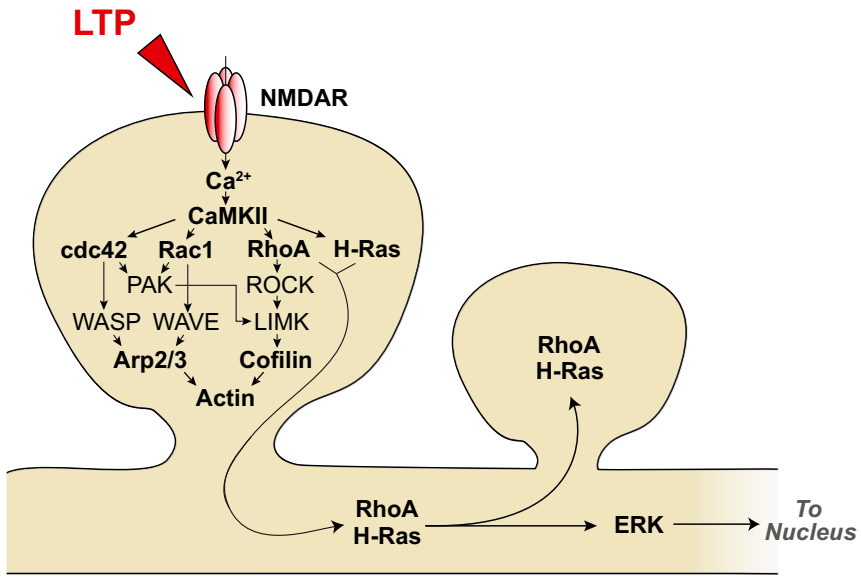

Figure 3. Schematic Diagram of Signaling Pathways Associated with Structural LTP

by downstream molecules of Rac and Cdc42. Loss of Arp2/3 completely blocks structural LTP but not LTD (Kim et al., 2013). Therefore, both cofilin and Arp2/3 seem to converge downstream of small GTPases to regulate structural LTP via actin remodeling (Figure 3).

Mechanisms and Roles of Signal Spreading

The fact that biochemical signals can spread from stimulated spines to their parent dendritic shafts is not surprising because diffusion is extremely efficient at a micrometer-length scale. For cytosolic and membrane-bound proteins, it takes only ~0.3 and ~5 s to diffuse out of the spine, respectively (Bloodgood and Sabatini, 2005; Harvey et al., 2008; Murakoshi et al., 2011). For diffusible transmembrane proteins like AMPAR, it takes ~30 s (Borgdorff and Choquet, 2002; Patterson et al., 2010). On the other hand, the sustained compartmentalization of signaling in spines requires specific mechanisms. For example, Cdc42 is as mobile as H-Ras and RhoA, but only Cdc42 activation is highly restricted in the stimulated spine (Murakoshi et al., 2011). One possible mechanism is to rapidly inactivate the molecules before their spreading (Yasuda and Murakoshi, 2011).

Spreading signals likely play important roles in many forms of heterosynaptic plasticity, such as the facilitation of LTP and heterosynaptic depression in the surrounding area (see above). In this respect, it is interesting that many subcellular compartments required for synaptic plasticity exist outside of the spine. For example, recycling endosomes containing AMPARs are often found in dendritic shafts and translocated into the spines during LTP (Park et al., 2006). Also, the protein synthesis machinery is located in the dendritic shaft (Buxbaum et al., 2014; Ostroff et al., 2002; Steward and Levy, 1982). This arrangement seems to be optimized for signal spreading over several micrometers. The spread of signaling may also contribute to the induction of plasticity in a clustered fashion and create a local accumulation of synaptic inputs that, in turn, results in the functional compartmentalization of dendritic segments (Branco and Häusser, 2010; Govindarajan et al., 2006; Larkum and Nevian, 2008). Clustered plasticity has been found in several paradigms in vivo. For example, sensory deprivation by whisker trimming can induce the accumulation of super-ecliptic pHluorin (SEP)-tagged GluA1 in spines located within a short stretch (~10 μm) of dendritic branches of layer 2/3 pyramidal neurons in the somatosensory cortex (Makino and Malinow, 2011). Similarly, acute whisker stimulation leads to an increase in the intensity of SEP-GluA1 in spines and adjacent dendritic shafts in a subset of dendrites of layer 2/3 pyramidal neurons in the somatosensory cortex (Zhang et al., 2015). Furthermore, motor learning-dependent spinogenesis in layer 5 pyramidal neurons in the motor cortex appears to be clustered in dendritic branches and shows a spatial correlation over ~1 μm (Fu et al., 2012). In addition, spontaneous activities of adjacent spines are frequently synchronized in CA3 pyramidal neurons in organotypic hippocampal slice cultures (Kleindienst et al., 2011; Takahashi et al., 2012). Further studies will be required to reveal whether heterosynaptic plasticity and synaptic crosstalk are associated with clustered plasticity and input synchronization.

New Protein Synthesis in Dendrites

It is known that local translation of mRNAs in dendrites plays an important role in maintaining L-LTP and L-LTD (Costa-Mattioli et al., 2009; Huber et al., 2000; Kang and Schuman, 1996; Sutton and Schuman, 2006). Because of the significance of protein synthesis in the maintenance of synaptic plasticity, several sensors for the visualization of newly synthesized proteins have been developed. For example, newly synthesized proteins can be imaged using destabilized GFP (dGFP) regulated by the UTR of the mRNA encoding a target protein (Aakalu et al., 2001). Because the lifetime of dGFP is short (~2 h), only newly synthesized proteins are visible. However, this method cannot be used for fused proteins because the stability of dGFP may be changed by the fusion. Theoretically, fluorescence recovery after photoconversion of photoconvertible fluorescent proteins or FRAP of fluorescent proteins fused with a target protein should report newly synthesized proteins. However, it appears that these procedures cause significant phototoxcity when applied over entire neurons (Lin et al., 2008). These limitations were overcome by the development of an engineered protein tag named time-specific tagging for the age measurement of proteins (TimeSTAMP). TimeSTAMP encodes the protease flanked by two cleavage sites, an epitope tag, and a protein of interest (Lin et al., 2008). The epitope tag is removed from the proteins of interest by the protease immediately after translation. The application of a specific inhibitor of the protease initiates the accumulation of the newly synthesized, epitope-tagged protein, which can be detected with subsequent immunostaining. In a more recent

version of TimeSTAMP, a protein of interest is fused with split yellow fluorescent protein (YFP) with the protease and its recognition sites (Butko et al., 2012). The protease cleavage separates the split YFP domains before the formation of the YFP chromophore. The application of the protease inhibitor allows chromophore formation, enabling highly sensitive, low-background imaging of the newly synthesized protein fused with YFP in living neurons. The PSD-95 coding sequence, including the 3′ UTR fused with fluorescent TimeSTAMP, revealed that PSD-95 is, indeed, newly synthesized in response to local dendritic stimulation of BDNF and mGluR5 and localized preferentially to stimulated synapses (Butko et al., 2012).

Although the functional significance of dendritic translation is evident, how local translation is regulated by synaptic activity has remained elusive. A recent study revealed a new mechanism that regulates dendritic translation by visualizing single endogenous β-actin mRNA molecules with single-molecule fluorescence in situ hybridization (FISH) (Buxbaum et al., 2014). It has been shown that ~50% of dendritic β-actin mRNA molecules are masked by forming complexes with RNA granules containing densely packed ribosomes. β-actin mRNA and rRNA in these complexes are inaccessible by the FISH probes and, presumably, inactive for translation. Chemically induced LTP increased the number of β-actin mRNA and rRNA molecules that can be probed with FISH, suggesting that mRNA unmasking occurred in dendrites. The same stimulation also increased the mobility of ribosomes and β-actin mRNA molecules in dendrites, indicating that mRNA and ribosomes are released from the complex. These results suggest that RNA granules containing mRNAs and ribosomes exist in a suppressed state along the dendrites and that LTP induction could prompt the disassembly of the complexes, releasing mRNA and ribosomes to induce local translation in dendrites.

Biochemical Computation between the Spine and Nucleus for Structural Plasticity

Several forms of LTP and memory that last longer than several hours require gene transcription as well as translation (Bliss and Collingridge, 1993; Costa-Mattioli et al., 2009; Cracco et al., 2005; Kelleher et al., 2004; Sutton and Schuman, 2006). Gene transcription is regulated by various protein kinase cascades, including the CaMKK-CaMKIV, Ras-Raf-MEK-ERK, and cAMP-PKA pathways. Activation of these kinases leads to the phosphorylation of transcription factors such as cAMP-responsive element-binding (CREB) and Elk-1 to produce new mRNAs required for L-LTP (Alberini, 2009). Conversely, CaN is also activated by neuronal activity and dephosphorylates and activates the transcription factor myocyte enhancer factor 2 (MEF2). This activates MEF2-dependent transcription, leading to the elimination of excitatory synapses (Barbosa et al., 2008; Cole et al., 2012; Flavell et al., 2006; Pulipparacharuvil et al., 2008). Therefore, activity-dependent transcription can regulate the persistence of synaptic plasticity as well as the structural refinement of synaptic connections. However, little is known about the mechanisms of long-distance signaling from the synapse to the nucleus and from the nucleus back to the synapse. To couple synaptic activities with changes in gene expression, there must be some mechanism that links local synaptic events in individual spines and signals to the nucleus. This may be mediated by somatic membrane depolarization caused by the activation of a population of synapses (Adams and Dudek, 2005) or the propagation of regenerative Ca^{2+} waves from the stimulated synapses to the nucleus mediated by the ER (Ch'ng and Martin, 2011). Additionally, recent studies have demonstrated that the signaling between the synapse and nucleus can be mediated by biochemical cascades.

Signal Spreading from Single Spines to the Nucleus

In response to single-spine stimulation, signaling mediated by RhoA, CaN, and H-Ras spreads over ~5–10 μm (Fujii et al., 2013; Harvey et al., 2008; Murakoshi et al., 2011; Figure 2B). The spreading of biochemical signaling may be extended further to much longer distances to activate signaling in the nucleus. This possibility has been explored recently using 2pFLIM and a FRET sensor for ERK activity (Zhai et al., 2013). Because ERK is a downstream effector molecule of H-Ras, the diffusion of H-Ras could cause long-distance spreading of ERK activity. It has been demonstrated that the induction of LTP at only a few (three to seven) spines is sufficient to activate ERK in the nucleus (Figure 2B). Furthermore, immunostaining showed that downstream transcription factors, including CREB and Elk1, are also activated in response to stimulation of a few spines in an ERK-dependent manner. These results suggest that the activation of a small number of spines has a profound impact on the activation of nuclear signaling that regulates gene transcription. The onset of nuclear ERK activation is 5–30 min after stimulation and shows a greater delay when distal dendrites are stimulated. The delay is consistent with the diffusion of cytosolic proteins from the spine to the nucleus, suggesting that the diffusion of ERK may be an important factor for the process. The signal can be integrated over surprisingly long times (more than 30 min) and space (~80 μm). Furthermore, the spatially dispersed inputs over multiple branches activated nuclear ERK much more efficiently than clustered inputs over one branch. The preference of sparse inputs over multiple dendrites appears to be caused by saturation of ERK activation in response to the stimulation of a few dendritic spines on a branch. In this situation, stimulating more than a few spines in one branch will not increase signaling to the nucleus. Instead, the number of stimulated branches is critical for increasing signals in the nucleus. Therefore, the dendritic branch seems to act as a biochemical computation unit, and supersensitive integration in each branch plays an important role in controlling synapse-to-nucleus signaling.

In addition to signal spreading via diffusion, energy-dependent transport via motor proteins seems to play important roles in synapse-to-nucleus signaling. One proposed mechanism is the transmission of signals via molecular messengers that are dissociated from the stimulated synapse and delivered to the nucleus. Interestingly, synapses contain various proteins with a nuclear localization signal (NLS) that are localized both in synapses and the nucleus (Jordan and Kreutz, 2009). Importin α is one of these proteins, and it functions as an adaptor that binds an NLS-containing cargo and forms a heterotrimeric complex with importin β1 to facilitate the transport of this complex into the nucleus following LTP-inducing stimuli (Goldfarb et al., 2004; Jeffrey et al., 2009; Thompson et al., 2004). Importantly, several

transcriptional regulators, including CREB, necrosis factor κB (NF-κB), and Jacob, have been shown to be translocated into the nucleus in response to synaptic activities via the importin-dependent pathway (Jordan and Kreutz, 2009; Karpova et al., 2013). For example, Jacob has been found to be a synapto-nuclear messenger containing an NLS. Following synaptic but not extrasynaptic NMDAR activation, Jacob is phosphorylated by ERK, which causes the dissociation of Jacob from spines, leading to its translocation into the nucleus in an importin α-dependent manner. The presence of phosphorylated Jacob in the nucleus increases CREB phosphorylation, inducing the expression of CRE-dependent genes (Karpova et al., 2013). Furthermore, CREB-regulated transcriptional coactivator 1 (CRTC1) is also translocated from the synapses to the nucleus and binds to CREB to upregulate CRE-dependent transcription (Kovács et al., 2007; Zhou et al., 2006). CRTC1 nuclear translocation requires the Ca^{2+}-CaN pathway, and the persistent accumulation of CRTC1 in the nucleus requires the cAMP pathway (Ch'ng et al., 2012; Nonaka et al., 2014).

It is likely that neither simple diffusion nor active transport are efficient enough to alter transcription in the nucleus. The volume of the nucleus is several thousand times bigger than that of a single spine, and, therefore, the impact of each spine should be very small. Because only a few spines can drive nuclear signal activation, there must be some mechanisms to amplify signal by orders of magnitude. A signaling cascade with multiple steps (for example, the classical Ras-Raf-MEK-ERK pathway) may be able to dramatically amplify signaling. A more robust mechanism would be regenerative signal amplification by positive feedback. For example, a computational model predicts that the duration of LTD in cerebellar Purkinje cells is prolonged by a positive feedback loop consisting of protein kinase C (PKC), mitogen-activated protein kinase (MAPK), and phospholipase A2 (PLA2) (Kuroda et al., 2001). Later it was shown that the reciprocal activations of PKC to MAPK and MAPK to PKC are required for cerebellar LTD and that this positive feedback loop causes PKC to be active more than 20 min (Tanaka and Augustine, 2008). This kind of mechanism could also be used for amplifying the signal at single spines to effect gene transcription in the nucleus.

Biochemical Signaling from the Nucleus Back to the Spine

Given that L-LTP is specific to stimulated spines, the newly transcribed and synthesized proteins must function specifically in the activated spines. Therefore, there must be specific interactions between the newly synthesized proteins and the activated spines during L-LTP. This can be explained by the synaptic tag-and-capture hypothesis. In this mechanism, LTP induction generates a protein "tag" (or a state of molecules) only at potentiated synapses, which can capture newly synthesized plasticity-related protein/products (PRPs) specifically induced by L-LTP (Frey and Morris, 1997; Redondo and Morris, 2011). Although the molecular identity is largely unknown, this hypothesis has provided a framework to account for the protein synthesis-dependent synaptic plasticity. PRPs that have been implicated in synaptic plasticity include Homer1a, Arc, and GluA1 (Redondo and Morris, 2011). Among these proteins, Homer1a, a postsynaptic scaffolding protein and a major immediate early gene, has been shown to be specifically recruited from the soma to the stimulated spine with synaptic activities, supporting the synaptic tag hypothesis (Okada et al., 2009). The synaptic tag can be a temporary state of the synapse that is represented by multiple proteins and their interactions, like the structure of the actin cytoskeleton (Redondo and Morris, 2011). For example, it is known that LTP induction causes the formation of a stable pool of F-actin (Honkura et al., 2008; Okamoto et al., 2004) that potentially exists as cofilin-actin co-helices (Bosch et al., 2014). This newly formed pool of F-actin can act as a synaptic tag (Okamoto et al., 2004, 2009; Ramachandran and Frey, 2009). Interestingly, synaptic tagging appears to occur not only at stimulated spines but also at non-stimulated spines. Following LTP induction, Arc, an immediate early gene necessary for spatial learning and fear memory (Guzowski et al., 2000; Plath et al., 2006; Ploski et al., 2008), is accumulated in non-stimulated spines and excluded from potentiated spines (Okuno et al., 2012). The amount of synaptic Arc was correlated negatively with the amount of surface GluA1 in synapses, consistent with previous studies suggesting that Arc weakens synapses by promoting endocytosis of AMPARs (Chowdhury et al., 2006). Therefore, inverse synaptic tagging by Arc may help to maintain the contrast of synaptic weight changes between active and inactive synapses during L-LTP by removing surface AMPARs from non-stimulated spines.

Concluding Remarks

We have described the mechanisms and roles of spatiotemporal regulation of biochemical signaling in neurons during spine structural plasticity. Recent advances in optical techniques have revealed new mechanisms of biochemical computation that underlie various forms of synaptic plasticity. Two-photon uncaging of neurotransmitters has enabled researchers to study the spatiotemporal regulation of homo-and heterosynaptic plasticity and synaptic crosstalk at the level of single spines (Figure 1). Imaging of signal transduction with FRET/FLIM techniques has allowed the spatiotemporal pattern of biochemical signaling initiated at single spines to be accessed directly. These studies have collectively provided many insights into the dynamic regulation of biochemical signaling in neuronal compartments during structural plasticity. In the temporal axis, it has been found that signaling is transmitted in multiple stages during structural LTP (Figures 2 and 3). First, a short Ca^{2+} signal (~0.1 s) is integrated by CaMKII activation over seconds to ~1 min. Second, the transient CaMKII signal is further relayed to several small GTPases and their downstream kinases, which leads to actin remodeling over the course of minutes to hours. Finally, PSDs and presynaptic structures are reorganized over hours. On the spatial axis, it has been revealed that biochemical computation occurs in multiple-length scales from a single spine to a short stretch of dendrite around the spine and a whole dendritic branch (Figures 2 and 3). Biochemical signaling can spread further into the nucleus and regulate gene transcription.

To understand the more complicated aspects of signal transduction, including positive and negative feedforward and feedback loops, it is necessary to manipulate signals with high spatiotemporal resolution while imaging signal transduction. In this area, various tools to regulate protein activities with light have been developed (Kennedy et al., 2010; Lee et al.,

2014; Levskaya et al., 2009; Tyszkiewicz and Muir, 2008; Wu et al., 2009; Yazawa et al., 2009; Zhou et al., 2012). By combining FRET/FLIM imaging with the optical manipulation of protein activities, the mechanisms underlying spatiotemporal signal regulation in neurons may be clarified. Another future challenge will be to find out how the operating principles of signal transduction during synaptic plasticity in vitro can be applied to learning and memory of animals in vivo. Imaging of spine structural plasticity during learning and memory in vivo has been performed by several groups (Holtmaat et al., 2006; Lai et al., 2012; Moczulska et al., 2013; Xu et al., 2009; Yang et al., 2009). Applying FRET-FLIM imaging in vivo will allow us to link findings based on controlled stimulation in slices with molecular mechanisms of learning and memory. Continued development of optical techniques will help to elucidate the operating principles of biochemical computation mediated by complicated signaling networks in neurons.

ACKNOWLEDGMENTS

We thank the member of the R.Y. lab for discussions; T. Yasuda, S. Soderling, and Y. Hayashi for critical reading; and H. Bito and Y. Hayashi for Figure 2. This study was supported by the Japan Society for the Promotion of Science (to J.N.) and the National Institute of Health (R01MH080047, R01NS068410).

REFERENCES

Aakalu, G., Smith, W.B., Nguyen, N., Jiang, C., and Schuman, E.M. (2001). Dynamic visualization of local protein synthesis in hippocampal neurons. Neuron *30*, 489–502.

Abraham, W.C. (2008). Metaplasticity: tuning synapses and networks for plasticity. Nat. Rev. Neurosci. *9*, 387.

Abraham, W.C., Christie, B.R., Logan, B., Lawlor, P., and Dragunow, M. (1994). Immediate early gene expression associated with the persistence of heterosynaptic long-term depression in the hippocampus. Proc. Natl. Acad. Sci. USA *91*, 10049–10053.

Adams, J.P., and Dudek, S.M. (2005). Late-phase long-term potentiation: getting to the nucleus. Nat. Rev. Neurosci. *6*, 737–743.

Alberini, C.M. (2009). Transcription factors in long-term memory and synaptic plasticity. Physiol. Rev. *89*, 121–145.

Araki, Y., Zeng, M., Zhang, M., and Huganir, R.L. (2015). Rapid dispersion of SynGAP from synaptic spines triggers AMPA receptor insertion and spine enlargement during LTP. Neuron *85*, 173–189.

Araya, R., Jiang, J., Eisenthal, K.B., and Yuste, R. (2006). The spine neck filters membrane potentials. Proc. Natl. Acad. Sci. USA *103*, 17961–17966.

Araya, R., Vogels, T.P., and Yuste, R. (2014). Activity-dependent dendritic spine neck changes are correlated with synaptic strength. Proc. Natl. Acad. Sci. USA *111*, E2895–E2904.

Barbosa, A.C., Kim, M.S., Ertunc, M., Adachi, M., Nelson, E.D., McAnally, J., Richardson, J.A., Kavalali, E.T., Monteggia, L.M., Bassel-Duby, R., and Olson, E.N. (2008). MEF2C, a transcription factor that facilitates learning and memory by negative regulation of synapse numbers and function. Proc. Natl. Acad. Sci. USA *105*, 9391–9396.

Barral, Y., Mermall, V., Mooseker, M.S., and Snyder, M. (2000). Compartmentalization of the cell cortex by septins is required for maintenance of cell polarity in yeast. Mol. Cell *5*, 841–851.

Bastrikova, N., Gardner, G.A., Reece, J.M., Jeromin, A., and Dudek, S.M. (2008). Synapse elimination accompanies functional plasticity in hippocampal neurons. Proc. Natl. Acad. Sci. USA *105*, 3123–3127.

Bliss, T.V., and Collingridge, G.L. (1993). A synaptic model of memory: long-term potentiation in the hippocampus. Nature *361*, 31–39.

Bliss, T.V., and Collingridge, G.L. (2013). Expression of NMDA receptor-dependent LTP in the hippocampus: bridging the divide. Mol. Brain *6*, 5.

Bloodgood, B.L., and Sabatini, B.L. (2005). Neuronal activity regulates diffusion across the neck of dendritic spines. Science *310*, 866–869.

Bloodgood, B.L., Giessel, A.J., and Sabatini, B.L. (2009). Biphasic synaptic Ca influx arising from compartmentalized electrical signals in dendritic spines. PLoS Biol. *7*, e1000190.

Borgdorff, A.J., and Choquet, D. (2002). Regulation of AMPA receptor lateral movements. Nature *417*, 649–653.

Bosch, M., Castro, J., Saneyoshi, T., Matsuno, H., Sur, M., and Hayashi, Y. (2014). Structural and molecular remodeling of dendritic spine substructures during long-term potentiation. Neuron *82*, 444–459.

Branco, T., and Häusser, M. (2010). The single dendritic branch as a fundamental functional unit in the nervous system. Curr. Opin. Neurobiol. *20*, 494–502.

Bredt, D.S., and Nicoll, R.A. (2003). AMPA receptor trafficking at excitatory synapses. Neuron *40*, 361–379.

Butko, M.T., Yang, J., Geng, Y., Kim, H.J., Jeon, N.L., Shu, X., Mackey, M.R., Ellisman, M.H., Tsien, R.Y., and Lin, M.Z. (2012). Fluorescent and photooxidizing TimeSTAMP tags track protein fates in light and electron microscopy. Nat. Neurosci. *15*, 1742–1751.

Buxbaum, A.R., Wu, B., and Singer, R.H. (2014). Single β-actin mRNA detection in neurons reveals a mechanism for regulating its translatability. Science *343*, 419–422.

Bywalez, W.G., Patirniche, D., Rupprecht, V., Stemmler, M., Herz, A.V., Pálfi, D., Rózsa, B., and Egger, V. (2015). Local postsynaptic voltage-gated sodium channel activation in dendritic spines of olfactory bulb granule cells. Neuron *85*, 590–601.

Ch'ng, T.H., and Martin, K.C. (2011). Synapse-to-nucleus signaling. Curr. Opin. Neurobiol. *21*, 345–352.

Ch'ng, T.H., Uzgil, B., Lin, P., Avliyakulov, N.K., O'Dell, T.J., and Martin, K.C. (2012). Activity-dependent transport of the transcriptional coactivator CRTC1 from synapse to nucleus. Cell *150*, 207–221.

Chazeau, A., Mehidi, A., Nair, D., Gautier, J.J., Leduc, C., Chamma, I., Kage, F., Kechkar, A., Thoumine, O., Rottner, K., et al. (2014). Nanoscale segregation of actin nucleation and elongation factors determines dendritic spine protrusion. EMBO J. *33*, 2745–2764.

Cheng, D., Hoogenraad, C.C., Rush, J., Ramm, E., Schlager, M.A., Duong, D.M., Xu, P., Wijayawardana, S.R., Hanfelt, J., Nakagawa, T., et al. (2006). Relative and absolute quantification of postsynaptic density proteome isolated from rat forebrain and cerebellum. Mol. Cell. Proteomics *5*, 1158–1170.

Chowdhury, S., Shepherd, J.D., Okuno, H., Lyford, G., Petralia, R.S., Plath, N., Kuhl, D., Huganir, R.L., and Worley, P.F. (2006). Arc/Arg3.1 interacts with the endocytic machinery to regulate AMPA receptor trafficking. Neuron *52*, 445–459.

Cole, C.J., Mercaldo, V., Restivo, L., Yiu, A.P., Sekeres, M.J., Han, J.H., Vetere, G., Pekar, T., Ross, P.J., Neve, R.L., et al. (2012). MEF2 negatively regulates learning-induced structural plasticity and memory formation. Nat. Neurosci. *15*, 1255–1264.

Costa-Mattioli, M., Sossin, W.S., Klann, E., and Sonenberg, N. (2009). Translational control of long-lasting synaptic plasticity and memory. Neuron *61*, 10–26.

Cracco, J.B., Serrano, P., Moskowitz, S.I., Bergold, P.J., and Sacktor, T.C. (2005). Protein synthesis-dependent LTP in isolated dendrites of CA1 pyramidal cells. Hippocampus *15*, 551–556.

Desmond, N.L., and Levy, W.B. (1983). Synaptic correlates of associative potentiation/depression: an ultrastructural study in the hippocampus. Brain Res. *265*, 21–30.

Doyère, V., Srebro, B., and Laroche, S. (1997). Heterosynaptic LTD and depotentiation in the medial perforant path of the dentate gyrus in the freely moving rat. J. Neurophysiol. *77*, 571–578.

Engert, F., and Bonhoeffer, T. (1999). Dendritic spine changes associated with hippocampal long-term synaptic plasticity. Nature *399*, 66–70.

Enoki, R., Hu, Y.L., Hamilton, D., and Fine, A. (2009). Expression of long-term plasticity at individual synapses in hippocampus is graded, bidirectional, and mainly presynaptic: optical quantal analysis. Neuron *62*, 242–253.

Ewers, H., Tada, T., Petersen, J.D., Racz, B., Sheng, M., and Choquet, D. (2014). A Septin-Dependent Diffusion Barrier at Dendritic Spine Necks. PLoS ONE *9*, e113916.

Flavell, S.W., Cowan, C.W., Kim, T.K., Greer, P.L., Lin, Y., Paradis, S., Griffith, E.C., Hu, L.S., Chen, C., and Greenberg, M.E. (2006). Activity-dependent regulation of MEF2 transcription factors suppresses excitatory synapse number. Science *311*, 1008–1012.

Frey, U., and Morris, R.G. (1997). Synaptic tagging and long-term potentiation. Nature *385*, 533–536.

Frost, N.A., Shroff, H., Kong, H., Betzig, E., and Blanpied, T.A. (2010). Single-molecule discrimination of discrete perisynaptic and distributed sites of actin filament assembly within dendritic spines. Neuron *67*, 86–99.

Fu, M., Yu, X., Lu, J., and Zuo, Y. (2012). Repetitive motor learning induces coordinated formation of clustered dendritic spines in vivo. Nature *483*, 92–95.

Fujii, H., Inoue, M., Okuno, H., Sano, Y., Takemoto-Kimura, S., Kitamura, K., Kano, M., and Bito, H. (2013). Nonlinear decoding and asymmetric representation of neuronal input information by CaMKIIα and calcineurin. Cell Rep. *3*, 978–987.

Goldfarb, D.S., Corbett, A.H., Mason, D.A., Harreman, M.T., and Adam, S.A. (2004). Importin alpha: a multipurpose nuclear-transport receptor. Trends Cell Biol. *14*, 505–514.

Govindarajan, A., Kelleher, R.J., and Tonegawa, S. (2006). A clustered plasticity model of long-term memory engrams. Nat. Rev. Neurosci. *7*, 575–583.

Govindarajan, A., Israely, I., Huang, S.Y., and Tonegawa, S. (2011). The dendritic branch is the preferred integrative unit for protein synthesis-dependent LTP. Neuron *69*, 132–146.

Grunditz, A., Holbro, N., Tian, L., Zuo, Y., and Oertner, T.G. (2008). Spine neck plasticity controls postsynaptic calcium signals through electrical compartmentalization. J. Neurosci. *28*, 13457–13466.

Gu, J., Lee, C.W., Fan, Y., Komlos, D., Tang, X., Sun, C., Yu, K., Hartzell, H.C., Chen, G., Bamburg, J.R., and Zheng, J.Q. (2010). ADF/cofilin-mediated actin dynamics regulate AMPA receptor trafficking during synaptic plasticity. Nat. Neurosci. *13*, 1208–1215.

Guzowski, J.F., Lyford, G.L., Stevenson, G.D., Houston, F.P., McGaugh, J.L., Worley, P.F., and Barnes, C.A. (2000). Inhibition of activity-dependent arc protein expression in the rat hippocampus impairs the maintenance of long-term potentiation and the consolidation of long-term memory. J. Neurosci. *20*, 3993–4001.

Harnett, M.T., Makara, J.K., Spruston, N., Kath, W.L., and Magee, J.C. (2012). Synaptic amplification by dendritic spines enhances input cooperativity. Nature *491*, 599–602.

Harris, K.M., and Stevens, J.K. (1989). Dendritic spines of CA 1 pyramidal cells in the rat hippocampus: serial electron microscopy with reference to their biophysical characteristics. J. Neurosci. *9*, 2982–2997.

Harvey, C.D., and Svoboda, K. (2007). Locally dynamic synaptic learning rules in pyramidal neuron dendrites. Nature *450*, 1195–1200.

Harvey, C.D., Yasuda, R., Zhong, H., and Svoboda, K. (2008). The spread of Ras activity triggered by activation of a single dendritic spine. Science *321*, 136–140.

Hayama, T., Noguchi, J., Watanabe, S., Takahashi, N., Hayashi-Takagi, A., Ellis-Davies, G.C., Matsuzaki, M., and Kasai, H. (2013). GABA promotes the competitive selection of dendritic spines by controlling local Ca2+ signaling. Nat. Neurosci. *16*, 1409–1416.

Hill, T.C., and Zito, K. (2013). LTP-induced long-term stabilization of individual nascent dendritic spines. J. Neurosci. *33*, 678–686.

Holtmaat, A., Wilbrecht, L., Knott, G.W., Welker, E., and Svoboda, K. (2006). Experience-dependent and cell-type-specific spine growth in the neocortex. Nature *441*, 979–983.

Honkura, N., Matsuzaki, M., Noguchi, J., Ellis-Davies, G.C., and Kasai, H. (2008). The subspine organization of actin fibers regulates the structure and plasticity of dendritic spines. Neuron *57*, 719–729.

Huber, K.M., Kayser, M.S., and Bear, M.F. (2000). Role for rapid dendritic protein synthesis in hippocampal mGluR-dependent long-term depression. Science *288*, 1254–1257.

Huganir, R.L., and Nicoll, R.A. (2013). AMPARs and synaptic plasticity: the last 25 years. Neuron *80*, 704–717.

Jeffrey, R.A., Ch'ng, T.H., O'Dell, T.J., and Martin, K.C. (2009). Activity-dependent anchoring of importin alpha at the synapse involves regulated binding to the cytoplasmic tail of the NR1-1a subunit of the NMDA receptor. J. Neurosci. *29*, 15613–15620.

Jordan, B.A., and Kreutz, M.R. (2009). Nucleocytoplasmic protein shuttling: the direct route in synapse-to-nucleus signaling. Trends Neurosci. *32*, 392–401.

Kang, H., and Schuman, E.M. (1996). A requirement for local protein synthesis in neurotrophin-induced hippocampal synaptic plasticity. Science *273*, 1402–1406.

Karpova, A., Mikhaylova, M., Bera, S., Bär, J., Reddy, P.P., Behnisch, T., Rankovic, V., Spilker, C., Bethge, P., Sahin, J., et al. (2013). Encoding and transducing the synaptic or extrasynaptic origin of NMDA receptor signals to the nucleus. Cell *152*, 1119–1133.

Kelleher, R.J., 3rd, Govindarajan, A., Jung, H.Y., Kang, H., and Tonegawa, S. (2004). Translational control by MAPK signaling in long-term synaptic plasticity and memory. Cell *116*, 467–479.

Kennedy, M.J., Hughes, R.M., Peteya, L.A., Schwartz, J.W., Ehlers, M.D., and Tucker, C.L. (2010). Rapid blue-light-mediated induction of protein interactions in living cells. Nat. Methods *7*, 973–975.

Kim, C.H., and Lisman, J.E. (1999). A role of actin filament in synaptic transmission and long-term potentiation. J. Neurosci. *19*, 4314–4324.

Kim, I.H., Racz, B., Wang, H., Burianek, L., Weinberg, R., Yasuda, R., Wetsel, W.C., and Soderling, S.H. (2013). Disruption of Arp2/3 results in asymmetric structural plasticity of dendritic spines and progressive synaptic and behavioral abnormalities. J. Neurosci. *33*, 6081–6092.

Kim, I.H., Wang, H., Soderling, S.H., and Yasuda, R. (2014). Loss of Cdc42 leads to defects in synaptic plasticity and remote memory recall. eLife *3*, 3.

Kleindienst, T., Winnubst, J., Roth-Alpermann, C., Bonhoeffer, T., and Lohmann, C. (2011). Activity-dependent clustering of functional synaptic inputs on developing hippocampal dendrites. Neuron *72*, 1012–1024.

Kopec, C.D., Real, E., Kessels, H.W., and Malinow, R. (2007). GluR1 links structural and functional plasticity at excitatory synapses. J. Neurosci. *27*, 13706–13718.

Korobova, F., and Svitkina, T. (2010). Molecular architecture of synaptic actin cytoskeleton in hippocampal neurons reveals a mechanism of dendritic spine morphogenesis. Mol. Biol. Cell *21*, 165–176.

Kovács, K.A., Steullet, P., Steinmann, M., Do, K.Q., Magistretti, P.J., Halfon, O., and Cardinaux, J.R. (2007). TORC1 is a calcium- and cAMP-sensitive coincidence detector involved in hippocampal long-term synaptic plasticity. Proc. Natl. Acad. Sci. USA *104*, 4700–4705.

Kovalchuk, Y., Eilers, J., Lisman, J., and Konnerth, A. (2000). NMDA receptor-mediated subthreshold Ca(2+) signals in spines of hippocampal neurons. J. Neurosci. *20*, 1791–1799.

Krucker, T., Siggins, G.R., and Halpain, S. (2000). Dynamic actin filaments are required for stable long-term potentiation (LTP) in area CA1 of the hippocampus. Proc. Natl. Acad. Sci. USA *97*, 6856–6861.

Kuroda, S., Schweighofer, N., and Kawato, M. (2001). Exploration of signal transduction pathways in cerebellar long-term depression by kinetic simulation. J. Neurosci. *21*, 5693–5702.

Kwon, H.B., and Sabatini, B.L. (2011). Glutamate induces de novo growth of functional spines in developing cortex. Nature *474*, 100–104.

Lai, C.S., Franke, T.F., and Gan, W.B. (2012). Opposite effects of fear conditioning and extinction on dendritic spine remodelling. Nature *483*, 87–91.

Lang, C., Barco, A., Zablow, L., Kandel, E.R., Siegelbaum, S.A., and Zakharenko, S.S. (2004). Transient expansion of synaptically connected dendritic spines upon induction of hippocampal long-term potentiation. Proc. Natl. Acad. Sci. USA *101*, 16665–16670.

Larkum, M.E., and Nevian, T. (2008). Synaptic clustering by dendritic signalling mechanisms. Curr. Opin. Neurobiol. *18*, 321–331.

Lee, S.J., Escobedo-Lozoya, Y., Szatmari, E.M., and Yasuda, R. (2009). Activation of CaMKII in single dendritic spines during long-term potentiation. Nature *458*, 299–304.

Lee, S., Park, H., Kyung, T., Kim, N.Y., Kim, S., Kim, J., and Heo, W.D. (2014). Reversible protein inactivation by optogenetic trapping in cells. Nat. Methods *11*, 633–636.

Levskaya, A., Weiner, O.D., Lim, W.A., and Voigt, C.A. (2009). Spatiotemporal control of cell signalling using a light-switchable protein interaction. Nature *461*, 997–1001.

Lin, M.Z., Glenn, J.S., and Tsien, R.Y. (2008). A drug-controllable tag for visualizing newly synthesized proteins in cells and whole animals. Proc. Natl. Acad. Sci. USA *105*, 7744–7749.

Lisman, J., Yasuda, R., and Raghavachari, S. (2012). Mechanisms of CaMKII action in long-term potentiation. Nat. Rev. Neurosci. *13*, 169–182.

Mainen, Z.F., Malinow, R., and Svoboda, K. (1999). Synaptic calcium transients in single spines indicate that NMDA receptors are not saturated. Nature *399*, 151–155.

Makino, H., and Malinow, R. (2011). Compartmentalized versus global synaptic plasticity on dendrites controlled by experience. Neuron *72*, 1001–1011.

Maletic-Savatic, M., Malinow, R., and Svoboda, K. (1999). Rapid dendritic morphogenesis in CA1 hippocampal dendrites induced by synaptic activity. Science *283*, 1923–1927.

Matsuzaki, M., Ellis-Davies, G.C., Nemoto, T., Miyashita, Y., Iino, M., and Kasai, H. (2001). Dendritic spine geometry is critical for AMPA receptor expression in hippocampal CA1 pyramidal neurons. Nat. Neurosci. *4*, 1086–1092.

Matsuzaki, M., Honkura, N., Ellis-Davies, G.C., and Kasai, H. (2004). Structural basis of long-term potentiation in single dendritic spines. Nature *429*, 761–766.

Meyer, D., Bonhoeffer, T., and Scheuss, V. (2014). Balance and stability of synaptic structures during synaptic plasticity. Neuron *82*, 430–443.

Moczulska, K.E., Tinter-Thiede, J., Peter, M., Ushakova, L., Wernle, T., Bathellier, B., and Rumpel, S. (2013). Dynamics of dendritic spines in the mouse auditory cortex during memory formation and memory recall. Proc. Natl. Acad. Sci. USA *110*, 18315–18320.

Murakoshi, H., Wang, H., and Yasuda, R. (2011). Local, persistent activation of Rho GTPases during plasticity of single dendritic spines. Nature *472*, 100–104.

Nägerl, U.V., Eberhorn, N., Cambridge, S.B., and Bonhoeffer, T. (2004). Bidirectional activity-dependent morphological plasticity in hippocampal neurons. Neuron *44*, 759–767.

Nagerl, U.V., Kostinger, G., Anderson, J.C., Martin, K.A., and Bonhoeffer, T. (2007). Protracted synaptogenesis after activity-dependent spinogenesis in hippocampal neurons. J. Neurosci. *27*, 8149–8156.

Noguchi, J., Matsuzaki, M., Ellis-Davies, G.C., and Kasai, H. (2005). Spine-neck geometry determines NMDA receptor-dependent Ca2+ signaling in dendrites. Neuron *46*, 609–622.

Nonaka, M., Kim, R., Fukushima, H., Sasaki, K., Suzuki, K., Okamura, M., Ishii, Y., Kawashima, T., Kamijo, S., Takemoto-Kimura, S., et al. (2014). Region-specific activation of CRTC1-CREB signaling mediates long-term fear memory. Neuron *84*, 92–106.

Oh, W.C., Hill, T.C., and Zito, K. (2013). Synapse-specific and size-dependent mechanisms of spine structural plasticity accompanying synaptic weakening. Proc. Natl. Acad. Sci. USA *110*, E305–E312.

Oh, W.C., Parajuli, L.K., and Zito, K. (2015). Heterosynaptic structural plasticity on local dendritic segments of hippocampal CA1 neurons. Cell Rep. *10*, 162–169.

Okada, D., Ozawa, F., and Inokuchi, K. (2009). Input-specific spine entry of soma-derived Vesl-1S protein conforms to synaptic tagging. Science *324*, 904–909.

Okamoto, K., Nagai, T., Miyawaki, A., and Hayashi, Y. (2004). Rapid and persistent modulation of actin dynamics regulates postsynaptic reorganization underlying bidirectional plasticity. Nat. Neurosci. *7*, 1104–1112.

Okamoto, K., Bosch, M., and Hayashi, Y. (2009). The roles of CaMKII and F-actin in the structural plasticity of dendritic spines: a potential molecular identity of a synaptic tag? Physiology (Bethesda) *24*, 357–366.

Okuno, H., Akashi, K., Ishii, Y., Yagishita-Kyo, N., Suzuki, K., Nonaka, M., Kawashima, T., Fujii, H., Takemoto-Kimura, S., Abe, M., et al. (2012). Inverse synaptic tagging of inactive synapses via dynamic interaction of Arc/Arg3.1 with CaMKIIβ. Cell *149*, 886–898.

Oliveira, A.F., and Yasuda, R. (2014). Neurofibromin is the major ras inactivator in dendritic spines. J. Neurosci. *34*, 776–783.

Ostroff, L.E., Fiala, J.C., Allwardt, B., and Harris, K.M. (2002). Polyribosomes redistribute from dendritic shafts into spines with enlarged synapses during LTP in developing rat hippocampal slices. Neuron *35*, 535–545.

Park, M., Salgado, J.M., Ostroff, L., Helton, T.D., Robinson, C.G., Harris, K.M., and Ehlers, M.D. (2006). Plasticity-induced growth of dendritic spines by exocytic trafficking from recycling endosomes. Neuron *52*, 817–830.

Patterson, M.A., Szatmari, E.M., and Yasuda, R. (2010). AMPA receptors are exocytosed in stimulated spines and adjacent dendrites in a Ras-ERK-dependent manner during long-term potentiation. Proc. Natl. Acad. Sci. USA *107*, 15951–15956.

Plath, N., Ohana, O., Dammermann, B., Errington, M.L., Schmitz, D., Gross, C., Mao, X., Engelsberg, A., Mahlke, C., Welzl, H., et al. (2006). Arc/Arg3.1 is essential for the consolidation of synaptic plasticity and memories. Neuron *52*, 437–444.

Ploski, J.E., Pierre, V.J., Smucny, J., Park, K., Monsey, M.S., Overeem, K.A., and Schafe, G.E. (2008). The activity-regulated cytoskeletal-associated protein (Arc/Arg3.1) is required for memory consolidation of pavlovian fear conditioning in the lateral amygdala. J. Neurosci. *28*, 12383–12395.

Pulipparacharuvil, S., Renthal, W., Hale, C.F., Taniguchi, M., Xiao, G., Kumar, A., Russo, S.J., Sikder, D., Dewey, C.M., Davis, M.M., et al. (2008). Cocaine regulates MEF2 to control synaptic and behavioral plasticity. Neuron *59*, 621–633.

Racz, B., and Weinberg, R.J. (2008). Organization of the Arp2/3 complex in hippocampal spines. The J. Neurosci. *28*, 5654–5659.

Ramachandran, B., and Frey, J.U. (2009). Interfering with the actin network and its effect on long-term potentiation and synaptic tagging in hippocampal CA1 neurons in slices in vitro. J. Neurosci. *29*, 12167–12173.

Redondo, R.L., and Morris, R.G. (2011). Making memories last: the synaptic tagging and capture hypothesis. Nat. Rev. Neurosci. *12*, 17–30.

Sabatini, B.L., Oertner, T.G., and Svoboda, K. (2002). The life cycle of Ca(2+) ions in dendritic spines. Neuron *33*, 439–452.

Schikorski, T., and Stevens, C.F. (1997). Quantitative ultrastructural analysis of hippocampal excitatory synapses. J. Neurosci. *17*, 5858–5867.

Sdrulla, A.D., and Linden, D.J. (2007). Double dissociation between long-term depression and dendritic spine morphology in cerebellar Purkinje cells. Nat. Neurosci. *10*, 546–548.

Segev, I., and Rall, W. (1988). Computational study of an excitable dendritic spine. J. Neurophysiol. *60*, 499–523.

Smith, K.R., Kopeikina, K.J., Fawcett-Patel, J.M., Leaderbrand, K., Gao, R., Schürmann, B., Myczek, K., Radulovic, J., Swanson, G.T., and Penzes, P. (2014). Psychiatric risk factor ANK3/ankyrin-G nanodomains regulate the structure and function of glutamatergic synapses. Neuron *84*, 399–415.

Sobczyk, A., and Svoboda, K. (2007). Activity-dependent plasticity of the NMDA-receptor fractional Ca2+ current. Neuron *53*, 17–24.

Star, E.N., Kwiatkowski, D.J., and Murthy, V.N. (2002). Rapid turnover of actin in dendritic spines and its regulation by activity. Nat. Neurosci. *5*, 239–246.

Steiner, P., Higley, M.J., Xu, W., Czervionke, B.L., Malenka, R.C., and Sabatini, B.L. (2008). Destabilization of the postsynaptic density by PSD-95 serine 73 phosphorylation inhibits spine growth and synaptic plasticity. Neuron *60*, 788–802.

Steward, O., and Levy, W.B. (1982). Preferential localization of polyribosomes under the base of dendritic spines in granule cells of the dentate gyrus. J. Neurosci. *2*, 284–291.

Sutton, M.A., and Schuman, E.M. (2006). Dendritic protein synthesis, synaptic plasticity, and memory. Cell *127*, 49–58.

Svoboda, K., Tank, D.W., and Denk, W. (1996). Direct measurement of coupling between dendritic spines and shafts. Science *272*, 716–719.

Tada, T., Simonetta, A., Batterton, M., Kinoshita, M., Edbauer, D., and Sheng, M. (2007). Role of Septin cytoskeleton in spine morphogenesis and dendrite development in neurons. Curr. Biol. *17*, 1752–1758.

Takahashi, N., Kitamura, K., Matsuo, N., Mayford, M., Kano, M., Matsuki, N., and Ikegaya, Y. (2012). Locally synchronized synaptic inputs. Science *335*, 353–356.

Takao, K., Okamoto, K., Nakagawa, T., Neve, R.L., Nagai, T., Miyawaki, A., Hashikawa, T., Kobayashi, S., and Hayashi, Y. (2005). Visualization of synaptic Ca^{2+} /calmodulin-dependent protein kinase II activity in living neurons. J. Neurosci. *25*, 3107–3112.

Takizawa, P.A., DeRisi, J.L., Wilhelm, J.E., and Vale, R.D. (2000). Plasma membrane compartmentalization in yeast by messenger RNA transport and a septin diffusion barrier. Science *290*, 341–344.

Takumi, Y., Ramírez-León, V., Laake, P., Rinvik, E., and Ottersen, O.P. (1999). Different modes of expression of AMPA and NMDA receptors in hippocampal synapses. Nat. Neurosci. *2*, 618–624.

Tanaka, K., and Augustine, G.J. (2008). A positive feedback signal transduction loop determines timing of cerebellar long-term depression. Neuron *59*, 608–620.

Tanaka, J., Horiike, Y., Matsuzaki, M., Miyazaki, T., Ellis-Davies, G.C., and Kasai, H. (2008a). Protein synthesis and neurotrophin-dependent structural plasticity of single dendritic spines. Science *319*, 1683–1687.

Thompson, K.R., Otis, K.O., Chen, D.Y., Zhao, Y., O'Dell, T.J., and Martin, K.C. (2004). Synapse to nucleus signaling during long-term synaptic plasticity; a role for the classical active nuclear import pathway. Neuron *44*, 997–1009.

Toni, N., Buchs, P.A., Nikonenko, I., Bron, C.R., and Muller, D. (1999). LTP promotes formation of multiple spine synapses between a single axon terminal and a dendrite. Nature *402*, 421–425.

Tønnesen, J., Katona, G., Rózsa, B., and Nägerl, U.V. (2014). Spine neck plasticity regulates compartmentalization of synapses. Nat. Neurosci. *17*, 678–685.

Tyszkiewicz, A.B., and Muir, T.W. (2008). Activation of protein splicing with light in yeast. Nat. Methods *5*, 303–305.

Van Harreveld, A., and Fifkova, E. (1975). Swelling of dendritic spines in the fascia dentata after stimulation of the perforant fibers as a mechanism of post-tetanic potentiation. Exp. Neurol. *49*, 736–749.

Wang, X.B., Yang, Y., and Zhou, Q. (2007). Independent expression of synaptic and morphological plasticity associated with long-term depression. J. Neurosci. *27*, 12419–12429.

Wiegert, J.S., and Oertner, T.G. (2013). Long-term depression triggers the selective elimination of weakly integrated synapses. Proc. Natl. Acad. Sci. USA *110*, E4510–E4519.

Wu, Y.I., Frey, D., Lungu, O.I., Jaehrig, A., Schlichting, I., Kuhlman, B., and Hahn, K.M. (2009). A genetically encoded photoactivatable Rac controls the motility of living cells. Nature *461*, 104–108.

Xie, Y., Vessey, J.P., Konecna, A., Dahm, R., Macchi, P., and Kiebler, M.A. (2007). The GTP-binding protein Septin 7 is critical for dendrite branching and dendritic-spine morphology. Curr. Biol. *17*, 1746–1751.

Xu, T., Yu, X., Perlik, A.J., Tobin, W.F., Zweig, J.A., Tennant, K., Jones, T., and Zuo, Y. (2009). Rapid formation and selective stabilization of synapses for enduring motor memories. Nature *462*, 915–919.

Yang, G., Pan, F., and Gan, W.B. (2009). Stably maintained dendritic spines are associated with lifelong memories. Nature *462*, 920–924.

Yasuda, R. (2006). Imaging spatiotemporal dynamics of neuronal signaling using fluorescence resonance energy transfer and fluorescence lifetime imaging microscopy. Curr. Opin. Neurobiol. *16*, 551–561.

Yasuda, R. (2012). Studying signal transduction in single dendritic spines. Cold Spring Harb. Perspect. Biol. *4*, 4.

Yasuda, R., and Murakoshi, H. (2011). The mechanisms underlying the spatial spreading of signaling activity. Curr. Opin. Neurobiol. *21*, 313–321.

Yasuda, R., Harvey, C.D., Zhong, H., Sobczyk, A., van Aelst, L., and Svoboda, K. (2006). Supersensitive Ras activation in dendrites and spines revealed by two-photon fluorescence lifetime imaging. Nat. Neurosci. *9*, 283–291.

Yazawa, M., Sadaghiani, A.M., Hsueh, B., and Dolmetsch, R.E. (2009). Induction of protein-protein interactions in live cells using light. Nat. Biotechnol. *27*, 941–945.

Yuste, R. (2011). Dendritic spines and distributed circuits. Neuron *71*, 772–781.

Yuste, R. (2013). Electrical compartmentalization in dendritic spines. Annu. Rev. Neurosci. *36*, 429–449.

Yuste, R., and Denk, W. (1995). Dendritic spines as basic functional units of neuronal integration. Nature *375*, 682–684.

Zakharenko, S.S., Zablow, L., and Siegelbaum, S.A. (2001). Visualization of changes in presynaptic function during long-term synaptic plasticity. Nat. Neurosci. *4*, 711–717.

Zhai, S., Ark, E.D., Parra-Bueno, P., and Yasuda, R. (2013). Long-distance integration of nuclear ERK signaling triggered by activation of a few dendritic spines. Science *342*, 1107–1111.

Zhang, Y., Cudmore, R.H., Lin, D.T., Linden, D.J., and Huganir, R.L. (2015). Visualization of NMDA receptor-dependent AMPA receptor synaptic plasticity in vivo. Nat. Neurosci. *18*, 402–407.

Zhou, Q., Homma, K.J., and Poo, M.M. (2004). Shrinkage of dendritic spines associated with long-term depression of hippocampal synapses. Neuron *44*, 749–757.

Zhou, Y., Wu, H., Li, S., Chen, Q., Cheng, X.W., Zheng, J., Takemori, H., and Xiong, Z.Q. (2006). Requirement of TORC1 for late-phase long-term potentiation in the hippocampus. PLoS ONE *1*, e16.

Zhou, X.X., Chung, H.K., Lam, A.J., and Lin, M.Z. (2012). Optical control of protein activity by fluorescent protein domains. Science *338*, 810–814.

Zhu, J.J., Qin, Y., Zhao, M., Van Aelst, L., and Malinow, R. (2002). Ras and Rap control AMPA receptor trafficking during synaptic plasticity. Cell *110*, 443–455.