Long-Term Potentiation: From CaMKII to AMPA Receptor Trafficking

Bruce E. Herring[1] and Roger A. Nicoll[1,2]

[1]Department of Cellular and Molecular Pharmacology and [2]Department of Physiology, University of California, San Francisco, California 94143; email: Bruce.Herring@ucsf.edu, Roger.Nicoll@ucsf.edu

Annu. Rev. Physiol. 2016. 78:351–65

The *Annual Review of Physiology* is online at physiol.annualreviews.org

This article's doi: 10.1146/annurev-physiol-021014-071753

Keywords

LTP, synapse, plasticity, spine, memory

Abstract

For more than 20 years, we have known that Ca^{2+}/calmodulin-dependent protein kinase (CaMKII) activation is both necessary and sufficient for the induction of long-term potentiation (LTP). During this time, tremendous effort has been spent in attempting to understand how CaMKII activation gives rise to this phenomenon. Despite such efforts, there is much to be learned about the molecular mechanisms involved in LTP induction downstream of CaMKII activation. In this review, we highlight recent developments that have shaped our current thinking about the molecular mechanisms underlying LTP and discuss important questions that remain in the field.

INTRODUCTION

Long-term potentiation (LTP): a long-lasting increase in the efficiency of glutamatergic synaptic transmission

***N*-Methyl-D-aspartate receptor (NMDAR):** a glutamate receptor that is required for LTP induction

Ca^{2+}/calmodulin-dependent protein kinase (CaMKII): a Ca^{2+}/calmodulin-activated enzyme that is necessary and sufficient for LTP induction

α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR): a glutamate receptor that is responsible for most excitatory neurotransmission in the central nervous system

How do we learn, and how does our brain store information? These are some of the most fascinating questions of our time. Learning and memory stem from the ability of our brains to change with experience. Synapses, the connections between neurons, are ideally suited for this purpose. The plasticity of synapses is truly remarkable and, since as early as the beginning of the last century, has been proposed to be a foundational element of learning and memory (1). This idea was formulated into a more concrete model by Donald Hebb in 1949. However, it wasn't until roughly 20 years later that the discovery of long-term potentiation (LTP) offered substantial evidence in favor of such a model (2, 3). This work showed that subjecting hippocampal excitatory synapses to brief, high-frequency stimulation produced a rapid and long-lasting increase in the strength of these synapses that can persist for many days (4). To this day LTP is believed to be one of the primary mechanisms giving rise to synaptic plasticity in the brain and remains an uncontested cellular model of learning and memory.

Since the discovery of LTP nearly 50 years ago, the precise cellular and molecular steps that give rise to LTP have been the subject of intense study. Although LTP occurs at excitatory synapses throughout the brain, LTP occurring at Schaffer collateral–CA1 synapses of the hippocampus represents the most robust and widely studied form of this phenomenon. For many years, the biggest debate concerning LTP centered on whether the observed increase in glutamatergic transmission efficiency arose from an increase in the presynaptic release of glutamate or from an increase in the sensitivity of the postsynaptic side of the synapse to glutamate. The strongest evidence in favor of an increase in presynaptic release of glutamate was an observed reduction in synaptic failure rate during LTP (5, 6). However, the discovery of silent synapses and their ability to be unsilenced during LTP provided a postsynaptic explanation for this observation (7, 8). This finding, coupled with the more recent observation that glutamate uncaging on single spines is sufficient to induce LTP, has largely solidified the LTP mechanism as postsynaptic (9). As such, attention has focused largely on elucidating the postsynaptic mechanisms responsible for LTP induction.

LTP induction at Schaffer collateral–CA1 synapses requires the activation of postsynaptic NMDA receptors (NMDARs) (10). Ca^{2+} influx through activated NMDARs in turn results in calmodulin-dependent activation of Ca^{2+}/calmodulin-dependent protein kinase (CaMKII) (11). Within approximately 10 s, this activation of CaMKII produces a rapid increase (12) in the number of AMPA receptors (AMPARs) at synapses. However, the molecular mechanism by which CaMKII accomplishes this task has remained a mystery for more than 20 years. Multiple forms of LTP, even at the same synapses in the CA1 region of the hippocampus, have been proposed. These different forms of LTP are thought to depend on a variety of factors, such as stimulus frequency/pattern, age of the animal, time after the induction of LTP, and dependence on protein synthesis (13). In this review, we focus on NMDAR-dependent LTP at synapses in the CA1 region of adult rodents during the first hour after induction and discuss our current knowledge regarding the molecular steps of LTP downstream of CaMKII activation. The goal of this review is to complement other recent reviews on this topic (11, 13–18).

CHALLENGING ASSUMPTIONS

Since the discoveries suggesting that CaMKII activity is both necessary and sufficient for the induction of LTP (19–22), tremendous efforts have been made to identify the substrates of this kinase that are required for the synaptic changes associated with LTP. During the 1990s, two lines of research on LTP evolved in parallel: the role of AMPAR trafficking on the one hand and the role of CaMKII on the other. Thus, a very attractive possibility was that CaMKII directly

phosphorylated the AMPAR subunits, resulting in an augmentation of their trafficking/function. In particular, converging lines of evidence focused on the critical role of the C terminus of the AMPAR subunit GluA1. Soderling and colleagues investigated the ability of CaMKII to phosphorylate AMPAR subunits. These researchers found that GluA1 was indeed phosphorylated by CaMKII and that LTP induction resulted in an upregulation of AMPAR subunit phosphorylation (23, 24). Phosphorylation mapping studies later identified Ser831 as a GluA1 carboxy-terminal (C-tail) residue phosphorylated by CaMKII (25, 26), and LTP induction indeed resulted in increases in CaMKII-mediated Ser831 phosphorylation (27). Over subsequent years, additional AMPAR subunit C-tail residues phosphorylated by PKC and PKA have been proposed to play roles in LTP (28, 29). In addition to the effects that CaMKII phosphorylation has on AMPAR trafficking, single-channel conductance is increased (30, 31). Further evidence pointing to a critical role for the GluA1 C-tail came from studies by Malinow and colleagues, who monitored the trafficking of overexpressed AMPAR subunits by measuring the degree of rectification of synaptic responses (32, 33). They found that GFP-tagged GluA1 homomers failed to traffic to synapses under basal conditions but could be driven to the synapse by LTP. GFP-tagged GluA2 homomers, however, did traffic to the synapse under basal conditions. Finally, genetic deletion of GluA1 prevented LTP (34), whereas mice lacking both GluA2 and GluA3 showed normal LTP (35). Such findings are consistent with a model in which phosphorylation of the C-tail of GluA1 is critical for LTP.

CaMKII phosphorylation of AMPAR accessory proteins has also been implicated in LTP. Most AMPARs are associated with transmembrane AMPAR-regulatory proteins (TARPs), which are a family of related proteins differentially expressed throughout the brain (36, 37). TARPs play critical roles in the folding, trafficking, and gating of AMPARs. Interestingly, the cytoplasmic C-tail has numerous closely spaced serines that are phosphorylated by CaMKII (38). The C-tail of stargazin, a TARP family member, interacts with negatively charged lipid bilayers in a phosphorylation-dependent manner such that phosphorylation reduces this interaction, freeing up the C-tail for other interactions (39). Overexpression of a phosphonull TARP impairs LTP (38) and thus supports a model in which phosphorylation of TARPs is involved in the translocation of AMPARs during LTP induction.

All of the data summarized above suggest a receptor-centric model in which modification of AMPARs/TARPs is responsible for their trafficking into the postsynaptic density (PSD) during LTP. This model has dominated the field of LTP for the past 20 years. The data also propose that AMPARs/TARPs are the immediate downstream target of CaMKII. However, although manipulations that block phosphorylation of the various residues in the C-tail of GluA1 impair LTP, none have been successful in eliminating LTP. Furthermore, LTP is still present in knock-in mice harboring alanine mutations that prevent phosphorylation of Ser831 and Ser845 (27). Thus, these findings raise the possibility that other mechanisms may play a role in LTP.

A recent study using a molecular replacement strategy has challenged the role of the GluA1 C-tail and TARPs in LTP (40). In these experiments, endogenous AMPAR subunits were replaced with recombinant AMPARs that contained GluA1 subunits lacking residues previously implicated in LTP. In all cases, including a construct in which the entire GluA1 C-tail was deleted, normal LTP was observed. Furthermore, replacement of endogenous synaptic AMPARs with kainate receptors (KARs) and their Neto auxiliary subunit, which are not normally expressed at these synapses and do not interact with TARPs (41), also supported normal LTP. Thus, these findings would appear to rule out an essential role for any of the AMPAR subunits, their C-tails, or their auxiliary subunits in LTP. How are we to reconcile these findings with the previous literature? This study used a near saturation induction protocol, which results in more reliable and robust LTP. With a weaker induction protocol, as was used in many of the previous studies, AMPAR/TARP phosphorylation may affect either the threshold or the magnitude of LTP. Nonetheless, normal

Transmembrane AMPA receptor proteins (TARPs): a protein family that binds to and regulates AMPAR folding, trafficking, and gating

Postsynaptic density (PSD): a protein-dense region on the postsynaptic side of glutamatergic synapses that concentrates and organizes glutamate receptors

Kainate receptor (KAR): a glutamate receptor distinguished by its response to kainate

Neto proteins: a protein family that binds to and regulates KAR trafficking and gating

levels of LTP can be generated in the absence of AMPARs. In light of these results, can a receptor-centric model of LTP be salvaged? For this model to survive, both AMPARs/TARPs and KARs/Netos would apparently have to undergo a similar posttranslational covalent modification during LTP. However, there is limited structural similarity between AMPARs/TARPs and KARs/Netos.

The two remaining and more parsimonious explanations for these data are what we refer to in this review as the PSD-centric model and the vesicle-centric model. In the PSD-centric model, the PSD is the target of CaMKII's action. CaMKII, via mechanisms that are currently unknown, creates slots in the PSD such that receptors, which are highly mobile in the membrane (42), are captured by the PSD. In this model, the slots must be rather promiscuous because they are unable to distinguish between AMPARs/TARPs and KARs/Netos. It is unclear what features these two receptors might have in common that would enable them to be recognized by these hypothetical slots. In the alternative vesicle-centric model, recycling endosomes are the downstream target of CaMKII's action during LTP. This model is parsimonious in that vesicles will deliver whatever receptor is expressed in them.

Structural LTP (sLTP): a sustained physical enlargement of spines that coincides with LTP

PSD-95: a scaffolding protein in the PSD that supports the localization of glutamate receptors at synapses

EMERGING HYPOTHESIS EXPLAINING LTP

Relationship Between Synapse Structure and Function

Given that posttranslational modification of AMPARs and their auxiliary subunits appears not to be essential for LTP, what can we currently say about mechanisms supporting LTP downstream of CaMKII activation? One of the models discussed above posits that CaMKII, by an unknown mechanism, creates slots in the PSD. One possibility is that such a phenomenon would involve structural changes. The idea that synaptic structural modifications contribute to LTP induction largely grew from a study published in 2004 by Matsuzaki et al. (43), who used two-photon glutamate uncaging to activate NMDARs on individual dendritic spines of CA1 pyramidal neurons. The vast majority of glutamatergic synapses in the brain are located on spines. These small protrusions on the dendrites of neurons are chemically and electrically segregated microcompartments that contain a number of specialized proteins that support the structure and function of glutamatergic synapses. Matsuzaki et al. found that repetitive stimulation of NMDARs on a single spine resulted in a rapid and long-lasting increase in the size of the stimulated spine; this increase required both NMDAR and CaMKII activation. The size of neighboring unstimulated spines was unaltered. This physical enlargement of stimulated dendritic spines, later termed structural LTP (sLTP), was accompanied by a significant increase in AMPAR-mediated currents that was also specific to the stimulated spine. Through the use of this method, observation of both synaptic size and synaptic AMPAR-mediated currents revealed that NMDAR-mediated increases in synaptic AMPAR content occurred in lockstep with the physical enlargement of the synapse; both events occurred with a delay of approximately 10 s (12). Thus, evidence from a number of labs (12, 43–48) suggests that LTP-inducing stimuli result in a physical enlargement of glutamatergic synapses that potentially allows the synapse to accommodate more AMPARs (**Figure 1**). A recent paper looked at this possibility in considerable detail (49). Bosch et al. (49) followed the changes in both the size of the spine and the size of the PSD following the induction of sLTP. In accordance with previous findings, the AMPAR content closely matched the spine volume changes. However, the size of the PSD increased only after a delay of approximately 60 min. In addition, there was no change in the content of several PSD scaffolding proteins (e.g., PSD-95) that enhance synaptic transmission and are believed to serve as slots for AMPARs during LTP (50–52). This finding casts doubt on a simple model in which LTP rapidly recruits slot proteins to the PSD. Rather,

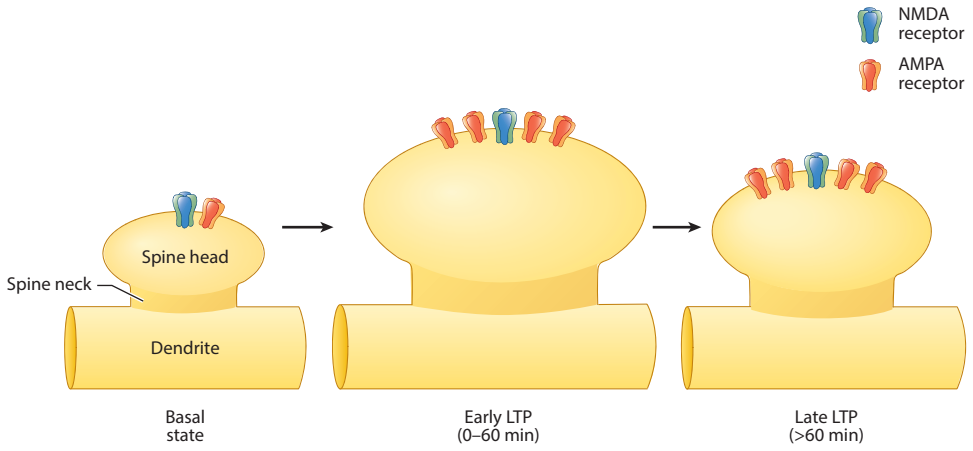

Figure 1

Structural and functional changes at glutamatergic synapses during long-term potentiation (LTP). Glutamatergic synapses are formed on the spine head. LTP induction causes significant increases in the size of the spine head and in the diameter of the spine neck. This physical enlargement of synapses is referred to as structural LTP. LTP induction also causes a significant increase in synaptic AMPA receptor expression, referred to as functional LTP. The time courses of the structural and functional changes that occur at glutamatergic synapses during LTP induction are identical.

this finding suggests that slot proteins are already present and that either (*a*) slots for AMPARs are unsaturated and the structural change in some way provides additional AMPARs that fill these slots or (*b*) structural changes modify slot proteins in such a way as to tether AMPARs. Some caution must be exercised in interpretation of the results of this study, however, given that the molecular tracking experiments performed relied on overexpressed proteins that have profound effects on synaptic function that occlude LTP [e.g., PSD-95 (50)].

What Gives Rise to Synaptic Structural Changes During LTP?

How does NMDAR and CaMKII activation during LTP increase the size of glutamatergic synapses? The primary cytoskeletal element of dendritic spines is actin. Actin filaments, produced by the polymerization of actin monomers, form a branched network within glutamatergic synapses and are responsible for the morphology of dendritic spines (53–56). Thus, it stands to reason that CaMKII activation during LTP promotes actin polymerization within the synapse, leading to the expansion of the actin cytoskeletal network in the spine, and such expansion in turn gives rise to synapse enlargement (**Figure 2**). Indeed, Matsuzaki et al. and others have found that latrunculin A, an inhibitor of actin polymerization, prevents NMDAR-mediated spine enlargement (43, 57) and LTP (58).

What molecular mechanisms might be involved in promoting synaptic actin polymerization during LTP induction? The Rho family of small GTPases (Rho GTPases) is a group of G proteins that promote actin polymerization through their ability to inhibit the negative actin polymerization regulators ADF (actin depolymerizing factor) and cofilin. Rho GTPases act as molecular switches in cells and have roles in a diverse array of cellular functions ranging from organelle development to cell movement. When bound to a GTP, these Rho GTPase proteins are active until they catalyze the hydrolysis of the bound GTP, resulting in inactivation of the Rho GTPase.

Rho GTPases: G proteins that regulate cytoskeletal dynamics at glutamatergic synapses

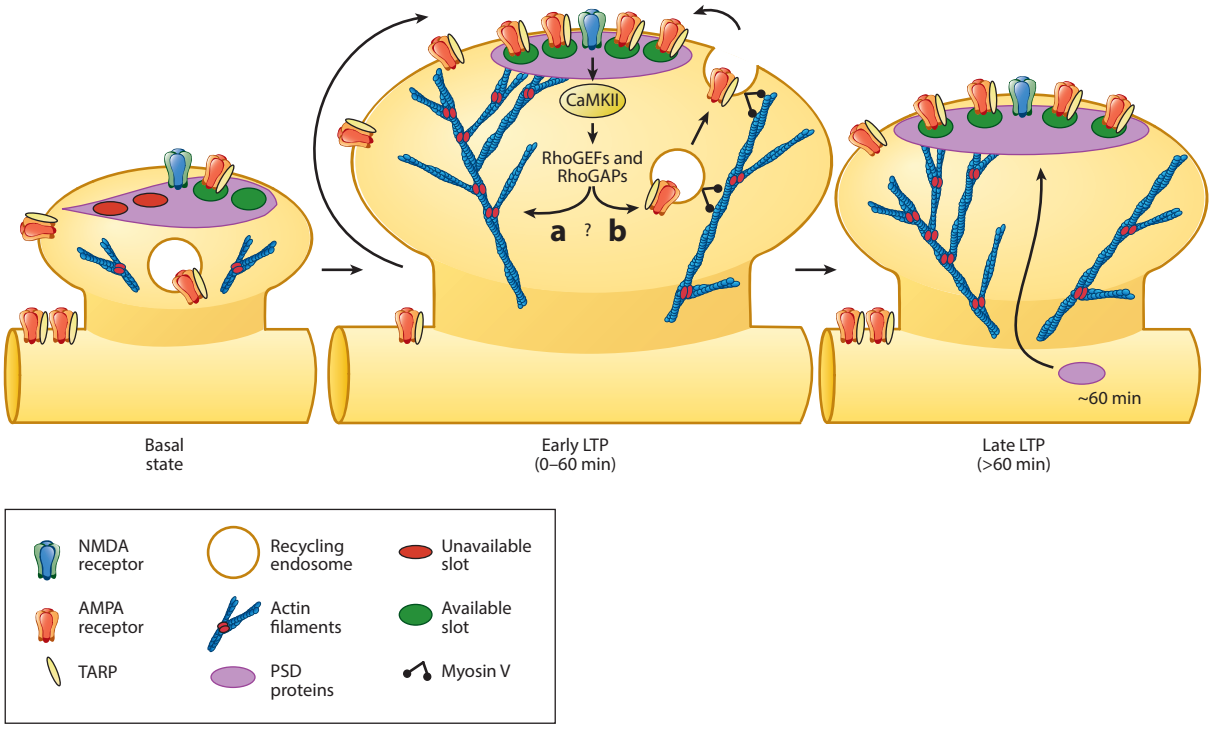

Figure 2

The cellular and molecular mechanisms involved in long-term potentiation (LTP). During LTP induction, NMDA receptor activation results in the Ca^{2+}-mediated activation of Ca^{2+}/calmodulin-dependent protein kinase (CaMKII). Activated CaMKII may then influence the activity of Rho GTPase–regulatory proteins [e.g., RhoGEFs (guanine nucleotide exchange factors that activate Rho GTPases) and/or RhoGAPs (GTPase-activating proteins that inhibit Rho GTPases)] to promote actin polymerization in the head and neck of dendritic spines. Increased actin polymerization in spines results in an increase in both the size of the spine head and the diameter of the spine neck. These structural changes may then give rise to an increase in synaptic AMPA receptor expression in two ways: (*a*) Increased actin polymerization in spines may result in the modification and/or reorganization of postsynaptic density (PSD) proteins in a way that converts unavailable slots (*red ovals*) into available slots (*green ovals*) for AMPA receptors. This reorganization may serve to capture freely diffusing extrasynaptic surface AMPA receptors. (*b*) Actin polymerization in spines may create additional tracks supporting the transport and ultimate fusion of AMPA receptor–containing vesicles in the spine head. At later stages, various PSD proteins are recruited to the PSD, and the PSD increases in size. TARP denotes transmembrane AMPAR-regulatory protein.

Commonly studied members of this family of proteins are Cdc42, Rac1, and RhoA. All three of these Rho GTPases are found at glutamatergic synapses and impact the density and morphology of dendritic spines (59, 60). A recent study using newly developed molecular FRET-based sensors and two-photon fluorescence lifetime imaging monitored the spatiotemporal activation of Cdc42 and RhoA during sLTP induction in individual spines by using two-photon glutamate uncaging (59). Remarkably, LTP induction in individual spines produced a rapid and relatively long lasting increase in the activation of both Cdc42 and RhoA in the stimulated spine, and this activation closely followed the time course of spine enlargement. Furthermore, inhibition of RhoA and Cdc42 activation significantly reduced the induction and maintenance of sLTP, respectively. Inhibition of these GTPases also blocked functional LTP without impacting CaMKII activation. Thus, both structural LTP and functional LTP appear to require the activation of Rho GTPases that promote actin polymerization.

REMAINING QUESTIONS

How Might Increasing Actin Polymerization Increase Synaptic AMPAR Expression?

Given the identical time courses of glutamatergic synapse enlargement and synaptic AMPAR accumulation during LTP, it seems reasonable that structural changes due to actin polymerization play a role in increasing the number of AMPARs in a synapse. How then might actin polymerization during LTP induction give rise to increases in synaptic AMPAR expression? Here we discuss in depth two models that we feel are currently supported by the most evidence: the PSD-centric model and the vesicle-centric model.

PSD-centric model. The PSD-centric model proposes that actin polymerization during LTP results in an increase in the number of slots in the PSD that can accommodate AMPARs. As mentioned above, recent work suggests that, whereas spine enlargement during LTP induction is time locked with the increase in synaptic AMPAR number, there is initially no increase in size of the PSD or in the number of putative slot proteins, e.g., PSD-95, in the PSD. How then might the AMPAR density of the PSD increase during LTP induction? One possibility is that prior to LTP some slots for AMPARs in the PSD are unavailable, and LTP induction is able to unmask these slots. How might this unmasking occur? Given that actin filaments are capable of interacting with proteins that make up the PSD (61), increased actin polymerization during LTP induction may cause a rearrangement of the existing PSD scaffolding molecules in some way as to make those slots that were previously inaccessible now available for AMPAR interaction. Surface AMPARs located on the perisynaptic membrane region of the dendrite are able to freely diffuse in and out of synapses (42). Such a rearrangement of the PSD scaffolding molecules by actin polymerization may allow the PSD to now more effectively capture surface AMPARs that diffuse into the synapse (**Figure 2**). Furthermore, increasing the number of AMPARs that can be inserted into the existing PSD may also exponentially increase the ability of the synapse to retain AMPARs in general. Through a process referred to as macromolecular crowding, increasing the density of proteins in the PSD may give rise to a greater number of intermolecular collisions that may effectively increase synaptic AMPAR retention times without the need for energetically expensive biochemical modifications (62). Observations of activity- and actin-dependent increases in the ability of synapses to retain surface AMPARs and other receptors (e.g., glycine receptors) are indeed consistent with such a model (63–66).

Vesicle-centric model. The vesicle-centric model proposes that actin polymerization during LTP promotes the fusion of AMPAR-containing vesicles in or near the spine, resulting in an increased number of surface AMPARs at the synapse. A long history suggests that exocytosis of AMPARs is involved in LTP (67, 68). Recycling endosomes containing AMPARs have been reported in spines (69) and dendritic shafts at the base of spines (70). Recent studies (71, 72) have also found that syntaxin-3, SNAP-47, VAMP2, and complexin, all components of the vesicle fusion machinery, are required for LTP expression, although a previous study claimed a role for syntaxin-4, rather than syntaxin-3 (69). Interestingly, baseline AMPAR transmission was not affected by inhibition of these proteins. Thus, the findings presented apparently require the existence of at least two distinct exocytic streams: one stream that is responsible for constitutive delivery of AMPARs to synapses to maintain basal synaptic transmission and another stream that delivers additional AMPARs to synapses during LTP induction.

What might be the mechanism that controls vesicle release during LTP induction? Calcium influx through the NMDAR is apparently not sufficient, because disrupting CaMKII function by

SNARE proteins: a family of proteins that mediate vesicle fusion

mutating the autophosphorylation site (T286A) blocks LTP but has no effect on NMDAR synaptic responses (22). Might the signaling pathways involved in sLTP (Rho GTPases) also be involved in regulated exocytosis? In a number of cell types, increasing actin filament generation promotes the trafficking of cargo-containing vesicles and ultimately their fusion with the plasma membrane (73, 74). Evidence also exists that Rho GTPases can directly interact with SNARE proteins and, once activated, promote the fusion of SNARE complex–associated vesicles (75). One might even propose that the structural changes create a mechanical force that directly causes vesicle fusion. Such a proposal has been made to explain the calcium-independent release of synaptic vesicles with hypertonic solutions (76). Finally, the actin motors myosin Va and Vb have been implicated in LTP (77, 78; but see Reference 79). Such motor proteins would presumably deliver recycling endosomes to the membrane for release (**Figure 2**).

Although studies suggest that fusion of AMPAR vesicles is involved in LTP, the origins of such vesicles remain unclear. The most attractive hypothesis is that AMPAR-containing vesicles reside within the head of the spine. If this is the case, newly formed actin filaments during LTP may promote fusion of the vesicles with the plasma membrane of the spine head. However, the presence of AMPAR-containing vesicles in spines remains controversial. Studies examining spine ultrastructure have indeed observed the presence of fusion-competent vesicles within spines (80). Furthermore, a study using light microscopy reported the colocalization of recycling endosome markers and internalized AMPAR subunits within spine heads (69). In addition, imaging studies using superecliptic pHluorin–tagged GluA1 (SEP-GluA1) have reported exocytotic events in spines (12). In contrast, multiple ultrastructural immunogold studies have had difficulty identifying the presence of intracellular compartments containing AMPARs within spines (81, 82). Rather than being found in the spine, AMPAR-containing vesicular compartments have been found in dendritic shafts near spines (82). This is consistent with reports that use SEP-GluA1 and show that exocytosis occurs primarily on the shafts (12, 83–85).

PSD-centric model versus vesicle-centric model. Recent studies have attempted to address the contribution of laterally diffusing AMPARs relative to newly exocytosed AMPARs during LTP by using CA1 pyramidal neurons transfected with SEP-GluA1 (12, 83). If the structural enlargement of synapses during LTP is permissive for the exocytosis of AMPAR-containing vesicles in the spine head, one might expect that the increase in synaptic AMPAR expression would lag behind the structural change. However, using SEP-GluA1 to visualize synaptic AMPAR insertion during LTP induction in individual spines, researchers showed that GluA1 expression and spine enlargement occur in lockstep (12). Fluorescence recovery after photobleaching performed by these two groups revealed that LTP results in a significant increase in the rate of SEP-GluA1 exocytosis in both spines and shafts immediately surrounding the potentiated spines. Patterson et al. (12) compared the time courses of the fluorescence following exocytosis in the spine of spontaneous events and those occurring during the induction of LTP. Spontaneous events were transient, whereas events driven by LTP were sustained. This finding implies that two processes occur during LTP induction: activity-dependent exocytosis and a modification of the PSD to capture the newly released (and presumably preexisting surface) receptors. By contrast, there was no difference in the decay of fluorescence in spontaneous and stimulated events in the shaft, indicating that the capturing effect of stimulation is restricted to the spine and/or PSD.

Interestingly, Patterson et al. (12) found that the change in spine fluorescence produced by exocytosis during LTP induction is not dependent on CaMKII activation and accounts for 10–30% of the total fluorescence change that occurs during LTP. Thus, it was presumed that the remaining 70–90% of the increase in synaptic AMPAR expression that occurs with LTP stems from lateral diffusion. As discussed above, because LTP is absent in the CaMKII T286A

knock-in mouse (22), the role of CaMKII-independent exocytosis in LTP is unclear. Additionally, the paucity of evidence for the presence of AMPAR-containing vesicles in spines raises the possibility that the overexpressed tagged receptors may be expressed in spine vesicles that normally lack them. Because endogenous AMPARs are found in vesicular compartments in dendritic shafts near spines, what role might these AMPARs play in LTP? There are two possible routes by which these AMPARs reach the synapse. First, the vesicles may be trafficked into the spine via the spine neck, where they then fuse and release AMPARs. However, it remains questionable whether NMDAR-dependent recruitment of dendritic shaft vesicles into the spine head could occur fast enough to contribute to LTP induction. Second, these AMPARs may be exocytosed onto the shaft during LTP induction and then diffuse into the spine. To address this possibility, Makino & Malinow (83) bleached a region of dendrite, including spines, and then induced LTP on one of the bleached spines. Whereas LTP induced the usual rapid spine enlargement, the SEP-GluA1 exocytosis in the nearby shaft occurred with a delay of approximately a minute. This finding reveals that shaft AMPARs are unlikely to play a role in the initial phase of LTP; rather, such AMPARs may replenish a depleted local pool of receptors.

Although both of these SEP-GluA1 imaging studies (12, 83) concluded that increases in synaptic AMPAR expression during LTP induction stem primarily from lateral diffusion of surface AMPARs into the synapse, the concern remains that in each case experiments were performed using neurons in which GluA1 was overexpressed. Thus, the relative contributions of these two AMPAR delivery mechanisms may differ for endogenous receptors. These imaging studies also appear to be at odds with the severe loss of LTP caused by the block of exocytosis (71, 72). Perhaps the block of exocytosis in these experiments, which were carried out 10–15 days after injection of lentiviral shRNA constructs targeting SNARE proteins, depleted a reserve pool of AMPARs and thus indirectly prevented LTP. Such an argument has been proposed to explain the lack of LTP in the GluA1 knockout mouse (40). Future studies will be necessary to definitively assess the relative contributions of vesicular fusion and lateral diffusion to the upregulation of endogenous synaptic AMPARs during LTP.

In addition, actin remodeling during LTP induction could inhibit endocytosis of synaptic AMPARs. Pharmacological promotion of actin polymerization, for example, prevents AMPAR endocytosis at synapses (65). Despite relatively little evidence currently in support of this idea, such a mechanism could also give rise to an increase in synaptic AMPAR expression during LTP induction.

What Direct Downstream Target of CaMKII Is Responsible for LTP?

How does CaMKII give rise to LTP? This is perhaps one of the most interesting questions, and yet its answer has frustratingly remained the most elusive. LTP induction appears to rely on CaMKII-dependent activation of a group of Rho GTPases that in turn promote an expansion of the actin cytoskeleton, an increase in spine size, and an increase in the number of AMPARs at the synapse. However, it remains unclear how elevated CaMKII activity during LTP gives rise to an increase in Rho GTPase activation. Rho GTPases are activated by guanine nucleotide exchange factors (GEFs) and are inactivated by GTPase-activating proteins (GAPs). GEFs bind to and activate Rho GTPases by stimulating the release of bound GDP and by allowing the binding of GTP to the Rho GTPase. GAPs, in contrast, inhibit Rho GTPase activity by binding to Rho GTPases associated with GTP and by facilitating the hydrolysis of GTP to form GDP. Recently, researchers showed that LTP induction using a chemLTP protocol results in CaMKII-dependent phosphorylation and in rapid removal of the synaptic GAP protein SynGAP1 from synapses (86). Expression of a recombinant mutant form of SynGAP1 that was unable to be phosphorylated by

Guanine nucleotide exchange factors (GEFs): a class of proteins that activate GTPases by promoting their association with GTP

CaMKII prevented both the structural enlargement of spines and increases in synaptic AMPAR expression observed in dissociated hippocampal neurons after the chemLTP protocol. Such data may point to SynGAP1 acting as a tonic break on Rho GTPase activation that is released upon LTP induction. However, in this study, the manipulations that prevented chemLTP induction had strong baseline effects on synapses, including a significant reduction in both the size of spines and the number of AMPARs found at synapses. Thus, concern exists as to whether the basic machinery required for LTP induction, such as synaptic NMDAR expression, may also be affected by these manipulations. Furthermore, increases in functional synaptic AMPAR insertion were examined 60 min following chemLTP, and thus determining whether this mechanism is involved in the early phase of LTP or only later on is difficult. SynGAP1 is also expressed most highly during early stages of neonatal development and may thus play a more prominent role in cortical synaptic development than in adult LTP expression (86).

GTPase-activating proteins (GAPs): a class of proteins that inhibit GTPase activity by stimulating the ability of GTPases to convert GTP to GDP

ChemLTP: various methods of inducing LTP-like phenomena by bath-applying agents that promote NMDAR activation

Enhancing the activity of GEFs that activate Rho GTPases (RhoGEFs) is another mechanism by which CaMKII may increase Rho GTPase activity during LTP induction (87). One RhoGEF protein that has been proposed to be involved in NMDAR-dependent synaptic potentiation is RasGRF2. RasGRF2 activates the Rho GTPase Rac1 (88). RasGRF2 is indeed expressed at glutamatergic synapses, and its function can be upregulated in an NMDAR activation–dependent fashion (88). However, mice lacking both members of the RasGRF family, RasGRF1 and RasGRF2, display largely normal LTP, suggesting that these proteins are for the most part dispensable in LTP induction (89). Another GEF protein proposed to be involved in NMDAR-dependent synaptic potentiation is Kalirin. Kalirin is heavily concentrated in glutamatergic synapses, and CaMKII phosphorylation increases the ability of Kalirin to activate Rho GTPases (90). Overexpression of the primary splice variant of Kalirin in the hippocampus and cortex, Kalirin-7, results in a significant increase in the size of dendritic spines (90). Moreover, overexpression of a mutant form of Kalirin-7 that prevents its phosphorylation by CaMKII blocks chemLTP-mediated increases in spine size without affecting baseline spine morphology (90). Diminished LTP has also been reported in a mouse line lacking only the Kalirin-7 isoform. These mice, however, exhibit reduced synaptic NMDAR expression, which may account for the LTP phenotype observed (91, 92). Furthermore, a different mouse line lacking all isoforms of Kalirin displays largely normal LTP (93). Thus, Kalirin proteins alone cannot account for LTP induction, and as a result, enthusiasm for Kalirin playing an important role in LTP has waned.

GEF and GAP proteins remain the most compelling candidates for the direct downstream targets of CaMKII involved in the induction of LTP. Although a growing number of such proteins have been proposed to be involved in LTP, no single member of this protein class has been convincingly shown to account for LTP induction. Thus, it remains a gnawing, unsettled mystery as to what GEFs, GAPs, or other Rho GTPase–regulatory proteins (e.g., GDP-dissociation inhibitors) are the immediate downstream target(s) of CaMKII. It is also possible that CaMKII phosphorylates Rho GTPases themselves, given that phosphorylation of these molecules can influence their activity (94).

SUMMARY AND CONCLUSIONS

Molecules potentially involved in the plasticity of glutamatergic synapses and thus in learning and memory are increasingly being implicated in neuropsychiatric and neurodegenerative disease (95, 96). Thus, it is now more important than ever that we strive to understand the cellular and molecular mechanisms that govern the strength of glutamatergic synaptic transmission. In this review, we attempt to summarize our most current understanding of LTP and to highlight some of the most important unanswered questions that remain in the field. Our relatively new appreciation

for the role that spine structure plays in the induction of LTP has undoubtedly shifted the focus of the field toward signal transduction cascades that influence both the structure and the molecular organization of synapses. The most pressing questions that now stand before us are: How does CaMKII trigger the activation of such signal transduction cascades, and how might the resulting changes in morphology and molecular organization of synapses promote the entry and capture of AMPARs in synapses? Our hope is that current techniques, coupled with emerging technologies that will ultimately allow for the visualization of synaptic molecular reorganization with nanometer resolution, will significantly aid our ability to answer such questions.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

LITERATURE CITED

1. Cajal SR. 1911. *Histologie du systeme nerveux de l'homme et des vertebres*, transl. N Swanson, LW Swanson. New York: Oxford Univ. Press
2. Lømo T. 1966. Frequency potentiation of excitatory synaptic activity in the dentate area of the hippocampal formation. *Acta Physiol. Scand.* 68:128
3. Bliss TV, Lømo T. 1973. Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. *J. Physiol.* 232:331–56
4. Abraham WC. 2003. How long will long-term potentiation last? *Philos. Trans. R. Soc. B* 358:735–44
5. Bekkers JM, Stevens CF. 1990. Presynaptic mechanism for long-term potentiation in the hippocampus. *Nature* 346:724–29
6. Malinow R, Tsien RW. 1990. Presynaptic enhancement shown by whole-cell recordings of long-term potentiation in hippocampal slices. *Nature* 346:177–80
7. Isaac JT, Nicoll RA, Malenka RC. 1995. Evidence for silent synapses: implications for the expression of LTP. *Neuron* 15:427–34
8. Liao D, Hessler NA, Malinow R. 1995. Activation of postsynaptically silent synapses during pairing-induced LTP in CA1 region of hippocampal slice. *Nature* 375:400–4
9. Kerchner GA, Nicoll RA. 2008. Silent synapses and the emergence of a postsynaptic mechanism for LTP. *Nat. Rev. Neurosci.* 9:813–25
10. Collingridge GL, Kehl SJ, McLennan H. 1983. Excitatory amino acids in synaptic transmission in the Schaffer collateral–commissural pathway of the rat hippocampus. *J. Physiol.* 334:33–46
11. Lisman J, Yasuda R, Raghavachari S. 2012. Mechanisms of CaMKII action in long-term potentiation. *Nat. Rev. Neurosci.* 13:169–82
12. Patterson MA, Szatmari EM, Yasuda R. 2010. AMPA receptors are exocytosed in stimulated spines and adjacent dendrites in a Ras-ERK-dependent manner during long-term potentiation. *PNAS* 107:15951–56
13. Nicoll RA, Roche KW. 2013. Long-term potentiation: peeling the onion. *Neuropharmacology* 74:18–22
14. Malinow R, Mainen ZF, Hayashi Y. 2000. LTP mechanisms: from silence to four-lane traffic. *Curr. Opin. Neurobiol.* 10:352–57
15. Huganir RL, Nicoll RA. 2013. AMPARs and synaptic plasticity: the last 25 years. *Neuron* 80:704–17
16. Colgan LA, Yasuda R. 2014. Plasticity of dendritic spines: subcompartmentalization of signaling. *Annu. Rev. Physiol.* 76:365–85
17. Bliss TV, Collingridge GL. 2013. Expression of NMDA receptor–dependent LTP in the hippocampus: bridging the divide. *Mol. Brain* 6:5
18. Soderling TR, Derkach VA. 2000. Postsynaptic protein phosphorylation and LTP. *Trends Neurosci.* 23:75–80
19. Silva AJ, Stevens CF, Tonegawa S, Wang Y. 1992. Deficient hippocampal long-term potentiation in alpha-calcium-calmodulin kinase II mutant mice. *Science* 257:201–6

20. Lledo PM, Hjelmstad GO, Mukherji S, Soderling TR, Malenka RC, Nicoll RA. 1995. Calcium/calmodulin-dependent kinase II and long-term potentiation enhance synaptic transmission by the same mechanism. *PNAS* 92:11175–79
21. Pettit DL, Perlman S, Malinow R. 1994. Potentiated transmission and prevention of further LTP by increased CaMKII activity in postsynaptic hippocampal slice neurons. *Science* 266:1881–85
22. Giese KP, Fedorov NB, Filipkowski RK, Silva AJ. 1998. Autophosphorylation at Thr286 of the alpha calcium-calmodulin kinase II in LTP and learning. *Science* 279:870–73
23. McGlade-McCulloh E, Yamamoto H, Tan SE, Brickey DA, Soderling TR. 1993. Phosphorylation and regulation of glutamate receptors by calcium calmodulin-dependent protein kinase-II. *Nature* 362:640–42
24. Barria A, Muller D, Derkach V, Griffith LC, Soderling TR. 1997. Regulatory phosphorylation of AMPA-type glutamate receptors by CaM-KII during long-term potentiation. *Science* 276:2042–45
25. Barria A, Derkach V, Soderling T. 1997. Identification of the Ca^{2+}/calmodulin-dependent protein kinase II regulatory phosphorylation site in the alpha-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate-type glutamate receptor. *J. Biol. Chem.* 272:32727–30
26. Mammen AL, Kameyama K, Roche KW, Huganir RL. 1997. Phosphorylation of the alpha-amino-3-hydroxy-5-methylisoxazole4-propionic acid receptor GluR1 subunit by calcium/calmodulin-dependent kinase II. *J. Biol. Chem.* 272:32528–33
27. Lee HK, Takamiya K, Han JS, Man H, Kim CH, et al. 2003. Phosphorylation of the AMPA receptor GluR1 subunit is required for synaptic plasticity and retention of spatial memory. *Cell* 112:631–43
28. Esteban JA, Shi SH, Wilson C, Nuriya M, Huganir RL, Malinow R. 2003. PKA phosphorylation of AMPA receptor subunits controls synaptic trafficking underlying plasticity. *Nat. Neurosci.* 6:136–43
29. Boehm J, Kang MG, Johnson RC, Esteban J, Huganir RL, Malinow R. 2006. Synaptic incorporation of AMPA receptors during LTP is controlled by a PKC phosphorylation site on GluR1. *Neuron* 51:213–25
30. Derkach VA. 2003. Silence analysis of AMPA receptor mutated at the CaM-kinase II phosphorylation site. *Biophys. J.* 84:1701–8
31. Kristensen AS, Jenkins MA, Banke TG, Schousboe A, Makino Y, et al. 2011. Mechanism of Ca^{2+}/calmodulin-dependent kinase II regulation of AMPA receptor gating. *Nat. Neurosci.* 14:727–35
32. Hayashi Y, Shi SH, Esteban JA, Piccini A, Poncer JC, Malinow R. 2000. Driving AMPA receptors into synapses by LTP and CaMKII: requirement for GluR1 and PDZ domain interaction. *Science* 287:2262–67
33. Shi S, Hayashi Y, Esteban JA, Malinow R. 2001. Subunit-specific rules governing AMPA receptor trafficking to synapses in hippocampal pyramidal neurons. *Cell* 105:331–43
34. Zamanillo D, Sprengel R, Hvalby O, Jensen V, Burnashev N, et al. 1999. Importance of AMPA receptors for hippocampal synaptic plasticity but not for spatial learning. *Science* 284:1805–11
35. Meng Y, Zhang Y, Jia Z. 2003. Synaptic transmission and plasticity in the absence of AMPA glutamate receptor GluR2 and GluR3. *Neuron* 39:163–76
36. Jackson AC, Nicoll RA. 2011. The expanding social network of ionotropic glutamate receptors: TARPs and other transmembrane auxiliary subunits. *Neuron* 70:178–99
37. Straub C, Tomita S. 2012. The regulation of glutamate receptor trafficking and function by TARPs and other transmembrane auxiliary subunits. *Curr. Opin. Neurobiol.* 22:488–95
38. Tomita S, Stein V, Stocker TJ, Nicoll RA, Bredt DS. 2005. Bidirectional synaptic plasticity regulated by phosphorylation of stargazin-like TARPs. *Neuron* 45:269–77
39. Sumioka A, Yan D, Tomita S. 2010. TARP phosphorylation regulates synaptic AMPA receptors through lipid bilayers. *Neuron* 66:755–67
40. Granger AJ, Shi Y, Lu W, Cerpas M, Nicoll RA. 2013. LTP requires a reserve pool of glutamate receptors independent of subunit type. *Nature* 493:495–500
41. Chen L, El-Husseini A, Tomita S, Bredt DS, Nicoll RA. 2003. Stargazin differentially controls the trafficking of alpha-amino-3-hydroxyl-5-methyl-4-isoxazolepropionate and kainate receptors. *Mol. Pharmacol.* 64:703–6
42. Opazo P, Sainlos M, Choquet D. 2012. Regulation of AMPA receptor surface diffusion by PSD-95 slots. *Curr. Opin. Neurobiol.* 22:453–60
43. Matsuzaki M, Honkura N, Ellis-Davies GC, Kasai H. 2004. Structural basis of long-term potentiation in single dendritic spines. *Nature* 429:761–66

44. Harvey CD, Svoboda K. 2007. Locally dynamic synaptic learning rules in pyramidal neuron dendrites. *Nature* 450:1195–200
45. Lee SJ, Escobedo-Lozoya Y, Szatmari EM, Yasuda R. 2009. Activation of CaMKII in single dendritic spines during long-term potentiation. *Nature* 458:299–304
46. Okamoto K, Nagai T, Miyawaki A, Hayashi Y. 2004. Rapid and persistent modulation of actin dynamics regulates postsynaptic reorganization underlying bidirectional plasticity. *Nat. Neurosci.* 7:1104–12
47. Kopec CD, Li B, Wei W, Boehm J, Malinow R. 2006. Glutamate receptor exocytosis and spine enlargement during chemically induced long-term potentiation. *J. Neurosci.* 26:2000–9
48. Tönnesen J, Katona G, Rózsa B, Nägerl UV. 2014. Spine neck plasticity regulates compartmentalization of synapses. *Nat. Neurosci.* 17:678–85
49. Bosch M, Castro J, Saneyoshi T, Matsuno H, Sur M, Hayashi Y. 2014. Structural and molecular remodeling of dendritic spine substructures during long-term potentiation. *Neuron* 82:444–59
50. Stein V, House DR, Bredt DS, Nicoll RA. 2003. Postsynaptic density-95 mimics and occludes hippocampal long-term potentiation and enhances long-term depression. *J. Neurosci.* 23:5503–6
51. Nakagawa T, Futai K, Lashuel HA, Lo I, Okamoto K, et al. 2004. Quaternary structure, protein dynamics, and synaptic function of SAP97 controlled by L27 domain interactions. *Neuron* 44:453–67
52. Ehrlich I, Klein M, Rumpel S, Malinow R. 2007. PSD-95 is required for activity-driven synapse stabilization. *PNAS* 104:4176–81
53. Honkura N, Matsuzaki M, Noguchi J, Ellis-Davies GCR, Kasai H. 2008. The subspine organization of actin fibers regulates the structure and plasticity of dendritic spines. *Neuron* 57:719–29
54. Bosch M, Hayashi Y. 2012. Structural plasticity of dendritic spines. *Curr. Opin. Neurobiol.* 22:383–88
55. Nimchinsky EA, Sabatini BL, Svoboda K. 2002. Structure and function of dendritic spines. *Annu. Rev. Physiol.* 64:313–53
56. Fortin DA, Srivastava T, Soderling TR. 2012. Structural modulation of dendritic spines during synaptic plasticity. *Neuroscientist* 18:326–41
57. Harvey CD, Yasuda R, Zhong HN, Svoboda K. 2008. The spread of Ras activity triggered by activation of a single dendritic spine. *Science* 321:136–40
58. Kim CH, Lisman JE. 1999. A role of actin filament in synaptic transmission and long-term potentiation. *J. Neurosci.* 19:4314–24
59. Murakoshi H, Wang H, Yasuda R. 2011. Local, persistent activation of Rho GTPases during plasticity of single dendritic spines. *Nature* 472:100–4
60. Nakayama AY, Harms MB, Luo LQ. 2000. Small GTPases Rac and Rho in the maintenance of dendritic spines and branches in hippocampal pyramidal neurons. *J. Neurosci.* 20:5329–38
61. Sheng M, Hoogenraad CC. 2007. The postsynaptic architecture of excitatory synapses: a more quantitative view. *Annu. Rev. Biochem.* 76:823–47
62. Santamaria F, Gonzalez J, Augustine GJ, Raghavachari S. 2010. Quantifying the effects of elastic collisions and non-covalent binding on glutamate receptor trafficking in the post-synaptic density. *PLOS Comput. Biol.* 6:e1000780
63. Ehlers MD, Heine M, Groc L, Lee MC, Choquet D. 2007. Diffusional trapping of GluR1 AMPA receptors by input-specific synaptic activity. *Neuron* 54:447–60
64. Allison DW, Gelfand VI, Spector I, Craig AM. 1998. Role of actin in anchoring postsynaptic receptors in cultured hippocampal neurons: differential attachment of NMDA versus AMPA receptors. *J. Neurosci.* 18:2423–36
65. Zhou Q, Xiao M, Nicoll RA. 2001. Contribution of cytoskeleton to the internalization of AMPA receptors. *PNAS* 98:1261–66
66. Charrier C, Ehrensperger MV, Dahan M, Levi S, Triller A. 2006. Cytoskeleton regulation of glycine receptor number at synapses and diffusion in the plasma membrane. *J. Neurosci.* 26:8502–11
67. Lledo PM, Zhang X, Südhof TC, Malenka RC, Nicoll RA. 1998. Postsynaptic membrane fusion and long-term potentiation. *Science* 279:399–403
68. Lu W, Man H, Ju W, Trimble WS, MacDonald JF, Wang YT. 2001. Activation of synaptic NMDA receptors induces membrane insertion of new AMPA receptors and LTP in cultured hippocampal neurons. *Neuron* 29:243–54

69. Kennedy MJ, Davison IG, Robinson CG, Ehlers MD. 2010. Syntaxin-4 defines a domain for activity-dependent exocytosis in dendritic spines. *Cell* 141:524–35
70. Kelly EE, Horgan CP, McCaffrey MW, Young P. 2011. The role of endosomal-recycling in long-term potentiation. *Cell. Mol. Life Sci.* 68:185–94
71. Jurado S, Goswami D, Zhang YS, Molina AJM, Südhof TC, Malenka RC. 2013. LTP requires a unique postsynaptic SNARE fusion machinery. *Neuron* 77:542–58
72. Ahmad M, Polepalli JS, Goswami D, Yang X, Kaeser-Woo YJ, et al. 2012. Postsynaptic complexin controls AMPA receptor exocytosis during LTP. *Neuron* 73:260–67
73. Bader MF, Doussau F, Chasserot-Golaz S, Vitale N, Gasman S. 2004. Coupling actin and membrane dynamics during calcium-regulated exocytosis: a role for Rho and ARF GTPases. *Biochim. Biophys. Acta Mol. Cell Res.* 1742:37–49
74. Papadopulos A, Martin S, Tomatis VM, Gormal RS, Meunier FA. 2013. Secretagogue stimulation of neurosecretory cells elicits filopodial extensions uncovering new functional release sites. *J. Neurosci.* 33:19143–53
75. Nevins AK, Thurmond DC. 2005. A direct interaction between Cdc42 and vesicle-associated membrane protein 2 regulates SNARE-dependent insulin exocytosis. *J. Biol. Chem.* 280:1944–52
76. Rosenmund C, Stevens CF. 1996. Definition of the readily releasable pool of vesicles at hippocampal synapses. *Neuron* 16:1197–207
77. Correia SS, Bassani S, Brown TC, Lise MF, Backos DS, et al. 2008. Motor protein–dependent transport of AMPA receptors into spines during long-term potentiation. *Nat. Neurosci.* 11:457–66
78. Wang ZP, Edwards JG, Riley N, Provance DW, Karcher R, et al. 2008. Myosin Vb mobilizes recycling endosomes and AMPA receptors for postsynaptic plasticity. *Cell* 135:535–48
79. Schnell E, Nicoll RA. 2001. Hippocampal synaptic transmission and plasticity are preserved in myosin Va mutant mice. *J. Neurophysiol.* 85:1498–501
80. Spacek J, Harris KM. 1997. Three-dimensional organization of smooth endoplasmic reticulum in hippocampal CA1 dendrites and dendritic spines of the immature and mature rat. *J. Neurosci.* 17:190–203
81. Baude A, Nuzzer Z, Molnar E, McIlhinney RAJ, Somogyi P. 1995. High-resolution immunogold localization of AMPA type glutamate receptor subunits at synaptic and non-synaptic sites in rat hippocampus. *Neuroscience* 69:1031–55
82. Tao-Cheng JH, Crocker VT, Winters CA, Azzam R, Chludzinski J, Reese TS. 2011. Trafficking of AMPA receptors at plasma membranes of hippocampal neurons. *J. Neurosci.* 31:4834–43
83. Makino H, Malinow R. 2009. AMPA receptor incorporation into synapses during LTP: the role of lateral movement and exocytosis. *Neuron* 64:381–90
84. Lin DT, Makino Y, Sharma K, Hayashi T, Neve R, et al. 2009. Regulation of AMPA receptor extrasynaptic insertion by 4.1N, phosphorylation and palmitoylation. *Nat. Neurosci.* 12:879–87
85. Yudowski GA, Puthenveedu MA, Leonoudakis D, Panicker S, Thorn KS, et al. 2007. Real-time imaging of discrete exocytic events mediating surface delivery of AMPA receptors. *J. Neurosci.* 27:11112–21
86. Araki Y, Zeng ML, Zhang MJ, Huganir RL. 2015. Rapid dispersion of SynGAP from synaptic spines triggers AMPA receptor insertion and spine enlargement during LTP. *Neuron* 85:173–89
87. Miller MB, Yan Y, Eipper BA, Mains RE. 2013. Neuronal Rho GEFs in synaptic physiology and behavior. *Neuroscientist* 19:255–73
88. Schwechter B, Rosenmund C, Tolias KF. 2013. RasGRF2 Rac-GEF activity couples NMDA receptor calcium flux to enhanced synaptic transmission. *PNAS* 110:14462–67
89. Jin SX, Feig LA. 2010. Long-term potentiation in the CA1 hippocampus induced by NR2A subunit–containing NMDA glutamate receptors is mediated by Ras-GRF2/Erk MAP kinase signaling. *PLOS ONE* 5:e11732
90. Xie Z, Srivastava DP, Photowala H, Kai L, Cahill ME, et al. 2007. Kalirin-7 controls activity-dependent structural and functional plasticity of dendritic spines. *Neuron* 56:640–56
91. Ma XM, Kiraly DD, Gaier ED, Wang Y, Kim EJ, et al. 2008. Kalirin-7 is required for synaptic structure and function. *J. Neurosci.* 28:12368–82
92. Kiraly DD, Lemtiri-Chlieh F, Levine ES, Mains RE, Eipper BA. 2011. Kalirin binds the NR2B subunit of the NMDA receptor, altering its synaptic localization and function. *J. Neurosci.* 31:12554–65

93. Xie Z, Cahill ME, Radulovic J, Wang J, Campbell SL, et al. 2011. Hippocampal phenotypes in kalirin-deficient mice. *Mol. Cell. Neurosci.* 46:45–54
94. Liu M, Bi F, Zhou X, Zheng Y. 2012. Rho GTPase regulation by miRNAs and covalent modifications. *Trends Cell Biol.* 22:365–73
95. Mandela P, Ma XM. 2012. Kalirin, a key player in synapse formation, is implicated in human diseases. *Neural Plast.* 2012:728161
96. Volk L, Chiu SL, Sharma K, Huganir RL. 2015. Glutamate synapses in human cognitive disorders. *Annu. Rev. Neurosci.* 38:127–49