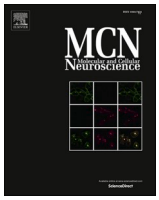
Molecular and Cellular Neuroscience 124 (2023) 103819

Contents lists available at ScienceDirect

Molecular and Cellular Neuroscience

journal homepage: www.elsevier.com/locate/ymcne



Review

Plasticity of postsynaptic nanostructure

W.J. Droogers, H.D. MacGillavry *

Division of Cell Biology, Neurobiology and Biophysics, Department of Biology, Faculty of Science, Utrecht University, 3584 CH, The Netherlands

ARTICLE INFO

Keywords:
Synapse
Synaptic plasticity
Postsynaptic density
Glutamate receptors
Super-resolution microscopy

ABSTRACT

The postsynaptic density (PSD) of excitatory synapses is built from a wide variety of scaffolding proteins, receptors, and signaling molecules that collectively orchestrate synaptic transmission. Seminal work over the past decades has led to the identification and functional characterization of many PSD components. In contrast, we know far less about how these constituents are assembled within synapses, and how this organization contributes to synapse function. Notably, recent evidence from high-resolution microscopy studies and *in silico* models, highlights the importance of the precise subsynaptic structure of the PSD for controlling the strength of synaptic transmission. Even further, activity-driven changes in the distribution of glutamate receptors are acknowledged to contribute to long-term changes in synaptic efficacy. Thus, defining the mechanisms that drive structural changes within the PSD are important for a molecular understanding of synaptic transmission and plasticity. Here, we review the current literature on how the PSD is organized to mediate basal synaptic transmission and how synaptic activity alters the nanoscale organization of synapses to sustain changes in synaptic strength.

1. Introduction

Excitatory synapses are remarkably heterogeneous in structure, molecular composition, and function. Even along single dendrites, the electrophysiological responses of individual synapses vary considerably (Holler et al., 2021; Matsuzaki et al., 2001) and correlate well with the morphological characteristics and molecular composition of synapses. The number of AMPA-type glutamate receptors found at hippocampal CA3-CA1 synapses for instance is highly variable but correlates strictly with synapse size (Nusser et al., 1998). Similarly, spine head area correlates with PSD area and AMPA receptor-mediated responses (Holler et al., 2021; Matsuzaki et al., 2001). Importantly, these postsynaptic structural aspects are modified by changes in synaptic activity. In particular, the long-term potentiation (LTP) and depression (LTD) of synapses are associated with prominent changes in synaptic composition and structure. Pioneering work at hippocampal synapses revealed the astonishing complexity of molecular pathways that underlie synaptic plasticity (Bosch and Hayashi, 2012; Nishiyama and Yasuda, 2015). Central to this process is the dynamic trafficking of AMPA receptors to and from the PSD to adjust synaptic strength (Huganir and Nicoll, 2013; Malinow and Malenka, 2002). Nevertheless, it is becoming increasingly clear that the absolute number of AMPA receptors at the PSD is not the sole determinant of synaptic efficacy. Since single presynaptic vesicle release events in the synaptic cleft lead to a narrow, transient peak in glutamate concentration, only a sub-population of the receptor pool that directly opposes the release site becomes maximally activated (Liu et al., 1999; Raghavachari and Lisman, 2004). Consequently, compacting AMPA receptors in a clustered configuration, *i.e.*, without changing the absolute number of receptors, is sufficient to potentiate excitatory postsynaptic currents (EPSCs) (MacGillavry et al., 2013; Savtchenko and Rusakov, 2014). Thus, even though canonically the expression of LTP is thought to be primarily mediated by an activity-induced increase in the number of postsynaptic AMPARs, synapses can be strongly potentiated by reorganizing the spatial distribution of synaptic components in the absence of an absolute increase in receptor number (Fig. 1).

In most synapses, there is indeed prominent nanoclustering of receptors. Super-resolution studies found that both AMPA receptors and scaffolding proteins such as PSD-95 are enriched in subsynaptic nanodomains (MacGillavry et al., 2013; Nair et al., 2013) that are transsynaptically aligned with presynaptic release sites into so-called nanocolumns (Tang et al., 2016). Recent exciting work is beginning to unravel how this heterogeneous organization of PSD components is established, and how this organization is modulated by plasticity-inducing stimuli. Chronic changes in synaptic activity that induce synaptic scaling profoundly alter the scaffold and receptor organization at synapses (Fukata et al., 2013; MacGillavry et al., 2013). Also, chemical LTD is associated with the reorganization and loss of synaptic PSD-95 (Compans et al., 2021; Tang et al., 2016). And, NMDA receptor-

* Corresponding author.
E-mail address: h.d.macgillavry@uu.nl (H.D. MacGillavry).

https://doi.org/10.1016/j.mcn.2023.103819
Received 1 November 2022; Received in revised form 19 January 2023; Accepted 23 January 2023
Available online 30 January 2023

mediated potentiation of synapses is associated with an increase in the number of nanodomains, and an increase in the alignment of presynaptic release machinery with PSD-95 (Hruska et al., 2018; Tang et al., 2016). Even further, environmental enrichment was found to be associated with an increase in structural reorganization of PSDs *in vivo*, suggesting that experience facilitates nano-structural remodeling of the PSD (Wegner et al., 2022). Here, we discuss how the nanoscale organization of the PSD determines the strength of synaptic transmission and explore how plasticity mechanisms could alter the organization of the PSD to express changes in synaptic strength (Fig. 2).

2. Subsynaptic distribution of AMPA and NMDA receptors

AMPA receptors are responsible for the fast excitatory responses to presynaptic stimuli, and the number of AMPA receptors close to glutamate release sites is an important determinant of synaptic strength. NMDA receptors act as synaptic coincident detectors and serve as a gate for the induction of synaptic plasticity and activation of NMDA receptors is key for the induction of the main forms of Hebbian synaptic plasticity (Mayer et al., 1984; Nowak et al., 1984). Importantly, considering the rapid decay of glutamate after vesicle release (Clements et al., 1992; Diamond and Jahr, 1997), and the low affinity for glutamate of in particular AMPA receptors (Traynelis et al., 2010), only receptors close to the release site are predicted to become activated (Raghavachari and Lisman, 2004; Santucci and Raghavachari, 2008). Indeed, these theoretical predictions have been confirmed by electrophysiological and calcium imaging studies that found that single release events do not saturate postsynaptic AMPA and NMDA receptors (Mainen et al., 1999; McAllister and Stevens, 2000; Nimchinsky et al., 2004). Thus, the subsynaptic distribution of AMPA and NMDA receptors directly impacts receptor activation and determines how the amplitude and variability of postsynaptic responses are modulated. The subsynaptic distribution of glutamate receptors has therefore been studied extensively using various methods in different preparations (Scheefhals and MacGillavry, 2018). In general, electron microscopy (EM) studies have pointed out that NMDA receptors are preferentially enriched in the center of the synapse with AMPA receptors residing more peripherally (Kharazia and Weinberg, 1997; Racca et al., 2000). The rapid developments in super-resolution microscopy in the past decade have further spurred the investigation of protein distributions at excitatory synapses. Particularly techniques such as single-molecule localization microscopy (SMLM), stimulated emission depletion (STED) and expansion microscopy now allow accurate investigation of synapse nanostructure in diverse cultured and *in vivo* preparations (Arizono et al., 2022; Sarkar et al., 2022). A general emerging notion from these studies is that the organization of the PSD is much more heterogeneous than previously was appreciated and that both AMPA and NMDA receptors are enriched in distinct nanodomains (Jezequel et al., 2017; Kellermayer et al., 2018; MacGillavry et al., 2013; Nair et al., 2013).

AMPA receptors are tetrameric complexes assembled from combinations of four subunits: GluA1–4. In the adult hippocampus, GluA1/2 and GluA2/3 hetero-tetramers are the most prevalent synaptic receptor types, with a smaller contribution of calcium-permeable GluA1 homotetramers (Lu et al., 2009). Moreover, native AMPA tetramers associate with a complement of auxiliary proteins that further diversify the functional properties and organization of synaptic AMPA receptors. Single-molecule tracking studies showed that AMPA receptors diffuse freely on the extrasynaptic membrane but are immobilized once they enter the PSD (Heine et al., 2008; Hoze et al., 2012; Tardin et al., 2003). In the PSD, AMPA receptors are anchored by scaffolding proteins in 80–100-nm sized nanodomains (MacGillavry et al., 2013; Nair et al., 2013). AMPAR nanodomains seem to generally localize laterally in the

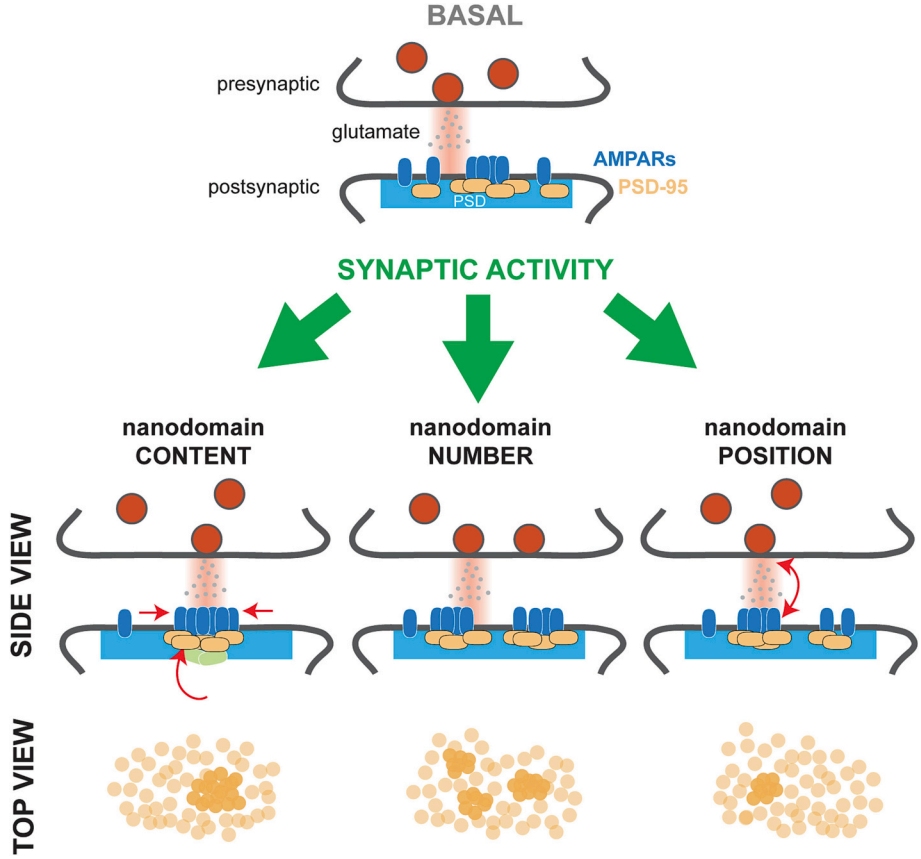


Fig. 1. Nanoscale reorganization of the PSD during synaptic plasticity. Synapses can change the content, number and position of postsynaptic nanodomains to increase or decrease neurotransmission efficacy in response to synaptic activity.

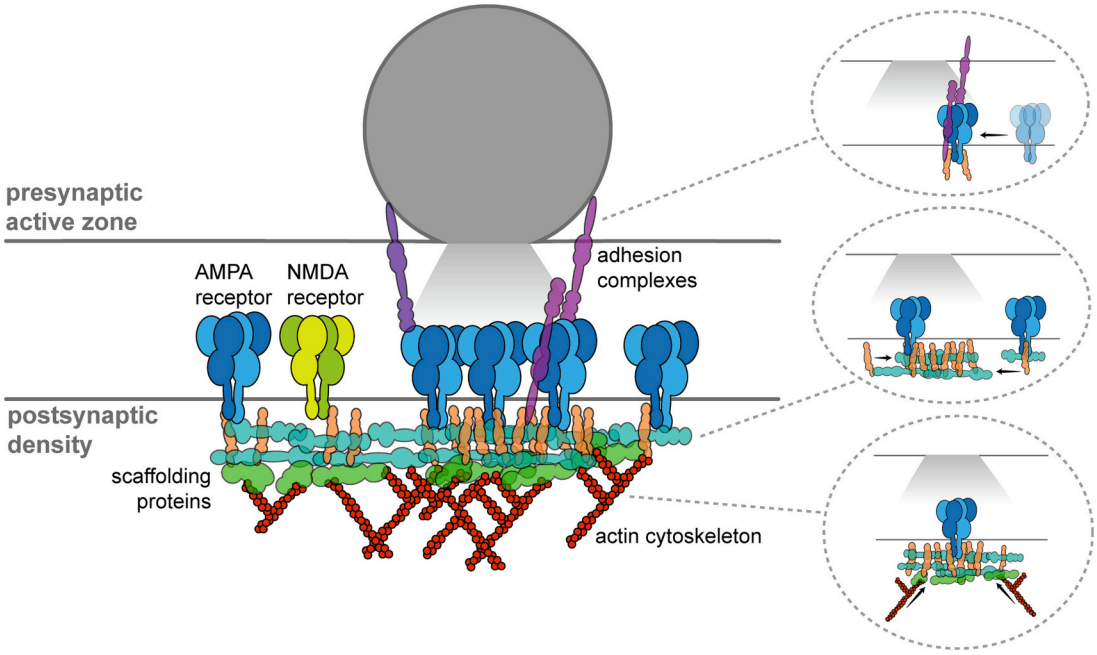


Fig. 2. Mechanisms of activity-induced formation and maintenance of postsynaptic nanodomains. Glutamate receptors and scaffolding proteins can be clustered in apposition of presynaptic release sites by transsynaptic adhesion complexes (top circle). Postsynaptic nanodomains can be formed by increased binding between scaffolding proteins (middle circle). Actin polymerization and nucleation can facilitate glutamate receptor clustering *via* scaffold-actin interactions (bottom circle).

synapse (Goncalves et al., 2020; Li et al., 2021). Super-resolution microscopy and EM studies have estimated that nanodomains contain 10–20 AMPA receptors (Compans et al., 2021; Getz et al., 2022; Martinez-Sanchez et al., 2021), and that synapses contain 1–3 nanodomains (Hruska et al., 2022; MacGillavry et al., 2013; Nair et al., 2013).

A key event in the potentiation of synapses is the subunit-specific recruitment of AMPA receptors to the postsynaptic membrane. The sequence diversity in the intracellular C-tail of GluA1/2 and GluA2/3 hetero-tetramers has drawn considerable attention, and models of subunit-specific rules for trafficking to and from synapses are based primarily on differences in intracellular interactions (Shi et al., 2001). Importantly however, the optogenetic, forced recruitment of GluA1 receptors to the PSD was shown to not affect the amplitude of miniature EPSCs (Sinnen et al., 2017), suggesting that the mere addition of receptors to a synapse does not necessarily potentiates transmission, but that adding receptors specifically to nanodomains aligned with presynaptic sites of vesicle release is required to enhance synaptic transmission. What dictates the precise positioning of receptors in subsynaptic nanodomains? The intracellular C-tails of AMPA receptors seem to be principally involved in surface trafficking, and do not mediate lateral diffusion or anchoring and positioning of AMPA receptors (Bats et al., 2007; Kerr and Blanpied, 2012). Rather, it is more likely that the AMPA receptor auxiliary proteins that interact with PSD scaffold proteins facilitate activity-driven changes in AMPA receptor positioning (Fig. 2). Phosphorylation of TARPs by CaMKII or PKC for instance promotes the interaction with PSD-95 and stabilizes AMPA receptors at the synapse (Chetkovich et al., 2002; Hafner et al., 2015; Opazo et al., 2010; Sainlos et al., 2011; Schnell et al., 2002; Watson et al., 2021), and is required for the expression of both electrically- and chemically-induced LTP (Rouach et al., 2005; Tomita et al., 2005). Further investigation into other auxiliary proteins is needed to determine the relative contribution of these proteins to subsynaptic AMPA receptor positioning.

NMDA receptors are predominantly hetero-tetramers comprising of the GluN1 subunit associated with GluN2 subunits or a combination of GluN2 and GluN3 subunits (Paoletti et al., 2013). In the hippocampus, GluN2A and GluN2B-containing receptors are most abundant and are thought to differentially govern plasticity. The relative abundance of GluN2A and GluN2B-containing receptors is regulated during development and plasticity, and influences not only the properties of NMDA currents, but also the ability to induce plasticity due to differential ability in recruiting signaling cascades. In mature hippocampal synapses, both GluN2A and GluN2B-containing receptors were found to form nanodomains (Kellermayer et al., 2018). Consistent with EM studies, this study showed that NMDA receptor nanodomains tend to reside in the center of the PSD and their number varied between 1 and 4 per synapse. GluN2A nanodomains were on average smaller and more numerous than GluN2B nanodomains. This study found little spatial overlap of GluN2A and GluN2B nanodomains (~30 %). This fraction could represent a population of tri-heterotetrameric, GluN2A- and GluN2B-containing NMDA receptors, although estimations from functional studies suggest a larger contribution of tri- heterotetrameric NMDA receptors to postsynaptic EPSCs (Gray et al., 2011; Rauner and Kohr, 2011). Intriguingly, the properties of GluN2B nanodomains, but not GluN2A nanodomains, scale along the length of the dendritic tree (Ferreira et al., 2020). GluN2B nanodomains at distal synapses were significantly smaller but contained a higher density of receptors than nanodomains at synapses close to the soma. Functionally, the distance-dependent scaling of GluN2B nanodomain properties was modulated by interactions with CaMKII and depended on neuronal network activity. These findings are not only of interest to understand mechanisms of synaptic plasticity but also bear relevance for understanding psychiatric disorders associated with NMDA dysfunction. For instance, autoantibodies isolated from patients with anti-NMDA encephalitis (Ladepeche et al., 2018), or from psychotic schizophrenia patients (Jezequel et al., 2017), were shown to severely disrupt the nanoscale organization and function of NMDA receptors.

The co-existence of functionally distinct receptor nanodomains at synapses strengthens the idea that the postsynaptic compartment is much more organized than previously anticipated. How the segregation of glutamate receptor subtypes contributes to the physiology of synapses and their ability to induce and express plasticity however remains largely unknown. The seemingly segregated nanodomain organization of AMPA and NMDA receptors (Hosokawa et al., 2021) suggests that

these distinct receptor domains could be coupled to functionally different release sites. Indeed, asynchronous and synchronous release sites were found to be preferentially aligned with AMPA receptors and NMDA receptors respectively (Li et al., 2021). This spatial segregation of synchronous and asynchronous transmission thus suggests that synapses can process AMPA receptor-mediated transmission and NMDA receptor-gated plasticity separately in both time and space, thereby corroborating the innate differences in glutamate receptor kinetics. More quantitative investigation of the distance between nanodomains within individual synapses could predict whether single glutamate release events can evoke exclusively AMPA- or NMDA-mediated synaptic currents.

3. Nanoscale organization of scaffolding proteins within the postsynaptic density

A defining hallmark of excitatory synapses is the PSD, a dense accumulation of proteins attached to the postsynaptic membrane that forms the structural platform for anchoring and positioning glutamate receptors. The PSD can be readily visualized as an electron-dense structure in EM and has been extensively characterized in ultrastructural studies. PSDs are generally disc-shaped structures that can be irregular in shape, with sizes ranging from 100 to 500 nm in diameter and 30–50 nm in thickness (Harris and Stevens, 1989). The structure and composition of the PSD is dynamic and can be rapidly and reversibly altered during synaptic plasticity (Araki et al., 2015; Bosch et al., 2014; Compans et al., 2021; Hruska et al., 2018; Steiner et al., 2008). Nevertheless, the activity-driven mechanisms that control this internal organization of the PSD are only starting to be elucidated.

Proteomics studies revealed that the PSD is composed of hundreds of different protein species with highly diverse stoichiometries (Cheng et al., 2006; Sheng and Hoogenraad, 2007). Multidomain scaffolding proteins define the shape, size and internal architecture of the PSD and are key for the function of excitatory synapses. The membrane-associated guanylate kinase (MAGUK) family member PSD-95 is one of the most abundant proteins in the PSD (Cheng et al., 2006) and anchors AMPARs at the synapse *via* interactions with auxiliary subunits such as TARPs (Bats et al., 2007; Schnell et al., 2002). In line with this, overexpression or depletion of PSD-95 severely impacts synaptic transmission and plasticity (Ehrlich et al., 2007; Stein et al., 2003; Sun and Turrigiano, 2011). PSD-95 is a prototypical scaffold protein that interacts with membrane proteins, a multitude of other scaffolds, signaling molecules and actin-binding proteins. PSD-95 is anchored at the synaptic membrane *via* palmitoylation (El-Husseini et al., 2002; El-Husseini et al., 2000a) and extends vertically into the PSD (Chen et al., 2008). The scaffold protein GKAP is situated deeper into the PSD and forms an intermediate scaffold that interacts with PSD-95 and couples it to Shank and Homer proteins that together form a stable multimeric platform (Hayashi et al., 2009; Naisbitt et al., 1999). This typical layered organization of scaffold proteins along the axo-dendritic axis has consistently been found by immuno-EM studies and SMLM studies (Dani et al., 2010; Petersen et al., 2003; Valtschanoff and Weinberg, 2001). This layered, but interconnected distribution allows intracellular actin dynamics to control membrane protein organization (Kerr and Blanpied, 2012) and could allow for layer-specific malleability of the PSD by differentially reorganizing scaffold proteins. To what extent the organization of individual scaffolding layers can be controlled independently remains unknown. Interestingly, it was found that Shank can interact with autoinhibited CaMKII, while activation of CaMKII promotes unbinding from Shank and conversely favors binding to GluN2B (Cai et al., 2021). These findings suggest that the regulated shuttling of CaMKII between different layers of the PSD could mediate layer-specific modulation. More broadly, activity-dependent post-translational modifications likely influence scaffold-scaffold interactions and impact scaffolding nanodomain compaction (Fig. 2).

PSD-95 is central in controlling the number of AMPA receptors at the synapse, and it is conceivable that PSD-95 instructs the lateral distribution of AMPA receptors through auxiliary subunits like TARPγ8 (Watson et al., 2021). Conventional microscopy approaches consistently found that the distributions of endogenous PSD-95 (using antibodies or genetic labeling approaches) and recombinantly expressed variants of PSD-95 are highly enriched in the PSD (Broadhead et al., 2016; El-Husseini et al., 2000b; Fortin et al., 2014; Willems et al., 2020). Spatial analysis of immunogold labeling EM for PSD-95 averaged across multiple PSDs indicated that PSD-95 is homogeneously distributed across the PSD, but quantitative analysis of immuno-EM data on isolated PSDs did report clustered distributions (DeGiorgis et al., 2006; Swulius et al., 2010). Moreover, EM tomography data show clear vertical filaments that most likely resemble PSD-95 proteins in their extended configuration. Indeed, PSD-95 knockdown leads to the loss of these vertical elements (Chen et al., 2011). Intriguingly, the loss of these vertical elements is not homogenous. Rather, PSD-95 knockdown leads to the loss of distinct 30–80-nm sized electron-dense patches that include PSD-95-associated horizontal elements and putative AMPA receptors. Similarly, a recent study combining EM tomography and SMLM showed that the signaling organizing protein AKAP79/150 is heterogeneously distributed within the PSD and also adopts a vertical, extended configuration at the PSD (Chen et al., 2022). These results further indicate that the subsynaptic distribution of PSD proteins is much more heterogeneous than was anticipated. Indeed, quantitative super-resolution studies revealed that the spatial distribution of PSD-95 within individual synapses is highly heterogeneous (Fukata et al., 2013; MacGillavry et al., 2013). At most synapses, PSD-95 is concentrated in distinct nanodomains, defined as clear peaks in the local density of PSD-95. The density of PSD-95 within these nanodomains is 3–4-fold higher than in the rest of the PSD (Droogers et al., 2022; MacGillavry et al., 2013). On average, synapses contain 1–3 scaffold nanodomains that are 80–100 nm in diameter. Strikingly, AMPARs are enriched in nanodomains of PSD-95 (MacGillavry et al., 2013; Nair et al., 2013), and their colocalization scales with synapse size (Droogers et al., 2022). Even further, nanodomains of other scaffold proteins like GKAP, Shank and Homer, are generally also aligned along the axo-dendritic axis suggesting that nanocolumns extend throughout the layers of the PSD (Tang et al., 2016).

Liquid-liquid phase separation (LLPS) as the driving force for compartmentalization in cellular processes is gaining considerable interest (Boeynaems et al., 2018). Also at synapses, LLPS has been described to play a role in organizing protein complexes into functional units (Feng et al., 2019). For the PSD, phase separation was first demonstrated using purified SynGAP and PSD-95 proteins. SynGAP was shown to bind PSD-95 as a trimer *via* multivalent interactions (Zeng et al., 2016). The mixing of these two components was sufficient to create highly concentrated liquid-like droplets. In a follow-up study, it was shown that mixing the core scaffolding proteins of the PSD also induced phase separation (Zeng et al., 2018). These condensates have PSD-like properties and were able to selectively solubilize the tail of the NMDA receptor, while excluding the tail of GABA receptors. Similarly, these scaffold condensates can capture TARP proteins *via* multivalent interactions in their C-tail (Zeng et al., 2019). These studies shed new light on how multivalent interactions could contribute to protein organization within the PSD, but how LLPS contributes to subsynaptic protein compartmentalization remains unknown.

4. Actin-PSD interactions shape synaptic nanoscale organization

The actin cytoskeleton in dendritic spines can dynamically interact with the PSD in several ways. Shank proteins interact with various actin-modulating proteins (Han et al., 2013; MacGillavry et al., 2016; Qualmann et al., 2004) and Shank3 was even found to bind F-actin directly (Salomaa et al., 2021). In addition, foci of high actin polymerization in the spine localize preferentially near the PSD and their number correlates with spine size (Frost et al., 2010). Functionally, these direct and indirect interactions of the actin cytoskeleton with the PSD allow actin

to support continuous dynamic changes in PSD morphology and control the subsynaptic distribution of scaffolds and receptors (Blanpied et al., 2008; Kerr and Blanpied, 2012; MacGillavry et al., 2016). Consistently, acute F-actin depolymerization preferentially destabilizes scaffolding proteins situated deep in the PSD (Kuriu et al., 2006), suggesting that actin dynamics can mold the PSD by exerting force on its cytoplasmic side. However, other actin-binding proteins such as α-actinin bind PSD-95 at its N-terminus, close to the plasma membrane (Matt et al., 2018), suggesting that actin could also exert force on the sub-membranous layers of the PSD. In addition, F-actin binds AKAP79/150 at its plasma membrane-associated N-terminus, thereby retaining it in the PSD (Gomez et al., 2002).

Prominent changes in actin polymerization and depolymerization are broadly thought to induce spine structural plasticity (Okamoto et al., 2004). Modest actin depolymerization indeed impairs the maintenance of LTP in the CA1 area of the hippocampus but leaves basic synaptic transmission intact (Krucker et al., 2000) and many actin regulators have been implicated in the expression of LTP (Yang and Liu, 2022). In addition, actin depolymerization is necessary for structural LTD and leads to loss of PSD-95 and AMPA receptors (Horne and Dell'Acqua, 2007; Zhou et al., 2004). Therefore, changes in actin dynamics likely aids in the expression of LTP and LTD, potentially in part by reshaping the subsynaptic AMPA receptor distribution. Given the direct interactions of F-actin with the PSD, activity-driven changes in actin dynamics could mediate the nanoscale reorganization of the PSD. Both α-actinin and the actin-nucleation factor Arp2/3 for instance are recruited to the spine upon structural LTP (Bosch et al., 2014). Indeed, super-resolution studies found that Arp2/3 locates in close proximity to PSD-95 nanodomains (Chazeau et al., 2014). Moreover, another actin-nucleation factor Abelson-interactor protein 1 (Abi1), which binds Shank3 (Wang et al., 2019), clusters in a single nanodomain that overlaps nearly completely with synaptic PSD-95 nanodomains. Since actin depolymerization leads to a decrease in the area and number of PSD-95 nanodomains (MacGillavry et al., 2013), it can be hypothesized that actin polymerization shapes and maintains the nanostructure of the PSD, whereas nucleation and anchoring of actin instructs activity-driven nanoscale reorganization (Fig. 2). Nevertheless, it remains to be investigated how actin-induced morphological changes of the PSD regulate positioning of AMPA receptors and mediate synaptic plasticity.

5. Transsynaptic coordination of synaptic efficacy

The alignment of postsynaptic receptor nanodomains with presynaptic release sites has been predicted to have profound consequences for the regulation of synaptic strength (Biederer et al., 2017; Tang et al., 2016). How is this precise spatial alignment established across the synaptic cleft? It is attractive to pose that adhesion complexes are involved in the transsynaptic linkage of pre- and postsynaptic nanodomains. Cryo-electron tomography visualized transsynaptic assemblies or 'sub-columns' that link presynaptic vesicles with putative receptors, lending direct structural support that transsynaptic complexes bridge the cleft to bidirectionally organize subsynaptic structures (Martinez-Sanchez et al., 2021). Molecular and functional studies have identified a diverse group of synaptic adhesion complexes (Jang et al., 2017). Most of these adhesion proteins, including the postsynaptic neuroligin (NL) family, SynCAM immunoglobulin proteins, and leucine-rich repeat transmembrane (LRRTM) proteins were first identified as drivers of synaptogenesis (Biederer et al., 2002; de Wit et al., 2009; Scheiffele et al., 2000). However, adhesion proteins are still abundant in mature synapses and are now acknowledged to also fulfill myriad functions in the transsynaptic coordination of synaptic transmission and plasticity (Biederer et al., 2017). In particular, the NL and LRRTM families, which both interact with PSD-95, are good candidates for organizing the nanostructure of synapses. In recent work, a role for NL1 was proposed in coordinating transsynaptic alignment of glutamate release and AMPA receptors. Using a dominant-negative approach and competing peptides it was shown that chronic disruption of neurexin-NL1 interactions alters transsynaptic nanostructure as resolved with SMLM (Haas et al., 2018). This supports a role for NL1 in transsynaptic alignment, although other effects of the chronic manipulation during development and maturation of synapses cannot be excluded. Compared to NL1, LRRTM2 was found to be more stably enriched in postsynaptic nanodomains that were more closely localized to the center of the synapse than NL1 (Chamma et al., 2016). In 3D SMLM experiments, LRRTM2 was indeed shown to be an intrinsic component of the nanocolumn, positioned in close alignment with both pre- and postsynaptic nanodomains (Ramsey et al., 2021). Furthermore, acute cleavage of the extracellular domain of LRRTM2 resulted in the progressive reduction of AMPA receptors aligned with presynaptic RIM1 nanodomains (Ramsey et al., 2021). This led to a concomitant reduction in the amplitude of evoked postsynaptic currents illustrating the functional role of intact nanocolumns formed by LRRTM2. The response amplitude of spontaneous release events did not change, which substantiates evidence for its distinct molecular mechanism and possibly involves separate nanocolumns (Ramirez and Kavalali, 2011).

Another intriguing possibility is that the N-terminal domain (NTD) of the AMPAR itself could be involved in transsynaptic interactions that control receptor positioning relative to the presynaptic release site (Fig. 2). This bulky extracellular domain makes up ~50% of the total receptor complex and extends prominently into the synaptic cleft, up to 15 nm, and thus effectively bridges the synaptic cleft. In addition, electrophysiological studies demonstrated that the NTD of AMPA receptor subunits contribute considerably to synaptic transmission and plasticity (Diaz-Alonso et al., 2017; Watson et al., 2017). This domain displays high sequence diversity between individual subunits (Greger et al., 2017) and in fact, removal of the NTD alters the subsynaptic distribution of AMPARs in a subunit-dependent manner (Watson et al., 2021). Deletion of the GluA1 NTD led to an increase in AMPA receptor nanodomain size and a decrease in receptor density. In contrast, removal of the GluA2 NTD did not affect AMPA receptor nanodomain organization. Whether this is mediated by a presynaptic interaction partner remains untested, but for instance presynaptic Pentraxins and C1ql family members have been found to interact with this domain (Lee et al., 2017; Martinelli et al., 2016). Moreover, the GluA1 NTD was found to interact with neuroplastin-65, a presynaptic adhesion molecule (Jiang et al., 2021). However, whether transsynaptic, NTD-mediated interactions instruct subsynaptic receptor positioning, or mediate activity-induced reorganization of synapse nanostructure remains unexplored. All in all, the AMPA receptor NTDs are likely to influence subsynaptic positioning of the receptor during synaptic plasticity.

6. Discussion/outlook

Numerous studies from the past two decades have implied that the synapse can change its efficacy by re-arranging the nanoscale organization of glutamate receptors. However, we have only begun to elucidate the mechanisms that determine and govern the functional nanoscale (re)organization, and even less is known about the signaling cascades that lead to these processes. Future studies will need to uncover the mechanisms necessary for the formation and maintenance of nanodomains, and to pinpoint the local signals that induce the coordinated reorganization of synapses during synaptic plasticity.

Currently however, a major challenge is to experimentally relate changes in the nanoscale organization of individual synapses to their function. Computational simulations have therefore been instrumental to unravel the functional aspects of synaptic nano-architecture (Franks et al., 2003; Raghavachari and Lisman, 2004; Savtchenko and Rusakov, 2014; Xie et al., 1997). Realistic simulations of synaptic transmission allow exploration of the parameter space in which a physiologically viable synapse can operate. In addition, synaptic efficacy can be measured quantitatively for an infinite number of experimental designs. For example, synaptic characteristics such as receptor number, type,

state and distribution, release site number and position, release probability and glutamate molecules per release event, can be varied freely. Furthermore, the movement and interaction probability of each synaptic component can be set according to literature and the research question that is being addressed. Thus, *in silico* simulations of synaptic transmission and plasticity can assess hypotheses about synaptic nanoorganization that wet-lab experiments cannot.

Further progress in the field will also be fueled by new developments in tools to reliably monitor and manipulate endogenous synaptic components to study the contribution of protein dynamics to the expression of synaptic plasticity. Current developments in CRISPR/Cas9-mediated genome editing now allow endogenous labeling of virtually any protein in neurons (Droogers et al., 2022; Fang et al., 2021; Gao et al., 2019; Willems et al., 2020; Zhong et al., 2021). These approaches hold great promise for resolving and manipulating the dynamic changes in subsynaptic protein distribution during synaptic plasticity as these tools overcome several experimental limitations associated with protein overexpression and antibody labeling such as undesired side-effects from protein overexpression or limited specificity of antibodies. Moreover, specifically for AMPA receptors, several knock-in mice have now been engineered to monitor endogenous surface receptor dynamics in slice systems as well as in *in vivo* learning paradigms (Getz et al., 2022; Graves et al., 2021). Even further, apart from monitoring protein distribution, these tools allow acute manipulation of protein distribution and dynamics. For example, inducible recruitment of AMPA receptor to synapses using chemical or optogenetic dimerization systems are very powerful tools to acutely control synaptic receptor content (Droogers et al., 2022; Sinnen et al., 2017). Also, changing receptor mobility in a whole culture, slice or brain using chemically induced dimerization of endogenous AMPA receptors was shown to acutely disrupt synaptic plasticity (Getz et al., 2022). These novel tools for manipulating subsynaptic receptor organization, acutely and chronically, will likely have broad applications *in vivo* and for behavioral studies, in addition to functional studies on the importance of nanodomain organization.

Disruptions in the molecular processes that control synaptic plasticity are a hallmark of cognitive disorders (Volk et al., 2015). In fact, mutations in more than 200 postsynaptic proteins have been associated with over 130 mental diseases (Bayes et al., 2011). Specifically, postsynaptic scaffolding proteins such as the Shank proteins have been implicated in autism spectrum disorder, schizophrenia and mental retardation (Berkel et al., 2010; Leblond et al., 2014). As glutamate receptor clustering close to release sites is regulated by postsynaptic scaffolding proteins, it is conceivable that impaired subsynaptic (re)organization of scaffolding proteins and glutamate receptors can lead to various synaptopathies. Future directions studying the possible pathogenic role of impaired subsynaptic (re)organization will provide a deeper understanding of disease mechanisms.

Declaration of competing interest

None.

Data availability

No data was used for the research described in the article.

Acknowledgements

We thank all members of the MacGillavry lab for their valuable input and discussion. This work was supported by the Dutch Research Council (OCENW.KLEIN.163).

References

Araki, Y., Zeng, M., Zhang, M., Huganir, R.L., 2015. Rapid dispersion of SynGAP from synaptic spines triggers AMPA receptor insertion and spine enlargement during LTP. Neuron 85, 173–189.
Arizono, M., Idziak, A., Quici, F., Nagerl, U.V., 2022. Getting sharper: the brain under the spotlight of super-resolution microscopy. Trends. Cell. Biol. 33 (2), 148–161.
Bats, C., Groc, L., Choquet, D., 2007. The interaction between stargazin and PSD-95 regulates AMPA receptor surface trafficking. Neuron 53, 719–734.
Bayes, A., van de Lagemaat, L.N., Collins, M.O., Croning, M.D., Whittle, I.R., Choudhary, J.S., Grant, S.G., 2011. Characterization of the proteome, diseases and evolution of the human postsynaptic density. Nat. Neurosci. 14, 19–21.
Berkel, S., Marshall, C.R., Weiss, B., Howe, J., Roeth, R., Moog, U., Endris, V., Roberts, W., Szatmari, P., Pinto, D., Bonin, M., Riess, A., Engels, H., Sprengel, R., Scherer, S.W., Rappold, G.A., 2010. Mutations in the SHANK2 synaptic scaffolding gene in autism spectrum disorder and mental retardation. Nat. Genet. 42, 489–491.
Biederer, T., Sara, Y., Mozhayeva, M., Atasoy, D., Liu, X., Kavalali, E.T., Sudhof, T.C., 2002. SynCAM, a synaptic adhesion molecule that drives synapse assembly. Science 297, 1525–1531.
Biederer, T., Kaeser, P.S., Blanpied, T.A., 2017. Transcellular nanoalignment of synaptic function. Neuron 96, 680–696.
Blanpied, T., Kerr, J., Ehlers, M., 2008. Structural plasticity with preserved topology in the postsynaptic protein network. Proc. Natl. Acad. Sci. U. S. A. 105, 12587–12592.
Boeynaems, S., Alberti, S., Fawzi, N.L., Mittag, T., Polymenidou, M., Rousseau, F., Schymkowitz, J., Shorter, J., Wolozin, B., Van Den Bosch, L., Tompa, P., Fuxreiter, M., 2018. Protein phase separation: a new phase in cell biology. Trends Cell Biol. 28, 420–435.
Bosch, M., Hayashi, Y., 2012. Structural plasticity of dendritic spines. Curr. Opin. Neurobiol. 22, 383–388.
Bosch, M., Castro, J., Saneyoshi, T., Matsuno, H., Sur, M., Hayashi, Y., 2014. Structural and molecular remodeling of dendritic spine substructures during long-term potentiation. Neuron 82, 444–459.
Broadhead, M.J., Horrocks, M.H., Zhu, F., Muresan, L., Benavides-Piccione, R., DeFelipe, J., Fricker, D., Kopanitsa, M.V., Duncan, R.R., Klenerman, D., Komiyama, N.H., Lee, S.F., Grant, S.G., 2016. PSD95 nanoclusters are postsynaptic building blocks in hippocampus circuits. Sci. Rep. 6, 24626.
Cai, Q., Zeng, M., Wu, X., Wu, H., Zhan, Y., Tian, R., Zhang, M., 2021. CaMKIIalpha-driven, phosphatase-checked postsynaptic plasticity via phase separation. Cell Res. 31, 37–51.
Chamma, I., Letellier, M., Butler, C., Tessier, B., Lim, K.H., Gauthereau, I., Choquet, D., Sibarita, J.B., Park, S., Sainlos, M., Thoumine, O., 2016. Mapping the dynamics and nanoscale organization of synaptic adhesion proteins using monomeric streptavidin. Nat. Commun. 7, 10773.
Chazeau, A., Mehidi, A., Nair, D., Gautier, J.J., Leduc, C., Chamma, I., Kage, F., Kechkar, A., Thoumine, O., Rottner, K., Choquet, D., Gautreau, A., Sibarita, J.B., Giannone, G., 2014. Nanoscale segregation of actin nucleation and elongation factors determines dendritic spine protrusion. EMBO J. 33, 2745–2764.
Chen, X., Winters, C., Azzam, R., Li, X., Galbraith, J., Leapman, R., Reese, T., 2008. Organization of the core structure of the postsynaptic density. Proc. Natl. Acad. Sci. U. S. A. 105, 4453–4458.
Chen, X., Nelson, C.D., Li, X., Winters, C.A., Azzam, R., Sousa, A.A., Leapman, R.D., Gainer, H., Sheng, M., Reese, T.S., 2011. PSD-95 is required to sustain the molecular organization of the postsynaptic density. J. Neurosci. 31, 6329–6338.
Chen, X., Crosby, K.C., Feng, A., Purkey, A.M., Aronova, M.A., Winters, C.A., Crocker, V. T., Leapman, R.D., Reese, T.S., Dell'Acqua, M.L., 2022. Palmitoylation of A-kinase anchoring protein 79/150 modulates its nanoscale organization, trafficking, and mobility in postsynaptic spines. Front. Synaptic Neurosci. 14, 1004154.
Cheng, D., Hoogenraad, C.C., Rush, J., Ramm, E., Schlager, M.A., Duong, D.M., Xu, P., Wijayawardana, S.R., Hanfelt, J., Nakagawa, T., Sheng, M., Peng, J., 2006. Relative and absolute quantification of postsynaptic density proteome isolated from rat forebrain and cerebellum. Mol. Cell. Proteomics 5, 1158–1170.
Chetkovich, D.M., Chen, L., Stocker, T.J., Nicoll, R.A., Bredt, D.S., 2002. Phosphorylation of the postsynaptic density-95 (PSD-95)/discs large/zona occludens-1 binding site of stargazin regulates binding to PSD-95 and synaptic targeting of AMPA receptors. J. Neurosci. 22, 5791–5796.
Clements, J.D., Lester, R.A., Tong, G., Jahr, C.E., Westbrook, G.L., 1992. The time course of glutamate in the synaptic cleft. Science 258, 1498–1501.
Compans, B., Camus, C., Kallergi, E., Sposini, S., Martineau, M., Butler, C., Kechkar, A., Klaassen, R.V., Retailleau, N., Sejnowski, T.J., Smit, A.B., Sibarita, J.-B., Bartol, T.M., Perrais, D., Nikoletopoulou, V., Choquet, D., Hosy, E., 2021. NMDAR-dependent long-term depression is associated with increased short term plasticity through autophagy mediated loss of PSD-95. In: Nature Communications. Nature Publishing Group, p. 2849.
Dani, A., Huang, B., Bergan, J., Dulac, C., Zhuang, X., 2010. Superresolution imaging of chemical synapses in the brain. Neuron 843–856.
de Wit, J., Sylwestrak, E., O'Sullivan, M., Otto, S., Tiglio, K., Savas, J., Yates, J.r., Comoletti, D., Taylor, P., Ghosh, A., 2009. LRRTM2 interacts with Neurexin1 and regulates excitatory synapse formation. Neuron 64, 799–806.
DeGiorgis, J.A., Galbraith, J.A., Dosemeci, A., Chen, X., Reese, T.S., 2006. Distribution of the scaffolding proteins PSD-95, PSD-93, and SAP97 in isolated PSDs. Brain Cell Biol. 35, 239–250.
Diamond, J.S., Jahr, C.E., 1997. Transporters buffer synaptically released glutamate on a submillisecond time scale. J. Neurosci. 17, 4672–4687.
Diaz-Alonso, J., Sun, Y.J., Granger, A.J., Levy, J.M., Blankenship, S.M., Nicoll, R.A., 2017. Subunit-specific role for the amino-terminal domain of AMPA receptors in synaptic targeting. Proc. Natl. Acad. Sci. U. S. A. 114, 7136–7141.

Droogers, W.J., Willems, J., MacGillavry, H.D., de Jong, A.P., 2022. Multiplex labeling and manipulation of endogenous neuronal proteins using sequential CRISPR/Cas9 gene editing. eNeuro 9. ENEURO.0056-22.2022.
Ehrlich, I., Klein, M., Rumpel, S., Malinow, R., 2007. PSD-95 is required for activity-driven synapse stabilization. Proc. Natl. Acad. Sci. U. S. A. 104, 4176–4181.
El-Husseini, A., Craven, S., Chetkovich, D., Firestein, B., Schnell, E., Aoki, C., Bredt, D., 2000a. Dual palmitoylation of PSD-95 mediates its vesiculotubular sorting, postsynaptic targeting, and ion channel clustering. J. Cell Biol. 148, 159–172.
El-Husseini, A., Schnell, E., Chetkovich, D., Nicoll, R., Bredt, D., 2000b. PSD-95 involvement in maturation of excitatory synapses. Science 290, 1364–1368.
El-Husseini, A.-D., Schnell, E., Dakoji, S., Sweeney, N., Zhou, Q., Prange, O., Gauthier-Campbell, C., Aguilera-Moreno, A., Nicoll, R., Bredt, D., 2002. Synaptic strength regulated by palmitate cycling on PSD-95. Cell 108, 849–863.
Fang, H., Bygrave, A.M., Roth, R.H., Johnson, R.C., Huganir, R.L., 2021. An optimized CRISPR/Cas9 approach for precise genome editing in neurons. elife 10.
Feng, Z., Chen, X., Zeng, M., Zhang, M., 2019. Phase separation as a mechanism for assembling dynamic postsynaptic density signalling complexes. Curr. Opin. Neurobiol. 57, 1–8.
Ferreira, J.S., Dupuis, J.P., Kellermayer, B., Benac, N., Manso, C., Bouchet, D., Levet, F., Butler, C., Sibarita, J.B., Groc, L., 2020. Distance-dependent regulation of NMDAR nanoscale organization along hippocampal neuron dendrites. Proc. Natl. Acad. Sci. U. S. A. 117, 24526–24533.
Fortin, D.A., Tillo, S.E., Yang, G., Rah, J.C., Melander, J.B., Bai, S., Soler-Cedeno, O., Qin, M., Zemelman, B.V., Guo, C., Mao, T., Zhong, H., 2014. Live imaging of endogenous PSD-95 using ENABLED: a conditional strategy to fluorescently label endogenous proteins. J. Neurosci. 34, 16698–16712.
Franks, K.M., Stevens, C.F., Sejnowski, T.J., 2003. Independent sources of quantal variability at single glutamatergic synapses. J. Neurosci. 23, 3186–3195.
Frost, N.A., Shroff, H., Kong, H., Betzig, E., Blanpied, T.A., 2010. Single-molecule discrimination of discrete perisynaptic and distributed sites of actin filament assembly within dendritic spines. Neuron 67, 86–99.
Fukata, Y., Dimitrov, A., Boncompain, G., Vielemeyer, O., Perez, F., Fukata, M., 2013. Local palmitoylation cycles define activity-regulated postsynaptic subdomains. J. Cell Biol. 202, 145–161.
Gao, Y., Hisey, E., Bradshaw, T.W.A., Erata, E., Brown, W.E., Courtland, J.L., Uezu, A., Xiang, Y., Diao, Y., Soderling, S.H., 2019. Plug-and-play protein modification using homology-independent universal genome engineering. Neuron 103 (583–597), e588.
Getz, A.M., Ducros, M., Breillat, C., Lampin-Saint-Amaux, A., Daburon, S., Francois, U., Nowacka, A., Fernandez-Monreal, M., Hosy, E., Lanore, F., Zieger, H.L., Sainlos, M., Humeau, Y., Choquet, D., 2022. High-resolution imaging and manipulation of endogenous AMPA receptor surface mobility during synaptic plasticity and learning. Sci. Adv. 8, eabm5298.
Gomez, L.L., Alam, S., Smith, K.E., Horne, E., Dell'Acqua, M.L., 2002. Regulation of A-kinase anchoring protein 79/150-cAMP-dependent protein kinase postsynaptic targeting by NMDA receptor activation of calcineurin and remodeling of dendritic actin. J. Neurosci. 22, 7027–7044.
Goncalves, J., Bartol, T.M., Camus, C., Levet, F., Menegolla, A.P., Sejnowski, T.J., Sibarita, J.B., Vivaudou, M., Choquet, D., Hosy, E., 2020. Nanoscale co-organization and coactivation of AMPAR, NMDAR, and mGluR at excitatory synapses. Proc. Natl. Acad. Sci. U. S. A. 117, 14503–14511.
Graves, A.R., Roth, R.H., Tan, H.L., Zhu, Q., Bygrave, A.M., Lopez-Ortega, E., Hong, I., Spiegel, A.C., Johnson, R.C., Vogelstein, J.T., Tward, D.J., Miller, M.I., Huganir, R.L., 2021. Visualizing synaptic plasticity in vivo by large-scale imaging of endogenous AMPA receptors. elife 10.
Gray, J.A., Shi, Y., Usui, H., During, M.J., Sakimura, K., Nicoll, R.A., 2011. Distinct modes of AMPA receptor suppression at developing synapses by GluN2A and GluN2B: single-cell NMDA receptor subunit deletion in vivo. Neuron 71, 1085–1101.
Greger, I.H., Watson, J.F., Cull-Candy, S.G., 2017. Structural and functional architecture of AMPA-type glutamate receptors and their auxiliary proteins. Neuron 94, 713–730.
Haas, K.T., Compans, B., Letellier, M., Bartol, T.M., Grillo-Bosch, D., Sejnowski, T.J., Sainlos, M., Choquet, D., Thoumine, O., Hosy, E., 2018. Pre-post synaptic alignment through neuroligin-1 tunes synaptic transmission efficiency. In: eLife. eLife Sciences Publications, Ltd.
Hafner, A.S., Penn, A.C., Grillo-Bosch, D., Retailleau, N., Poujol, C., Philippat, A., Coussen, F., Sainlos, M., Opazo, P., Choquet, D., 2015. Lengthening of the stargazin cytoplasmic tail increases synaptic transmission by promoting interaction to deeper domains of PSD-95. Neuron 86, 475–489.
Han, K., Holder Jr., J.L., Schaaf, C.P., Lu, H., Chen, H., Kang, H., Tang, J., Wu, Z., Hao, S., Cheung, S.W., Yu, P., Sun, H., Breman, A.M., Patel, A., Lu, H.C., Zoghbi, H.Y., 2013. SHANK3 overexpression causes manic-like behaviour with unique pharmacogenetic properties. Nature 503, 72–77.
Harris, K.M., Stevens, J.K., 1989. Dendritic spines of CA 1 pyramidal cells in the rat hippocampus: serial electron microscopy with reference to their biophysical characteristics. J. Neurosci. 9, 2982–2997.
Hayashi, M.K., Tang, C., Verpelli, C., Narayanan, R., Stearns, M.H., Xu, R.M., Li, H., Sala, C., Hayashi, Y., 2009. The postsynaptic density proteins Homer and Shank form a polymeric network structure. Cell 137, 159–171.
Heine, M., Groc, L., Frischknecht, R., Béïque, J., Lounis, B., Rumbaugh, G., Huganir, R., Cognet, L., Choquet, D., 2008. Surface mobility of postsynaptic AMPAwRs tunes synaptic transmission. Science 320, 201–205.
Holler, S., Kostinger, G., Martin, K.A.C., Schuhknecht, G.F.P., Stratford, K.J., 2021. Structure and function of a neocortical synapse. Nature 591, 111–116.
Horne, E.A., Dell'Acqua, M.L., 2007. Phospholipase C is required for changes in postsynaptic structure and function associated with NMDA receptor-dependent long-term depression. J. Neurosci. 27, 3523–3534.
Hosokawa, T., Liu, P.W., Cai, Q., Ferreira, J.S., Levet, F., Butler, C., Sibarita, J.B., Choquet, D., Groc, L., Hosy, E., Zhang, M., Hayashi, Y., 2021. CaMKII activation persistently segregates postsynaptic proteins via liquid phase separation. Nat. Neurosci. 24, 777–785.
Hoze, N., Nair, D., Hosy, E., Sieben, C., Manley, S., Herrmann, A., Sibarita, J.B., Choquet, D., Holcman, D., 2012. Heterogeneity of AMPA receptor trafficking and molecular interactions revealed by superresolution analysis of live cell imaging. Proc. Natl. Acad. Sci. U. S. A. 109, 17052–17057.
Hruska, M., Henderson, N., Le Marchand, S.J., Jafri, H., Dalva, M.B., 2018. Synaptic nanomodules underlie the organization and plasticity of spine synapses. Nat. Neurosci. 21, 671–682.
Hruska, M., Cain, R.E., Dalva, M.B., 2022. Nanoscale rules governing the organization of glutamate receptors in spine synapses are subunit specific. Nat. Commun. 13, 920.
Huganir, R.L., Nicoll, R.A., 2013. AMPARs and synaptic plasticity: the last 25 years. Neuron 80, 704–717.
Jang, S., Lee, H., Kim, E., 2017. Synaptic adhesion molecules and excitatory synaptic transmission. Curr. Opin. Neurobiol. 45, 45–50.
Jezequel, J., Johansson, E.M., Dupuis, J.P., Rogemond, V., Grea, H., Kellermayer, B., Hamdani, N., Le Guen, E., Rabu, C., Lepleux, M., Spatola, M., Mathias, E., Bouchet, D., Ramsey, A.J., Yolken, R.H., Tamouza, R., Dalmau, J., Honnorat, J., Leboyer, M., Groc, L., 2017. Dynamic disorganization of synaptic NMDA receptors triggered by autoantibodies from psychotic patients. Nat. Commun. 8, 1791.
Jiang, C.H., Wei, M., Zhang, C., Shi, Y.S., 2021. The amino-terminal domain of GluA1 mediates LTP maintenance via interaction with neuroplastin-65. Proc. Natl. Acad. Sci. U S A 118.
Kellermayer, B., Ferreira, J.S., Dupuis, J., Levet, F., Grillo-Bosch, D., Bard, L., Linares-Loyez, J., Bouchet, D., Choquet, D., Rusakov, D.A., Bon, P., Sibarita, J.B., Cognet, L., Sainlos, M., Carvalho, A.L., Groc, L., 2018. Differential nanoscale topography and functional role of GluN2-NMDA receptor subtypes at glutamatergic synapses. Neuron 100, 106–119 e107.
Kerr, J.M., Blanpied, T.A., 2012. Subsynaptic AMPA receptor distribution is acutely regulated by actin-driven reorganization of the postsynaptic density. J. Neurosci. 32, 658–673.
Kharazia, V.N., Weinberg, R.J., 1997. Tangential synaptic distribution of NMDA and AMPA receptors in rat neocortex. Neurosci. Lett. 238, 41–44.
Krucker, T., Siggins, G.R., Halpain, S., 2000. Dynamic actin filaments are required for stable long-term potentiation (LTP) in area CA1 of the hippocampus. Proc. Natl. Acad. Sci. U. S. A. 97, 6856–6861.
Kuriu, T., Inoue, A., Bito, H., Sobue, K., Okabe, S., 2006. Differential control of postsynaptic density scaffolds via actin-dependent and -independent mechanisms. J. Neurosci. 26, 7693–7706.
Ladepeche, L., Planaguma, J., Thakur, S., Suarez, I., Hara, M., Borbely, J.S., Sandoval, A., Laparra-Cuervo, L., Dalmau, J., Lakadamyali, M., 2018. NMDA receptor autoantibodies in autoimmune encephalitis cause a subunit-specific nanoscale redistribution of NMDA receptors. Cell Rep. 23, 3759–3768.
Leblond, C.S., Nava, C., Polge, A., Gauthier, J., Huguet, G., Lumbroso, S., Giuliano, F., Stordeur, C., Depienne, C., Mouzat, K., Pinto, D., Howe, J., Lemiere, N., Durand, C. M., Guibert, J., Ey, E., Toro, R., Peyre, H., Mathieu, A., Amsellem, F., Rastam, M., Gillberg, I.C., Rappold, G.A., Holt, R., Monaco, A.P., Maestrini, E., Galan, P., Heron, D., Jacquette, A., Afenjar, A., Rastetter, A., Brice, A., Devillard, F., Assouline, B., Laffargue, F., Lespinasse, J., Chiesa, J., Rivier, F., Bonneau, D., Regnault, B., Zelenika, D., Delepine, M., Lathrop, M., Sanlaville, D., Schluth-Bolard, C., Edery, P., Perrin, L., Tabet, A.C., Schmeisser, M.J., Boeckers, T.M., Coleman, M., Sato, D., Szatmari, P., Scherer, S.W., Rouleau, G.A., Betancur, C., Leboyer, M., Gillberg, C., Delorme, R., Bourgeron, T., 2014. Meta-analysis of SHANK mutations in autism spectrum disorders: a gradient of severity in cognitive impairments. PLoS Genet. 10, e1004580.
Lee, S.J., Wei, M., Zhang, C., Maxeiner, S., Pak, C., Calado Botelho, S., Trotter, J., Sterky, F.H., Sudhof, T.C., 2017. Presynaptic neuronal pentraxin receptor organizes excitatory and inhibitory synapses. J. Neurosci. 37, 1062–1080.
Li, S., Raychaudhuri, S., Lee, S.A., Brockmann, M.M., Wang, J., Kusick, G., Prater, C., Syed, S., Falahati, H., Ramos, R., Bartol, T.M., Hosy, E., Watanabe, S., 2021. Asynchronous release sites align with NMDA receptors in mouse hippocampal synapses. Nature communications. Nat. Res. 1–13.
Liu, G., Choi, S., Tsien, R.W., 1999. Variability of neurotransmitter concentration and nonsaturation of postsynaptic AMPA receptors at synapses in hippocampal cultures and slices. Neuron 22, 395–409.
Lu, W., Shi, Y., Jackson, A.C., Bjorgan, K., During, M.J., Sprengel, R., Seeburg, P.H., Nicoll, R.A., 2009. Subunit composition of synaptic AMPA receptors revealed by a single-cell genetic approach. Neuron 62, 254–268.
MacGillavry, H.D., Song, Y., Raghavachari, S., Blanpied, T.A., 2013. Nanoscale scaffolding domains within the postsynaptic density concentrate synaptic AMPA receptors. Neuron 78, 615–622.
MacGillavry, H.D., Kerr, J.M., Kassner, J., Frost, N.A., Blanpied, T.A., 2016. Shank-cortactin interactions control actin dynamics to maintain flexibility of neuronal spines and synapses. Eur. J. Neurosci. 43, 179–193.
Mainen, Z.F., Malinow, R., Svoboda, K., 1999. Synaptic calcium transients in single spines indicate that NMDA receptors are not saturated. Nature 399, 151–155.
Malinow, R., Malenka, R.C., 2002. AMPA receptor trafficking and synaptic plasticity. Annu. Rev. Neurosci. 25, 103–126.
Martinelli, D.C., Chew, K.S., Rohlmann, A., Lum, M.Y., Ressl, S., Hattar, S., Brunger, A.T., Missler, M., Sudhof, T.C., 2016. Expression of C1ql3 in discrete neuronal populations controls efferent synapse numbers and diverse behaviors. Neuron 91, 1034–1051.
Martinez-Sanchez, A., Laugks, U., Kochovski, Z., Papantoniou, C., Zinzula, L., Baumeister, W., Lučić, V., 2021. Trans-synaptic assemblies link synaptic vesicles and

neuroreceptors. In: Science Advances. American Association for the Advancement of Science.
Matsuzaki, M., Ellis-Davies, G.C., Nemoto, T., Miyashita, Y., Iino, M., Kasai, H., 2001. Dendritic spine geometry is critical for AMPA receptor expression in hippocampal CA1 pyramidal neurons. Nat. Neurosci. 4, 1086–1092.
Matt, L., Kim, K., Hergarden, A.C., Patriarchi, T., Malik, Z.A., Park, D.K., Chowdhury, D., Buonarati, O.R., Henderson, P.B., Gokcek Sarac, C., Zhang, Y., Mohapatra, D., Horne, M.C., Ames, J.B., Hell, J.W., 2018. alpha-Actinin anchors PSD-95 at postsynaptic sites. Neuron 97, 1094–1109 e1099.
Mayer, M.L., Westbrook, G.L., Guthrie, P.B., 1984. Voltage-dependent block by Mg2+ of NMDA responses in spinal cord neurones. Nature 309, 261–263.
McAllister, A.K., Stevens, C.F., 2000. Nonsaturation of AMPA and NMDA receptors at hippocampal synapses. Proc. Natl. Acad. Sci. U. S. A. 97, 6173–6178.
Nair, D., Hosy, E., Petersen, J.D., Constals, A., Giannone, G., Choquet, D., Sibarita, J.-B., 2013. Super-resolution imaging reveals that AMPA receptors inside synapses are dynamically organized in nanodomains regulated by PSD95. J. Neurosci. 13204–13224.
Naisbitt, S., Kim, E., Tu, J., Xiao, B., Sala, C., Valtschanoff, J., Weinberg, R., Worley, P., Sheng, M., 1999. Shank, a novel family of postsynaptic density proteins that binds to the NMDA receptor/PSD-95/GKAP complex and cortactin. Neuron 23, 569–582.
Nimchinsky, E.A., Yasuda, R., Oertner, T.G., Svoboda, K., 2004. The number of glutamate receptors opened by synaptic stimulation in single hippocampal spines. J. Neurosci. 24, 2054–2064.
Nishiyama, J., Yasuda, R., 2015. Biochemical computation for spine structural plasticity. Neuron 87, 63–75.
Nowak, L., Bregestovski, P., Ascher, P., Herbet, A., Prochiantz, A., 1984. Magnesium gates glutamate-activated channels in mouse central neurones. Nature 307, 462–465.
Nusser, Z., Lujan, R., Laube, G., Roberts, J.D., Molnar, E., Somogyi, P., 1998. Cell type and pathway dependence of synaptic AMPA receptor number and variability in the hippocampus. Neuron 21, 545–559.
Okamoto, K., Nagai, T., Miyawaki, A., Hayashi, Y., 2004. Rapid and persistent modulation of actin dynamics regulates postsynaptic reorganization underlying bidirectional plasticity. Nat. Neurosci. 7, 1104–1112.
Opazo, P., Labrecque, S., Tigaret, C.M., Frouin, A., Wiseman, P.W., De Koninck, P., Choquet, D., 2010. CaMKII triggers the diffusional trapping of surface AMPARs through phosphorylation of stargazin. Neuron 67, 239–252.
Paoletti, P., Bellone, C., Zhou, Q., 2013. NMDA receptor subunit diversity: impact on receptor properties, synaptic plasticity and disease. Nat. Rev. Neurosci. 14, 383–400.
Petersen, J.D., Chen, X., Vinade, L., Dosemeci, A., Lisman, J.E., Reese, T.S., 2003. Distribution of postsynaptic density (PSD)-95 and Ca2+/calmodulin-dependent protein kinase II at the PSD. J. Neurosci. 23, 11270–11278.
Qualmann, B., Boeckers, T.M., Jeromin, M., Gundelfinger, E.D., Kessels, M.M., 2004. Linkage of the actin cytoskeleton to the postsynaptic density via direct interactions of Abp1 with the ProSAP/Shank family. J. Neurosci. 24, 2481–2495.
Racca, C., Stephenson, F.A., Streit, P., Roberts, J.D., Somogyi, P., 2000. NMDA receptor content of synapses in stratum radiatum of the hippocampal CA1 area. J. Neurosci. 20, 2512–2522.
Raghavachari, S., Lisman, J.E., 2004. Properties of quantal transmission at CA1 synapses. J. Neurophysiol. 92, 2456–2467.
Ramirez, D.M., Kavalali, E.T., 2011. Differential regulation of spontaneous and evoked neurotransmitter release at central synapses. Curr. Opin. Neurobiol. 21, 275–282.
Ramsey, A.M., Tang, A.H., LeGates, T.A., Gou, X.Z., Carbone, B.E., Thompson, S.M., Biederer, T., Blanpied, T.A., 2021. Subsynaptic positioning of AMPARs by LRRTM2 controls synaptic strength. Sci. Adv. 7.
Rauner, C., Kohr, G., 2011. Triheteromeric NR1/NR2A/NR2B receptors constitute the major N-methyl-D-aspartate receptor population in adult hippocampal synapses. J. Biol. Chem. 286, 7558–7566.
Rouach, N., Byrd, K., Petralia, R.S., Elias, G.M., Adesnik, H., Tomita, S., Karimzadegan, S., Kealey, C., Bredt, D.S., Nicoll, R.A., 2005. TARP gamma-8 controls hippocampal AMPA receptor number, distribution and synaptic plasticity. Nat. Neurosci. 8, 1525–1533.
Sainlos, M., Tigaret, C., Poujol, C., Olivier, N.B., Bard, L., Breillat, C., Thiolon, K., Choquet, D., Imperiali, B., 2011. Biomimetic divalent ligands for the acute disruption of synaptic AMPAR stabilization. Nat. Chem. Biol. 7, 81–91.
Salomaa, S.I., Miihkinen, M., Kremneva, E., Paatero, I., Lilja, J., Jacquemet, G., Vuorio, J., Antenucci, L., Kogan, K., Hassani Nia, F., Hollos, P., Isomursu, A., Vattulainen, I., Coffey, E.T., Kreienkamp, H.J., Lappalainen, P., Ivaska, J., 2021. SHANK3 conformation regulates direct actin binding and crosstalk with Rap1 signaling. Curr. Biol. 31, 4956–4970 e4959.
Santucci, D.M., Raghavachari, S., 2008. The effects of NR2 subunit-dependent NMDA receptor kinetics on synaptic transmission and CaMKII activation. PLoS Comput. Biol. 4, e1000208.
Sarkar, D., Kang, J., Wassie, A.T., Schroeder, M.E., Peng, Z., Tarr, T.B., Tang, A.H., Niederst, E.D., Young, J.Z., Su, H., Park, D., Yin, P., Tsai, L.H., Blanpied, T.A., Boyden, E.S., 2022. Revealing nanostructures in brain tissue via protein decrowding by iterative expansion microscopy. Nat. Biomed. Eng. 6, 1057–1073.
Savtchenko, L.P., Rusakov, D.A., 2014. Moderate AMPA receptor clustering on the nanoscale can efficiently potentiate synaptic current. Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci. 369, 20130167.
Scheefhals, N., MacGillavry, H.D., 2018. Functional organization of postsynaptic glutamate receptors. Mol. Cell. Neurosci. 91, 82–94.
Scheiffele, P., Fan, J., Choih, J., Fetter, R., Serafini, T., 2000. Neuroligin expressed in nonneuronal cells triggers presynaptic development in contacting axons. Cell 101, 657–669.
Schnell, E., Sizemore, M., Karimzadegan, S., Chen, L., Bredt, D., Nicoll, R., 2002. Direct interactions between PSD-95 and stargazin control synaptic AMPA receptor number. Proc. Natl. Acad. Sci. U. S. A. 99, 13902–13907.
Sheng, M., Hoogenraad, C., 2007. The postsynaptic architecture of excitatory synapses: a more quantitative view. Annu. Rev. Biochem. 76, 823–847.
Shi, S., Hayashi, Y., Esteban, J.A., Malinow, R., 2001. Subunit-specific rules governing AMPA receptor trafficking to synapses in hippocampal pyramidal neurons. Cell 105, 331–343.
Sinnen, B.L., Bowen, A.B., Forte, J.S., Hiester, B.G., Crosby, K.C., Gibson, E.S., Dell'Acqua, M.L., Kennedy, M.J., 2017. Optogenetic control of synaptic composition and function. Neuron 93, 646–660 e645.
Stein, V., House, D.R., Bredt, D.S., Nicoll, R.A., 2003. Postsynaptic density-95 mimics and occludes hippocampal long-term potentiation and enhances long-term depression. J. Neurosci. 23, 5503–5506.
Steiner, P., Higley, M.J., Xu, W., Czervionke, B.L., Malenka, R.C., Sabatini, B.L., 2008. Destabilization of the postsynaptic density by PSD-95 serine 73 phosphorylation inhibits spine growth and synaptic plasticity. Neuron 60, 788–802.
Sun, Q., Turrigiano, G.G., 2011. PSD-95 and PSD-93 play critical but distinct roles in synaptic scaling up and down. J. Neurosci. 31, 6800–6808.
Swulius, M.T., Kubota, Y., Forest, A., Waxham, M.N., 2010. Structure and composition of the postsynaptic density during development. J. Comp. Neurol. 518, 4243–4260.
Tang, A.H., Chen, H., Li, T.P., Metzbower, S.R., MacGillavry, H.D., Blanpied, T.A., 2016. A trans-synaptic nanocolumn aligns neurotransmitter release to receptors. Nature 536, 210–214.
Tardin, C., Cognet, L., Bats, C., Lounis, B., Choquet, D., 2003. Direct imaging of lateral movements of AMPA receptors inside synapses. EMBO J. 22, 4656–4665.
Tomita, S., Stein, V., Stocker, T.J., Nicoll, R.A., Bredt, D.S., 2005. Bidirectional synaptic plasticity regulated by phosphorylation of stargazin-like TARPs. Neuron 45, 269–277.
Traynelis, S.F., Wollmuth, L.P., McBain, C.J., Menniti, F.S., Vance, K.M., Ogden, K.K., Hansen, K.B., Yuan, H., Myers, S.J., Dingledine, R., 2010. Glutamate receptor ion channels: structure, regulation, and function. Pharmacol. Rev. 62, 405–496.
Valtschanoff, J.G., Weinberg, R.J., 2001. Laminar organization of the NMDA receptor complex within the postsynaptic density. J. Neurosci. 21, 1211–1217.
Volk, L., Chiu, S.L., Sharma, K., Huganir, R.L., 2015. Glutamate synapses in human cognitive disorders. Annu. Rev. Neurosci. 38, 127–149.
Wang, L., Pang, K., Han, K., Adamski, C.J., Wang, W., He, L., Lai, J.K., Bondar, V.V., Duman, J.G., Richman, R., Tolias, K.F., Barth, P., Palzkill, T., Liu, Z., Holder, J.L., Zoghbi, H.Y., 2019. An autism-linked missense mutation in SHANK3 reveals the modularity of Shank3 function. Mol. Psychiatry 25, 2534–2555.
Watson, J.F., Ho, H., Greger, I.H., 2017. Synaptic transmission and plasticity require AMPA receptor anchoring via its N-terminal domain. elife 6.
Watson, J.F., Pinggera, A., Ho, H., Greger, I.H., 2021. AMPA receptor anchoring at CA1 synapses is determined by N-terminal domain and TARP gamma8 interactions. Nat. Commun. 12, 5083.
Wegner, W., Steffens, H., Gregor, C., Wolf, F., Willig, K.I., 2022. Environmental enrichment enhances patterning and remodeling of synaptic nanoarchitecture as revealed by STED nanoscopy. elife 11.
Willems, J., de Jong, A.P.H., Scheefhals, N., Mertens, E., Catsburg, L.A.E., Poorthuis, R. B., de Winter, F., Verhaagen, J., Meye, F.J., MacGillavry, H.D., 2020. ORANGE: a CRISPR/Cas9-based genome editing toolbox for epitope tagging of endogenous proteins in neurons. PLoS Biol. 18, e3000665.
Xie, X., Liaw, J.S., Baudry, M., Berger, T.W., 1997. Novel expression mechanism for synaptic potentiation: alignment of presynaptic release site and postsynaptic receptor. Proc. Natl. Acad. Sci. U. S. A. 94, 6983–6988.
Yang, Y., Liu, J.J., 2022. Structural LTP: signal transduction, actin cytoskeleton reorganization, and membrane remodeling of dendritic spines. Curr. Opin. Neurobiol. 74, 102534.
Zeng, M., Shang, Y., Araki, Y., Guo, T., Huganir, R.L., Zhang, M., 2016. Phase transition in postsynaptic densities underlies formation of synaptic complexes and synaptic plasticity. Cell 166, 1163–1175 e1112.
Zeng, M., Chen, X., Guan, D., Xu, J., Wu, H., Tong, P., Zhang, M., 2018. Reconstituted postsynaptic density as a molecular platform for understanding synapse formation and plasticity. Cell 174, 1172–1187 e1116.
Zeng, M., Diaz-Alonso, J., Ye, F., Chen, X., Xu, J., Ji, Z., Nicoll, R.A., Zhang, M., 2019. Phase separation-mediated TARP/MAGUK complex condensation and AMPA receptor synaptic transmission. Neuron 104, 529–543 e526.
Zhong, H., Ceballos, C.C., Massengill, C.I., Muniak, M.A., Ma, L., Qin, M., Petrie, S.K., Mao, T., 2021. High-fidelity, efficient, and reversible labeling of endogenous proteins using CRISPR-based designer exon insertion. elife 10.
Zhou, Q., Homma, K.J., Poo, M.M., 2004. Shrinkage of dendritic spines associated with long-term depression of hippocampal synapses. Neuron 44, 749–757.