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Glutamate Receptor Dynamics in Dendritic Microdomains

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Abstract

Among diverse factors regulating excitatory synaptic transmission, the abundance of postsynaptic glutamate receptors figures prominently in molecular memory and learning-related synaptic plasticity. To allow for both long-term maintenance of synaptic transmission and acute changes in synaptic strength, the relative rates of glutamate receptor insertion and removal must be tightly regulated. Interactions with scaffolding proteins control the targeting and signaling properties of glutamate receptors within the postsynaptic membrane. In addition, extrasynaptic receptor populations control the equilibrium of receptor exchange at synapses and activate distinct signaling pathways involved in plasticity. Here, we review recent findings that have shaped our current understanding of receptor mobility between synaptic and extrasynaptic compartments at glutamatergic synapses, focusing on AMPA and NMDA receptors. We also examine the cooperative relationship between intracellular trafficking and surface diffusion of glutamate receptors that underlies the expression of learning-related synaptic plasticity.

Introduction

Information storage and plasticity require that synapses carry out two opposing tasks: maintaining stable long-term synaptic connections while at the same time remaining plastic and allowing for rapid changes in synaptic strength. A major challenge has been to understand how the large array of synaptic proteins that govern these opposing processes are regulated to selectively establish, maintain, and modify the strength of synapses. Increasing evidence has shown that synaptic complexes once viewed as static are, in fact, highly dynamic structures that rapidly exchange receptors and scaffold proteins. At excitatory synapses in the mammalian brain, much work has focused on the proteins that regulate the stability and signaling properties of synapses on dendritic spines. These femtoliter-sized protrusions receive excitatory glutamatergic input from directly apposing presynaptic terminals (Bourne and Harris, 2008). Occupying the small portion of the spine membrane directly opposite the contacting presynaptic terminal is the postsynaptic density (PSD), an electron-dense protein network containing glutamate receptors and various membrane proteins anchored to cytoskeletal scaffolding molecules (Okabe, 2007; Sheng and Hoogenraad, 2007). Within the PSD, most rapid excitatory synaptic transmission occurs through two types of glutamate receptors, the N-methyl-D-aspartate (NMDA)-type receptor and the α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA)-type receptor. Homo- or heterotetrameric AMPA receptors are assembled from combinations of GluR1-4 subunits, while NMDA receptors are heterotetramers composed of two NR1 subunits, two NR2 subunits selected from among four gene products (NR2A-D), and occasionally an NR3 subunit (NR3A-B) (Kohr, 2006; Greger et al., 2007). The differential expression and trafficking of these glutamate receptor subunits directly affects their targeting to and retention within synaptic compartments and, thus, the

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magnitude of synaptic transmission (Kennedy and Ehlers, 2006; Derkach et al., 2007; Lau and Zukin, 2007; Shepherd and Huganir, 2007). The equally important topic of receptor trafficking at inhibitory synapses has been recently reviewed (Kneussel and Loebrich, 2007; Michels and Moss, 2007) and will not be discussed in detail.

Emanating from classic studies on the vertebrate neuromuscular junction (NMJ), the concept of the stable synapse was inferred from imaging studies of the nicotinic acetylcholine receptor (nAChR), which established that the half-life of nAChRs residing in the NMJ was many days (Berg and Hall, 1975) and that the total number of nAChRs per NMJ remains relatively constant throughout the lifetime of an animal (Pestronk et al., 1980). Furthermore, the concentration of junctional nAChRs was found to greatly exceed that of extrajunctional receptors (Fertuck and Salpeter, 1976), indicating that nAChRs are trapped and stabilized within synapses. Similar findings of apparently stable synapses were also made in the central nervous system, where glycine receptors are enriched within synapses (Triller et al., 1985; Seitanidou et al., 1988). The relatively high concentration of neurotransmitter receptors within the postsynaptic specialization and the interactions between these receptors and intracellular scaffold proteins both led to the predominant view that synaptic receptors are tightly fixed within the synapse. However, evidence for dynamic receptor populations and receptor exchange at synapses has long existed. Early studies of nAChR diffusion in the developing NMJ showed that nAChRs fluorescently labeled with α-bungarotoxin undergo spontaneous redistribution and could become integrated into newly assembled NMJs (Anderson and Cohen, 1977), providing clear evidence that extrajunctional receptors can aggregate into nascent NMJs on the plasma membrane. Building on these findings was the observation that functional nAChRs replace locally inactivated receptors through lateral diffusion (Young and Poo, 1983). The recovery of acetylcholine responses at the sites of local inactivation did not occur when receptor diffusion was perturbed by cross-linking surface receptors. In addition, fluorescently labeled surface nAChRs in developing muscle fibers can exist as either freely moving pools diffusely distributed in the plasma membrane or as relatively immobile pools contained within concentrated patches (Axelrod et al., 1978). Importantly, the relatively immobile nAChRs contained in patches could be dispersed by electrical stimulation (Axelrod et al., 1978), showing that receptor aggregation is directly affected by synaptic activity. Subsequently, time-lapse imaging in vivo found that nAChRs undergo rapid and reversible activity-dependent exchange through perijunctional domains (Akaaboune et al., 1999, 2002). Taken together, these important studies demonstrated that nAChRs exchange between distinct membrane microdomains through a process involving lateral diffusion in the plasma membrane.

Later work at central excitatory synapses would show that glutamate receptors are present in multiple locations throughout the cell, suggesting that glutamate receptors may also undergo lateral diffusion and exchange between membrane compartments. A powerful combination of imaging and electrophysiological studies identified functional glutamate receptor populations localized within synapses and in extrasynaptic domains. For example, immunohistochemical studies demonstrated synaptic and extrasynaptic glutamate receptor distribution throughout the dendritic arborization (Rogers et al., 1991; Blackstone et al., 1992; Petralia and Wenthold, 1992; Martin et al., 1993a, 1993b; Molnar et al., 1993, 1994; Aoki et al., 1994; Baude et al., 1994; Siegel et al., 1994; Kharazia et al., 1996a, 1996b; Perez-Otano et al., 2006). In addition, application of glutamate receptor agonists to membrane patches isolated from both dendritic and somatic regions of hippocampal neurons revealed AMPA receptor-mediated currents, implying that functional glutamate receptors exist outside of synapses (Jonas and Sakmann, 1992; Spruston et al., 1995; Andrasfalvy and Magee, 2001). Later work using subcellular immunogold labeling of NMDA and AMPA receptor subunits provided high-resolution electron microscopy (EM) visualization of receptors at extrasynaptic sites on spines, dendrites, somata, and within intracellular compartments (Baude et al., 1995; Kharazia et al., 1996a; He et al., 1998; Nusser et al., 1998; Petralia and Wenthold, 1999; Takumi et al., 1999b). In addition,

EM studies revealed that synaptic glutamate receptors occupy distinct regions within the postsynaptic membrane (Baude et al., 1995; Kharazia et al., 1996a; Nusser et al., 1998; Takumi et al., 1999a; Racca et al., 2000; He et al., 2001; Perez-Otano et al., 2006; Masugi-Tokita et al., 2007). Taken together, these EM studies clearly demonstrated the existence of multiple receptor populations occupying diverse regions of the plasma membrane and intracellular membrane compartments. However, whether these glutamate receptor populations represented static pools or were able to exchange between synaptic, extrasynaptic, and intracellular compartments would not be entirely appreciated until the development of highly sensitive optical and biochemical techniques for tracking receptors.

Techniques to Track Glutamate Receptor Mobility

The total pool of extrasynaptic glutamate receptors available to enter into synapses is ultimately established by the relative rates of synthesis, degradation, endocytosis, and exocytosis. Before discussing the cooperative relationship between intracellular trafficking and surface diffusion of glutamate receptors (see below), we first describe the methods used to track receptor exchange and lateral mobility at the postsynaptic membrane and the factors that influence surface mobility of glutamate receptors.

As a result of thermal agitation, all receptors are naturally mobile within a lipid membrane and undergo random Brownian motion. However, in a typical cell membrane, receptor mobility is strongly influenced by physical obstacles and reversible biochemical interactions (Kusumi et al., 2005). To better understand how these barriers affect receptor diffusion, a variety of methods have been developed to optically track glutamate receptor movement, yielding important insight into the physical interactions and local environment of a receptor (Triller and Choquet, 2005; Groc et al., 2007b). One method involves tagging heterologously expressed receptors with variants of green fluorescent protein (GFP) and measuring fluorescent recovery after photobleaching (FRAP). By measuring FRAP, it is possible to quantify the proportion of receptors that are exchangeable in a given area, based on the extent of fluorescence recovery, which in turn provides important information on the bulk dynamics of a receptor population. Furthermore, this technique makes it possible to calculate the apparent diffusion coefficient of the mobile and exchangeable receptor population (Reits and Neefjes, 2001; Chen et al., 2006), although this is necessarily an averaged value that can be highly sensitive to the availability of exchangeable pools of unbleached molecules, the specific geometry of the bleached area, and the properties of the interface with neighboring structures. Importantly, a slow rate of fluorescence recovery of a labeled receptor can indicate the presence of many different physical barriers, such as reversible chemical interactions, temporary cytoskeletal corrals and pickets, or restricted membrane geometry (Choquet and Triller, 2003). It is also possible to specifically track the surface population of glutamate receptors using the pH-sensitive GFP variant superecliptic pHluorin (SEP), which fluoresces at the neutral pH of the extracellular space but is quenched within acidic internal compartments (Ashby et al., 2004, 2006; Kopec et al., 2006; Park et al., 2006; Yudowski et al., 2006; Yudowski et al., 2007; Heine et al., 2008). SEP-tagged receptors have also been effectively used to measure relative rates of endocytosis and exocytosis (Ashby et al., 2004; Bogdanov et al., 2006; Kopec et al., 2006; Park et al., 2006; Yudowski et al., 2006, 2007).

One drawback of measuring diffusion with tagged receptors is the requirement for expression of exogenous receptors, typically at levels where the stoichiometry, protein interactions, or intracellular trafficking may not accurately reflect endogenous receptors. For example, increasing the pool of extrasynaptic receptors could change the equilibrium of receptor exchange at synapses and influence multiple types of plasticity (Passafaro et al., 2001; Shi et al., 2001; Tovar and Westbrook, 2002; Ashby et al., 2004; Holcman and Triller, 2006; Oh et al., 2006; Ehlers et al., 2007; Yudowski et al., 2007). However, it should be noted that

overexpression of AMPA receptor subunits does not increase AMPA excitatory postsynaptic currents (EPSCs) (Shi et al., 2001) or change the proportion of mobile GluR2-containing AMPA receptors (Tardin et al., 2003; Ashby et al., 2006). Finally, it is important to consider that the size of the illumination spot in FRAP experiments is typically larger than the synapse, which prohibits an unequivocal distinction between synaptic or extrasynaptic receptor exchange.

Single-particle tracking (SPT) is a powerful technique to track the movement of individual receptors in real time with high temporal and spatial resolution. Unlike FRAP (or the complementary technique of photoactivation using photoswitchable fluorescent proteins), which measures the bulk exchange of a population of molecules, SPT can be used to measure the diffusion of individual receptors, or at least that of individual probes bound to diffusing receptors. Fluorescently coupled antibodies directed against extracellular receptor epitopes allow the visualization and mapping of receptor trajectories. Diffusion coefficients can be derived by plotting the receptor mean square displacement (MSD) over time, which can also distinguish free versus confined diffusion. In the case of Brownian or free diffusion, the MSD plot over time appears linear, whereas for confined receptor movement the MSD plot will curve to a quasi-maximum (Qian et al., 1991; Kusumi et al., 1993; Saxton, 1993) (Figures 1A and 1B). As receptors move between different membrane microdomains, they can undergo alternating periods of free and confined diffusion. Several different types of physical barriers could confine receptor movements. In the case of receptor “corralling,” rapidly moving receptors will diffuse in a semiconfined space, an effect that does not necessarily lead to changes in the instantaneous diffusion coefficient. However, confined receptors that undergo a local decrease in the diffusion coefficient indicate the presence of either reversible biochemical interactions (e.g., receptor-PSD protein interactions) or nonchemical interactions arising from molecular crowding and collisions with other molecules in or near the membrane. SPT also allows quantification of receptor exchange rates between distinct compartments and, conversely, dwell times, providing important information on the equilibria between receptor populations, which define their statistical thermodynamic distribution within the plasma membrane.

Initial SPT experiments in neurons utilized 500 nm antibody-coated latex beads that were directed against subunits of glycine receptors or AMPA receptors (Meier et al., 2001; Borgdorff and Choquet, 2002) and provided a first glimpse of individual glutamate receptor mobility in the extrasynaptic neuronal membrane. An improvement over latex bead tracking was the use of antibodies conjugated to organic dyes (e.g., Cy3, Cy5). The smaller antibody-receptor complex allows for optical tracking of receptor movement within more spatially restricted domains, such as the synaptic cleft. Such experimental paradigms have demonstrated that extrasynaptic AMPA receptors exchange laterally in and out of the PSD and are mobile within the PSD (Tardin et al., 2003). The major limitation to using single organic dyes is their rapid photobleaching, which prevents the acquisition of receptor trajectories over time periods longer than a few seconds.

The introduction of semiconductor quantum dots (QDs) has circumvented many of these limitations (Dahan et al., 2003). QDs are resistant to photobleaching (Michalet et al., 2005; Triller and Choquet, 2005), allowing tracking of labeled receptors for minutes. Importantly, the characteristic “blinking” of individual QDs enables the identification of single molecules. In addition, the high signal-to-noise ratio allows one to fit the fluorescent signal to a two-dimensional Gaussian function to identify the centroid of the object with a pointing accuracy typically between 5 and 50 nm. This pointing accuracy is below the diffraction limited resolution of the light microscope, which makes possible high-precision nanometer-scale spatial tracking of receptors within a compartment. This technique has been used to map areas

explored by AMPA receptors within the PSD, demonstrating that individual receptors remain confined to nanometer-scale subdomains of the synapse (Ehlers et al., 2007) (Figure 1C).

There are several limitations to SPT of glutamate receptors. The labeling method itself may influence diffusive properties or restrict access to sterically confined membrane domains. For example, both the size of the antibody-receptor complex and the potential cross-linking of receptors could alter the rate of receptor endocytosis and their signaling properties. In addition, the mobility of attached probes (e.g., antibody-receptor complexes and QDs) could be slowed or impeded by extracellular obstacles and barriers. Such factors are especially important when tracking receptors within restricted synaptic compartments. Indeed, synaptic receptors tracked using organic-dye-conjugated antibodies have faster mobility than QD-conjugated antibodies (Groc et al., 2004). Nonetheless, QDs do enter into and exchange at synapses (Dahan et al., 2003). Moreover, in cultured hippocampal neurons, QDs loaded into synaptic vesicles are released during action potential stimuli and diffuse rapidly out of the synaptic cleft, indicating diffusional access to the synapse (Zhang et al., 2007). Recent advances in reducing the size of QDs and engineering small monovalent affinity ligands coupled to QDs should allow for better access to the synapse (Howarth et al., 2006).

Several techniques have also been devised to track the exchange of functional glutamate receptors at synapses. One method, known as electrophysiological tagging, involves expression of receptor subunits with altered channel properties. For example, in the case of certain AMPA receptor subunits, mutations that “tag” the expressed receptor by altering current rectification can be directly measured. After delivering stimuli that alter synaptic properties, it is then possible to identify newly inserted synaptic AMPA receptors with distinct channel properties relative to the original synaptic receptor population (Hayashi et al., 2000; Zhu et al., 2000; Shi et al., 2001; Barria and Malinow, 2002; Esteban et al., 2003). A second electrophysiological technique to track functional receptor movements utilizes pharmacological inactivation of synaptic receptors with use-dependent blockers (e.g., MK-801 for NMDA receptors), allowing for temporal measurements of endogenous receptor exchange at synapses (Tovar and Westbrook, 2002; Harris and Pettit, 2007; Zhao et al., 2008). Another method for functionally tracking native glutamate receptors has been the direct covalent attachment of a photoreactive antagonist, whose proximity to the ligand-binding site is determined by absorption of a UV photon (Adesnik et al., 2005; Nilsen and England, 2007). Two-photon uncaging of glutamate is yet another powerful electrophysiological technique for mapping glutamate receptor responses. This method has been used to measure the strength of responses along dendrites, which ultimately reveals the location and density of functional glutamate receptors (Pettit et al., 1997; Pettit and Augustine, 2000; Matsuzaki et al., 2001; Smith et al., 2003).

No single technique can reveal the entire complex behavior of a dynamic receptor population; each has caveats. It is only when SPT, FRAP, and electrophysiological techniques are combined and interpreted as a whole that a clear and more complete picture of glutamate receptor dynamics begins to emerge.

Ongoing Glutamate Receptor Exchange at Synapses

Several different techniques have demonstrated that glutamate receptors exchange between synaptic and extrasynaptic compartments. Quantification of the bulk movements of surface SEP-GluR2 AMPA receptors using FRAP revealed that ~50% of surface GluR2 in spines is exchangeable, with a recovery phase that plateaus within 10–15 min (Ashby et al., 2006). This fluorescence recovery of SEP-GluR2 in spines is likely a result of surface lateral diffusion rather than plasma membrane insertion, as incubation of neurons with anti-GFP antibodies, a treatment that alters lateral diffusion of SEP-labeled surface receptors, slowed the fluorescence recovery of SEP-GluR2 in spines (Ashby et al., 2006). A similar fluorescence recovery rate

was obtained following photobleaching of spine-localized EYFP-GluR1 (Sharma et al., 2006). In addition, a recent FRAP study in intact hippocampal brain slices found that 30% of spine- and 60% of shaft-localized SEP-GluR2 is exchangeable (Heine et al., 2008), indicating that AMPA receptors are mobile in both dissociated culture and brain slices. Therefore, a major fraction of surface AMPA receptors in spines exist in a mobile population that undergoes exchange within minutes. Such rapid exchange of glutamate receptors at spines could provide the basis for acute changes in synaptic strength. Yet, the finding that approximately half the GluR2 AMPA receptors in spines do not exchange on this timescale indicates that a population of receptors is confined or immobile within synapses.

Both the exchange of mobile glutamate receptors and stabilization within synapses have been demonstrated by SPT experiments. Latex bead tracking of GluR2 revealed a wide range of diffusion coefficients for extrasynaptic AMPA receptors in hippocampal neurons, with a tendency for slower diffusion rates as neurons mature (Borgdorff and Choquet, 2002). Organic-dye-labeled antibodies subsequently allowed for the tracking of GluR2 in both synaptic and extrasynaptic compartments and for visualizing receptor exchange at synapses (Tardin et al., 2003). Similar to latex bead tracking, a wide range of diffusion coefficients exists for extrasynaptic GluR2. Within synaptic regions, two populations were detected, one apparently immobile pool and a second mobile population, consistent with the 50% mobile pool detected by FRAP methods (Ashby et al., 2006; Sharma et al., 2006). The MSD plot of GluR2 trajectories in synaptic and extrasynaptic domains revealed confined movements in synapses compared to primarily free Brownian diffusion in extrasynaptic domains (Figures 1A and 1B). Importantly, extrasynaptic GluR2 AMPA receptors can enter into synaptic regions (Figures 1A and 3A), providing evidence that extrasynaptic receptors can act as a readily available pool to supply synapses.

The mobility of individual NMDA receptors has been directly visualized at synapses using a combination of FRAP, single dye molecule, and QD tracking. Under basal conditions, extrasynaptic NR1 has a median diffusion coefficient that is four times slower than extrasynaptic GluR2, while the median diffusion coefficient of synaptic NR1 is similar to synaptic GluR2 (Groc et al., 2004). In addition, the fluorescence recovery of synaptic EYFP-NR1 following photobleaching plateaus at 15% after 5 min versus 40% for EYFP-GluR1 (Sharma et al., 2006), indicating that the bulk mobile pool of NMDA receptors at synapses is reduced compared to that of AMPA receptors (Sharma et al., 2006), perhaps due to stronger or more abundant receptor-scaffold interactions.

Electrophysiological approaches have also shown that glutamate receptors undergo lateral diffusion and exchange. Lateral diffusion of native AMPA receptors has been demonstrated with the photoreactive AMPA receptor antagonist ANQX. Following irreversible AMPA receptor inactivation at the somatic plasma membrane by focal UV irradiation, AMPA-mediated currents recover within tens of seconds (Adesnik et al., 2005; Nilsen and England, 2007), indicating that functional extrasynaptic AMPA receptors are mobile in the plasma membrane. Using the irreversible NMDA receptor open-channel blocker MK-801 together with synaptic stimulation of young cultured hippocampal neurons to selectively inactivate synaptic NMDA receptors, initial studies found that 65% of NMDA receptors activated by synaptically released glutamate exchanged within 7 min (Tovar and Westbrook, 2002). The recovery from MK-801 block was not a result of receptor insertion into the plasma membrane, as blocking extrasynaptic receptors with coapplication of NMDA and MK-801 prevented synaptic recovery. Thus, recovery of synaptic NMDA responses following MK-801 block requires exchange from extrasynaptic sources, which are likely NR2B-containing NMDA receptors (Zhao et al., 2008). The time course of recovery measured from MK-801 block appears faster (65% in 7 min) than detected by FRAP methods (15% in 5 min) with the NR1 subunit (Sharma et al., 2006). Possible reasons for this difference could be that GFP-tagged

NR1 exchanges more slowly than endogenous NR1 or that synaptically activated NMDA receptors make up the majority of exchangeable receptors. A similar electrophysiological approach in acute hippocampal slices found essentially no recovery of synaptic NMDA current after MK-801 synaptic blockade and subsequent washout in CA1 pyramidal neurons, suggesting that a stable pool of extrasynaptic NMDA receptors does not rapidly exchange with synaptic receptors (Harris and Pettit, 2007). The reason for this apparent discrepancy is not yet clear but could involve developmental differences between cell cultures and brain slices or the presence of astrocytes, secreted factors, or extracellular matrix in brain slices that restrict NMDA receptor mobility (Groc et al., 2007a). Thus, a variety of experimental approaches have documented distinct diffusional properties of glutamate receptors distinguished by receptor type, subunit composition, membrane localization, and developmental stage.

Spine and Dendrite Geometry Influence Glutamate Receptor Diffusion

Perhaps the most evident factor limiting glutamate receptor diffusion is the tortuous geometry of dendrites and dendritic spines. Biophysical models of receptor influx into spines predict that changes in spine neck length are an important mechanism to control receptor exchange between the spine head and the dendritic shaft and, therefore, synaptic receptor content (Holcman and Triller, 2006). Photobleaching experiments with SEP-tagged glutamate receptors also reveal a strong influence of spine neck geometry on glutamate receptor diffusion (Ashby et al., 2006). Following photobleaching, the recovery of SEP-GluR2 fluorescence in spines is much slower than flat surfaces, and the recovery in large mushroom spines (containing thin necks) is slower than stubby spines with thick necks (Ashby et al., 2006). Therefore, the spine neck acts as a barrier to glutamate receptor diffusion. However, other factors could contribute, as large mature spines may have stronger receptor-PSD interactions, and other mechanisms for maintaining or recapturing mobile glutamate receptors could conceivably covary with spine geometry. Nonetheless, spine neck geometry does influence the diffusion of membrane-associated proteins that are not associated with the PSD. In particular, the diffusion of membrane-bound GFP in dendritic spines is retarded relative to the dendritic shaft, suggesting that escape of membrane-associated proteins from spines is also influenced by spine neck diameter (Richards et al., 2004).

In addition to the effects of spine neck geometry on synaptic glutamate receptor exchange, the mere presence of spines would be expected to strongly influence receptor diffusion along the length of a dendrite. Before extrasynaptic glutamate receptors can enter into spines, they first must travel some distance along the dendritic membrane. As with freely diffusing cytosolic proteins (Santamaria et al., 2006), a pool of extrasynaptic receptors moving laterally along a dendrite is likely to encounter a “minefield” of dendritic spines operating as diffusional traps. Indeed, biophysical models of the behavior of receptors inserted into the somatic plasma membrane and diffusing from proximal to distal sites predict that spines will cause a steep decay profile in steady-state receptor concentration along the dendrite by acting as diffusional sinks (Bressloff and Earnshaw, 2007). Therefore, although a significant fraction of glutamate receptors may be inserted at the soma (Adesnik et al., 2005), it seems unlikely that receptors inserted into the soma could supply spiny dendrites several hundred microns away. Moreover, the somatic source model predicts a decreasing proximal-to-distal gradient of receptors. However, a reverse proximal-to-distal increasing gradient is observed for AMPA receptors in CA1 pyramidal neurons (Magee and Cook, 2000; Andrasfalvy and Magee, 2001; Smith et al., 2003). Such considerations highlight the important role of intracellular membrane trafficking coupled with receptor lateral diffusion for delivery of glutamate receptors to distal dendritic locations.

Nanoarchitecture of the PSD and Spatial Confinement of Glutamate Receptors

In addition to the effect of spine neck geometry on receptor mobility, the arrangement and composition of the PSD will directly control the establishment of diffusion barriers (corrals and pickets) and submembranous receptor-binding sites that in turn determine receptor dwell time and thus abundance within synapses. Indeed, electron microscope images of PSDs clearly indicate the presence of physical obstacles that could potentially lead to receptor confinement (Figures 1D and 1E). PSDs have a disc-like shape with a diameter of ~100–500 nm and a thickness of 30–50 nm (Carlin et al., 1980; Harris et al., 1992; Spacek and Harris, 1997; Valtschanoff and Weinberg, 2001). Serial-section EM reconstruction and freeze-fracture EM indicate that PSD surface area ranges from 0.008–0.54 μm^2 (Harris and Stevens, 1989; Tanaka et al., 2005). This large range in PSD surface area corresponds to variations in spine size; indeed, PSD surface area positively correlates with spine head size (Harris and Stevens, 1989; Okabe, 2007). The variability in PSD surface area is an important factor to consider when analyzing single molecule trajectories or interpreting FRAP data, as large differences in PSD area could naturally lead to variation in the mobility and confinement of synaptic receptors.

Stoichiometry of Glutamate Receptors and PSD Scaffold Proteins

PSD size and stability are controlled by scaffolding proteins that assemble into this complex structure (Okabe, 2007; Sheng and Hoogenraad, 2007). The most abundant PSD proteins are involved in signal transduction and scaffold formation; for example, calcium/calmodulin-dependent protein kinase II (CaMKII)-α and -β make up 7.4% and 1.3% of the PSD mass (Peng et al., 2004; Cheng et al., 2006). These measurements are consistent with EM images of immunogold-labeled biochemically isolated PSDs, which reveal CaMKII holoenzyme towers studded over the cytosolic face of the PSD (Petersen et al., 2003; Gaertner et al., 2004) (Figure 1D). Also abundant are PDZ scaffold proteins that tether glutamate receptors to the PSD through reversible chemical interactions. Both mass spectrometry and immunogold EM estimate between 60 to 400 of these major scaffold molecules per PSD (e.g., PSD-95, GKAP/SAPAP, SAP97, shank, and homer) (Peng et al., 2004; Chen et al., 2005; Cheng et al., 2006). Importantly, estimates of the number of synaptic scaffolds by quantitative light microscopy using calibration of fluorescence signal from GFP-tagged molecules yielded comparable results, opening promising possibilities for similar quantifications in real time (Sugiyama et al., 2005).

The number of glutamate receptors per synapse appears to be less than PSD scaffold proteins; mass spectrometry, immunogold EM, and electrophysiological experiments estimate AMPA receptor numbers in the range of ~5–200 (Nusser et al., 1998; Matsuzaki et al., 2001; Smith et al., 2003; Peng et al., 2004; Tanaka et al., 2005; Cheng et al., 2006; Masugi-Tokita et al., 2007). Unlike AMPA receptors, NMDA receptor numbers are less variable and have a smaller positive correlation with spine head size (Takumi et al., 1999b; Racca et al., 2000). Ca^{2+} imaging studies estimate a very small number (~1–5) of NMDA receptors activated by synaptic stimulation at CA1 hippocampal synapses (Nimchinsky et al., 2004), suggesting that there are fewer NMDA receptors than AMPA receptors at mature synapses.

The overabundance of scaffold proteins relative to glutamate receptors is consistent with the availability of free receptor positions or “slots” for changes in synaptic strength (Lisman and Raghavachari, 2006). The presence and spatial arrangement of such slots may determine how receptors partition between synaptic and extrasynaptic domains. Although the nature of these slots is not known, both immunogold freeze-fracture replica labeling and single-particle QD imaging reveal that AMPA receptors are contained within intrasynaptic microdomains,

suggesting that these nanoscale compartments may concentrate receptor-binding proteins (Ehlers et al., 2007; Masugi-Tokita et al., 2007) (Figures 1C and 1E). In addition, the placement of AMPA receptor microdomains relative to presynaptic release sites could be an important factor controlling synaptic strength (Raghavachari and Lisman, 2004).

Structural Organization of the PSD and Glutamate Receptor Exchange

In addition to differences in the absolute number of receptors at synapses, AMPA and NMDA receptors may display distinct spatial distributions within and outside of the PSD (Baude et al., 1995; Kharazia et al., 1996a; Nusser et al., 1998; Takumi et al., 1999a; Racca et al., 2000; He et al., 2001). For example, AMPA receptors appear either concentrated at the periphery or homogeneous throughout the PSD (Masugi-Tokita et al., 2007), while NMDA receptors are more compactly distributed at the center of the PSD (Kharazia et al., 1996a; Racca et al., 2000). These differences likely reflect distinct constraints on receptor diffusion consistent with FRAP experiments showing that GluR1 has a faster rate of fluorescence recovery compared to NR1 (Sharma et al., 2006). Differences in the distribution and mobility between receptor types could be a result of distinct binding partners of their cytosolic tails. NMDA receptors have large cytosolic tails of 600 amino acids or more, whereas AMPA receptor tails are from 50 to 100 amino acids in length; longer cytosolic tails could lead to both multiple and stronger scaffold interactions. Also suggesting stronger PSD interactions is the observation that NMDA receptors are insoluble following detergent extraction, whereas AMPA receptors remain soluble (Allison et al., 1998). Conversely, the difference in glutamate receptor intra-PSD distribution could result from differential interactions of extracellular domains. Indeed, extracellular domains of AMPA receptors associate with N-cadherin (Nuriya and Huganir, 2006; Saglietti et al., 2007; Silverman et al., 2007), which may localize to the edge of the PSD (Uchida et al., 1996; Saglietti et al., 2007). In addition, N-terminal extracellular domains of AMPA receptors bind to neuronal pentraxins (O'Brien et al., 1999, 2002; Mi et al., 2002; Xu et al., 2003; Sia et al., 2007), whose spatial distribution at synapses is not known but could contribute to altered receptor mobility or distribution.

Beyond the border of the PSD is a loosely defined "perisynaptic" region, located within ~100–200 nm of the PSD edge. The perisynaptic region is compositionally (and likely functionally) distinct from more distant extrasynaptic domains and contains a cohort of glutamate receptor types, including group I metabotropic glutamate receptors (mGluRs) and NR3A-containing NMDA receptors, as well as the scaffold protein homer (Baude et al., 1993; Lujan et al., 1997; Tu et al., 1999; Perez-Otano et al., 2006) (Figure 2). The perisynaptic region may have functional significance in controlling glutamate receptor exchange. For example, SPT demonstrated that GluR2-containing AMPA receptors shift into the perisynaptic domain upon bath application of glutamate (Tardin et al., 2003), a manipulation that causes loss of synaptic AMPA receptors (Carroll et al., 1999b). Conversely, synapse-potentiating stimuli (bicuculline/glycine) cause a reduction in the number of perisynaptic receptors (Tardin et al., 2003). On the other hand, imaging of SEP-GluR2 showed a rapid loss of extrasynaptic AMPA receptors that precedes the loss of synaptic receptors following activation of NMDA receptors (Ashby et al., 2004). These data suggest that the perisynaptic region may be a transition zone or reservoir for receptor trafficking into or out of the PSD, a process that may be controlled by the cytoskeleton, scaffold density, and binding capacity of the PSD.

Many unanswered questions remain as to what types of scaffold interactions regulate glutamate receptor exchange between synaptic and perisynaptic regions and how these interactions are regulated by synaptic activity. For AMPA receptors, extracellular interactions with N-cadherin may provide an important control over mobility in the perisynaptic region (Nuriya and Huganir, 2006; Saglietti et al., 2007). In addition to AMPA receptors, the perisynaptic localization and mobility of the group I mGluRs are also regulated by synaptic activity. Normally, mGluR5

undergoes either fast or slow mobility, corresponding to receptors existing in dispersed or clustered states, respectively (Serge et al., 2002). Addition of the group I selective mGluR agonist DHPG increases mGluR5 diffusion, which may occur through uncoupling of G_q from mGluR5, thus leading to the loss of underlying cytoskeletal interactions (Serge et al., 2002). Cytoskeletal tethering of mGluRs to the perisynaptic region may occur through homer binding (Figure 2), as mGluR5 confinement increases following homer-1b overexpression and confined mGluR5 receptors exist in clusters containing homer (Serge et al., 2002). Therefore, activity-dependent interactions between glutamate receptors and perisynaptic scaffold proteins provide an important control over glutamate receptor retention and transit through the perisynaptic zone.

Beyond the perisynaptic membrane is the “extrasynaptic” region, consisting of the remaining spine membrane, spine neck, dendrites, and soma (Figure 2). Although the precise dimensions are somewhat arbitrary, synaptic, perisynaptic, and extrasynaptic regions comprise compositionally and functionally distinct membrane domains, which lead to different diffusional behavior of receptors and activation of downstream signaling pathways. In the case of NMDA receptors, subunit composition may lead to differential targeting between these compartments. Specifically, NR2A-containing receptors are abundant at synapses, while NR2B-containing receptors are often more enriched in extrasynaptic domains, although both are found in synaptic and extrasynaptic domains (Watanabe et al., 1992;Williams et al., 1993;Sheng et al., 1994;Wang et al., 1995;Li et al., 1998;Tovar and Westbrook, 1999;Sans et al., 2000a;Barria and Malinow, 2002;Thomas et al., 2006). Extrasynaptic targeting of NMDA receptor subunits may occur through the NMDA receptor-binding protein GIPC, which associates with surface and intracellular receptors but is excluded from synapses (Yi et al., 2007) (Figure 2). Interestingly, nonconventional NMDA receptors containing NR3A are also enriched at the edge of the PSD and in perisynaptic domains (Perez-Otano et al., 2006) (Figure 2). Correspondingly, selective activation of synaptic versus extrasynaptic receptors may trigger distinct signaling cascades, thereby producing very different effects on synapse plasticity and neuronal survival (Sala et al., 2000;Sattler et al., 2000;Lu et al., 2001;Hardingham and Bading, 2002;Hardingham et al., 2002;Ehlers, 2003;Massey et al., 2004;Morishita et al., 2007;Sun and June Liu, 2007). Thus, microdomains at andnear the synapse mayact as distinct platforms for signaling.

In addition to their lateral organization, the PSDs at glutamatergic synapses have a laminar organization that may also influence receptor exchange. Early direct freeze deep etch EM of excitatory synapses in the cochlear nucleus revealed a meshwork of 4 nm thick actin filaments in receptor-rich domains of the post-synaptic membrane attached to a subjacent lattice of 8–9 nm filaments (Gulley and Reese, 1981). This tighter filament lattice at the postsynaptic plasmalemma was proposed to limit receptor mobility. Immunogold labeling has revealed the laminar distance of specific PSD proteins from the postsynaptic membrane into the spine interior. The cytoplasmic C-terminal tail of AMPA and NMDA receptors define the first molecular layer between the postsynaptic membrane and the underlying scaffold proteins (Valtschanoff and Weinberg, 2001). In stepwise order, starting closest to the plasma membrane, GluR1, CaMKII, ProSAP2, VASP, cortactin, and actin extend from 17 to 110 nm into the spine cytosol (Rostaing et al., 2006). These findings comport with previous results showing that the tail of the NR2 subunit extends 12 nm into the cytosol, whereas GKAP, shank, and homer reside ~25 nm deep (Valtschanoff and Weinberg, 2001; Petralia et al., 2005). To date, little is known about how this laminar organization is established and regulated or how the architecture of these layers influences receptor stabilization. Interestingly, molecular exchange of the “deep” scaffold proteins GKAP and shank is sensitive to actin depolymerization, whereas the exchange of the membrane-associated scaffold PSD-95 is not (Allison et al., 1998; Kuriu et al., 2006). Recent electron tomography studies have identified vertical filaments containing PSD-95 as a major structural feature of the PSD (Chen et al., 2008). The precise vertical and

horizontal arrangement of scaffolds could easily be envisioned to trap or tether glutamate receptors to defined sites within the PSD to regulate receptor exchange (Rostaing et al., 2006; Chen et al., 2008) (Figures 1D and 2).

Glutamate Receptor Microdomains within the PSD

Both EM images and single-particle tracking experiments support restricted receptor movements within the postsynaptic membrane. For example, the mobility of individual AMPA receptors is confined within subregions of the synaptic membrane (Ehlers et al., 2007). Super-resolution analysis of receptor diffusion within the postsynapse demonstrated that only 20% of the PSD area in active synapses is accessible to individual QD-labeled GluR1 AMPA receptors (Ehlers et al., 2007) (Figure 1C). In contrast, 80% of the PSD is accessible to single diffusing GluR1 receptors at inactive synapses, suggesting that there are activity-dependent barriers to receptor diffusion within synapses. The confinement of GluR1 to subregions inside active synapses could be due to the increased presence of scaffold binding sites (or “slots”) acting as receptor traps, to altered arrangement of the scaffold network, to changes in underlying cytoskeletal organization, to altered affinities of receptor-scaffold interactions, or some combination of these factors. The limited access of diffusing GluR1 receptors to most of the PSD area is fully consistent with EM studies showing the presence of proteinaceous clumps and voids in isolated PSDs (Petersen et al., 2003) (Figure 1D). CaMKII holoenzymes form large towers on the cytosolic face of the PSD membrane, separated by deep valleys, which could conceivably act as channels for receptor lateral diffusion within the synaptic membrane or may serve to define receptor diffusion boundaries. Below the membrane, the core of the PSD is dominated by vertically oriented filaments containing PSD-95, which contact NMDA receptors and AMPA receptors (Chen et al., 2008). Further supporting subdomain confinement of AMPA receptors in the PSD is the nonhomogeneous clumped distribution of AMPA receptors viewed en face in freeze-fracture electron micrographs (Masugi-Tokita et al., 2007) (Figure 1E). Although the biophysical nature of these PSD subdomains is not clear, the three-dimensional architecture of the PSD likely has strong effects on receptor mobility. One attractive possibility is that constriction and expansion of diffusional channels or scaffold barriers could control receptor entry, intra-PSD mobility, and stabilization. Indeed, CaMKII undergoes translocation into spines following NMDA receptor stimulation (Shen and Meyer, 1999), an event that could trigger rearrangement of PSD structure.

Due to their low affinity for glutamate and differences in activation and desensitization kinetics, the packing density and spatial arrangement of AMPA receptors within the PSD could be an important contributor to synaptic strength, independent of absolute receptor number. Modeling studies indicate that AMPA receptor density within the PSD is more important than the overall size of the PSD (Franks et al., 2003; Raghavachari and Lisman, 2004; Lisman and Raghavachari, 2006). In cultured hippocampal neurons, the variability in miniature excitatory postsynaptic current (mEPSC) amplitude is proportional to the quantity of neurotransmitter released (McAllister and Stevens, 2000), indicating that AMPA receptors are not saturated by quantal release. It thus follows that alterations in the density of AMPA receptors and their positioning relative to glutamate release sites could dramatically alter the postsynaptic response. Indeed, local diffusion can replace desensitized AMPA receptors and thereby fine tune synaptic transmission (Heine et al., 2008). Determining the impact of receptor packing density and subsynaptic confinement remains a difficult experimental problem. It will be important in the future to understand how receptor lateral diffusion is regulated to organize receptors into assembled clusters. In addition, increased filling of receptor slots could produce increased molecular crowding and retention of glutamate receptors through non-chemical interactions, which may be especially important for persistent trapping of newly mobilized receptors during synaptic potentiation. The long-term persistence or depletion of ensembles of

synaptic receptors may provide a basis for enduring information storage at the single-synapse level (Shouval and Kalantzis, 2005).

Glutamate Receptor Interactions in the PSD

In addition to their confinement by physical barriers established by the overall architecture of the PSD, a wide array of receptor-interacting proteins have been identified that control the stability and residence time of glutamate receptors within the postsynaptic membrane. With the notable exception of PSD-95 family members, NMDA receptors and AMPA receptor/TARP complexes bind to different sets of synaptic scaffold proteins (Figure 2). For NR3A-containing NMDA receptors, targeting to perisynaptic regions requires an interaction with syndapin-1/PACSIN-1 (Perez-Otano et al., 2006). For NR3A-lacking NMDA receptors, synaptic targeting requires binding of the NR2 subunit C-terminal tails to members of the PSD-95 family of proteins (Niethammer et al., 1996) (Figure 2). This family of proteins includes PSD-93/chapsyn-110, PSD-95, SAP-97/hDlg, and SAP-102 (reviewed in Lau and Zukin, 2007), and all contain PDZ domains. Notably, genetic deletion of NR2 C-terminal tails leads to a deficit in synaptic targeting (Mori et al., 1998;Steigerwald et al., 2000;Mohrmann et al., 2002). A significant portion of this targeting defect is likely due to disrupted binding to PDZ scaffolds like PSD-95, because selective mutation of the PDZ-binding domain in NR2B prevents synaptic incorporation (Prybylowski et al., 2005).

In addition to binding NR2 subunits of NMDA receptors, PSD-95 family members bind to AMPA receptors via their auxiliary TARP subunits (Chen et al., 2000; Chetkovich et al., 2002; Schnell et al., 2002; Dakoji et al., 2003; Tomita et al., 2004). The canonical TARP protein stargazin/γ2 (and likely the other TARPs) functions at multiple levels of the secretory pathway to control surface expression of AMPA receptors (Chen et al., 2000, 2003; Schnell et al., 2002; Cuadra et al., 2004; Vandenberghe et al., 2005; Matsuda et al., 2008). Although TARP-dependent trafficking to the extrasynaptic membrane does not require PSD-95 binding, this interaction is required for subsequent stabilization of AMPA receptor/TARP to the synapse (Schnell et al., 2002). This was directly supported by single-particle tracking experiments in hippocampal neurons revealing that stargazin-dependent interactions between AMPA receptors and PSD-95 control receptor diffusion and trapping at the PSD (Bats et al., 2007). Both GluR1- and GluR2-labeled QDs have reduced mobility at PSD-95-labeled synaptic sites compared to regions outside of PSD-95 clusters. Confinement of AMPA receptors within PSD-95 puncta involves stargazin, as overexpression of a stargazin mutant that cannot interact with PSD-95 increases the mobile fraction and decreases the dwell time of receptors within synapses (Bats et al., 2007). QD tracking of HA-tagged stargazin also revealed reduced mobility at PSD-95 clusters (Bats et al., 2007). Therefore, TARPs and AMPA receptors diffuse together in synaptic and extrasynaptic sites, and binding of TARPs to PSD-95 stabilizes synaptic AMPA receptors.

In addition to binding PSD-95 family members via TARPs, a distinct set of PDZ proteins bind AMPA receptor subunits directly. Both protein interacting with C kinase (PICK1) (Xia et al., 1999) and glutamate receptor interacting protein/AMPA receptor binding protein (GRIP/ABP) (Dong et al., 1997; Srivastava et al., 1998) are PDZ-domain-containing proteins that bind to the C terminus of GluR2 AMPA receptors and regulate their targeting to synapses (Figure 2) (Xia et al., 1999; Chung et al., 2000; Daw et al., 2000; Osten et al., 2000; Kim et al., 2001; Seidenman et al., 2003; Hanley and Henley, 2005). PICK1 and GRIP bind the same extreme C-terminal domain of GluR2, although their binding is differentially regulated by phosphorylation of GluR2 at serine 880 (Hirbec et al., 2003; Seidenman et al., 2003; Lu and Ziff, 2005; Lin and Huganir, 2007). These distinct binding properties of PICK1 and GRIP/ABP are important for AMPA receptor removal during long-term depression (LTD) (Xia et al., 2000; Kim et al., 2001; Chung et al., 2003; Steinberg et al., 2006) and for rapid plasticity

of Ca^{2+}-permeable AMPA receptors at cerebellar stellate cell synapses (Liu and Cull-Candy, 2000; Gardner et al., 2005; Liu and Cull-Candy, 2005). Activation of Ca^{2+}-permeable AMPA receptors (which lack GluR2) can lead to incorporation of GluR2-containing AMPA receptors that have a smaller single-channel conductance, a linear current-voltage relationship, and are impermeable to Ca^{2+} (Isaac et al., 2007). PICK1 is also important for expression of long-term potentiation (LTP), and overexpression of PICK1 occludes LTP by increasing levels of synaptic GluR2-lacking AMPA receptors (Terashima et al., 2008). Although the precise trafficking functions of PICK1 are not completely resolved, PICK1 contains a BAR domain that binds phospholipids on curved membranes (Peter et al., 2004; Jin et al., 2006) and thus may participate in targeting GluR2 AMPA receptors to microdomains of membrane curvature. PICK1 also binds actin and the Arp2/3 complex and may regulate the assembly of actin at sites of GluR2 endocytosis (Rocca et al., 2008).

Interestingly, studies in mice lacking GluR2 and/or GluR3 indicate that the GluR2 C terminus is not required for synaptic insertion (Panicker et al., 2008), and GluR2/3-lacking AMPA receptors are endocytosed in a manner indistinguishable from GluR2-containing AMPA receptors in wild-type neurons (Biou et al., 2008), although targeted in vivo mutations in the GluR2 PDZ-binding domain eliminate cerebellar LTD (Steinberg et al., 2006). Taken together, these findings support an emerging concept that AMPA receptor-binding proteins have subunit preferences that allow precise, and perhaps redundant, control over synaptic stabilization and membrane trafficking of distinct AMPA receptor subtypes.

Postsynaptic scaffold proteins are themselves dynamic. Not all PSD scaffold proteins have a similar retention time within the PSD, and it is likely that networks of protein interactions will affect scaffold stability and their ability to retain receptors. Recent studies have measured exchange kinetics and dynamics of PSD proteins (Friedman et al., 2000; Bresler et al., 2004; Gray et al., 2006; Kuriu et al., 2006; Sharma et al., 2006; Tsuriel et al., 2006). When the exchange kinetics of different PSD proteins were compared by FRAP, CaMKII had the greatest mobile pool (~82%), followed by GluR1 (~56%), PSD-95 (~45%), and NR1 (~38%) (Sharma et al., 2006). Another study compared the stability of four different postsynaptic scaffold proteins—PSD-95, GKAP, shank, and homer-1c/Zip45—and found that each protein had distinct exchange rates in the PSD as measured by FRAP (Kuriu et al., 2006). PSD-95 had the least mobile population (~13%), followed by GKAP (~36%), shank (~35%), and homer-1c/Zip45 (~62%). Given that PSD-95 is relatively stable in the PSD (Kuriu et al., 2006; Sharma et al., 2006) and occupies a position immediately subjacent to the postsynaptic membrane (Valtschanoff and Weinberg, 2001), one might have thought that loss of PSD-95 would have a dramatic effect on the retention of less stable PSD proteins. However, disruption of PSD-95 did not affect the dynamics of GKAP, shank, or Zip45 (Kuriu et al., 2006), suggesting that multiple interactions and internal scaffold redundancy control the exchange of individual scaffold molecules. On the other hand, acute pharmacological disruption of F-actin rapidly eliminates the dynamic fraction of GKAP, shank, and homer-1c/Zip45 (Kuriu et al., 2006). Although it is generally accepted that increased expression of core PSD scaffolds including PSD-95 and shank increase synaptic AMPA receptor content (El-Husseini et al., 2000; Sala et al., 2001; Stein et al., 2003; Ehrlich and Malinow, 2004; Beique et al., 2006; Ehrlich et al., 2007), it remains to be determined how ongoing exchange of PSD scaffolds impacts glutamate receptor dynamics, which protein interactions are facilitated or impaired during scaffold exchange, and whether scaffold exchange regulates or reflects PSD nanocompartmentalization.

The Cortical Actin Cytoskeleton Controls Receptor Diffusion

In all eukaryotic cells, the membrane-associated cortical F-actin cytoskeleton is a key determinant of membrane protein diffusion by providing points of direct cytoskeletal anchoring and organizing membrane microdomains (Kusumi et al., 2005). In spines, depolymerization

of F-actin increases the exchange of membrane-anchored GFP (Richards et al., 2004), suggesting that the submembranous actin cytoskeleton constrains lateral diffusion. In addition, the actin cytoskeleton determines spine morphology (Fischer et al., 1998), which in turn influences receptor diffusion (Ashby et al., 2006; Holcman and Triller, 2006). Actin dynamics are tightly tuned by synaptic activity and may confer a rapid mechanism to control receptor diffusion (Star et al., 2002; Okamoto et al., 2004, 2007). At inhibitory synapses, SPT studies show that acute depolymerization of F-actin increases glycine receptor exchange between synaptic and extrasynaptic compartments, leading to a decreased dwell time within inhibitory synapses and ultimately a loss of synaptic receptors (Charrier et al., 2006). There is evidence that the actin cytoskeleton tethers glutamate receptors at both synaptic and nonsynaptic sites. For example, depolymerization of F-actin causes a 40% decrease in the number of synaptic AMPA and NMDA receptor clusters (Allison et al., 2000). Nonsynaptic NMDA receptor clusters persist, while nonsynaptic AMPA receptor clusters disperse following F-actin depolymerization (Allison et al., 2000), indicating that extrasynaptic AMPA receptor clustering is controlled by interactions with the actin cytoskeleton (Figure 2). Interestingly, F-actin filaments can directly contact the PSD (Landis and Reese, 1983; Rostaing et al., 2006) and associate with diverse protein elements of the PSD (Okabe, 2007; Sheng and Hoogenraad, 2007), including scaffold complexes containing shank and cortactin (Okabe, 2007; Sheng and Hoogenraad, 2007) (Figure 2). Likewise, pharmacological disruption of F-actin eliminates the dynamic fraction of several core PSD scaffold proteins, including GKAP and shank, which associate with AMPA and NMDA receptors (Kuriu et al., 2006). Submicron variation in actin organization may account for differences in glutamate receptor mobility in synaptic, perisynaptic, and extrasynaptic membrane microdomains.

Glutamate Receptor Mobility and Synaptic Activity

Activity Level Controls Glutamate Receptor Movement

Synaptic activity can influence glutamate receptor diffusion through control of membrane trafficking, spine morphology, cytoskeletal organization, and the affinity of interactions with PSD scaffold proteins. Indeed, chemically induced LTP and LTD, as well as chronic modifications of synaptic activity influence surface AMPA receptor diffusion (Borgdorff and Choquet, 2002; Tardin et al., 2003; Ashby et al., 2004; Groc et al., 2004; Sharma et al., 2006; Ehlers et al., 2007). Besides the direct influence on receptor diffusion consequent to plasticity-associated changes of spine morphology (Ashby et al., 2006), LTP-inducing stimuli reduce the diffusion of soluble molecules between spines and shafts, indicating local activity-dependent diffusional coupling of the spine neck with the dendritic shaft (Bloodgood and Sabatini, 2005).

AMPA receptor diffusion is highly sensitive to local changes in Ca^{2+}. For example, GluR2 pauses reversibly near synapses, and local elevation of dendritic Ca^{2+} by ionophore uncaging decreases GluR2 extrasynaptic mobility by 80%, while buffering intracellular Ca^{2+} with BAPTA increases GluR2 mobility (Borgdorff and Choquet, 2002). Surprisingly, GluR2 mobility is also arrested following Ca^{2+} uncaging in young neurons (DIV1–4) lacking synapses (Borgdorff and Choquet, 2002), indicating that decreased receptor movement is not merely a result of spine or PSD entrapment. Therefore, extrasynaptic AMPA receptors can be confined by Ca^{2+}-dependent scaffolding interactions or remodeling of the submembranous cytoskeleton. In addition, increases in Ca^{2+} levels triggered by physiological synaptic stimulation decrease the mobility of GluR1- and GluR2-containing AMPA receptors, an effect that has important implications for short-term plasticity. For example, the decreased mobility of AMPA receptors following synaptic stimulation is associated with increased paired-pulse depression (PPD) (Heine et al., 2008). Although the recovery from fast synaptic depression is widely believed to be dominated by a presynaptic component (Zucker and Regehr, 2002), a recent study has demonstrated that lateral exchange of desensitized synaptic AMPA receptors

for naive functional AMPA receptors also contributes to recovery from PPD (Heine et al., 2008). Conditions that reduce surface AMPA receptor mobility (e.g., antibody cross-linking, receptor clustering, or increases in Ca^{2+}) prevent the normal recovery from PPD by preventing exchange with desensitized receptors (Heine et al., 2008). These findings highlight the important role of AMPA receptor diffusion and synaptic exchange on a rapid millisecond timescale. Moreover, these data suggest that subtle changes in the mobility and dwell time of synaptic AMPA receptors may have a dramatic impact on the exchange of desensitized receptors and expression of short-term synaptic plasticity.

Changes in AMPA receptor mobility are also important for long-term forms of synaptic plasticity. In synaptic compartments, diffusion of Cy-5-labeled synaptic GluR2 subunits is accelerated by bath application of glutamate (Tardin et al., 2003). This effect is similar to population dynamics of SEP-GluR2, as NMDA receptor-induced chemical LTD causes a loss of synaptic GluR2 AMPA receptors, which is preceded by endocytosis of extrasynaptic GluR2 AMPA receptors (Ashby et al., 2004). These findings suggest a model whereby LTD expression involves destabilization of scaffold interactions, followed by increased mobility of synaptic AMPA receptors, and finally receptor release into the extrasynaptic compartment for endocytosis.

Given that chemically induced LTD correlates with an increased mobility of synaptic AMPA receptors, does LTP decrease AMPA receptor mobility at synapses? Such a mechanism could provide a basis for the net increase in synaptic AMPA receptors during LTP (Shi et al., 1999; Hayashi et al., 2000; Lu et al., 2001; Kopec et al., 2006; Sharma et al., 2006). Within 5 min after chemical LTP induction, the percentage of mobile synaptic GluR2 AMPA receptors increases but returns to baseline levels after 40 min, suggesting that newly incorporated synaptic GluR2 receptors are mobile but soon become stabilized within synapses (Tardin et al., 2003). These results are consistent with FRAP experiments showing that chemical LTP stimuli increase the proportion of exchangeable EYFP-GluR1 in spines but decrease the rate of recovery following photobleaching (Sharma et al., 2006), suggesting that, while the number of mobile GluR1 AMPA receptors increases, synaptic receptor exchange decreases, ultimately allowing for synaptic trapping of AMPA receptors. In addition, SPT analysis revealed that the percentage of perisynaptic GluR2 receptors is reduced following chemical LTP induction (Tardin et al., 2003), suggesting that the perisynaptic pool of AMPA receptors is depleted as receptors enter into the PSD (Tardin et al., 2003). Still to be resolved are differences in rapid activity-dependent diffusional behavior of AMPA receptors containing GluR1 and GluR2. Nonetheless, these results suggest that the perisynaptic region maintains tight control over the synaptic exchange of AMPA receptors in response to activity.

Exchange of AMPA receptors at synapses during long-term plasticity could conceivably occur by altering AMPA receptor-TARP interactions or TARP-PSD-95 interactions, and phosphoregulation could provide the basis for these reversible interactions. For example, serine 295 phosphorylation of PSD-95 is associated with increased synaptic targeting of AMPA receptors, while dephosphorylation of serine 295 in PSD-95 is important for synaptic depression (Kim et al., 2007). In addition, protein kinase A (PKA)-mediated phosphorylation of the stargazin C terminus prevents its interaction with PSD-95 (Chetkovich et al., 2002), suggesting that surface mobility of AMPA receptors during LTD could be increased by PKAphosphorylation ofstargazin. Stargazin phosphorylation is controlled by additional kinases and phosphatases, whose activities are dependent on NMDA receptor activation (Tomita et al., 2005). For example, phosphorylation of TARPs by CaM-KII and protein kinase C (PKC) is important for LTP and synaptic targeting of AMPA receptors, while dephosphorylation by PP1/PP2B is important for LTD, indicating that the phosphorylation state of TARPs allows for bidirectional plasticity by controlling AMPA receptor exchange at synapses (Tomita et al., 2005). Phosphorylation of AMPA receptors may also facilitate their

delivery to the plasma membrane, thereby increasing the pool of extrasynaptic receptors and thus altering the equilibrium of synaptic receptor exchange. For example, chemical LTP induction increases the phosphorylation ofserine 845 onGluR1 (Lee etal., 2000), which accelerates receptor recycling (Ehlers, 2000) and increases the extrasynaptic pool of GluR1 (Oh et al., 2006).

In contrast to AMPA receptors, the mobility of NMDA receptors in synaptic and extrasynaptic compartments is unaffected by inhibiting network activity (TTX) or by KCl depolarization (Groc et al., 2004), consistent with FRAP data showing that NR1 dynamics are unaffected by chemical LTP induction (Sharma et al., 2006). However, activation of PKC with phorbol ester dramatically increased the diffusion coefficient of both extrasynaptic NR1 (12-fold) and synaptic (5-fold) receptors (Groc et al., 2004). These findings demonstrate that NMDA receptor mobility is more restricted than that of AMPA receptors, both in synaptic and extrasynaptic compartments, and is not significantly affected by synaptic activity, perhaps reflecting stronger scaffold interactions than AMPA receptors. In addition, the difference in mobility between NMDA and AMPA receptors could result from the more peripheral localization of AMPA receptors in synapses relative to NMDA receptors, as detected by immunogold labeling (Kharazia et al., 1996a; Tanaka et al., 2005; Masugi-Tokita et al., 2007).

Input-Specific Activity and Local Control of AMPA Receptor Diffusion

In addition to acute cell-wide manipulations of neural activity, the long-term activity state of a single synapse influences local receptor abundance and exchange (Harms et al., 2005; Ehlers et al., 2007; Hou et al., 2008a). Single-molecule imaging of GluR1 AMPA receptors using QDs has shown that active synapses more efficiently trap AMPA receptors than nearby inactive synapses rendered silent by expression of the tetanus toxin light chain (Ehlers et al., 2007). The median diffusion coefficient of GluR1 was reduced at active synapses as compared to inactive synapses, and the dwell time of GluR1 was significantly longer at active synapses (Ehlers et al., 2007) (Figures 3A and 3B). At inactive synapses GluR1 explores a much greater area of the postsynaptic membrane compared to nearby active synapses, although GluR1 at both types of synapses exhibited confined movement (Ehlers et al., 2007) (Figures 3C). During a 1 min time course, 20% of GluR1 exited active synapses, and 75% of GluR1 exited silent synapses and, in certain instances, GluR1 receptors that exited silent synapses entered into active synapses and became immediately trapped (Ehlers et al., 2007) (Figures 3A and 3B). Interestingly, acute blockade of glutamate receptors and action potentials did not alter GluR1 diffusion at either active or silenced synapses, indicating that activity-dependent changes in local receptor diffusional properties require prolonged differences in spontaneous synaptic activity and thus could represent an enduring biophysical modification. More broadly, these observations support a “capitalistic synapse” model whereby excitatory synapses compete for a limiting pool of diffusible GluR1 AMPA receptors based on local levels of vesicular release from presynaptic terminals, suggesting a diffusional basis for input-specific strengthening of synapses (Figure 3C).

In addition to AMPA receptors, PSD scaffold proteins also exist as a limited pool that is selectively retained in larger synapses. Two-photon imaging of GFP-tagged PSD-95 in vivo showed that a subset of PSD-95 puncta are stable for days, whereas a separate population turns over rapidly and is exchanged with nearby spines (Gray et al., 2006). Large PSDs capture more PSD-95 than smaller PSDs, and changes in PSD size correlate with PSD-95 retention time. Therefore, in a situation analogous to GluR1 AMPA receptor trapping by active synapses (Ehlers et al., 2007), neighboring PSDs also seem to compete for a limited pool of PSD-95. In addition to PSD-95 exchange, the scaffold protein ProSAP2/shank3 also undergoes activity-regulated exchange between neighboring synapses on a timescale of minutes (Tsuriel et al., 2006). In a separate study, pharmacological enhancement of neuronal activity increased GKAP

content at synapses but, conversely, caused loss of shank and homer-1c/Zip45 (Kuriu et al., 2006). The ability of active synapses to capture AMPA receptors (Ehlers et al., 2007) and large synapses to sequester PSD scaffold proteins (Isaac, 2003; Perez-Otano and Ehlers, 2004; Petralia et al., 2005; Gray et al., 2006), suggests that stable differences in synaptic strength are maintained by dynamic equilibria between local pools of exchangeable synaptic proteins, the size and dynamics of which are determined by local synaptic activity.

Glutamate Receptor Mobility for Synapse Maturation and Development

During brain development, excitatory synapses throughout the CNS undergo stereotyped changes in molecular and physiological properties. Such changes result in part from alterations in glutamate receptor trafficking, subunit expression, and changes in postsynaptic scaffolding proteins that alter receptor residence time (Isaac, 2003; Perez-Otano and Ehlers, 2004; Petralia et al., 2005). In the case of AMPA receptors at CA1 hippocampal synapses, GluR4 is expressed early during neuronal maturation and recruited to synapses displaying spontaneous activity (Zhu et al., 2000). Synaptic trapping of GluR4 involves an extracellular binding to pentraxins (Sia et al., 2007), a process that may be important for recruitment of AMPA receptors to the postsynaptic specialization during synaptogenesis. In the case of NMDA receptors, NR2B-containing NMDA receptors are expressed early and are later replaced at synapses by NR2A-containing receptors, which eventually predominate at synapses (Carmignoto and Vicini, 1992; Watanabe et al., 1992; Williams et al., 1993; Sheng et al., 1994; Wang et al., 1995; Li et al., 1998; Rumbaugh and Vicini, 1999; Tovar and Westbrook, 1999; Sans et al., 2000b; Barria and Malinow, 2002). The basis for these developmental subunit changes at synapses may result from a differential expression of scaffolding proteins, such as SAP102 and PSD-95 (Sans et al., 2000a), as the C-terminal domains of NR2 subunits possess distinct binding preferences and interact with distinct sets of scaffold proteins and signaling molecules (Kornau et al., 1995; Niethammer et al., 1996; Sans et al., 2000a). As NR2 subunit expression regulates NMDA receptor targeting and trafficking during development (Mori et al., 1998; Steigerwald et al., 2000; Roche et al., 2001; Mohrmann et al., 2002; Lavezzari et al., 2004; Prybylowski et al., 2005), one might expect subunit-specific differences in NMDA receptor mobility. Indeed, QD tracking of NR2A- versus NR2B-containing NMDA receptors has demonstrated slower mobility of surface NR2A (Groc et al., 2006, 2007a). NR2A diffusion coefficients in both synaptic and extrasynaptic compartments are slower than those of NR2B (Groc et al., 2006), and a greater proportion of NR2A is immobile in these two compartments. Conversely, the dwell time of NR2B in synapses is 3-fold shorter at DIV15 than at DIV8, and the dwell time of NR2A was significantly longer than NR2B at DIV15. Electrophysiological data also reveal increased mobility of NR2B-containing receptors and exchange at synapses. In 3-week-old hippocampal slices, mobile NR2B-containing NMDA receptors are responsible for the recovery of sEPSCs following MK-801 block (Zhao et al., 2008). Surprisingly, after the NR2B-mediated recovery, an LTP stimulation protocol leads to induction of LTD at these synapses, indicating that NMDA receptor subunit switching can lead to a novel type of metaplasticity (Zhao et al., 2008). Taken together, these observations demonstrate that subunit-specific differences in NMDA receptor synaptic targeting correlate with distinct diffusional and synaptic properties.

In addition to PSD scaffold proteins, the extracellular matrix also influences subunit-specific synaptic targeting of NMDA receptors. For example, developmental acceleration in synaptic NR1/2B receptor diffusion correlates with increased expression of reelin (Groc et al., 2007a), an extracellular matrix protein involved in developmental maturation and synaptic plasticity, which carries out these functions through binding to β1 integrin (Rodriguez et al., 2000) and lipoprotein receptors (Dityatev and Schachner, 2006; Qiu et al., 2006). SPT of NR2B-containing receptors revealed that antibody-mediated inhibition of reelin decreases the mobility of native NR2B receptors and increases synaptic dwell time, whereas addition of

recombinant reelin to young hippocampal neurons accelerates synaptic maturation by reducing NR2B-mediated synaptic currents and receptor dwell time within synapses (Groc et al., 2007a).

Providing further evidence for mobile NMDA receptors during synapse maturation is the finding that NMDA receptor subunits exchange at neonatal synapses but not more mature synapses (Bellone and Nicoll, 2007). Although LTP-inducing stimuli do not affect the exchange kinetics of EYFP-NR1 in cultured hippocampal neurons (Sharma et al., 2006), LTP induction at neonatal CA1 synapses can lead to subunit switching of synaptic NR2A receptors for NR2B receptors within seconds. This effect can endure for at least 1 hr and can be reversed by depotentiating stimuli (Bellone and Nicoll, 2007). Although the precise molecular mechanisms are not yet clear, this reversible switch between NR2B- and NR2A-containing receptors supports tight regulation of NMDA receptor trafficking.

One possible mechanism that could lead to preferential down-regulation of NR2B- versus NR2A-containing receptors is their differential binding to the clathrin adaptor AP-2. The C terminus of NR2B contains a tyrosine-based AP-2 clathrin internalization sequence (Lavezzari et al., 2003) adjacent to its PSD-95-binding site, suggesting that NR2B receptor interactions with PSD proteins prevent NMDA receptor downregulation by clathrin-mediated endocytosis. Indeed, NR2B interactions with PSD-95, SAP97, and PSD-93 inhibit NR2B endocytosis (Roche et al., 2001; Lavezzari et al., 2003). In addition, Fyn kinase-mediated phosphorylation of the NR2B AP-2-binding motif prevents endocytosis and thus may be a mechanism to increase synaptic targeting of NR2B (Prybylowski et al., 2005). The NR2A subunit also contains a tyrosine-based sorting motif, but this sequence is not required for endocytosis. Instead, a dileucine-based AP-2 internalization motif is utilized (Prybylowski et al., 2005). Following endocytosis, NMDA receptors can be sorted for either recycling or degradation (Lavezzari et al., 2004; Scott et al., 2004). The membrane-proximal sorting signals of both NR1 and NR2 drive NMDA receptors into lysosomes for degradation, whereas the NR2 distal sorting motifs direct NMDA receptors to a recycling pathway (Scott et al., 2004). Currently, it is not known how these sorting motifs are differentially utilized, although the NR2 distal motif is sufficient to drive recycling even in the presence of both the NR1 and NR2 proximal degradation motifs (Scott et al., 2004).

An additional factor controlling NMDA receptor channel properties, surface expression, and synaptic exchange during development is the NR3A subunit. Unlike NR2 subunits, the nonconventional NMDA receptor subunit NR3A has a strict window of expression during development, which peaks around postnatal day 8 and begins to decline at postnatal day 12 into adulthood in most brain regions in the rat (Wong et al., 2002). When associated with NR1/NR2 NMDA receptors, NR3A attenuates NMDA responses by reducing single-channel conductance (Das et al., 1998; Sasaki et al., 2002). NR3A knockout mice have enhanced NMDA receptor responses, and expression of NR3A in oocytes reduces the unitary conductance of NMDA receptors (Das et al., 1998). In addition, NR3A-containing channels have decreased Ca^{2+} permeability and are less sensitive to Mg^{2+} (Sasaki et al., 2002). The decreased sensitivity of NR3A-containing receptors to Mg^{2+} reduces the voltage dependence of these NMDA receptors, potentially altering the Hebbian learning rules for plasticity during synaptogenesis. Interestingly, the postnatal decline in NR3A expression corresponds to sensitive periods of plasticity in several brain regions. Endocytosis and downregulation of NR3A occurs in an activity-dependent manner and is mediated by direct interaction of the NR3A intracellular domain with the dynamin-associated F-BAR protein syndapin-1/PACSIN-1, whose upregulation also coincides with the developmental loss of NR3A (Perez-Otano et al., 2006). Thus, activity-dependent endocytosis of NR3A by syndapin-1/PACSIN-1 may contribute to experience-driven developmental changes in synaptic NMDA receptors.

Spine-Localized Endocytic Cycling Regulates Surface Glutamate Receptor Distribution

Glutamate receptors are transported through diverse intracellular compartments (Kennedy and Ehlers, 2006); this vesicular transport to and from the plasma membrane tunes the abundance of receptors in both synaptic and extrasynaptic regions (Lau and Zukin, 2007; Shepherd and Huganir, 2007). Initial evidence for dynamic glutamate receptor trafficking demonstrated that AMPA receptors undergo continual endocytosis and exocytosis, even in the absence of synaptic activity (Carroll et al., 1999b; Luscher et al., 1999; Shi et al., 1999; Beattie et al., 2000; Ehlers, 2000; Lin et al., 2000; Man et al., 2000) (Figures 4 and 5). Endocytosis of glutamate receptors occurs through a dynamin-dependent pathway (Carroll et al., 1999a; Luscher et al., 1999), and both dendritic spines and shafts contain the full assembly of clathrin-coat proteins and adaptors that sort, trap, and remove glutamate receptors from the cell surface (Blanpied et al., 2002; Racz et al., 2004; Lu et al., 2007). Both NMDA and AMPA receptor subunits contain sorting sequences that allow them to bind directly to the cargo-sorting adaptor AP-2 (Roche et al., 2001; Lee et al., 2002; Lavezzari et al., 2003; Prybylowski et al., 2005; Kastning et al., 2007). Endocytosis of glutamate receptors is dependent on dynamin function, based on evidence that an inhibitory peptide perturbing dynamin function blocks internalization (Luscher et al., 1999). In addition, snapshots of endocytosis captured by immunogold EM demonstrate internalization of GluR2/3 subunits within spine clathrin-coated pits (Petralia et al., 2003). Although most dendritic spines of hippocampal neurons contain endocytic machinery (Blanpied et al., 2002; Racz et al., 2004; Lu et al., 2007), it currently remains unknown whether endocytosis of glutamate receptors occurs preferentially in spines or dendritic shafts and whether the location of receptor endocytosis influences recycling or degradation.

Dendritic spines appear to contain all of the core endocytic machinery (clathrin, AP-2, dynamin-2, epsin, Hip1) at a location that has been designated the endocytic zone (EZ) (Blanpied et al., 2002; Racz et al., 2004; Yao et al., 2006; Lu et al., 2007), which consists of stable clathrin coats located lateral to the PSD (Figures 4A–4C). This positioning may maintain tight spatial control over glutamate receptor levels in response to synaptic activity, providing a mechanism for input-specific plasticity. Unlike the dynamic clathrin-coated pits observed in immature neurons and other cell types (Blanpied et al., 2002; Ehrlich et al., 2004; Newpher et al., 2005), the spine EZ resembles the stable behavior of nonterminal clathrin-coated pits (Merrifield et al., 2005), which can persist for tens of minutes (Blanpied et al., 2002). Given this long-term stability, the EZ could also act as a secondary diffusion trap on the spine membrane to help prevent lateral escape of receptors from the spine. This trapping may also prevent diffusion of cargo into neighboring synapses, which, in addition to receptor-scaffold interactions, would provide a synergistic mechanism for tuning postsynaptic receptor levels on a spine-by-spine basis (Figure 4C).

How does the endocytic machinery target to spines, and what is its functional role in synaptic transmission? A recent study has shown that dynamin-3 couples EZs to PSDs (Figures 2 and 4C) (Lu et al., 2007). Whereas the brain-specific dynamin-1 localizes to presynaptic terminals and the ubiquitous dynamin-2, which mediates endocytosis in all cell types, is thought to be both pre- and postsynaptic, dynamin-3 is enriched at the postsynaptic specialization and is the only isoform known to bind to the postsynaptic adaptor homer (Brakeman et al., 1997; Tu et al., 1998; Xiao et al., 1998; Gray et al., 2003, 2005). Consistent with a direct scaffolding function, disrupting dynamin-3 expression or its interaction with homer uncouples the EZ from the PSD, leading to a marked decrease in PSDs with an adjacent EZ (Lu et al., 2007). The functional consequences of EZ loss from the PSD are counterintuitive. Rather than increasing synaptic AMPA receptor levels, as would be expected for blocking receptor endocytosis, spatial uncoupling of the EZ depletes synaptic AMPA receptors and decreases the amplitude

and frequency of mEPSCs (Lu et al., 2007). These results suggest a model where the EZ, by allowing for spine-localized endocytic cycling, serves as a trap to recapture and maintain the extrasynaptic pool of AMPA receptors, thereby limiting the escape of AMPA receptors from spines (Lu et al., 2007) (Figure 4C). Consistent with this notion, spine-localized recycling endosomes are important for maintaining a mobilizable pool of synaptic AMPA receptors (Park et al., 2004).

There are many unanswered questions regarding the functions of the spine EZ. What types of postsynaptic cargo are trafficked by the EZ? Does the EZ contribute to spatial differences in AMPA receptor abundance across dendritic segments? Interestingly, in nonneuronal cells, different types of cargo influence clathrin-coated pit dynamics and rates of endocytosis. For example, clathrin-coated pits containing G protein-coupled receptors have very long lifetimes, which are thought to occur because of cargo-cytoskeleton interactions (Puthenveedu and von Zastrow, 2006). A similar cargo-scaffold interaction could occur at the spine EZ, perhaps with perisynaptic mGluRs that also bind directly to the PSD scaffold homer (Brakeman et al., 1997; Tu et al., 1998; Xiao et al., 1998). In addition to cytoskeletal interactions, the most obvious contributing factor to coated-pit formation is the lipid composition of the plasma membrane. AP-2, AP180/CALM, epsins, amphiphysin, endophilins, and dynamin all interact with phosphatidylinositol-4-5 bisphosphate, $PI(4,5)P_2$ (Traub, 2003; Legendre-Guillemin et al., 2004; Wenk and De Camilli, 2004; Itoh and De Camilli, 2006). Does the spine membrane somehow maintain a region of high $PI(4,5)P_2$, which ultimately recruits the EZ, and if so, how does this lipid microdo-main become established adjacent to the PSD?

Cholesterol/sphingolipid-rich lipid rafts exert important control over endocytosis and surface targeting of glutamate receptors (Hering et al., 2003; Hou et al., 2008b). AMPA receptors associate with mobile lipid rafts on the cell surface, and this association is controlled by NMDA receptor activation (Hou et al., 2008b). Interestingly, lipid raft depletion results in elevated AMPA receptor endocytosis and decreased AMPA receptor exocytosis (Hering et al., 2003; Hou et al., 2008b), indicating that the positioning of lipid rafts could lead to tight spatial control of receptor insertion and removal, an effect that could be especially important within dendritic spines to alter the abundance of synaptic AMPA receptors. It remains unknown how surface diffusion of glutamate receptors is affected by interactions with lipid rafts and what role lipid rafts play in the lateral exchange of synaptic glutamate receptors.

A major remaining question is the role of the EZ in synaptic plasticity. Clathrin-mediated endocytosis is required for downregulation of AMPA receptors and expression of LTD (Carroll et al., 1999b; Beattie et al., 2000; Man et al., 2000). In fact, perturbing endocytosis by dynamin inhibition leads to increased AMPA receptor currents, while blocking exocytosis leads to decreased AMPA receptor-mediated currents (Luscher et al., 2000). This led to a simple idea that exocytosis increases AMPA receptors for LTP, and endocytosis downregulates receptors for LTD. However, these experiments have relied on global inhibition of exo/endocytosis throughout the cell; it is not clear whether the location of receptor endocytosis leads to different outcomes for the expression of plasticity. Given the finding that a loss of spine EZs decreases synaptic AMPA receptor levels (Lu et al., 2007), an obvious question is whether LTP or LTD can occur at synapses that lack an adjacent EZ (Figure 5).

Once internalized, AMPA receptors and NMDA receptors can be sorted in endosomes for either recycling or degradation (Kennedy and Ehlers, 2006; Lau and Zukin, 2007; Shepherd and Huganir, 2007) (Figure 5). Differential AMPA receptor sorting is dependent upon the type of receptor activated. Stimulation of AMPA receptors alone leads to their downregulation, whereas activation of NMDA receptors initially leads to AMPA receptor endocytosis (10 min) and later recycling back to the cell surface (30 min) (Ehlers, 2000). It is not clear how this sorting occurs, although the phosphorylation state of the receptor may be an important factor.

For example, dephosphorylation of serine 845 of GluR1 is associated with LTD and may be the sorting signal that leads to lysosomal degradation (Lee et al., 1998; Ehlers, 2000), while phosphorylation of GluR1 at serine 845 accompanies reinsertion into the spine membrane via trafficking from recycling endosomes (Ehlers, 2000; Lee et al., 2000; Esteban et al., 2003). Following induction of LTD, Ca^{2+}-dependent activation of Rab5 leads to the formation of AMPA receptor-containing endocytic vesicles, likely derived from the EZ, which are then delivered to the early endosome (Brown et al., 2005) (Figure 5). GluR1 dephosphorylation also occurs following Rab5 activation, which could provide the sorting signal needed for GluR1 delivery to lysosomes. Therefore, expression of LTD requires activation of a Rab5-dependent sorting pathway and AMPA receptor dephosphorylation. It remains unknown whether AMPA receptors destined for recycling or degradation are internalized through the spine EZ.

The EZ is typically visualized as a single discrete patch of clathrin adjacent to the PSD (Blanpied et al., 2002; Lu et al., 2007). An interesting possibility is that the trafficking or lateral diffusion of receptors in spines is inherently directed, polarized, or channeled due to the asymmetric positioning of the EZ relative to the PSD. If the EZ acts as a polarized center for receptor endocytosis (Blanpied et al., 2002; Lu et al., 2007) and spine-localized recycling endosomes direct the exocytosis of cargo at distinct spine membrane domains (Park et al., 2006), this could establish a gradient for receptor movement in spines (Figure 5). Such a polarized cargo delivery system in spines could produce specific entry and exit sites within the PSD. Elucidation of the exact sites of receptor exocytosis, which is still debated, will be an important step in the evaluation of this model. Indeed, whereas cargo from recycling endosomes can be directly exocytosed in spines (Park et al., 2006), AMPA receptors can either accumulate in spines (Kopec et al., 2006) or be exocytosed in the dendritic shaft (Yudowski et al., 2007).

Glutamate Receptor Exocytosis and Synaptic Incorporation

It remains unknown whether there are dedicated sites of glutamate receptor exocytosis or whether these events occur randomly. The spatial positioning of exocytic sites could be important to control the rate at which receptors enter into synapses, as receptors inserted more closely to the PSD would encounter fewer obstacles preventing diffusion into the PSD. Not only would receptor insertion near the PSD be important for the timing of plasticity induction, but spine-localized insertion events could target receptors to individual spines for input-specific plasticity. Conversely, random insertion of receptors into all sites throughout the cell could contribute to global changes in the available pool of extrasynaptic receptors. Several different approaches have been used to map the locations of AMPA receptor and recycling endosome cargo insertion. One method employed thrombin cleavage of exogenously expressed HA-tagged AMPA receptors (Passafaro et al., 2001), allowing temporal and spatial mapping of newly inserted receptors (Rosenberg et al., 2001), although not in real time. Using the thrombin cleavage assay, GluR2 insertion and synaptic accumulation occurred more quickly than GluR1 insertion (Passafaro et al., 2001). Consistent with electrophysiological studies (Shi et al., 2001), swapping the C-terminal tails of GluR1 and GluR2 conferred the corresponding trafficking properties to the other subunit, indicating that C-terminal domains dictate insertion kinetics and synaptic targeting, presumably by directing the sorting of receptors to specific intracellular compartments or by interactions with scaffolding proteins.

Pharmacological approaches have also been used to track the location of receptor insertion. Using a photoreactive irreversible antagonist of AMPA receptors to rapidly and focally block surface AMPA receptors, the time course of receptor insertion was measured (Adesnik et al., 2005). Fast exchange of intracellular receptors with surface receptors occurred within the soma, but very little synaptic exchange was detected. SEP-tagging of glutamate receptors has also been used to visualize insertion of AMPA receptors into the spine membrane and dendrites,

demonstrating differences in the insertion rates of distinct glutamate receptor subunits (Kopec et al., 2006; Yudowski et al., 2007). Under basal conditions SEP-tagged GluR2, NR2B, and NR2A have higher synaptic enrichment than GluR1. Following induction of chemical LTP, both SEP-tagged GluR1 and GluR2 subunits accumulate at the spine surface, whereas NMDA receptor subunit levels do not change (Kopec et al., 2006). However, these experiments did not directly visualize exocytic events, and thus the exact sites of exocytosis could not be determined.

Rapid time-lapse imaging of SEP-GluR1 exocytosis demonstrated that local insertion of GluR1 AMPA receptors in the dendritic shaft is important to target receptors to the spine membrane (Yudowski et al., 2007). SEP-GluR1 exocytosis occurs under basal conditions in the somata and dendrites, and potentiating stimuli increase the frequency of these individual exocytic events. Some exocytic events involved membrane insertion of GluR1 and rapid lateral dispersion, which subsequently led to a short-lived increase of surface GluR1 within 2–5 μm of the insertion site (Yudowski et al., 2007). Other persistent events were characterized by slow lateral dispersion of GluR1 following exocytosis, which led to a less obvious increase in local surface receptor concentration (Yudowski et al., 2007). Importantly, the transient insertion events in dendritic shafts were able to supply nearby spines by short-range lateral diffusion. Therefore, even in the absence of direct spine insertion, receptors can still enter into nearby synapses from dendritic shafts. Direct GluR1 insertion events were not detected in spines (Yudowski et al., 2007), although similar stimuli evoke robust spine exocytosis from recycling endosomes (Park et al., 2006). The finding that newly inserted GluR2 accumulates at synapses faster than GluR1 (Passafaro et al., 2001) suggests that AMPA receptors with different subunit compositions may be inserted in different locations. An important caveat here is that it is not clear whether heterologously expressed AMPA receptor subunits, which are typically homomers and in the case of GluR1 signal differently due to high Ca^{2+}-permeability, undergo intracellular trafficking that differs from endogenous heteromeric receptors. Indeed, the high rate of synthesis of recombinant receptors expressed from foreign expression plasmids would favor cargo loading in the biosynthetic secretory pathway far in excess of that for endogenous receptors, whose rate of synthesis is much lower. Nonetheless, local exocytosis of AMPA receptors in dendrites or spines coupled with short-range diffusion to the postsynaptic membrane provides a general basis for short-range targeting of AMPA receptor to synapses.

Perhaps the strongest evidence for spine-localized exocytosis is the exocytic trafficking from dendritic recycling endosomes (Park et al., 2004, 2006) (Figure 5). The translocation of recycling endosomes into spines correlates with spine enlargement and spine-localized exocytosis (Park et al., 2006), and blocking recycling endosome transport reduces insertion of synaptic GluR1 for LTP (Park et al., 2004). Furthermore, time-lapse imaging of transferrin receptor (TfR)-SEP, which populates recycling endosomes at steady state, shows a rapid insertion of recycling endosome cargo into the spine membrane (Park et al., 2006). Vesicles derived from recycling endosomes may be targeted to the postsynaptic membrane by the exocyst (Gerges et al., 2006). In addition to mobilized recycling endosomes, endosomal compartments are also found in the dendritic shaft and extend between several spines (Cooney et al., 2002), suggesting that a single endosome could deliver cargo to multiple spines. The mechanisms responsible for sorting endosomal cargo traffic between neighboring spines remain to be determined.

Chemically induced LTP leads to increased mobilization of recycling endosomes into spines and exocytosis of endosomal cargo during spine growth (Park et al., 2004, 2006). These findings provide an attractive explanation for how spines increase size and AMPA receptor content concomitantly during the expression of LTP (Matsuzaki et al., 2004). However, given the differences in the rate of insertion and targeting of GluR1 and GluR2 into synapses (Passafaro et al., 2001), it is possible that AMPA receptor subunits are sorted through different

endosomes or undergo sorting within endosomes. Studies on exogenous homomeric AMPA receptors have found large differences in the endosomal sorting of GluR1 and GluR2 (Lee et al., 2004), although these differences were not seen with endogenous receptors (Ehlers, 2000). Notably, endocytic cycling of AMPA receptors is intact in neurons from mutant mice lacking both GluR2 and GluR3 (Biou et al., 2008), suggesting that these two subunits are not absolutely required for endosomal trafficking.

Do recycling endosomes contain both GluR1 and GluR2 AMPA receptor subunits to the same degree, or do different populations of endosomes carry different receptor subunits? Recent evidence indicates that a single spine may contain multiple endosomal pathways with distinct trafficking functions. One population of endosomes is Rab11 dependent and transports AMPA receptors into synapses (Brown et al., 2007), consistent with the observation that Rab11-dependent endosomes deliver GluR1 AMPA receptors for expression of LTP (Park et al., 2004). An additional class of endosomes controlled by Rab4 mediates constitutive recycling within spines (Brown et al., 2007). These findings are consistent with the morphological heterogeneity of recycling endosomes, tubules, and vesicles observed in dendrites and spines (Cooney et al., 2002; Park et al., 2006). Taken together, these data suggest that dendritic spines are highly complex autonomous units, which contain many of the trafficking compartments needed to maintain spine morphology, receptor targeting, and synapse function. Despite these advances and the potential involvement of distinct classes of motor proteins (Setou et al., 2002; Wu et al., 2002; Hoogenraad et al., 2005; Osterweil et al., 2005; Lise et al., 2006; Correia et al., 2008), there remains a fundamental lack of information on the molecular machinery that transports endosomal compartments and their cargo in spines and the mechanisms by which such machinery is activated or modified during synaptic plasticity.

Integrating Models for Glutamate Receptor Trafficking and Diffusion during Synaptic Plasticity

Many different trafficking events contribute to synaptic glutamate receptor abundance. How do these pathways converge in a cooperative manner for synaptic plasticity? As mentioned above, Ca^{2+} fluxes control the dynamics of endocytosis, exocytosis, and lateral diffusion. In presynaptic terminals, Ca^{2+} influx controls the assembly dynamics of clathrin-coated pits and endocytic adaptors via regulation of phosphorylation and dephosphorylation (Nichols et al., 1994; Bauerfeind et al., 1997; Marks and McMahon, 1998; Slepnev et al., 1998; Lai et al., 1999; Cousin and Robinson, 2001; Tan et al., 2003; Anggono et al., 2006). Ca^{2+}-dependent activation of calcineurin leads to the dephosphorylation of multiple endocytic proteins that drive synaptic vesicle recycling. Most prominently, dynamin-1 undergoes calcineurin-dependent dephosphorylation at serines 774 and 778 during synaptic vesicle endocytosis (Tan et al., 2003), and this dephosphorylation increases its association with the F-BAR protein syndapin-1 (Anggono et al., 2006). Rephosphorylation of dynamin-1 at these same sites by Cdk5 sustains the cycle of phosphorylation and dephosphorylation and is required for synaptic vesicle endocytosis (Tan et al., 2003; Tomizawa et al., 2003). It is tempting to speculate that a similar series of events could occur during endocytosis in dendritic spines. Calcineurin activity is required for expression of LTD and endocytosis of AMPA receptors (Beattie et al., 2000; Ehlers, 2000). It is possible that dephosphorylation of postsynaptic cargo and adaptors that regulate EZ dynamics is controlled by synaptic activity and Ca^{2+} levels. Although dynamin-1 is almost exclusively presynaptic, the related dynamin-3 contains conserved phosphorylation sites (Graham et al., 2007) and is concentrated in dendritic spines where it is required for spatial coupling of the EZ to the PSD (Lu et al., 2007). Moreover, syndapin-1/PACSIN-1, which associates with dynamin-1 in a phosphorylation-regulated manner in presynaptic terminals (Anggono et al., 2006), is also found in postsynaptic compartments (Perez-Otano et al., 2006). Postsynaptic exocytosis is also enhanced by influx of Ca^{2+} driven by potentiating stimuli (Maletic-Savatic et al., 1998; Park et al., 2006). Although the SNARE-

dependent exocytosis of synaptic vesicles in presynaptic terminals evoked by Ca^{2+} requires synaptotagmin (Yoshihara and Littleton, 2002; Rizo et al., 2006), the exact targets of Ca^{2+} regulation for spine exocytosis have yet to be determined.

As Ca^{2+} entry into spines can potentially drive both endocytosis and exocytosis, how are the relative rates of each balanced to express long-term potentiation and depression? Presumably, LTD effectors such as calcineurin are activated by smaller but more sustained calcium transients than the targets of AMPA receptor exocytosis activated during LTP. Moreover, it is likely that, much like AMPA receptors (Lee et al., 2000), the core machinery for endocytosis and exocytosis is itself a reversible target for kinase and phosphatase signaling during LTP and LTD. Indeed, LTD-inducing stimuli activate the endocytic Rab family GTPase Rab5 (Brown et al., 2005), whereas LTP-inducing stimuli augment generalized endosomal recycling (Park et al., 2004) (Figure 5). Thus, although both types of trafficking machinery are regulated by Ca^{2+}, the magnitude and kinetics of stimulation will ultimately determine which types of trafficking events are activated, and this activation will likely be reflected by a change in the rates of ongoing endocytosis or exocytosis or by the precise spatial location of membrane trafficking events. In addition, because exocytosis and endocytosis are not thought to occur immediately within the PSD, scaffold proteins within the PSD must also release or accept receptors in response to synaptic activity, whose exchange rate at the PSD could easily be influenced by tuning the available pool of extrasynaptic receptors through simple mass action.

In the case of LTD-inducing stimuli, endocytosis of extrasynaptic receptors would lessen the extrasynaptic pool and decrease the number of free receptors that could enter synapses, eventually leading to depletion of synaptic receptors. In addition, increased mobility of synaptic receptors driven by reversible chemical interactions would lead to decreased synaptic AMPA receptors and reduced dwell times at synapses (Figure 5). In the case of LTP induction, increased AMPA receptor membrane insertion along with decreased mobility of synaptic receptors could lead to receptor accumulation and increased dwell times at synapses (Figure 5). Further, activity-dependent regulation of the number of available scaffold binding sites (or “slots”) or their affinity for AMPA receptors may be the key gatekeepers of synaptic receptor content, whose effects would also be amplified through molecular crowding (Lisman and Raghavachari, 2006).

Similar to experimental data (Luscher et al., 1999), mathematical modeling suggests that blocking endocytosis will double the PSD AMPA receptor number within 1 hr, while blocking exocytosis halves synaptic AMPA receptors levels in less than 10 min (Earnshaw and Bressloff, 2006). The modeling also found that, during expression of plasticity, GluR1 insertion alone could not account for the increase of synaptic receptors that occurs during LTP. Additional mechanisms, such as increasing the number of PSD scaffold positions and synaptic affinity, would also be required. Modeling of LTD expression led to a similar conclusion, where decreases in synaptic receptor levels were only achieved in the long term if the number of synaptic slot positions in the PSD were also decreased (Earnshaw and Bressloff, 2006). One possibility is that receptor-scaffold complexes may be added coordinately, which would allow for large-scale changes in receptor abundance (Lisman and Raghavachari, 2006). Thus, both exocytosis of AMPA receptors and addition of receptor-binding proteins may contribute to the increase of GluR1-containing AMPA receptors that occurs during LTP. Such analysis also emphasizes the importance of receptor density (rather than absolute number) and nanopositioning as potential determinants of synaptic strength. Given the very low affinity of AMPA receptors for glutamate, the precise positioning or packing of AMPA receptors relative to presynaptic release sites may impact synaptic strength.

Conclusions and Remaining Questions

Advances in imaging technology now permit direct analysis of glutamate receptor movements in the plasma membrane. Besides supporting earlier findings demonstrating receptor exchange at synapses, these techniques have also allowed a glimpse of receptor behavior inside synapses. The relatively few types of receptors characterized by SPT have provided an expanded understanding of glutamate receptor behavior. In future studies, it will be important to analyze the mobility of the remaining glutamate receptors. In addition, other postsynaptic membrane proteins, such as adhesion molecules and ion channels that control plasticity, have yet to be analyzed in detail. Multiprobe imaging experiments or high-density single-molecule imaging techniques would allow simultaneous tracking of individual receptors in a whole population (Manley et al., 2008), providing a better understanding of how individual receptor movements reflect the behavior of the population. In addition, developing smaller probes to label receptors would facilitate tracking of single receptors as they undergo diffusion, endocytosis, and exocytosis (Lasne et al., 2006). Combined tracking of receptor lateral mobility and intracellular membrane trafficking will give a greater understanding of the dynamic movements of glutamate receptors and allow for visualization of receptor cycling in individual spines. Moreover, smaller probes have the advantage of increased synaptic access, which will allow for a more accurate and thorough analysis of synaptic receptor interactions.

At the interface of lateral diffusion and intracellular trafficking is spine endocytosis and exocytosis. We have only scratched the surface of possible functions of the spine endocytic zone (EZ). Are specific receptors excluded from EZ internalization? How does plasticity induction change the function and activity of the EZ? Does the spine EZ trap glutamate receptors? Does the size or position of the EZ affect trapping? Does the EZ provide a reserve pool of receptors under conditions where receptor-binding sites in the PSD, or slots, have become saturated? In addition, how often do such receptor slots change in the PSD and will it be possible to label free slots? What are the signaling pathways that regulate scaffold-receptor binding properties? Finally, is there a nanoarchitecture within the PSD that concentrates or rarefies receptors within synaptic subdomains, or perhaps generates receptor entry and exit points?

Even less is clear regarding the exocytosis of glutamate receptors. Where does such exocytosis occur? How is it regulated? What is the relevant trafficking machinery, and how does it interface with plasticity signaling? What is the spatial distribution of exocytic sources and endocytic sinks for diffusible pools of extrasynaptic glutamate receptors at individual synapses or across dendritic segments? Which intracellular compartments transport various types of AMPA and NMDA receptors?

Filling in these large gaps will move us closer to a more complete cell-biological and biophysical understanding of postsynaptic plasticity. Given the ever-expanding appreciation of glutamate receptor trafficking in the development and plasticity of diverse neural circuits, the pathological plasticity that occurs during addiction (Nestler, 2002; Lau and Zukin, 2007), neurobehavioral disorders including autism and mental retardations (Pardo and Eberhart, 2007), chronic pain syndromes (Bleakman et al., 2006), the diminished synaptic function during aging and early Alzheimer's disease (Almeida et al., 2005; Chang et al., 2006; Hsieh et al., 2006; Lau and Zukin, 2007; Shankar et al., 2007), the neural damage accompanying stroke and brain injury (Hardingham et al., 2002), and the altered states of mood and perception in depression and schizophrenia (Tsai and Coyle, 2002; Lau and Zukin, 2007), uncovering the molecular mechanisms underlying glutamate receptor mobility promises to unleash new insight into how the brain develops computes, stores, and suffers.

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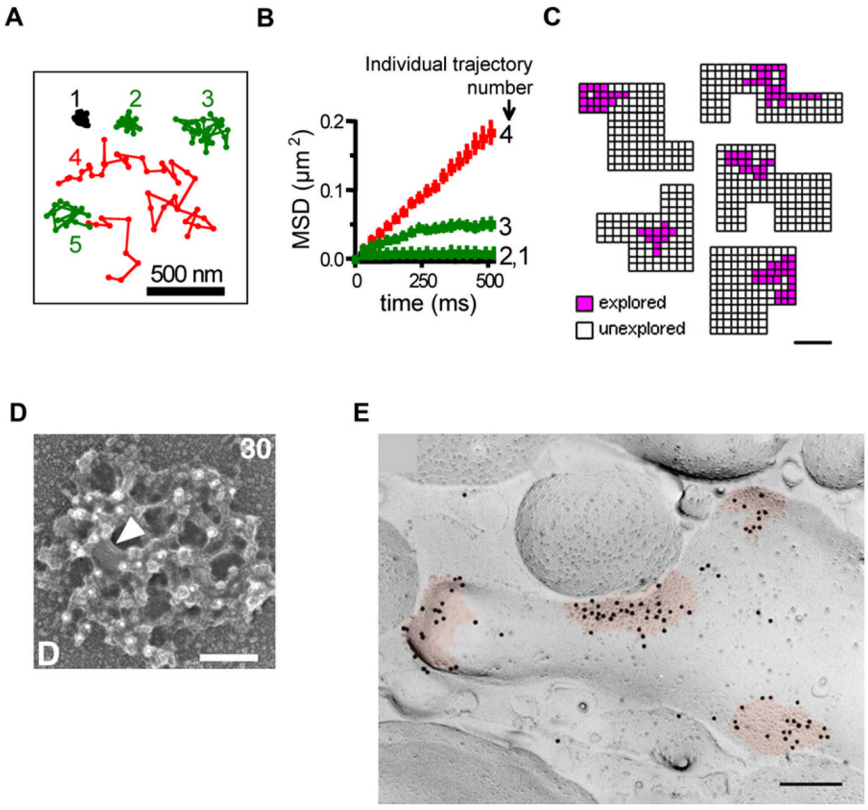

Figure 1. Nanoarchitecture of the PSD and Single-Particle Tracking of AMPA Receptors in Synaptic and Extrasynaptic Compartments

(A) AMPA receptor trajectories within synaptic and extrasynaptic compartments. As a control, immobilized Cy5-anti-GluR2 was fixed onto a coverslip (1). Examples 2–5 are trajectories from tracking of single Cy5-anti-GluR2 bound to AMPA receptors on living dendrites. The trajectories recorded in synaptic and extrasynaptic regions are shown in green and red, respectively. Examples 2 and 3 remained within synaptic sites, example 4 remained in the extrasynaptic membrane, and example 5 began in the extrasynaptic region and entered into a synaptic site.

(B) Plots of the mean square displacement (MSD) versus time corresponding to the examples shown in (A). Trajectories 2 and 3 remaining in synaptic regions had varying degrees of confinement and were less mobile than trajectory 4. Error bars are equal to the SEM.

(A) and (B) are adapted from Tardin et al. (2003); reprinted with permission from Nature Publishing Group, copyright 2003.

(C) Individual GluR1-QDs are restricted to subdomains within active synapses. Five individual synaptic regions defined as a set of connected pixels are indicated. Individual pixels divided into 0.0016 μm^2 subdomains were coded based on the presence (pink) or absence (white) of a GluR1-QD residing in that location at any time during the imaging period as defined by the centroid of a 2D Gaussian function fit to the GluR1-QD fluorescent signal. Scale bar, 200 nm. Adapted from Ehlers et al. (2007); reprinted with permission from Elsevier, copyright 2007.

(D) CaMKII immunogold labeling (white dots) on the cytoplasmic surface of a biochemically isolated PSD. Shown is the cytoplasmic surface of the PSD. A membrane patch is indicated by the arrowhead. Scale bar, 100 nm. Adapted from Petersen et al. (2003); reprinted with permission from the Society for Neuroscience, copyright 2003.

(E) AMPA receptor distribution at synapses (colored in red) in the molecular layer of the cerebellum shown by SDS-digested freeze-fracture replica labeling. Intra-membrane particles

are shown on the E face of the PSD and contain dark immunogold particles for pan-AMPA receptors (GluR1-4). Scale bar, 100 nm. Adapted from Masugi-Tokita et al. (2007); reprinted with permission from the Society for Neuroscience, copyright 2007.

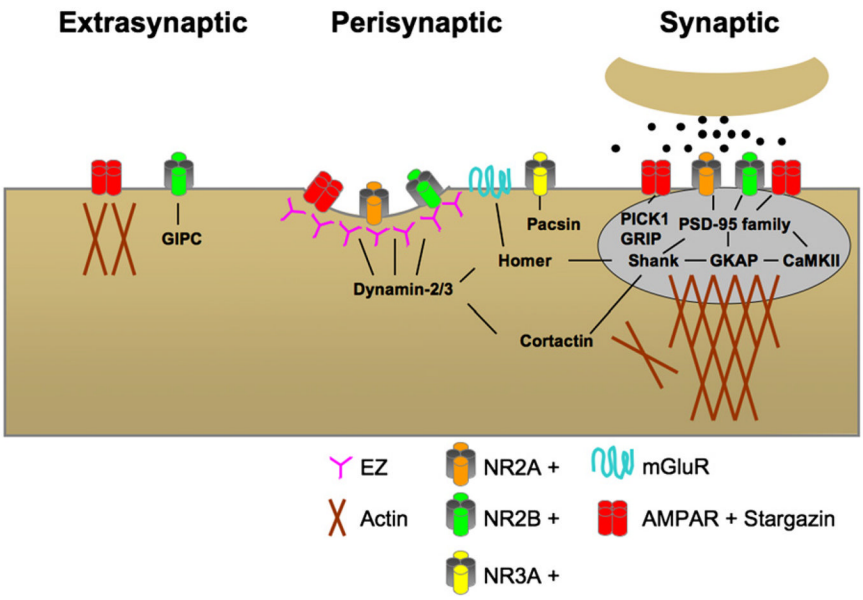

Figure 2. Glutamate Receptor Interactions in Synaptic, Perisynaptic, and Extrasynaptic Compartments

Distinct protein networks engage AMPA receptors and NMDA receptors in synaptic, perisynaptic, and extrasynaptic compartments. Direct protein interactions are shown by connecting lines. See text for details.

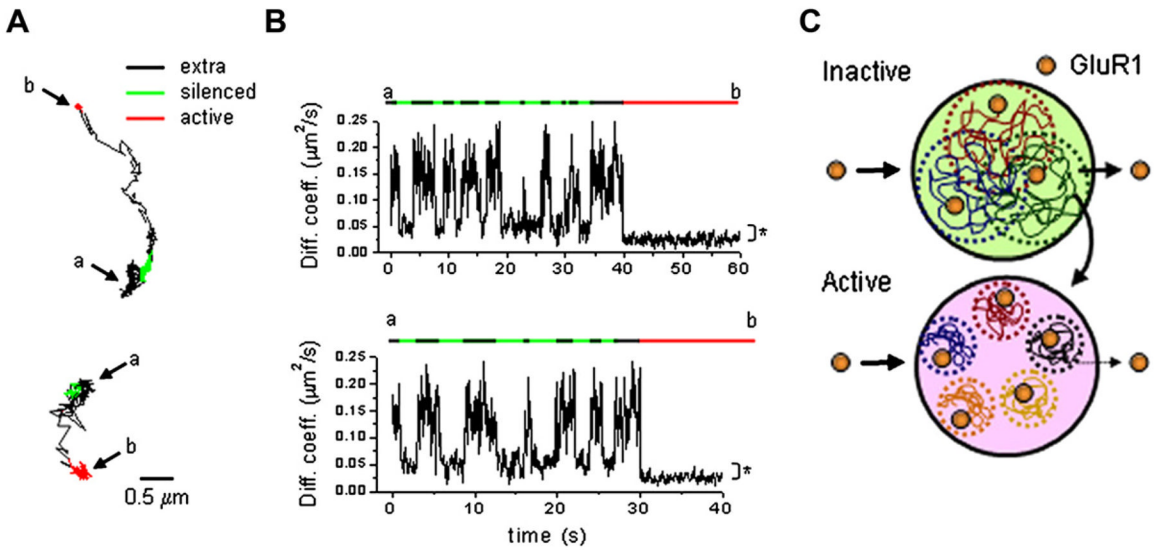

Figure 3. Active Synapses Capture GluR1-QDs by Diffusional Exchange

(A) Diffusion of GluR1 from silenced to active synapses. Trajectories of GluR1-QDs originating in a silenced synapse (green), escaping from the synapse (black, extrasynaptic), and transiting to an active synapse (red). The trajectory starts at point (a) and ends at point (b).

(B) Plots of the instantaneous diffusion coefficient versus time for the examples in (A). Bars correspond to extrasynaptic (black), silenced synapses (green), and active synapses (red).

(C) A model for GluR1 lateral diffusion at active and inactive synapses viewed en face. Input-specific spontaneous synaptic activity reduces receptor mobility, limits exchange with the extrasynaptic membrane, and confines GluR1 within small subdomains of the postsynaptic membrane. This diffusional trap leads to GluR1 accumulation at active synapses.

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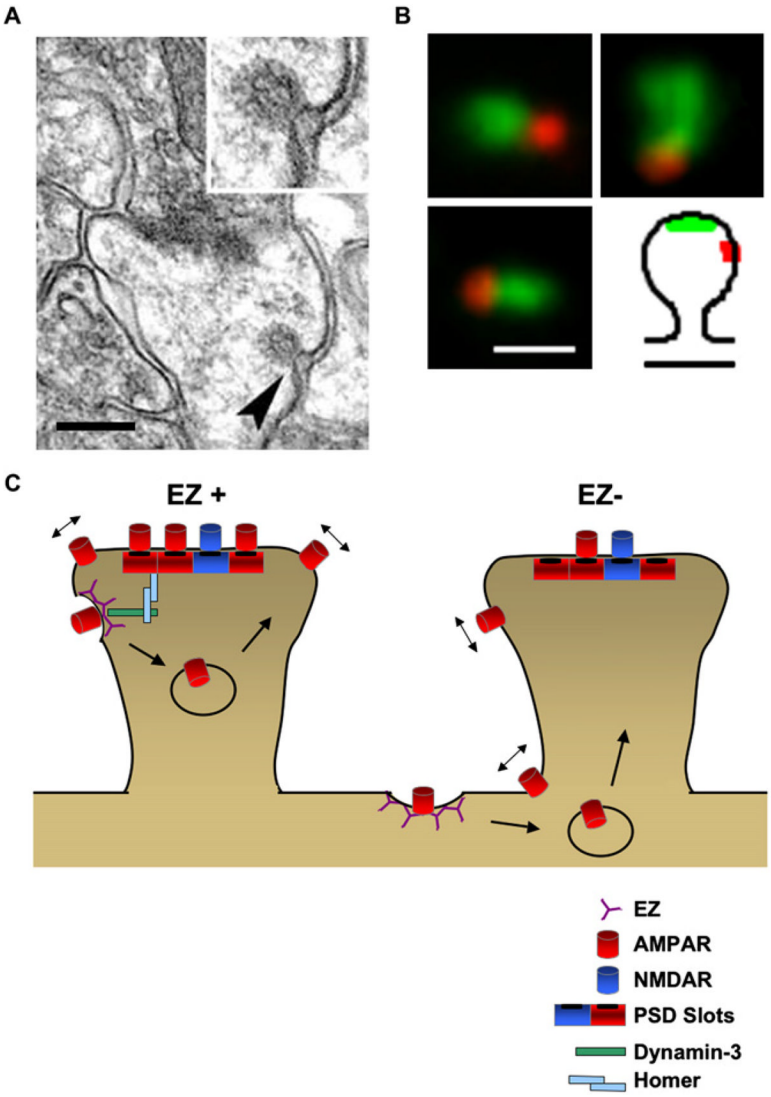

Figure 4. Spine-Localized Endocytic Zones Maintain Synaptic Glutamate Receptors

(A) Electron micrograph of a dendritic spine from CA1 hippocampus of adult brain. The arrowhead points to an invaginating clathrin-coated pit located adjacent to the electron-dense PSD. Scale bar, 200 nm. Adapted from Racz et al. (2004); reprinted with permission from Nature Publishing Group, copyright 2004.

(B) Examples of endocytic zones in spines marked by clathrin-DsRed (red), which are distinct from PSD-95-EGFP (green). Scale bar, 1 μm. Adapted from Blanpied et al. (2002).

(C) (Left) Endocytic zone positive (EZ+) spines maintain a local supply of extrasynaptic AMPA receptors. The spine EZ acts as a diffusional trap to recapture AMPA receptors that have escaped the synapse. Endocytic cycling through spines maintains a pool of local extrasynaptic AMPA receptors leading to increased filling of AMPA receptor slots in synapses. (Right) In the absence of an endocytic zone, receptors that escape the synapse are not recaptured, leading to a decrease in the extrasynaptic receptor abundance in spines and subsequently loss of AMPA receptors from the synapse. Model based on Lu et al. (2007).

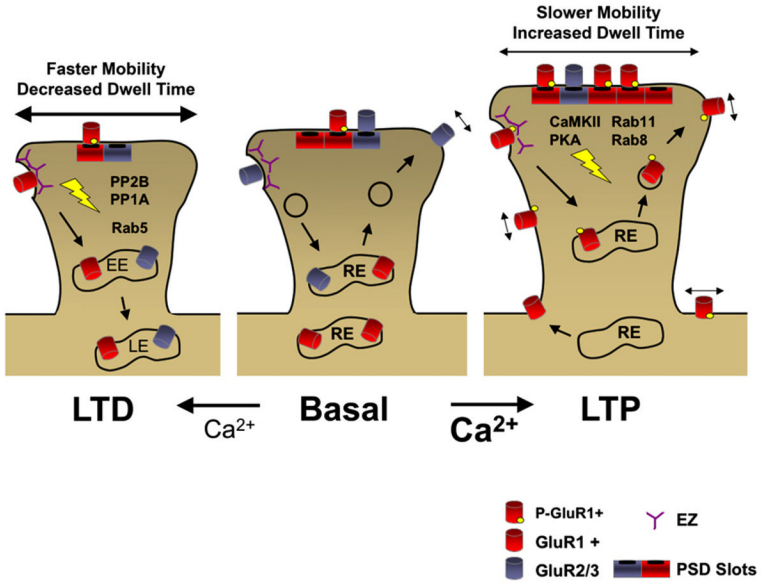

Figure 5. Integrating Models for Receptor Trafficking and Diffusion during Synaptic Plasticity

Induction of LTP by Ca^{2+} influx through NMDA receptors leads to activation (lightning bolt) of PKA and CaMKII, which in turn promotes the mobilization of recycling endosomes (RE) into spines, exocytosis from recycling endosomes, and appearance of AMPA receptors at the spine membrane. The number of available slots in the PSD increases through unknown mechanisms, which can be filled by increased levels of extrasynaptic AMPA receptors. Receptor diffusion inside synapses decreases due to stronger scaffold interactions and/or receptor confinement. The EZ may also contribute to LTP by maintaining local recycling of AMPA receptors and preventing their escape from the spine membrane. On the other hand, induction of LTD leads to activation of protein phosphatases (lightning bolt), including PP2B and PP1, triggering clathrin-, dynamin-, and Rab5-dependent endocytosis of AMPA receptors, likely at the spine EZ. Receptor downregulation occurs by trafficking through early (EE) and late endosomes (LE). Loss of synaptic slot positions through unknown mechanisms reduces AMPA receptor capacity and increases the diffusion of synaptic AMPA receptors. EZ, endocytic zone; P-GluR1, phosphorylated GluR1.