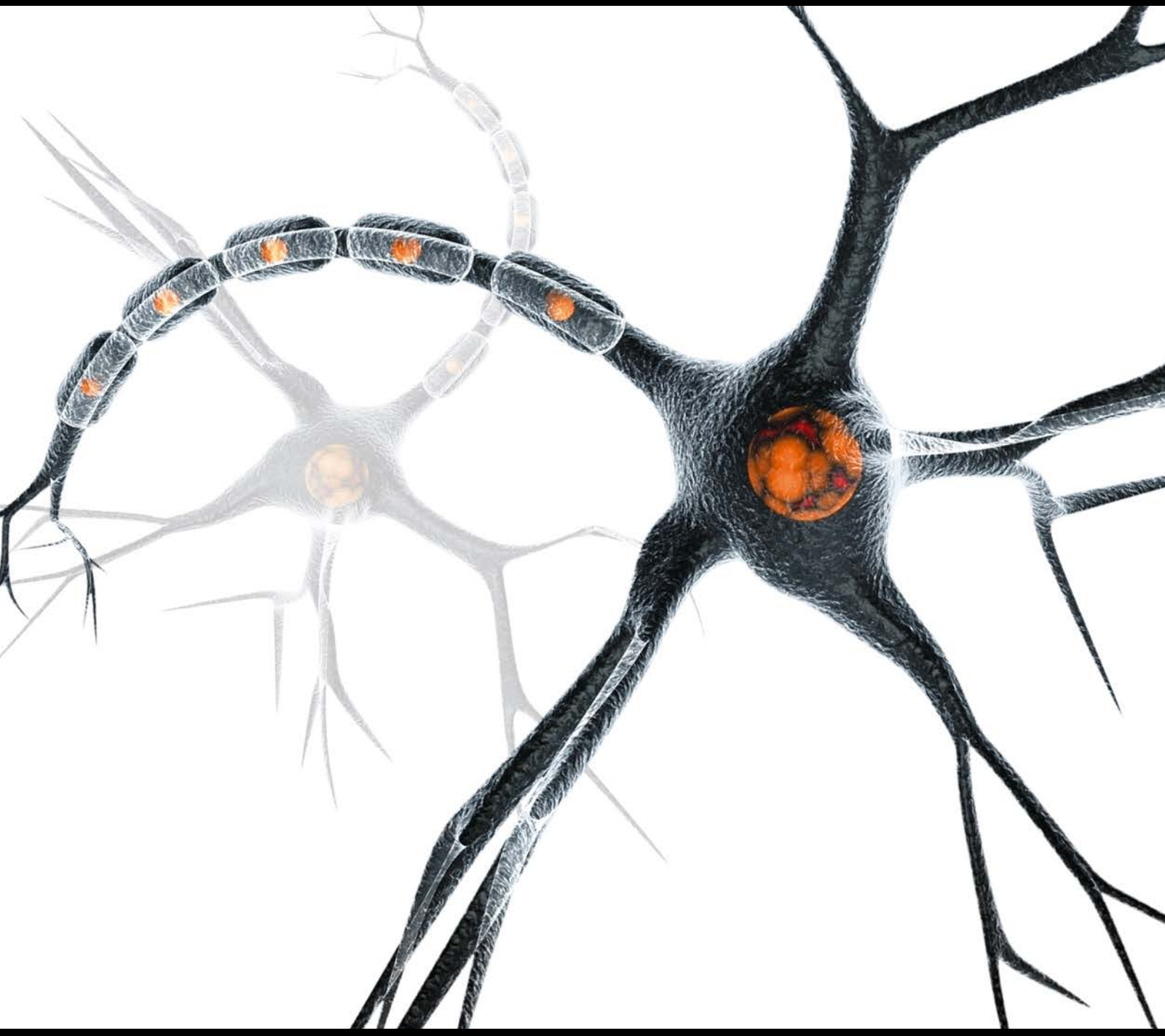
Neural Plasticity

Glutamate Receptors in Alzheimer's Disease: Mechanisms and Therapies

Guest Editors: Victor Anggono, Li-Huei Tsai, and Jürgen Götz



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Editorial Board

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Editorial

Glutamate Receptors in Alzheimer's Disease: Mechanisms and Therapies

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Alzheimer's disease (AD) manifests as a progressive loss in memory, cognition, and language that is commonly associated with elevated levels of amyloid-beta (Aβ) peptide and hyperphosphorylated tau in the brain. There is currently no cure for AD as its causes remain poorly understood. Accumulating evidence suggests that synaptic dysfunction is a major contributor early in disease pathogenesis prior to neuronal loss. Glutamatergic neurotransmission is particularly vulnerable to the neurotoxic effects of various assemblies of Aβ and hyperphosphorylated tau. Indeed, these toxic species act in synergy and severely disrupt excitatory synaptic transmission, synaptic plasticity, and network activity.

The chronology of how Aβ impairs neuronal function is not completely understood. However, accumulating evidence suggests that Aβ causes an initial hyperexcitability of neurons due to spillover and excessive levels of glutamate in the extrasynaptic space, leading to overstimulation of NMDA receptors and subsequently synaptic loss and cell death. The clearance of extracellular glutamate is primarily accomplished by astrocytic glutamate transporters, a process that is disrupted by Aβ. In this issue, Y.-L. Lan et al. present a new view that enhancing the interaction and synergy between aquaporin-4 and glutamate transporter-1 may confer a neuroprotective effect against excitotoxicity and neuronal death in AD.

Synaptic depression is a consequence of reduced numbers of glutamate receptors in the postsynaptic density and the eventual loss of synapses. S. Guntupalli et al. provide a comprehensive view of key signal transduction pathways underlying Aβ-induced endocytosis of the AMPA-type glutamate receptors, most of which are commonly shared with mechanisms that lead to long-term depression. Although the effects of Aβ in Hebbian-type synaptic plasticity, such as long-term potentiation and depression, are well established, its role in influencing homeostatic synaptic plasticity has only begun to emerge. S.-S. Jang and H.-J. Chung discuss the idea that disruption of Hebbian plasticity in AD is due to aberrant metaplasticity, a form of homeostatic plasticity that sets the threshold for future synaptic plasticity. In addition, they provide comprehensive views on the mechanisms underlying aberrant metaplasticity in AD.

Actin cytoskeleton and synaptic adhesion molecules play crucial roles in maintaining the integrity and structural plasticity of synapses. H. Stefen et al. focus on the actin cytoskeleton and discuss the current understanding of how Aβ disrupts actin dynamics, leading to impairments in the trafficking of AMPA, NMDA, and metabotropic glutamate receptors in the postsynaptic compartment. Several classes of synaptic adhesion molecules are known to mediate the attachment of presynaptic and postsynaptic membranes to ensure that the synaptic junction stays intact during synaptic plasticity. I. Leshchyns'ka and V. Sytnyk offer scientific evidence to illustrate that Aβ interacts with synaptic adhesion molecules and affects their expression and synaptic localization, all of which impair the function and integrity of synapses, leading to the disruption of neuronal networks in AD.

The striatal-enriched tyrosine phosphatase (STEP) plays a key role in regulating AMPA and NMDA receptor trafficking

in synaptic plasticity. M. Kamceva et al. provide an update on recent advances in this area, including a discussion of the substrates and signaling pathways of STEP associated with Aβ toxicity, as well as the potential therapeutic benefits of STEP inhibition in mouse models of AD. S.-S. Jang et al. have also investigated the effects of epileptic seizures, which are commonly associated with AD, in regulating the expression of STEP and its substrates. They found that a single episode of electroconvulsive seizures potently increases STEP expression, concomitant with decreases in the levels of NMDA receptors and dephosphorylation of extracellular signal regulated kinase 1/2 (ERK1/2). Interestingly, electroconvulsive seizures also increase Aβ production. This study suggests that seizure-induced upregulation of Aβ and STEP leads to NMDA receptor internalization and ERK1/2 inactivation in the hippocampus.

By highlighting the molecular mechanisms that underlie Aβ-induced defects in glutamate receptor trafficking, synaptic plasticity, and network activity, we hope that this special issue will provide a better mechanistic understanding of the etiology of the cognitive and memory deficits associated with AD. We also trust that this special issue will spark further research that will contribute to ongoing efforts to identify meaningful therapeutic targets for the treatment of AD.

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Review Article

Synaptic Cell Adhesion Molecules in Alzheimer's Disease

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Alzheimer's disease (AD) is a neurodegenerative brain disorder associated with the loss of synapses between neurons in the brain. Synaptic cell adhesion molecules are cell surface glycoproteins which are expressed at the synaptic plasma membranes of neurons. These proteins play key roles in formation and maintenance of synapses and regulation of synaptic plasticity. Genetic studies and biochemical analysis of the human brain tissue, cerebrospinal fluid, and sera from AD patients indicate that levels and function of synaptic cell adhesion molecules are affected in AD. Synaptic cell adhesion molecules interact with Aβ, a peptide accumulating in AD brains, which affects their expression and synaptic localization. Synaptic cell adhesion molecules also regulate the production of Aβ via interaction with the key enzymes involved in Aβ formation. Aβ-dependent changes in synaptic adhesion affect the function and integrity of synapses suggesting that alterations in synaptic adhesion play key roles in the disruption of neuronal networks in AD.

1. Synaptic Cell Adhesion Molecules

Cell adhesion molecules (CAMs) are cell surface glycoproteins located at the cell surface plasma membrane of neurons and other cells. CAMs have a large extracellular domain and are either transmembrane proteins or attached to the plasma membrane via a glycosylphosphatidylinositol (GPI) anchor. The extracellular domains of CAMs mediate cell adhesion by either forming homophilic adhesion bonds via binding to the same molecules on cell surface membranes of adjacent cells or interacting heterophilically with other proteins on the cell surface membranes of adjacent cells or in the extracellular matrix [1].

CAMs accumulating at synapses between neurons are often called synaptic CAMs and represent members of the major families of cell adhesion molecules, including immunoglobulin superfamily (IgSF) CAMs, cadherins, integrins, neuroligins, and neurexins, and also other cell surface proteins, which mediate cell adhesion, such as cellular prion protein (PrP^c) and amyloid precursor protein (APP) (Figure 1).

Synaptic CAMs perform numerous functions at synapses (Figure 1). In developing neurons, CAMs promote mechanical stabilization of the contacts between axons and dendrites of neurons [2] and formation of synapses [3]. Synaptic CAMs also play key roles in the establishment of neurotransmission by recruiting other synaptic components, such as synaptic scaffolding proteins, which interact with the intracellular domains of synaptic CAMs, and associated neurotransmitter receptors (Figure 1), and by inducing the maturation of the neurotransmitter release machinery [4]. In mature neurons, CAMs play a role in the stabilization of the synapse ultrastructure [5–7], regulation of the neurotransmitter release [8, 9], and synaptic remodeling and plasticity [10–13]. The multiple roles of synaptic CAMs in regulation of synapse formation and function have been described in a number of recent reviews [14, 15] and are discussed here mostly in the context of Alzheimer's disease.

Alzheimer's disease (AD) is a neurodegenerative brain disorder, which predominantly affects the aging population. One of the earliest signs of AD is the loss of synapses [16]. Synapse loss in AD has been linked at least partly to the toxicity induced by Aβ, a peptide that accumulates in the brains of AD patients [17–19]. Synaptic cell adhesion is directly involved in AD pathogenesis, since APP is a precursor protein of the Aβ peptide and also a synaptic cell adhesion molecule playing a role in regulation of synaptic morphology, synaptic plasticity, and hippocampus-dependent behavior [20]. Functions of APP in synapses and molecular mechanisms of Aβ formation are the subject of a

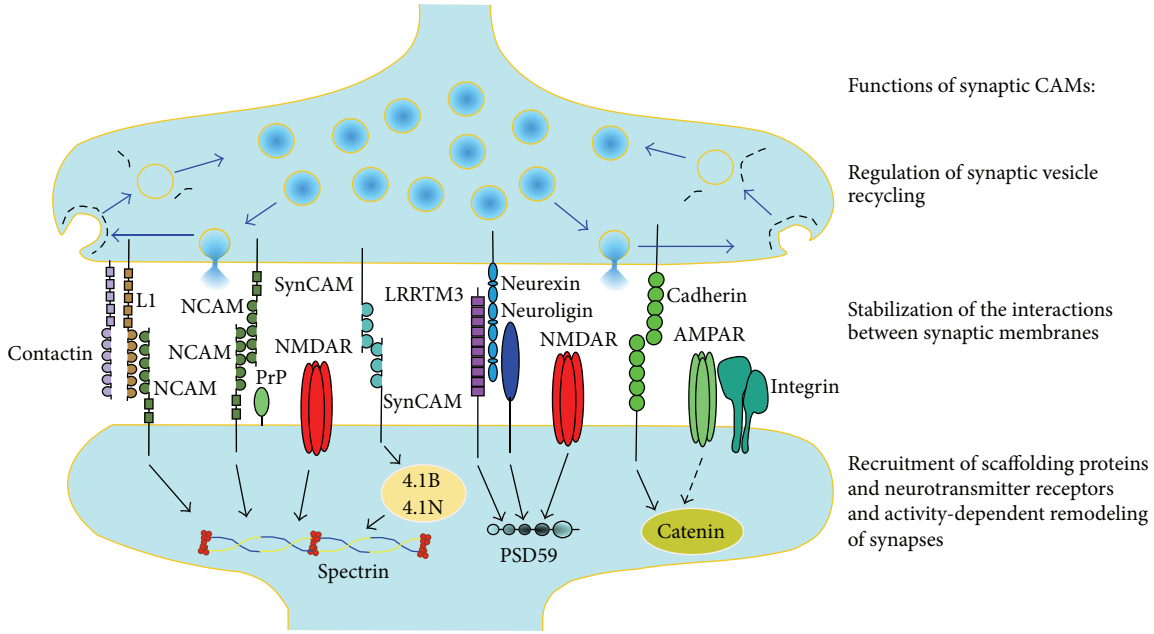

Figure 1: Schematic diagram illustrating examples of synaptic CAMs in glutamatergic synapses. Synaptic CAMs accumulate in synaptic membranes where they form homophilic (e.g., NCAM-NCAM, SynCAM-SynCAM, and cadherin-cadherin) or heterophilic (e.g., L1-NCAM, neuroligin-neurexin, and LRRTM3-neurexin) adhesion bonds, which are important for stabilization of the interactions between synaptic membranes. Presynaptically, CAMs are involved in regulation of synaptic vesicle recycling (blue arrows), which is mediated by coat proteins (black dashed lines) assembled on synaptic membranes to reform synaptic vesicles after exocytosis and neurotransmitter release. Postsynaptically, intracellular domains of synaptic CAMs interact with scaffolding and adaptor proteins (examples of interactions are shown with black arrows), such as spectrin, postsynaptic density protein 95 (PSD95), proteins 4.1B and 4.1N, or catenin, which link synaptic CAMs to postsynaptic glutamate receptors, the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPAR) and N-methyl-D-aspartic acid receptors (NMDAR). Interactions with synaptic CAMs promote the recruitment of scaffolding proteins and neurotransmitter receptors to synapses and are involved in the activity-dependent remodeling of synapses. NCAM: neural cell adhesion molecule, SynCAM: synaptic cell adhesion molecule, PrP: cellular prion protein, and LRRTM3: Leucine-rich-repeat- (LRR-) containing transmembrane protein 3.

number of recent reviews [21–23] and are not discussed here. In this review, we summarize current data on the changes in the levels and function of other synaptic CAMs in AD brains and their complex interactions with Aβ suggesting that abnormal function in different synaptic CAMs can be an important factor contributing to synapse dysfunction in AD.

2. Genetic Association between CAMs and AD

The involvement of CAMs in AD is suggested by genome-wide association studies (GWAS). Significantly altered expression of CAM pathway genes in AD was found in the samples from the cerebellum and temporal cortex of AD-affected individuals and AD-nonaffected controls [24]. Besides APP, among synaptic CAMs found to be associated with the risk of AD, PRNP gene coding for PrP^c has been identified as an AD susceptibility gene by systematic meta-analysis of AD genetic association studies [25]. The methionine/valine (M/V) polymorphism at codon 129 within the PRNP gene, which represents a known risk factor for Creutzfeldt-Jakob disease (CJD), has also been reported to be a risk factor for early onset AD [26–28].

Single nucleotide polymorphisms (SNPs) in the neural cell adhesion molecule 2 (NCAM2), a synaptic IgSF CAM highly expressed in hippocampal synapses, have been reported as a risk factor related to the progression of AD in the Japanese population [29]. SNPs in the NCAM2 gene also show association with levels of Aβ in the cerebrospinal fluid in humans, suggesting that NCAM2 is involved in the pathogenic pathway to the senile plaques that concentrate in AD brains [30]. In another large GWAS involving over 16,000 individuals, SNPs in contactin-5, another member of the synaptic IgSF CAMs localizing to the presynaptic membranes [31], were shown to be significantly associated with AD [32]. The junctional adhesion molecule 2 (JAM2) is another member of IgSF potentially linked to AD. SNPs in JAM2 were found to be significantly associated with AD [33]. JAM2 is localized to tight junctions in epithelial and endothelial cells but is also expressed in retinal ganglion cells [34]. The link between JAM2 and AD is also suggested by a study reporting chromosomal 21 region duplication spanning 0.59 Mb and comprising JAM2, APP, and some other genes in a patient with AD [35]. Whether JAM2 functions in the regulation of synapses in neurons is, however, not known. The association with AD was also observed for SNPs in the gene coding for the leucine-rich repeat transmembrane neuronal 3 (LRRTM3) synaptic CAM, which is highly expressed in the hippocampus [36]. Meta-analysis of five GWAS also identified the gene coding for neurexin-3 as a gene playing a role in susceptibility to AD in males [37].

3. Changes in the Levels of Synaptic CAMs in AD

Changes in the levels of synaptic CAMs in AD brains have been reported in a number of studies performed over the last 25 years. Reduced levels of the largest NCAM isoform with the longest intracellular domain, NCAM180, but not total NCAM levels have been reported in one of the early studies comparing samples from control and AD frontal cortex by quantitative crossed immunoelectrophoresis [38] suggesting changes in the expression of NCAM in AD. In later studies, analysis of control and AD brain sections by immunohistochemistry with antibodies against NCAM found significantly fewer NCAM positive neurons in the frontal cortex of AD-affected individuals when compared to normal aging individuals [39]. In agreement, the levels of NCAM were shown to be reduced in frontal and temporal cortex from AD patients by ELISA [40]. Interestingly, there was little difference in the levels of NCAM in the occipital cortex and hippocampus of control and AD patients [39, 41]. However, immunohistochemical analysis of the AD hippocampus using antibodies against polysialic acid (PSA), a unique carbohydrate attached predominantly to NCAM, revealed an increase in the immunoreactivity and numbers of PSA-NCAM positive neurons in AD hippocampus and especially in the dentate gyrus indicating changes in the posttranslational processing of NCAM [42]. PSA-NCAM is highly expressed in the developing nervous system, but its expression in the mature nervous system is restricted to brain areas undergoing plastic changes [43], suggesting that an increase in PSA-NCAM in AD is related to extensive neuronal remodeling in AD brains.

Levels of contactin-2, a GPI anchored IgSF CAM also called transient axonal glycoprotein 1 (TAG-1), were shown by Western blot to be reduced in the temporal lobe of AD patients [44]. Contactin-2 is cleaved by β-site amyloid precursor protein-cleaving enzyme 1 (BACE1) and its levels in AD brains inversely correlate with BACE1 levels and amyloid plaque density [44] suggesting that an increase in BACE1 activity observed in late-onset AD [45–49] results in increased contactin-2 cleavage. BACE-1 also cleaves other synaptic CAMs, such as IgSF CAM L1 and the close homologue of L1 (CHL1) [50, 51]. The intracellular domain of L1 is also cleaved by γ-secretase in human carcinoma cells [52], and γ-secretase induced proteolytic cleavage of L1 is increased in a mouse model of AD, which carries human APP with the pathogenic Swedish mutation and the L166P mutated human presenilin-1 [53]. Changes in the activity of BACE-1 and γ-secretase may therefore affect the expression of a number of other synaptic CAMs in AD brains.

In addition to IgSF CAMs, levels of PrP^c analyzed by Western blot were also found to be decreased in the hippocampus of patients with sporadic AD but not with familial AD [54]. Levels of PrP^c are also lower in the temporal cortex samples of AD patients [54, 55]. Levels of N-cadherin are also reduced in the temporal cortex of AD patients [56]. In contrast, Western blot analyses have not revealed significant changes in the levels of contactin-5 in the temporal cortex of AD patients [55] and levels of full length N-cadherin in the superior frontal gyrus of AD patients [57]. Levels of platelet endothelial cell adhesion molecule 1 (PECAM1), an IgSF CAM, were also similar in frontal and temporal cortex of control subjects and moderate to severe AD patients [58]. Therefore, expression of only a subset of synaptic CAMs appears to be affected in AD and only in some brain regions.

Interestingly, in a recent study, levels of NCAM2 were shown by Western blot to be increased in the hippocampus of AD patients but strongly reduced in synaptosomes isolated from this brain region [59] (Figure 2). Levels of NCAM2 were not significantly affected in the temporal cortex and cerebellum of AD patients. These observations indicate that changes in the total protein levels or the lack of such changes does not necessarily correlate with the changes in the subcellular localization and function of synaptic CAMs. Changes in the levels of other synaptic CAMs at synapses in AD brains and whether alterations in the overall levels of other synaptic CAMs reflect changes in their synaptic localization remain to be investigated in the future studies.

4. Changes in the Levels of the Proteolytic Products of CAMs in AD

In addition to changes in the levels of the full length synaptic CAMs, changes in the levels of the proteolytic products of synaptic CAMs have also been found in AD brains. Interestingly, changes in the proteolytic products of synaptic CAMs do not necessarily correlate with the changes in the total protein levels. While the total levels of N-cadherin appear to be unaffected in the superior frontal gyrus of AD patients, the levels of ectodomain-shed C-terminal fragment of N-cadherin are increased [57]. The levels of the extracellular domains of NCAM2 proteolytically released from the neuronal cell surface are increased in AD hippocampus [59] (Figure 2). This increase in the levels of proteolytic products of NCAM2 inversely correlates with the levels of full length NCAM2 at synapses, while the total levels of NCAM2 are also increased in AD hippocampus [59] (Figure 2). It is therefore possible that changes in the proteolytic products of synaptic CAMs in AD brains reflect changes in their proteolysis at specific subcellular locations, such as synapses, rather than changes in the overall turnover of these proteins.

A number of studies indicate that the proteolytic products of CAMs are also present at varying levels in the cerebrospinal fluid (CSF) and serum of humans. Western blot analyses with antibodies specific to different portions of these molecules show that these proteolytic products are detectable with the antibodies against the epitopes within their extracellular domains while the intracellular domains are not detectable [60]. These observations indicate that the proteolytic products of CAMs in CSF and serum represent fragments of the extracellular domains of CAMs possibly released to CSF by shedding from the cell surface of neurons in the brain. Proteolytic products of several CAMs have been reported to be increased in CSF and serum of AD patients. For example, CSF levels of L1 analyzed by ELISA have been reported to be significantly increased in AD [60]. This study also reported an increase in the CSF levels of NCAM, which, however, was not

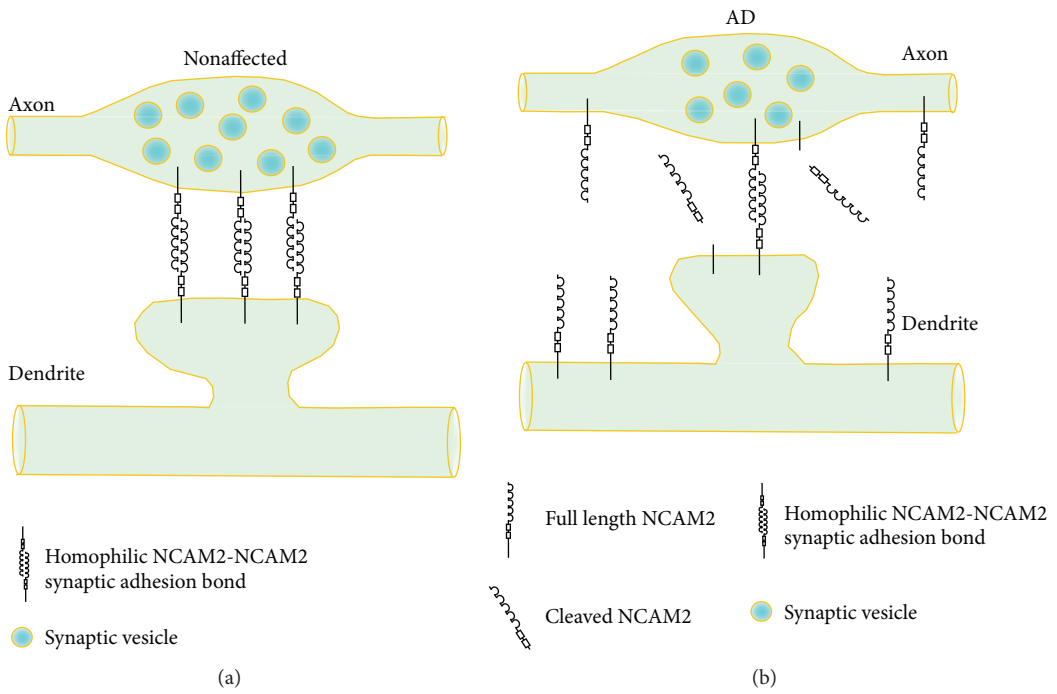

Figure 2: Changes in NCAM2-mediated synaptic adhesion in AD-affected hippocampus. In AD-nonaffected hippocampus (a), NCAM2 accumulates in synapses and plays a role in the synapse maintenance. In AD-affected hippocampal synapses (b), levels of full length NCAM2 are decreased. This decrease is accompanied by an increase in the levels of the proteolytic cleavage products of NCAM2. The overall expression of NCAM2 is also increased probably due to the increase in the levels of extrasynaptic NCAM2.

statistically significant when compared to normal controls. Increased levels of several proteolytic products of NCAM were also found in the sera of AD patients [61]. In contrast, ELISA analysis has not revealed significant differences in the levels of neuronal cell adhesion molecule (NrCAM), L1 family member, in CSF samples from healthy controls and AD patients [62].

Analysis of the levels of the proteolytic products of CAMs has therefore been proposed to be useful in diagnostics of AD. It should be noted, however, that levels of the proteolytic products of such CAMs as NCAM or L1 in CSF and serum samples of healthy individuals and AD patients overlap considerably [60, 61]. Also, changes in CSF levels of these products are often not specific to AD. For example, levels of the proteolytic products of L1 are also increased in vascular dementia and dementia of mixed type [60]. Levels of the proteolytic products of NCAM are increased in CSF of people suffering from schizophrenia [63] and bipolar mood disorder type I or recurrent unipolar major depression [64], but not in bipolar mood disorder type II patients [64]. However, levels of NCAM are not changed in the serum of patients with autism, although levels of NCAM180 protein but not mRNA are reduced in the brains of these patients [65]. Also, in contrast to AD, CSF levels of L1 are decreased in schizophrenia [63]. Therefore, analysis of specific isoforms and cleavage products derived via different proteolysis pathways might be required to establish proteolytic products of CAMs as markers of specific neurologic conditions including AD.

5. Synaptic CAMs as Receptors for Aβ Oligomers

A number of observations indicate that synaptic CAMs act as receptors for Aβ oligomers at the synaptic sites. The extracellular domain of L1 but not the extracellular domain of CHL1 interacts with Aβ in a label-free binding assay [66] (Figure 3). The fibronectin type III homologous repeats 1–3 of the extracellular domain of L1 mediate this effect. Interestingly, the recombinant extracellular domain of L1, but not the recombinant extracellular domain of CHL1, inhibits aggregation of Aβ *in vitro*. Furthermore, overexpression of L1 by injection of adenoassociated virus encoding L1 decreases the Aβ plaque load, levels of Aβ42, Aβ42/40 ratio, and astrogliosis in a mouse model of AD, which carries human APP with the pathogenic Swedish mutation and the L166P mutated human presenilin-1 [66]. The extracellular domain of NCAM2 also binds to Aβ oligomers both *in vitro* and *in vivo* in cultured mouse hippocampal neurons and in the hippocampus of Aβ generating transgenic mice overexpressing human APP containing the pathogenic Swedish mutation [59].

Aβ oligomers also directly associate with the N-terminus of PrP^c both *in vitro* and in the human AD brain, with the binding sites located within residues 23–27 and 95–110 of PrP^c [67–71]. Interaction of PrP^c with Aβ is a function of Aβ load in the brain and does not depend on PrP^c levels [71]. The pathological relevance of this interaction remains, however, to be established. PrP^c interacts with other synaptic proteins,

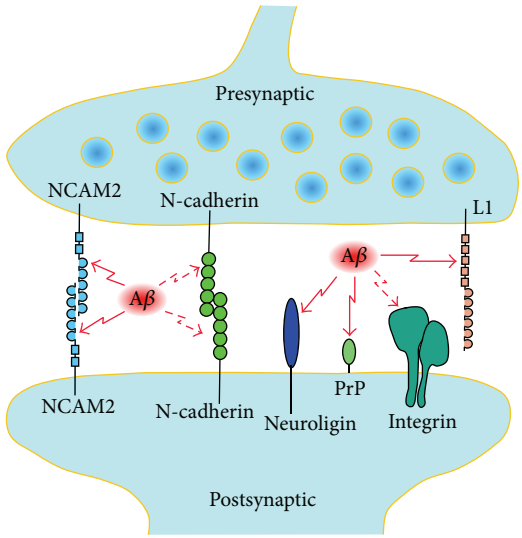

Figure 3: Synaptic CAMs function as receptors for Aβ. Schematic representation of a synapse showing presynaptic and postsynaptic CAMs, which bind to Aβ. Direct interaction with Aβ has been demonstrated for NCAM2, neuroligin, PrP^c, and L1 (solid red arrows). Binding of Aβ to integrins and N-cadherins is suggested by indirect observations and remains to be confirmed in a direct binding assay (dashed red arrows).

including N-methyl-D-aspartic acid- (NMDA-) type glutamate receptors [72] and NCAM [73], and binding of Aβ oligomers to PrP^c can interrupt the physiological interactions of PrP^c at synapses, resulting in disturbed neuronal communication [74]. PrP^c deficient mice are resistant to the neurotoxic effect of Aβ oligomers, and antibodies against PrP^c or PrP^c peptides prevent Aβ oligomer-induced neurotoxicity indicating that PrP^c is involved in the molecular pathways activated by Aβ oligomers to induce neuronal cell death [75]. PrP^c traps and concentrates Aβ in an oligomeric form and disassembles mature Aβ fibers [70]. The cleavage fragment of PrP^c containing binding sites for Aβ strongly suppresses Aβ toxicity in cultured mouse hippocampal neurons and *in vivo* in mice after intracerebroventricular injections of Aβ [69, 76]. However, memory impairment induced by injection of Aβ oligomers is not reduced in PrP^c knockout mice [77], ablation or overexpression of PrP^c has no effect on the impairment of hippocampal synaptic plasticity in a transgenic model of AD [78], and synaptic depression, reduction in spine density, or blockade of LTP is induced by Aβ in organotypic hippocampal slice neurons from both wild type and PrP^c knockout mice [79]. Therefore, the Aβ-mediated synaptic defects do not require PrP^c.

Neuroligin-1 is enriched in excitatory synapses and its extracellular domain binds to Aβ *in vitro* and in cultured rat hippocampal neurons and rat cerebral cortex [80, 81]. Aβ does not interact with neuroligin-2, which is enriched in inhibitory synapses. Neuroligin-1 acts as a nucleating factor during the Aβ aggregation process, stimulating the formation of Aβ oligomers [81]. The soluble extracellular α/β-hydrolase-fold (ChE-like) domain of neuroligin-1 reduces the Aβ-induced reduction in synaptic density in cultured rat hippocampal neurons and in field excitatory postsynaptic potentials (fEPSP) in rat hippocampal slices possibly by competing with the synaptic neuroligin-1 for binding to Aβ [80].

Indirect observations also suggest that Aβ interacts with integrins since Aβ toxicity was inhibited in human neurons pretreated with adhesion-blocking antibodies against different subunits of integrins, and in particular β1, α2, and αV [82]. Inhibition of $\alpha 1\beta 1$ integrin has also been shown to reduce Aβ toxicity in rat hippocampal cultures [83]. Aβ toxicity was also inhibited by disintegrin echistatin, a peptide isolated from snake venom that has been shown to inhibit RGD-dependent integrins such as $\alpha V\beta 1$ and by integrin ligands such as vitronectin, fibronectin, and superfibronectin suggesting that integrin ligands compete with Aβ for binding to integrins [82]. Application of Aβ also reduces the overall expression of N-cadherin in cultured mouse cortical neurons suggesting that Aβ can bind to N-cadherins [56], although a reduction in N-cadherin proteolysis after application of Aβ has been reported in another study [84].

Interestingly, some CAMs have been shown to interact also with APP. The association of N-cadherin with APP in mouse brains has been shown by coimmunoprecipitation experiments [85]. In an unbiased search for the binding partners of APP using time-controlled transcardiac perfusion cross-linking followed by high stringency immunoaffinity purification and tandem mass spectrometry, several other cell adhesion molecules were identified including PrP^c and IgSF CAMs Thy-1, contactin, NCAM1, and neurofascin [86]. In spite of homology to NCAM2, NCAM1 binds to a region of APP which is different to the Aβ-containing region [87] indicating that these interactions may play a role in physiological functions of both molecules. In agreement, contactin-2 has been shown to be a functional ligand of APP. Binding of contactin-2 to APP increases the release of the intracellular domain of APP through γ-secretase-dependent cleavage [88]. Contactin-2 competitively inhibits the binding of APP to transforming growth factor β2 (TGFβ2) [89]. Binding of TGFβ2 to APP induces neuronal cell death [90] and this effect is inhibited by TAG-1 [89] suggesting that TAG-1 regulates interactions of APP with extracellular ligands.

6. Synaptic CAMs in Regulation of Aβ Production

BACE1 is a potential therapeutic target for AD since BACE1 cleavage of APP is the rate limiting step in Aβ production [91]. Synaptic cell adhesion molecules have been shown to play a role in regulation of BACE1 activity. In a high-throughput siRNA screen assessing 15,200 genes for their role in Aβ secretion, LRRTM3 has been identified as a neuronal gene that promotes APP processing by BACE1 [92]. Knockdown of LRRTM3 expression using siRNA results in reduced secretion of Aβ in cultured cells and primary neurons, while overexpression of LRRTM3 increases Aβ secretion [92] suggesting that LRRTM3 promotes BACE1 activity.

In contrast, overexpression of PrP^c results in inhibited BACE1-mediated cleavage of APP and reduced Aβ production, while Aβ production is increased in the brains of

PrP^c knockout mice and in cultured N2a cells after siRNA mediated knockdown of PrP^c expression [93] suggesting that PrP^c inhibits BACE1 activity. In agreement, in a follow-up study, PrP^c has been shown to interact with the prodomain of BACE1 in the trans-Golgi network and regulate targeting of BACE1 to the cell surface and endosomes where it preferentially cleaves APP [94]. PrP^c reduces BACE1-mediated cleavage of wild type APP, but not human APP with the Swedish and Indiana familial mutations, suggesting that PrP^c may play a role in sporadic AD but not in familial AD [94]. Interestingly, the region at the extreme N-terminus of PrP^c, which is critical for the interaction or PrP^c with BACE-1 and PrP^c-dependent inhibition of APP-cleaving activity [93], also contains the binding site for Aβ oligomers [67]. These observations suggest that PrP^c can play a protective role (inhibition of BACE1) and pathogenic role (binding of toxic Aβ oligomers) in AD and also suggest that the protective function of PrP^c can be affected by Aβ oligomers.

An increase in Aβ secretion is also observed in cells cotransfected with N-cadherin [85, 95]. N-cadherin promotes cell surface expression of γ-secretase and increases accessibility of γ-secretase to APP [95]. Altogether, these observations thus indicate that synaptic CAMs are involved in regulation of the key enzymes involved in Aβ production.

7. Effects of Disruptions of Synaptic Adhesion on the Synapse Integrity in AD

Inhibition of N-cadherin function by blocking INP peptides, which mimic a short sequence in the EC1 domain of N-cadherin and thus impair the homophilic transsynaptic interaction of N-cadherin molecules, accelerates the Aβ-induced synapse impairment characterized by a reduction in the frequency of the AMPA receptor-mediated miniature excitatory postsynaptic currents (AMPA mEPSCs) and reduced density of synaptic boutons along dendrites in cultured cortical neurons [57]. Similar effects are observed when N-cadherin function is inhibited by expression of the dominant-negative, truncated N-cadherin lacking the extracellular cadherin domains, or by overexpression of the ectodomain-shed C-terminal fragment of human N-cadherin, which accumulates in AD brains. It is noteworthy that the ectodomain-shed C-terminal fragment of human N-cadherin is further cleaved by γ-secretase, and inhibition of γ-secretase activity also accelerates the Aβ-induced synapse impairment [57].

Inhibition of N-cadherin function alone has no effect on the numbers of synapses and frequency of AMPA mEPSCs [57]. Interestingly, disruption of NCAM2-mediated synaptic adhesion using recombinant extracellular domains of NCAM2 (NCAM2-ED) results in a reduction in synapse density along dendrites of hippocampal neurons and dispersion of AMPA receptors from synapses [59]. NCAM2-ED accumulates in AD hippocampus and its effect on the synapse integrity is similar to and not additive with the effect of Aβ [59]. It is therefore possible that Aβ-dependent proteolysis of NCAM2 is one of the initial synapse-destabilizing effects of Aβ, which is then followed by disruption of N-cadherin containing adhesion complexes resulting in the complete synapse disassembly.

The complex formed by Aβ and neuroligin-1 also contains GluN2B but not GluN2A subunits of NMDA receptors [80] suggesting that Aβ can directly affect the function of neuroligin-1 in anchoring NMDA receptors at synapses. Whether binding of Aβ to other synaptic CAMs directly contributes to the synapse loss has to be investigated in the future studies.

8. Future Directions

While a number of observations indicate that synaptic cell adhesion molecules are affected in AD, our understanding of the molecular and cellular mechanisms underlying these changes and their role in the disease progression is still very incomplete. Further studies assessing levels of synaptic CAMs specifically at synapses are needed to understand whether changes in the overall levels of these CAMs reflect changes in the synaptic adhesion. Whether an increase in the levels of specific proteolytic products of CAMs in CSF and sera of AD patients reflect the Aβ-dependent proteolysis of CAMs at synapses is an interesting possibility which can be analyzed in the future studies. Since synaptic CAMs play key roles in the maintenance of synapse integrity and function by interacting with synaptic scaffolding proteins and neurotransmitter receptors, further analysis of the effects of Aβ-dependent disruption of synaptic adhesion at the synaptic level may help to understand the molecular mechanisms of the initial stages of AD. Furthermore, a number of reports showing that the Aβ toxicity can be reduced by targeting synaptic CAMs indicate that synaptic CAMs deserve further consideration as molecular targets in designing new treatments of AD.

Competing Interests

The authors declare that they have no competing interests.

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Review Article

Role of Striatal-Enriched Tyrosine Phosphatase in Neuronal Function

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Striatal-enriched protein tyrosine phosphatase (STEP) is a CNS-enriched protein implicated in multiple neurologic and neuropsychiatric disorders. STEP regulates key signaling proteins required for synaptic strengthening as well as NMDA and AMPA receptor trafficking. Both high and low levels of STEP disrupt synaptic function and contribute to learning and behavioral deficits. High levels of STEP are present in human postmortem samples and animal models of Alzheimer's disease, Parkinson's disease, and schizophrenia and in animal models of fragile X syndrome. Low levels of STEP activity are present in additional disorders that include ischemia, Huntington's chorea, alcohol abuse, and stress disorders. Thus the current model of STEP is that optimal levels are required for optimal synaptic function. Here we focus on the role of STEP in Alzheimer's disease and the mechanisms by which STEP activity is increased in this illness. Both genetic lowering of STEP levels and pharmacological inhibition of STEP activity in mouse models of Alzheimer's disease reverse the biochemical and cognitive abnormalities that are present. These findings suggest that STEP is an important point for modulation of proteins required for synaptic plasticity.

1. Introduction

There are 107 protein tyrosine phosphatases (PTPs) in the human genome and many of these play important roles in cellular function [1]. Striatal-enriched protein tyrosine phosphatase (STEP), encoded by the *PTPN5* gene, is a CNS-enriched member of the PTP family [2]. PTPs are divided into tyrosine-specific phosphatases and dual-specificity phosphatases, with tyrosine-specific phosphatases further divided into intracellular PTPs and receptor-like PTPs [3]. STEP is an intracellular PTP, expressed throughout the CNS with the exception of the cerebellum [4].

Dysfunction in a growing number of PTPs contributes to the etiology of several diseases [5–7] and, as a result, PTPs, including STEP, have emerged as attractive targets for drug discovery [8, 9]. The current model of STEP function is that it normally opposes synaptic strengthening by dephosphorylating key synaptic substrates. Substrates include subunits of glutamate receptors N-methyl-D-aspartate receptors (NMDARs) and α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid receptors (AMPARs), leading to internalization of these receptor complexes [10–13]. Thus, increased expression of STEP disrupts synaptic function and is associated with a number of neuropsychiatric disorders, such as Alzheimer's disease [14–17]. Pharmacological inhibition of STEP would be predicted to alleviate synaptic dysfunction in Alzheimer's disease, and the successful effort in this area is reviewed below.

2. Domain Structure of Major STEP Isoforms

Like other PTPs, STEP contains a signature consensus sequence [I/V]HCxAGxxR[S/T]G at its C-terminus that is required for catalytic function and an upstream kinase-interacting motif (KIM) that is involved in binding to all known substrates [18–22]. The STEP family is alternatively spliced from a single STEP gene (*PTPN5*) and has two major isoforms, $STEP_{61}$ and $STEP_{46}$, which are differentially expressed in brain regions and at developmental times [18, 23, 24]. $STEP_{61}$ is found in multiple brain regions that include the striatum, central nucleus of the amygdala, optic nerve, hippocampus, neocortex, spinal cord, olfactory tubercle and bulb, and lateral amygdala, while $STEP_{46}$ is expressed in striatum, nucleus accumbens, amygdala, and the optic nerve [23, 25].

$STEP_{61}$ is abundantly expressed at birth and throughout adulthood, while $STEP_{46}$ is not expressed until postnatal day 6 and increases over the first postnatal month when it plateaus to adult levels [24, 26]. STEP isoforms are found in both excitatory and inhibitory neurons [27], as well as in glia [25, 28].

$STEP_{61}$ contains 172 additional amino acids at its amino-terminus compared to $STEP_{46}$. The region contains two hydrophobic domains that are required to target $STEP_{61}$ to the endoplasmic reticulum and the postsynaptic density of dendritic spines [23, 29]; in contrast, $STEP_{46}$ is primarily cytosolic [18]. $STEP_{61}$ has two polyproline-rich regions that, in addition to the KIM domain, are involved in substrate binding and contribute to substrate specificity: the first polyproline domain is necessary for binding to Fyn [30], while the second is necessary for binding of Pyk2 [21].

Two additional alternatively spliced isoforms of STEP exist: $STEP_{38}$ and $STEP_{20}$ [4, 18, 31, 32]. While $STEP_{61}$ and $STEP_{46}$ both contain the signature consensus PTP sequence, $STEP_{38}$ and $STEP_{20}$ do not and are catalytically inactive [31]. Although these STEP isoforms remain to be fully characterized, they both contain KIM domains, suggesting that they may serve as variants that associate with target substrates and protect them from dephosphorylation. Both of these inactive STEP isoforms contain a 10-amino acid sequence at their carboxyl termini that is introduced during splicing and serves an unknown function.

3. Posttranslational Regulation of STEP

It is important to briefly review the posttranslational regulation of STEP as it informs us of potential mechanisms in disease. STEP activity is regulated by several mechanisms that include phosphorylation, dimerization, proteolytic cleavage, ubiquitination, and local translation (for more extensive review, see [33]). Two of these mechanisms of normal STEP regulation, phosphorylation and ubiquitination, are important to note when understanding STEP dysregulation in Alzheimer's disease, which is discussed below.

Phosphorylation by protein kinase A (PKA) reduces STEP activity in two ways. PKA directly phosphorylates $STEP_{61}$ and $STEP_{46}$ at a regulatory serine within their KIM domains [34], introducing steric hindrance that prevents STEP from binding to its substrates. PKA also reduces STEP activity indirectly by phosphorylating DARPP-32, a potent inhibitor of protein phosphatase 1 (PP1). PP1 normally dephosphorylates STEP at the regulatory serine residue within the KIM domain; thus, inhibition of PP1 maintains STEP phosphorylation and reduces levels of the dephosphorylated, active STEP protein [35].

4. STEP Substrates

4.1. Mitogen-Activated Protein Kinase (MAPK) Family. The discovery of STEP substrates was an important advance in the understanding of the possible function of STEP in regulating neuronal signaling. Two members of the MAPK family of proteins are STEP substrates, the extracellular signal-regulated kinases 1 and 2 (ERK1/2) and p38 [36–39]. ERK1/2 is implicated in synaptic plasticity and memory formation via its roles in stabilizing dendritic spines, initiating local protein synthesis in dendrites and spines, and involvement in nuclear transcription [40, 41]. STEP dephosphorylates the regulatory Tyr^{204} or Tyr^{187} residues in their respective activation loops, thereby inactivating ERK1/2.

The role of STEP regulation of ERK1/2 signaling has been studied in numerous ways, including infusion of a membrane-permeable TAT- (transactivator of transcription-) STEP cysteine to serine mutant [TAT-STEP (C to S)]. This mutant isoform is catalytically inactive, as the cysteine residue is required for substrate dephosphorylation. However, TAT-STEP (C to S) still binds to its substrates but does not release them, as dephosphorylation is required for substrate release; thus, TAT-STEP (C to S) inhibits downstream signaling pathways [10, 37]. ERK1/2 is necessary for the development of synaptic strengthening and the consolidation of fear memories in the lateral amygdala (LA). Infusion of TAT-STEP (C to S) into the LA rats did not affect the acquisition of fear memories, but there was no consolidation of these memories [42]. STEP knockout (KO) mice further established a relationship between STEP and ERK1/2, as these mice have significant elevation of phospho-ERK1/2 and increased phosphorylation of the downstream targets of ERK1/2, the transcription factors CREB and Elk1 [43, 44]. Moreover, STEP KO mice have facilitated amygdala-dependent learning (fear conditioning [45]) and facilitated hippocampal-dependent learning (Morris water maze [44]). These studies suggested that STEP normally regulates the duration of ERK1/2 signaling and also suggested the hypothesis that elevated levels of STEP might disrupt synaptic plasticity and memory formation [37].

The MAPK, p38, is also a STEP substrate but in contrast to ERK1/2 is involved in regulation of cell death pathways and NMDAR-mediated excitotoxicity [46, 47]. Excess glutamate stimulation activates extrasynaptic GluN2B-containing NMDARs, which results in phosphorylation of p38; p38 then phosphorylates target proteins involved in cell death pathways [48]. STEP normally dephosphorylates Tyr^{182} in the activation loop of p38, inactivating the protein [13, 48]. In addition, a number of studies have used molecular, kinetic, and structural analyses to gain insights into small differences in the KIM-containing PTPs that affect their binding to ERK2 and p38 [49–52]. Notably, both ERK1/2 and p38 regulate STEP expression levels through modulation of two phosphorylation sites adjacent to the KIM domain and dephosphorylation of these sites leads to the ubiquitination and degradation of STEP, suggesting a feedback mechanism to decrease STEP expression when ERK1/2 and p38 levels are low [53].

A study by Xu and colleagues [48] shed light on how STEP might regulate both p38 and ERK1/2, two proteins with very different and opposing functions. The differential regulation of these kinases by STEP depended on whether synaptic or extrasynaptic NMDARs were stimulated. $STEP_{61}$ is rapidly ubiquitinated and degraded following synaptic NMDAR stimulation, resulting in activation of ERK1/2 (but not p38 signaling) and activation of synaptic strengthening and neuronal survival pathways. With increased glutamate signaling, extrasynaptic NMDARs are engaged and promote activation of calpain and the cleavage of $STEP_{61}$ within the KIM domain.

The cleavage of the substrate-binding domain results in a STEP variant ($STEP_{33}$) that is unable to bind to and inactivate its substrates. Thus, stimulation of extrasynaptic NMDARs results in cleavage of $STEP_{61}$ and activation of p38 and cell death pathways. Using a peptide corresponding to the calpain cleavage site that prevents $STEP_{61}$ cleavage, there was a significant protection of neurons from glutamate-mediated excitotoxicity [48].

4.2. GluN2B and GluA2. Early studies demonstrated that dopamine signaling regulates STEP activity [34]. As mentioned above, stimulation of dopamine D1 receptors leads to activation of PKA and the phosphorylation and inactivation of STEP. Stimulation of D2 receptors has the opposite effects by reducing phosphorylation of the regulatory serine residue within the KIM domain and promoting the dephosphorylation of STEP substrates [34]. Thus, the hypothesis emerged that perhaps STEP lay between dopamine signaling and glutamate signaling through the ability of dopamine to regulate STEP activity and thereby regulate the tyrosine phosphorylation and surface expression of both NMDA and AMPA receptor complexes [10, 12, 44, 54].

Glutamate is the most abundant excitatory neurotransmitter within the CNS and binds to both metabotropic and ionotropic glutamate receptors to promote numerous cell signaling pathways in neurons [55, 56]. NMDARs are ligand-gated ion channels composed of two GluN1 and two GluN2 subunits. Activation of NMDARs requires both glutamate and glycine binding to the receptor as well as postsynaptic membrane depolarization. These receptors are selectively permeable to Ca^{2+} ions, which activate signaling molecules needed for long-term potentiation (LTP) and long-term depression (LTD) [57, 58]. STEP regulates the phosphorylation of the GluN2B subunit of NMDARs via two parallel pathways, the direct dephosphorylation of GluN2B (Tyr^{1472}) as well as inactivation of the nonreceptor tyrosine kinase Fyn that phosphorylates GluN2B at that site [30, 59]. When dephosphorylated by STEP, the Tyr^{1472} residue of GluN2B binds to clathrin adaptor proteins and promotes internalization of GluN1/GluN2B receptors [60]. Congruent with this observation, the surface expression of GluN1/GluN2B receptor complexes is increased in STEP KO mice [14, 44].

The effect of STEP on NMDAR function is significant. High levels of STEP decrease NMDAR excitatory postsynaptic currents (EPSCs) and prevent the occurrence of high-frequency stimulation LTP [54]. When STEP was inhibited with a functional-inhibiting STEP antibody, NMDAR EPSCs were enhanced and LTP occluded. The administration of a noncompetitive NMDAR agonist dizocilpine (MK801) and a Src family kinase inhibitory peptide prevents these effects, suggesting a role of STEP as a "tonic brake" on LTP by opposing Src family kinase-mediated enhancement of NMDARs activity [54].

As noted above, STEP is rapidly ubiquitinated and degraded after synaptic NMDAR stimulation [48], consistent with the emerging model that STEP activity must be decreased for LTP to occur. This is consistent with a recent study that found a role for STEP in the regulation of homeostatic synaptic plasticity [61]. Prolonged neuronal activity results in the upregulation of STEP that increases removal of NMDA and AMPA receptors from synaptic membranes. Prolonged neuronal inhibition had the opposite effect, leading to the hypothesis that fine-tuning of STEP activity is necessary for maintaining proper levels of these glutamate receptors at synapses.

AMPARs are also implicated in synaptic strengthening and memory consolidation. These receptors are ligand-gated ion channels composed of subunits GluA1 to GluA4. They regulate fast synaptic transmission that depolarizes postsynaptic membranes and activates NMDARs [56, 62]. AMPAR trafficking occurs in LTD and appears to be regulated by tyrosine phosphatases that include STEP [12, 63, 64]. STEP was found to regulate the Tyr dephosphorylation of the GluA2 subunit, leading to internalization of GluA1/GluA2 receptor complexes following mGluR stimulation [12].

Local translation of STEP is increased after activation of mGluRs by the agonist DHPG (S-3,5-dihydroxyphenylglycine). This results in the tyrosine dephosphorylation of the GluA2 subunit and internalization of GluA1/GluA2 receptor complexes [12]. DHPG induces the dephosphorylation of GluA2 and internalization of AMPARs, which is decreased by the substrate-trapping protein TAT-STEP (C to S). Further, STEP KO neuronal cultures do not undergo DHPG-mediated AMPAR endocytosis, which is restored with the addition of wild type TAT-STEP protein to the STEP KO cultures. These findings suggested that, following mGluR stimulation, STEP is activated to dephosphorylate GluA2 receptors, promoting their internalization. As suggested by this model, the surface expression of GluA1/GluA2-containing AMPARs is elevated in STEP KO mice [12, 44].

5. STEP Dysregulation in Alzheimer's Disease

The dysregulation of STEP and glutamate receptors is implicated in several neuropsychiatric disorders, including Alzheimer's disease (AD) [65, 66]. In AD, abnormally high levels of Aβ bind to and activate α7nAChRs [67–70], causing calcium influx and activation of calcineurin and PP1 and the dephosphorylation of STEP at the regulatory serine site within the KIM domain [10]. The ability of STEP to bind to its target proteins is increased and STEP substrates are dephosphorylated. To confirm that Aβ binding to α7nAChRs and activation of PP1 were leading to activation of STEP, neuronal cultures derived from α7nAChR KO mice and treated with Aβ were used to test whether activation of STEP was prevented in the absence of α7nAChRs. In fact, there was only a partial reduction STEP activation, suggesting that another mechanism was involved in activating STEP in AD.

Both mouse models of AD and neuronal cultures treated with Aβ were examined and found to have an accumulation of active STEP [14, 71–73]. The increase in STEP was shown not to be due to transcription or translation, suggesting that perhaps the normal degradation of STEP was disrupted. One of the effects of Aβ is inhibition of the proteasome [74, 75]. Since STEP is normally degraded through the ubiquitin proteasome pathway, an increase in STEP activity was found

to be due to an Aβ disruption of the ubiquitin proteasome pathway. In summary, an increase in the dephosphorylation of STEP coupled with a decrease in its degradation leads to the significant increase in STEP activity in AD.

6. Studies of STEP in Mouse Models of AD

Tg-2576. The Tg-2576 AD model mouse line is a transgenic mouse line that overexpresses the 695-amino acid isoform of human amyloid precursor protein (APP). APP is an integral membrane protein, proteolysis of which generates the amyloid fibrillar form of Aβ, the primary component in amyloid plaques in AD brains. The mutated APP present in this mouse line contains $Lys^{670} \rightarrow Asn$ and $Met^{671} \rightarrow Leu$ mutations [76] and these mutations in APP are found in early onset familial AD [77–79]. At 3 months of age, Tg-2576 mice perform normally in cognitive tasks and Aβ levels are indistinguishable from control animals. However, the Tg-2576 mice show cognitive impairments by 10 months of age [76]. STEP levels are normal at the earlier time points but are significantly elevated at later time points [72].

3xTg-AD. The 3xTg-AD transgenic mouse line possesses three separate mutations. First, the 3xTg-AD line has the same APP mutation present in Tg-2576 mice. Second, 3xTg-AD mice have a presenilin mutation, one of the proteins comprising the γ-secretase complex responsible for cleaving APP at the C-terminus of the Aβ domain. Third, tau is mutated in the 3xTg-AD line. Tau is a microtubule-associated protein acting to stabilize microtubules by binding to tubulin. Tau is hyperphosphorylated in AD, which causes paired helical filaments and destabilization of microtubules. These paired helical filaments are found in neurofibrillary tangles in patients with AD [80].

The 3xTg-AD mouse line has several phenotypes consistent with symptoms of human AD [81, 82]. Working memory and hippocampal memory deficits are documented, as are circadian rhythm abnormalities that are often present in early stages of AD. These behavioral and cognitive deficits are seen in combination with Aβ plaque aggregation and neurofibrillary tangles, which include paired helical filaments of hyperphosphorylated tau protein [81, 82]. STEP levels were again found to be normal at earlier time points when cognitive function was unaffected but were significantly elevated at time points when cognitive deficits were present. Moreover, crossing 3xTg-AD mice with STEP KO mice reversed the cognitive deficits [14, 72].

7. STEP Inhibition and AD Mouse Models

The elevation of STEP in AD as well as the finding that genetic reduction of STEP reversed cognitive deficits in an AD mouse model validated STEP as a target for drug discovery. A high throughput screen led to the discovery of an inhibitor of STEP, 8-(trifluoromethyl)-1,2,3,4,5-benzopentathiepin-6-amine hydrochloride (TC-2153) [83]. Cortical neurons treated with TC-2153 exhibit significant increase in the Tyr phosphorylation of STEP substrates GluN2B, Pyk2, and ERK1/2. Mice injected with TC-2153 also showed increased Tyr phosphorylation of STEP substrates. Phosphatase assays were performed comparing inhibition of STEP to a panel of PTPs, including two highly related PTPs, He-PTP and PTP-SL. TC-2153 was more selective towards STEP compared with these other PTPs. Furthermore, STEP is only found in the cortex, whereas the highly related He-PTP is found in the spleen and PTP-SL in the cerebellum, tissues that lack STEP. WT and STEP KO mice were injected with TC-2153 or vehicle and the Tyr phosphorylation of ERK1/2 and Tyr phosphorylation of Pyk2 were compared in various organs. Significant increases in pERK1/2 and pPyk2 were observed only in the frontal cortex and hippocampus, but not in tissues outside of the brain or the cerebellum, where other members of the PTP family dephosphorylate ERK1/2 and Pyk2 but do not appear to be inhibited by TC-2153.

To determine the mechanism by which TC-2153 inhibits STEP, glutathione (GSH) was added in *in vitro* assays. It decreased the activity of TC-2153 by two orders of magnitude, implying an oxidative mechanism for STEP inhibition. STEP was then incubated with TC-2153 to monitor enzyme activity. Following 24 h of dialysis, STEP remained inhibited, suggesting that TC-2153 led to the formation of a covalent bond, although STEP activity could be recovered following incubation with GSH or DTT.

High-resolution tandem mass spectrometry was performed to determine whether TC-2153 modified the active site cysteine of STEP. WT STEP and a STEP mutant in which the catalytic cysteine was changed to serine were compared. Analysis of the catalytic Cys^{472} of STEP in the absence of TC-2153 revealed a disulfide bridge between Cys^{465} and Cys^{472} which was not present in the STEP (C to S) mutant. Incubation of WT STEP with TC-2153 revealed the presence of a *de novo* trisulfide within the Cys^{465}/Cys^{472} bridge, which was not observed in WT STEP alone or in the mutated STEP. These results suggested that the active site cysteine is modified by TC-2153 and that sulfur(s) from the benzopentathiepin core is retained.

TC-2153 was effective in reversing cognitive deficits in both 6- and 12-month-old 3xTg-AD mice [83]. In the novel object recognition task (NOR), mice were injected with either vehicle or TC-2153 three hours prior to the training phase and tested for memory recall after 24 hours. Post hoc analysis suggested that discrimination indexes for object memory in the AD-TC group were significantly higher than those of the AD-Veh group, while TC-2153-treated WT mice did not differ from the Veh-treated WT mice. Of interest, no significant changes were found for beta amyloid or phospho-tau levels in 12-month-old 3xTg-AD mice, suggesting that inhibition of STEP activity was sufficient to reverse cognitive deficits.

The reference memory version of the Morris water maze was then conducted [83]. 3xTg-AD mice were injected daily with TC-2153, 3 hours prior to training for peak efficacy. This STEP inhibition resulted in a reversal of memory deficits on days 5 and 6 of the task in 3xTg-AD mice. To quantify memory status, the number of mouse entries in a circular zone located around the platform, or the target zone, and in the opposite quadrants was evaluated during probe trial

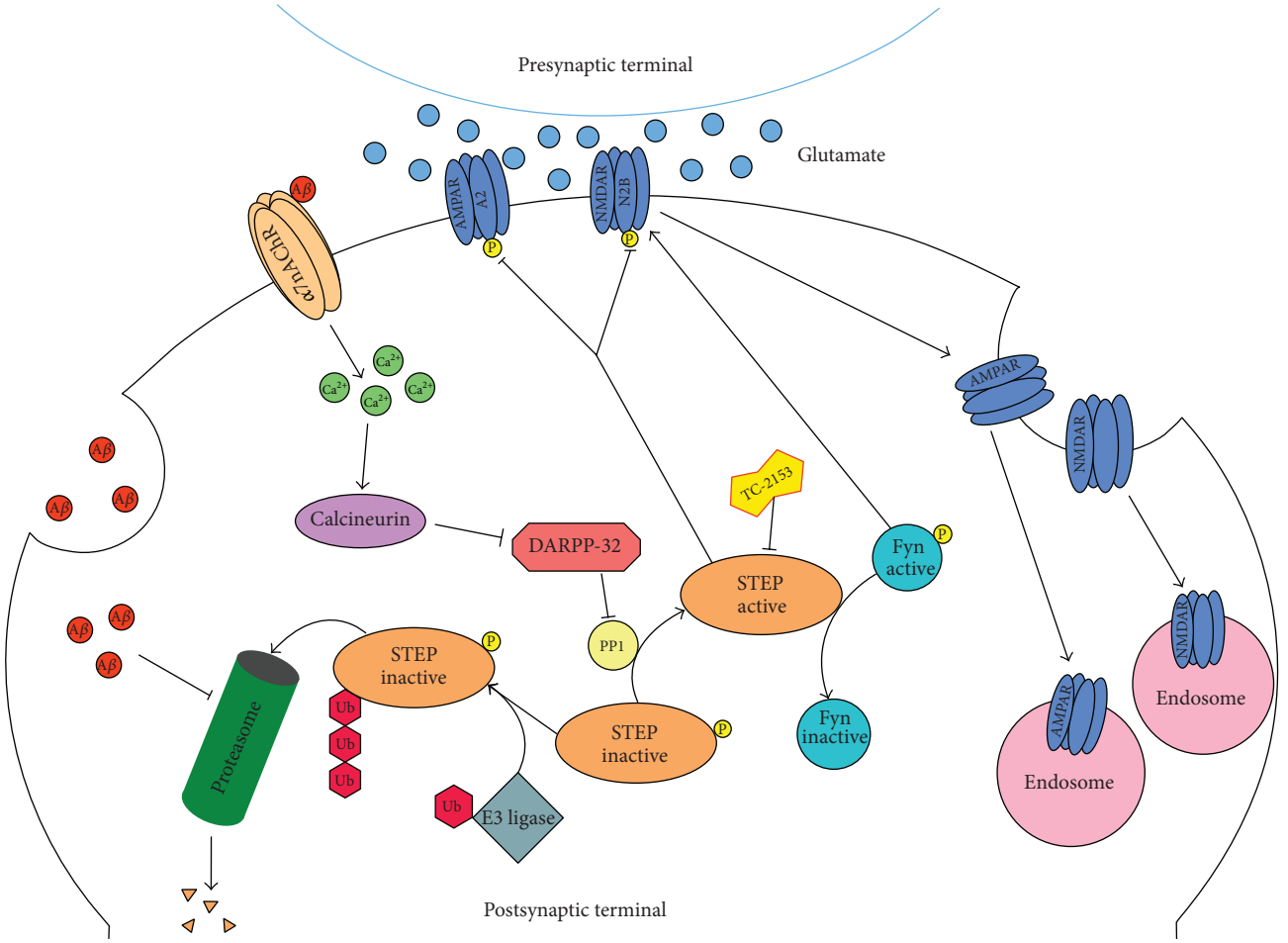

FIGURE 1: STEP signaling pathways associated with Alzheimer's disease. The binding of Aβ to α7nAChRs results in activation of calcineurin (PP2B), inhibition of DARPP-32, and activation of PP1. PP1 dephosphorylates $STEP_{61}$ at a regulatory serine within the substrate-binding domain (Ser^{221}). Dephosphorylation of this serine residue increases the affinity of STEP for its substrates. In a parallel pathway, Aβ inhibits the proteasome, thereby blocking the degradation of $STEP_{61}$. Both mechanisms result in an accumulation of active $STEP_{61}$. The increase in active $STEP_{61}$ results in increased dephosphorylation of GluN2B Tyr^{1472} and internalization of GluN2B-containing NMDARs. In addition, dephosphorylation of Fyn results in its inactivation. Thus, active $STEP_{61}$ directly dephosphorylates GluN2B and at the same time inactivates the kinase that phosphorylates $STEP_{61}$ at Tyr^{1472}.

24 hours after the last acquisition day. AD mice showed no preference for the target zone, in contrast to AD mice treated with TC-2153, which spent as much time as WT mice in the target zone.

8. Conclusion

STEP acts by dephosphorylating regulatory tyrosine residues in substrates that include subunits of both NMDA and AMPA glutamate receptors, thereby leading to internalization of these receptor complexes (see Figure 1). Additional targets of STEP include the kinases ERK1/2, Fyn, and Pyk2 that are inactivated by dephosphorylation of regulatory tyrosines within their activation loop, thus modulating downstream signaling pathways. When STEP activity is elevated, as occurs in Alzheimer's disease, the increased internalization of glutamate receptors disrupts synaptic function and contributes to the cognitive deficits that are present. Importantly, the STEP inhibitor TC-2153 significantly improves cognitive function in 3xTg-AD mice.

Although this review focused on Alzheimer's disease, STEP activity is elevated in several additional disorders, including Parkinson's disease [17], drug abuse [84–86], fragile X syndrome [16], and schizophrenia [15]. Moreover, a series of papers recently showed that low levels of STEP also disrupt synaptic function in several additional disorders, including Huntington's chorea [87, 88], cerebral ischemia [89], alcohol abuse [90–92], and stress disorders [93–95]. Thus the current model suggests that both high and low levels of STEP activity disrupt signaling pathways and contribute to neuropsychiatric and neurodegenerative disorders, making STEP an important focus of future research.

Competing Interests

The authors declare that they have no competing interests.

Authors' Contributions

Marija Kamceva and Jessie Benedict contributed equally to this work.

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Review Article

Regulation of the Postsynaptic Compartment of Excitatory Synapses by the Actin Cytoskeleton in Health and Its Disruption in Disease

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Disruption of synaptic function at excitatory synapses is one of the earliest pathological changes seen in wide range of neurological diseases. The proper control of the segregation of neurotransmitter receptors at these synapses is directly correlated with the intact regulation of the postsynaptic cytoskeleton. In this review, we are discussing key factors that regulate the structure and dynamics of the actin cytoskeleton, the major cytoskeletal building block that supports the postsynaptic compartment. Special attention is given to the complex interplay of actin-associated proteins that are found in the synaptic specialization. We then discuss our current understanding of how disruption of these cytoskeletal elements may contribute to the pathological events observed in the nervous system under disease conditions with a particular focus on Alzheimer's disease pathology.

1. Introduction

Memories are coded in the ensemble activity of small groups of neurons distributed throughout the brain. Glutamate is the primary excitatory neurotransmitter in the brain and the majority of synaptic connections between the glutamatergic neurons are made on dendritic spines. These specialized dendritic protrusions are supported by an actin-rich cytoskeletal protein matrix that not only provides structural support but also is essential for the delivery and anchoring of neurotransmitter receptors and other molecules involved in synaptic transmission. The synapse's capacity for change allows for memory formation and adaption to the environment. This synaptic remodelling is a dynamic process involving trafficking of neurotransmitter receptors into or out of the synaptic complex. These modifications require regulated disassembly and reassembly of the actin cytoskeleton. Orchestrating the controlled breakdown and reassembly of the actin cytoskeleton requires coordinated activity of an array of actin-associated proteins.

Alzheimer's disease (AD) is a neurodegenerative brain disorder that erodes memories and clouds thinking, gradually destroying one's sense of self. A loss of synaptic connectivity is thought to underlie the cognitive symptoms of AD. Synapse loss is observed in early stages of the pathology [1] and the correlation between synapse loss and severity of cognitive impairment is well established [2–4]. The early emergence of altered network connectivity has been confirmed by subsequent functional imaging studies [5, 6].

Cellular and murine models of AD have provided insight into the cellular mechanisms that underlie the loss of synaptic function in AD. It has become increasingly apparent that actin cytoskeletal function is disrupted in the pathology. Here we review the literature, describing the contribution of actin-associated proteins to synaptic function, and highlight recent findings implicating their involvement in AD pathology. Given the central role of the actin cytoskeleton in maintaining and modifying glutamatergic synaptic connections, proteins that modify or stabilize the cytoskeletal structures are potential therapeutic targets in the treatment of AD.

2. Structural and Functional Organization of the Postsynaptic Compartment of Excitatory Synapses

The majority of synaptic contacts between excitatory neurons are made on dendritic spines. These small structures house the postsynaptic molecules necessary for synaptic transmission. The prototypical spine contains a bulbous head (0.01–1 μm^3) and is connected to its parent dendrite via a thin (0.1 μm diameter) spine neck [7], which restricts diffusion between the two compartments, allowing concentration and segregation of signalling molecules [8, 9]. At the distal end of the spine head, directly across the synaptic cleft from the active zone of the presynaptic bouton, is an electron-dense postsynaptic density (the PSD), within which neurotransmitter receptors, cell adhesion molecules, cell signalling molecules, and a myriad of molecules involved in synapse stability are embedded [10, 11].

Spines are diverse in both shape and intracellular constituents. The neck length and width and head size vary along a continuum even within the same section of dendrite [12]. Despite this continuum they are usually classified based on the relative size of the spine head and neck [13, 14]. Mushroom spines have a large head and thin neck. Thin spines have a long neck and small head. Stubby spines are short with no obvious neck constriction. Synaptic function and structure are tightly intertwined and the different shapes are thought to reflect differences in synaptic strength and developmental stage [15–18]. Spine head volume has been found to be tightly correlated with PSD area [19] and the number of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs) in the postsynaptic membrane [20]. Thus, spines with largest heads form the strongest synaptic connections. These mushroom spines are less motile and more persistent than thin spines [21–23]. Large spines are also more likely to contain organelles including endoplasmic reticulum and mitochondria [24], likely due to increased metabolic demands associated with maintaining the expanded synaptic machinery.

The morphology of dendritic spines is highly dynamic. Spines undergo functional and morphological changes during development and in response to neuronal activity. Nascent spines emerge as thin or filopodia-like protrusions [23, 25, 26]. Most of these newly formed spines will disappear, whereas others that find synaptic partners will undergo a morphological transition into the more stable "mushroom" spines [26]. Once established, spines continue to be sculpted by neuronal activity. As discussed above, spine morphology and function are linked with the spine head volume tightly correlated with the number of AMPARs in the postsynaptic membrane. Therefore, the trafficking of neurotransmitter receptors to the plasma membrane is essential for synapse maturation and activity-dependent changes in synapse strength thought to underlie memory formation. Note that memories and their synaptic substrates can persist for years, well beyond the lifetime of the proteins responsible for synaptic transmission. Thus, even synapse maintenance requires cycling of proteins in and out of the membrane for protein replenishment.

The synaptic cytoskeleton not only is necessary for the structural support of the synaptic connections but also is critical for the cycling of neurotransmitter receptor and other proteins between the plasma membrane and endoplasmic compartment. The primary cytoskeletal component found in dendritic spines is actin [27]. Actin is present in two forms: filamentous actin (F-actin), which is the insoluble, polymer form that makes up the cytoskeleton, and its soluble monomeric building block, globular actin (G-actin). F-actin in spines displays a compartment specific organization with more linear oriented actin filaments in the spine neck and a branched organization in the head compartment [28].

Time-lapse studies suggest that spines initially develop as filopodia, which grow the mushroom-shaped head, characteristic of mature spines through branching of actin filaments (comprehensively reviewed by Yoshihara et al. [26] and Hotulainen and Hoogenraad [29]). These studies also highlight the dynamic nature of the actin cytoskeleton. Spine shape changes follow changes in actin dynamics and have been observed occurring within seconds [30], responding to chemical or electrical stimulation [31]. Live-cell imaging of green fluorescent protein labelled actin (GFP-actin) has indicated that actin is organized into structurally and functionally distinct F-actin populations within the postsynaptic compartment [15, 30]. Dynamic and stable pools of F-actin were identified that consisted of differing rates of actin treadmilling [30]. The dynamic pool of F-actin was shown to have an actin turnover rate <1 min and is believed to be involved in generating force to expand the spine head and AMPAR insertion at the PSD. A stable pool of F-actin was localized to the base of the spine and had a slower turnover rate of approximately 17 min. The stable pool was suggested to provide resistance against the force generated by the active pool, maintaining the stability of the spine [15]. Honkura and colleagues also identified a third pool of F-actin, an enlargement pool, which was required for spine head enlargement during the induction of long-term potentiation (LTP).

A major challenge in the analysis of structural and functional changes at synapses is the extremely small size, with the spine heads being less than a micron in diameter. Advances in superresolution microscopy provide us with some fundamental understanding of actin dynamics in dendritic spines. Photoactivated localization microscopy (PALM) revealed a greater velocity of actin movement in the spine head and a net flow of G-actin from dendrite into the spine [32]. Stimulated emission depletion (STED) imaging shows not only size changes, but also small shape changes that are difficult to detect with other methods. Stimulation often causes spines to take on a cup-like shape [33].

In more recent years, superresolution imaging has been implemented in various studies to more accurately examine dendritic spine properties such as morphology and diffuse dynamics of proteins, calcium, and small molecules [34]. For instance, Lu and colleagues [35] were able to visualise single molecule dynamics of the actin cytoskeleton modulating kinase calcium/calmodulin dependent kinase II (CAMKII) [36] within dendritic spines by using PALM. By implementing this high resolution technique Lu and team were able to distinguish multiple subpopulations of CAMKII within the spine

head based on motility. Furthermore, STED imaging in combination with fluorescence recovery after photobleaching (FRAP) was able to show that, upon stimulation of spine-specific LTP, as spine heads become larger, spine necks become shorter and wider [37]. In addition to this finding, Tønnesen and colleagues showed that spines which appear stubby in two-photon imaging are mushroom headed with short necks when visualised with STED. These studies highlight the importance of using superresolution imaging techniques when investigating characteristics of spines.

3. Regulation of the Postsynaptic Actin Cytoskeleton

The actin cytoskeleton in eukaryotic cells is regulated by a host of actin-associated proteins. The complex actions of these proteins govern actin cytoskeleton dynamics, enabling functional and structural diversity of F-actin populations within dendritic spines. Actin regulators facilitate assembly, disassembly, branching, stabilization, and reorganization of the cytoskeleton, all critical requirements for synaptic plasticity.

Assembly of actin filaments and as such the actin cytoskeleton requires the formation of rod-like actin polymers known as actin filaments or filamentous actin (F-actin) from actin monomers (globular actin, G-actin). The process by which G-actin is accommodated into the fast growing (barbed) end of an actin filament and dispersed from the opposite pointed end is referred to as actin treadmilling. Various actin-associated proteins are involved in regulating the assembly of actin filaments. In the following section we will focus our attention on a select number of key regulators, including the actin sequestering protein profilin, the actin nucleators formin and actin-related proteins 2 and 3 complex (Arp2/3), the actin depolymerizing factor (ADF)/cofilin, the actin motor protein myosin, and the actin stabilizing protein tropomyosin.

3.1. Actin Sequestering and Nucleating Proteins. Actin filament nucleation can occur *de novo* or as filament branches that nucleate on preexisting filaments. Formins are a superfamily of proteins with at least 15 different proteins found in mammalian cells that promote the *de novo* nucleation of unbranched actin filaments (for reviews, see [38, 39]). Their activity is regulated by small GTPases thereby controlling the assembly of new actin filaments [40–42]. Formins play a critical role in supporting the early morphogenesis of filopodial spines [43], and it localizes to fine, filopodial structures that are found at the distal part of more mature spines [44].

Arp2/3 promotes nucleation of F-actin daughter branches of existing F-actin mother filaments [45]. Actin filaments within filopodia were found to originate from branch points in lamellipodia that were generated by Arp2/3 [46]. Arp2/3 complex is detected in the central region of the spine head approximately 200–400 nm from the PSD indicating a local segregation of morphologically distinct actin filament populations [47].

Depletion of Arp2/3 complex in both B35 neuroblastoma cells and primary hippocampal neurons was found to decrease growth cone F-actin and reduce lamellipodia protrusion and contraction [46]. In addition to this, cells with deficient levels of Arp2/3 had lamellipodia that were narrower and contained actin networks that were less complex and contained fewer branches [46]. Conversely, in a study by Yang and colleagues [48], inhibition of Arp2/3 using the reversible Arp2/3 inhibitor CK-666 unexpectedly resulted in an increase in actin retrograde flow, which was significantly reduced upon inhibition of myosin II, suggesting that Arp2/3 restricts myosin II mediated retrograde flow of actin [48].

Activation of Arp2/3 occurs via the activity of nucleation promoting factors (NPFs) such as neural Wiskott-Aldrich syndrome (N-WASP), WASP family verprolin-homologous protein (WAVE, also known as SCAR), and WASP and SCAR homolog (WASH) [49]. Arp2/3 and WASH have been implicated in early endosome morphology and function. Through immunocytochemical analysis of fibroblast-like cells, WASH was found to extensively associate with early endosome markers EEA1 and Rab5 and weakly associate with recycling endosome marker Rab11 [50]. siRNA-mediated knockdown of WASH resulted in larger and more elongated EEA1 positive structures compared to controls [50]. Knockdown of WASH reduced trafficking of epidermal growth factor (EGF) to late endosomes, an effect also observed in response to actin-polymerization disruption. However, knockdown of WASH was not found to affect reuptake or recycling of transferrin, implying specificity of WASH to the endocytic degradation pathway [50]. These results suggest that WASH activity affects endosomal trafficking of cargo, most likely via Arp2/3 mediated actin dynamics.

Profilin is thought to promote F-actin elongation at the barbed end by accelerating the nucleotide exchange of ADP for ATP on G-actin. Recent findings suggest that profilin has separable roles in G-actin regulation [51]. Suarez and colleagues found that profilin is required for actin contractile ring formation in fission yeast through interactions with formin as well as limiting Arp2/3-mediated actin branching by sequestering G-actin [51]. Profilin is thought to favour formin mediated F-actin elongation over Arp2/3 while reducing the ability of both formin and Arp2/3 to nucleate filaments [51].

3.2. Actin Filament Depolymerizing Proteins. Turnover and disassembly of F-actin occur through the actions of ADF/cofilin. With the continual addition of monomeric ATP-bound actin to the barbed end of the filament, previously incorporated actin monomers become progressively distal to the barbed end to form the body of the filament. As this occurs, the ATP is hydrolysed to ADP [52]. The resulting ADP-bound actin subunits are still able to maintain filament stability due to the presence of an inorganic phosphate ($P_{(i)}$). It is believed that once $P_{(i)}$ has been released cofilin is able to bind to the filament, inducing severing and depolymerization [53].

Severing of ADP-actin from the pointed end of filaments is facilitated by the actions of ADF/cofilin in its dephosphorylated active state [54]. ADF/cofilin interacts directly with actin filaments, and its activity is regulated by the actions of LIM-Kinase 1 (LIMK-1) and Slingshot (SSH) phosphatase.

Phosphorylation of cofilin at its serine 3 site by LIMK-1 inhibits ADF/cofilin severing of actin filaments and increases F-actin content in actin-rich regions of neurons [55, 56]. Conversely, dephosphorylation as well as activation of cofilin by SSH results in severing of actin filaments. Based on cryoelectron microscopy three-dimensional reconstructions of cofilin bound to actin filaments, Galkin and colleagues [57] postulate that filaments decorated with cofilin undergo a conformational change whereby the actin protomers bound to cofilin rotate in a manner that induces greater flexibility of the filament. Once rotated, the filament exposed regions that were then vulnerable to severing through further ADF/cofilin actions [57]. The ability of ADF/cofilin to disassemble F-actin networks has been suggested to be integral to the enlargement of dendritic spine heads, possibly by creating new barbed ends from severed filaments [58]. The activity of cofilin can be modified by upstream signalling proteins such as Cdc42, which has been shown to promote cofilin activation [59].

Gelsolin is an actin-associated protein predominantly activated by Ca^{2+} [60]. When activated by Ca^{2+}, gelsolin undergoes conformational changes that expose actin binding sites [60–62]. Active gelsolin severs and then caps F-actin at barbed ends, resulting in the disassembly of the F-actin network and prevention of further polymerization [63–65]. However, in the presence of phosphatidylinositol 4,5-bisphosphate (PIP_2), gelsolin activity is largely abolished [66]. Furthermore, a study by Hartwig and colleagues [67] found that inhibition of gelsolin by PIP_2 application increased the prevalence of barbed ends suggesting that PIP_2 facilitates the removal of gelsolin caps from F-actin barbed ends. With the inhibition of gelsolin, further polymerization of actin filaments is enabled [68]. Overexpression of gelsolin in a PC12 neuronal-like cell line differentiated with nerve growth factor led to an increase in neurite length and motility rate compared to controls possibly through increased F-actin turnover [69]. Importantly, gelsolin function is required for the morphological transition of filopodia to spines [70].

3.3. Actin Stabilizing Proteins and "Gatekeepers" of Actin Filament Dynamics and Stability. Drebrin A (DA) is the adult isoform of drebrin and is found in mature neurons at postsynaptic sites of dendritic spines [71]. Drebrin A binds to F-actin, inhibiting depolymerization of the filament predominantly at the barbed end [72, 73]. An atomic force spectroscopy study by Sharma and colleagues [74] found that drebrin binding resulted in a twisting of F-actin conformation that induced stiffening of the filament. The conformational twist induced by drebrin was found to occur in a manner that was opposite to cofilin induced twisting in F-actin structure, suggesting that drebrin and cofilin have antagonistic effects of F-actin structure [74].

Drebrin has been shown to compete with cofilin for F-actin binding sites [75]. Cosedimentation experiments showed that DA and cofilin are able to simultaneously bind to F-actin and inhibit the actions of each other [75]. Neurons transfected with DA-GFP were found to have dendritic spines that had significantly lengthened necks compared to controls [71]. Binding of drebrin to F-actin was also shown to inhibit myosin V binding. *In vitro* motility assays showed an impairment of F-actin binding to myosin V coated glass surfaces when in the presence of drebrin A [72]. However, F-actin that was able to bind to myosin V in the presence of drebrin did not show any impairment in F-actin-myosin sliding. This suggests that drebrin may also modulate myosin V activity.

Tropomyosins (Tpms) are a family of actin-associated proteins and key regulators of the actin cytoskeleton. In mammals, TPM1, TPM2, TPM3, and TPM4 genes have been shown to be responsible for the generation of more than 40 known tropomyosin isoforms [76] with gene products from TPM1, TPM3, and TPM4 found in neuronal cells. The primary structure of Tpm proteins consists of paired α-helices arranged in a coiled-coil manner [77, 78]. Although the primary structure of tropomyosin is highly conserved between the various isoforms, alternatively spliced exons allow functional diversity and differential localization within the cell [76, 79, 80].

Studies that have examined the interaction between Tpm and actin filaments have shown that Tpm isoforms have distinct F-actin regulatory effects as well as differential affinities to associate with F-actin [76, 78, 81] and facilitating functional diversity of cytoskeletal F-actin [82]. Tpm3.1, a tropomyosin isoform derived from the TPM3 gene, is involved in F-actin stabilization and reduced cell motility [76, 82] whereas Tpm1.12, derived from the TPM1 gene, promotes F-actin-ADF interactions resulting in F-actin severing [76].

Further studies exploring the effects of increased Tpm3.1 protein levels found an increase in the length and branching of dendrites and axons, along with increased growth cone size [83] and an increased pool of filamentous actin in growth cones [84]. A subsequent study showed altered growth cone dynamics in response to the knockdown of Tpm3.1 and Tpm3.2 [85]. These results suggest that this particular tropomyosin isoform may be involved in the stabilization of the actin cytoskeleton in neurons. Guven and colleagues [79] have detected Tpm3 gene products in the postsynaptic compartment of mature cultured neurons suggesting a potential role in the maintenance of synaptic connections.

Another mode of actin filament regulation and stabilization is through actin capping proteins, which bind to the barbed end of actin, preventing further elongation. This process limits the length of actin filaments. Actin capping protein (CP) has been found to be associated with the actin and Arp2/3 network in spine heads [28], and the level has been observed to be elevated during stages of synapse formation. Knockout of CP in rat hippocampal cultures has been shown to lead to altered spine morphology and a reduction in synapse density [86]. Another protein with actin capping function, Eps8, has been found to play a role in actin-based motility, such as filopodia growth and numbers [87]. Eps8 is also enriched in the PSD, and as in the case of CP knockdown, reduced expression levels alter spine morphology [88]. The actin severing protein gelsolin can also cap actin filaments, depending on calcium ion concentration [89]; however this has not been studied in detail in neurons.

3.4. Actin Motor Proteins. Myosins form a superfamily of actin motor proteins. The family contains motors with diverse

functions that range from building contractile elements (conventional family members) such as the sarcomere of muscle cells to driving intracellular transport of vesicles (unconventional family members). Characteristic to all myosins is the presence of catalytic head or motor domains that bind and hydrolyse ATP to produce motility. The tail of myosins can either align with other myosin molecules to form myosin filaments (e.g., the formation of the thick filaments in muscle, built by muscle isoforms of myosin II) or bind to different cargos allowing transport of these cargos along actin filaments. For a review on myosin function and diversity, see Hartman and Spudich [90]. The motor domains of the myosins bind to F-actin [91] and have been shown to travel along the filament in a hand-over-hand type movement using various imaging techniques [92–94]. The unconventional myosins V and VI have been implicated in vesicle trafficking within dendritic spines [95, 96].

Together these actin-associated proteins provide diverse regulation of the cytoskeleton within the postsynaptic compartment of dendritic spines.

4. Learning and Memory as Actin Cytoskeleton-Dependent Process

Long-term changes in connections between neurons are thought to be the basis of memory formation. At most synapses in the brain, activity-dependent synaptic plasticity is triggered by a rise in postsynaptic calcium, which triggers a series of downstream effectors that can initiate different forms of synaptic plasticity, including long-term potentiation (LTP) and long-term depression (LTD) of synaptic transmission [97]. Although the loci of expression can vary between pre- and postsynaptic structures depending on the synapse, developmental stage, and induction protocol, at forebrain glutamatergic synapses synaptic plasticity often manifests as a change in the number of AMPA receptors expressed at the synapse [98].

As discussed above, synaptic proteins are continuously shuttled in and out of the plasma membrane; thus the number of AMPARs at the synapse is governed by the relative rate of receptor exocytosis and endocytosis, resulting in concomitant spine head volume changes. Several studies have shown that LTP is accompanied by an increase in spine volume [22, 31, 99] whereas LTD is accompanied by a reduction in spine volume [31, 100].

Actin dynamics are integral to both structural and functional synaptic plasticity. The LTP-associated spine enlargement is associated with an increase in F-actin [101] that persisted for several weeks. Furthermore, blocking actin polymerization via application of latrunculin and cytochalasin toxins impairs LTP and spine enlargement [102–106]. Conversely, LTD is associated with a relative decrease in F-actin [31] and stabilization of the actin cytoskeleton impairs LTD and the associated reduction in spine volume [107]. Application of drugs impacting actin stability also disrupts a variety of associative learning tasks (see [108] for review).

Measurements of fluorescence recovery of photobleached green fluorescent protein-tagged actin (GFP-actin) in the spine head indicate that most of the actin in the spine head is found in filaments that rapidly turn over, with only a small fraction of actin being assembled in the form of stable filaments [30]. Actin dynamics are altered by neuronal activity. High frequency stimulation, which induces LTP and enlarges the spine head, is associated with an increase in proportion of actin present as F-actin in the spine head [31]. Conversely, LTD-inducing low-frequency stimulation was found to reduce the spine head volume and the F-actin : G-actin ratio [31].

Using photoactivatable GFP-actin, Honkura and colleagues [15] tracked the spatial temporal movement of actin. Their data provide evidence for three functional pools of F-actin: a dynamic pool at the tip of the spine head, a stable pool at the base of the spine, and an "enlargement pool" that emerges following repeated stimulation of the spine head with glutamate. The persistence of the enlargement pool in the spine head was associated with structural LTP.

In addition to actin, several actin-associated molecules show activity-dependent changes in phosphorylation state and distribution. LTP induces a transient increase in spines immunopositive for phosphorylated (deactivated) cofilin [109]. A recent study by Bosch et al. [110] describes the spatiotemporal dynamics of GFP tagged actin and several GFP tagged actin-associated proteins following the induction of LTP. They found that structural LTP was associated with the rapid translocation of actin and several actin-associated proteins into the spine head. Of particular note was cofilin, which became concentrated in the spine head.

Structural plasticity is not limited to the neurons. Actin-rich perisomatic astrocytic processes [111] at glutamatergic synapses show activity-dependent remodelling, expanding in concert with spine enlargement following the induction of LTP [112].

5. The Actin Cytoskeleton as Key Regulator in Glutamate Receptor Trafficking

Synaptic function directly correlates with the composition of neurotransmitter receptor integration at the postsynaptic membrane. The cellular and molecular mechanisms of ionotropic receptor trafficking have been extensively reviewed [113–115]; here we will focus on regulatory mechanisms by which the cytoskeleton controls the neurotransmitter receptor expression profile in excitatory synapses. See Figure 1 for a schematic summarising the mechanisms involved in glutamate receptor trafficking.

5.1. AMPA Receptor Trafficking. AMPARs are the primary ionotropic glutamate receptors responsible for fast excitatory synaptic transmission. At the postsynaptic membrane, regulation of AMPAR levels determines the strength of synaptic transmission. During LTP induction, AMPARs are inserted into the PSD and are removed during LTD. AMPARs comprise a combination of four subunits GluA1–4 (GluR1–4) [120] forming heterotetrameric channels [121]. However, the majority of synaptic AMPARs have GluA1/2 subunit composition [122].

AMPAR trafficking to and from the plasma membrane depends on actin dynamics. Prolonged treatment (24 h) of

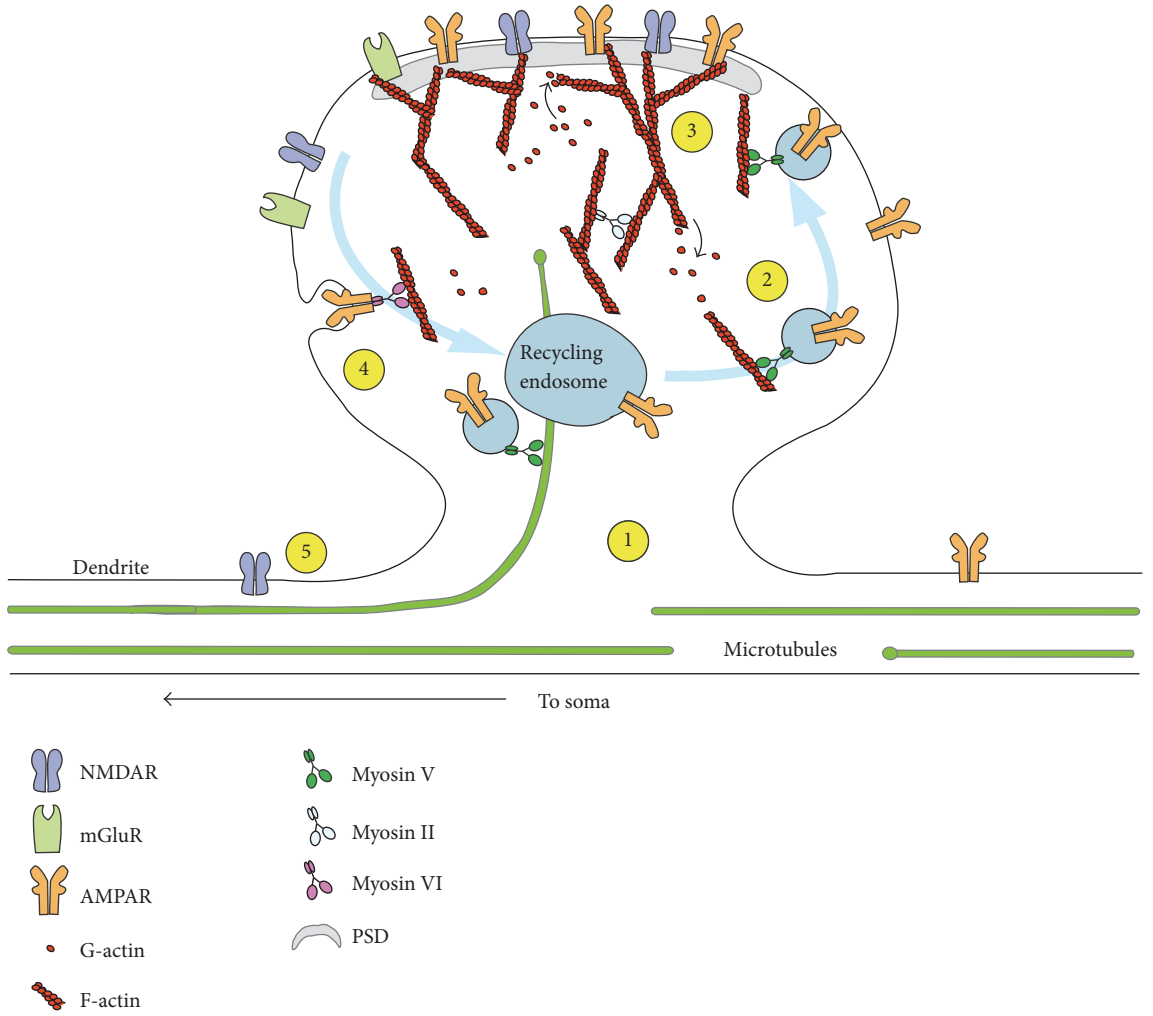

Figure 1: Schematic representation of the cytoskeleton-dependent trafficking of neurotransmitter receptors. Depicted are the key structures of the synapse and associated cytoskeletal molecules. Numbers indicate the following steps: (1) Myosin V traffics vesicles with receptors from the soma to the distal dendritic sites via microtubules (MT) [116]. MT plus ends are indicated by green circles. (2) Once within spines, myosin V transports receptors to plasma membrane via actin filaments [117]. (3) Anchoring of receptors in the PSD relies on myosin II contractile force on actin cytoskeleton in combination with constant actin treadmilling/turnover [48]. Lateral diffusion of receptors to and from the PSD to presynaptic regions can occur. (4) Receptor internalization involves myosin VI activity. Myosin VI transports internalized receptors to endosomal organelles, facilitating recycling of receptors back to the membrane or to degradation pathways [95]. (5) Receptors can also travel between the PSD and peripheral sites [118, 119].

cultured primary hippocampal neurons with latrunculin A, an inhibitor of F-actin nucleation, attenuates the expression of GluA1 AMPAR subunit in dendritic spines [123]. Hippocampal neurons, treated with latrunculin A prior to tetraethylammonium (TEA) LTP induction, prevented insertion of GluR1 during TEA LTP. Furthermore, treatment with 1 μM jasplakinolide prior to TEA also inhibited the insertion of GluR1 in response to TEA LTP induction [124]. Furthermore, treatment with jasplakinolide, an F-actin stabilizer, prevented AMPAR endocytosis during intense NMDAR activation [124]. These results suggest that expression of AMPARs at the postsynaptic membrane requires both polymerization and disassembly of the actin cytoskeleton, and F-actin stabilization is required to anchor AMPARs at the plasma membrane. Overall these studies show that AMPAR trafficking and anchoring require actin cytoskeletal dynamics and remodelling.

AMPAR trafficking processes are facilitated by actin cytoskeleton dynamics. GTPases such as Rho Ras and Rac activate downstream effectors that in turn stimulate or inhibit the activity of actin-associated proteins thereby regulating actin cytoskeletal dynamics [125]. Based on this, it is not surprising that actin-associated proteins and their upstream activators are able to affect trafficking of glutamate receptor subunits.

Vasodilator-Stimulated Phosphoprotein (VASP) regulates and modulates synaptic strength through actin polymerization. VASP has been shown to bind to both actin and profilin, promoting F-actin polymerization and preventing barbed end capping [126–128]. VASP has also been found

to be essential for synapse maintenance. Overexpression of VASP increased dendritic spine volume, F-actin content, and the expression GluR1 [129]. Conversely, knockdown of VASP reduced the density of dendritic spines and synapses and GluR1 subunits within the spine [129].

Arp2/3 gates the trafficking of endosomal vesicles and internalization of AMPARs. Arp2/3 is inhibited by protein interacting with C kinase 1 (PICK1) which is in turn inhibited by the activity of GTPase ADP-ribosylation factor 1 (Arf1). Inhibition of Arp2/3 by PICK1 overexpression facilitates endocytosis of AMPAR subunits during LTD [130]. Similarly, blocking Arf1 results in AMPAR endocytosis [131].

Further studies implied a role of PICK1 in LTD. Knockdown of PICK1 prevented NMDAR LTD induced removal of GluA1 subunits from the plasma membrane. More specifically, a time course analysis of AMPAR endocytosis in response to NMDAR-mediated LTD showed that PICK1 knockdown did not prevent the initial endocytosis of AMPARs but failed to retain the receptors intracellularly. Recycling of AMPARs back to the plasma membrane during knockdown of PICK1 inhibited NMDAR-induced LTD [132].

Myosin motor proteins are critical for AMPAR trafficking. Myosin motors Va and Vb are considered efficient organelle transporters, their long lever arms allowing them to travel along the top of actin filaments in a step-like fashion rather than spiralling around the filament [133]. Interference of myosin Va and Vb function has been shown to impact neuronal cell shape and function, including changes in the composition of the PSD and modulation of LTD and LTP induction [133]. Myosin Va binds directly with the C-terminal of GluA1 subunits [117] and is required for transportation of AMPARs from the dendritic shaft into spines. Furthermore, myosin Va is implicated in the induction of LTP. Imaging of GFP-GluR1 showed that depletion of myosin Va resulted in reduced expression of GluR1 to synapses in response to CAMKII mimicked LTP. Electrophysiological experiments using siRNA knockdown of myosin Va abolished LTP induction as indicated by AMPAR mediated responses [117]. In a separate study by Wagner and colleagues [134], myosin Va was found to be critically involved in the trafficking of smooth endoplasmic reticulum (ER) into the spines of Purkinje neurons. Attenuation of myosin Va motility along actin filaments inhibited the insertion of smooth ER tubules into the spines of Purkinje neurons [134]. Reduction of ER tubule insertion minimised transient Ca^{2+}-release upon mGluR activation, a process required for LTD within the cerebellum [134]. No changes were observed in fast AMPAR mediated Ca^{2+}-transients. These studies suggest that myosin Va is involved in various functional aspects of synaptic plasticity, including the trafficking of smooth ER into spines and the exocytosis of AMPARs.

Myosin Vb localizes to different regions of neurons in an age dependent manner. At 7–14 days *in vitro* (DIV) this particular motor protein was detected in the soma and dendrites while being absent at synapses [116]. At more mature ages, 21–28 DIV, myosin Vb was predominantly detected in the soma but was also observed in dendritic spines that were positive for synaptic markers synaptophysin and PSD95 [116]. Lisé and colleagues suggest that these results imply that myosin Vb is responsible for the initial transportation of cargo from the soma to distant dendritic sites early on in neuronal development and then remains locally at synaptic regions where it is involved in delivery and recycling of cargo to the synapse once the neuron has matured. A more recent study by Wang and colleagues [96] also detected myosin Vb enrichment in dendritic spines using immunocytochemical techniques. In addition to this, Wang et al. also determined that myosin Vb is involved in trafficking of recycling endosomes into spines. Recycling endosomes are a source of AMPARs and are responsible for directing these receptors back to the plasma membrane during LTP induction [135, 136].

The role of myosin Vb in the transportation of glutamate receptor subunits within the postsynaptic compartment was determined using transfection of functionally deficient or dominant negative versions of myosin Vb and the small GTPase Rab11 [116]. Expression of myosin Vb C-terminal tail constructs, fused to GFP, resulted in a reduction of GluR1 subunit clustering at sites, positive for synaptophysin. This suggests that full length myosin Vb is required for delivery of GluR1 subunits to the synapse [116]. Furthermore, Lisé and colleagues found that expression of this construct did not alter the localization of GluR2/3 subunits, implying that myosin Vb may specifically regulate pools of GluR1 homomeric AMPARs. Myosin Vb is thought to associate with GluR1 through RabII coupling [116]. RabII is a recycling endosome protein that binds to myosin Vb via C-terminal amino acids 1797–1846 [137]. Neurons transfected with myosin Vb mutants that lack the C-terminal domain required for RabII binding had reduced GluR1 clustering and surface expression, suggesting that myosin Vb trafficking of GluR1 is mediated by RabII binding [116]. Furthermore, binding of RabII requires a conformational change in the myosin Vb protein that occurs in response to Ca^{2+} [96].

Unlike myosin V, myosin VI has been reported to travel along F-actin towards the pointed/minus end of the filament [138, 139]. From coimmunoprecipitate assays myosin VI has been shown to associate with GluR1 and GluR2 subunits [95]. Furthermore, myosin VI has been shown to form a complex with GluR1 and the scaffolding protein SAP97, suggesting a functional link between the actin cytoskeleton AMPAR subunits and the postsynaptic scaffold [140]. Hippocampal neurons deficient in myosin VI had greatly reduced levels of internalized AMPARs after AMPA stimulation compared to controls suggesting that myosin VI is important for endocytosis of AMPARs. In addition to this Osterweil and colleagues [95] confirmed that myosin VI trafficking of AMPARs occurs via clathrin-mediated endocytosis.

Cofilin in AMPAR Trafficking. Using live imaging recordings, Gu et al. [141] found that inhibition of LIMK1 resulted in enhanced trafficking of GluR1 and inhibition of SSH resulted in diminished GluR1 trafficking to the spine surface during TEA-induced LTP. Due to the relationship between LIMK1 and SSH and cofilin, these results suggest that cofilin activation is required for expression of AMPARs during LTP. As there were no significant changes in spine head to neck ratios before and after 6 h treatment with LIMK1 and SSH inhibiting peptides, Gu and colleagues concluded that cofilin

mediates AMPAR trafficking independent of the actions of cofilin on cytoskeletal spine structure. Consistent with this finding, Yuen and colleagues [142] also found a reduction in AMPAR excitatory postsynaptic current (EPSC) amplitude and frequency in response to siRNA knockdown of SSH.

In an experiment investigating the role of cofilin in an aversive conditioning paradigm, it was observed that cofilin was temporally enhanced in the infralimbic cortex of rats after extinction of an aversive memory [143]. Furthermore, elevation of cofilin levels resulted in an increase in surface expression of GluA1 and GluA2 subunits and facilitated extinction learning. Conversely, inhibition of cofilin during extinction training prevented the insertion of these subunits at the plasma membrane and impaired extinction learning [143].

These studies imply that the F-actin severing properties of cofilin are required for AMPAR recruitment at the plasma membrane during LTP. This finding would fit well with the suggested cofilin mediated cytoskeletal reorganization during LTP posed by Chen and colleagues [58] whereby cofilin severing of F-actin allows for enlargement of dendritic spine volume during LTP. As AMPAR expression at the postsynaptic membrane and spine volume are tightly correlated it is plausible that cofilin activity mediates these processes.

Drebrin in AMPAR Trafficking. Electrophysiological recordings demonstrated that expression of DA-GFP in neurons enhances excitatory transmission compared to GFP controls. Inhibition of drebrin A by DA antisense oligonucleotides further supported a role of DA in synaptic transmission as inhibition of DA resulted in a decrease in excitatory transmission, indicated by decreases in miniature EPSC (mEPSC) amplitude and frequency, compared to controls [71]. Drebrin knockdown was found to impair AMPAR mediated mEPSC amplitude and frequency in hippocampal neurons. In addition, mEPSC amplitude and frequency in response to glutamate-induced LTP were reduced during drebrin knockdown. These results suggest that drebrin is involved in AMPAR trafficking and insertion at the plasma membrane [144].

4.1N in AMPAR Trafficking. Protein 4.1N is a homolog of 4.1R, a protein found to be an integral component of the cytoskeleton in erythrocytes. 4.1N has been found to localize in various regions of the brain including the CA1–CA3 areas of the hippocampus [145]. Furthermore, immunocytochemical analyses showed colocalization of 4.1N with PSD95, suggesting that 4.1N localizes to sites of synaptic connection [145]. Like its erythrocyte homolog, 4.1N is thought to associate with the actin cytoskeleton [145].

Using coimmunoprecipitation and deletion of various amino acids in GluR1 proteins, 4.1N was found to associate with GluR1 at the membrane proximal region of the C-terminal domain at amino acids 812–823 [146]. Truncations of GluR1 at this membrane proximal region resulted in GluR1 becoming incapable of associating with 4.1N and having decreased expression at the plasma membrane [146] suggesting that 4.1N activity is required for expression of GluR1 subunits in the plasma membrane.

5.2. NMDA Receptor Trafficking. N-Methyl-D-aspartate receptors (NMDARs) are glutamate receptors, present in smaller numbers than AMPARs, which serve to modulate excitatory transmission by affecting AMPAR expression [113]. Activation of AMPARs leads to removal of the Mg^{2+} block of NMDARs, allowing for calcium influx. This calcium influx affects a variety of pathways which regulate the expression of AMPARs.

NMDARs have a heterotetrameric structure, usually consisting of two GluN1 and two GluN2 subunits. The subunits are produced and assembled in the endoplasmic reticulum in the cell body and then moved to the spine by various kinesins, moving along microtubules [113]. While transport to the spine involves only microtubules and not actin, short distance transport in the spine head to the PSD seems to be dependent on actin and myosin motor proteins [147]. However, the exact proteins and mechanisms involved are still unclear. Myosin IIb has been shown to be involved in insertion of NMDARs at the membrane, but it is not believed that it is responsible for transporting it to the membrane [148]. At the surface of the synapse, NMDARs are associated with scaffolding proteins of the PSD [149].

Endocytosis of NMDA receptors can be triggered by low-frequency stimulation [150]. Receptor clusters can move laterally in and out of the synaptic site. When NMDARs were irreversibly blocked with a drug, recovery of NMDAR-mediated current was observed, suggesting receptors migrated to the synaptic site from the periphery [118]. Actin dynamics have also been shown to affect NMDAR placement at the synapse [123].

There is evidence that actin dynamics have a regulatory role in NMDAR function. Alpha-actinin-2, an actin binding protein present in the PSD, competes with calmodulin for binding to NMDARs [151]. Alpha-actinin is a protein that binds to both NMDARs [152] and components of the PSD [153] and has a role in spine morphology [154].

Severing of actin filaments or preventing polymerization of actin has been found to induce a rundown of NMDAR current. Inactivation of RhoA which promotes actin polymerization increases rundown of NMDAR current [155]. Similarly, administering cytochalasin, a drug blocking actin polymerization, also induces rundown [156]. Conversely, knockout of gelsolin enhances NDMAR current [157]. Modification of actin can also affect placement of receptors in the postsynapse. Latrunculin A, an inhibitor of actin polymerization, modifies localization of NMDA receptors [123].

Another way actin may be involved in NMDAR trafficking is through its function in microtubule extension. Drebrin, an actin stabilizing protein, interacts with both actin microfilaments and microtubules and promotes entry of microtubules into dendritic spines [158]. NMDARs also have a part in mediating spine morphology. Deletion of the NR1 subunit has been found to reduce spine density and increase head size [159]. The actin severing protein cofilin is required in spines for NMDAR-induced remodelling [160].

5.3. mGluR Receptor Trafficking. As well as the ionotropic glutamate receptors, there are also metabotropic receptors (mGluRs) present at the postsynaptic membrane. Unlike

NMDARs and AMPARs, metabotropic receptors are G-protein coupled receptors. They produce their effects through signalling pathways involving inositol phosphate (IP), diacylglycerol (DAG), activation of protein kinase C (PKC), and release of intracellular Ca^{2+} stores. There are three families of mGluRs: Groups I, II, and III. In neuronal tissue, Groups II and III are located on the presynaptic membrane, while Group I is located on the postsynaptic membrane. Group I includes mGluR1 and mGluR5. They are comprised of GluA1 and GluA5 receptor subunits. Little is known about synthesis and trafficking of mGluRs to the spine, but there is evidence that, like NMDARs, they are associated with the PSD [161].

mGluRs can move laterally on the postsynaptic membrane. It has been found that transport of mGluR5 on the membrane surface involves being bound to microtubules, and the movement of these microtubules was dependent on actin flow. Preventing actin polymerization through application of cytochalasin D disrupted the movement of mGluR5 on the membrane [119]. In cultured hippocampal neurons, mGluRs have been found to be located at perisynaptic regions of excitatory synapses [162]. mGluR function can be modified by signalling molecules. One example is Rab8, a small GTPase involved in vesicular trafficking. It binds to the C-terminal tail of mGluRs and inhibits the production of IP and the release of intracellular Ca^{2+} [163] Rab8 expression resulted in inhibition of mGluR1 endocytosis, maintaining cell surface expression of the receptors [163]. mGluRs are responsible for activity-dependent synaptic plasticity through their role in regulating trafficking of other glutamate receptors. It has been found that blocking both mGluR1 and mGluR5 prevented induction of LTD, suggesting that mGluRs have a role in mediating AMPAR endocytosis [164].

6. Disruption of the Actin Cytoskeleton and Neurotransmitter Receptor Trafficking in Disease

While current research on pathological mechanisms of AD encompasses the study of a diverse range of potential mechanisms, a central theme underlying AD pathology is the loss of synaptic connectivity. Neural connections within the brain underlie the most basic and fundamental requirements for successfully interacting with the world around us. The loss of these neural circuits can catastrophically impair one's ability to function independently, as observed in AD. Although various gene mutations have been implicated in familial forms of AD [165] the causes behind the onset of AD pathology are as yet unknown.

A considerable effort in Alzheimer's disease research has been to identify the brain regions most vulnerable to degeneration. Over the last two decades the literature has reported significant hippocampal deterioration in early AD pathology [1, 166–169]. Hippocampal volume is often used as a diagnostic tool for AD as the level of deterioration positively correlates with the severity of AD symptoms [170, 171]. Other features of the medial temporal lobe, wherein lies the hippocampus, such as cortical thickness, have also been described as reliable indicators of AD pathology [172–174]. These hippocampal measures have also been successful as an indicator of AD vulnerability in presymptomatic patients [175, 176].

The molecular mechanisms of AD pathogenesis are still not well understood. The two major pathological hallmarks of AD are the extracellular accumulation of proteolytic derivations of amyloid precursor protein (APP) called amyloid-β (Aβ) peptides and intracellular aggregation of tau protein fibrils. Accumulation of these abnormal proteins is thought to be responsible for the breakdown of synapses, decreases in spine density, and impairment of synaptic plasticity [177–179]. Although studies have highlighted interplay between these two pathological markers [180–182], evidence suggests that the accumulation of Aβ oligomers accelerates tau aggregation and synaptic loss [183, 184]. The biosynthesis of Aβ arises as a residual product from α-secretase failing to cleave APP [185]. In the absence of α-secretase activity, β- and γ-secretases cleave APP, generating Aβ peptides. Large oligomeric peptides have been shown to be neurotoxic in comparison to small Aβ oligomers and soluble monomers, which have been implicated in neuroprotective processes [186, 187].

6.1. Pathological Role of Soluble and Aggregated Forms of Amyloid β Peptide. Amyloid fibrils are aggregations of long, insoluble fibres of Aβ peptide. Protofibrils (shorter, soluble aggregations that are precursors to fibrils) and fibrils have been observed to have an overexcitatory effect on neurons and interactions with NMDA receptors [188]. Amyloid plaques, large extracellular deposits of Aβ fibrils, are the most obvious form of pathophysiology, associated with Alzheimer's disease. Behavioural deficits of dementia have previously been found to be correlated with the size of the cortical area affected by plaques [189]. In the study by Cummings and Cotman, deposition of Aβ was found to strongly correlate with scores on the Mini-Mental State Exam (MMSE), the Blessed Information Memory Concentration (IMC) test, and Clinical Dementia Rating (CDR) with higher deposition resulting in poorer scores. In brain slice cultures, plaque-covered areas contained only few dendritic spines and spine volume was reduced in the areas around the plaque [190]. However, synaptic deficits can occur in the absence of plaques [191] and the extent of plaque formation does not always correlate with the degree of neurodegeneration or clinical status of AD [192, 193]. More recent studies are still debating as to whether or not plaque formation is responsible for behavioural deficits in AD. In some of these studies, behavioural deficits could be rescued in response to a reduction in amyloid plaques [194, 195]. However, these studies also observed reductions in soluble Aβ, which is believed to be highly toxic when in oligomeric form [187]. Cramer and colleagues [196] used a mouse model of AD to show that increasing levels of apolipoprotein E (apoE) can lead to a reduction of soluble and insoluble Aβ. ApoE is involved in the proteolytic degradation of soluble forms of Aβ. Acute increases in apoE resulted in significant reductions in both soluble Aβ and plaque quantity. AD-associated learning deficits were also reduced in both the Morris water maze and contextual fear conditioning paradigms upon treatment with apoE [196]. However, these behavioural measurements were found to only correlate with reduced levels of soluble

Aβ and not with plaque removal. Plaque formation can be present without cognitive decline [197]. This shifted the focus to an increased interest in understanding the pathological role of soluble forms of Aβ. Soluble Aβ forms include monomers, dimers, and larger oligomers of Aβ protein. Soluble Aβ is localized to the postsynaptic compartment in both animal models of Alzheimer's disease [198] and human patients [199]. Aβ oligomers cause alterations to pre- and postsynaptic morphology, including spine shrinkage and collapse [200]. LTP has been found to be inhibited in brain slices after application of oligomers sourced from cell cultures [201], dimers extracted from human AD brains [178], and synthetic Aβ oligomers [202]. Aβ can also facilitate LTD. Application of oligomers from different sources allowed LTD to be induced in conditions that are normally insufficient to do so [203].

6.2. The Effects of Amyloid β on the Regulation of the Actin Cytoskeleton. There are many conflicting pathways in which Aβ is proposed to alter actin cytoskeletal dynamics. The predominant theories involve modulation of cofilin activity. Conflicting evidence is found throughout the literature that suggests that cofilin is either activated or inhibited in response to Aβ toxicity (see Figure 2). Petratos and colleagues [204] reported an increase in active RhoA in SH-SY5Y cells treated with Aβ. RhoA activates Rho kinase II (ROCKII), which leads to the deactivation of myosin light chain kinase, dephosphorylating and thereby inhibiting the actions of LIMK [205]. Another pathway in which Aβ is proposed to increase cofilin activation is through inhibition of Rac1. RhoA activation requires deactivation of Rac1 [206]. Therefore it is suggested that Aβ induced increases of RhoA antagonistically decrease levels of Rac1. Rac1 inhibition reduces PAK1 signalling, which reduces the phosphorylation and activation of LIMK [207, 208]. This pathway is supported by findings of decreased PAK1 in the brains of AD patients [209]. Inactivation of LIMK in both pathways would result in reduced phosphorylation and as such the activation of cofilin [56]. Increased activation of cofilin may then disrupt receptor trafficking through disassembly of the actin cytoskeleton [208] and/or formation of cofilin rods [210]. Conversely, various studies suggest that Aβ ultimately inhibits cofilin activation through alternate signalling pathways. Mendoza-Naranjo and colleagues [211] found an increase in levels of GTPase Cdc42 in hippocampal neurons treated with fibrillar Aβ. Cdc42-PAK1-LIMK signalling cascades result in decreased cofilin activation [56, 212] which would have implications for actin cytoskeleton dynamics.

The formation of cofilin rods, abnormal aggregates of bound actin and cofilin, has been shown to disrupt vesicle transport and cause accumulation of Aβ and APP [210, 213, 214]. Blocking intracellular trafficking by cofilin aggregation induces synaptic loss in hippocampal neurons [215]. Hippocampal neurons transfected with wild-type GFP-cofilin resulted in the formation of cofilin-actin rods. Transfections using cofilin mutants, phosphomimetic GFP-cofilin and constitutively active GFP-cofilin, resulted in no or reduced cofilin rod formation, respectively. This suggests that both active and inactive cofilin are required for the formation of cofilin rods, a requirement potentially fulfilled by the contradicting pathways mentioned above. As these mutations involved the phosphorylation Ser3 site on cofilin it is believed that the formation of cofilin rods is critically dependent on this site [215]. Immunostaining detected rod localization in distal dendrites and occasionally in axons. An additional observation was made in that decreased MAP2 fluorescence was apparent in regions containing rod formation compared to neighbouring regions absent of rods. The authors suggest that this could imply impairment of microtubule integrity [215]. Formation of cofilin rods from endogenous levels of cofilin was observed in response to glutamate treatment or neurotoxic ATP depletion [215].

Immunostaining of RFP-cofilin and GFP-Rab5, a small GTPase that localizes to early endosomes [216], showed that, in areas where cofilin rods appeared, early endosomes positive for Rab5 were either largely absent or positioned at the distal or proximal ends of rods suggesting that they were immobilised at these regions. This finding was similar for imaging of GFP-mitochondria, where localization was found to be either largely absent in regions containing rods or trapped between rods. These results imply that rod formation disrupts intracellular organelle distribution [215]. This was further confirmed using live imaging techniques. Prior to rod formation, mitochondria appeared to be able to freely move within the cell; however, after rod formation mitochondria trajectory was significantly restricted and slower than that before rod formation [215].

Rod formation was also found to affect synaptic transmission and induce synaptic loss. Markers of pre- and postsynaptic regions were significantly reduced in areas containing rods. In addition to this, dendritic spine density was also decreased in rod rich areas. The cofilin rod reduction in spine density was in part supported by electrophysiological recordings of hippocampal neurons expressing varying quantities of cofilin rods. A significant reduction in mEPSC frequency but not amplitude was observed in neurons with exceptionally high levels of rods [215]. Neurons with mild levels of rods had mEPSC frequencies and amplitudes that were comparable to controls. These results suggest that aggregation of cofilin rod formation induces synaptic loss, which eventually leads to the loss of neuronal function [215].

Decreased levels of gelsolin were found in AD patients' plasma. The level of decrease was correlated to progression of disease, as measured with a Mini-Mental Status Examination [219]. Gelsolin has also been found to be involved with removal of Aβ. Gelsolin forms a complex with Aβ, making it less neurotoxic [220].

6.3. The Effects of Amyloid β on the Trafficking of Neurotransmitter Receptors. It is plausible then that neurotoxic stimulation via soluble Aβ activity drives the formation of cofilin rod formation in AD pathology and through this pathway disrupts synaptic properties such as AMPAR trafficking via early endosomes. AMPARs can be internalized to early endosomal organelles where they are then transferred to recycling endosomes for reinsertion at the plasma membrane, or to late endosomes for degradation depending on the type of synaptic stimulation [221]. AMPAR stimulation induces endocytosis

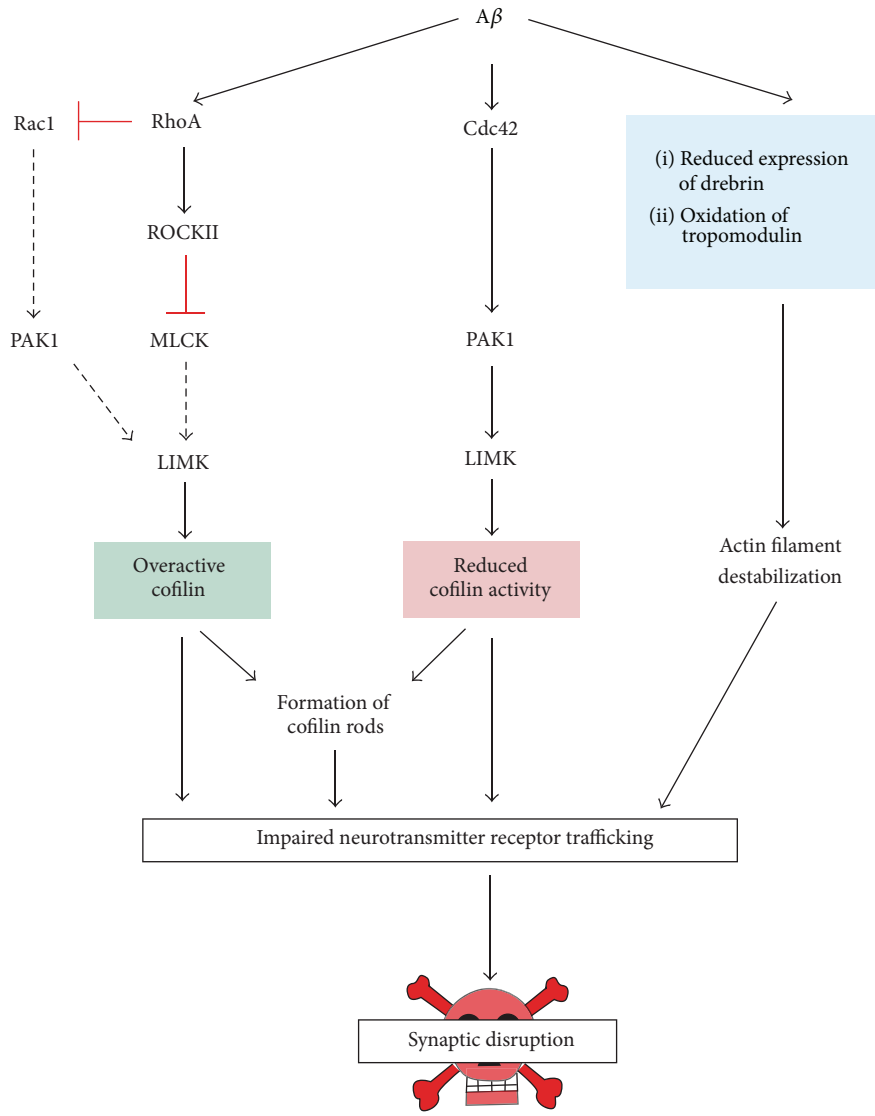

FIGURE 2: Amyloid-β disrupts the actin cytoskeleton and receptor trafficking through multiple pathways. There are many conflicting pathways through which Aβ is proposed to alter the actin cytoskeleton. These may involve both up- and downregulation of cofilin activity. Activation of RhoA by Aβ [204] antagonistically inhibits Rac1 [206], both leading to increased cofilin activity. In contrast, Aβ can cause decrease in cofilin activity via activation of Cdc42 [211]. Both active and inactive cofilin are thought to be required for the formation of cofilin rods, which lead to impairment of intracellular transport [215]. Alternatively or in addition to this, altered expression and/or processing of actin filament stabilizing proteins [217, 218] may impact the trafficking of neurotransmitter receptors.

of AMPARs to early endosomes followed by transferral to late endosomal organelles and subsequent degradation [135, 221]. NMDAR excitation on the other hand results in AMPAR internalization to early endosomes and then to recycling endosomes ultimately leading to the reinsertion at the plasma membrane [135, 221]. As Aβ is thought to weakly activate NMDARs [222], it is postulated that AMPARs are endocytosed to early endosomes but are possibly unable to be transferred to recycling endosomes due to cofilin-actin rod obstructions. This would result in weakening and eventual loss of synapses as is observed in both neurons with extensive cofilin-actin rod formation and also neurons afflicted by Aβ. Recent research confirms the importance of recycling endosome location on synaptic potential. Positioning of endosomes has been found to be important for AMPAR trafficking and synapse architecture, with the removal of recycling endosomes from the spine resulting in decreased surface AMPAR levels [223].

A potential way by which synapses are destabilized is the loss or functional disruption of actin stabilizing proteins at the synapses. Drebrin has been found to be reduced in the brains of AD and Down syndrome patients [217]. Drebrin reduction is also associated with impaired synaptic plasticity [224] and altered movement of NMDAR clusters to synapses [225]. In brains from AD cases, Tpms were found in neurofibrillary tangles (NFT), intracellular protein aggregates of abnormally phosphorylated tau protein [226, 227]. However, studies are limited, because antibodies used were not specific

for Tm isoforms: identities of specific isoforms in NFTs are unknown. Interestingly, Tpm3.1 is a major target of oxidative damage in AD, suggesting that disruption of Tpm3.1 may contribute to pathological changes in the disease [218]. Pathways by which Aβ affects the nucleation of actin filaments may be closely connected to those disrupting microtubule dynamics. Knockdown of the formin mDia1 in NIH3T3 cells reduces Aβ induced pathological stabilization of MTs [228].

7. Conclusions

In this paper we discussed the current understanding of the role that the actin cytoskeleton plays in the regulation of the postsynaptic compartment, how it drives structural changes, how it supports neurotransmitter receptor trafficking and synaptic function, and how these processes are disrupted in neurodegenerative diseases. Currently, there are no efficient treatments for stopping or even reversing the pathological mechanisms in neurodegenerative diseases such as AD. A more detailed understanding of the regulatory mechanisms of the postsynaptic cytoskeleton may allow us to develop new strategies for protecting synaptic connections and to increase their resistance to pathological effects in the disease. In particular, it remains to be fully understood how the trafficking of glutamate receptors is disrupted by the presence of Aβ. As many actin-associated proteins exist in the cell in antagonistic relationships with other actin-associated proteins, it would be interesting to know the extent to which alteration of one regulatory protein affects others. Furthermore, studies involving *in vivo* techniques would provide a more accurate picture of how actin cytoskeleton dynamics influence the trafficking of AMPARs and ultimately synaptic plasticity. In addition to these studies, new advances in superresolution imaging could be implemented to examine not only the mobility of AMPARs in response to alterations in various actin-associated proteins but also changes in the distribution and localization of these proteins. Eventually, this leads us to the question of whether we can develop strategies that target specifically the synaptic actin cytoskeleton *in vivo*. Most actin cytoskeleton targeting drugs are rather unspecific for the actin filament populations that are manipulated. More recent approaches have shown that specific subpopulations of actin filaments can now be directly manipulated [229]. To exploit the use of these drugs for the potential therapeutic use in treating neurological disease, a detailed understanding of how different actin filament populations at synapses are formed, maintained, and turned over will be essential considerations for future studies.

Competing Interests

The authors declare that they have no competing interests.

Authors' Contributions

Holly Stefen and Chanchanok Chaichim contributed equally to this work.

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Research Article

Seizure-Induced Regulations of Amyloid-β, $STEP_{61}$, and $STEP_{61}$ Substrates Involved in Hippocampal Synaptic Plasticity

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Alzheimer's disease (AD) is a neurodegenerative disorder characterized by progressive cognitive decline. Pathologic accumulation of soluble amyloid-β (Aβ) oligomers impairs synaptic plasticity and causes epileptic seizures, both of which contribute to cognitive dysfunction in AD. However, whether seizures could regulate Aβ-induced synaptic weakening remains unclear. Here we show that a single episode of electroconvulsive seizures (ECS) increased protein expression of membrane-associated STriatal-Enriched protein tyrosine Phosphatase ($STEP_{61}$) and decreased tyrosine-phosphorylation of its substrates N-methyl D-aspartate receptor (NMDAR) subunit GluN2B and extracellular signal regulated kinase 1/2 (ERK1/2) in the rat hippocampus at 2 days following a single ECS. Interestingly, a significant decrease in ERK1/2 expression and an increase in APP and Aβ levels were observed at 3-4 days following a single ECS when $STEP_{61}$ level returned to the baseline. Given that pathologic levels of Aβ increase $STEP_{61}$ activity and $STEP_{61}$-mediated dephosphorylation of GluN2B and ERK1/2 leads to NMDAR internalization and ERK1/2 inactivation, we propose that upregulation of $STEP_{61}$ and downregulation of GluN2B and ERK1/2 phosphorylation mediate compensatory weakening of synaptic strength in response to acute enhancement of hippocampal network activity, whereas delayed decrease in ERK1/2 expression and increase in APP and Aβ expression may contribute to the maintenance of this synaptic weakening.

1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by progressive and irreversible cognitive decline [1]. Although AD pathology shows amyloid plaques that consist of insoluble amyloid-β (Aβ) [2], the abnormal accumulation of soluble Aβ oligomeric peptides correlates closely with cognitive dysfunction in early AD and disrupts synaptic plasticity [3], which is widely believed to underlie learning and memory [3, 4]. Specifically, soluble Aβ oligomers at pathologic levels inhibit long-term potentiation (LTP) and enhance long-term depression (LTD) of excitatory synaptic strength in the hippocampus, a brain region susceptible for neurodegeneration in AD [3]. Interestingly, the pathological accumulation of amyloid precursor protein (APP) and oligomeric Aβ also causes aberrant neuronal hyperexcitability in cortical and hippocampal neuronal networks of AD mouse models [5–9], consistent with the fact that humans and animal models with early-onset autosomal dominant familial AD have epileptic seizures [10–21]. Experimental inhibition of epileptic seizures prevents memory loss in AD transgenic model mice [17], suggesting that Aβ-induced aberrant increases in neuronal network activity also contribute to cognitive dysfunction in AD. However, whether seizures could regulate Aβ-induced synaptic weakening remains unclear.

STriatal-Enriched protein tyrosine Phosphatase 61 ($STEP_{61}$) has recently emerged as a key regulator of Aβ-induced synaptic weakening [11, 22–26] and as a postsynaptic density protein highly regulated by hyperexcitability in hippocampal neurons [27]. Application of Aβ oligomers to cortical cultures reduces surface expression of glutamate-gated ionotropic receptors including N-methyl D-aspartate receptors (NMDARs) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs) by upregulating $STEP_{61}$ activity [11, 22, 24–26]. While $STEP_{61}$ levels are

elevated in the cortices of several AD mouse models [22, 28], genetic ablation of $STEP_{61}$ blocks Aβ-induced reduction in surface AMPAR and NMDAR expression [22, 25] and prevents memory loss and LTP impairment in AD mouse models [25, 26], suggesting a critical role of $STEP_{61}$ in mediating Aβ-induced synaptic weakening and cognitive dysfunction in AD. We have also reported that prolonged enhancement of hippocampal network activity in primary culture leads to elevated $STEP_{61}$ expression and $STEP_{61}$-dependent reduction in tyrosine- (Tyr-) phosphorylation of its substrates, NMDAR subunit GluN2B and AMPAR subunit GluA2 [27].

Given that $STEP_{61}$ level is elevated in human AD which displays epileptic seizures as a comorbid condition [10–13], we hypothesize that hippocampal neuronal hyperexcitability induced by seizures will increase $STEP_{61}$ level, leading to compensatory downregulation of synaptic strength by dephosphorylating GluN2B, GluA2, and ERK1/2, key proteins critical for synaptic plasticity. To test this hypothesis, we induced a single electroconvulsive seizure (ECS) or chronic ECS (a single ECS each day for 7 consecutive days) in adult rats to elevate hippocampal network activity *in vivo* [29–32] and examined protein expression of $STEP_{61}$ and Aβ, as well as tyrosine-phosphorylation of $STEP_{61}$ substrates GluN2B, GluA2, and ERK1/2. ECS is an animal model for electroconvulsive therapy (ECT), which provides an efficient and relatively fast acting treatment for depression, anxiety, and other psychiatric conditions in humans [33]. During ECS, sufficient current administration reliably elicits nonrecurring stage 4-5 tonic-clonic seizures [29–32]. We chose ECS to globally elevate brain activity *in vivo* because a single ECS does not induce cell death or notable structural remodeling [33] which are evident in pilocarpine- or kainate-induced chronic epilepsy models [34, 35]. Furthermore, ECS does not involve invasive surgical methodologies as often used in kindling following intracranial electrode placement [34, 35].

We discovered that a single ECS increases the expression of membrane-associated $STEP_{61}$ and decreases Tyr^{1472}-phosphorylation of GluN2B and $Tyr^{204/187}$-phosphorylation of ERK1/2 in the hippocampus at 48 hours (h) following a single ECS. Interestingly, upregulation of APP and Aβ levels was observed at 72–96 h following a single ECS when $STEP_{61}$ level returned to the baseline. Chronic ECS results in a transient increase in APP and Aβ expression at 48 h and Aβ expression at 96 h following chronic ECS but did not alter $STEP_{61}$ expression and Tyr-phosphorylation of its substrates. Furthermore, a persistent decrease in GluN2B expression was observed over a course of 96 h following chronic ECS. These results suggest that elevated expression of APP, Aβ, and $STEP_{61}$ and dephosphorylation of GluN2B and ERK1/2 may contribute to compensatory weakening of synaptic strength in response to seizure-induced hippocampal network hyperexcitability.

2. Material and Methods

2.1. Materials. Antibodies used include anti-$STEP_{61}$ (catalogue SC-23892, Santa Cruz), anti-GluN2B (#14544, Cell Signaling), anti-ERK1/2 (SC-154, Santa Cruz), anti-GluA2 (#5306, Cell Signaling), anti-APP (#SC-28365, Santa Cruz), and anti-β-actin (#4967, Cell Signaling). Phosphorylation site specific antibodies used include anti-GluN2B-$pTyr^{1472}$ which recognizes phosphorylated Tyr-1472 of GluN2B (P1516-1472, PhosphoSolutions), anti-ERK1/2-$pThr^{202}/Tyr^{204}$ which recognizes phosphorylated Thr^{202}/Tyr^{204} of ERK1 and Thr^{185}/Tyr^{187} of ERK2 (#9106, Cell Signaling), anti-GluA2-p3Y which recognizes phosphorylated Tyr^{869}, Tyr^{873}, and Tyr^{876} (3Y) of GluA2 (#3921S, Cell Signaling), and anti-GluA2-pY^{876} which recognizes phosphorylated Tyr^{876} of GluA2 (#4027S, Cell Signaling).

2.2. Animals. The Institutional Animal Care and Use Committee at the University of Illinois at Urbana-Champaign approved all experimental procedures involving animals in this study.

2.3. Electroconvulsive Seizure (ECS). Male Sprague-Dawley rats (bred in house; strain origin: Charles River Laboratories) were weaned at postnatal day (P) 28, housed in groups of 2–4 male littermates, and weighed 3 times per week. All animals were maintained in standard conditions with a 12-hour (h) light-dark cycle and ad libitum access to food and water. Male rats were used to eliminate potential confounding sex differences. Rats received either a single ECS or chronic ECS (a single ECS each day for 7 consecutive days) as previously described [30, 36, 37] with the following modification. All ECS were induced between 7:30 and 10:00 a.m. in adult rats weighing 200–250 grams. One at a time, rats were connected via ear-clip electrodes to a pulse generator (Ugo Basile, Comerio, Italy), and a 0.5 sec, 100 pulses/sec, 55 mA shock was delivered to elicit a stage 4-5 seizure. All ECS lasted <10 sec, after which rats were returned to their home cage. Sham "no seizure" animals (NS) were handled identically, including ear-clip electrodes attachment, but no current was delivered. One experiment for a single ECS or chronic ECS included one NS rat and one ECS-treated rat per each time point following the last ECS.

2.4. Whole Brain Lysate Preparation. At specific time point following a single ECS or chronic ECS, animals were sacrificed by CO_2 inhalation and rapidly decapitated. The hippocampi were dissected from their brains and homogenized in ice-cold homogenization buffer (solution A) containing (in mM) 320 sucrose, 1 $NaHCO_3$, 1 $MgCl_2$, 0.5 $CaCl_2$, 1 $NaVO_3$, 10 $Na_4O_7P_2$, 50 NaF, and Halt protease inhibitors (Thermo Fisher Scientific) (1.25 mL total volume per pair of hippocampi). The crude membrane fraction (P2) was isolated from the hippocampi homogenates as previously described [38] with the following modification. After centrifuging for 10 min at 1,400 g, the postnuclear supernatants were separated (S1) from insoluble tissue and nuclear pellet (P1). The pellets were reconstituted in ice-cold solution A (1.25 mL total volume per pair of hippocampi) and centrifuged for additional 10 min at 710 g. The resultant supernatant was combined with the S1 fraction, and the entire volume was

then spun at 13,800 g for 10 min. The supernatant (S2) was removed, and the remaining pellet (P2 membrane fraction) was resuspended in ice-cold solution B containing (in mM) 320 sucrose, 1 NaHCO, 1 $NaVO_3$, 10 $Na_4O_7P_2$, 50 NaF, and protease inhibitor cocktails (1 mL total volume per pair of hippocampi). BCA assay (Pierce) analysis was performed to determine protein concentrations across samples, which were subsequently normalized to 1 mg/mL in solution B. The S1, S2, and P2 lysates were stored at −80°C until use.

2.5. Western Blot Analysis. After adding SDS sample buffer, the lysates (S1, P2, and S2) were heated at 37°C or 75°C for 30 min. Lysate samples were run on SDS-polyacrylamide gel electrophoresis (SDS-PAGE) gels and transferred to polyvinyl difluoride (PVDF) membrane (Millipore). Each gel contained lysates from one experiment for a single ECS or chronic ECS, including from one NS rat and one ECS-treated rat per each time point following the last ECS. Immunoblot analysis was performed as previously described [39, 40] with the following modifications. Each blot was blocked in 5% milk and 0.1% Tween-20 in Tris buffered saline (TBS) for 1 h and then incubated in primary antibodies in washing buffer (1% milk and 0.1% Tween-20 in TBS) overnight at 4°C. Primary antibodies used include anti-$STEP_{61}$ (1 : 200), anti-GluN2B (1 : 1000), anti-GluA2 (1 : 1000), anti-APP (1 : 200), anti-β-actin (1 : 1000), anti-GluN2B-$pTyr^{1472}$ (1 : 1000), anti-GluA2-p3Y (1 : 1000), anti-GluA2-pY^{876} (1 : 500), and anti-ERK1/2- $pThr^{202}/Tyr^{204}$ (1 : 1000). After incubating in HRP-conjugated secondary antibody in washing buffer for 1 h, blots were visualized with enhanced chemifluorescence substrate (ECL, Thermo Fisher Scientific) and developed with a Konica SRX-101A film processor. Densitometric quantification was performed with ImageJ Software (National Institutes of Health) as previously described [39, 40]. The band intensity of a protein of interest was divided by the β-actin band intensity per each time point. The ratio of NS control group was taken as 100%, and the ratio of ECS-treated group at each time point was normalized to the ratio of NS control to obtain the % of relative protein expression.

2.6. Statistical Analysis. All data shown represent the mean value ± SEM. The number of rats is expressed as sample size n. Statistical analyses were performed with either Microsoft Excel or Origin (version 8.5; OriginLab). For most data sets, a priori value ($^*p$) < 0.05 was considered statistically significant following one-way ANOVA and *post hoc* ANOVA tests (Fisher's test). For Figure 1(a), Student's t-test was used due to low sample size (n = 2-3 rats per postnatal day). For the statistical analysis of the levels of $Tyr^{204/187}$-phosphorylated ERK1/2 in Figure 4(a) and Aβ in Figure 5(a), Student's t-test was used because one-way ANOVA and *post hoc* ANOVA tests (Fisher's test) were not adequate to perform in the data sets that contained a large variability when 5 sets of independent experiments for a single ECS were compared. For Student's t-test, a priori value ($^\#p$) < 0.05 was considered statistically significant.

3. Results and Discussion

3.1. A Single ECS but Not Chronic ECS Transiently Increased $STEP_{61}$ Expression in the Hippocampus. To test whether elevation of hippocampal network activity *in vivo* regulates $STEP_{61}$ level, ECS was induced in rats and their hippocampal membrane fractions were collected for western blot analysis. First, we examined the developmental expression of $STEP_{61}$ in male rats (Figure 1(a)). Hippocampal $STEP_{61}$ expression began to increase at P12 compared to P3–P10 (332.8 ± 16.3% of P10, $p < 0.005$, t-test compared to P10) (Figure 1(a)). Although highly variable, $STEP_{61}$ expression steadily increased from P12 to P28 and reached a plateau at P42 with statistical significance (543.5 ± 18.0% of P10, $p < 0.05$, t-test compared to P10 and P12) (Figure 1(a)). Since hippocampal $STEP_{61}$ expression stabilized by P42 (Figure 1(a)), ECS were induced only in male rats that were at >P42 and weighed 220–240 g. For the induction of ECS, male rats received a single electric shock (0.5 sec, 100 pulses/sec, 55 mA) for once (a single ECS) or 7 consecutive days (chronic ECS) as previously described [30, 36] (Figures 1(b) and 1(c)). "No seizure" animals (NS) were handled identically, but no current was delivered. $STEP_{61}$ protein level in the crude membrane P2 fractions of hippocampus progressively increased up to 169.9 ± 28.3% by 48 h following induction of a single ECS compared to NS groups ($p < 0.01$, Figure 1(d); see Supplemental Figure 1 of the Supplementary Material available online at http://dx.doi.org/10.1155/2016/2123748). Interestingly, elevated $STEP_{61}$ level decreased back to the level of NS group by 72 h after a single ECS (Figure 1(d), Supplemental Figure 1). Although an increasing trend has been observed for $STEP_{61}$ expression over the course of 96 h following chronic ECS, this trend did not reach statistical significance due to a large standard deviation (Figure 1(e), Supplemental Figure 1). A single ECS or chronic ECS did not alter $STEP_{61}$ expression in postnuclear supernatant (S1) fraction (Supplemental Figure 2). Taken together, these data indicate that a single ECS but not chronic ECS caused a transient but significant increase in membrane-associated $STEP_{61}$ expression in the hippocampus *in vivo*.

3.2. A Single ECS but Not Chronic ECS Transiently Decreased Tyr^{1472}-Phosphorylation of GluN2B in the Hippocampus. Enriched in the postsynaptic density, $STEP_{61}$ dephosphorylates NMDAR subunit GluN2B at Tyr^{1472}, leading to internalization of GluN2B-containing NMDAR [11, 22, 24, 25, 41, 42]. We hypothesized that a single ECS-induced increase in $STEP_{61}$ expression would decrease Tyr^{1472}-phosphorylation of GluN2B in the hippocampus. Consistent with our hypothesis, western blot analysis of hippocampal P2 lysates revealed a significant reduction in the level of Tyr^{1472}-phosphorylated GluN2B (GluN2B-pY^{1472}) compared to NS group from 48 to 72 h following a single ECS (Supplemental Figure 3), with the most reduction seen at 48 h (Figure 2(a), 27.0 ± 12.7% of NS, $p < 0.005$) when $STEP_{61}$ expression was transiently enhanced (Figure 1(d)). The level of Tyr^{1472}-phosphorylated GluN2B was returned to the level of NS group by 96 h after a single ECS (Figure 2(a), Supplemental Figure 3)

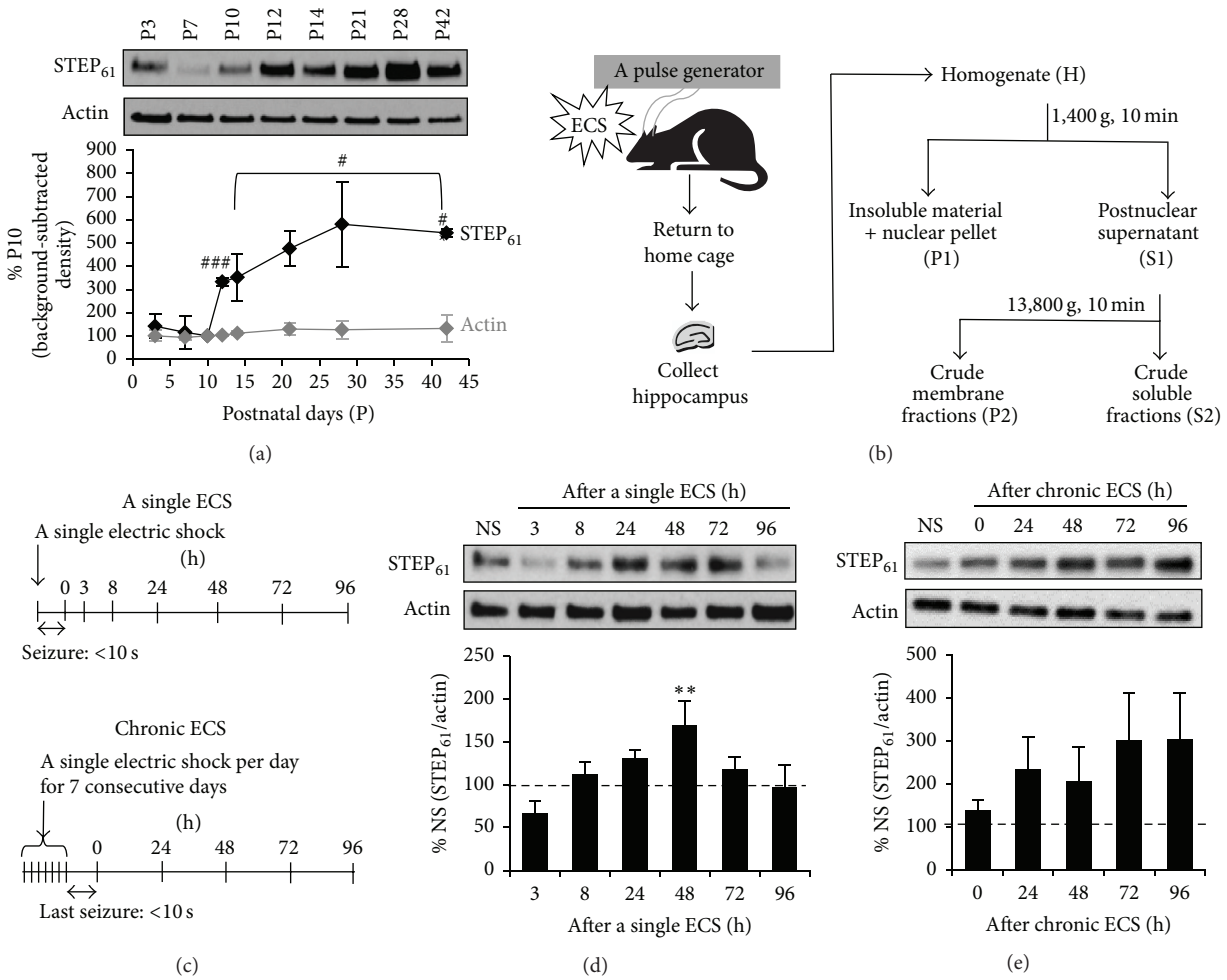

FIGURE 1: A single ECS but not chronic ECS transiently increases membrane-associated $STEP_{61}$ expression in the hippocampus. (a) Hippocampal expression of $STEP_{61}$ during postnatal development. Immunoblot analysis and quantification of $STEP_{61}$ and β-actin from the crude membrane (P2) fractions of rat hippocampi were obtained at postnatal days (P) 3, 7, 10, 12, and 14 ($n = 3$ rats per time point) and 21, 28, and 42 ($n = 2$ rats per time point). Background-subtracted western blot band intensities of $STEP_{61}$ and β-actin were normalized to those of P10 group, which was taken as 100%. $STEP_{61}$ expression significantly increased from P10 to P12 by 3-fold ($^{###}p < 0.005$, t-test) and to P42 by 5-fold ($^{#}p < 0.05$, t-test). (b) Schematic workflow of an experiment from ECS induction in rats to biochemical fractionation of their hippocampi. (c) Schematic experimental design of a single ECS and chronic ECS (a single ECS per day for 7 consecutive days). (d-e) Immunoblot analysis of $STEP_{61}$ in the hippocampal crude membrane (P2) fraction following a single ECS ((d) $n = 5$ rats per time point) and chronic ECS ((e) $n = 6$ rats per time point). Time points shown represent the duration after the induction of a single ECS (d) or chronic ECS (e) prior to brain removal. The ratio of the $STEP_{61}$ band intensity over the β-actin band intensity was calculated per each time point and normalized to the ratio of "no seizure" (NS) sham group, which was taken as 100%. Data shown represent the mean band intensity ± SEM. (c) A single ECS transiently increases $STEP_{61}$ expression in the hippocampus ($^{**}p < 0.01$). (d) Chronic ECS does not significantly alter $STEP_{61}$ expression in the hippocampus.

when elevated $STEP_{61}$ level decreased back to the level of NS group (Figure 1(d)). Total GluN2B expression did not change following a single ECS (Figure 2(a), Supplemental Figure 3). Although chronic ECS did not alter the level of Tyr^{1472}-phosphorylated GluN2B compared to NS control (Figure 2(b), Supplemental Figure 3), there was a modest but significant reduction in total GluN2B expression from 0 h to 24 h and 72 h to 96 h following chronic ECS compared to NS control (Figure 2(b), 96 h: 63.8 ± 6.7%, $p < 0.005$). These data indicate that a single ECS transiently reduced Tyr^{1472}-phosphorylation of GluN2B whereas chronic ECS persistently reduced total GluN2B expression in the hippocampus.

3.3. A Single ECS but Not Chronic ECS Increased the Level of Tyr^{876}-Phosphorylated GluA2 in the Hippocampus. $STEP_{61}$ reduces Tyr-phosphorylation of AMPAR subunit GluA2 and mediates AMPAR internalization upon acute stimulation of group 1 metabotropic glutamate receptors (mGluR) and application of Aβ [24, 26]. Although it is unclear which specific Tyr residue (s) in GluA2 is directly dephosphorylated by $STEP_{61}$, AMPAR internalization is reported to involve dephosphorylation of Tyr^{869}, Tyr^{873}, and Tyr^{876} (3Tyr) within the intracellular GluA2 C-terminal region (GluA-p3Y) [43]. We therefore hypothesized that a single ECS-induced increase in $STEP_{61}$ expression would decrease

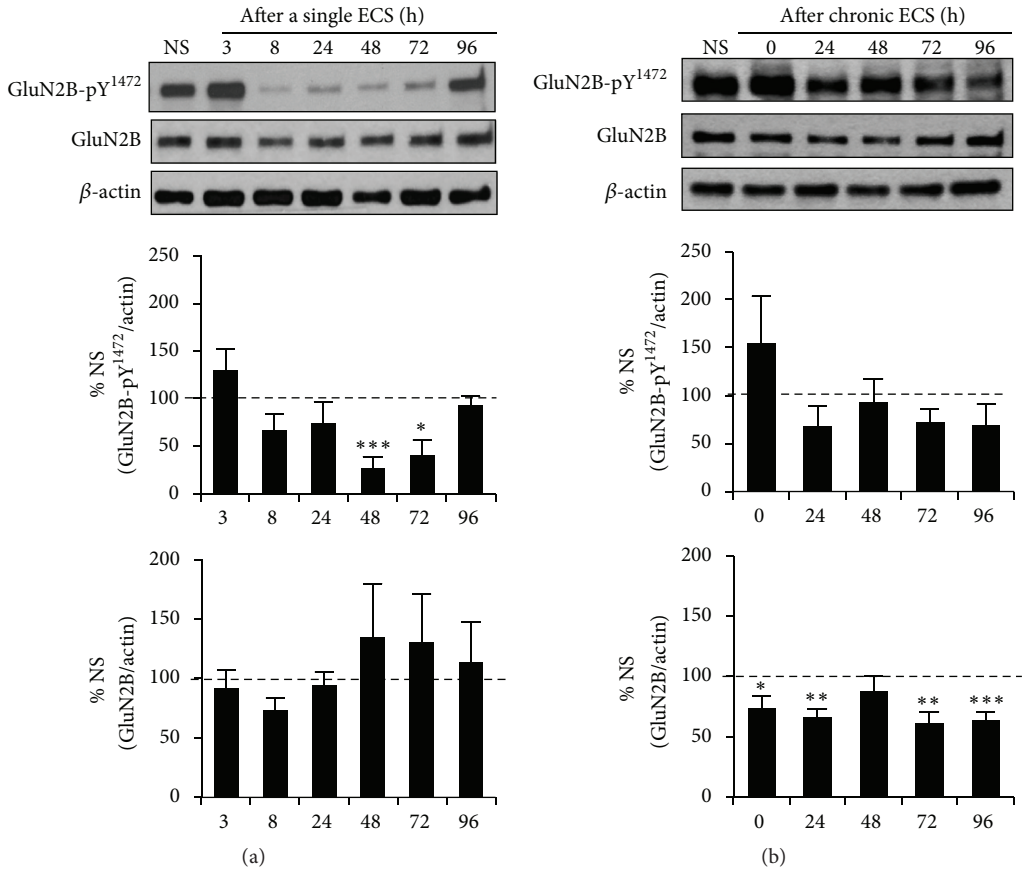

Figure 2: A single ECS but not chronic ECS transiently decreases the level of Tyr^{1472}-phosphorylated GluN2B in the hippocampus. Immunoblot analysis for the phosphorylation of GluN2B at Tyr^{1472} (Y^{1472}) and total GluN2B expression in the hippocampal crude membrane (P2) fraction following a single ECS ($n = 5$ rats per time point) (a) and chronic ECS ($n = 6$ rats per time point) (b). The ratio of the Tyr^{1472}-phosphorylated GluN2B band intensity over the β-actin band intensity (top graphs) and the ratio of total GluN2B band intensity over the β-actin band intensity (bottom graphs) were calculated per each time point and normalized to that of "no seizure" (NS) sham group. Data shown represent the mean band intensity ± SEM. (a) A single ECS transiently decreases the level of Tyr^{1472}-phosphorylated GluN2B in the hippocampus at 48 h ($^{***}p < 0.005$) and 72 h ($^{*}p < 0.05$) following a single ECS. (b) Chronic ECS significantly decreases total GluN2B expression over the time course of 96 h in the hippocampus ($^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.005$).

the level of 3Tyr-phosphorylated GluA2 as well as Tyr^{876}-phosphorylated GluA2 in the hippocampus. There was an increasing trend for the level of 3Tyr-phosphorylated GluA2 over the course of 96 h following a single ECS compared to NS control, although this increase did not reach statistical significance due to a large standard deviation (Figure 3(a), Supplemental Figure 4). To our surprise, the level of Tyr^{876}-phosphorylated GluA2 was unaltered at 48 h following a single ECS (Figure 3(b), Supplemental Figure 4, 77.8 ± 21.4% of NS, $p > 0.05$) when $STEP_{61}$ expression was significantly increased compared to NS control (Figure 1(d)). Instead, the level of Tyr^{876}-phosphorylated GluA2 was significantly increased by 2-fold at 96 h following a single ECS (Figure 3(b), 178.6 ± 27.5% of NS, $p < 0.05$) when $STEP_{61}$ expression was similar to that of NS control (Figure 1(d)). A single ECS had no effect on total GluA2 expression (Figure 3(c), Supplemental Figure 4). Chronic ECS did not alter the levels of 3Tyr-phosphorylated GluA2, Tyr^{876}-phosphorylated GluA2, and total GluA2 (Figures 3(d)–3(f), Supplemental Figure 5). These data indicate that a single ECS regulates Tyr^{876}-phosphorylation of GluA2 in the hippocampus.

3.4. A Single ECS and Chronic ECS Differently Altered $Tyr^{204/187}$-Phosphorylation of ERK1/2 in the Hippocampus. $STEP_{61}$-mediated dephosphorylation of ERK1/2 at $Tyr^{204/187}$ inactivates ERK1/2, opposing synaptic strengthening during LTP [44, 45]. Thus, we next tested whether a single ECS-induced increase in $STEP_{61}$ expression would decrease $Tyr^{204/187}$-phosphorylation of ERK1/2 in the hippocampus. There was an initial increasing trend for the level of $Tyr^{204/187}$-phosphorylated ERK1/2 (ERK-$pY^{204/187}$) until 24 h following a single ECS (Figure 4(a), $p > 0.05$). At 48 h following a single ECS when $STEP_{61}$ expression was significantly increased (Figure 1(d)), the level of $Tyr^{204/187}$-phosphorylated ERK1/2 was markedly reduced to 31.9 ± 10.2% of NS ($p < 0.005$, Figure 4(a), Supplemental Figure 6). As $STEP_{61}$ level reduced to those of NS groups from 48 h to 96 h

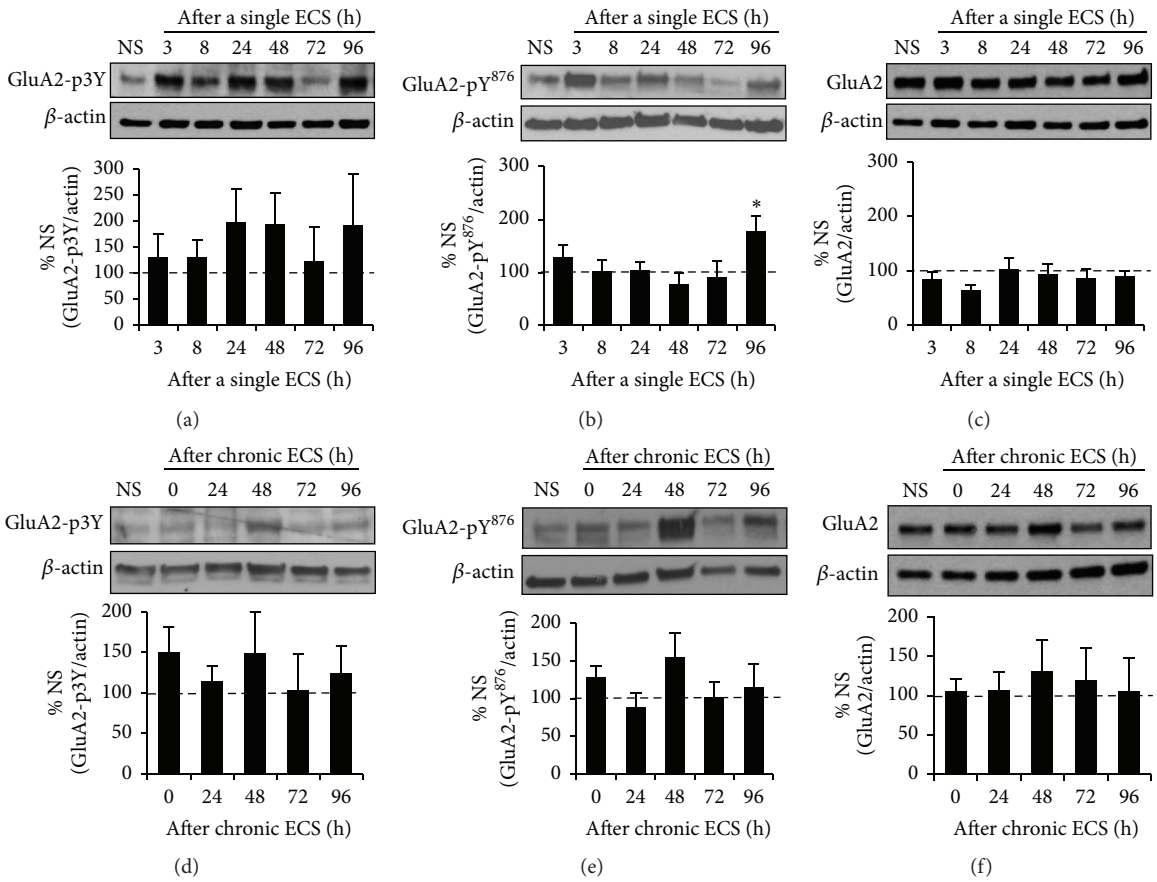

Figure 3: A single ECS but not chronic ECS increases the level of Tyr^{876}-phosphorylated GluA2 in the hippocampus. Immunoblot analysis for phosphorylation of GluA2 at Tyr^{876} (Y^{876}) or 3Tyr (3Y: Tyr^{869}, Tyr^{873}, and Tyr^{876}) and total GluA2 expression in the hippocampal crude membrane (P2) fraction following a single ECS ($n = 5$ rats per time point) (a–c) and chronic ECS ($n = 5$ rats for 72 h time point and $n = 6$ rats per all other time points) (d–f). The ratio of the phosphorylated GluA2 band intensity over the β-actin band intensity (a-b, d-e) and the ratio of total GluA2 band intensity over the β-actin band intensity (c, f) were calculated per each time point and normalized to that of "no seizure" (NS) sham group. Data shown represent the mean band intensity ± SEM. (a–c) A single ECS increases the level of Tyr^{876}-phosphorylated GluA2 at 96 h time point ((b) $^{*}p < 0.05$) but does not alter the level of 3Tyr-phosphorylated GluA2 and total GluA2. (d–f) Chronic ECS does not change Tyr-phosphorylation of GluA2 (d-e) and total GluA2 expression (f).

after a single ECS (Figure 1(d)), the level of $Tyr^{204/187}$-phosphorylated ERK1/2 also gradually increased to the level of NS groups (Figure 4(a), Supplemental Figure 6). Total ERK1/2 expression was significantly reduced at 72 h to 96 h following a single ECS (Figure 4(a), Supplemental Figure 6; 72 h: 84.2 ± 6.4% of NS, $p < 0.05$, 96 h: 81.1 ± 4.0% of NS, $p < 0.05$). Interestingly, chronic ECS caused about a 6-fold increase in the level of $Tyr^{204/187}$-phosphorylated ERK1/2 at 0 h time point compared to NS group (Figure 4(b), Supplemental Figure 6; 0 h: 580.5 ± 273.6% of NS, $p < 0.05$). However, this initial increase was decreased to the level of NS group by 24 h after chronic ECS (Figure 4(b), $p < 0.05$ between 0 h and 24 h). There was no change in total ERK1/2 expression in the hippocampus following chronic ECS (Figure 4(b), Supplemental Figure 6). Collectively, these results show that a single ECS and chronic ECS dynamically modulate $Tyr^{204/187}$-phosphorylation of ERK1/2.

3.5. A Single ECS and Chronic ECS Increased the Expression of APP and Aβ Oligomers in the Hippocampus. The Aβ peptide is derived from the cleavage of the amyloid precursor protein (APP) by β-secretase and γ-secretase at the Golgi and to a lesser extent endoplasmic reticulum [46]. Aβ oligomers have been shown to reduce surface expression of NMDARs and AMPARs by upregulating $STEP_{61}$ activity [11, 22, 24–26]. Furthermore, an Aβ-mediated disruption of the proteasome leads to increased $STEP_{61}$ levels in human AD brains and AD mouse models [11, 22, 25, 26]. Since Aβ is produced and secreted from neurons in response to synaptic activity [47–49], we next examined whether ECS could increase the production of Aβ peptides by performing western blotting in crude soluble S2 fractions of the hippocampus. Upon induction of a single ECS, the level of Aβ oligomers increased by 3-fold compared to the NS group at 72 h (Figure 5(a), Supplemental Figure 7; Aβ-72 h: 345.3 ± 75.8%, $p < 0.05$) when $STEP_{61}$ level is similar to that of NS group (Figure 1(d)).

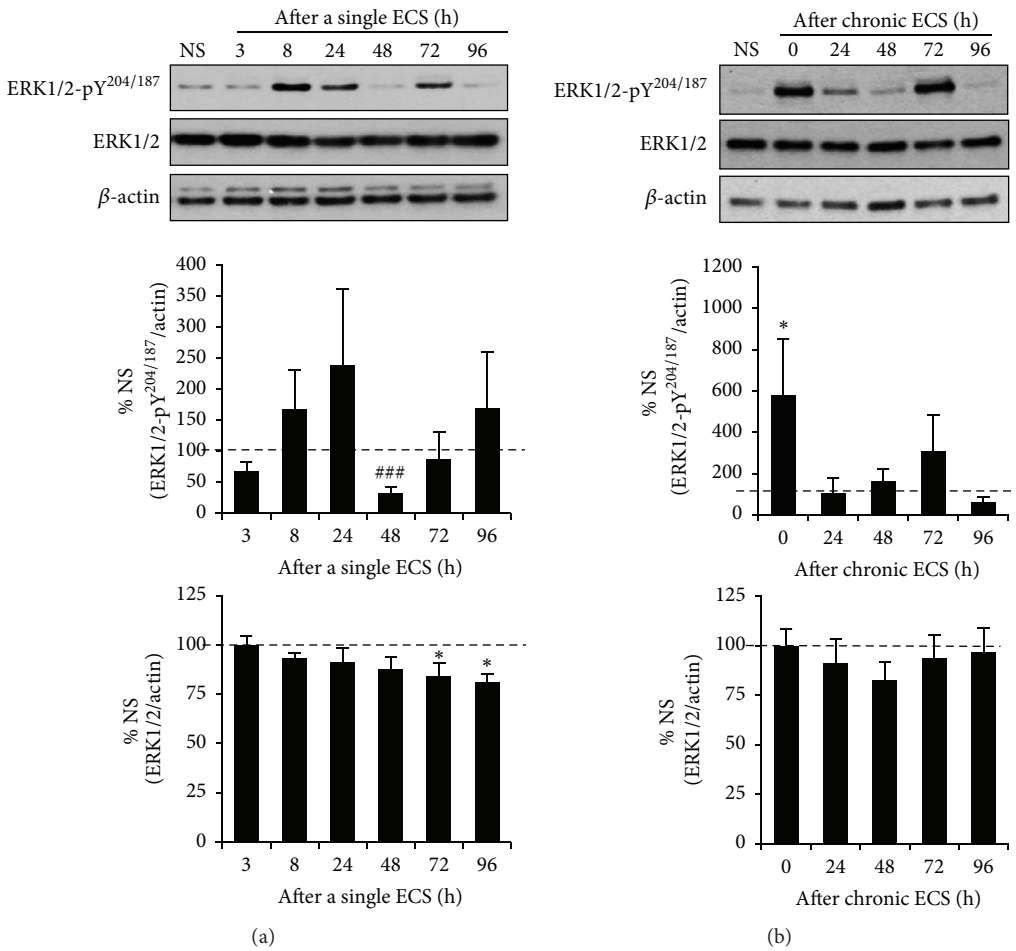

Figure 4: A single ECS and chronic ECS differently alter $Tyr^{204/187}$-phosphorylation of ERK1/2 in the hippocampus. Immunoblot analysis for the phosphorylation of ERK1/2 at $Tyr^{204/187}$ ($Y^{204/187}$) and total ERK1/2 expression in the hippocampal crude membrane (P2) fraction following a single ECS ($n = 5$ rats per time point) (a) and chronic ECS ($n = 6$ rats per time point) (b). The ratio of the $Tyr^{204/187}$-phosphorylated ERK1/2 band intensity over the β-actin band intensity (top graphs) and the ratio of total ERK1/2 band intensity over the β-actin band intensity (bottom graphs) were calculated per each time point and normalized to that of "no seizure" (NS) sham group. Data shown represent the mean band intensity ± SEM. (a) A single ECS transiently decreases the level of $Tyr^{204/187}$-phosphorylated ERK1/2 in the hippocampus at 48 h ($^{###}p < 0.005$, t-test) and total ERK1/2 expression at 72–96 h ($^{*}p < 0.05$) following a single ECS. (b) Chronic ECS significantly increases the level of $Tyr^{204/187}$-phosphorylated ERK1/2 at 0 h following chronic ECS ($^{*}p < 0.05$) but has no effect on total GluN2B expression in the hippocampus.

APP expression was also increased by 3-fold at 72–96 h following a single ECS (Figure 5(a), Supplemental Figure 7; APP-72 h: 406.5 ± 83.0%, $p < 0.005$, APP-96 h: 363.6 ± 81.9%, $p < 0.01$). Chronic ECS also caused a 2- to 3-fold increase in the expression of Aβ oligomers at 48 h and 96 h (Figure 5(b), Supplemental Figure 8; Aβ-48 h: 235.4 ± 44.7%, $p < 0.05$, Aβ-96 h: 283.5 ± 43.1%, $p < 0.01$) and a 2-fold increase in the expression of APP at 48 h following chronic ECS (Figure 5(b), Supplemental Figure 8; 228.0 ± 34.3% $p < 0.005$). These data indicate that both a single ECS and a chronic ECS led to a delayed increase in the levels of APP and Aβ oligomers in the hippocampus.

3.6. The Physiologic Consequences of $STEP_{61}$ Regulation in the Hippocampus by ECS. We show that $STEP_{61}$ level was markedly increased in rat hippocampus at 48 h after a single induction of ECS (Figure 1(d)), which induces global elevation of hippocampal neuronal activity [33]. Consistent with this increase in $STEP_{61}$ expression, the level of Tyr^{1472}-phosphorylated GluN2B was reduced at 48–72 h following a single ECS without altering total GluN2B expression (Figure 2(a)). Considering that $STEP_{61}$-mediated dephosphorylation of GluN2B leads to internalization of GluN2B-containing NMDARs [11], upregulation of $STEP_{61}$ (Figure 1(d)) may serve as a compensatory mechanism to reduce surface density of NMDARs in the hippocampus in response to seizures (Figure 6). Consistent with the previous report on ECS-induced decreases in PSD-95 and GluN2A/B expression [50], chronic ECS caused a persistent decrease in total GluN2B expression over the course of 96 h

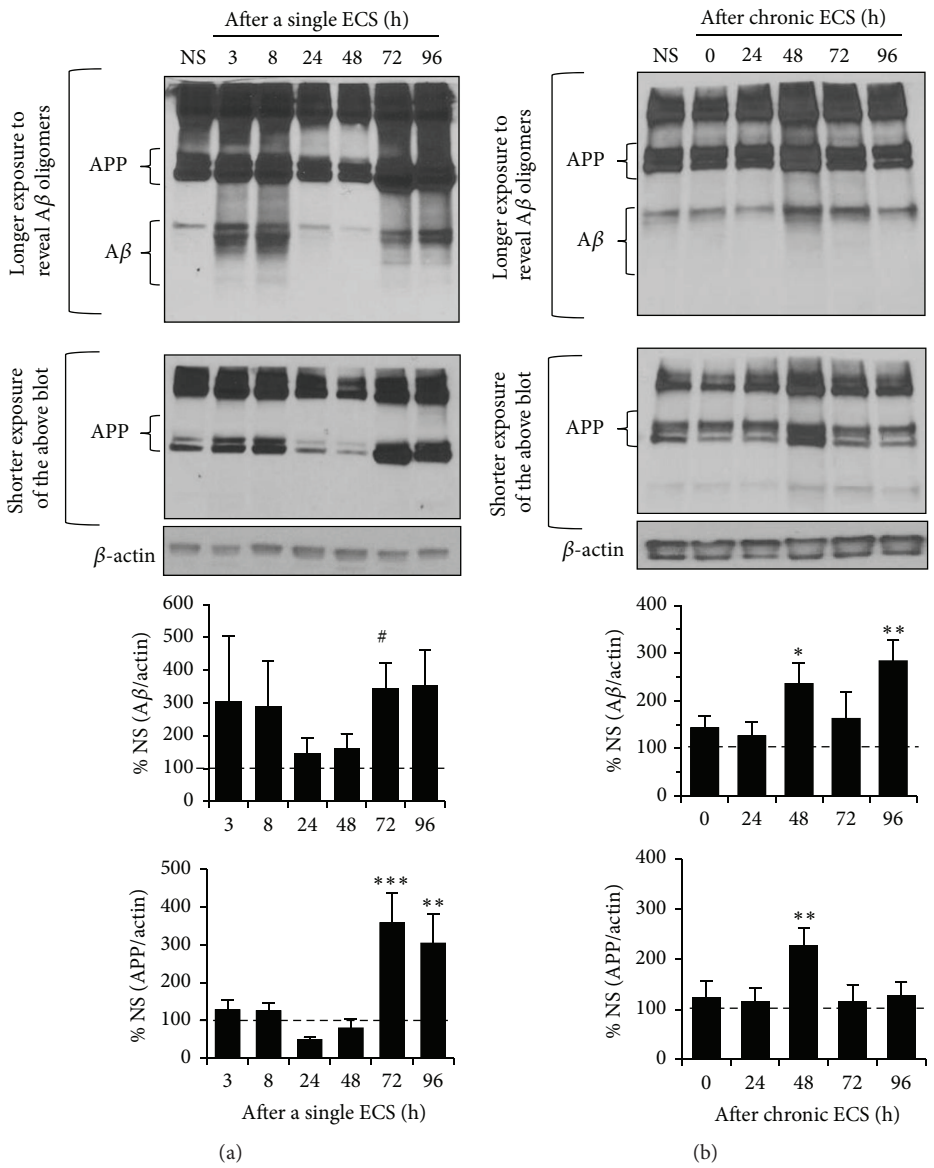

Figure 5: A single ECS and chronic ECS increase APP and oligomeric Aβ expression in the hippocampus. Immunoblot analysis of APP and oligomeric Aβ in the hippocampal crude soluble (S2) fraction following a single ECS ($n = 5$ rats per time point) (a) and chronic ECS ($n = 6$ rats per time point) (b). The ratio of the Aβ band intensity over the β-actin band intensity (top graphs) and the ratio of the APP band intensity over the β-actin band intensity (bottom graphs) were calculated per each time point and normalized to that of "no seizure" (NS) sham group. Data shown represent the mean band intensity ± SEM. (a) A single ECS increases Aβ expression at 72 h ($^{\#}p < 0.05$, t-test) and APP expression at 72–96 h following a single ECS ($^{**}p < 0.01$, $^{***}p < 0.005$). (b) Chronic ECS increases Aβ expression at 48 h and 96 h, as well as APP expression at 48 h following chronic ECS ($^{*}p < 0.05$, $^{**}p < 0.01$).

following chronic ECS (Figure 2(b)). NMDAR activation requires coincident binding of glutamate and membrane depolarization produced by opening of AMPARs [51]. Hence, although chronic ECS did not alter GluA2 level (Figure 3(f)), a persistent decline in GluN2B expression (Figure 2(b)) could facilitate synaptic weakening in response to repetitive seizures.

$STEP_{61}$ mediates AMPAR internalization upon mGluR activation and Aβ application by dephosphorylating GluA2 [24, 26], suggesting a possibility that a single ECS-induced increase in $STEP_{61}$ expression could lead to synaptic weakening by decreasing Tyr-phosphorylation of GluA2. Unexpectedly, the level of Tyr^{876}-phosphorylated GluA2 was enhanced at 96 h following a single ECS (Figure 3(b)). No significant changes were seen in the level of 3Tyr-phosphorylated GluA2 and total GluA2 following a single ECS (Figures 3(a) and 3(c)). Since the level of Tyr^{876}-phosphorylated GluA2 was unaltered at 48 h following a single ECS when $STEP_{61}$ was increased (Figures 1(d) and 3(b)), Tyr^{876} of GluA2 might not have been directly regulated by $STEP_{61}$. It is also possible that

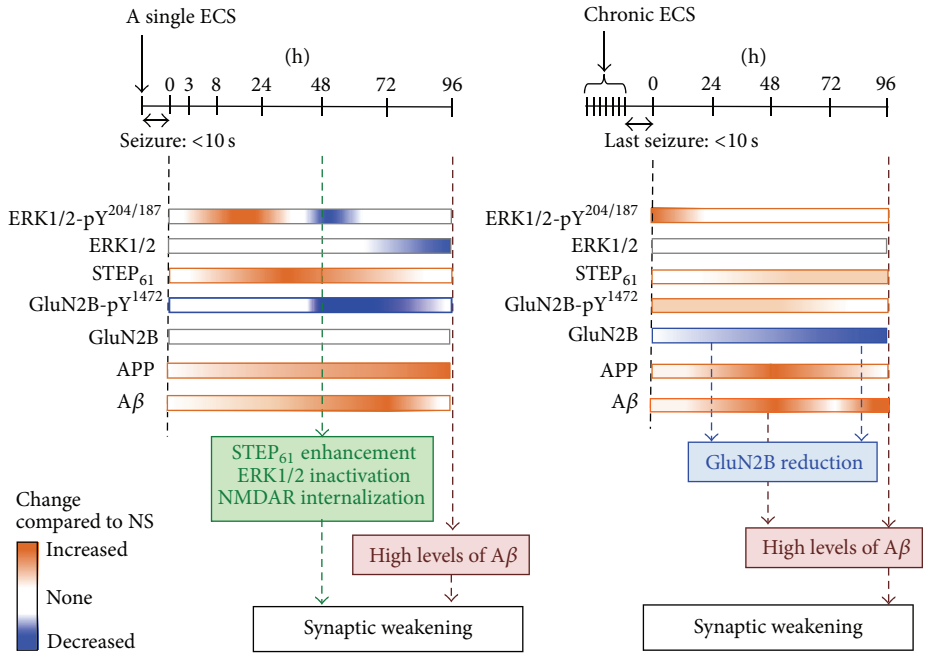

FIGURE 6: Model by which seizure-induced changes in Aβ, $STEP_{61}$, and Tyr-phosphorylation of $STEP_{61}$ lead to synaptic weakening in the hippocampus. A single ECS increases $STEP_{61}$ expression and decreases Tyr-phosphorylation of NMDAR subunit GluN2B and ERK1/2 in the hippocampus at 48 h time point, leading to synaptic weakening via NMDAR internalization and ERK1/2 inactivation. A delayed decrease in ERK1/2 expression as well as a delayed enhancement of APP and Aβ expression at 72–96 h following a single ECS maintains this synaptic weakening. Chronic ECS-induced increase in APP expression and Aβ production as well as persistent decrease in total GluN2B level leads to synaptic weakening.

$STEP_{61}$ dephosphorylates a specific residue within the 3Tyr motif, but that kinase-mediated phosphorylation of another residue within the same motif could mask the $STEP_{61}$ effect. Identification of specific phosphorylation sites regulated by $STEP_{61}$ may aid future studies to dissect the role of $STEP_{61}$ in ECS-induced regulation of GluA2 Tyr-phosphorylation.

The level of $Tyr^{204/187}$-phosphorylated ERK1/2 was markedly decreased at 48 h following a single ECS (Figure 4(a)), when $STEP_{61}$ expression was at its peak (Figure 1(d)). Since $STEP_{61}$-mediated dephosphorylation of ERK1/2 at $Tyr^{204/187}$ inactivates ERK1/2 [44, 45], our results suggest a significant reduction in ERK1/2 activity by ECS-induced upregulation of $STEP_{61}$. The total ERK1/2 expression was also reduced at 72–96 h following a single ECS (Figure 4(a)). Considering that activation of ERK1/2 drives synaptic delivery of AMPAR [52] and activity-dependent regulation of gene transcription during LTP [53], ERK1/2 inactivation at 48 h and ERK1/2 reduction at 72–96 h following a single ECS would also facilitate synaptic weakening (Figure 6). Interestingly, chronic ECS caused a 6-fold increase in the level of $Tyr^{204/187}$-phosphorylated ERK1/2 at 0 h following chronic ECS compared to NS control, which was returned to the level of NS control by 24 h after chronic ECS (Figure 4(b)). These temporal changes in ERK1/2 activity are consistent with previous reports in cultured neurons that ERK1/2 undergoes rapid activation in response to glutamate stimulation, followed by a $STEP_{61}$-dependent delayed inactivation to baseline [44, 54, 55]. Taken together, our results suggest that upregulation of $STEP_{61}$ and downregulation of its substrates critical for synaptic plasticity may provide efficient means to mediate synaptic weakening (Figure 6).

3.7. The Physiologic Consequences of APP and Aβ Regulation in the Hippocampus by ECS. Previous studies have shown that application of Aβ oligomers activates $STEP_{61}$, which subsequently leads to internalization of NMDAR and AMPAR [11, 22]. We speculate that the delayed 3-fold increase in APP and Aβ expression at 72–96 h following a single ECS (Figure 5(a)) would enhance $STEP_{61}$ activity, leading to a persistent reduction in NMDAR and AMPAR surface expression at these time points when $STEP_{61}$ levels returned back to NS control levels (Figure 6). Such persistent decrease in synaptic strength is expected to lead to the elimination of synapses [56–58]. Indeed, decreases in synapse density are evident in the hippocampi of patients with early AD and correlate strongly with memory impairment [59–61]. Furthermore, a single ECS has been shown to cause memory deficits in rats when it was administered right after the hippocampus-dependent learning experience [50, 62], consistent with clinical observations of retrograde amnesia as one severe side effect for electroconvulsive therapy in humans [63]. Hence, it will be interesting to test if seizure-induced increase in APP and Aβ expression and downregulation of NMDAR and ERK1/2 through $STEP_{61}$ could be the basis of cognitive deficits in early AD and ECT.

Interestingly, a transient 2-fold increase in APP and Aβ expression was observed at 48 h after chronic ECS compared to NS control, which was followed by a 3-fold increase in Aβ expression at 96 h time point (Figure 5(b)). The initial increase in APP expression could be the basis for the delayed increase in Aβ levels. Animals administered with chronic ECS display increased dentate granule cell neurogenesis [64] and molecular layer mossy fiber sprouting [65, 66]. Since APP regulates neurite outgrowth as well as cell adhesion and promotes neuronal survival [67–70], chronic ECS-induced increase in APP expression may regulate hippocampal neurogenesis and mossy fiber sprouting. Interestingly, similar 3-4-fold increase in APP levels has been found in the postmortem temporal lobe from patients with early AD [71] and from patients with intractable temporal lobe epilepsy with abnormal neurite outgrowth [72]. The AD transgenic mouse models with elevated APP expression display spontaneous seizures, sharp wave discharges, and mossy fiber sprouting as well as ectopic expression of inhibitory neuropeptides in their hippocampus [5, 14, 15, 17–21]. Importantly, hippocampal neurons in transgenic APP-overexpressing AD mice display hyperexcitability well before plaque formation [6]. Since Aβ application increases the activity of excitatory neurons in acute brain slices [20] and neuronal activity stimulates synthesis and synaptic release of Aβ [47–49], we speculate that chronic ECS-induced initial increase in APP expression may result in neuronal hyperexcitability, which in turn causes heightened Aβ expression, ultimately leading to pathologic positive feedback loop of Aβ production [73].

3.8. The Mechanisms Underlying ECS-Induced Expression of $STEP_{61}$ and APP. Previous studies have shown that $STEP_{61}$ expression is regulated by multiple mechanisms. $STEP_{61}$ is locally translated in dendrites upon mGluR5 activation through a mechanism dependent on ERK1/2 phosphorylation [24]. Interestingly, fragile X mental retardation protein (FMRP) binds to and inhibits translation of STEP mRNA [74] whereas genetic ablation of FMRP leads to increased STEP expression [75, 76]. Hence, it is possible that the initial increasing trend in ERK1/2 phosphorylation induced by a single ECS (Figure 4(a)) could increase $STEP_{61}$ expression by 48 h (Figure 1(d)) by triggering local dendritic synthesis of $STEP_{61}$ upon FMRP inhibition. In addition, $STEP_{61}$ undergoes proteasome-dependent degradation upon polyubiquitination [77], suggesting another possibility that a single ECS could elevate $STEP_{61}$ expression by inhibiting proteasomal $STEP_{61}$ degradation. Lastly, both a single ECS and a chronic ECS stimulate robust induction of immediate early genes and subsequent downstream genes important for neural plasticity [30, 31, 66, 78–83]. Considering that 48 h blockade of neuronal activity or NMDAR in cultured hippocampal neurons leads to a significant reduction in $STEP_{61}$ mRNA and protein expression [27], a single ECS may stimulate transcription of $STEP_{61}$ in the hippocampus through NMDAR activation. Further investigation is needed to investigate if enhancement of hippocampal network activity upon a single ECS increases $STEP_{61}$ protein level by enhancing transcription and translation of $STEP_{61}$ and/or inhibiting its proteasomal degradation.

It is unclear how a single and a chronic ECS caused a delayed increase in APP and Aβ expression in the hippocampus (Figure 5). Previous studies have shown that APP synthesis and processing are stimulated by interleukin-1 (IL-1) [84–86], which is synthesized and released from activated microglia [87, 88]. Consistently, neuronal expression of APP is associated with heightened IL-1 immunoreactivity in human temporal lobe epilepsy [72]. Interestingly, activated microglia are found in the hippocampus 24 h after a single or repeated ECS, and the number of activated microglial cells remained increased for weeks after ECS [89]. Though highly speculative, it is possible that ECS-induced persistent activation of microglia could stimulate IL-1 synthesis and release, leading to delayed APP production in neurons following a single ECS or a chronic ECS.

Chronic ECS did not induce significant alterations in the levels of $STEP_{61}$, GluA2, and ERK1/2 compared to NS groups (Figures 1–4). While chronic ECS is therapeutically used to reduce stress [33], it is also possible that the "no seizure" (NS) animals might have been hyperstressed by the repeated exposures to handling and the ECS apparatus, even though current was not delivered. Considering the interdependence of stress and $STEP_{61}$ expression [90], heightened stress in the NS animals in combination with dampened stress levels in chronic ECS-received rats may account for the lack of effects on $STEP_{61}$ regulation following chronic ECS administration.

4. Conclusion

Here, we show that a single ECS transiently increases protein expression of membrane-associated $STEP_{61}$ and decreases Tyr-phosphorylation of NMDAR subunit GluN2B and ERK1/2 in the hippocampus. A delayed decrease in the levels of ERK1/2 as well as a delayed enhancement of APP and Aβ expression is also seen in the hippocampus following a single ECS. Chronic ECS treatment also leads to a persistent decrease in GluN2B level and a transient increase in APP and Aβ production. To our knowledge this is the first study reporting the temporal expression of APP, Aβ, $STEP_{61}$ and its substrates at various time points following a single ECS and chronic ECS. We propose that this regulation causes a transient weakening of synaptic strength to combat global enhancement of hippocampal neuronal activity induced by ECS. This regulation may also contribute to hippocampus-dependent memory loss induced by ECS, supporting antiepileptic drugs as potential therapy for cognitive dysfunction in early AD [17]. Given that Aβ-induced increase in $STEP_{61}$ expression is involved in NMDAR and AMPAR internalization during synaptic weakening in AD [11, 22, 24–26], the work reported here emphasizes the need to dissect the detailed molecular mechanisms underlying activity-dependent regulation of $STEP_{61}$. These mechanistic insights may help to explain the heightened $STEP_{61}$ expression present in AD [11, 22, 25] and fragile X syndrome [76] which have epileptic seizures as comorbid conditions.

Competing Interests

The authors declare that they have no competing interests.

Authors' Contributions

Sung-Soo Jang and Sara E. Royston contributed equally. Gunhee Lee and Shuwei Wang contributed equally.

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Review Article

Amyloid-β-Induced Dysregulation of AMPA Receptor Trafficking

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Evidence from neuropathological, genetic, animal model, and biochemical studies has indicated that the accumulation of amyloid-beta (Aβ) is associated with, and probably induces, profound neuronal changes in brain regions critical for memory and cognition in the development of Alzheimer's disease (AD). There is considerable evidence that synapses are particularly vulnerable to AD, establishing synaptic dysfunction as one of the earliest events in pathogenesis, prior to neuronal loss. It is clear that excessive Aβ levels can disrupt excitatory synaptic transmission and plasticity, mainly due to dysregulation of the AMPA and NMDA glutamate receptors in the brain. Importantly, AMPA receptors are the principal glutamate receptors that mediate fast excitatory neurotransmission. This is essential for synaptic plasticity, a cellular correlate of learning and memory, which are the cognitive functions that are most disrupted in AD. Here we review recent advances in the field and provide insights into the molecular mechanisms that underlie Aβ-induced dysfunction of AMPA receptor trafficking. This review focuses primarily on NMDA receptor- and metabotropic glutamate receptor-mediated signaling. In particular, we highlight several mechanisms that underlie synaptic long-term depression as common signaling pathways that are hijacked by the neurotoxic effects of Aβ.

1. Introduction

Alzheimer's disease (AD) is the most common cause of dementia among the aging population. Early memory deficits and progressive loss of higher cognitive functions are common clinical features of AD patients. Pathologically, AD is characterized by insoluble aggregates of extracellular amyloid-beta (Aβ) peptides (senile plaques) and intracellular filaments composed of hyperphosphorylated tau (neurofibrillary tangles) in the brain. Strong evidence from human genetics and transgenic mouse models has implicated Aβ in the etiology and pathogenesis of AD [1]. Aβ peptides are derived from β-secretase- and γ-secretase-mediated sequential proteolytic cleavage of the amyloid-precursor protein (APP), with $A\beta_{1-40}$ and $A\beta_{1-42}$ being the most abundant species [2]. Many human mutations associated with familial AD, such as those that are found in genes encoding APP and the catalytic subunit of γ-secretase, presenilin (PS1 and PS2), promote amyloidogenic processing of APP, leading to enhanced Aβ production [3]. Recent studies have shown that soluble oligomeric forms of Aβ (ranging from dimers and trimers to dodecamers) exert potent and acute neurotoxic effects on the structure and function of synapses, including reduced excitatory synaptic transmission, loss of dendritic spines, and aberrant neuronal network activity [4, 5]. These deleterious effects could contribute to the cognitive deficit and memory loss associated with AD, indicating that "synaptic failure" is likely to be one of the earliest events that occurs in the pathogenesis of AD prior to neuronal loss [6–8].

The majority of fast excitatory synaptic transmission in the mammalian central nervous system is mediated by the release of glutamate from the presynaptic terminal and its binding to glutamate receptors on the postsynaptic membrane. The ionotropic glutamate receptors consist of AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid), NMDA (*N*-methyl-*D*-aspartate), and kainate receptors. Among these, AMPA receptors (AMPARs) are the principal receptors that mediate fast excitatory synaptic

transmission in the mammalian brain. They are tetrameric assemblies of two dimers of four potential subunits (GluA1–GluA4) encoded by distinct genes, *GRIA1–GRIA4*. The predominant AMPARs expressed in the hippocampal and cortical pyramidal neurons are composed of GluA1/GluA2 and GluA2/GluA3 subunits [9]. Brief periods of high neuronal activity open NMDA receptors (NMDARs) and induce Ca^{2+} influx, leading to a long-lasting increase in synaptic efficacy, known as long-term potentiation (LTP), which is characterized by an increase in the number of AMPARs on the postsynaptic membrane and spine growth. In contrast, repetitive low frequency stimulation leads to the removal of synaptic AMPARs to produce long-term depression (LTD), that is, a decrease in synaptic strength. It has long been postulated that these forms of synaptic plasticity represent a cellular correlate of learning and memory [10].

One of the key mechanisms underlying synaptic plasticity is the tight control of AMPAR number at synapses. This requires a balance between the biosynthesis (number of receptors being produced), membrane trafficking (the movement of receptors to and from the plasma membrane via exocytosis and endocytosis), and degradation of receptors (receptor turnover), all of which are dynamically regulated by AMPAR interacting proteins as well as by various posttranslational modifications that occur on their cytoplasmic carboxyl terminal domains [11, 12]. Aberrant trafficking of AMPARs usually leads to impaired synaptic plasticity and deficits in learning and memory [11]. Importantly, several studies have demonstrated a role for Aβ in promoting AMPAR endocytosis and hence synaptic depression [13–16]. This review focuses primarily on NMDAR and metabotropic glutamate receptor- (mGluR-) mediated signaling. In particular, we highlight several mechanisms that underlie synaptic LTD as common signaling pathways that are hijacked by the neurotoxic effects of Aβ. Several pharmacological agents that target these pathways and are efficacious in inhibiting or reversing the neurotoxic effects of Aβ on glutamatergic neurotransmission and synaptic plasticity are also discussed.

2. Aβ Alters Synaptic Plasticity *In Vitro* and *In Vivo*

The ability of neurons to modulate their synaptic strength is widely believed to be a cellular correlate of learning and memory. NMDAR-dependent LTP and LTD are two major forms of synaptic plasticity that are best studied in the hippocampus, a region of the brain that is both critical for memory formation and highly vulnerable to Aβ toxicity. It is well established that synthetic soluble Aβ oligomers [17, 18] or those secreted from cell lines overexpressing APP [19] acutely and potently block hippocampal LTP at high concentration. More recent studies have further shown that soluble Aβ dimers, but not Aβ monomers, either prepared by chemical cross-linking or extracted directly from postmortem AD brains, are extremely potent in inhibiting hippocampal LTP both *in vitro* and *in vivo* [4, 20]. Congruent with the LTP hypothesis of long-term memory, injection of these soluble Aβ oligomers into the rat hippocampus disrupts cognitive function and learned behavior [4, 21]. Most transgenic AD mouse models overexpressing different familial AD mutations, such as Tg2576 (APP_{Swe}; K670N/M671L), PDAPP (APP_{Ind}; V717F), 3xTg (APP_{swe}, Tau P301L, and PS1 M146V), and 5xFAD (APP_{swe}, $APP_{Florida}$; I716V, APP_{London}; V717I, PS1 M146L, and PS1 L286V), generally display impairments in LTP and cognition [22–26]. Notably, some AD transgenic mice show abnormal LTP and learning deficits well in advance of plaque formation [22, 27, 28]. Collectively, these results lend support to the idea that soluble oligomeric Aβ plays a key role in disrupting synaptic plasticity. More importantly, studies performed in human subjects have also revealed deficits in LTP-like cortical plasticity in mild-to-moderate AD patients [29–31].

Consistent with the fact that Aβ induces an impairment in LTP, soluble Aβ oligomers have been demonstrated to facilitate the expression of LTD in the hippocampus [4, 17, 32]. Although the exact mechanisms underlying Aβ-induced LTD remain equivocal, they have been shown to involve internalization of NMDA- and AMPA-type glutamate receptors, dendritic spine shrinkage, and eventual synaptic loss [14, 16, 33, 34].

3. Mechanisms Underlying Aβ-Induced Deficits in AMPAR Function

Dynamic trafficking of AMPARs to and from synapses is a critical mechanism underlying the induction of synaptic plasticity. Defects in the endocytosis and lysosomal trafficking pathways are known to contribute significantly to AD pathogenesis [35]. Consistent with this notion, overexpression of APP and a high concentration of soluble oligomeric Aβ are able to induce the removal of surface AMPARs at synapses, leading to synaptic depression and inhibition of LTP [14, 19, 36, 37]. Mechanistically, these neurotoxic effects of Aβ are mediated by high levels of glutamate at synapses as a result of a disrupted glutamate reuptake process [32] that subsequently leads to aberrant activation of NMDARs, mGluRs, and the cellular prion protein (PrP^C), as well as elevated levels of AMPAR ubiquitination. Activation of these signaling pathways in turn promotes synaptic depression, via common pathways shared with LTD as summarized in Figure 1, which are discussed in detail in the following sections.

3.1. NMDARs. NMDAR-dependent LTD induced by low frequency stimulation or by direct application of NMDA (chemically induced LTD) triggers Ca^{2+} entry into the postsynaptic compartment and activates protein phosphatase 2B (PP2B, also known as calcineurin), which in turn leads to the activation of protein phosphatase 1 (PP1) [38, 39]. PP1 and PP2B are known to mediate NMDAR-induced AMPAR internalization by dephosphorylating the GluA1 subunit of AMPARs at Ser-845 [40, 41], a protein kinase A (PKA) site that is crucial for maintaining the stability of AMPARs at perisynaptic sites and LTP [42–44]. NMDAR-dependent LTD also induces the p38 mitogen activated protein kinase (p38 MAPK) signaling pathway via the activation of Rap small GTPases, leading to the removal of AMPARs [45, 46].

Emerging evidence demonstrates that toxic levels of Aβ aberrantly enhance the activity of NMDARs in favor of LTD induction, thereby preventing LTP [32, 37, 47]. In cultured

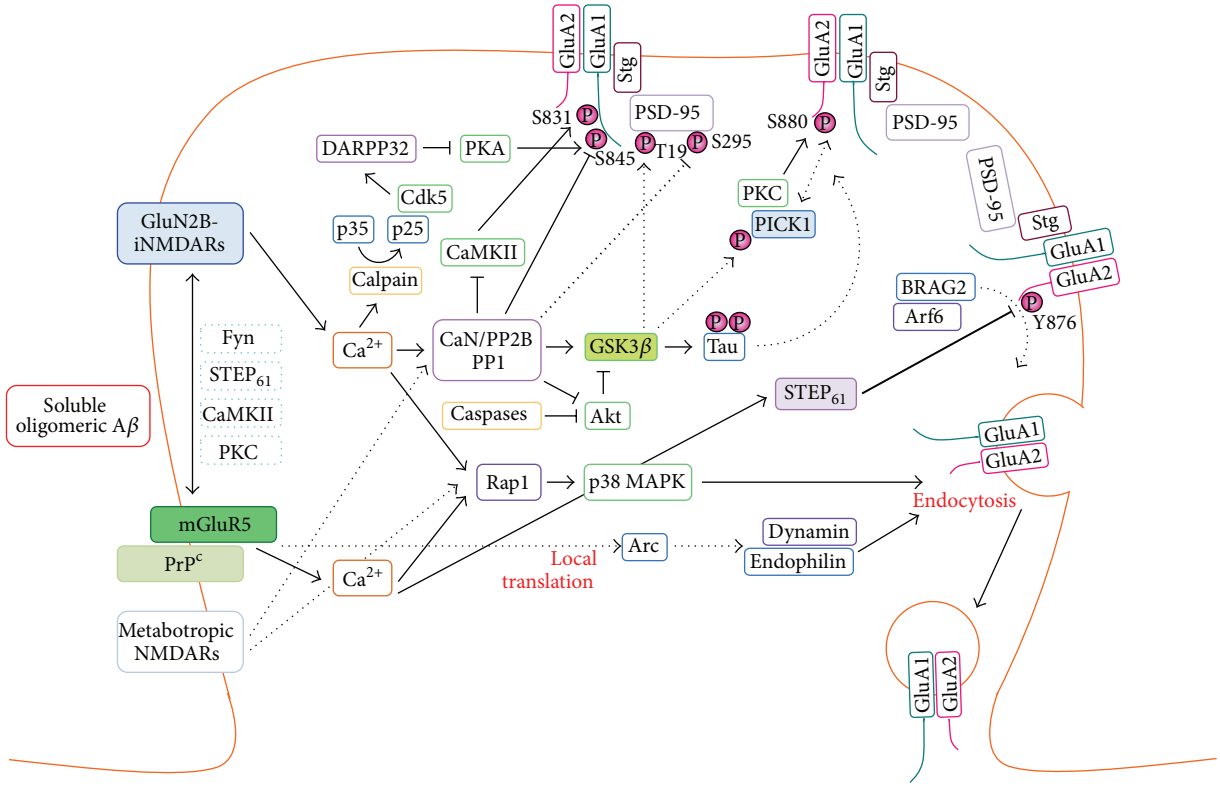

FIGURE 1: Signaling mechanisms involved in Aβ-induced AMPAR internalization. Soluble Aβ oligomers activate ionotropic NMDA (iNMDA) and metabotropic glutamate (mGlu) receptors, leading to an increase in intracellular Ca^{2+}. Ca^{2+} subsequently activates a number of signal transduction cascades involving protein phosphatases (calcineurin, PP1, and $STEP_{61}$) and protein kinases (Cdk5, PKC, and GSK3β) to modulate the phosphorylation of AMPAR subunits, as well as intracellular signaling and scaffolding molecules. Activation of these pathways, which are commonly shared with LTD, promotes AMPAR internalization and synaptic depression. The cross talk between NMDAR and mGluR5 signaling can be modulated by factors such as Fyn, CaMKII, PKC, and $STEP_{61}$. The involvement of metabotropic NMDARs in mediating the neurotoxic effects of Aβ, which do not involve the flux of Ca^{2+}, has recently been proposed, albeit this remains controversial. Dotted arrows indicate events that are inferred from the study of LTD and have not been shown to be directly involved in Aβ-mediated signaling. Thicker lines indicate common pathways, while colored boxes indicate potential therapeutics targets for AD.

neurons and acute brain slices, soluble oligomeric Aβ induces excessive influx of Ca^{2+} through the GluN2B-containing extrasynaptic NMDARs, which subsequently activates the Rap-p38 MAPK signaling pathway, as well as the protein phosphatases, PP1 and calcineurin [13, 14, 16, 32, 37, 48–50]. One of the consequences of Aβ-induced activation of calcineurin is reduced phosphorylation of Ser-845, which induces AMPAR endocytosis and impairs the synaptic incorporation of these receptors [16]. Consistent with this finding, $APP_{Swe,Ind}$ transgenic mice display lower levels of Ser-845 phosphorylation, a phenomenon that correlates well with the loss of AMPARs on the cell surface and deficits in initial learning and memory [16].

Another key substrate of PP1 the activity of which is required for the expression of NMDAR-dependent LTD is glycogen synthase kinase-3β (GSK3β) [51]. PP1 can activate GSK3β by a direct dephosphorylation mechanism, as well as via the modulation of the upstream caspase–Akt signaling pathways, which are also crucial for AMPAR internalization and LTD [51, 52]. Interestingly, both GSK3β and caspases are enzymes that have been widely implicated in AD. Indeed, it has been demonstrated that inhibition of LTP by Aβ is mediated by the caspase 3, Akt1, and GSK3β signaling pathway [32, 53]. Paradoxically, however, GSK3β activity has also been reported to play a role in maintaining AMPAR synaptic expression under basal conditions as its inhibition leads to the loss of surface AMPAR expression by controlling the rate of AMPAR internalization [54]. However, during NMDAR-dependent LTD, GSK3β may preferentially phosphorylate other substrates including the key scaffolding protein in excitatory synapses, postsynaptic density-95 (PSD-95). PSD-95 stabilizes AMPARs at synapses through its interaction with transmembrane AMPAR regulatory proteins (TARPs), auxiliary subunits of AMPARs [55]. Overexpression of PSD-95 promotes synaptic maturation and enhances synaptic strength, whereas PSD-95 knockdown results in the opposite effects [56–60]. It appears that GSK3β phosphorylation of PSD-95 at Thr-19, following its dephosphorylation at Ser-295 by PP1, destabilizes and mobilizes PSD-95 away from the PSD, resulting in increased AMPAR internalization [61, 62].

Whether or not the phosphorylation status of PSD-95 is modulated by oligomeric Aβ via the GSK3β and PP1 signaling pathways remains to be determined.

GSK3β is also a major kinase that phosphorylates the microtubule-associated protein tau [63, 64]. Aβ causes tau hyperphosphorylation and mislocalization from axons to somatodendritic compartments, where it accumulates and mediates Aβ-induced downregulation of surface AMPARs [65–68]. Recent studies have shown that NMDAR-induced GSK3β phosphorylation of tau at Ser-396 is required for hippocampal LTD by enhancing the interaction between the GluA2 subunits of AMPARs with the protein interacting with C-kinase 1 (PICK1) [69, 70], a process that is fundamental for AMPAR internalization and/or intracellular retention during LTD [71–76]. Furthermore, phosphorylation of PICK1 by GSK3β at Ser-416 has also been reported to augment this interaction [77].

GluA2 can be phosphorylated by protein kinase C (PKC) at Ser-880 and by the protein tyrosine kinase of the sarcoma (Src) family at Tyr-876, both of which are required for AMPAR internalization and LTD [78–80]. GluA2 phosphorylation at these sites differentially regulates the interaction of the subunit with PICK1 and glutamate receptor interacting proteins (GRIP) 1 and 2 [80, 81]. GRIP1 plays an important role in stabilizing AMPARs at synapses and is essential for LTD [72, 79]. Given that phosphorylation of GluA2 weakens the interaction of the subunit with GRIP1, but not PICK1, it has been postulated that LTD involves destabilization and detachment of GluA2 from synapses, allowing AMPARs to be internalized. In accord with the role of Aβ in inducing aberrant AMPAR endocytosis, one study has observed that oligomeric Aβ increases PKC-mediated phosphorylation of GluA2 at Ser-880 and subsequently reduces surface expression of AMPARs in cultured hippocampal neurons [15]. More importantly, several molecular and pharmacological manipulations that inhibit GluA2 internalization potently prevent Aβ-induced synaptic depression and rescue memory impairment in AD mice. These include the GluA2-R845A mutant [14], GluA2-3Y peptides [82], and a small molecule PICK1 inhibitor [83].

A new mechanism underlying the pathological action of Aβ that involves the cyclin-dependent kinase 5- (Cdk5-) activating peptide, p25, has recently been described by Seo et al. [26]. Elevated levels of p25 have been implicated in many neurodegenerative diseases, including AD [84]. In their study, Seo et al. found that Aβ induces calpain-mediated cleavage of p35 into p25 in the hippocampus, a process that requires the activity of GluN2B-containing NMDARs and Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII). The Aβ-induced elevation in p25/Cdk5 activity subsequently enhances the phosphorylation of dopamine- and cyclic adenosine monophosphate-regulated neuronal phosphoprotein (DARPP-32) at Thr-75, thereby inhibiting the activity of PKA [85]. In a synergistic manner, Aβ also triggers dephosphorylation of DARPP-32 at Thr-34, presumably by calcineurin, thereby releasing its inhibition on PP1 [86, 87]. These converging mechanisms eventually lead to the loss of GluA1 phosphorylation at Ser-845 and induce AMPAR internalization and synaptic depression. Remarkably, genetic inhibition of p25 generation rescues LTP and memory deficits in 5xFAD transgenic mice [26].

In addition to promoting the internalization of AMPARs, oligomeric Aβ can also act through mechanisms that prevent the forward trafficking of AMPARs towards the plasma membrane. Aβ has been shown to cause aberrant redistribution of CaMKII from the synaptic to the cytosolic fraction both in cultured neurons and in the brain of APP_{swe} transgenic mice [88]. CaMKII can potentiate AMPAR-mediated transmission via (a) phosphorylation of GluA1 at Ser-831 to enhance AMPAR channel conductance, (b) phosphorylation of the TARP, stargazin, to facilitate synaptic recruitment of AMPARs, and (c) potentiation of the Ras-ERK (extracellular signal-regulated kinase) pathway to promote AMPAR insertion into the plasma membrane [45, 89–91]. Consistent with the role of CaMKII in synaptic potentiation, exposure of soluble Aβ oligomers reduces surface GluA1 clusters in cultured neurons, concomitant with decreased AMPAR synaptic responses in cortical pyramidal neurons recorded from acute brain slices of APP_{swe} transgenic mice [88].

Aβ has been shown to interact with NMDARs [92, 93] and to reduce their surface expression through endocytosis [33]. Aβ-induced internalization of NMDARs involves dephosphorylation of the GluN2B subunit at Tyr-1472 by $STEP_{61}$ (striatal-enriched protein tyrosine phosphatase 61), the expression of which is upregulated in several AD mouse models, as well as in the postmortem prefrontal cortex of AD patients [33, 94–96]. The fact that Aβ enhances the internalization of NMDARs seems counterintuitive given the role of NMDARs in mediating AMPAR endocytosis, spine loss, and ultimately excitotoxicity in neurons. Recent studies on the putative oligomeric Aβ receptor, PrP^C, have provided insights into two potential mechanisms that regulate NMDAR function [97, 98]. Firstly, soluble oligomeric Aβ binding to PrP^C activates the tyrosine kinase Fyn, which initially phosphorylates GluN2B and transiently enhances NMDAR function, before the $STEP_{61}$ level increases and dephosphorylates GluN2B [99]. Secondly, Aβ disrupts the ability of PrP^C to limit excessive NMDAR activity in a copper-dependent manner, potentially by chelating copper ions and preventing them from binding to PrP^C, thereby producing large nondesensitizing steady-state NMDAR currents [100]. Albeit controversial, loss of PrP^C function has been reported to prevent Aβ-induced LTP and memory impairment in mice [98, 101–105]. Despite this, the role of PrP^C in regulating AMPAR trafficking has not been directly examined.

Recent studies by Kessels and colleagues have challenged the central role of NMDAR-mediated Ca^{2+} influx in Aβ-induced synaptic depression [106]. It is well established that the neurotoxic effects of oligomeric Aβ on synapses can be blocked by the NMDAR antagonist, D-APV (D-2-amino-5-phosphonopentanoic acid), which prevents glutamate binding and blocks the activation of NMDARs. However, noncompetitive NMDAR antagonists that block ion flow through the receptor, such as MK-801, ketamine, and 7-chlorokynurenic acid, are not able to rescue Aβ-mediated synaptic depression [106, 107]. A similar finding was recently reported for oligomeric Aβ-induced dendritic

spine loss [108]. Consistent with the idea that Aβ operates through shared pathways with LTD, metabotropic, but not ionotropic, NMDAR function has been shown to be required for NMDAR-dependent LTD in the hippocampus by activating the p38 MAPK signaling pathway [109]. In fact, ligand binding to the extracellular domain of NMDARs induces conformational change and movement of their cytoplasmic tails, allowing PP1 to dephosphorylate CaMKII together with other signaling molecules that contribute to synaptic depression [110, 111]. Although the role of metabotropic NMDARs remains controversial [46], it does offer an explanation for the fact that the FDA-approved NMDAR antagonist, memantine, has poor efficacy in treating early-stage AD [112]. Further research is warranted, as delineating the metabotropic NMDAR signaling pathway may shed light on new strategies for the development of future AD drugs.

3.2. mGluRs. mGluRs belong to the G-protein-coupled receptor superfamily that modulates neuronal excitability, synaptic transmission, and plasticity in the central nervous system [113]. Group I mGluRs, which consist of two members, mGluR1 and mGluR5, predominantly localize to the postsynaptic membrane and are canonically coupled to $G\alpha_{q/11}$ to activate phospholipase Cβ (PLCβ) that catalyzes the hydrolysis of phosphoinositides into inositol 1,4,5-triphosphate (IP_3) and diacylglycerol (DAG). Subsequently, these second messengers trigger the release of Ca^{2+} from intracellular stores and activate PKC, respectively. Group I mGluRs, and more specifically mGluR5, are the predominant receptors that mediate mGluR-dependent LTD in the hippocampus and have been widely implicated in AD [114].

It is well established that mGluR-dependent LTD requires the internalization of GluA2-containing AMPARs, leading to a long-term reduction in the number of surface AMPARs [115, 116]. One of the mechanisms that regulates mGluR-induced AMPAR endocytosis involves the phosphorylation of GluA2 at Ser-880 by PKC, a process that is facilitated by PICK1 [117–119]. However, in the CA1 region of the hippocampus, internalization of AMPARs does not require PKC but instead relies on the dephosphorylation of GluA2 at Tyr-876 by $STEP_{61}$ [120–122]. Dephosphorylation of GluA2 stimulates the binding of BRAG2 (brefeldin resistant Arf GEF 2), which in turn activates the small GTPase Arf6 through augmentation of its GEF (guanine-nucleotide exchange factor) activity and promotes AMPAR endocytosis [122]. In accordance with this model, it has been reported that Aβ-induced internalization of AMPARs requires $STEP_{61}$ activity [95]. Genetic deletion of $STEP_{61}$ restores the number of AMPARs on the postsynaptic membrane, enhances LTP, and improves cognitive function in AD mice [95, 123]. A new small molecule inhibitor of $STEP_{61}$, TC-2153, has recently been shown to reverse cognitive deficits in 3xTg AD mice [124]. Like NMDAR-dependent LTD, mGluR-mediated LTD also involves the Rap1-p38 MAPK signal transduction pathway to facilitate AMPAR internalization via the formation of the GDI-Rab5 complex [125–127]. In addition, a role for ERK in mGluR-dependent LTD has also been reported [128].

One unique feature of mGluR-dependent LTD is its requirement for rapid translation of preexisting mRNAs (local protein translation) in dendrites [129]. mGluR-dependent *de novo* protein synthesis can be regulated through multiple pathways, including the PI3K-Akt-mTOR (mammalian target of rapamycin) and ERK signaling pathways that converge on the initiation and elongation factors of protein translation [130]. Several mRNA encoding proteins that regulate AMPAR trafficking are locally translated during mGluR-dependent LTD, including the activity-regulated cytoskeleton-associated protein (Arc), microtubule-associated protein 1B (MAP1B), and STEP [121, 131–133]. All of these proteins are known to facilitate the internalization of AMPARs. MAP1B is a known GRIP1 binding protein [134]. Given that GRIP1 stabilizes AMPARs at synapses, the newly synthesized MAP1B may sequester GRIP, hence loosening its interaction with GluA2. On the other hand, Arc interacts with the endocytic proteins, endophilin and dynamin, and is able to enhance dynamin polymerization and GTPase activity, thereby promoting AMPAR endocytosis [135, 136]. Interestingly, soluble oligomeric Aβ rapidly induces Arc expression in neurons, which may contribute to the loss of AMPARs from the plasma membrane [137]. Moreover, Arc also regulates the endosomal trafficking of APP and BACE1, as well as PS1, a mechanism that is essential for the activity-dependent production of Aβ in the brain, and genetic deletion of Arc reduces the Aβ load in APP_{swe};PS1ΔE9 transgenic AD mice [138]. This may serve as a positive feedback mechanism underlying the overproduction of Aβ in the pathophysiology of AD.

Studies from several laboratories have implicated the mGluR-dependent signaling pathway in the neurotoxic effects of Aβ on synaptic function [4, 14, 139–143]. Notably, genetic and pharmacological inhibition of mGluR5 prevents oligomeric Aβ-induced impairment in LTP, spine loss, and cognitive deficits in AD mouse models [139, 142–145]. More recently, a seminal study by Strittmatter and colleagues identified an interaction between mGluR5 and PrP^C, which together act as a coreceptor for oligomeric Aβ [144]. They also revealed an essential role for mGluR5 and PrP^C coupling in the pathology of AD [146]. Mechanistically, mGluR5 links PrP^C to key intracellular signaling molecules, such as Homer1b/c, Pyk2, Fyn, and CaMKII, all of which play major roles in synaptic plasticity [144, 146, 147]. When neurons are exposed to oligomeric Aβ, the PrP^C-mGluR5 complex mediates the aberrant activation of Pyk2, Fyn, and CaMKII, causing altered neuronal states that lead to impaired LTP [99, 144, 146]. It is interesting to note that Aβ also induces a biphasic alteration in CaMKII activity, resembling that of Fyn, in a PrP^C-mGluR5-dependent manner, and that this is accompanied by the increased association of mGlu5 with CaMKII [146]. Given that mGluR5 activation enhances NMDAR forward trafficking through CaMKII-mediated phosphorylation of GluN2B at Ser-1303 [148], it is hypothesized that Aβ-induced enhancement of the association between mGluR5 and CaMKII may prevent synaptic potentiation. Furthermore, pharmacological activation of mGluR5 in the presence of PrP^C causes a redistribution of

CaMKII into the cytoplasm [147], which may have an impact on AMPAR trafficking.

Interestingly, the cross talk between mGluR5 and NMDAR signaling is bidirectional. Not only can mGluR5 potentiate NMDAR currents through CaMKII and PKC signaling pathways [148, 149], but also activation of NMDARs can potentiate mGluR5 responses under physiological conditions [150, 151]. This involves the NMDAR-dependent activation of calcineurin that dephosphorylates mGluR5 and reduces receptor desensitization. However, a high concentration of NMDA can induce PKC-dependent mGluR5 phosphorylation and inhibit mGluR5 responses [152]. Although the interaction of mGluR5 and NMDARs has been implicated in synaptic plasticity and various animal behaviors [153–156], their alteration in the presence of Aβ binding to PrP^C and how this impacts on AMPAR trafficking remain unclear.

3.3. Protein Ubiquitination. Posttranslational ubiquitination, a regulatory signal that controls protein trafficking and turnover, has recently emerged as an important mechanism that regulates AMPAR function [157, 158]. All AMPAR subunits undergo activity-dependent ubiquitination in cultured neurons, a process that is Ca^{2+}-dependent and requires the activity of L-type voltage-gated Ca^{2+} channels [159–161]. The primary E3 ligases that catalyze the ubiquitination of GluA1 and GluA2 subunits are Nedd4-1 and RNF167, respectively [160, 162]. While the role of protein ubiquitination on the GluA1 and GluA2 subunits in ligand-induced AMPAR endocytosis remains controversial, it is well accepted that ubiquitination of AMPARs regulates the intracellular sorting of receptors into late endosomes for degradation [159–161, 163]. Under normal conditions, the degradation of AMPARs is required for protein homeostasis to ensure turning over of old or used receptors in order to maintain healthy levels of AMPARs in neurons. However, when the ubiquitin pathway is hijacked (e.g., by elevated levels of Aβ), there is an excessive downregulation of AMPARs and synaptic depression. Indeed, a new finding has demonstrated a role for naturally secreted and synthetic Aβ in promoting the ubiquitination of AMPARs by Nedd4-1 [164]. Interestingly, knocking down Nedd4-1 rescued Aβ-induced synaptic deficits, including reduced glutamatergic synaptic transmission, decreased levels of surface AMPARs, and the loss of dendritic spines. These findings have important implications in targeting ubiquitin E3 ligases as potential drug targets for the treatment of AD.

4. Concluding Remarks

Research over the past decade has provided strong evidence that the cognitive deficit associated with AD is caused by the neurotoxic effects of soluble Aβ oligomers on synaptic function. Increasing evidence indicates that the trafficking of AMPARs, which is essential to multiple forms of synaptic and structural plasticity in the brain, is aberrantly dysregulated by oligomeric Aβ and manifests as impairments in LTP, learning, and memory. It is particularly encouraging to learn that pharmacological and genetic manipulations that block endocytosis or enhance the forward trafficking of AMPARs can rescue LTP and reverse cognitive deficits in AD mice. Given that Aβ-induced AMPAR internalization requires the same adaptor proteins as the conventional trafficking pathway, it will be challenging to minimize unwanted side effects. Hence, further research is needed to identify specific targets for improving the memory deficits associated with AD. Rapid progress has been made in delineating the molecular mechanisms and signaling pathways underlying the loss of AMPARs from the plasma membrane induced by oligomeric Aβ (Figure 1). The discovery of PrP^C as a receptor for soluble Aβ oligomers that signals through NMDA and mGluR5 receptor has underscored the importance of glutamatergic signaling in the etiology of AD. It is likely that these receptors act cooperatively to mediate the synaptotoxic effects of Aβ, highlighting the need for further investigation of the associated signaling mechanisms with a view to developing more effective therapeutic strategies for the treatment of AD.

Competing Interests

The authors declare that they have no competing interests.

Authors' Contributions

Sumasri Guntupalli and Jocelyn Widagdo contributed equally to this work.

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Review Article

Emerging Link between Alzheimer's Disease and Homeostatic Synaptic Plasticity

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Alzheimer's disease (AD) is an irreversible brain disorder characterized by progressive cognitive decline and neurodegeneration of brain regions that are crucial for learning and memory. Although intracellular neurofibrillary tangles and extracellular senile plaques, composed of insoluble amyloid-β (Aβ) peptides, have been the hallmarks of postmortem AD brains, memory impairment in early AD correlates better with pathological accumulation of soluble Aβ oligomers and persistent weakening of excitatory synaptic strength, which is demonstrated by inhibition of long-term potentiation, enhancement of long-term depression, and loss of synapses. However, current, approved interventions aiming to reduce Aβ levels have failed to retard disease progression; this has led to a pressing need to identify and target alternative pathogenic mechanisms of AD. Recently, it has been suggested that the disruption of Hebbian synaptic plasticity in AD is due to aberrant metaplasticity, which is a form of homeostatic plasticity that tunes the magnitude and direction of future synaptic plasticity based on previous neuronal or synaptic activity. This review examines emerging evidence for aberrant metaplasticity in AD. Putative mechanisms underlying aberrant metaplasticity in AD will also be discussed. We hope this review inspires future studies to test the extent to which these mechanisms contribute to the etiology of AD and offer therapeutic targets.

1. Introduction

Neurons communicate with each other at specialized intercellular junctions, called synapses. The strength of synaptic transmission can be dynamically and persistently altered in response to changes in neuronal activity. In the book *The Organization of Behavior*, Donald Hebb postulated that connections between neurons that are simultaneously active are strengthened [1]. Such "Hebbian plasticity" was first demonstrated at excitatory glutamatergic synapses in rabbit hippocampus by the seminal work of Bliss and Lomo [2]. High frequency stimulation of presynaptic axons in the perforant pathway induces stronger and long-lasting excitatory postsynaptic potentials (EPSPs) in neurons of the postsynaptic dentate gyrus [2]. This long-term potentiation (LTP) of excitatory synaptic strength lasts hours to months [2] and can be induced electrically in brain slices as well as *in vivo* in behaving animals [2, 3]. Hence, the associative and input-specific synaptic plasticity such as LTP and its counterpart long-term depression (LTD) is thought to underlie cellular correlates of learning and memory [4–7].

Hebbian plasticity also represents a positive feedback mechanism. Once LTP is induced, saturated synapses undergo further potentiation with greater ease than before the LTP induction, leading to unstable runaway excitation [8–10]. Similarly, continuous synaptic depression during LTD could result in unnecessary synaptic silencing and elimination [8–10]. In order to sense and counteract destabilizing effects of LTP and LTD, neurons employ negative feedback processes called homeostatic synaptic plasticity [11–14]. This adaptive plasticity offers a compensatory refinement of synaptic strength to maintain the stability of network activity within a physiologic limit [13–15]. For example, prolonged elevation of neuronal activity results in compensatory downscaling of synaptic strength to prevent hyperexcitation, whereas prolonged suppression of neuronal activity leads to compensatory upscaling of synaptic strength to prevent synapse silencing and elimination [13–15]. Without this

homeostatic mechanism, the capacity of an active synapse would get saturated due to unconstrained potentiation, limiting its ability to store information (i.e., memory). Homeostatic synaptic plasticity is therefore a vital partner of Hebbian synaptic plasticity.

Defects in homeostatic synaptic plasticity could, in principle, cause abnormal Hebbian plasticity at synapses, leading to pathologic levels of synaptic potentiation or elimination in neurologic diseases. For example, Alzheimer's disease (AD) is characterized by progressive and irreversible memory impairment [16] and associated with inhibition of LTP and enhancement of LTD in the hippocampus [17–27]. While physiologic levels of soluble amyloid-β (Aβ) oligomers have been shown to enhance synaptic activity and LTP [28, 29], pathologic levels of soluble Aβ oligomers impair LTP and enhance LTD in acute hippocampal slices [30–33]. Such impairment in Hebbian synaptic plasticity correlates strongly with memory impairment in early AD when Aβ plaques and neuronal degeneration are minimal [34–36]. Recent studies suggest that this abnormal Hebbian plasticity is due to pathologic engagement or disruption of metaplasticity [27, 32, 37], a form of homeostatic synaptic plasticity that controls the induction threshold of LTP and LTD [38]. Interestingly, Aβ-induced aberrant hyperexcitability is found in cortical and hippocampal neuronal networks of human AD and mouse models of AD [39–45]. Further, epileptiform electrical seizures and neuronal activity stimulate Aβ synthesis and its release from the neurons in the hippocampus [46–48]. Indeed, a pathologic positive feedback loop between Aβ production and neuronal hyperexcitability would favor LTP inhibition and LTD induction.

These studies have provided a possible link between abnormal metaplasticity and cognitive dysfunction in AD pathogenesis, although our knowledge on the underlying mechanisms is limited. An understanding of the molecular mechanisms through which altered metaplasticity contributes to AD synaptopathology will be crucial in decoding the etiology of AD and may facilitate "correcting metaplasticity" as a putative novel therapy to restore Hebbian synaptic plasticity and treat cognitive dysfunction in early AD. In this paper, we review recent studies demonstrating aberrant metaplasticity in AD and discuss the possible underlying mechanisms focused on glutamate receptor regulation.

2. Abnormal Hebbian Synaptic Plasticity in AD

AD is a neurodegenerative disorder characterized by progressive and irreversible cognitive decline [16]. It is the 6th leading cause of death in the United States and the most common cause of dementia, which affects over 44 million people worldwide [49]. The molecular hallmarks of AD are amyloid plaques (extracellular deposits consisting of aggregated insoluble Aβ) and neurofibrillary tangles (intracellular filamentous aggregates of hyperphosphorylated tau) in the hippocampus and cortices [50–52], the brain regions critical for learning and memory. Interestingly, genetic suppression of endogenous tau blocks cognitive dysfunction in AD animal models, in which Aβ expression has been increased using a transgene [53–55], suggesting that tau acts downstream of Aβ in AD pathogenesis.

Importantly, soluble Aβ peptides rather than insoluble amyloid plaques have emerged to play critical roles in the early stages of AD pathogenesis. First, amyloid plaques are found at later stages after memory loss is already evident in humans and AD animal models with genetically elevated Aβ level [17–27]. Second, intracranial injection of soluble Aβ oligomers is sufficient to cause memory loss [29, 33, 56–59]. Third, rare early-onset autosomal dominant familial AD (FAD) is associated with increased levels of soluble Aβ due to mutations in genes whose protein products are involved in Aβ production and processing [60, 61]. Aβ peptides are generated by successive proteolysis of amyloid-β precursor protein (APP), a large transmembrane glycoprotein that is initially cleaved by the β-site APP-cleaving enzyme 1 (BACE1) and subsequently by γ-secretase in the transmembrane domain [62–64]. The FAD mutations are found in APP and presenilins [60, 61], which are catalytic components of γ-secretase [65]. Lastly, a major genetic risk factor for most AD (i.e., sporadic AD) is polymorphic ε4 allele of apolipoprotein E [66, 67]. The encoded ApoE4 is less efficient in clearing Aβ than the common ApoE3, suggesting a strong association between sporadic AD and increased levels of soluble Aβ [68].

How could pathologic levels of soluble Aβ oligomers cause cognitive dysfunction? The first clue came from the studies in AD mouse models with genetically elevated Aβ [17–27]. Before the development of amyloid plaques is evident, these AD mouse models display severe impairment of hippocampal LTP [17–27]. Furthermore, LTD is induced in these AD mouse hippocampi with subthreshold stimulations, which normally cannot induce LTD in wild-type control mice [17–27]. Subsequent studies have shown that direct application of soluble Aβ oligomers (synthetic, cell-culture secreted, or AD brain-derived) at pathologic levels inhibits LTP and enhances LTD in acute hippocampal slice [30–33]. A persistent and unchecked decrease in synaptic strength is expected to lead to the pathologic elimination of synapses [69–71]. Indeed, decreases in synapse density are evident in hippocampi of patients with early AD [72–75]. Therefore, abnormal Hebbian synaptic plasticity is thought to be the basis of memory loss in early AD when amyloid plaques and neuronal degeneration are minimal [34–36].

3. Is Abnormal Hebbian Synaptic Plasticity due to Defective Homeostatic Synaptic Plasticity in AD?

Decades of studies cited above have compared the magnitudes of LTP and LTD in AD transgenic mouse models to determine if pathologic levels of soluble Aβ oligomers affect Hebbian synaptic plasticity. However, the absolute changes in the LTP and LTD magnitudes vary with age and AD mouse model [147], suggesting age- and strain-dependent differences for the induction threshold of LTP and LTD in these animals. The induction thresholds of LTP and LTD can be modified as a consequence of previous postsynaptic neuronal activity (Figure 1) [10, 148, 149]. LTP induction is favorable in neurons whose previous synaptic and intrinsic

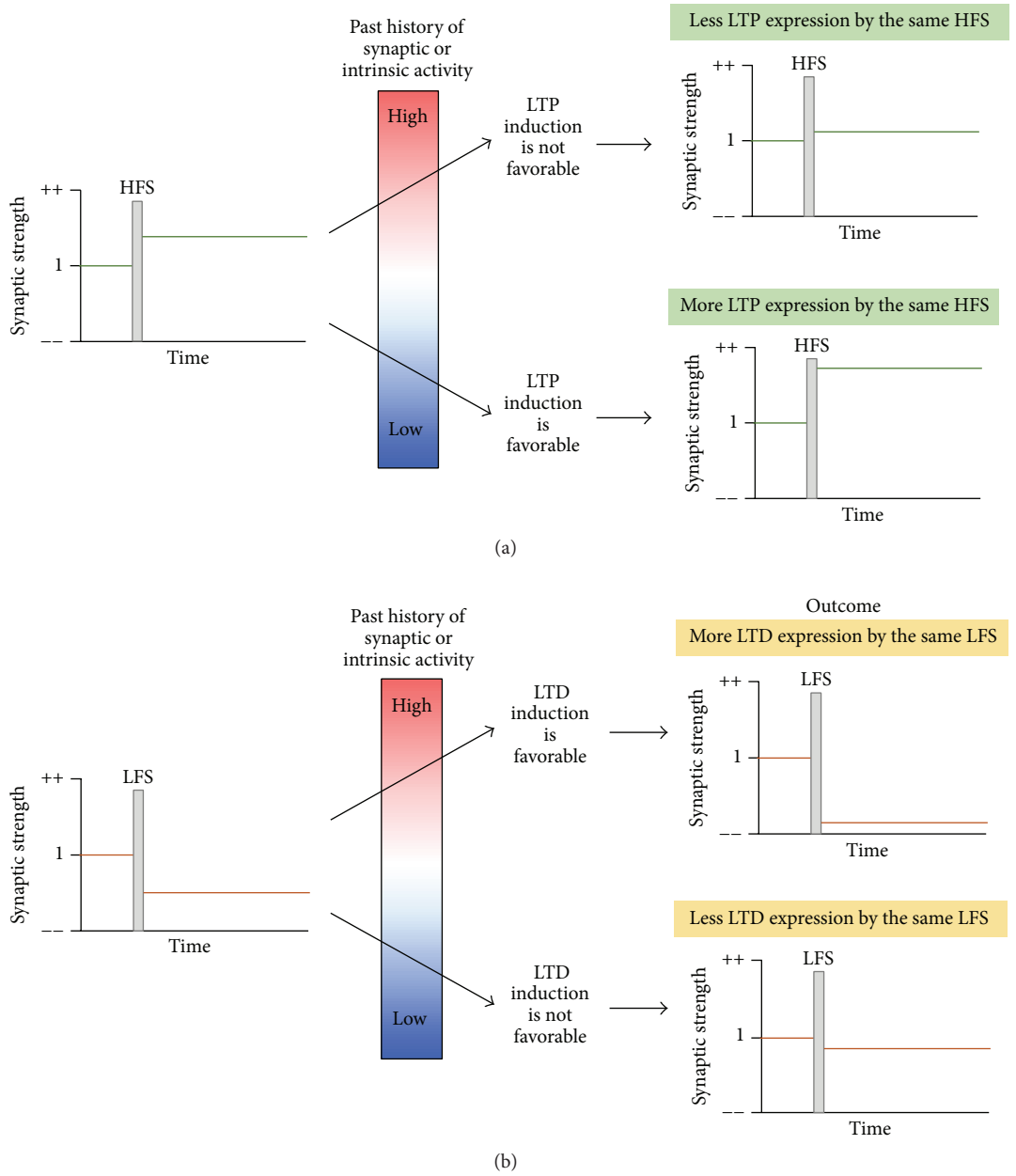

Figure 1: Metaplasticity. The induction threshold of LTP and LTD can be modified as a consequence of overall past synaptic or intrinsic activity of postsynaptic neurons. Such compensatory adjustment called "metaplasticity" provides stability to neuronal networks that support Hebbian synaptic plasticity. (a) LTP induction by conventional high frequency stimulation (HFS) is favorable in the neurons whose previous synaptic and intrinsic activities were low. (b) LTD induction by conventional low frequency stimulation (LFS) is favorable in the neurons whose previous synaptic and intrinsic activities were high.

activities were low, whereas LTD induction is preferred when the previous activities were high [10, 148, 149]. Such compensatory adjustment of the induction thresholds for LTP and LTD, called "metaplasticity," occurs as a form of homeostatic synaptic plasticity and provides stability to neuronal networks and supports Hebbian synaptic plasticity [10].

Hence, it is possible that the abnormal Hebbian synaptic plasticity in AD could arise from the defects in metaplasticity. Several studies have provided supporting evidence for this hypothesis. Aberrant neuronal hyperexcitability has been observed in cortical and hippocampal neuronal networks of patients with early AD [150] and FAD AD mouse models with heightened levels of APP and Aβ [21, 41–44, 151]; this is also consistent with reports that patients with early AD and FAD animal models exhibit epileptic seizures [21, 45, 152–160]. Pharmacological inhibition of epileptic seizures inhibits memory loss in AD mouse models [156], implicating critical roles of aberrant neuronal hyperexcitability in cognitive dysfunction presented early in AD pathogenesis [39, 40]. Hence, Aβ-induced cognitive dysfunction in early AD may

result from the inability of neurons to adapt to persistent increases in overall neural network activity rather than the absolute changes in LTP and LTD magnitudes.

Additional support for this hypothesis comes from the report that soluble Aβ oligomers result in excessive activation of N-methyl D-aspartate receptors (NMDARs) containing GluN2B subunits, causing LTP inhibition and LTD facilitation via ERK and CREB signaling pathways [161]. GluN2B-selective antagonists effectively prevent Aβ-induced LTP inhibition [161–163], suggesting that early activation of extrasynaptic NMDARs primes the synapse to inhibit LTP induction and facilitate LTD induction. Consistent with this notion, GluN2B-selective antagonists prevent priming-induced inhibition of LTP [164]. The beneficial effects of the partial NMDAR antagonist memantine in AD also support the possible role of metaplasticity in AD-associated synaptic dysfunction because memantine does not block LTP acutely but restores LTP induction impaired by tonic NMDAR activation [165, 166].

A recent study by Megill et al. has provided direct evidence for impaired metaplasticity in an AD transgenic mouse model [27]. This study examined frequency- and age-dependent synaptic plasticity in the APP/PS1 AD mouse model [27], which has two FAD-linked mutations (a Swedish mutation in APP and a deletion FAD mutation in exon 9 of presenilin-1) [167]. These mutations increase total Aβ production, resulting in a higher level of aggregation-prone Aβ42 peptides, and accelerated AD pathology [168] and age-dependent cognitive deficits [169, 170]. While the wild-type mice show a shift of the induction threshold to favor LTP and suppress LTD at the hippocampal CA1 Schaffer collateral synapses with age, the APP/PS1 transgenic mice fail to undergo this normal developmental metaplasticity [27]. As a result, the magnitudes of LTP and LTD remained the same in the APP/PS1 transgenic mice from when they were young (1 month of age) until they were adult (6 months of age). When the absolute magnitudes of LTP and LTD were compared, the adult APP/PS1 mice display LTP inhibition and LTD facilitation compared to age-matched wild-type mice [27]. Although electrophysiological characterization of other AD mouse models with elevated Aβ levels should be performed to see if impaired developmental metaplasticity is a general phenomenon for AD, these findings suggest that the Hebbian synaptic plasticity defects in AD could be due to the inability of neurons to undergo developmental metaplasticity (Figure 2).

4. Putative Mechanisms Underlying Defective Homeostatic Synaptic Plasticity in AD

How can pathologic levels of soluble Aβ oligomers cause aberrant metaplasticity in AD? One way to mediate metaplasticity is to alter the induction mechanisms of LTP and LTD by regulating the function of NMDARs [10, 171–174] because calcium (Ca^{2+}) influx through NMDARs at the postsynaptic density (PSD) is critical for inductions of NMDAR-dependent LTP and LTD [175–177]. An effective means to alter Ca^{2+} current per unit charge through NMDAR is to change subunit composition of NMDAR [177]. Such a change influences Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) interaction with NMDARs and has been shown to control Hebbian synaptic plasticity [178]. For example, GluN2B-containing NMDARs bind to CaMKII with high affinity whereas those containing GluN2A interact with CaMKII with low affinity. Consistent with their decreased affinity for CaMKII, altering synaptic NMDARs from GluN2B-containing receptors to GluN2A-containing ones markedly reduces LTP induction [178]. Developmental metaplasticity in the visual cortex has also been suggested to involve experience-dependent changes in the GluN2 subunit composition of NMDARs that influence the induction thresholds of LTP and LTD [173, 179].

Soluble Aβ oligomers have been shown to decrease glutamate reuptake and subsequently increase extracellular glutamate levels [32, 180]. Such glutamate spillover would activate extracellular NMDARs, which are mostly composed of GluN2B-containing NMDARs at mature synapses [175, 176]. Indeed, soluble Aβ oligomers enhance activation of GluN2B-containing NMDARs more rapidly than synaptic depression and such actions would prime excitatory synapses to inhibit LTP induction and favor LTD induction [161]. However, NMDAR subunit composition and current are similar between wild-type mice and APP/PS1 mice at all ages [27], suggesting that developmental metaplasticity defect in the APP/PS1 mice is not due to altered NMDAR function during the induction of LTP and LTD.

Another way to induce metaplasticity is to alter the expression mechanisms of LTP and LTD by regulating α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs) [181], which mediate the majority of excitatory synaptic current upon glutamate binding [182]. A major postsynaptic expression mechanism for LTP is the synaptic recruitment of AMPARs from a perisynaptic reserve pool and their subsequent stabilization at excitatory synapses, whereas that for LTD is the removal and internalization of synaptic AMPARs [175, 182]. Insertion and removal of synaptic AMPARs during the expression of LTP and LTD, respectively, are tightly regulated processes by phosphorylation of AMPAR subunit GluA1 at Ser-845 and Ser-831 [183–185]. Phosphorylation of GluA1 at Ser-845 by protein kinase A (PKA) is necessary for synaptic targeting of GluA1 driven by CaMKII [186], whereas dephosphorylation at Ser-845 mediates GluA1 internalization [183, 187, 188] and NMDAR-dependent LTD [185]. In addition, Ser845 phosphorylation of GluA1 mediates synaptic insertion of Ca^{2+}-permeable GluA1-containing AMPARs during synaptic scaling in cultured dissociated cortical neurons upon chronic activity deprivation [92] and homeostatic synaptic scaling in the visual cortex upon sensory deprivation [189, 190]. Phosphorylation of GluA1 at Ser831 by protein kinase C (PKC) [191] and CaMKII [192, 193] increases following LTP induction [184, 194] and supports LTP expression [183–185]. Although GluA1 phosphorylation at Ser-845 and Ser-831 has been shown to reduce the induction threshold for LTP [195, 196], adult APP/PS1 mice display normal levels of GluA1 phosphorylation and perisynaptic AMPARs compared

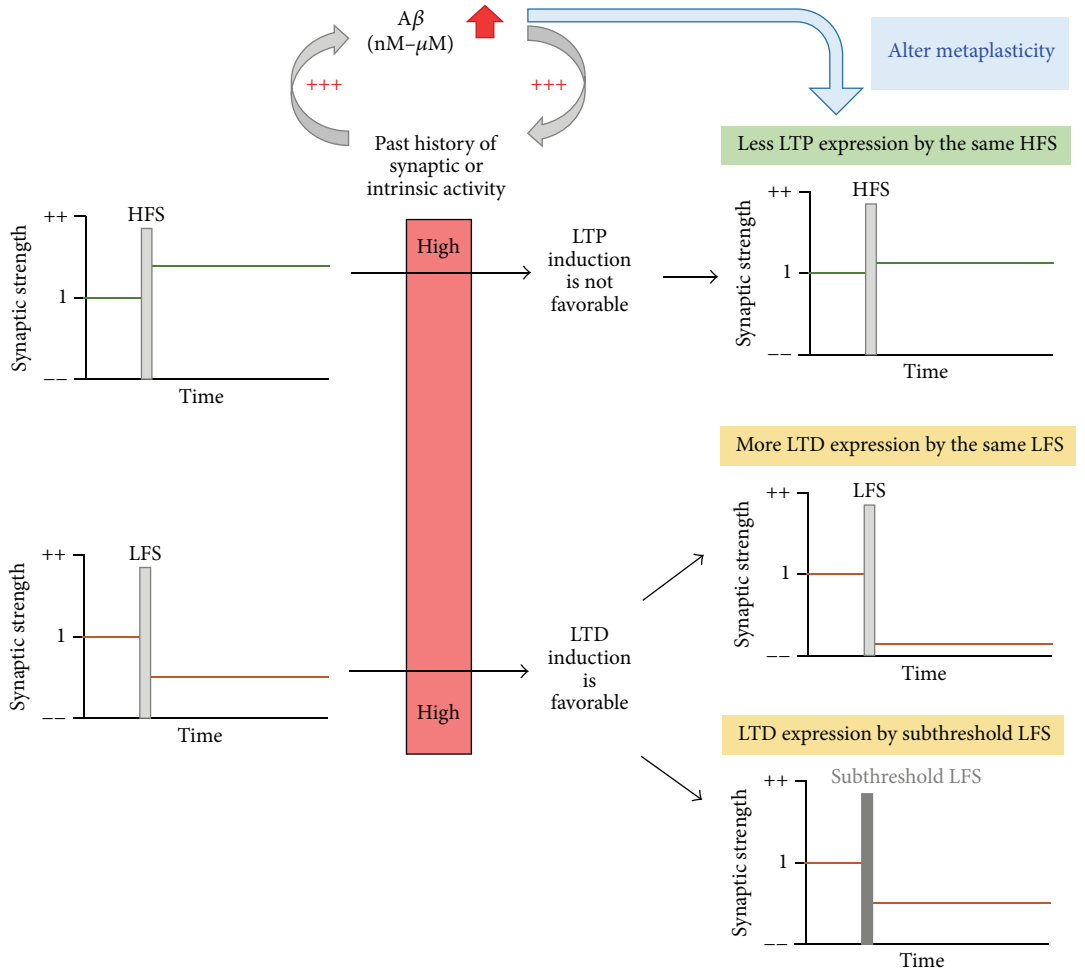

Figure 2: Aberrant metaplasticity in AD. Aβ increases the activity of excitatory neurons, which in turn stimulates synthesis and release of Aβ in a positive feedback loop, leading to pathologic accumulation of Aβ. Neuronal hyperexcitability or early activation of GluN2B-containing NMDAR by heightened Aβ expression induces aberrant metaplasticity, leading to inhibition of LTP by HFS and enhancement of LTD in the hippocampus by LFS or normal LTD induction by subthreshold LFS.

to those of wild-type mice [27]. Hence, the developmental metaplasticity defect in APP/PS1 mice is not due to insufficient AMPAR availability for synaptic insertion; rather, it is due to regulation of AMPAR trafficking by means other than GluA1 phosphorylation.

Metaplasticity is a form of homeostatic synaptic plasticity in which the magnitude and polarity of synaptic plasticity are adjusted accordingly based on the past history of synaptic and neural activity [38]. Since metaplasticity can occur at a single synapse [197, 198], it is tempting to speculate that pathologic levels of Aβ may impair developmental metaplasticity by altering postsynaptic expression mechanisms of homeostatic synaptic plasticity (Figure 3). Homeostatic synaptic plasticity has been extensively investigated using primary dissociated culture of neocortical and hippocampal neurons (Table 1). In these studies, prolonged blockade of network activity for 48 hours (h) with the sodium channel blocker tetrodotoxin (TTX) induces a significant increase in AMPAR-mediated miniature excitatory postsynaptic current (mEPSC) amplitude and synaptic AMPAR density, indicating the postsynaptic expression of homeostatic synaptic scaling [78, 80, 94, 138, 199–201]. Conversely, mEPSC amplitudes are scaled down in dissociated neuronal culture after prolonged enhancement of network activity by KCl depolarization or blocking inhibitory neurotransmission with antagonists for A-type gamma-aminobutyric acid ($GABA_A$) receptors, such as bicuculline [78, 80, 90, 199, 202, 203]. Interestingly, many of the crucial mediators of homeostatic synaptic plasticity have also been implicated in AMPAR regulation during LTP and LTD expression and AD pathology (Table 1). Taken together, these correlated functional roles raise an intriguing possibility that pathologic accumulation of Aβ may impair molecular mechanisms involved in homeostatic synaptic plasticity, which manifests as disruption of Hebbian synaptic plasticity in AD (Figure 3).

4.1. AMPAR Scaffolding Proteins. Glutamate receptor-interacting protein 1 (GRIP1) and PICK1 (protein interacting with C-kinase 1) are PDZ (postsynaptic density 95/discs large/zona occludens) domain-containing proteins that

Table 1: Molecular mechanisms and players involved in AD and the expression of homeostatic synaptic plasticity.

	Roles in synaptic scaling	Involvement in AD	References
	AMPAR scaffolding proteins		
GRIP1	Synaptic accumulation and removal of GRIP1 mediate synaptic scaling and downscaling, respectively, by regulating synaptic AMPAR targeting.		[76, 77]
PICK1	PICK1 degradation mediates synaptic scaling.	PICK1 interaction with GluA2 mediates Aβ-induced synaptic depression.	[78, 79]
	Regulators of AMPAR trafficking		
Arc/Arg3.1	Downregulation of Arc/Arg3.1 mediates synaptic scaling by increasing surface AMPAR density. Upregulation of Arc/Arg3.1 mediates synaptic downscaling by promoting AMPAR endocytosis.	Arc/Arg3.1 expression is elevated in AD and mediates activity-dependent generation of Aβ by binding to presinilin-1 and regulating γ-secretase trafficking.	[80–82]
Homer1a	Downregulation of Homer1a mediates synaptic scaling, whereas upregulation of Homer1a mediates synaptic downscaling by regulating surface AMPAR density and Tyr-phosphorylation.		[83]
	Regulators of synaptic AMPAR density		
PSD-95	Synaptic accumulation of PSD-95 mediates synaptic scaling, whereas its interaction with TARP mediates synaptic downscaling.	Pathological level of Aβ leads to PSD-95 degradation.	[84–89]
PSD-93	PSD-93 mediates synapticscaling.		[84]
GKAP	Synaptic accumulation and removal of GKAP mediate synaptic scaling and downscaling, respectively, by regulating surface AMPAR density.	Pathological level of Aβ leads to GKAP degradation.	[90, 91]
	Posttranslation modification of AMPAR		
Calcineurin	Reduced calcineurin activity mediates synaptic scaling via GluA1-Ser845 dephosphorylation and subsequent synaptic trafficking of Ca^{2+}-permeable AMPARs.	In AD mouse model, increased activity of calcineurin induces dephosphorylation and synaptic removal of the GluR1 subunit of AMPAR.	[92, 93]
$STEP_{61}$	Downregulation of $STEP_{61}$ mediates synaptic scaling, whereas enhanced $STEP_{61}$ upon chronic activity induces dephosphorylation of GluN2B and GluA2.	$STEP_{61}$ expression is elevated in AD and mediates Aβ-induced dephosphorylation and internalization of NMDARs and AMPARs, whereas inhibition of $STEP_{61}$ prevents cognitive deficits and impaired hippocampal LTP in AD mouse models.	[94–98]
PP1	Downregulation of PP1 inhibitor-2 (I-2) mediates synaptic downscaling by reducing surface AMPARs.	Inhibition of PP1 blocks Aβ-induced impairment in hippocampal LTP.	[99, 100]
DHHC2	Translocation of DHHC2 to PSD mediates synaptic scaling by enhancing synaptic targeting of PSD95 and AMPAR.		[86]
Nedd4-1	Upregulation of Nedd4-1 mediates synaptic downscaling by reducing surface AMPAR density.	Nedd4-1 expression is elevated in AD.	[101, 102]
SUMO-1 and Ubc9	SUMOylation of Arc/Arg3.1 mediates synaptic scaling.	SUMO-conjugating enzyme, Ubc9, enhances SUMOylation and rescues Aβ-induced deficits in hippocampal LTP and learning and memory.	[103, 104]

Table 1: Continued.

	Roles in synaptic scaling	Involvement in AD	References
	Local dendritic translation of AMPAR		
eEF2	Increased eEF2 activity mediates synaptic scaling by stimulating local dendritic synthesis.		[105]
miRNA-92a	Inhibition of miRNA-92A mediates synaptic scaling by stimulating local dendritic synthesis of GluA1.		[106]
Retinoic acid (RA)	Increased RA activity mediates synaptic scaling by stimulating local dendritic synthesis of GluA1 through RA receptor.	RA regulates the expression of APP processing genes, attenuates Aβ deposition, and rescues memory deficits in AD mouse model.	[107–114]
	Secreted factors		
BDNF	Downregulation of BDNF mediates synaptic scaling.	Downregulation of BDNF levels is associated with the degree of synaptic and cognitive deficits during the progression of AD.	[81, 115–117]
TNFα	TNFα mediates synaptic scaling in primary neuronal culture and visual cortex upon activity deprivation.	TNFα contributes to AD-related brain neuroinflammation and amyloidogenesis via β-secretase regulation.	[118–127]
	Cell adhesion molecules		
β3 integrin	Enhanced surface expression of β3 integrin inhibits the small GTPase Rap1 and mediates synaptic scaling by stabilizing synaptic.		[128, 129]
MHC-1	MHC-1 mediates TTX-induced synaptic scaling in hippocampal cultured neurons.		[130]
N-Cadherin	N-Cadherin interaction with β-catenin mediates synaptic scaling and downscaling by regulating GluA1-containing AMPARs.	Inhibition of N-Cadherin interaction with β-catenin accelerates Aβ-induced synaptic impairments.	[131–135]
EphA4	Increased Eph4 activity mediates synaptic downscaling by stimulating ubiquitin-dependent proteasome degradation of GluA1.	Soluble Aβ oligomers upregulate EphA4 whereas genetic ablation or inhibition of EphA4 prevents hippocampal LTP impairment in AD transgenic model mice.	[136, 137]
	Transcriptional regulation		
CaMKK-CaMK4	Reduced activity of the CaMKK/CaMK4 signaling pathway mediates synaptic scaling, whereas its stimulation mediates synaptic downscaling.		[138–140]
MSK1	MSK1 mediates TTX-induced synaptic scaling in hippocampal neurons by increasing surface AMPAR density.	MSK1 activity is elevated in AD.	[141, 142]
MeCP2	MeCP2 mediates synaptic scaling in visual cortex upon visual deprivation *in vivo*.		[143]
	Other proteins		
Plk2	Increase in Plk2 activity mediates synaptic downscaling.		[144, 145]
Cdk5	Increase in Cdk5 activity mediates synaptic downscaling.	Enhanced Cdk5 activity in AD contributes to Tau phosphorylation and toxicity.	[144, 146]

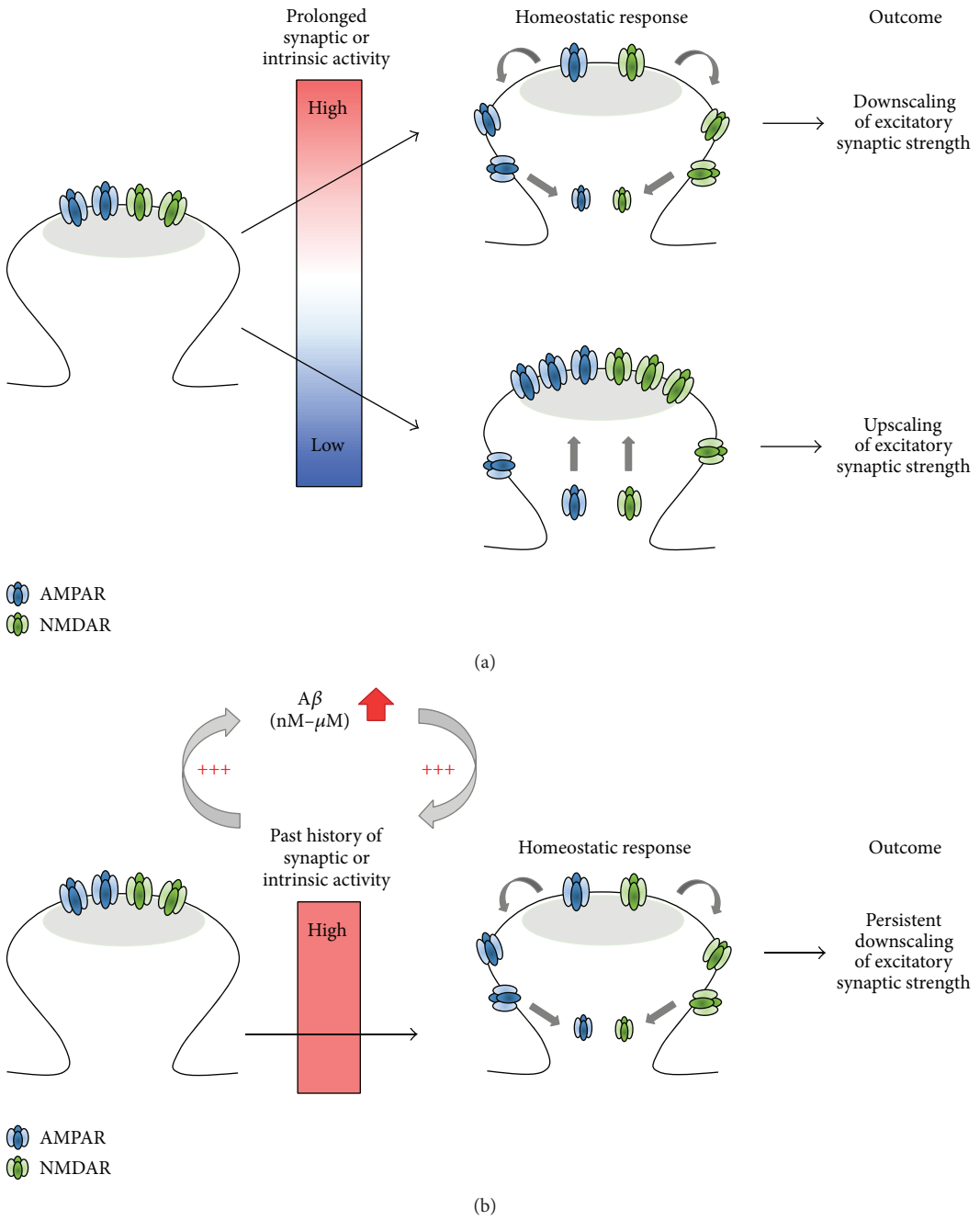

Figure 3: Postsynaptic expression mechanisms in normal and AD synapses. (a) In normal synapses, chronic activity blockade leads to synaptic scaling expressed by a compensatory increase in synaptic AMPAR density and current, whereas chronic activity elevation leads to synaptic downscaling expressed by a compensatory decrease in synaptic AMPAR density and current. (b) In AD, Aβ increases neuronal excitability and/or synaptic activity, leading to induction of synaptic downscaling. Because pathologic feedback loop continues to produce and release Aβ, synaptic downscaling becomes persistent and opposes the postsynaptic expression mechanisms for LTP.

regulate AMPAR trafficking by binding to the same intracellular C-terminus of GluA2 [204, 205]. GRIP1 binding to the unphosphorylated GluA2 C-terminus promotes synaptic targeting of AMPARs [206] whereas PICK1 can bind to both phosphorylated and unphosphorylated GluA2 [206] and mediates activity-dependent endocytosis of GluA2-containing AMPARs and stabilizes them in intracellular pools [207–209]. Recent studies have reported that chronic activity deprivation increases GRIP1 abundance at excitatory synapses and its interaction with GluA2, leading to synaptic targeting of AMPARs in cortical cultured neurons [76, 77]. In contrast, chronic enhancement of neuronal activity removes GRIP1 from excitatory synapses, which decreases surface AMPARs at synapses [76]. Compared to bidirectional modulation of synaptic GRIP1 expression in homeostatic synaptic plasticity, PICK1 expression is only altered by

chronic activity blockade [78]. The TTX-induced synaptic scaling accompanies lysosome-mediated PICK1 degradation and can be occluded by genetic knock-out or shRNA knock-down of PICK1 [78]. Interestingly, pathologic levels of Aβ oligomers fail to reduce surface GluA2 expression and excitatory synaptic transmission in PICK knock-out neurons [79], indicating that GluA2 interaction with PICK1 mediates Aβ-induced synaptic depression. Thus, Aβ-dependent modulation of PICK1 and GRIP1 levels may likely contribute to aberrant developmental metaplasticity in AD.

AMPARs at excitatory synapses are also regulated by scaffolding proteins of the membrane associated guanylate kinase (MAGUK) family, which includes PSD-95, PSD-93, and SAP102 [210]. Chronic activity blockade increases synaptic accumulation of PSD95 and SAP102, whereas chronic activity enhancement decreases synaptic accumulation of PSD95 alone [84–86]. Double knock-down of PSD95/PSD93 or triple knock-down of PSD95/PSD93/SAP102 completely blocks chronic inactivity-induced increase in mEPSC amplitude [84], suggesting that PSD95 and PSD93 mediate synaptic scaling. In contrast, synaptic downscaling requires the PDZ1/2 domains of PSD-95 [84], which interact with transmembrane AMPAR regulatory proteins [TARPs] [211, 212]. Since TARPs link PSD-95 to AMPARs and promote synaptic insertion and stabilization of AMPARs [211, 212], these findings raise the possibility that reduced PSD95-TARP interaction may contribute to synaptic downscaling. Importantly, decreased PSD-95 expression is evident in AD mouse models [87] and Aβ application in cortical neuronal culture leads to downregulation of PSD-95 expression and dispersal of Shank1 [88, 89], another scaffolding protein enriched in excitatory glutamatergic synapses [213]. Interestingly, synaptic accumulation of guanylate kinase-associated protein (GKAP), which links Shank1 to PSD95 [214, 215], is increased upon chronic inhibition of neural activity and decreased by chronic excitation [90]. Such regulation of synaptic GKAP targeting contributes to bidirectional homeostatic scaling of excitatory synaptic strength [90]. Consistent with reports that pathological levels of Aβ increase degradation of PSD-95 and GKAP [89, 91], diminished interactions between PSD-95, TARP, and GKAP could dysregulate homeostatic synaptic plasticity in AD.

4.2. AMPAR Trafficking Regulators. Multiple proteins regulate synaptic AMPAR density by controlling their trafficking. One of them is Arc/Arg3.1, which is an immediate early gene. Arc/Arg3.1 mRNAs accumulate at excitatory synapses, where they are locally translated following synaptic simulation [216, 217]. Arc/Arg3.1 protein facilitates AMPAR internalization from the postsynaptic membrane by interacting with endocytosis mediators, endophilin2/3 and dynamin [218]. Chronic activity blockade of hippocampal or cortical cultured neurons has been shown to decrease mRNA and protein expression of Arc/Arg3.1 [80, 81]. Further, genetic ablation of Arc/Arg3.1 increases basal mEPSC amplitude and surface density of GluA1 and occludes TTX-induced increase in synaptic scaling [80]. Conversely, chronic elevation of neuronal activity increases Arc/Arg3.1 levels and decreases surface density of GluA1, whereas this regulation is absent in Arc/Arg3.1 knock-out neurons [80]. In addition to the critical roles of Arc/Arg3.1 in homeostatic synaptic plasticity, Arc/Arg3.1 expression is elevated in the medial prefrontal cortex of human AD patients [82], suggesting that elevated Arc/Arg3.1 expression may likely lead to AMPAR internalization during AD pathogenesis. In support of this notion, Arc/Arg3.1 is required for metabotropic glutamate receptor- (mGluR-) dependent LTD [219], a form of LTD that is also induced by application of Aβ oligomers [33]. Furthermore, Arc/Arg3.1 has been shown to mediate activity-dependent generation of Aβ by binding to presinilin-1 and regulating γ-secretase trafficking [82]. Based on these reports, persistent elevated Arc/Arg3.1 expression may act in multiple ways to disrupt synaptic homeostasis in AD by enhancing Aβ production and reducing synaptic AMPAR density.

Another immediate early gene, Homer1a, also contributes to homeostatic synaptic plasticity in Arc/Arg3.1-independent pathway [83]. Homer1a interrupts crosslinking action of constitutively expressed forms of Homer [220], thereby activating group I mGluRs in the absence of glutamate [221]. Chronic elevation of activity enhances Homer1a mRNA and protein expression, whereas chronic inactivity reduces Homer1a expression in cortical cultured neurons [83]. Importantly, mGluR inhibition or genetic ablation of Homer1a blocks bidirectional scaling of mEPSC amplitude and surface AMPAR density [83], implicating mGluR signaling and Homer1a in homeostatic synaptic plasticity. Interestingly, elevated tyrosine phosphorylation of GluA2 has been observed in Homer1a knock-out neurons [83] whereas tyrosine phosphorylation of GluA2 is decreased following group 1 mGluR stimulation through striatal enriched protein phosphatase ($STEP_{61}$) [95]. Although the specific Tyr residues on GluA2 regulated by $STEP_{61}$ are unknown, the downregulation of GluA2 tyrosine phosphorylation decreases surface expression of GluA2-containing AMPARs [222, 223]. Our recent study has demonstrated that chronic activity deprivation decreases protein and mRNA expression of $STEP_{61}$ and increases Tyr-phosphorylation of its substrates, including the NMDAR subunit GluN2B and the AMPAR subunit GluA2 in hippocampal cultured neurons [94]. Increasing $STEP_{61}$ activity blocks the increases in mEPSC amplitude and Tyr-phosphorylation of GluN2B and GluA2 induced by chronic activity blockade [94], suggesting that downregulation of $STEP_{61}$ is crucial for mediating homeostatic synaptic scaling. Conversely, chronic activity enhancement increases $STEP_{61}$ expression and decreases Tyr-phosphorylation of GluN2B and GluA2 [94]. Interestingly, elevated $STEP_{61}$ expression is observed in cortices of human AD patients and causes dephosphorylation and internalization of AMPARs in AD mouse models [95–97]. Further, genetic ablation or pharmacologic inhibition of $STEP_{61}$ prevents cognitive deficits and impaired hippocampal LTP in AD mouse models [96–98]. Given that $STEP_{61}$ may also participate in metaplasticity [224], persistent elevation of $STEP_{61}$ and Homer1a may disrupt developmental metaplasticity in AD.

In addition, alterations in Ca^{2+} influx modulates Ca^{2+}-dependent activation of kinases such as Polo-like kinase 2 (Plk2) and Cyclin D kinase 5 (Cdk5) as well as protein

phosphatases including calcineurin and protein phosphatase-1 (PP1) during homeostatic synaptic plasticity [93, 144, 145]. The increases in Plk2 and Cdk5 activity are thought to contribute to synaptic downscaling [144, 145] and AD pathogenesis [146]. Calcineurin-induced dephosphorylation of GluA1 at Ser845 has also been implicated in homeostatic synaptic plasticity [92] and AD [93]. Since PP1 activity downstream of calcineurin stimulation is required for LTD [225, 226], calcineurin may contribute to metaplasticity by regulating phosphorylation status of proteins which alters synaptic AMPAR density and function. In addition, Ca^{2+} influx through L-type voltage-gated Ca^{2+} channels (VGCCs) has been shown to increase PP1 activity via Ser43-phosphorylation of PP1 inhibitor-2 (I-2) following chronic activity elevation in hippocampal cultured neurons [99]. Furthermore, selective inhibition of PP1 blocks downscaling of surface AMPAR expression and mEPSC amplitude induced by chronic activity [99] as well as Aβ-induced impairment in hippocampal LTP [100], providing PP1 as another candidate signaling protein that may contribute to aberrant metaplasticity in AD.

4.3. Posttranslational Modification of AMPAR. Recent studies have revealed posttranslational modifications in addition to phosphorylation as important regulatory mechanisms of AMPAR expression during homeostatic synaptic plasticity. One such modification is palmitoylation, which mediates covalent attachment of palmitic acid [227]. The TTX-induced chronic silencing of network activity causes palmitoylation enzyme DHHC2 to be translocated from the dendrite to the postsynaptic density, resulting in homeostatic accumulation of PSD-95 and AMPARs at excitatory synapses [86]. Given that AMPAR trafficking is dynamically regulated by subunit-selective palmitoylation [228–230], these studies implicate palmitoylation of AMPAR subunits in the mechanism of synaptic scaling. Synaptic scaling also involves SUMOylation [103], which mediates covalent attachment of small ubiquitin-like modifiers (SUMO) [231]. Although there is no direct evidence for SUMOylation of AMPAR subunits [232], the TTX-induced elevation of surface AMPAR expression requires SUMOylation of Arc/Arg3.1 [103], a known regulator of AMPAR endocytosis [218]. Interestingly, reduced SUMOylation is observed in adult AD model mice [104]. While inhibition of SUMOylation blocks hippocampal LTP and hippocampal-dependent learning and memory in wild-type mice, the upregulation of SUMOylation by supplying its conjugating enzyme, Ubc9, rescues Aβ-induced deficits in hippocampal LTP and learning and memory [104]. Hence, reduced SUMOylation of Arc/Arg3.1 may contribute to defective developmental metaplasticity in AD.

Lastly, AMPARs are subjected to activity-dependent ubiquitination by the E3 ubiquitin ligase Nedd4-1, leading to their internalization and degradation in lysosomes [233–235]. Chronic elevation of neuronal activity increases Nedd4-1 protein levels, whereas shRNA-mediated knock-down of Nedd4-1 blocks the homeostatic reduction of surface AMPAR expression and mEPSC amplitudes induced by chronic activity [101], indicating that Nedd4-1 is required for homeostatic downscaling of excitatory synaptic strength. Given that elevated Nedd4-1 expression is found in human AD brain tissues [102], dysregulation of Nedd4-1 levels and subsequent impairment in homeostatic synaptic plasticity may play a role in AD etiology.

4.4. Regulation of Transcription and Translation. Ca^{2+} influx through NMDARs or L-type VGCCs activates signaling pathways that regulate transcriptions of genes important for neural development and plasticity. Consistent with this assertion, downscaling of excitatory synaptic strength induced by prolonged excitation of hippocampal CA1 neurons requires Ca^{2+} influx through L-type VGCCs and transcription activated downstream of CaMKK/CaMK4 signaling pathways [139]. TTX-induced synaptic scaling also requires transcription and translation; however, the mechanism involves a decrease in somatic Ca^{2+} influx through L-type VGCCs and subsequent reduction in CaMKK/CaMK4 signaling pathways [138, 140]. Recently, chronic inactivity was shown to increase transcription of genes encoding AMPARs and proteins that regulate AMPAR trafficking by decreasing cytosine methylation of genes [236]. Consistently, inhibition of DNA methylation alone induces synaptic scaling [236]. Furthermore, loss of methyl-CpG-binding protein-2 (MeCP2) prevents synaptic scaling in the visual cortex upon visual deprivation *in vivo* [143]. Taken together, these studies suggest that bidirectional homeostatic synaptic plasticity involves epigenetic modulation of genes whose protein products regulate excitatory synaptic transmission.

Our laboratory recently identified genes regulated by chronic alterations of neuronal activity in hippocampal neurons using unbiased gene expression analysis [81]. We identified several immediate early genes as well as genes associated with gene ontology terms "synaptic transmission" and "regulation of synaptic plasticity" [81]. One of the immediate early genes encodes brain-derived neurotrophic factor (BDNF) [81]. BDNF, which is secreted in an activity-dependent manner [237], regulates synaptic transmission and plasticity and promotes neuronal survival and transcription [238, 239]. We showed that BDNF mRNA expression is decreased in cultured hippocampal neurons upon chronic activity blockade using TTX treatment [81]. Importantly, inhibition of TrkB receptor signaling alone causes synaptic scaling in a similar extent as prolonged TTX treatment [115] whereas exogenous BDNF application prevents TTX-induced synaptic scaling [115] presumably through activation of mitogen- and stress-activated kinase 1 (MSK1) [141]. Interestingly, downregulation of BDNF is associated with the degree of synaptic and cognitive deficits during AD progression [116, 117, 240] and MSK1 activity is also elevated in AD [142]. These studies raise a possibility that aberrant BDNF-TrkB-MSK1 signaling pathway may disrupt synaptic homeostasis in AD.

In addition to the importance of transcriptional regulation, dendritic protein synthesis may serve as a mechanism to locally maintain the stability of synaptic strength. Chronic silencing of excitatory synaptic inputs stimulates dendritic protein synthesis by increasing the activity of eukaryotic elongation factor-2 (eEF2) [105]. Furthermore, simultaneous treatment of hippocampal neurons with TTX (to block action potentials) and APV (to block NMDAR-mediated miniature

synaptic transmission) increases the expression of GluA1 homomers by stimulating local dendritic translation of GluA1 mRNAs [107, 241–243]. This synaptic scaling is mediated by microRNA-92a, which is a small noncoding RNA that inhibits translation of GluA1 mRNAs by binding to their 3′ untranslated region (UTR) [106]. Other studies have also reported that retinoic acid (RA) signaling via RA receptor-α (RARα) interaction with the 5′ UTR of GluA1 mRNA contributes to synaptic scaling following prolonged cotreatment with TTX and APV by stimulating local dendritic synthesis of GluA1 [107–109]. Importantly, RAR signaling has been shown to regulate the expression of genes related to APP processing [110–113], attenuate Aβ deposition, and rescue memory deficits in AD mouse models [114], suggesting that alteration of RA signaling pathways may contribute to impaired metaplasticity in AD.

4.5. Cell Adhesion Molecules (CAMs). β3 integrin is a cell adhesion molecule (CAM) enriched in excitatory synapses [244, 245] and controls synaptic currents mediated by GluA2-containing AMPARs [128]. Synaptic scaling induced by chronic activity blockade is associated with enhanced surface expression of β3 integrin in hippocampal neurons and is absent in β3 integrin knock-out neurons [128, 129]. Pharmacological perturbation of β3 integrin enhances GluA2 internalization and reduces synaptic AMPAR currents by activating the small GTPase Rap1 [128], which has been implicated in homeostatic downscaling of excitatory synapses [144, 246]. In addition to β3 integrin, class I major histocompatibility complex (MHC-1) proteins, which are found postsynaptically at excitatory synapses, also contribute to synaptic scaling following chronic activity blockade [130]. Although the role of β3 integrin and MHC-1 in AD pathogenesis remains unknown, their neuronal expression is regulated by glia-derived tumor necrosis factor α (TNFα) [128, 247, 248], which is involved in AD pathology in humans and AD mouse models [118–124]. TNFα elevates AMPAR-mediated mEPSC amplitude through activation of TNFα receptor during synaptic scaling [125, 126] whereas TNFα knock-out mice lack synaptic scaling in their visual cortex [127] but display normal LTP [127, 249, 250]. Hence, TNFα may influence metaplasticity through β3 integrin and MHC-1, and such a signaling pathway may be disrupted in AD.

N-Cadherin is another CAM that is enriched at excitatory synapses and has been implicated in AD as well as homeostatic synaptic plasticity. N-Cadherin promotes APP dimerization, modulates Aβ secretion, and reduces surface expression of presinilin-1 [131, 132]. N-Cadherin also binds to the extracellular domains of GluA1 in a Ca^{2+}-dependent manner and regulates GluA1 surface expression [251, 252]. Although N-Cadherin interaction with the actin cytoskeleton [133, 253] contributes to dendritic spine enlargement during LTP expression [253–257], the interaction between N-Cadherin and β-catenin mediates bidirectional homeostatic synaptic plasticity by regulating GluA1-containing AMPARs [133, 134]. Given that inhibition of N-Cadherin interaction with β-catenin accelerates Aβ-induced synaptic impairments [135], dysregulation of N-Cadherin may likely impair homeostatic synaptic plasticity in AD. Ephrin receptor tyrosine kinase subfamily EphA4 is another CAM implicated in homeostatic synaptic plasticity. Increased activity of EphA4 mediates homeostatic downscaling by stimulating ubiquitin-dependent proteasome degradation of GluA1 [136]. Interestingly, soluble Aβ oligomers induce EphA4 activation, whereas genetic ablation or inhibition of EphA4 prevents hippocampal LTP impairment in AD transgenic model mice [137], raising an interesting possibility that Aβ-induced enhancement in EphA4 activity may impair metaplasticity in AD by regulating AMPAR degradation.

5. Conclusions

Recent studies have uncovered an exciting link between pathologic accumulation of Aβ and aberrant metaplasticity, a form of homeostatic synaptic plasticity that controls the induction threshold for LTP and LTD. Specifically, these studies have suggested a novel hypothesis that aberrant metaplasticity may contribute to LTP inhibition and LTD enhancement in AD. However, the molecular mechanism underlying Aβ-dependent alteration of metaplasticity remains largely unknown. Since many molecular players involved in homeostatic synaptic plasticity have been shown to regulate synaptic AMPAR density in Hebbian synaptic plasticity, it is tempting to speculate that pathologic levels of Aβ mediate their effect via a common mechanism shared between Hebbian and homeostatic plasticity at excitatory synapses. Challenges lie ahead in understanding how the molecular players and pathways reviewed here work together to express homeostatic plasticity at excitatory synapses and how Aβ disrupts homeostatic synaptic plasticity in AD. Future studies designed to tackle these challenges should offer substantial insights into the homeostatic control of excitatory synaptic strength in normal brain and AD brain. These studies may also facilitate the search for targeted therapeutic interventions to correct aberrant metaplasticity in AD, thus reversing persistent synaptic weakening and cognitive dysfunction in AD.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Review Article

The Neuroprotective Effect of the Association of Aquaporin-4/Glutamate Transporter-1 against Alzheimer's Disease

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Alzheimer's disease (AD) is a progressive neurodegenerative disorder that is characterized by memory loss and cognitive dysfunction. Aquaporin-4 (AQP4), which is primarily expressed in astrocytes, is the major water channel expressed in the central nervous system (CNS). This protein plays an important role in water and ion homeostasis in the normal brain and in various brain pathological conditions. Emerging evidence suggests that AQP4 deficiency impairs learning and memory and that this may be related to the expression of glutamate transporter-1 (GLT-1). Moreover, the colocalization of AQP4 and GLT-1 has long been studied in brain tissue; however, far less is known about the potential influence that the AQP4/GLT-1 complex may have on AD. Research on the functional interaction of AQP4 and GLT-1 has been demonstrated to be of great significance in the study of AD. Here, we review the interaction of AQP4 and GLT-1 in astrocytes, which might play a pivotal role in the regulation of distinct cellular responses that involve neuroprotection against AD. The association of AQP4 and GLT-1 could greatly supplement previous research regarding neuroprotection against AD.

1. Introduction

Aquaporin-4 (AQP4) is the predominant aquaporin expressed in the brain that provides the major route for water movement across plasma membranes [1]. Most of the studies that have been conducted on the physiological role of AQP4 have primarily focused on its contributions to water homeostasis and to certain pathophysiological processes such as ischemia, cerebral edema, epilepsy, K^+ spatial buffering, and the development or integrity of the blood-brain barrier [2]. Therefore, AQP4 has been considered to be a critical modulator for both water and ion homeostasis in the brain. Recently, it has been reported that AQP4 plays a role in synaptic plasticity and that AQP4 deficiency impaired synaptic plasticity, spatial memory, and associative fear memory [3, 4]. However, little is known about the potential role of AQP4 in synaptic plasticity, especially in memory-associated areas in the brain such as the hippocampal CA1 region [5] and the parietal cortex [6].

Glutamate is the principal excitatory amino acid neurotransmitter in the mammalian brain. Glutamate-activated N-methyl-D-aspartate (NMDA) receptors increase Ca^{2+} influx into the cytosol, which triggers changes in neuronal metabolism and gene expression that are necessary for brain function. However, excessive release of glutamate could induce cell death through excitotoxicity, oxidative stress, or both, which leads to the development of neurofibrillary tangles, and, in turn, AD [7]. Therefore, the maintenance of a physiological level of extracellular glutamate is important for normal synaptic transmission and for the prevention of excitotoxicity. Clearance of glutamate from the extracellular space is accomplished primarily by transporter-mediated uptake of

widely localized astrocytic glutamate transporters (GLTs) [8]. Within many brain regions, the dominant glutamate transporter is glutamate transporter-1 (GLT-1), while the glutamate/aspartate transporter (GLAST) is only highly expressed in the molecular layer of the cerebellum [9]. Previous studies have shown that glutamate uptake is reduced after Aβ infusion, which results in high extracellular glutamate concentrations, and, consequently, excitotoxicity [10, 11]. Conversely, enhancement of GLT-1 may be beneficial for the treatment of certain neurodegenerative disorders such as AD [12].

Glutamate is released into the synaptic cleft during neural activity, and water is actively transported by GLTs along with the reuptake of glutamate. In addition, the passive water permeability of GLTs is increased in the presence of glutamate and during depolarization of the cell [13]. It has been reported that one consequence of elevated glutamate levels is cell swelling, which in this case occurs primarily in astrocytes [14]. Furthermore, reports [15] have also suggested that astrocytic uptake of glutamate is important for the initiation of swelling. Together with the notion that AQP4 is the predominant water channel protein in the mammalian brain and is believed to be important for water homeostasis in the brain, the interaction between AQP4 and GLT-1 is thought to be an intimate spatial relationship [16–18]. It is believed that the finding of the colocalization of AQP4 and GLT-1 may be of great significance in terms of the treatment of various neurodegenerative disorders such as AD, and therefore, more significant research efforts should be directed toward this area.

2. The Localization of AQP4 and GLT-1 in Astrocytes

MacAulay et al. have provided a series of indications [13, 19] that, at the pial and vascular interfaces, the AQP4 pool is concentrated in the subpial and perivascular astrocytic endfeet. On the contrary, the water transport properties of the astroglial cell membrane that faces the neuropil are determined predominantly by GLTs. If this is true, GLTs may also impact the regulation of water balance, ionic balance, and various other agents in the synapse that are closely related to synaptic activity. Unfortunately, these two studies were performed in oocytes and not in intact brain tissue. Therefore, more efforts should be directed toward the clarification of the distinct distribution and physiological roles of AQP4 and GLTs for water homeostasis in the central nervous system. The coexpression of AQP4 and GLTs was discovered in the early 1990s by Nielsen et al. [20], who demonstrated that AQP4 is prominently expressed in astrocytes where GLTs are localized. Since then, a great deal of interest and excitement arose in the exploration of whether the parallel location of AQP4 and GLTs reflects a functional obligatory coupling. However, regrettably, the object of this study was GLAST. After that, additional evidence further revealed the close relationship between AQP4 and GLT-1, and it seems that the AQP4 and GLT-1 in the astrocyte cell membrane might exert effects on the nervous system as a functional complex [16–18]. Recent data further suggested that GLT-1 and AQP4 exist in astrocytic membranes as a macromolecular complex, as neuromyelitis optica-immunoglobulin G autoantibodies directed against AQP4 result in the concomitant loss of GLT-1 [16, 21]. Presently, using brain slices from individuals with ischemic stroke, Mogoanta et al. [22] conducted the first study on AQP4 and GLT-1 integration at the tissue level. They found that an increase in the colocalization of AQP4 and GLT-1 is a reaction to ischemia. They presented the idea that the application of the AQP4/GLT-1 regulator can ameliorate the therapeutic effects of stroke. With these coherent findings, an interesting hypothesis could be proposed in that there might be a correlation between the association of AQP4/GLT-1 and AD.

According to previous studies, AQP4 and GLT-1 might be concentrated in separate membrane domains. AQP4 is highly concentrated in those astrocytic membrane domains that face blood vessels and the pia, while GLT-1, in contrast, functions and is enriched in those astrocytic membrane domains that face the neuropil and synapses [20, 23]. However, this does not actually lead to the conclusion that it is impossible for GLT-1 to be coenriched with AQP4 in the perivascular space or in the subpial endfoot membranes where AQP4 is expressed in abundance. Intriguingly, the most recent study has offered an amendment to the ideas regarding the distribution of GLT-1. Schreiner et al. [24] demonstrated that, in neonatal and juvenile animals, discrete clusters of GLT-1 were also detected within the perivascular endfeet and that these clusters seem to preferentially colocalize with GFAP. Hence, the idea that the two molecules colocalize to form a supramolecular complex could not be denied simply because the prime functions of AQP4 and GLT-1 are assigned to different membrane domains. Moreover, it is known [20] that the perisynaptic region often features low densities of AQP4 and that the macromolecular AQP4/GLT-1 complexes at this site could actually exert the most important effects in anti-AD neuroprotection.

3. Synergistic Protective Effect of AQP4 and GLT-1 against Glutamate-Induced Neuronal Injury by Aβ

The regulatory potential of AQP4 on the function of GLT-1 with respect to synaptic plasticity and memory is clear. The finding of the physical interaction of AQP4 with GLT-1 suggests that this complex may be relevant for GLT-1 signaling mechanisms that are initiated within the neuronal membrane. In astrocytes, GLT-1 exists in a macromolecular complex with AQP4 [25], and in primary cultured astrocytes, both the expression of GLT-1 and glutamate uptake are downregulated by AQP4 deficiency [18]. These data indicate that AQP4 is not only coenriched with GLT-1, but could also regulate GLT-1 function. The study by Zeng et al. [18] provided the first direct evidence that AQP4 plays an important role in the function of GLTs. Through an analysis of cellular morphology, real time reverse transcriptase polymerase chain reaction (RT-PCR), and quantitative densitometry, they investigated the regulatory role of AQP4 in GLTs in primary cultured astrocytes from AQP4 knockout mice. They demonstrated that a lack of AQP4 downregulated astrocytic expression of

GLT-1 but not that of GLAST [18]. Therefore, it is thought that the knockout of AQP4 might disturb the direct physical contact between AQP4 and GLT-1 as well as their signal transduction pathway and the functions of GLT-1. However, this might be just one part of the entire explanation. A direct consequence of AQP4 knockout, or merely a consequence of water and ion imbalances that result from an AQP4 knockout, may also explain GLT-1 downregulation. A previous study revealed that AQP4 deficiency could downregulate GLT-1 expression and glutamate uptake in cortical astrocytes [18]. Furthermore, the findings of Li et al. [4] implied that AQP4 plays a role in synaptic plasticity in the amygdala via the regulation of GLT-1 expression. Similarly, Yang et al. [17] found that upregulation of GLT-1 expression by Ceftriaxone (a β-lactam antibiotic), which is known to upregulate GLT-1 in astrocytes, can rescue AQP4 deficiency-induced impairment of synaptic plasticity [26]. This suggests that AQP4 functions as the modulator of synaptic plasticity and memory and that chronic GLT-1 upregulation rescues hippocampal memory deficits induced by the AQP4 knockout. Further studies are needed to clarify this correlation and to demonstrate why the decreased expression of AQP4 might induce a decrease in GLT-1.

It is well known that astrocytes play a significant role in synaptic plasticity, which is defined as the ability of neurons to change their synaptic strength [27]. Additionally, the Aβ cascade is crucial in the etiology of AD, as deficits in the clearance of Aβ from the brain parenchyma are considered to be potential contributors to the onset of sporadic AD [28]. Low levels of glutamate or endogenous synaptic activity may enhance dendritic spine growth [29, 30]. In contrast, excessive glutamate can precipitate the loss of dendrites and spines [29, 31]. Moreover, normal endogenous levels of Aβ may increase physiological synaptic glutamate release [32]. It has also been demonstrated that Aβ induces glutamate release from astrocytes, which in turn results in neuronal extracellular NMDAR (eNMDAR) activation; this then leads to both molecular and functional changes and thus heralds synaptic damage [33]. Glutamate translocation is a multistep process that moves glutamate against its concentration gradient and that utilizes energy stored in the Na^+/K^+ electrochemical gradient [34]. Conventionally, the direction of transport is inward under physiological conditions, but glutamate is transported in the outward direction in excitotoxic conditions when the extracellular $[Na^+]$/intracellular $[K^+]$ ratio decreases and/or when the intracellular $[Na^+]$/extracellular $[K^+]$ ratio increases [34]. Such GLT-1 reversal was also shown in ischemic glutamate release [35]. Thus, it remains to be clarified whether GLT-1 activators could facilitate glutamate clearance under excitotoxic conditions, or if this would instead intensify reverse transport [34], because GLT-1 reversal might lead to neuronal damage. Furthermore, the Na^+/K^+-ATPase that maintains these ion gradients is compromised in the injured brain [36, 37]. Therefore, the Na^+/K^+-ATPase may have important regulatory potential in glutamate transport under physiological conditions.

Like GLT-1, AQP4 in the brain is involved in synaptic plasticity [38] and may also play important roles in antineurotoxicity. Intriguingly, in addition to the demonstration that AQP4 deletion increases NADH fluorescence in areas furthest away from cerebral microvessels, the study conducted by Thrane et al. [39] provided the first line of evidence that AQP4 impacts the oxygenation of brain tissue. This study suggests that K^+ uptake is suppressed in AQP4 knockout mice as a consequence of decreased oxygen delivery to tissue that is located the furthest from a vascular oxygen source. This finding might expedite research regarding neuroprotection against AD toxicity via the regulation of ion imbalance by AQP4. Thus, the inhibition of oxygen delivery in the setting of AQP4 deficiency might represent another important factor in the regulation of the Na^+/K^+-ATPase and ultimately the neuroprotection by GLT-1 against AD toxicity. Low-density lipoprotein receptor-related protein-1 (LRP1) is expressed in the perivascular endfeet of astrocytes and in brain microvascular endothelial cells; it mediates a continuous efflux of brain Aβ into the circulation [40]. Increased LRP1 expression has been identified in fine astrocyte processes that surround senile plaques (SPs) (AD is characterized pathologically by abnormal accumulation of extracellular aggregates of Aβ in the form of SPs [41], and a major component of these SPs is $A\beta_{1-42}$) [42]. LRP1 has also been shown to be necessary for Aβ-induced astrocyte activation [43], and thus it can be concluded that LRP1 is involved in Aβ clearance, which is mediated by activated astrocytes both in vivo and in vitro [44]. Yang et al. [44] demonstrated that AQP4 deficiency decreases LRP1 upregulation and Aβ uptake, which reduces astrocyte activity. Therefore, AQP4 may be significant in the upregulation of LRP1 and in the clearance of Aβ. AQP4 and GLT-1 are speculated to share an intimate spatial relationship and to be a part of the same supramolecular complex. It has been demonstrated that $A\beta_{1-42}$ in AD induces rapid GLT-1 mislocalization and internalization in astrocytes. This results in a marked reduction in the rate at which astrocytes clear synaptically released glutamate from the extracellular space, which inhibits the functions of GLT-1 [45]. Moreover, the disruption of this macromolecular complex might be partly attributed to GLT-1 mislocalization and internalization in astrocytes (Figure 1). Although further clarification is still needed, various recent studies have shown functional interactions of AQP4 and GLT-1; more efforts should be directed toward the elucidation of the physiological relevance of this complex to help clarify the promising neuroprotective effects of these proteins against AD. Most importantly, the hypothesis may be put forth that the interaction of AQP4 and GLT-1 in astrocytes may play a pivotal role in the regulation of distinct cellular responses that are directed toward neuronal preservation and neuroprotection against AD.

4. The Disruption of the Association of AQP4/GLT-1 in Alzheimer's Disease

Results obtained from studies that have included AQP4 knockout astrocytes have provided direct evidence for the interaction of AQP4 and GLT-1 [18]. Additionally, the interaction between AQP4 and GLT-1 is thought to be an intimate spatial relationship, where these two proteins are part of the same supramolecular complex [16–18]. Despite this evidence,

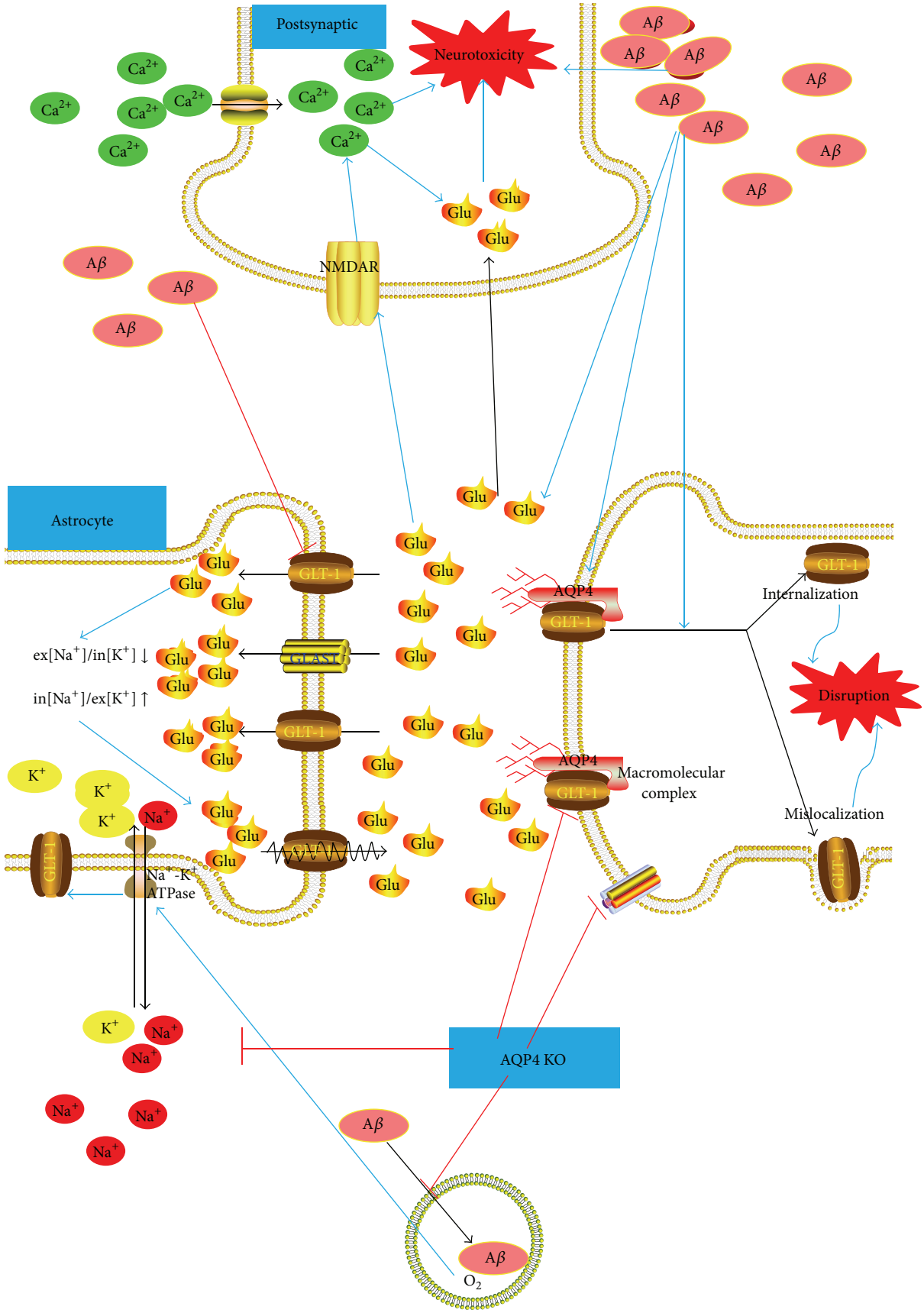

Figure 1: Continued.

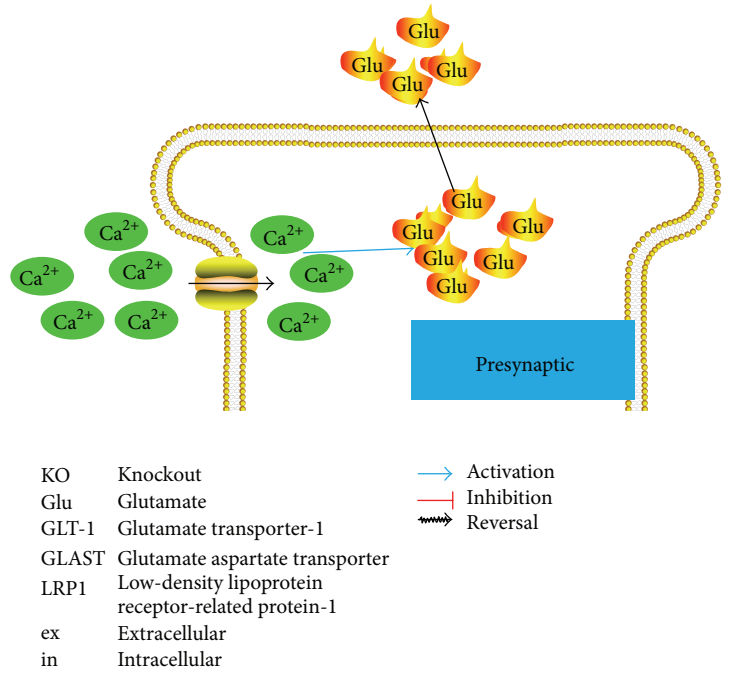

Figure 1: Neuroprotective effect of the association of AQP4/GLT-1 against glutamate-induced neuronal injury by Aβ. (1) Aβ targets. Aβ may increase the release of physiological synaptic glutamate. This in turn results in neuronal NMDAR activation, which leads to synaptic damage and neurotoxicity. Glutamate uptake by astrocytes is also reduced after Aβ infusion, which results in high extracellular glutamate concentrations, and, consequently, excitotoxicity. Glutamate dyshomeostasis could play a role in the pathogenesis of AD, and in this process, GLT-1 has demonstrated to undergo oxidative damage by exposure to Aβ. (2) Glutamatergic system. GLT-1 is the major glutamate transporter that is responsible for various essential neuroprotective functions that include the prevention of glutamate-mediated injury of neurons and synapses; it accomplishes this through the transport of glutamate in the inward direction under normal conditions. In excitotoxic conditions, when the extracellular $[Na^+]$/intracellular $[K^+]$ ratio is decreased and/or when the intracellular $[Na^+]$/extracellular $[K^+]$ ratio is increased, glutamate is transported in the outward direction, such that GLT-1 reversal might further intensify glutamate-induced synaptic damage. (3) Consequences of AQP4 knockout. It is known that AQP4 deficiency may downregulate both GLT-1 expression and glutamate uptake, but it may also downregulate LRP1, which is involved in Aβ clearance. Moreover, AQP4 deficiency could cause the inhibition of oxygen delivery from brain microvessels to the Na^+/K^+-ATPase. This might represent another important factor in the regulation of Na^+/K^+-ATPase function and ultimately the neuroprotection of GLT-1 against AD toxicity. (4) Neuroprotective effects of the association of AQP4 and GLT-1. As a functional complex, AQP4 and GLT-1 in astrocyte cell membranes could exert neuroprotective effects, and the mislocalization and internalization of GLT-1 in astrocyte membranes, as promoted by Aβ, might be responsible for the disruption of this macromolecular complex. This leads to a marked reduction in the rate at which astrocytes clear glutamate from the extracellular space, and, most importantly, to the development of AD.

an in-depth morphometric characterization of the relationship of GLT-1 with AQP4 with respect to the pathology of AD is still lacking.

The study conducted by Yang et al. [46] showed a significant increase in AQP4 expression in AD mice at the age of 9 months compared with wild type according to SDS-Page and western blot. The tg-ArcSwe mouse model of Alzheimer's disease was used in this study, as this model displays perivascular plaques as well as plaques that are confined to the neuropil. However, many studies have found that GLT-1 is significantly reduced or damaged in AD; more recent studies have further confirmed that both the GLT-1 mRNA and protein levels are reduced in AD [47–52] according to RT-PCR and western blot experiments, respectively. In addition because GLT-1 undergoes oxidative damage by exposure to Aβ [53–57], this raises the possibility that glutamate dyshomeostasis plays a role in the pathogenesis of AD. Intriguingly, contradictory results have been obtained in regard to GLT-1 expression in AD mice, which still need to be clarified. Previously, in the late 1990s, Beckstrøm and colleagues in their elegant study of AD patients aged 69 to 94 [58] observed individual differences in the levels of glutamate transporters. Therefore, this provided a rejection of a straightforward correlation between reduced glutamate transporter expression and AD [59]. Because this study was performed in human tissues, their results might be more convincing. Furthermore, a more recent study performed by Kulijewicz-Nawrot et al. also indicated that astrocytic GLT-1 expression in 3xTg-AD mice showed no significant difference at any age when they were compared with control animals [60]; these data were obtained by histograms and representative western blots. The authors found GLT-1 expression in the mPFC to be generally unchanged, which might suggest the preservation of glutamate uptake or possible differences in transporter expression between subjects [60]. Their results were in line with Beckstrøm et al.'s findings, which highlight

the variability in transporter expression. These contradictory results may be due to different methodologies and techniques used. Moreover, it was previously noted that $A\beta_{1-42}$ in AD induces rapid internalization and mislocalization of GLT-1 in astrocytes, which results in a marked reduction in the rate at which astrocytes clear synaptically released glutamate from the extracellular space [45]. Thus, it is thought that a disruption of the association of AQP4/GLT-1 might be partly attributed to GLT-1 mislocalization or internalization. This combined with the loss of GLT-1 in AD brains as well as in animal models [47, 51, 52] may be observed in neuronal membranes in AD. This may be of great functional significance to the neuroprotective effect of AQP4 and GLT-1, as this contributes to neuronal impairment. Furthermore, glutamate transporter variants may also be responsible for the reduced glutamate uptake and thus the neurotoxicity in AD [49]. All of these factors might cause the disruption of the AQP4/GLT-1 association in AD.

5. Conclusion and Future Studies

There is a general agreement that AQP4 may exert neuroprotective effects against $A\beta$ toxicity through its binding to GLT-1 at the plasma membrane. In addition, this water channel has been shown to contribute to GLT-1 function. Because it is plausible that the involvement of AQP4 in the modulation of $A\beta$ toxicity might be related to channel activation, then porin interactions with glutamate-activated GLT-1 may be crucial in the maintenance of AQP4 activation. Increasing evidence suggests that AQP4 and GLT-1 in the plasma membrane might be designated as dynamic signaling platforms; the interaction of these two proteins has a potential role in the regulation of distinct cellular responses that are directed toward neuronal preservation and neuroprotection against AD. It is enticing to speculate that a disruption of the AQP4/GLT-1 association, which has been observed in neuronal membranes in AD brains, may contribute to neuronal impairment. Furthermore, various studies have detailed the interaction of AQP4 with other signaling proteins that are involved in neuronal maintenance. Along these lines, the association of AQP4/GLT-1 with other proteins such as Kir4.1 [61, 62] may represent a relevant macromolecular complex in the plasma membrane. Thus, further explorations of these potential porin modifications at the neuronal membrane as a consequence of protein rearrangements in the plasma membrane might be a worthy pursuit [63]. This would provide a better understanding of the consequences of impaired protein associations that are related to AD.

Conflict of Interests

The authors declare no competing financial interests.

Acknowledgments

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