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Routes, destinations and delays: recent advances in AMPA receptor trafficking

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Abstract

Postsynaptic AMPA-type glutamate receptors (AMPA receptors) mediate most fast excitatory synaptic transmission and are crucial for many aspects of brain function, including learning, memory and cognition. The number, synaptic localization and subunit composition of synaptic AMPA receptors are tightly regulated by network activity and by the history of activity at individual synapses. Furthermore, aberrant AMPA receptor trafficking is implicated in neurodegenerative diseases. AMPA receptors therefore represent a prime target for drug development and the mechanisms that control their synaptic delivery, retention and removal are the subject of extensive research. Here, we review recent findings that have provided new insights into AMPA receptor trafficking and that might lead to the development of novel therapeutic strategies.

Introduction

AMPA-type glutamate receptors (AMPA receptors) are highly mobile proteins that undergo constitutive and activity-dependent translocation to, and removal from, synapses. Increases in synaptic AMPA receptor function through changes in their number, composition and/or properties result in the long-term potentiation (LTP) of synaptic efficacy. Conversely, removal of synaptic AMPA receptors provides a mechanism for long-term depression (LTD) [1]. Regulated AMPA receptor trafficking is also involved in slower, non-Hebbian mechanisms of plasticity [2].

AMPA receptors are tetrameric complexes of combinations of four separate subunits (GluA1–4). There are multiple routes for the delivery and removal of synaptic AMPA receptors and their respective contribution depends on the precise subunit composition and specific signaling cues. Significant recent progress has been made towards understanding the molecular control of AMPA receptor trafficking. Owing to space constraints and the focus on recent advances in this review, many important preceding studies are not discussed; for more extensive reviews of the field, we recommend excellent earlier publications [3–10].

Mechanisms of synaptic AMPA receptor delivery

Neuronal morphology requires proteins synthesized in the soma to travel considerable distances to distal synapses. Although one report has suggested that AMPA receptors are inserted in the soma and travel laterally along the dendritic membrane to synapses [11], the majority of evidence indicates that most AMPA receptors undergo kinesin- [12–15] and/or dynein- [16] mediated vesicular trafficking in dendrites. The Ca^{2+} -sensitive motor protein, Myosin Vb, is

also involved in the dendritic vesicular trafficking of GluA1-containing AMPARs [17] (Figure 1d).

The synaptic delivery of AMPARs requires the constitutive and activity-dependent transfer from intracellular compartments and the precise sites of insertion are a matter of ongoing debate (Box 1). On activation, NMDA-type glutamate receptors (NMDARs) allow Ca^{2+} to enter the cell, activating proteins involved in LTP (Figure 1a). Myosin Va is involved in the transport of AMPAR-containing recycling endosomes to sites of exocytosis [18] and it binds to Ca^{2+} /calmodulin-dependent protein kinase II (CaMKII)-phosphorylated GluA1 via the adaptor protein Rab11 (Figure 1b) to mediate short-range endosomal transport from the dendritic shaft to the spine head during LTP [19] (Figure 1c). On LTP induction, AMPARs undergo PKA-dependent insertion at perisynaptic sites (Figure 1f), are stabilized in the membrane by actin polymerization and are then translocated to the synapse for full expression of LTP [20].

Signaling mechanisms underlying localized AMPAR insertion are beginning to emerge. Ultrastructural analysis of thalamic synapses has identified a multistep process involving reserve, deliverable and synaptic pools of GluA1-containing AMPARs that is regulated by two small GTPases, Ras and Rap2 [21]. Endosomal recycling back to the plasma membrane is mediated, in part, by Rab4 and Rab11 [22]. Glutamate receptor-interacting protein (GRIP)-associated protein-1 (GRASP-1) is an effector of Rab4. It can simultaneously bind Rab4 and syntaxin 13, which is associated with Rab11-positive recycling endosomes, regulating AMPAR recycling and plasticity in dendrites [23]. The Ras/extracellular signal-regulated kinase (ERK) signaling pathway (see Glossary) acts as a diffusive signal from the synaptic site of potentiation that leads to AMPAR insertion into the dendritic membrane up to 3 μM away [24] (Figure 1h). It has also been proposed that endocytosis within spines contributes to synaptic AMPAR recruitment by generating a net inward membrane drift to enhance membrane protein delivery [25]. Interestingly, RNA editing of GluA2 (see below) has been shown to be important for exocytosis [26].

Removal of synaptic AMPARs

Interplay between phosphorylation and dephosphorylation is crucial for controlling AMPAR surface expression and endocytosis (Table 1). Protein kinase A (PKA) is anchored at the postsynaptic density (PSD) by a kinase anchoring protein 150 (AKAP150) (Figure 2c) and disruption of this interaction causes mislocalization of PKA and deficits in synaptic transmission and behavior [27]. AKAP150 also binds directly to a major scaffolding PSD protein, PSD-95. Preventing this interaction inhibits NMDAR-dependent, but not constitutive or metabotropic glutamate receptor (mGluR)-dependent, AMPAR endocytosis (Figure 2c) [28]. By contrast, another study argues that it is solely the interaction between AKAP150 and calcineurin (also known as protein phosphatase 2B, PP2B) that is required for NMDAR-dependent AMPAR endocytosis [29].

Protein interacting with C kinase 1 (PICK1) has been proposed to regulate synaptic AMPAR removal [30,31]. Ca^{2+} enhances PICK1 binding to GluA2 and a vesicle fusion protein β -SNAP to promote endocytosis of AMPARs containing GluA2 phosphorylated at serine-880 by protein kinase C (PKC) (Figure 2f) [32]. Additionally, PICK1 binds to and inhibits the Arp2/3 complex to enhance AMPAR internalization, possibly by reducing membrane tension maintained by actin treadmilling at the plasma membrane (Figure 2g) [33]. Recent reports, however, have questioned this interpretation. PICK1 knockdown does not interfere with NMDAR-dependent AMPAR endocytosis but increases AMPAR recycling back to the surface [34]. Furthermore, although PICK1 does not regulate the initial phase of NMDAR-induced AMPAR endocytosis, PICK1 knockdown blocks LTD and NMDAR-dependent, but

not mGluR-dependent, intracellular AMPAR accumulation, suggesting that PICK1 is necessary for the intracellular retention of AMPARs endocytosed in response to NMDAR activation [35].

Phosphorylation of tyrosine-876 in GluA2 regulates binding to the postsynaptically enriched guanine-nucleotide exchange factor (GEF) brefeldin-resistant Arf GEF 2 (BRAG2), which then activates the coat-recruitment factor ADP-ribosylation factor 6 (Arf6) to promote AMPAR endocytosis (Figure 2a). Deletion of BRAG2 or inhibition of the GluA2–BRAG2 interaction prevents AMPAR endocytosis and blocks both NMDAR- and mGluR-dependent LTD [36]. NMDAR stimulation induces activation of a small GTPase RalA and simultaneously dephosphorylates the endocytic adaptor RalBP1 (Figure 2d). RalA and PSD-95 then cooperatively recruit dephosphorylated RalBP1 into spines to promote AMPAR endocytosis (Figure 2e) [37]. Myotubularin-related protein 2 (MTMR2) is a phosphatase that interacts with PSD-95. MTMR2 knockdown decreases synaptic number and function by increasing GluA2 endocytosis and lysosomal degradation [38].

Mechanisms of synaptic AMPAR retention

One seemingly counterintuitive mechanism to retain AMPARs within the spine involves local exo- and endocytosis. Clathrin-positive endocytic zones are located adjacent to the PSD via the binding of dynamin-3 to the PSD-enriched protein Homer (Figure 2i). Inhibiting this interaction causes the loss of spine clathrin, decreased local endocytosis, impaired synaptic AMPAR cycling and reduced synaptic transmission [39]. Thus, localized endocytosis acts to capture and recycle AMPARs as they diffuse from the PSD. Clearly, this model requires local insertion, and a corresponding exocytic domain enriched in the t-SNARE {target SNARE [soluble N-ethylmaleimide-sensitive factor (NSF) attachment protein receptor]} syntaxin 4 that directs membrane fusion has recently been identified (Figure 1g). Disruption of syntaxin 4 impairs both spine exocytosis and LTP [40]. Crosstalk between recycling and lateral diffusion during LTP allows increased exocytosis of AMPARs and their subsequent immobilization at the PSD [41]. This dynamic retention of AMPARs provides a highly responsive system in which small alterations in the insertion:internalization ratio can modify synaptic transmission.

The constitutively active atypical PKC isoform protein kinase M zeta (PKM ζ) is the only known protein that is both necessary and sufficient to maintain synaptic potentiation [42,43]. LTP induction increases PKM ζ synthesis, which increases and maintains synaptic AMPARs. Inhibition of PKM ζ *in vivo* with ζ -inhibitory peptide (ZIP) does not block LTP induction, rather it prevents maintenance and can erase established memories without affecting the formation of new short-term memories [44]. Mechanistically, disruption of the GluA2–NSF interaction prevents PKM ζ -induced LTP and inhibiting the interaction between GluA2 and PICK1 occludes PKM ζ -mediated potentiation [45] (Figure 11). Furthermore, preventing GluA2 endocytosis in the amygdala prevents ZIP-induced memory loss [46].

Cell adhesion molecules also participate in synaptic AMPAR stabilization. The trans-synaptic cell adhesion molecule N-cadherin plays an important role in plasticity [47]. Overexpression of N-cadherin increases surface GluA1 [48] and a complex consisting of N-cadherin, δ -catenin, AMPAR-binding protein (ABP) and GRIP anchors AMPARs at synaptic or perisynaptic sites via C-terminal interactions [49]. N-cadherin also interacts with the extracellular N-terminal domain of GluA2 and disruption of this interaction prevents GluA2-mediated spine enlargement in cultured hippocampal neurons [50].

Trans-synaptic adhesion between neuroligins and neuroligins contributes to synapse formation, maturation and function [51]. Aggregating neuroligin with neuroligin-coated microbeads in young hippocampal primary cultures clusters postsynaptic proteins, including

GluA2-containing AMPARs [52]. Furthermore, disrupting neurexin–neuroligin interactions prevents AMPAR accumulation at both young and mature synapses [53]. Additionally, as discussed below, the stabilization of AMPAR by $\beta 3$ integrins is involved in homeostatic plasticity [54].

Transmembrane AMPA receptor regulatory proteins (TARPs) regulate AMPAR trafficking and channel kinetics [55,56]. The prototypic TARP, stargazin (γ -2) is an AMPAR auxiliary subunit that stabilizes synaptic AMPARs through interactions with PSD-95 [57]. Phosphorylation of synaptic TARPs is activity regulated and AMPAR currents are enhanced by overexpression of a phosphomimetic TARP mutant [58,59]. Phosphorylated stargazin interacts electrostatically with negatively charged lipids and this interaction competes with stargazin binding to PSD-95. The addition of positively charged lipids dissociates PSD-95 from lipid bilayers, increases stargazin binding to PSD-95 and enhances synaptic AMPAR activity [60]. Also, CaMKII-dependent phosphorylation of stargazin (Figure 1j) retains AMPARs at postsynaptic sites by reducing AMPAR diffusion (Figure 1k) [61]. Whereas no subunit selectivity has been reported for stargazin, the atypical TARP γ -5 selectively regulates calcium-permeable AMPARs (CP-AMPARs; discussed in more detail below) [62].

Recently, two proteins that might play a similar role to TARPs have been isolated by proteomics. Coexpression of AMPAR with transmembrane cornichon homolog (CNIH) proteins in *Xenopus* oocytes alters receptor surface expression and kinetics [63]. A subsequent report, however, suggests that CNIH are ER chaperones that have little or no effect on endogenous AMPAR trafficking in neurons [64]. Cytosine-knot AMPAR modulating protein 44 (CKAMP44) is a single transmembrane brain-specific protein enriched in the dentate gyrus region of the hippocampus, which co-immunoprecipitates with GluA1-3 and localizes to dendritic spines. Initial studies suggest that CKAMP44 promotes AMPAR desensitization and prolongs recovery from desensitization [65]

Post-transcriptional and post-translational modifications

As discussed in part above, the phosphorylation status of AMPARs is a crucial determinant of their trafficking and function. Although multiple kinases and phosphatases are involved, a working model is that activity-dependent phosphorylation of GluA1 delivers AMPARs to synapses in LTP, whereas GluA1 dephosphorylation is a signal for internalization and LTD. By contrast, GluA2 phosphorylation generally leads to internalization and its dephosphorylation is important in synaptic retention. (Table 1; for more detail, see [5,8,66]).

Surprisingly, given this prevailing model, the genetic ablation of single or defined combinations of AMPAR sub-units in individual CA1 hippocampal neurons indicates that approximately 80% of AMPAR synaptic transmission is mediated by GluA1/2 heteromers [67]. Furthermore, removal of GluA1, GluA2 and GluA3 eliminates all AMPAR currents, but does not alter NMDAR currents or dendritic morphology [67]. This suggests that all hetero- and homomeric subunit combinations can be recruited to CA1 synapses and might presage a reevaluation of the relationship between GluA1/2 and GluA2/3 receptors in basal trafficking and synaptic plasticity. Indeed, these results have been interpreted to indicate a fundamental subunit-independent trafficking mechanism, possibly mediated by TARPs [67].

The mRNA encoding most GluA2 subunits in adults is edited to replace glutamate with an arginine residue (Q/R). Incorporation of this edited subunit reduces Ca^{2+} permeability and has a major impact on AMPAR properties [68–70]. A fundamental functional distinction between AMPARs containing an edited form of GluA2 and those lacking GluA2 is that they are impermeable and permeable to calcium, respectively. Whereas most AMPARs contain an edited GluA2 subunit, there is a subpopulation of GluA2-lacking receptors (i.e. CP-AMPARs) and considerable effort has been directed to elucidating their role in synaptic

transmission and plasticity. Although it has been proposed that CP-AMPA trafficking plays an important role in LTP [71,72], this remains controversial because the stringent age dependence of plasticity pathways makes it difficult to compare between studies and different LTP induction protocols yield seemingly conflicting results (Box 2).

Other post-translational modifications involved in AMPAR exocytosis include palmitoylation of GluA1 at cysteine-811, which modulates PKC phosphorylation at serine-816 and serine-818 (Table 2). This, in turn, enhances association with a cytoskeletal protein, 4.1N, leading to increased membrane insertion [73] (Figure 1e). Glycosylation of GluA1 and GluA2 by *O*-linked β -*N*-acetylglucosamine (*O*-GlcNac) (Table 2) also plays a regulatory role in exocytosis and plasticity since inhibition leads to synaptic potentiation [74] and *O*-GlcNac modification of dephosphorylated GluA1 and GluA2 can modulate LTP and LTD, respectively [75].

AMPA degradation

Proteasomes are recruited to spines following NMDAR activation and proteasomal function is regulated by neuronal activity [76]. However, there is not yet a consensus on the role of proteasomal activity in AMPAR endocytosis and degradation. CaMKII stimulates proteasomal activity by phosphorylation of the Rpt6 proteasome subunit and is necessary for the activity-dependent recruitment of proteasomes to dendritic spines [77,78]. Inhibition of Na^+/K^+ ATPase decreases AMPAR expression through internalization and proteasomal degradation [79]. PSD-95 is ubiquitinated by the E3 ligase Mdm2 in response to NMDAR activation and proteasome inhibitors or mutations that block PSD-95 ubiquitination prevent NMDAR-induced AMPAR endocytosis and LTD (Figure 2b) [80]. However, another study reported that, although proteasome-dependent regulation of PSD-95 levels are important for GluA1 endocytosis, no polyubiquitinated PSD-95 could be detected, suggesting the requirement for an intermediate protein [81]. Recently, it has been reported that, although NMDAR-induced AMPAR endocytosis involves ubiquitination, it does not require proteasome function [82]. Furthermore, as indicated above, preventing the interaction of MTMR2 with PSD-95 increases GluA2 endocytosis and lysosomal degradation [38].

If and where AMPAR subunits are ubiquitinated is also the subject of debate. In *Caenorhabditis elegans*, ubiquitin ligases regulate the trafficking of the AMPA-type receptor GLR-1 [83]. In rat hippocampal neurons, agonist activation induces the Ca^{2+} -sensitive ubiquitination of GluA1 by the E3 ligase, neural-precursor cell-expressed developmentally downregulated gene 4-1 (Nedd4-1) to target surface-expressed GluA1 for endocytosis and lysosomal degradation [82] (Table 2). The GluA2 subunit is not ubiquitinated under these conditions and neither GluA1 nor GluA2 are ubiquitinated following NMDAR-mediated AMPAR internalization. Interestingly, this pathway is age dependent, as older neurons manifest basal levels of GluA1 ubiquitination that are comparable with AMPAR-dependent ubiquitination in younger neurons [84]. Recently, it was shown that EphA4 activation during homeostatic plasticity leads to GluA1 ubiquitination and degradation, in this case via the ubiquitin/proteasome system (Figure 2j)[85,86]. Thus, although there is agreement that ubiquitination is a key process in AMPAR trafficking and degradation, much work remains to be done to clarify the lysine residues responsible and the pathways involved.

AMPA trafficking and structural plasticity

Cytoskeletal actin dynamics play key roles in AMPAR trafficking and link structural and functional plasticity. The Rho GEF Kalirin-7 is recruited to synapses by ephrinB–EphB trans-synaptic signaling. It activates Rac1, a small spine-enriched Rho GTPase and its effector p21 activated kinase (PAK) to regulate the actin cytoskeleton and spine enlargement [87]. Kalirin-7 also interacts directly with GluA1 to modulate AMPAR surface expression

[88]. The Ras and Rap GTPase family and their downstream effector cascades also regulate AMPAR trafficking [21]. Synaptic activity increases the level of active GTP-bound Ras stimulating the MEK–ERK pathway, which in turn leads to increased AMPAR surface expression via phosphorylation of GluA1 at serine-845 and GluA2 at serine-841 [89].

As already mentioned, PICK1 binds to the Arp2/3 complex [33], which catalyzes actin branching and network formation [90]. PICK1 knockdown increases spine size and prevents NMDAR-mediated spine shrinkage, as does overexpression of a PICK1 mutant that cannot bind Arp2/3. Thus, PICK1 appears to regulate both AMPAR trafficking and spine size [91].

Homeostatic plasticity and AMPAR trafficking

Synaptic excitability is modulated to compensate for changes in network activity by regulating synaptic AMPARs through homeostatic plasticity. The immediate early gene product *Arc/Arg3.1* is central to this process [2,92]. *Arc/Arg3.1* regulates experience-driven synaptic scaling in the visual cortex [93] and specifically regulates AMPAR trafficking at thin spines *in vivo* [94]. Furthermore, *Arc/Arg3.1* overexpression or knockdown regulates basal AMPAR endocytosis (positively or negatively, respectively) yet does not alter NMDAR-dependent LTD [95], although *Arc/Arg3.1* has been implicated in the slower mGluR-dependent LTD [96]. This regulation occurs at the single synapse level [97]. Mechanistically, *Arc/Arg3.1* might interact with components of endocytic machinery, such as endophilin and dynamin, to control AMPAR internalization (Figure 2h) [98]. *Arc/Arg3.1* levels are regulated by ubiquitination and proteasomal degradation and preventing *Arc/Arg3.1* ubiquitination leads to its accumulation and decreased synaptic AMPARs [99].

Other pathways implicated in homeostatic plasticity are also emerging. Decreased synaptic activity increases retinoic acid synthesis, which enhances synaptic transmission by increased translation and surface delivery of GluA1-containing AMPARs [100]. This is mediated by fragile X mental retardation protein (FMRP), an RNA-binding protein that regulates dendritic AMPAR synthesis. FMRP has been associated with fragile X syndrome (FXS), the most common cause of inherited mental impairment, suggesting that the phenotype of this neurodevelopmental disorder might involve defects in homeostatic plasticity [101]. Decreased network activity also increases postsynaptic β -3-integrin levels, which stabilize synaptic AMPARs by Rap1 regulation of GluA2 endocytosis [54]. Furthermore, pololike kinase 2 (Plk2) is implicated in synaptic scaling via a non-catalytic interaction with NSF that homeostatically regulates GluA2-containing synaptic AMPARs [102]. Reduced synaptic activity also increases the expression of β -CaMKII, which induces presynaptic vesicle recycling and increases postsynaptic GluA1 surface expression, suggesting the presence of retrograde signaling [103]. Thus, multiple processes participate in synaptic scaling and this aspect of AMPAR trafficking is currently the focus of intense research activity.

AMPAR trafficking in disease

Many neurological and neurodegenerative disorders involve synaptic abnormalities. Manipulation of AMPAR trafficking provides an attractive potential therapeutic target for conditions ranging from addiction [104] to Alzheimer's disease (AD) [105,106]. Importantly, disruption of AMPAR trafficking by soluble amyloid beta ($A\beta$) oligomers is believed to be a major factor in synaptic dysfunction in AD [107]. Multiple experimental paradigms have been developed to model the pathology of AD and they share common general features, such as reduced synaptic AMPARs and aberrations in both LTP [108] and LTD [108,109]. In addition, overexpression of APP in cultured hippocampal neurons causes a deficit in presynaptic neurotransmitter vesicle recycling [110].

There are functional similarities between LTD and A β -induced AMPAR internalization [111]. Synaptic localization of CaMKII is altered both in APP transgenic mice and in hippocampal cultures treated with A β oligomers [112]. CaMKII knockdown occludes, and CaMKII overexpression blocks, the effect of long-term exposure of A β on AMPAR surface expression [112]. LTD and the A β -induced loss of synaptic AMPARs share other signaling mechanisms, including p38, calcineurin and GSK3 β [109]. Inhibition of calcineurin-mediated AMPAR endocytosis prevents A β -induced AMPAR internalization and spine loss [113]. Similarly, GSK3 β inhibition prevents A β effects on steady-state AMPAR surface expression and delivery of AMPARs into spines following LTP [114]. Interestingly, the same study reported a spine-specific correlation between the presence of mitochondria and resistance to A β -induced effects on AMPAR trafficking. A β also interferes with AMPAR trafficking by competition with proteolytic maturation of brain-derived neurotrophic factor (BDNF), which is required for synaptic potentiation associated with an *in vitro* model of eyeblink classical conditioning [115].

The identity of endogenous ligand(s) for A β is the subject of some controversy. A β oligomers bind to the cellular prion protein (PrP^C), but this accounts for only half of the total oligomer binding [116]. However, although A β oligomers interact with PrP^C, studies using higher concentrations of A β showed no difference between wild-type and PrP^C-knockout mice, suggesting that this interaction does not mediate the effects of A β oligomers [117]. A β oligomers also bind directly to the extracellular fibronectin repeat of the EphB2 receptor. This interaction leads to proteasomal degradation of EphB2, which in turn reduces LTP [118]. Overexpression of EphB2 in a mouse model of AD restores wild-type LTP and reverses memory impairment [118]. Intriguingly, A β oligomers preferentially label GluA2-positive spines and crosslinking experiments suggest that the A β oligomers bind in close proximity to GluA2-containing complexes [113]. Furthermore, AMPAR antagonists inhibit A β oligomer binding and synaptic loss [113], raising the as yet unsubstantiated possibility that A β binds directly to a GluA2 protein complex.

Another neurological disorder linked with aberrant AMPAR trafficking is FXS. FMRP is a translational repressor that colocalizes with other components of translational machinery in dendrites [119]. FMRP-knockout mice display a variety of phenotypes, including higher basal translation of GluA1 and GluA2 subunits [120] and lower surface expression of AMPARs [121]. Some forms of synaptic plasticity are affected in the mutant mice, probably in a developmentally regulated manner [122]. These include mGluR5-dependent LTP in the amygdala [121], muscarinic receptor (mAChR)-dependent LTD in CA1 region [123] and hippocampal homeostatic plasticity [101].

A key question is what mechanisms are responsible for the FMRP null phenotype. FMRP in dendrites binds to Arc/Arg3.1 mRNA and FMRP removal abolishes the rapid translation of Arc/Arg3.1 during mGluR5-dependent LTD [124]. RNAi knockdown of FMRP in hippocampal neuronal cultures increases AMPAR constitutive endocytosis and occludes mGluR-dependent AMPAR internalization, which can be reversed by blocking the constitutive activity of mGluR5s [125]. Decreased FMRP also causes excessive phosphoinositide 3-kinase (PI3K) activity and PI3K antagonists rescue synaptic protein synthesis and return AMPAR internalization rates to normal [126]. In the prefrontal cortex, FMRP has been implicated in the regulation of AMPAR surface expression via dopaminergic signaling [127]. This pathway is disrupted in FMRP-null mice owing to dopamine receptor D1 hyperphosphorylation by the G-protein coupled receptor kinase GRK2 and is restored on pharmacological inhibition of GRK2.

Future directions

There has been remarkable progress in the field of AMPAR trafficking over the past several years. Ever increasing details of how AMPARs are inserted into and removed from the plasma membrane have been elucidated, as well as uncovering details of how AMPARs diffuse laterally to and from the synapses. Impressive though these advances are, more work is needed before it will be possible to apply this knowledge to design therapeutic strategies for correcting defects in higher brain function (Box 3). For instance, if memory is encoded by synaptic plasticity, which in turn is defined by the properties of synaptic AMPARs, how is this maintained for years, given the lability of synaptic AMPARs? The answers to questions such as this, which require the integration of molecular neurobiology, network activity, behavior and cognitive approaches, represent formidable but fundamentally important long-term challenges.

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Glossary

4.1N	neuronal isoform of erythrocyte membrane cytoskeleton protein involved in maintenance of the actin cytoskeleton and activity-dependent surface expression of GluA1.
Amyloid beta (Aβ)	36–42 residue peptides formed from amyloid precursor protein (APP) when cleaved by β -secretase. Soluble A β oligomers might be causative agents in AD.
Activity-regulated cytoskeleton-associated protein (aka Arg3.1)	immediate early gene (IEG) mRNA localized at active synapses. Arc/Arg3.1 protein expression decreases surface AMPARs by promoting endocytosis.
Ephrins and Eph receptors	bidirectional receptor tyrosine kinase signaling system that mediates multiple neuronal processes, including cell migration, neurite outgrowth and synapse formation.
Exocyst complex	octameric complex of proteins that directs exocytic vesicles to defined plasma membrane sites.
Homer	adaptor/scaffold protein enriched in the PSD that binds multiple proteins, tethering them into signaling complexes. The Homer1a isoform is induced by neuronal activity.
Integrins	cell surface signaling receptors that activate kinases and also act as cell adhesion molecules as well as attaching to components of the extracellular matrix.
Kalirin-7 (huntingtin-associated protein interacting protein, HAPIP)	isoform of Rho GEF involved in synapse remodeling and implicated in schizophrenia and AD.
N-cadherins	Ca ²⁺ -dependent transmembrane cell adhesion proteins that bring regions of membranes from separate cells (e.g. pre- and

	postsynaptic) in close juxtaposition. The intracellular domain binds catenins that regulate the actin cytoskeleton.
Neurexin–neuroligin	presynaptic neurexins are receptors for postsynaptic neuroligin. The neurexin–neuroligin interaction is required for synapse formation and promotes AMPAR accumulation.
N-ethylmaleimide-sensitive factor	homohexameric ATPase that is crucial for presynaptic neurotransmitter release and is involved in postsynaptic AMPAR trafficking.
p21 activated kinases	kinases activated by the small GTP binding proteins CDC42 and Rac1. Involved in a wide range of cellular signaling activities.
Palmitoylation	fatty acid (usually palmitic acid) post-translational modification of cysteine residues in membrane proteins that alter the properties/functions of the substrate protein.
Protein interacting with C kinase 1 (PICK1)	multifunctional adaptor protein that interacts with many PDZ ligand proteins, including AMPAR subunits, to regulate their distribution and function.
Protein kinase M zeta (PKMζ)	N-terminal truncated form of the atypical PKC ζ proposed as a memory substrate responsible for maintaining LTP.
Ras–ERK signaling pathway	phosphorylation signaling pathway that communicates events occurring at the cell surface detected by receptor tyrosine kinases via activation of small Ras family GTPases to complex intracellular kinase cascades (e.g. the ERK pathway), which invoke appropriate cellular responses.
Stargazin (γ-2)	the first TARP to be characterized. It is classified as an AMPAR auxiliary subunit.
Total internal reflection fluorescence (TIRF) microscopy	specialized microscope that uses an evanescent wave to excite fluorophores immediately adjacent to the glass–water interface, allowing selective visualization of events in the plasma membrane.

References

1. Shepherd JD, Huganir RL. The cell biology of synaptic plasticity: AMPA receptor trafficking. *Annu. Rev. Cell Dev. Biol.* 2007; 23:613–643. [PubMed: 17506699]
2. Turrigiano GG. The self-tuning neuron: synaptic scaling of excitatory synapses. *Cell.* 2008; 135:422–435. [PubMed: 18984155]
3. Malinow R, Malenka RC. AMPA receptor trafficking and synaptic plasticity. *Annu. Rev. Neurosci.* 2002; 25:103–126. [PubMed: 12052905]
4. Palmer CL, et al. The molecular pharmacology and cell biology of α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors. *Pharmacol. Rev.* 2005; 57:253–277. [PubMed: 15914469]
5. Wang JQ, et al. Phosphorylation of AMPA receptors: mechanisms and synaptic plasticity. *Mol. Neurobiol.* 2005; 32:237–249. [PubMed: 16385140]
6. Nicoll RA, et al. Auxiliary subunits assist AMPA-type glutamate receptors. *Science.* 2006; 311:1253–1256. [PubMed: 16513974]
7. Kennedy MJ, Ehlers MD. Organelles and trafficking machinery for postsynaptic plasticity. *Annu. Rev. Neurosci.* 2006; 29:325–362. [PubMed: 16776589]

8. Santos SD, et al. Regulation of AMPA receptors and synaptic plasticity. *Neuroscience*. 2009; 158:105–125. [PubMed: 18424006]
9. Derkach VA, et al. Regulatory mechanisms of AMPA receptors in synaptic plasticity. *Nat. Rev. Neurosci.* 2007; 8:101–113. [PubMed: 17237803]
10. Nakagawa T. The biochemistry, ultrastructure and subunit assembly mechanism of AMPA receptors. *Mol. Neurobiol.* 2010; 42:161–184. [PubMed: 21080238]
11. Adesnik H, et al. Photoinactivation of native AMPA receptors reveals their real-time trafficking. *Neuron*. 2005; 48:977–985. [PubMed: 16364901]
12. Perestenko PV, Henley JM. Characterization of the intracellular transport of GluR1 and GluR2 alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor subunits in hippocampal neurons. *J. Biol. Chem.* 2003; 278:43525–43532. [PubMed: 12909632]
13. Setou M, et al. Glutamate-receptor-interacting protein GRIP1 directly steers kinesin to dendrites. *Nature*. 2002; 417:83–87. [PubMed: 11986669]
14. Shin H, et al. Association of the kinesin motor KIF1A with the multimodular protein liprin-alpha. *J. Biol. Chem.* 2003; 278:11393–11401. [PubMed: 12522103]
15. Ko J, et al. Interaction between liprin-alpha and GIT1 is required for AMPA receptor targeting. *J. Neurosci.* 2003; 23:1667–1677. [PubMed: 12629171]
16. Kapitein LC, et al. Mixed microtubules steer dynein-driven cargo transport into dendrites. *Curr. Biol.* 2010; 20:290–299. [PubMed: 20137950]
17. Lise MF, et al. Involvement of myosin Vb in glutamate receptor trafficking. *J. Biol. Chem.* 2006; 281:3669–3678. [PubMed: 16338934]
18. Wang Z, et al. Myosin Vb mobilizes recycling endosomes and AMPA receptors for postsynaptic plasticity. *Cell*. 2008; 135:535–548. [PubMed: 18984164]
19. Correia SS, et al. Motor protein-dependent transport of AMPA receptors into spines during long-term potentiation. *Nat. Neurosci.* 2008; 11:457–466. [PubMed: 18311135]
20. Yang Y, et al. Delivery of AMPA receptors to perisynaptic sites precedes the full expression of long-term potentiation. *Proc. Natl. Acad. Sci. U.S.A.* 2008; 105:11388–11393. [PubMed: 18682558]
21. Kiehl A, et al. Activity patterns govern synapse-specific AMPA receptor trafficking between deliverable and synaptic pools. *Neuron*. 2009; 62:84–101. [PubMed: 19376069]
22. Stenmark H. Rab GTPases as coordinators of vesicle traffic. *Nat. Rev. Mol. Cell Biol.* 2009; 10:513–525. [PubMed: 19603039]
23. Hoogenraad CC, et al. Neuron specific Rab4 effector GRASP-1 coordinates membrane specialization and maturation of recycling endosomes. *PLoS Biol.* 2010; 8:e1000283. [PubMed: 20098723]
24. Patterson MA, et al. AMPA receptors are exocytosed in stimulated spines and adjacent dendrites in a Ras-ERK-dependent manner during long-term potentiation. *Proc. Natl. Acad. Sci. U.S.A.* 2010; 107:15951–15956. [PubMed: 20733080]
25. Jaskolski F, et al. Dynamin-dependent membrane drift recruits AMPA receptors to dendritic spines. *J. Biol. Chem.* 2009; 284:12491–12503. [PubMed: 19269965]
26. Araki Y, et al. Plasma membrane insertion of the AMPA receptor GluA2 subunit is regulated by NSF binding and Q/R editing of the ion pore. *Proc. Natl. Acad. Sci. U.S.A.* 2010; 107:11080–11085. [PubMed: 20534470]
27. Tunquist BJ, et al. Loss of AKAP150 perturbs distinct neuronal processes in mice. *Proc. Natl. Acad. Sci. U.S.A.* 2008; 105:12557–12562. [PubMed: 18711127]
28. Bhattacharyya S, et al. A critical role for PSD-95/AKAP interactions in endocytosis of synaptic AMPA receptors. *Nat. Neurosci.* 2009; 12:172–181. [PubMed: 19169250]
29. Jurado S, et al. A calcineurin/AKAP complex is required for NMDA receptor-dependent long-term depression. *Nat. Neurosci.* 2010; 13:1053–1055. [PubMed: 20694001]
30. Dev KK, Henley JM. The schizophrenic faces of PICK1. *Trends Pharmacol. Sci.* 2006; 27:574–579. [PubMed: 17011050]
31. Hanley JG. PICK1: a multi-talented modulator of AMPA receptor trafficking. *Pharmacol. Ther.* 2008; 118:152–160. [PubMed: 18353440]

32. Hanley JG, Henley JM. PICK1 is a calcium-sensor for NMDA-induced AMPA receptor trafficking. *EMBO J.* 2005; 24:3266–3278. [PubMed: 16138078]
33. Rocca DL, et al. Inhibition of Arp2/3-mediated actin polymerization by PICK1 regulates neuronal morphology and AMPA receptor endocytosis. *Nat. Cell Biol.* 2008; 10:259–271. [PubMed: 18297063]
34. Lin DT, Huganir RL. PICK1 and phosphorylation of the glutamate receptor 2 (GluR2) AMPA receptor subunit regulates GluR2 recycling after NMDA receptor-induced internalization. *J. Neurosci.* 2007; 27:13903–13908. [PubMed: 18077702]
35. Citri A, et al. Calcium binding to PICK1 is essential for the intracellular retention of AMPA receptors underlying long-term depression. *J. Neurosci.* 2010; 30:16437–16452. [PubMed: 21147983]
36. Scholz R, et al. AMPA receptor signaling through BRAG2 and Arf6 critical for long-term synaptic depression. *Neuron.* 2010; 66:768–780. [PubMed: 20547133]
37. Han K, et al. Regulated RalBP1 binding to RalA and PSD-95 controls AMPA receptor endocytosis and LTD. *PLoS Biol.* 2009; 7:e1000187. [PubMed: 19823667]
38. Lee HW, et al. The phosphoinositide 3-phosphatase MTMR2 interacts with PSD-95 and maintains excitatory synapses by modulating endosomal traffic. *J. Neurosci.* 2010; 30:5508–5518. [PubMed: 20410104]
39. Lu J, et al. Postsynaptic positioning of endocytic zones and AMPA receptor cycling by physical coupling of dynamin-3 to Homer. *Neuron.* 2007; 55:874–889. [PubMed: 17880892]
40. Kennedy MJ, et al. Syntaxin-4 defines a domain for activity-dependent exocytosis in dendritic spines. *Cell.* 2010; 141:524–535. [PubMed: 20434989]
41. Petrini EM, et al. Endocytic trafficking and recycling maintain a pool of mobile surface AMPA receptors required for synaptic potentiation. *Neuron.* 2009; 63:92–105. [PubMed: 19607795]
42. Ling DSF, et al. Protein kinase Mzeta is necessary and sufficient for LTP maintenance. *Nat. Neurosci.* 2002; 5:295–296. [PubMed: 11914719]
43. Sacktor TC. PKMzeta, LTP maintenance and the dynamic molecular biology of memory storage. *Prog. Brain Res.* 2008; 169:27–40. [PubMed: 18394466]
44. Pastalkova E, et al. Storage of spatial information by the maintenance mechanism of LTP. *Science.* 2006; 313:1141–1144. [PubMed: 16931766]
45. Yao Y, et al. PKM zeta maintains late long-term potentiation by N-ethylmaleimide-sensitive factor/GluR2-dependent trafficking of postsynaptic AMPA receptors. *J. Neurosci.* 2008; 28:7820–7827. [PubMed: 18667614]
46. Miguez PV, et al. PKMzeta maintains memories by regulating GluR2-dependent AMPA receptor trafficking. *Nat. Neurosci.* 2010; 13:630–634. [PubMed: 20383136]
47. Tai CY, et al. Cadherins and synaptic plasticity. *Curr. Opin. Cell Biol.* 2008; 20:567–575. [PubMed: 18602471]
48. Nuriya M, Huganir RL. Regulation of AMPA receptor trafficking by N-cadherin. *J. Neurochem.* 2006; 97:652–661. [PubMed: 16515543]
49. Silverman JB, et al. Synaptic anchorage of AMPA receptors by cadherins through neural plakophilin-related arm protein AMPA receptor-binding protein complexes. *J. Neurosci.* 2007; 27:8505–8516. [PubMed: 17687028]
50. Saglietti L, et al. Extracellular interactions between GluR2 and N-cadherin in spine regulation. *Neuron.* 2007; 54:461–477. [PubMed: 17481398]
51. Craig AM, Kang Y. Neurexin-neuroligin signaling in synapse development. *Curr. Opin. Neurobiol.* 2007; 17:43–52. [PubMed: 17275284]
52. Heine M, et al. Activity-independent and subunit-specific recruitment of functional AMPA receptors at neurexin/neuroligin contacts. *Proc. Natl. Acad. Sci. U.S.A.* 2008; 105:20947–20952. [PubMed: 19098102]
53. Thyagarajan A, Ting AY. Imaging activity-dependent regulation of neurexin-neuroligin interactions using trans-synaptic enzymatic biotinylation. *Cell.* 2010; 143:456–469. [PubMed: 20933261]

54. Cingolani LA, et al. Activity-dependent regulation of synaptic AMPA receptor composition and abundance by beta3 integrins. *Neuron*. 2008; 58:749–762. [PubMed: 18549786]
55. Ziff EB. TARPs and the AMPA receptor trafficking paradox. *Neuron*. 2007; 53:627–633. [PubMed: 17329203]
56. Kato AS, et al. TARPs differentially decorate AMPA receptors to specify neuropharmacology. *Trends Neurosci*. 2010; 33:241–248. [PubMed: 20219255]
57. Bats C, et al. The interaction between Stargazin and PSD-95 regulates AMPA receptor surface trafficking. *Neuron*. 2007; 53:719–734. [PubMed: 17329211]
58. Tomita S, et al. Bidirectional synaptic plasticity regulated by phosphorylation of stargazin-like TARPs. *Neuron*. 2005; 45:269–277. [PubMed: 15664178]
59. Kessels HW, et al. Roles of stargazin and phosphorylation in the control of AMPA receptor subcellular distribution. *Nat. Neurosci*. 2009; 12:888–896. [PubMed: 19543281]
60. Sumioka A, et al. TARP phosphorylation regulates synaptic AMPA receptors through lipid bilayers. *Neuron*. 2010; 66:755–767. [PubMed: 20547132]
61. Opazo P, et al. CaMKII triggers the diffusional trapping of surface AMPARs through phosphorylation of stargazin. *Neuron*. 2010; 67:239–252. [PubMed: 20670832]
62. Soto D, et al. Selective regulation of long-form calcium-permeable AMPA receptors by an atypical TARP, gamma-5. *Nat. Neurosci*. 2009; 12:277–285. [PubMed: 19234459]
63. Schwenk J, et al. Functional proteomics identify cornichon proteins as auxiliary subunits of AMPA receptors. *Science*. 2009; 323:1313–1319. [PubMed: 19265014]
64. Shi Y, et al. Functional comparison of the effects of TARPs and cornichons on AMPA receptor trafficking and gating. *Proc. Natl. Acad. Sci. U.S.A.* 2010; 107:16315–16319. [PubMed: 20805473]
65. von Engelhardt J, et al. CKAMP44: a brain-specific protein attenuating short-term synaptic plasticity in the dentate gyrus. *Science*. 2010; 327:1518–1522. [PubMed: 20185686]
66. Lee HK. Synaptic plasticity and phosphorylation. *Pharmacol. Ther.* 2006; 112:810–832. [PubMed: 16904750]
67. Lu W, et al. Subunit composition of synaptic AMPA receptors revealed by a single-cell genetic approach. *Neuron*. 2009; 62:254–268. [PubMed: 19409270]
68. Isaac JT, et al. The role of the GluR2 subunit in AMPA receptor function and synaptic plasticity. *Neuron*. 2007; 54:859–871. [PubMed: 17582328]
69. Liu SJ, Zukin RS. Ca21-permeable AMPA receptors in synaptic plasticity and neuronal death. *Trends Neurosci*. 2007; 30:126–134. [PubMed: 17275103]
70. Cull-Candy S, et al. Regulation of Ca21-permeable AMPA receptors: synaptic plasticity and beyond. *Curr. Opin. Neurobiol.* 2006; 16:288–297. [PubMed: 16713244]
71. Plant K, et al. Transient incorporation of native GluR2-lacking AMPA receptors during hippocampal long-term potentiation. *Nat. Neurosci*. 2006; 9:602–604. [PubMed: 16582904]
72. Guire ES, et al. Recruitment of calcium-permeable AMPA receptors during synaptic potentiation is regulated by CaM-kinase I. *J. Neurosci*. 2008; 28:6000–6009. [PubMed: 18524905]
73. Lin DT, et al. Regulation of AMPA receptor extrasynaptic insertion by 4.1N, phosphorylation and palmitoylation. *Nat. Neurosci*. 2009; 12:879–887. [PubMed: 19503082]
74. Kanno T, et al. Regulation of AMPA receptor trafficking by O-glycosylation. *Neurochem. Res.* 2010; 35:782–788. [PubMed: 20165912]
75. Din N, et al. The function of GluR1 and GluR2 in cerebellar and hippocampal LTP and LTD is regulated by interplay of phosphorylation and O-GlcNAc modification. *J. Cell. Biochem.* 2010; 109:585–597. [PubMed: 20052678]
76. Bingol B, Schuman EM. Activity-dependent dynamics and sequestration of proteasomes in dendritic spines. *Nature*. 2006; 441:1144–1148. [PubMed: 16810255]
77. Djakovic SN, et al. Regulation of the proteasome by neuronal activity and calcium/calmodulin-dependent protein kinase II. *J. Biol. Chem.* 2009; 284:26655–26665. [PubMed: 19638347]
78. Bingol B, et al. Autophosphorylated CaMKIIalpha acts as a scaffold to recruit proteasomes to dendritic spines. *Cell*. 2010; 140:567–578. [PubMed: 20178748]

79. Zhang D, et al. Na,K-ATPase activity regulates AMPA receptor turnover through proteasome-mediated proteolysis. *J. Neurosci.* 2009; 29:4498–4511. [PubMed: 19357275]
80. Colledge M, et al. Ubiquitination regulates PSD-95 degradation and AMPA receptor surface expression. *Neuron.* 2003; 40:595–607. [PubMed: 14642282]
81. Bingol B, Schuman EM. A proteasome-sensitive connection between PSD-95 and GluR1 endocytosis. *Neuropharmacology.* 2004; 47:755–763. [PubMed: 15458847]
82. Citri A, et al. N-methyl-D-aspartate receptor- and metabotropic glutamate receptor-dependent long-term depression are differentially regulated by the ubiquitin-proteasome system. *Eur. J. Neurosci.* 2009; 30:1443–1450. [PubMed: 19821836]
83. Park EC, et al. The ubiquitin ligase RPM-1 and the p38 MAPK PMK-3 regulate AMPA receptor trafficking. *PLoS ONE.* 2009; 4:e4284. [PubMed: 19172179]
84. Schwarz LA, et al. Activity-dependent ubiquitination of GluA1 mediates a distinct AMPA receptor endocytosis and sorting pathway. *J. Neurosci.* 2010; 30:16718–16729. [PubMed: 21148011]
85. Fu AKY, et al. EphA4 signaling mediates APCCdh1-dependent down-regulation of AMPAR in homeostatic plasticity. *Nat. Neurosci.* 2011; 14:181–189. [PubMed: 21186356]
86. Kantamneni S, et al. Ubiquitin regulation of neuronal excitability. *Nat. Neurosci.* 2011; 14:126–128. [PubMed: 21270777]
87. Penzes P, Jones KA. Dendritic spine dynamics – a key role for kalirin-7. *Trends Neurosci.* 2008; 31:419–427. [PubMed: 18597863]
88. Xie Z, et al. Kalirin-7 controls activity-dependent structural and functional plasticity of dendritic spines. *Neuron.* 2007; 56:640–656. [PubMed: 18031682]
89. Stornetta RL, Zhu JJ. Ras and Rap signaling in synaptic plasticity and mental disorders. *Neuroscientist.* 2011; 17:54–78. [PubMed: 20431046]
90. Soderling SH. Grab your partner with both hands: cytoskeletal remodeling by Arp2/3 signaling. *Sci. Signal.* 2009; 2:pe5. [PubMed: 19176514]
91. Nakamura Y, et al. PICK1 inhibition of the Arp2/3 complex controls dendritic spine size and synaptic plasticity. *EMBO J.* 2011; 30:719–730. [PubMed: 21252856]
92. Bramham CR, et al. The immediate early gene *arc/arg3.1*: regulation, mechanisms and function. *J. Neurosci.* 2008; 28:11760–11767. [PubMed: 19005037]
93. Gao M, et al. A specific requirement of *Arc/Arg3.1* for visual experience-induced homeostatic synaptic plasticity in mouse primary visual cortex. *J. Neurosci.* 2010; 30:7168–7178. [PubMed: 20505084]
94. Peebles CL, et al. *Arc* regulates spine morphology and maintains network stability *in vivo*. *Proc. Natl. Acad. Sci. U.S.A.* 2010; 107:18173–18178. [PubMed: 20921410]
95. Shepherd JD, et al. *Arc/Arg3.1* mediates homeostatic synaptic scaling of AMPA receptors. *Neuron.* 2006; 52:475–484. [PubMed: 17088213]
96. Waung MW, et al. Rapid translation of *Arc/Arg3.1* selectively mediates mGluR-dependent LTD through persistent increases in AMPAR endocytosis rate. *Neuron.* 2008; 59:84–97. [PubMed: 18614031]
97. Beique JC, et al. *Arc*-dependent synapse-specific homeostatic plasticity. *Proc. Natl. Acad. Sci. U.S.A.* 2011; 108:816–821. [PubMed: 21187403]
98. Chowdhury S, et al. *Arc/Arg3.1* interacts with the endocytic machinery to regulate AMPA receptor trafficking. *Neuron.* 2006; 52:445–459. [PubMed: 17088211]
99. Greer PL, et al. The Angelman Syndrome protein Ube3A regulates synapse development by ubiquitinating *arc*. *Cell.* 2010; 140:704–716. [PubMed: 20211139]
100. Aoto J, et al. Synaptic signaling by all-trans retinoic acid in homeostatic synaptic plasticity. *Neuron.* 2008; 60:308–320. [PubMed: 18957222]
101. Soden ME, Chen L. Fragile X protein FMRP is required for homeostatic plasticity and regulation of synaptic strength by retinoic acid. *J. Neurosci.* 2010; 30:16910–16921. [PubMed: 21159962]
102. Evers DM, et al. Plk2 attachment to NSF induces homeostatic removal of GluA2 during chronic overexcitation. *Nat. Neurosci.* 2010; 13:1199–1207. [PubMed: 20802490]

103. Groth RD, et al. β -Ca21/CaM-dependent kinase type II triggers upregulation of GluA1 to coordinate adaptation to synaptic inactivity in hippocampal neurons. *Proc. Natl. Acad. Sci. U.S.A.* 2011; 108:828–833. [PubMed: 21187407]
104. Bowers MS, et al. AMPA receptor synaptic plasticity induced by psychostimulants: the past, present and therapeutic future. *Neuron*. 2010; 67:11–24. [PubMed: 20624588]
105. Tang BL. Neuronal protein trafficking associated with Alzheimer disease: from APP and BACE1 to glutamate receptors. *Cell Adh. Migr.* 2009; 3:118–128. [PubMed: 19372755]
106. Opazo P, Choquet D. A three-step model for the synaptic recruitment of AMPA receptors. *Mol. Cell. Neurosci.* 2010; 46:1–8. [PubMed: 20817097]
107. Walsh DM, Selkoe DJ. A beta oligomers – a decade of discovery. *J. Neurochem.* 2007; 101:1172–1184. [PubMed: 17286590]
108. Shankar GM, et al. Amyloid-beta protein dimers isolated directly from Alzheimer's brains impair synaptic plasticity and memory. *Nat. Med.* 2008; 14:837–842. [PubMed: 18568035]
109. Li S, et al. Soluble oligomers of amyloid beta protein facilitate hippocampal long-term depression by disrupting neuronal glutamate uptake. *Neuron*. 2009; 62:788–801. [PubMed: 19555648]
110. Ting JT, et al. Amyloid precursor protein overexpression depresses excitatory transmission through both presynaptic and postsynaptic mechanisms. *Proc. Natl. Acad. Sci. U.S.A.* 2007; 104:353–358. [PubMed: 17185415]
111. Hsieh H, et al. AMPAR removal underlies Abeta-induced synaptic depression and dendritic spine loss. *Neuron*. 2006; 52:831–843. [PubMed: 17145504]
112. Gu Z, et al. β -Amyloid impairs AMPA receptor trafficking and function by reducing Ca21/calmodulin-dependent protein kinase II synaptic distribution. *J. Biol. Chem.* 2009; 284:10639–10649. [PubMed: 19240035]
113. Zhao WQ, et al. Inhibition of calcineurin-mediated endocytosis and alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors prevents amyloid beta oligomer-induced synaptic disruption. *J. Biol. Chem.* 2010; 285:7619–7632. [PubMed: 20032460]
114. Rui Y, et al. Inhibition of AMPA receptor trafficking at hippocampal synapses by β -amyloid oligomers: the mitochondrial contribution. *Mol. Brain*. 2010; 3:10. [PubMed: 20346152]
115. Zheng Z, et al. Oligomeric amyloid- β inhibits the proteolytic conversion of brain-derived neurotrophic factor (BDNF), AMPA receptor trafficking and classical conditioning. *J. Biol. Chem.* 2010; 285:34708–34717. [PubMed: 20807770]
116. Lauren J, et al. Cellular prion protein mediates impairment of synaptic plasticity by amyloid-beta oligomers. *Nature*. 2009; 457:1128–1132. [PubMed: 19242475]
117. Kessels HW, et al. The prion protein as a receptor for amyloid-beta. *Nature*. 2010; 466:E3–4. discussion E4–5. [PubMed: 20703260]
118. Cisse M, et al. Reversing EphB2 depletion rescues cognitive functions in Alzheimer model. *Nature*. 2011; 469:47–52. [PubMed: 21113149]
119. Giorgi C, et al. The EJC factor eIF4AIII modulates synaptic strength and neuronal protein expression. *Cell*. 2007; 130:179–191. [PubMed: 17632064]
120. Muddashetty RS, et al. Dysregulated metabotropic glutamate receptor-dependent translation of AMPA receptor and postsynaptic density-95 mRNAs at synapses in a mouse model of fragile X syndrome. *J. Neurosci.* 2007; 27:5338–5348. [PubMed: 17507556]
121. Suvrathan A, et al. Characterization and reversal of synaptic defects in the amygdala in a mouse model of fragile X syndrome. *Proc. Natl. Acad. Sci. U.S.A.* 2010; 107:11591–11596. [PubMed: 20534533]
122. Pilpel Y, et al. Synaptic ionotropic glutamate receptors and plasticity are developmentally altered in the CA1 field of Fmr1 knockout mice. *J. Physiol.* 2009; 587:787–804. [PubMed: 19103683]
123. Volk LJ, et al. Multiple Gq-coupled receptors converge on a common protein synthesis-dependent long-term depression that is affected in fragile X syndrome mental retardation. *J. Neurosci.* 2007; 27:11624–11634. [PubMed: 17959805]
124. Park S, et al. Elongation factor 2 and fragile X mental retardation protein control the dynamic translation of Arc/Arg3.1 essential for mGluR-LTD. *Neuron*. 2008; 59:70–83. [PubMed: 18614030]

125. Nakamoto M, et al. Fragile X mental retardation protein deficiency leads to excessive mGluR5-dependent internalization of AMPA receptors. *Proc. Natl. Acad. Sci. U.S.A.* 2007; 104:15537–15542. [PubMed: 17881561]
126. Gross C, et al. Excess phosphoinositide 3-kinase subunit synthesis and activity as a novel therapeutic target in fragile X syndrome. *J. Neurosci.* 2010; 30:10624–10638. [PubMed: 20702695]
127. Wang H, et al. FMRP acts as a key messenger for dopamine modulation in the forebrain. *Neuron.* 2008; 59:634–647. [PubMed: 18760699]
128. Boehm J, et al. Synaptic incorporation of AMPA receptors during LTP is controlled by a PKC phosphorylation site on GluR1. *Neuron.* 2006; 51:213–225. [PubMed: 16846856]
129. Oh MC, Derkach VA. Dominant role of the GluR2 subunit in regulation of AMPA receptors by CaMKII. *Nat. Neurosci.* 2005; 8:853–854. [PubMed: 15924137]
130. Lee HK, et al. Identification and characterization of a novel phosphorylation site on the GluR1 subunit of AMPA receptors. *Mol. Cell. Neurosci.* 2007; 36:86–94. [PubMed: 17689977]
131. Delgado JY, et al. NMDA receptor activation dephosphorylates AMPA receptor glutamate receptor 1 subunits at threonine 840. *J. Neurosci.* 2007; 27:13210–13221. [PubMed: 18045915]
132. Oh MC, et al. Extrasynaptic membrane trafficking regulated by GluR1 serine 845 phosphorylation primes AMPA receptors for long-term potentiation. *J. Biol. Chem.* 2006; 281:752–758. [PubMed: 16272153]
133. Thomas GM, et al. Rapid and bi-directional regulation of AMPA receptor phosphorylation and trafficking by JNK. *EMBO J.* 2008; 27:361–372. [PubMed: 18188153]
134. Seidenman KJ, et al. Glutamate receptor subunit 2 Serine 880 phosphorylation modulates synaptic transmission and mediates plasticity in CA1 pyramidal cells. *J. Neurosci.* 2003; 23:9220–9228. [PubMed: 14534256]
135. Hayashi T, et al. Differential regulation of AMPA receptor subunit trafficking by palmitoylation of two distinct sites. *Neuron.* 2005; 47:709–723. [PubMed: 16129400]
136. Gerges NZ, et al. Dual role of the exocyst in AMPA receptor targeting and insertion into the postsynaptic membrane. *EMBO J.* 2006; 25:1623–1634. [PubMed: 16601687]
137. Corera AT, et al. Long-term potentiation in isolated dendritic spines. *PLoS ONE.* 2009; 4:e6021. [PubMed: 19547754]
138. Yudowski GA, et al. Real-time imaging of discrete exocytic events mediating surface delivery of AMPA receptors. *J. Neurosci.* 2007; 27:11112–11121. [PubMed: 17928453]
139. Makino H, Malinow R. AMPA receptor incorporation into synapses during LTP: the role of lateral movement and exocytosis. *Neuron.* 2009; 64:381–390. [PubMed: 19914186]
140. Adesnik H, Nicoll RA. Conservation of glutamate receptor 2-containing AMPA receptors during long-term potentiation. *J. Neurosci.* 2007; 27:4598–4602. [PubMed: 17460072]
141. Gray EE, et al. Long-term potentiation in the hippocampal CA1 region does not require insertion and activation of GluR2-lacking AMPA receptors. *J. Neurophysiol.* 2007; 98:2488–2492. [PubMed: 17652419]
142. Lu Y, et al. Age-dependent requirement of AKAP150-anchored PKA and GluR2-lacking AMPA receptors in LTP. *EMBO J.* 2007; 26:4879–4890. [PubMed: 17972919]
143. Gallo V, Ghiani CA. Glutamate receptors in glia: new cells, new inputs and new functions. *Trends Pharmacol. Sci.* 2000; 21:252–258. [PubMed: 10871893]

Box 1**Where does synaptic AMPAR exocytosis occur?**

In contrast to the well-defined localization of presynaptic exocytosis that mediates neurotransmitter release, the corresponding postsynaptic exocytic mechanisms that deliver neurotransmitter receptors, including AMPARs, to synapses have not yet been fully elucidated. There have been several studies using electrophysiology and recombinant fluorophore-tagged AMPAR subunits to visualize the insertion and trafficking of AMPARs in near real-time but, as outlined below, the exact locations of synaptic AMPAR exocytosis remain the subject of debate. The three main possibilities are: (i) insertion directly into the PSD; (ii) insertion into the dendritic spine membrane adjacent to, but outside, the PSD; and (iii) insertion into the dendritic shaft membrane outside the spine.

PSD

- AMPARs accumulate within the spine in the vicinity of the PSD following inhibition of the exocyst complex [136].
- Stimulation leads to increases in surface AMPAR content in synaptosomes [137].

Dendritic spine

- Two-photon imaging of GluA1 N-terminally fused to superecliptic phluorin (SEP) (SEP–GluA1) during spine-specific stimulation in hippocampal slices indicated that most of the activity-dependent insertion occurred either within or very close to spines [24].
- Confocal dual color imaging of SEP–GluA1 and mCherry-labeled transferrin receptor (mCherry–TfR; a marker for recycling endosomes) in dissociated hippocampal neurons demonstrated precise colocalization of mCherry–TfR and newly appearing SEP fluorescence within morphologically defined spines [40].

Dendritic shaft

- Epifluorescence imaging of SEP–GluA1 in dissociated neurons led to the observation of individual insertion events in the shaft. *Post hoc* labeling for PSD-95 indicated that insertion in unstimulated neurons did not colocalize with the PSD [138].
- The existence of perisynaptic AMPARs has been demonstrated by electrophysiological recordings in hippocampal slice preparations through the inhibition of glutamate reuptake. A rapid increase in perisynaptic AMPAR EPSCs was observed after theta burst stimulation [20].
- TIRF microscopy of SEP–GluA1 in unstimulated dissociated hippocampal neurons indicated that GluA1 insertion in the shaft occurred with very little SEP–GluA2 insertion [73].
- Chemically induced LTP and single spine stimulation in hippocampal slices, combined with dual imaging of dendritic morphology, revealed that SEP–GluA1 was preferentially inserted into the shaft, with very little insertion of SEP–GluA2 [139].

Box 2**Are CP-AMPARs involved in synaptic plasticity?**

The involvement and relative importance of CP-AMPARs in synaptic plasticity is currently a question of debate in the field. Evidence in support of this role has been suggested by several studies, as has evidence refuting their involvement. Their contributions to synaptic plasticity mechanisms might also occur at specific developmental time points only, and/or only in response to particular LTP induction protocols. Recent studies addressing this question are summarized below.

Yes

- LTP induced by a pairing protocol in the CA1 region of acute hippocampal slices from 2–3-week-old rats or mice was associated with a rapid insertion of GluA2-lacking AMPARs (i.e. CP-AMPARs) into potentiated synapses, which were replaced by GluA2-containing AMPARs after 25 min. This insertion was physiologically relevant, because pharmacological blockade of CP-AMPARs 3 min, but not 20 min, after stimulation, inhibited LTP [71].
- Inhibition of CaMKI inhibited the recruitment of CP-AMPARs during the early phase of LTP (induced by theta burst stimulation) in hippocampal slices derived from 4–6-week-old rats. By contrast, no involvement of CP-AMPARs was observed following LTP that was induced by high frequency stimulation [72].

No

- No evidence for the involvement of CP-AMPARs in LTP was observed following pairing protocol in hippocampal slices derived from 2–3-week-old rats [140].
- The insertion and activation of CP-AMPARs was found to not be involved in CA1-dependent LTP induced by high frequency stimulation in hippocampal slices of 8–12-week-old mice [141].

Sometimes

- Inhibitors of CP-AMPARs were demonstrated to block effectively single tetanus-induced hippocampal LTP in an age-dependent manner. CP-AMPARs were required for LTP in 2-week-old mice and in mice older than 8 weeks, but they were not required in mice aged 3–4 weeks [142].
- Prolonged silencing of individual spines was found to lead to synapse-specific insertion of CP-AMPARs in cultured dissociated hippocampal neurons. This suggests a link between synaptic accumulation of CP-AMPARs and homeostatic plasticity, which was demonstrated to be dependent on Arc/Arg3.1 [97].

Box 3**Outstanding questions**

- **Complexity of trafficking pathways**

Current evidence indicates multiple alternative routes for the delivery and removal of synaptic AMPARs. A more detailed understanding of the interrelationship, crosstalk and integration between these pathways is essential to intervene effectively and specifically in these processes to modify the availability of synaptic AMPARs. For example, what determines which pathways are utilized under basal, stimulated and pathophysiological conditions?

- **mRNA trafficking and local translation**

How is the dendritic and synaptic trafficking of mRNAs encoding AMPAR subunits and associated proteins regulated? How is local translation regulated by activity and what is the interrelationship between local protein synthesis and protein trafficking?

- **AMPAR assembly**

What are the mechanisms regulating the RNA editing, subunit composition and assembly of newly synthesized AMPARs and what processes control their endoplasmic reticulum exit and forward traffic to the membrane?

- **Regulation of CP-AMPARs**

What signals recruit GluA2-lacking AMPARs (i.e. CP-AMPARs) to synapses during LTP and how are the AMPAR subunits rearranged? Is there a reservoir of CP-AMPARs or are internalized receptors disassembled and reconfigured in the absence of the GluA2 subunit? Upon synaptic activity, what processes allow CP-AMPARs to be replaced with GluA2-containing AMPARs while maintaining LTP?

- **AMPAR trafficking and structural plasticity**

What are the mechanistic relationships between AMPAR trafficking and structural plasticity mediated by reorganization of the spine cytoskeleton?

- **Sorting of internalized AMPARs**

Do the sites of internalization and different endocytic pathways segregate AMPARs into distinct internal compartments and what determines the sorting of internalized receptors into recycling or degradative pathways? What are the cues and pathways that initiate AMPAR degradation? What are the sites for direct ubiquitination of AMPAR subunits and/or are AMPAR-associated proteins the primary targets for E3 ubiquitin ligases?

- **Non-neuronal AMPARs**

In addition to being expressed in neurons, non-neuronal cells, such as Bergmann glia, which play key roles in neuron–glial signaling, have been demonstrated to express AMPARs [62,143]. How is AMPAR trafficking regulated in these cells? Are the same signaling and trafficking pathways involved as in neurons?

- **Relationship of AMPAR trafficking to long-term memory storage**

AMPA receptors constantly traffic in and out of the synapse, yet information encoded within the synaptic network can persist for years. How this is achieved remains unknown, but an understanding of the mechanisms involved will be crucial for ultimately relating AMPAR trafficking to higher brain functions, such as learning, memory and cognition.

Figure 1.

Molecular processes involved in directing AMPAR trafficking in LTP. (a) Presynaptic glutamate release activates NMDARs, leading to Ca^{2+} influx in the postsynaptic cell. (b) Calcium activates CaMKII, leading to the phosphorylation of GluA1. (c) Receptors containing phosphorylated GluA1 are coupled via the Rab11 adaptor complex to the Ca^{2+} -activated motor protein MyoVa, which transports them over a short range along actin filaments from dendritic shafts to the spine head. (d) MyoVb is activated by Ca^{2+} and transports AMPAR along the actin cytoskeleton to sites of exocytosis. (e) PKC phosphorylation of GluA1 at S816 and S818 increases its affinity for the cytoskeletal adaptor protein 4.1N, which is required for membrane insertion and links AMPARs to the actin cytoskeleton. (f) PKA phosphorylation of GluA1 at S845 leads to AMPAR insertion at extrasynaptic and perisynaptic sites, ready for delivery to synapses. (g) Sites of exocytosis are enriched in syntaxin 4, which mediates membrane fusion events. (h) Diffusive Ras—ERK signaling is required for exocytosis on dendrites and spines up to 3 μm from the synaptic site of potentiation. (i) PKC phosphorylation of CP-AMPA receptors at perisynaptic sites leads to their transfer to synaptic sites. These are later replaced by edited GluA2-containing receptors. (j) Phosphorylation of stargazin ($\gamma 2$) by CaMKII. (k) The interaction between phosphorylated stargazin and PSD-95 traps AMPARs at synapses. (l) PKM ζ maintains AMPARs at synapses by downregulating GluA2-containing receptor internalization, possibly via NSF-mediated disassembly of GluA2–PICK1 complexes.

Figure 2.

Mechanisms of AMPARs endocytosis. Endocytosis in the spine might be localized to the clathrin-enriched endocytic zones adjacent to the PSD, shown here in green. The left spine depicts mechanisms involved in NMDAR-dependent endocytosis, whereas the right spine represents endocytosis not involving NMDAR activation, namely basal conditions, mGluR activation and synaptic scaling. (a) Phosphorylation of GluA2 at Tyr876 regulates its binding to BRAG2, which in turn activates the small GTPase Arf6 to internalize AMPARs on induction of both NMDAR- and mGluR-dependent LTD. (b) Ubiquitination by the E3 ligase Mdm2 and the subsequent proteasomal degradation of PSD-95 might be required for NMDAR-dependent endocytosis of AMPARs, although whether PSD-95 itself and/or an intermediate is polyubiquitinated for degradation remains to be further investigated. (c) Interactions between the synaptic anchoring protein, AKAP150 and calcineurin are required for NMDAR-dependent endocytosis of AMPARs. The binding of AKAP150 to PSD-95 is also required for NMDAR-dependent LTD. AKAP150 is involved in the localization of PKA and other proteins involved in the synaptic trafficking of AMPARs. (d) NMDAR stimulation activates RalA and dephosphorylates the endocytic adaptor, RalBP1. (e) RalA and PSD-95 target dephosphorylated RalBP1 to sites of endocytosis. (f) PICK1 interacts with the GluA2 subunit, promoting phosphorylation at Ser880 by PKC, leading to AMPAR endocytosis during NMDAR-LTD (however, alternative intracellular retention roles for PICK1 have also been proposed, as discussed in the main text). (g) PICK1 binds the Arp2/3 complex, an actin-nucleating protein, inhibiting its activity. This leads to a net reduction in the actin polymerization rate in treadmilling actin near the membrane, reducing membrane tension, which might promote AMPAR internalization subsequent to NMDAR stimulation [note, as for (f)]. (h) Arc/Arg3.1 interacts with components of endocytic machinery, such as endophilin and dynamin, to regulate constitutive endocytosis as well as internalization associated with mGluR activation and synaptic scaling. (i) The postsynaptic scaffold/adaptor protein, Homer, interacts with dynamin, coupling endocytic zones to the PSD. Loss of the Homer/dynamin interaction results in loss of clathrin and endocytosis at the PSD. (j) Eph4

activation leads to the ubiquitination and degradation of GluA1-containing receptors during homeostatic plasticity.

Table 1
Phosphorylation of GluA1 and GluA2 subunits and functional consequences on AMPAR trafficking

Site	Kinase	Functional roles	Refs
GluA1 – Ser816	PKC	Enhances binding to protein 4.1N to facilitate AMPAR membrane insertion	[73]
GluA1 – Ser818	PKC	Recruits AMPARs to PSDs; enhances binding to 4.1N; crucial for LTP-driven incorporation of AMPARs	[128]
GluA1 – Ser831	PKC, CamKII	Increases conductance of GluR1 homomers, but effect is lost in GluA1/GluA2 heteromers	[129]
GluA1 – Thr840	PKC, p70S6 kinase	PKC site in <i>in vitro</i> assays. High levels of basal phosphorylation in cells via p70S6 kinase and dephosphorylation might have a role in LTD	[130,131]
GluA1 – Ser845	PKA	Exocytosis of GluA1 to extrasynaptic sites on the plasma membrane increased synaptic AMPARs. Primes AMPARs for synaptic delivery by CamKII at Ser831	[132]
GluA2L ^a – Thr912	JNK1	Controls reinsertion of internalized GluA2L following NMDA treatment, without affecting basal GluA2L trafficking	[133]
GluA2 – Tyr876	Src	Controls binding to BRAG2 leading to activation of Arf6 and internalization during LTD	[36]
GluA2 – Ser880	PKC	Decreases GluA2 affinity for the anchoring protein GRIP, and increases its affinity for PICK1 involved in LTD	[134]

^a AMPAR subunits are categorized into long (L)- and short (S)-tailed classes according to their intracellular C-terminal domains. GluA2 and GluA4 mRNAs undergo alternative splicing that generates both L and S versions, whereas only GluA1L and GluA3S are produced. These different C-termini contain specific post-translational and protein interaction motifs, which specify binding to distinct sets of interacting proteins [4].

Table 2

Post-translational modifications (palmitoylation, glycosylation and ubiquitination^a) of GluA1 and GluA2 subunits and functional consequences on AMPAR trafficking^b

Site	Enzyme	Functional roles	Refs
Palmitoylation			
<ul style="list-style-type: none">GluA1 – Cys610GluA2 – Cys836GluA2 – Cys811	Palmitoyl acyl transferase family; consensus sequence asp-his-his-cys (DHHC)	Regulates interaction with 4.1N; modulates PKC phosphorylation of GluA1 and association with 4.1N	[73,135]
Glycosylation			
<ul style="list-style-type: none">GluA1 – predicted Ser818, 831 and 845GluA2 – predicted Ser 856	Addition: OGT; Removal: OGNase	Glycosylation by addition of the single sugar <i>O</i> -GlcNAc; proposed GluA1 sites modified by <i>O</i> -GlcNAc during hippocampal LTD and GluA2 <i>O</i> -GlcNAc modified during cerebellar LTP	[74,75]
Ubiquitination			
<ul style="list-style-type: none">GluA1 – lysine residue [identities of precise residue(s) unclear]GluA1 – one or more of four C-terminal lysines are ubiquitinated (precise residues not yet determined)	<ul style="list-style-type: none">APC E3 Ubiquitin ligase complex and activator Cdh1Nedd4-1 E3 ubiquitin ligase	<ul style="list-style-type: none">Homeostatic regulation of GluA1 proteasomal degradationRegulates trafficking of GluA1 to the late endosome for lysosomal degradation	<ul style="list-style-type: none">[85][84]

^aSee Table 1 for phosphorylation modifications of these subunits.

^bAbbreviations: APC, anaphase promoting complex; OGT, *O*-GlcNAc transferase; OGNase, *O*-GlcNAcase; *O*-GlcNAc, *O*-linked N-acetylglucosamine.