Review

Spine microdomains for postsynaptic signaling and plasticity

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Changes in the molecular composition and signaling properties of excitatory glutamatergic synapses onto dendritic spines mediate learning-related plasticity in the mammalian brain. This molecular adaptation serves as the most celebrated cell biological model for learning and memory. Within their micron-sized dimensions, dendritic spines restrict the diffusion of signaling molecules and spatially confine the activation of signal transduction pathways. Much of this local regulation occurs by spatial compartmentalization of glutamate receptors. Here, we review recently identified cell biological mechanisms regulating glutamate receptor mobility within individual dendritic spines. We discuss the emerging functions of glutamate receptors residing within sub-spine microdomains and propose a model for distinct signaling platforms with specialized functions in synaptic plasticity.

Introduction

Compartmentalized signal transduction enables cells to temporally and spatially restrict their responses to local extracellular cues. A striking example of spatially confined signaling occurs along the dendrites of principal neurons of the mammalian nervous system, where thousands of tiny dendritic spines contact presynaptic axon terminals (Figure 1a,b). First described by Ramón y Cajal a century ago, dendritic spines are semi-autonomous signaling units that receive presynaptic input in the form of released neurotransmitter, which in turn activates receptors embedded in the postsynaptic density (PSD), an electron-dense protein matrix also containing adhesion molecules, scaffold proteins and signaling enzymes [1,2] (Figure 1c). The primary neurotransmitter receptors at excitatory synapses in the mammalian brain are glutamate receptors. The various glutamate receptor subtypes have distinct biophysical properties, activate different signal transduction pathways, and exhibit specific spatial distributions. The regulated addition and removal of glutamate receptors at the PSD serves as an important mechanism to alter synaptic strength and is the prevailing molecular model for information storage and learning-related plasticity [3,4]. Local trafficking of glutamate receptors within individual dendritic spines enables neurons to respond to synaptic activity on a spine-by-spine basis, with even adjacent synapses able to respond to released neurotransmitter with greatly different efficacies [5]. Dendritic spines contain organelles, membrane compartments and distinct plasma membrane domains involved in receptor trafficking and signaling, which for the purposes of this review will be referred to as 'spine microdomains'. These spine microdomains include PSDs, endocytic zones (EZs), intracellular trafficking organelles, and the synaptic, extrasynaptic and perisynaptic regions of the spine membrane, all of which enable spines to behave in a semi-autonomous manner [5–7] (Box 1).

In this review, we highlight recent literature providing novel insight into the mechanisms of glutamate receptor mobility within individual spines, focusing on the influence of PSD nanostructure and trafficking between sub-spine microdomains. We also discuss specialized signaling properties of glutamate receptors residing in synaptic, perisynaptic and extrasynaptic spine microdomains, noting the unique contribution of each to multiple forms of synaptic plasticity.

Postsynaptic scaffolds and lateral mobility of glutamate receptors

Organization of the PSD and nanoscale compartments for glutamate receptors

The PSD is the most prominent spine microdomain in which glutamate receptors concentrate to mediate excitatory synaptic transmission (Box 1). The specific number and density of glutamate receptors within the PSD exert major control over the strength of synaptic transmission by amplifying or dampening the postsynaptic response to presynaptically released glutamate [8,9]. Structurally, the PSD is a network of proteinaceous filaments and large interconnected protein assemblies [2,10], a property that probably enables glutamate receptors to be retained within the PSD. For example, the retention of glutamate receptors within the PSD could occur by receptor corralling inside nanometer-sized compartments. In addition, glutamate receptors could accumulate within the PSD through molecular crowding and collisions with PSD membrane proteins, as well as reversible interactions with highly abundant scaffold molecules that might function as receptor-binding 'slots' [5,11,12]. Both biochemical and imaging studies demonstrate that PSD scaffold proteins (e.g. PSD-95, GKAP, SAP97, Shank and Homer; 60–400 molecules of each per synapse) outnumber glutamate receptors (1–200 glutamate receptors per synapse) [1,13–18], thus, potentially providing a structural basis for changes in synaptic strength through a reserve of receptor-binding or receptor-confining domains.

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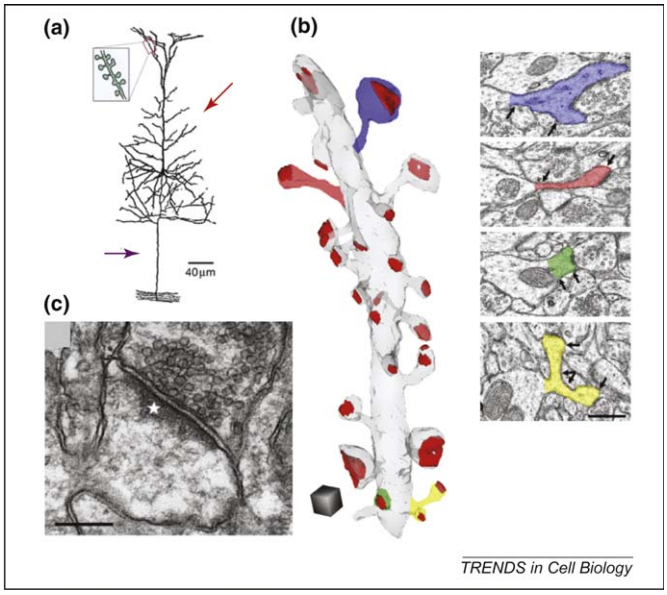

Figure 1. Subcellular anatomy of neuronal dendrites and spines. **(a)** Trace of a pyramidal cortical neuron labeled by Golgi staining adapted from Ramon y Cajal, 1911. The dendritic arbor (red arrow) is studded with small dendritic spines, which receive excitatory presynaptic inputs (inset). The axon (purple arrow) extends from the cell body and carries action potentials to contacting postsynaptic neurons. Scale bar, 40 μm. Adapted from Ref. [123]; reprinted with permission from Nature Publishing Group, copyright 2004. **(b)** A 3D rendering of a hippocampal dendrite (gray) reconstructed from serial section electron micrographs, demonstrating variable spine morphology along a dendrite. From top to bottom, the insets show an example of a mushroom spine (purple), thin spine (red), stubby spine (green), and branched spine (yellow). Spine geometry, and particularly the caliber of the spine neck, can have important effects on synaptic strength and signaling by restricting the diffusion of glutamate receptors and signaling enzymes between spines and dendrites [30]. Scale bar, 1 μm^3 cube for the reconstructed dendrite and 0.5 μm for the single EM sections. Adapted from Ref. [124]; reprinted with permission from Annual Reviews, copyright 2008. **(c)** Electron micrograph revealing glutamatergic synapse organization. Shown is an excitatory synapse onto a dendritic spine of a CA1 pyramidal neuron in stratum radiatum hippocampus. Within the dendritic spine, the PSD (white star) is positioned directly opposite to the presynaptic terminal containing abundant synaptic vesicles. Scale bar, 200 nm. Adapted from Ref. [125]; reprinted with permission from the Federation of European Neuroscience Societies and Blackwell Publishing Ltd, copyright 2006.

The nature of receptor-binding 'slots' or receptor-confining domains remains unknown, although evidence indicates compartmentalization within the PSD. For example, N-methyl-D-aspartate (NMDA)-type receptors (NMDARs) are often centrally located within the PSD, whereas α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA)-type receptors (AMPARs) seem homogenous or are found at the edge of the PSD [19] (Box 1). Therefore, different glutamate receptor subtypes can be separated into distinct PSD compartments. Indeed, GluR1-containing AMPARs are confined within intrasynaptic domains of <100 nm [20], and freeze fracture EM reveals clusters of AMPARs inside the PSD [14]. Electron micrographs of biochemically isolated PSDs also demonstrate a compartmentalized structure studded with large protein complexes containing Ca^{2+}/calmodulin-dependent protein kinase type II (CaMKII) and PSD-95 on the cytoplasmic surface, which could serve as receptor binding platforms [10,21,22].

In addition to the absolute number of glutamate receptors inside synapses, packing density and spatial positioning within the PSD can be important determinants of synaptic strength [8,9]. Given their low affinity for glutamate, AMPARs might only be activated if they are aligned with presynaptic release sites [23]. For example, modeling data demonstrate that the area of AMPAR activation upon presynaptic glutamate release has similar dimensions to the millimolar glutamate concentrations surrounding a vesicle release site [8]; therefore, AMPARs not directly aligned with release sites have a low probability of opening. In addition to AMPARs, the spatial positioning of NMDARs in the PSD can also be important for their activation. Based on modeling predictions, the activation of NR2A-containing NMDARs is insensitive to their spatial position relative to the glutamate release site, whereas NR2B-containing NMDARs demonstrate a hot spot of activation centered on the glutamate release site [9]. Therefore, in a manner similar to AMPARs, the activation of NR2B-containing NMDARs could depend on the location of the receptor relative to presynaptic release sites [9].

A recent study provides potential clues as to how the spatial positioning and density of glutamate receptors can be established inside the PSD [24]. Using high-resolution optical tagging of PSD subregions, it was found that the PSD-95 scaffold of the PSD behaves as a topologically stable matrix with very little internal molecular movement, although the overall structure of the matrix is plastic and flexible [24]. By stretching or compressing the PSD-95 matrix, actin-based elasticity of the PSD might enable glutamate receptors to become locally concentrated without the need for adding or removing receptors. In addition, local elasticity could enable glutamate receptor clusters to become aligned with release sites of the presynaptic active zone, an effect that could have a large influence on postsynaptic responses to released glutamate. Indeed, reducing AMPAR surface mobility by antibody cross-linking alters postsynaptic responses to trains of action potentials, indicating that small-scale positioning and lateral movement of AMPARs tune synaptic strength [25]. The precise nature and regulation of these small displacements of AMPARs is not clear, but the flexible nature of the PSD and the fluctuating density of the PSD-95 scaffold within the PSD [24] might allow for nanoscale glutamate receptor clustering and the formation of signaling hotspots [2,8,9,14,20].

Activity-dependent mobility of glutamate receptors at synapses

Glutamate receptors exchange laterally throughout the spine membrane [11,26] and, for AMPARs, the rate of exchange at synapses is highly sensitive to local activity. For example, Ca^{2+} uncaging reduces the lateral mobility of extrasynaptic GluR2, whereas sequestering Ca^{2+} increases GluR2 mobility [27]. Furthermore, physiological synaptic stimuli reduce AMPAR diffusion [25]. Therefore, Ca^{2+}-dependent cytoskeletal remodeling or direct receptor interactions could influence receptor diffusion [28]. Changes in AMPAR mobility also occur during the expression of synaptic plasticity. Indeed, pharmacological stimuli that mimic long-term potentiation (LTP; Box 2) decrease the rate of GluR1 exchange at synapses [29], which can result from changes in spine morphology [30] or PSD–receptor interactions [12]. Conversely, stimuli that induce synaptic

Box 1. Glutamate receptors at excitatory synapses

Information processing in the CNS is mediated by rapid responses of ionotropic glutamate receptors that include AMPA-type receptors, kainate (KA) receptors, and NMDA-type receptors [102,126]. Within dendritic spines, glutamate receptors concentrate at the PSD, the most prominent spine microdomain (Figure I). AMPARs mediate most rapid excitatory transmission in the brain and consist of homo- or hetero-tetramers assembled from GluR1–4 subunits, of which differential subunit composition dictates ion permeability and synaptic targeting [126]. Kainate receptors, which mediate a smaller portion of excitatory transmission, consist of tetrameric assemblies of GluR5–7 and KA1–2 subunits [127]. NMDARs have crucial signaling roles for synapse plasticity and are heterotetramers composed of two NR1 subunits and any two of four different NR2 subunits (NR2A–D). During early brain development, a subpopulation of NMDARs contains NR3A or NR3B, subunits that substitute for NR2. NR2 and NR3 subunits influence the channel properties and distribution of NMDARs between synaptic and nonsynaptic regions [102]. In addition to ionotropic receptors, eight different G-protein coupled receptors (mGluR1–8) divided into three groups (group I, II and III) comprise the metabotropic glutamate receptors (mGluRs), each signaling to specific downstream effectors [119]. The differential expression and assembly of glutamate receptor subtypes throughout the brain and at individual synapses enable diverse forms of postsynaptic signaling and synaptic transmission, facilitating the expression of multiple forms of synaptic plasticity [3,4]. Furthermore, the specific number and density of glutamate receptors exert major control over the strength of synaptic transmission by amplifying or dampening the postsynaptic response to presynaptically released glutamate.

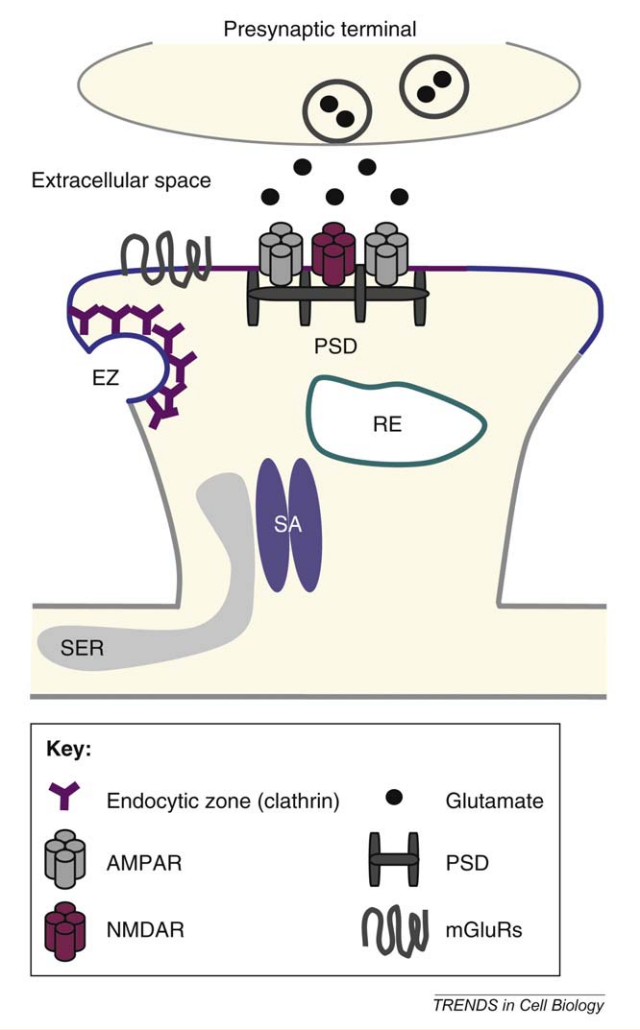

Figure I. Spine microdomains. Schematic showing dendritic spine organization and subspine microdomains. Shown are the extrasynaptic (light gray line), perisynaptic (blue line) and synaptic (purple line) plasma membranes within spines. The EZ is shown in purple and a spine-localized recycling endosome (RE) is shown in green. Both the smooth endoplasmic reticulum (SER) and the spine apparatus (SA) extend into the spine. The PSD, comprising scaffolding proteins including PSD-95, shank and homer, is directly opposite the presynaptic terminal (dark gray), where release of glutamate occurs (small black circles). Within the PSD reside NMDARs (magenta) and AMPARs (gray) that occupy distinct nanodomains. Group I metabotropic glutamate receptors (mGluRs) reside at the perisynaptic domains lateral to the PSD.

depression (e.g. bath application of glutamate) increase the mobility of synaptic GluR2 [31] (Figure 2).

The long-term activity state of single synapses also influences receptor diffusion. Active synapses trap GluR1-containing AMPARs more efficiently than do neighboring inactive synapses at which presynaptic glutamate release has been blocked, and GluR1-containing AMPARs have faster mobility and explore a greater area within inactive synapses [20], indicating that inactive synapses have fewer obstacles or binding sites confining AMPAR diffusion. Similarly, large PSDs capture more diffusing PSD-95 than smaller PSDs, and large PSDs retain PSD-95 longer than small PSDs [32], indicating that increases in PSD size could allow for increased trapping of AMPARs at active synapses. Moreover, acute increases in excitatory synaptic activity increase ongoing structural plasticity and PSD-95 scaffold dynamics within the PSD [24]. Therefore, presynaptic release and postsynaptic receptor activation influence glutamate receptor stabilization and PSD scaffold dynamics, providing a potential mechanistic basis for activity-dependent targeting and retention of AMPARs at individual synapses (Figure 2).

Unlike AMPARs, synaptic NMDAR numbers are less variable and demonstrate less of a correlation with spine size [33,34]. Therefore, although NMDARs exchange at synapses [26,35], their mobility is insensitive to activation or inhibition of network activity at mature synapses [36]. However, in neonatal synapses, plasticity-inducing stimuli can drive switching of NR2A- and NR2B-containing NMDARs [37]. In more mature neurons, NR2A lateral diffusion is slower than that of NR2B in both synaptic and extrasynaptic membranes, probably facilitating the preferential accumulation of synaptic NR2A as neurons mature [38].

Mechanisms controlling glutamate receptor mobility at synapses

Glutamate receptors bind to scaffold proteins through PDZ domains of the PSD-95 family members (PSD-93/chapsyn-110, PSD-95, SAP-97/hDlg and SAP-102) [4]. Although

Box 2. LTP and LTD

The hippocampus is an area of the brain important for learning and memory. Three major anatomical domains within the hippocampus are the dentate gyrus (DG), CA3 and CA1 [128] (Figure Ia). The excitatory synapses made between neurons in these regions can undergo experience-dependent plasticity, a basic cellular substrate for learning and memory. Axons from CA3 pyramidal neurons, termed Schaffer collaterals (SCs), make robust excitatory synaptic contact with the dendrites of CA1 pyramidal neurons. The SC–CA1 synapse in hippocampus is the best-studied glutamatergic synapse in the mammalian brain. Long-lasting increases in synaptic strength or LTP can be triggered with brief, high frequency stimulation of Schaffer collaterals projecting to CA1 pyramid cells in hippocampus [128] (Figure Ib). LTP of excitatory postsynaptic potentials can persist for hours or days and is associated with an increased abundance of AMPARs at synapses. Conversely, long-lasting decreases in synaptic strength or LTD can occur when CA1 synapses are stimulated at a slow rate (1 Hz) for long periods (10–15 min) [129] (Figure Ib). The decrease in synaptic strength is associated with a decrease in synaptic AMPARs. The abundance of glutamate receptors inside synapses is a major component of synaptic strength. The duration and frequency of synaptic stimulation leads to the differential activation of signaling pathways which control the trafficking of glutamate receptors to and from synapses. LTP-inducing stimuli drive exocytosis and incorporation of AMPARs at the postsynaptic membrane (Figure Ic), whereas LTD-inducing stimuli can lead to endocytosis and loss of synaptic AMPARs (Figure Id) (for reviews, see Refs [5,7]).

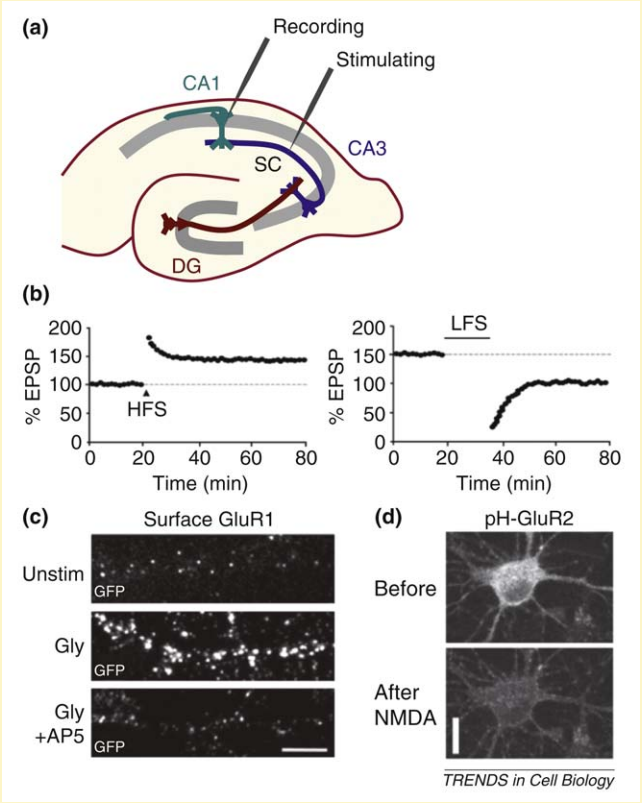

Figure I. **(a)** Schematic of the hippocampus showing the dentate gyrus (DG), CA3 and CA1 regions. Gray bars indicate cell body layers. Shown is a typical plasticity-induction protocol performed in hippocampal slices. SCs projecting from the CA3 neurons are stimulated while recordings are made from CA1 pyramidal neurons to monitor changes in synaptic strength. **(b)** Graph demonstrating the induction of LTP (left) and LTD (right) at SC–CA1 synapses in a hippocampal slice. LTP is observed as a long-lasting increase in the slope of the excitatory postsynaptic potentials (EPSP) after brief high frequency stimulation (HFS), whereas LTD is observed as a decrease in the EPSP after low frequency stimulation (LFS). **(c)** Example of AMPAR insertion upon chemically induced LTP (200 μM glycine, 0 mM Mg^{2+}) [66]. The insertion of GluR1-containing AMPARs after glycine treatment requires the activation of NMDARs (bottom) as shown by the block of insertion by the NMDAR antagonist AP5. Scale bar, 5 μm. Adapted with permission from Ref. [66] and reprinted with permission from AAAS. **(d)** An example showing loss of surface AMPARs upon chemically induced LTD (20 μM NMDA) [51]. Five minutes after chemical LTD induction, surface AMPAR levels are decreased, as measured by pH-GluR2, a pH-sensitive GFP variant that only fluoresces at the neutral cell surface. Scale bar, 10 μm. Adapted from Ref. [51] and reprinted with permission from the Society for Neuroscience.

NMDARs bind directly to PSD-95 family members, AMPARs indirectly associate with PSD-95 through auxiliary transmembrane AMPAR regulatory proteins (TARPs) [39]. Given that both NMDARs and AMPARs associate with PSD-95 family members, what are the factors responsible for differences in glutamate receptor subtype targeting to synapses? In the case of NMDARs, binding preferences towards distinct PSD-95 family members might contribute to differences in the targeting of NR2B- and NR2A-containing NMDARs to synapses [26]. For AMPARs, post-translational modification of scaffolds and receptors influence synaptic targeting. For example, phosphorylation of PSD-95 at Ser295 is associated with increased targeting of AMPARs, whereas dephosphorylation is important for synaptic depression [40]. Furthermore, phosphorylation of the auxiliary AMPAR subunit and TARP family member stargazin (also known as γ2) allows for bidirectional plasticity by controlling AMPAR exchange at synapses [41]. AMPARs themselves are targets of phosphoregulation. For example, PKA-dependent phosphorylation of GluR1 at Ser845 is increased upon LTP induction [42] (Figure 2), occurs as AMPARs recycle [43], is required for synaptic potentiation [44], and produces a larger pool of extrasynaptic AMPARs [45] that could be available to enter into synapses [23,46]. NMDAR stabilization at synapses is also regulated by phosphorylation because phosphorylation of NR2B prevents its interactions with the endocytic adaptor AP-2 and increases synaptic targeting [47]. Although multiple signaling enzymes control the phosphorylation state of glutamate receptors, it is not well understood how these various phosphorylation mechanisms are engaged and interact under varying conditions of synapse activation.

In addition to reversible interactions with PSD-95 family members, AMPAR exchange at synapses is controlled by other PDZ proteins [3]. Multi-PDZ domain GRIP proteins (also known as ABP) can anchor AMPARs inside the PSD [48], whereas PICK1 has been found to drive AMPAR recycling [49–52] and facilitate the exchange of extrasynaptic AMPARs into synapses [53]. Because GluR2 and GluR3, but not GluR1, bind PICK1 and GRIP [54,55], receptor interactions with PDZ scaffolds could help account for the targeting of distinct receptor subtypes. However, neurons from mice lacking both GluR2 and GluR3 display normal AMPAR endocytosis and recycling to the plasma membrane [56], indicating the presence of additional targeting mechanisms. Beyond PDZ interactions, extracellular AMPAR binding to both N-cadherin [57–59] and

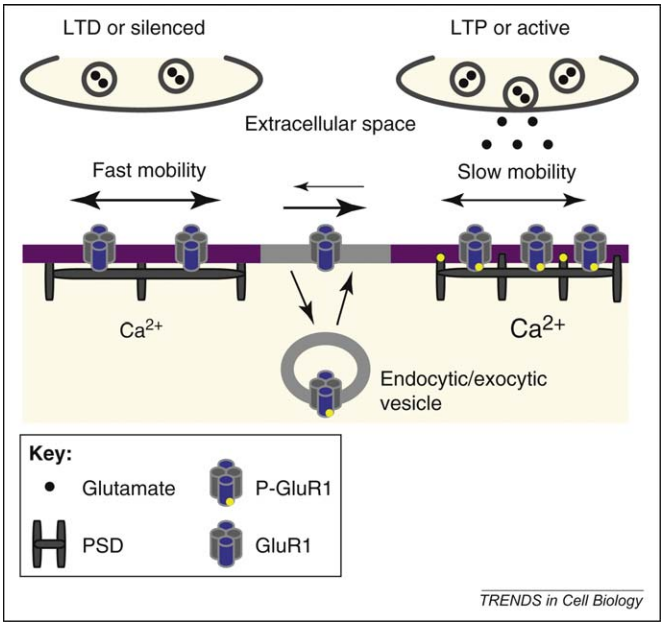

Figure 2. Activity-dependent mobility of AMPARs at synapses. Model for AMPAR mobility between synaptic (purple) and extrasynaptic (gray) membranes. AMPARs (blue and gray cylinders) show greater mobility and less confinement in the PSD (dark gray vertical and horizontal bars) within silenced or depressed synapses (left), and have slower mobility and greater confinement in the PSD within active or potentiated synapses (right), thus shifting the equilibrium of receptor exchange towards active synapses. The phosphorylation state (small yellow dots) of PSD scaffold proteins and AMPARs might regulate the synaptic stability of AMPARs. Intracellular trafficking of AMPARs and exocytosis increase the local mobile pool of AMPARs available to enter into synapses. Presynaptic terminals are shown in dark gray and released glutamate is indicated by the small black dots.

neuronal pentraxins [60] regulates receptor clustering and exchange at synapses. NMDAR mobility and retention in the PSD is also controlled by extracellular matrix interactions. The increased lateral mobility of NR2B-containing NMDARs during development coincides with the expression of reelin, an extracellular matrix protein important for synaptic maturation and plasticity [61].

Many questions remain as to how and where glutamate receptor movement is regulated. For example, where within the PSD or the spine do relevant post-translational modifications or protein interactions that control glutamate receptor exchange at synapses occur? If such regulation occurs in multiple spine microdomains, how do the diverse modifications and interactions cooperate in a synergistic manner for synaptic targeting?

Lateral spine membrane domains for glutamate receptor trafficking and signaling

As with all integral membrane proteins, glutamate receptors diffuse laterally throughout spine microdomains and across dendritic segments [11]. To counteract the loss of receptors from synapses resulting from lateral diffusion, glutamate receptors undergo directed intracellular trafficking. Both experimental and modeling data demonstrate that intracellular trafficking is crucial for maintaining the abundance of glutamate receptors at synapses, and for activity-dependent changes in AMPAR abundance during the expression of synaptic plasticity [7,62,63]. One major pathway for controlling the abundance of synaptic AMPARs is local endocytic recycling [43,64–68]. Within dendritic spines, AMPAR internalization is thought to occur at the EZ, a stable clathrin-coated membrane domain located adjacent to the PSD [69,70] (Box 1). Long-term stability and coupling between the EZ and PSD results from endocytic protein interactions with the PSD, in particular the direct interaction between dynamin-3 and the multimeric PSD adaptor homer [64], and could conceivably occur through cargo interactions to the underlying spine cytoskeleton [71]. Although the EZ would be predicted to remove glutamate receptors from the spine membrane, as occurs during expression of long-term depression (LTD) (Box 2), forced loss of EZs by dynamin-3 disruption instead leads to loss of synaptic AMPARs [64]. Although at first glance paradoxical, these results indicate that the major function of EZ is to capture and recycle a local pool of mobile AMPARs in the extrasynaptic spine membrane, thus counteracting the continual escape of AMPARs from spines by lateral diffusion [64].

Further supporting the notion of AMPAR recycling in spines is the presence of spine-localized recycling endosomes (REs). Activity-dependent translocation of REs into spines is required to supply a mobilizable pool of AMPARs to synapses during long-term synaptic plasticity [65,66,68]. Although trafficking of AMPARs from REs is crucial for expression of LTP [66], it is less clear how plasticity-inducing stimuli activate or augment the trafficking machinery to allow for AMPAR exocytosis. Recent findings indicate that the actin-binding motor protein myosin Vb (MyoVb) mediates basal trafficking of AMPARs to the dendritic membrane [72] and further acts as a spine-localized Ca^{2+} sensor that responds to Ca^{2+} elevation by associating with Rab11–FIP2 adaptors on REs [68]. This Ca^{2+}-dependent association leads to the transport or tethering of REs in spines, thereby enabling local AMPAR exocytosis and expression of LTP [68]. In addition, the related class V myosin, MyoVa, might also contribute to AMPAR targeting and the expression of LTP [73], although MyoVa mutant mice display normal postsynaptic receptor composition and plasticity at glutamatergic synapses [74,75], indicating that MyoVb is the primary motor for transporting AMPARs. Appealingly, Ca^{2+}-dependent activation of MyoVb provides a direct means to couple postsynaptic activity with spine-localized endosomal trafficking.

It remains unknown whether or not a stable exocytic zone is coupled to the PSD for direct receptor insertion. Although spine-localized exocyst components could direct insertion of AMPARs in close proximity to the PSD [76], and exocytosis of RE cargo has been observed in spines [65], insertion of heterologously expressed superecliptic pHluorin-tagged GluR1 (SEP–GluR1) was not detected in spines [77], perhaps owing to the limited abundance or differential sorting of SEP–GluR1 into spine-mobilized REs. However, an exocytosis-dependent increase in the perisynaptic population of AMPARs precedes LTP expression [46], indicating that spine-localized exocytosis could increase the abundance of perisynaptic AMPARs available to enter into synapses.

In addition to intracellular trafficking of AMPARs within spines, the combined presence of the EZ and PSD in spines probably increases the trapping and local concentration of surface AMPARs in the spine membrane itself, thereby increasing the available pool of extrasynap-

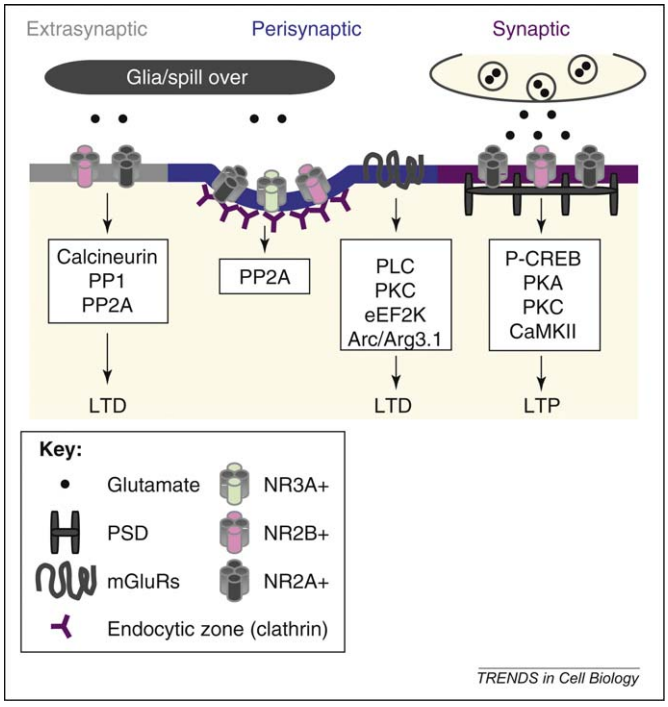

Figure 3. Signaling platforms in the spine membrane. Model showing putative signaling pathways and forms of synaptic plasticity activated by glutamate receptors in synaptic, perisynaptic and extrasynaptic spine microdomains. Extrasynaptic NMDARs, possibly NR2B- or NR2A-containing, are activated by glutamate from nearby glia cells, synaptic spillover, or extracellular glutamate accumulation. Protein phosphatases (calcineurin, PP1, PP2A) are activated, leading to LTD. Activation of perisynaptic glutamate receptors, probably group I metabotropic mGluRs, signals to phospholipase C (PLC) and PKC, leading to mGluR-dependent LTD. mGluR-LTD also requires protein synthesis, including the synthesis of Arc/Arg3.1, resulting from phosphorylation of eEF2K. NR3A-containing NMDARs bind PP2A, which might localize this enzyme near specific receptor populations. The EZ is indicated by purple clathrin trimers. Activation of synaptic NMDARs, either NR2A- or NR2B-containing, is coupled to phosphorylation of CREB and activation of PKA, PKC and Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), leading to LTP.

tic AMPARs that can enter into synapses through lateral diffusion. Furthermore, a large and stable clathrin-coated structure near the PSD could function as an additional glutamate receptor signaling platform, analogous to the differential signaling of membrane receptors that enter clathrin-coated pits and lipid rafts [78]. Potential candidates of EZ signaling could be perisynaptic mGluRs and NR3A-containing NMDARs [79,80], both of which interact with the endocytic machinery and influence postsynaptic responses to synaptic activity [5].

Signaling functions of glutamate receptors in sub-spine microdomains

Separable signaling functions of synaptic and extrasynaptic glutamate receptors

Distinct classes of glutamate receptors activate different signal transduction pathways. Moreover, the frequency and duration of glutamate receptor activation determines the type of signaling pathways that are activated and the form of plasticity that is expressed. For example, high-frequency stimulation triggers NMDAR-dependent LTP through activation of several kinases including CaMKII, protein kinase A (PKA), and protein kinase C (PKC), which phosphorylate AMPARs and TARPs, directly influencing their trafficking and channel properties [3,41,42,81] (Figure 3). By contrast, low-frequency stimulation triggers NMDAR-dependent LTD by activation of Ca^{2+}-dependent phosphatases such as calcineurin, which dephosphorylate substrates (e.g. GluR1) and triggers AMPAR downregulation [3,42] (Figures 2,3).

For NMDARs, the precise spatial location of the receptor during glutamate stimulation can determine the mode of signaling and plasticity [26,82] (Figure 3). For example, whereas activation of extrasynaptic NMDARs leads to dephosphorylation of CREB and LTD and to cell death, activation of synaptic NMDARs promotes phosphorylation of CREB and LTP and cell survival [82–86]. This spatial segregation means that signaling to downstream effectors of NMDAR-mediated excitotoxicity can be inhibited without disrupting NMDAR prosurvival and plasticity functions [87].

Signaling through extrasynaptic glutamate receptors could occur through either NR2A- or NR2B-containing NMDARs. Although the activation of extrasynaptic NR2B-containing NMDARs is implicated in LTD expression and cell death [83,85], recent modeling data indicate that extrasynaptic NR2B-containing NMDARs would have a low probability of opening outside of the presynaptic active zone compared with NR2A-containing NMDARS [9]. Therefore, it is likely that both NR2A- and NR2B-containing NMDARs are involved in signaling at extrasynaptic domains, and the activation of each subtype would be determined by the duration and frequency of stimulation. Unlike NMDARs, extrasynaptic AMPAR activation is probably absent or less common at most central synapses owing to their much lower glutamate affinity [23] and the low concentrations of extracellular glutamate [88].

Given the importance of NMDAR distribution to downstream signaling and plasticity, what are the determinants of NMDAR localization? As described earlier, NMDARs are stabilized at synapses through direct binding to PSD-95 family members, whereas targeting of NMDARs to extrasynaptic domains occurs through nonsynaptic proteins, including G α-interacting protein (GAIP)-interacting protein C terminus (GIPC) [89], and both AP-2 and syndapin-1 clathrin adaptors at sites of endocytosis [47,79,90]. NR2 C-terminal domains are important for synaptic localization [91,92], and probably contribute to differential targeting between synaptic and extrasynaptic compartments. NR2A has lower mobility at synapses than NR2B [38], although both subunits can be detected in synaptic and extrasynaptic compartments [82] (Figure 3). In addition, NR2A and NR2B differ substantially in their binding affinities for CaMKII, with NR2B exhibiting higher binding than NR2A; this could account for differences in NMDAR-dependent signaling and plasticity activated by these distinct receptor subtypes [93–96]. Therefore, specific signaling properties of NR2A- and NR2B-containing NMDARs are not simply a result of synaptic versus extrasynaptic localization, but also reflect a different assemblage of associated signaling molecules and inherent differences in channel biophysical properties [97].

The expression and differential usage of NR2 subunits has important implications for the forms of synaptic plasticity that are expressed. NR2A-containing receptors

have a lower affinity for glutamate, faster deactivation kinetics, higher channel open probability, and a more pronounced Ca^{2+}-dependent desensitization than NR2B-containing receptors [98]. Outside-out patch recordings of single NMDAR channels indicate that NR2A-containing receptors have a higher probability of opening upon brief synaptic-like pulses of glutamate compared to NR2B-containing receptors [99]. Mathematical modeling and simulations of synaptic responses to the low frequencies typically used to induce LTD predict a larger contribution of NR2B-containing NMDARs to Ca^{2+} influx and total charge transfer than NR2A-containing NMDARs. Conversely, under the tetanic high-frequency stimulation often used to induce LTP, the charge transfer mediated by NR2A-containing NMDARs greatly outweighs that of NR2B-containing NMDARs [99]. Furthermore, inhibition of NR2B-containing NMDARs in hippocampal slices has been reported to prevent the induction of LTD, but not LTP, whereas inhibition of NR2A-containing NMDARs blocks induction of LTP, but not LTD [100]. In addition, NR2A- but not NR2B-containing NMDARs promote the insertion of GluR1 for expression of LTP [101]. A caveat of these experiments is the incomplete specificity of NR2 subtype-specific pharmacological inhibition [102], which argues for more selective genetic manipulations. However, it is well established that the slower decay kinetics of NR2B-containing NMDARs produces enhanced summation of the NMDAR-mediated response to trains of stimuli, which can be further accentuated by the recruitment of extrasynaptic NMDARs by glutamate spillover during repeated release events [103–105]. Such enhanced summation is associated with an increased ability to induce LTP, probably owing to augmented or sustained Ca^{2+} influx [106,107]. Therefore, the distinct spatial distribution and biophysical properties of NR2A- and NR2B-containing NMDARs allow for diverse forms of signal transduction and synaptic plasticity. Notably, the relative contribution of NR2A- and NR2B-containing NMDARs at glutamatergic synapses is subject to both long-term and acute regulation by activity [37,95]. The mechanisms underlying activity-dependent regulation of NR2 subunits, and whether such mechanisms occur at single synapses or as a function of more global circuit activity, remain topics of active inquiry.

What are the potential sources of glutamate that could activate extrasynaptic NMDARs? Glial cells might provide a source of extracellular glutamate by controlling the clearance of extracellular glutamate or by directly releasing glutamate [108]. Glutamate from glia can support small tonic currents through extrasynaptic NMDARs [109], which can influence slow network oscillations [110]. In addition, glia secrete glutamate in an activity-dependent manner (Figure 3), and NR2B surface distribution is spatially related to glutamate vesicle release sites on astrocytes [108], indicating that nonsynaptic sources of glutamate activate extrasynaptic NMDARs and influence their localization. It remains to be determined whether or not extrasynaptic glutamate controls the abundance of extrasynaptic NMDARs.

In addition to glia, high frequency synaptic stimulation can lead to glutamate spillover and trigger NMDAR responses [111,112]. At AMPAR-only synapses, postsynaptic NMDAR responses can be detected when activation is strong enough to drive glutamate spillover [113]. In cerebellar stellate cells, activation of non-synaptic NMDARs by glutamate spillover induces an increase in GluR2-containing receptors and reduces the contribution of Ca^{2+}-permeable GluR2-lacking receptors [114]. Furthermore, extrasynaptic NMDAR activation influences the clustering of potassium channels that control somatodendritic excitability [115]. Therefore, extrasynaptic NMDARs might have an important function in sensing strong synaptic stimulation by detecting glutamate spillover.

Signaling through perisynaptic glutamate receptors

Residing within 100–200 nm of the PSD edge is the perisynaptic membrane, enriched in mGluRs and NR3A-containing NMDARs (Figure 3), as well as their associated scaffold and trafficking proteins (e.g. homer and syndapin-1) [79,80]. It is not clear if the perisynaptic domain represents a stable signaling domain, or simply a transit region in which glutamate receptors exchange between synaptic and extrasynaptic membranes. Given their unique localization, NR3A-containing NMDARs might signal from the perisynaptic domain [79]. Unlike NR1 and NR2 subunits, NR3A lacks a PDZ ligand sequence in its C terminus and does not bind to PSD-95 [116]. Thus, replacement of NR2 subunits by NR3A in the hetero-oligomer will reduce the valency of PDZ scaffold interactions. Moreover, perisynaptically localized NR3A binds directly to the F-BAR-domain-containing endocytic adaptor syndapin-1, which could regulate the function of the EZ by controlling recruitment of the endocytic machinery or actin cytoskeleton [79]. In addition, the C terminus of NR3A interacts with protein phosphatase 2A (PP2A), which dephosphorylates NR1 [117] and regulates NMDAR trafficking [118]. PP2A could also potentially dephosphorylate components of the nearby EZ and prime the EZ for endocytosis.

The group I mGluRs are also found in perisynaptic domains and, by coupling to G_q heterotrimeric G proteins, control the phospholipase C (PLC)-mediated production of diacylglycerol (DAG) and inositol-1,4,5-triphosphate (IP3), and thus activate downstream effectors including PKC [119] (Figure 3). mGluR-mediated synaptic depression (mGluR-LTD) involves endocytosis of AMPARs by local synthesis of the activity-regulated gene product Arc/Arg3.1 [120,121], which can directly bind dynamin-2 and endophilin-3 to facilitate endocytosis of AMPARs [122].

mGluR activation can also lead to extrasynaptic NMDAR downregulation [104]. At the developing calyx of Held synapse in auditory brainstem, pairing presynaptic stimulation with postsynaptic depolarization leads to downregulation of summated NMDAR excitatory postsynaptic current (EPSC) amplitudes, but not the amplitudes of single NMDAR-mediated miniature excitatory postsynaptic currents (mEPSCs), suggesting a preferential downregulation of perisynaptic or extrasynaptic NMDARs [104]. Expression of this downregulation requires Ca^{2+} influx, mGluR activation, ongoing synaptic activity, and dynamin-mediated endocytosis [104]. Therefore, co-activation of NMDARs and mGluRs through strong synaptic stimu-

lation could reduce the abundance of extrasynaptic NMDARs, indicating that mGluRs can exert control over extrasynaptic NMDAR levels and downstream signaling.

Concluding remarks and remaining questions

Many questions remain as to how receptor mobility is regulated to drive formation of clusters and signaling hotspots within the PSD. For example, how does PSD structure concentrate or separate receptors within synaptic nanodomains? How is the intrasynaptic positioning of nanodomains regulated by the actin cytoskeleton? How is the exchange of PSD scaffold proteins coupled to glutamate receptor exchange, and how do scaffold dynamics influence PSD morphology and architecture? What confines receptors? Are there crucial receptor-binding PSD slots and, if so, how dynamic are these structures?

Beyond the PSD, much remains to be learned about the role of lateral spine microdomains. Where does exocytosis occur in spines, and what Ca^{2+}-regulated factors influence AMPAR exocytosis? Does the spine EZ zone trap glutamate receptors and does the size or position of the EZ affect the efficiency of trapping? Do extrasynaptic receptors in spines and shafts have similar biophysical and signaling properties?

Studying the core cell biological machinery of dendrites and spines is crucial to understanding how the brain carries out complex functions such as learning and memory. Progress in recent years has shown that many forms of synaptic plasticity use cell biological mechanisms that have been conserved in diverse cell types and over evolution, but have been subverted for specialized function in highly differentiated CNS neurons. The evolutionary origin of such specialization is a topic worthy of investigation. However, we are only now beginning to understand how spine microdomains, glutamate receptor signaling, and localized membrane trafficking act together in information storage and synaptic learning. Uncovering such mechanisms holds promise for developing a cell biological understanding of neural computation and brain function in both health and disease.

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