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AMPA receptor trafficking and LTP: Carboxy-termini, amino-termini and TARPs

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Abstract

AMPA receptors (AMPARs) are fundamental elements in excitatory synaptic transmission and synaptic plasticity in the CNS. Long term potentiation (LTP), a form of synaptic plasticity which contributes to learning and memory formation, relies on the accumulation of AMPARs at the postsynapse. This phenomenon requires the coordinated recruitment of different elements in the AMPAR complex. Based on recent research reviewed herein, we propose an updated AMPAR trafficking and LTP model which incorporates both extracellular as well as intracellular mechanisms.

Keywords

AMPA receptor; Long-term potentiation; LTP; AMPAR amino-terminal domain; TARP; Synaptic plasticity

Introduction

It is a pleasure and honor to contribute to this special issue of *Neuropharmacology* that celebrates the landmark paper by Jeff Watkins and Dick Evans (Watkins and Evans, 1981). In reviewing the history of glutamatergic synaptic transmission this paper is a testament to the extraordinary power of pharmacology. Remarkably the cloning of the ionotropic glutamate receptors beautifully confirmed their pharmacological classification. AMPA receptors (AMPARs), the subject of this review, are expressed throughout the central nervous system, being present on virtually all central neurons. AMPARs mediate the vast majority of fast excitatory transmission in the brain and changes in the synaptic content of AMPARs underlie multiple forms of synaptic plasticity. Among these forms of synaptic plasticity, long-term potentiation (LTP) of excitatory synaptic transmission constitutes one

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of the most important molecular and cellular mechanisms underlying learning and memory formation (Martin et al., 2000; Nicoll, 2017). The mechanisms controlling (sub)synaptic AMPAR content directly determine synaptic strength, underlie LTP and several other forms of synaptic plasticity and are therefore tightly regulated.

Ever since its discovery (Bliss and Lomo, 1973; Lomo, 1966), multiple forms of LTP have been reported to exist throughout the CNS and the animal lifespan (Box 1). There is general agreement that NMDA receptor (NMDAR)-dependent LTP, the most widespread and functionally relevant form of LTP, relies on the synaptic incorporation of AMPARs (Kauer et al., 1988; Muller et al., 1988). This review focuses exclusively on the mechanisms of NMDAR-dependent LTP (Box 1). Admittedly, we do not list all the relevant work on AMPAR trafficking and LTP. Rather, we focus on some of the recent findings regarding the role played by different elements in the AMPAR complex which call for an updated subunit composition-dependent synaptic AMPAR trafficking model. Although it is likely that the molecular logic governing AMPAR trafficking discussed herein applies to multiple synapses, circuits and brain regions, we will discuss findings focusing on the hippocampal synapses formed between CA3 Schaffer collaterals and CA1 pyramidal neurons, one of the most paradigmatic excitatory synapses in the mammalian brain.

AMPAR composition

AMPARs are assembled as heteromers comprising pore forming subunits (GluA1-GluA4) and auxiliary subunits. In most cases, the pore forming core is arranged as a dimer of edited GluA2 (GluA2 (R)), which makes the receptor calcium-impermeable and resistant to blockade by polyamines (but see (Bowie, 2012) for exceptions), and two other subunits (Traynelis et al., 2010). Selective knockout of endogenous AMPAR subunits coupled with electrophysiological characterization of the resulting AMPAR mediated EPSC revealed that, in pyramidal neurons in the mature hippocampal CA1 region, AMPARs are formed by two GluA2 (R) subunits and 2 either GluA1 (80%) or GluA3 (20%) subunits. Hence, approximately 80% of the receptors are GluA1/GluA2 heteromers and the remaining ones are GluA2/GluA3 heteromers (Lu et al., 2009). Clearly, the subunit composition of AMPARs depends on cell type, among other factors. For instance, interneurons, depending on subtype, express different levels of GluA1-4 (Angulo et al., 1997; Geiger et al., 1995). Recent cryo-EM structural data from native AMPARs emphasize this heterogeneity with the identification of a sizable pool of heterotrimeric receptors containing 2 GluA2 (R) subunits in positions B and D, and GluA1 and GluA3 subunits occupying the A and C positions, respectively (Zhao et al., 2019).

The AMPAR subunits have four structural subdivisions: an intracellular carboxy-terminal domain (CTD), a transmembrane domain (TM), an extracellular ligand binding domain (LBD) and, lastly, the amino-terminal domain (ATD). While the LBD and the TM, involved in glutamate binding and channel pore formation, respectively, show high sequence identity among AMPAR subunits and are highly evolutionarily conserved, the ATD and the CTD are highly sequence diverse (Garcia-Nafria et al., 2016). The CTD is the most sequence divergent part of the receptor and has been extensively studied in the past decades for its role in subunit selective AMPAR trafficking (Henley and Wilkinson, 2016; Huganir and Nicoll,

2013). The ATD comprises about 50% of the AMPAR protein and is crucial for receptor assembly: it guides subunit dimerization and is also involved in receptor oligomerization (Traynelis et al., 2010). In contrast to the NMDAR, where the critical roles played by the ATD in receptor function are well established, a role for the AMPAR ATD has remained elusive until recently, when its crucial function regulating receptor synaptic docking has emerged.

AMPAR synaptic clustering. General trafficking rules

AMPAR trafficking can be divided into 3 main steps (Opazo and Choquet, 2011). First, the biogenesis and oligomerization of the receptor complex, which includes assembly of the pore-forming subunits and the auxiliary subunits, e.g., TARPs and cornichons, and occurs in the endoplasmic reticulum (Schwenk and Fakler, 2020) and the constitutive exocytosis of the receptor, which maintains the pool of surface membrane receptors available to synapses. Second, the lateral diffusion of surface receptors to the synapse. The third step is the synaptic capture of laterally diffusing AMPARs by synaptic “AMPAR slots”, the nature of which is likely to entail interactions with synaptic scaffolding proteins, as discussed later in this review, and whose abundance or availability can be strongly influenced by changes in activity (Huganir and Nicoll, 2013; Opazo et al., 2012). Hence, surface AMPARs are localized in two different pools: extrasynaptic and synaptic. The extrasynaptic pool of receptors represents a sizable pool. For instance, nucleated patches of somatic membrane, which lack excitatory synapses, generate nAs of AMPAR current (Zamanillo et al., 1999) and dendritic outside out patch recordings (Andrasfalvy and Magee, 2004) have demonstrated the existence of a pool of dendritic extrasynaptic AMPARs. This reservoir of mobile AMPARs can laterally diffuse to the synapse to support activity-dependent increases in the postsynaptic AMPAR complement, such as with LTP induction (Granger et al., 2013; Lledo et al., 1998; Makino and Malinow, 2009; Patterson et al., 2010; Penn et al., 2017).

Synaptic AMPAR complexes are positioned strategically in the PSD via interactions with a variety of synaptic scaffolding proteins and possibly *trans*-synaptic adhesion proteins and other synaptic organizing elements. Given the relatively low affinity of AMPARs for glutamate it is critical that these receptors be positioned immediately across from the presynaptic active zone to maximize their responsiveness to presynaptic glutamate (Lisman and Raghavachari, 2006). In fact, the emerging model predicts that the density of receptors in the area directly opposed to the active zone release site, rather than the total number of receptors in the PSD, determines postsynaptic responses (Biederer et al., 2017).

Synapse size is directly correlated to its strength (Holler et al., 2021) and the number of receptors (Chen et al., 2015; Matsuzaki et al., 2001; Takumi et al., 1999). This provides a link between structural and physiological long-term potentiation (Bosch et al., 2014; Matsuzaki et al., 2004; Meyer et al., 2014). There is growing evidence that the PSD is not homogeneous but instead is comprised by several AMPAR enriched nanodomains (Hruska et al., 2018; MacGillavry et al., 2013; Nair et al., 2013) precisely aligned with presynaptic release sites in independent nanocolumns (Tang et al., 2016). These *trans*-synaptic nanocolumns might act as functional units which scale up or down in response to (and supporting) plasticity (Chen et al., 2018). Therefore, to understand synaptic plasticity

it is essential to unveil the mechanisms whereby AMPARs are recruited and clustered within nanodomains at the synapse, which might involve the concerted action of several AMPAR domains, and multiple associated proteins inside and out of the cell.

Role of the GluA1 CTD in AMPAR synaptic clustering and LTP

Early studies using GluA1 and GluA2 knockout mice suggested a differential requirement of these two subunits for LTP. Whereas GluA1 is essential (Zamanillo et al., 1999) GluA2 is not (Jia et al., 1996). Based on the findings that the CTDs display the highest subunit divergence and are the least structured domains of the AMPARs, and that they are embedded in the protein matrix of the PSD, it was natural to focus on these domains as the substrate for subunit specific control of AMPAR trafficking and LTP. Two lines of research were pursued. The first focused on the role of PDZ domain interactions and the second focused on the role of phosphorylation.

A possible role of PDZ binding interactions playing a role in AMPAR function was first suggested by the finding that the PDZ binding motif (PBM) on the C-terminus of GluA1 bound to the MAGUK SAP97 (Leonard et al., 1998). Hayashi et al. reported that mutations in the PBM blocked LTP (Hayashi et al., 2000). However, it was later found that a knock in mouse lacking the PBM of GluA1 had normal LTP (Kim et al., 2005). More recent studies showed that the GluA1 PBM is not essential for AMPAR synaptic clustering (Granger et al., 2013; Kerr and Blanpied, 2012; Watson et al., 2020). Furthermore, deletion of the entire CTD does not impede LTP (Díaz-Alonso et al., 2020; Granger et al., 2013). Thus, a role for the PBM or for that matter the entire CTD of GluA1, as discussed below, remains unclear.

Different types of posttranslational modifications occurring at the GluA1 CTD, which may determine its trafficking behavior, have been identified. Phosphorylation is, undoubtedly, the most exhaustively characterized and a large body of literature has emerged on identifying kinases and their phosphorylation of the GluA1 CTD (Diering and Huganir, 2018; Roche et al., 1996; Shepherd and Huganir, 2007).

The possible role of AMPAR phosphorylation in determining the trafficking of the receptor grew out of the realization that kinases, and in particular Ca^{2+} /calmodulin-dependent protein kinase II (CaMKII), play a critical role in LTP (Herring and Nicoll, 2016). CaMKII is the most abundant protein in the PSD, the alpha and beta isoforms combined representing ~8.5% of the protein content in the PSD (Sheng and Hoogenraad, 2007). CaMKII plays an essential role in excitatory synaptic transmission and plasticity. It supports both AMPAR and NMDAR basal synaptic transmission (Incontro et al., 2018) and LTP (Silva et al., 1992). LTP and CaMKII potentiate synaptic transmission by selectively enhancing AMPAR mediated synaptic transmission (Lledo et al., 1995; Pettit et al., 1994) and CaMKII occludes LTP (Ehrlich and Malinow, 2004; Poncer et al., 2002; Stein et al., 2003). Finally, it has recently been reported that brief activation of a photoactivatable CaMKII initiates LTP (Shibata et al., 2021). However, the specific mechanisms through which CaMKII mediates LTP are still a matter of debate (Nicoll, 2017).

CaMKII phosphorylates the GluA1 CTD (Barria et al., 1997a; Mammen et al., 1997), and this phosphorylation is increased during LTP (Barria et al., 1997b). Other phosphorylation

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sites in the GluA1 CTD mediated by different kinases have also been identified and a role in LTP was proposed (Esteban et al., 2003; Hayashi et al., 2000). However, whether GluA1 phosphorylation plays a necessary role in LTP is unclear, given several factors. Among these, i) Single point mutations in the best characterized phosphorylated residues, serine 831 and serine 845, show no impact on LTP in knock-in studies (Lee et al., 2010). The simultaneous mutation of these residues results in impaired LTP in adult, but not young mice (Lee et al., 2003). In overexpression studies, mutation of serine 845, but not serine 831, blocked LTP (Esteban et al., 2003). ii) Whether the phosphorylation levels of these residues in vivo are consistent with an essential role of these modifications in synaptic plasticity is controversial. A study found that less than 1% of GluA1 is phosphorylated at serine 831 and less than 0.1% at serine 845, which, given the number of receptors per synapse means that only between 0 and 1 AMPAR are phosphorylated at any given time per synapse on average (Hosokawa et al., 2015). In contrast, another study found that 15–20% of GluA1 is phosphorylated at either of these residues in basal conditions, and that stimulation can increase this proportion to up to 50% (Diering et al., 2016). iii) LTP can occur in the absence of the GluA1 CTD (Díaz-Alonso et al., 2020; Granger et al., 2013; Liu et al., 2020). A recent study challenged this notion using knock-in mouse lines in which the GluA1 and GluA2 CTDs were swapped. GluA1/A2 CTD mice showed impaired LTP (Zhou et al., 2018), although a subsequent study revealed that this effect depends on the induction protocol and the age of the mice employed (Box 1) (Liu et al., 2020). Surprisingly, these mice also show severely impaired hidden platform learning and memory in the Morris water maze, in striking contrast with GluA1 KO mice (Zamanillo et al., 1999) and with knock-in mice in which the GluA1 CTD is truncated (Díaz-Alonso et al., 2020). These findings suggest that perhaps the grafting the GluA2 CTD fundamentally changes GluA1 trafficking or function.

In summary, the phosphorylation of residues in the GluA1 CTD happens in vivo, but it is not essential for LTP. However, it could well play a modulatory role in some synaptic plasticity mechanisms in a synapse type, induction protocol and age dependent fashion (Box 1).

Role of the ATD in AMPAR synaptic clustering and LTP

Although the ATD comprises almost half of the AMPAR subunit polypeptide, its function is far from fully understood. During receptor biogenesis, in the ER, the ATD guides the formation of dimers (Ayalon and Stern-Bach, 2001; Clayton et al., 2009; Jin et al., 2009) and then the oligomerization, preferentially forming GluA2(R) containing, heteromeric receptors (Rossmann et al., 2011). However, the fact that the heterologous expression of GluA1 receptors lacking the ATD appear to function normally, clearly indicates that it is not required for basic AMPAR function (Díaz-Alonso et al., 2017; Tomita et al., 2007). What might its role be?

An initial awareness of the crucial role of the ATD in synaptic trafficking came from the reevaluation of the long-standing subunit specific, receptor-centric AMPAR trafficking model (Shi et al., 2001). This model, which has dominated the field during the last 20 years, predicts that GluA1-containing AMPARs are excluded from the synapse in the absence of a strong synaptic stimulation. The experiments leading to the proposal of this model

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relied on the overexpression of an ATD-tagged GluA1 subunit. The original results showed that GFP-GluA1 is excluded from the synapse in basal conditions, hence baseline AMPAR mediated EPSCs were interpreted to be mediated by GluA2/A3 containing AMPARs. Upon induction of LTP or expression of a constitutively active CaMKII mutant, AMPAR-mediated EPSCs showed synaptic rectification in GFP-GluA1 expressing cells (Hayashi et al., 2000; Shi et al., 2001), which indicates that GluA2(R)-lacking AMPARs (presumably homomeric overexpressed GFP-GluA1 receptors) are recruited to the synapse. To note, LTP in adult WT cells does not change rectification, i. e., does not involve the insertion of endogenous calcium permeable homomeric GluA1 receptors (Adesnik and Nicoll, 2007; Hayashi et al., 2000).

Hence, the receptor-centric model assumed that GluA1-containing AMPAR do not contribute to basal synaptic transmission and are only recruited during LTP expression. However, this notion was challenged in experiments where WT GluA1 was overexpressed in CA1 pyramidal neurons in hippocampal slice cultures. Baseline AMPAR mediated synaptic currents show strong rectification, indicating the effective insertion of overexpressed homomeric GluA1 receptors (Díaz-Alonso et al., 2017; Granger et al., 2013; Watson et al., 2017), but see (Nabavi et al., 2014), hence the ability of GluA1 containing receptors to participate in constitutive synaptic trafficking. Further corroborating this notion, chronic activity blockade with TTX does not prevent synaptic insertion of GluA1 (Díaz-Alonso et al., 2017; Watson, 2020). In contrast, cells overexpressing GFP-tagged GluA1 do not show rectification (Díaz-Alonso et al., 2017; Hayashi et al., 2000; Watson, 2020). Therefore, the ATD GFP tag fundamentally changes the synaptic trafficking behavior of the GluA1 subunit. GluA2 trafficking does not seem to be affected to the same degree as GluA1 by the presence of a GFP tag. We did not observe a significant difference in the trafficking of WT and GFP-tagged GluA2 (Díaz-Alonso et al., 2017), in agreement with (Nabavi et al., 2014). Watson et al. also report significant rectification in GFP-GluA2(Q) expressing cells, indicating that this modified subunit is readily expressed at the synapse. However, the increase in EPSC size observed with overexpression of WT GluA2 is absent (Watson et al., 2017).

Interestingly, in the original experiments, the exclusion of GFP-tagged GluA1 from synapses is not observed in dissociated neuron cultures (Shi et al., 1999), suggesting that perhaps the less complex extracellular environment does not pose a significant steric hindrance to synaptic docking of ATD-tagged receptors and further indicating that extracellular interactions involving the AMPAR ATD and some yet to be identified synaptic cleft moieties are key to the docking of AMPARs.

Reassuringly, the recent results with the GFP-AMPAR subunit fully recapitulate previous results. Both expression of constitutively active CaMKII and induction of LTP were able to promote synaptic insertion of GFP-GluA1 (Díaz-Alonso et al., 2017; Hayashi et al., 2000). How the signaling cascade triggered by CaMKII is able to circumvent the trafficking restriction imposed by the GFP tag remains to be elucidated. As further discussed later, AMPAR trafficking and synaptic anchoring have two main requisites. First, an intact extracellular ATD. Second, interactions between TARP auxiliary subunits and scaffolding proteins in the PSD (Chen et al., 2000; Watson, 2020; Zeng et al., 2019). CaMKII-dependent

AMPAR trafficking is more likely to directly involve the second, although indirect action over the ATD, via a transmembrane or secreted factor, for instance, is also plausible.

Altogether, these observations suggest that the GFP tag can interfere with AMPAR ATD-dependent synaptic clustering, and constitute a cautionary note in experiments relying on extracellular GFP or similar tags to image AMPAR trafficking. The AMPAR extracellular region projects far into the synaptic cleft, at least halfway towards the presynaptic membrane (Greger et al., 2017). Therefore, it can participate in molecular interactions with extracellular elements, thereby contributing to the *trans-synaptic* clustering of AMPARs (Fig. 1). Indeed, although receptor function, assembly and surface delivery are intact in the absence of the ATD (Díaz-Alonso et al., 2017; Watson et al., 2017), ATD-lacking AMPAR subunit enrichment in dendritic spines is partially impaired compared to full length subunits. Furthermore, their localization in nanoclusters within the PSD appears more diffuse, in particular that of GluA1 (Watson, 2020). Finally, the truncation of the ATD leads to a severe impairment in GluA1 subunit synaptic trafficking and LTP (Díaz-Alonso et al., 2017; Jiang et al., 2021; Watson et al., 2017). In stark contrast, ATD-lacking GluA2 can rescue constitutive AMPAR trafficking and LTP (Díaz-Alonso et al., 2017; Jiang et al., 2021). A chimeric GluA1/A2ATD construct can rescue constitutive transmission in an AMPAR null background, but can't support LTP (Jiang et al., 2021), further pointing to the ATD as a critical domain controlling GluA1, but not GluA2 trafficking. Given the dominance of the GluA1 ATD in heteromeric AMPAR trafficking (Díaz-Alonso et al., 2017), we hypothesize that the GluA1 ATD is essential for AMPAR trafficking and LTP in physiological conditions (Figs. 1 and 3).

Why is the GluA1 ATD particularly important for AMPAR synaptic docking? There are several possibilities. First, an affinity reason: the AMPAR ATDs are highly sequence-divergent, with very little amino acid conservation in the surfaces exposed to the cleft. Hence, interactions with extracellular elements are potentially subunit-specific (Garcia-Nafria et al., 2016). Second, a structural reason: the ATD of subunits in positions A and C, typically occupied by GluA1 and/or GluA3, are more exposed to protein-protein interactions in the synaptic cleft compared to GluA2 subunits, almost exclusively found in positions B and D (Zhao et al., 2019). In summary, AMPAR synaptic clustering depends, perhaps, on subunit-specific and activity-regulated interactions with extracellular moieties. The nature of these "extracellular AMPAR slots" (Choquet, 2018; Diering and Huganir, 2018) is not yet fully clear and could involve, for instance, proteins anchored to the pre- or postsynapse, *trans-synaptic* adhesion proteins or proteins secreted into the cleft by neurons and glia (Fig. 1). There is some evidence for such interactions. Neuronal pentraxins are a family with 3 members, the secreted glycoproteins NP1/NPTX1 and NP2/NPTX2 (also referred to as neuronal activity-regulated pentraxin (Narp), and Neuronal pentraxin receptor NPR/NPTXR. NP1 can stabilize AMPAR at the synapse by interacting with the ATD. This mechanism seems to be particularly important for GluA4 containing AMPA receptors, such as those expressed by parvalbumin positive interneurons in the hippocampus (Pelkey et al., 2016; Sia et al., 2007). Glycans, secreted by astrocytes, stimulate synaptic AMPAR clustering by promoting NP1 release from the presynaptic terminal (Farhy-Tselnicker et al., 2017). Recently, Suzuki et al. designed a synthetic synaptic organizer molecule consisting of a combination of structural elements from NP1 and cerebellin which holds promise for

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the treatment of a variety of excitatory synaptic dysfunctions (Suzuki et al., 2020). NP2 is an immediate-early gene (Tsui et al., 1996) and early studies already showed that it may play a role in synaptic plasticity by modulating AMPAR trafficking (O'Brien et al., 1999). NPR is able to cluster the secreted pentraxins at the synapse and has recently been shown to stimulate synaptogenesis presynaptically (Lee et al., 2017). Triple NP1/NP2/NPR knockout mice display severely impaired synaptogenesis onto GluA4 overexpressing cultured astrocytes (Sia et al., 2007). However, AMPAR clustering in cultured hippocampal neurons shows no substantial defect in triple KO vs WT (Bjartmar et al., 2006).

Unbiased proteomics-based approaches may lead to the discovery of more ATD binding proteins relevant to AMPAR docking and synaptic plasticity. A recent analysis of GluA1 ATD interacting proteins in the rat brain found that the cell adhesion molecule neuroplastin 65 can interact with the GluA1 ATD. This study shows that this interaction occurs in *cis*, i.e., in the postsynaptic membrane, and that it is necessary for GluA1 synaptic trafficking, whereas it does not affect that of the GluA2 subunit (Jiang et al., 2021).

The extracellular matrix regulates various forms of synaptic plasticity (Dityatev et al., 2010) and may participate in the dynamic control of AMPAR mobility, by imposing a more or less restrictive steric impediment to receptor lateral diffusion (Frischknecht et al., 2009), by modulating the availability of ATD interacting elements in the cleft or by directly interacting with the ATD (Fig. 1). Brevican, a key component of perineuronal nets which wrap parvalbumin positive interneurons, regulates GluA1 synaptic clustering and synaptic plasticity (Favuzzi et al., 2017). Whether the extracellular matrix controls synaptic AMPAR clustering via similar mechanisms in other cell types has not been yet elucidated. Other proteins shown to interact with GluA1 extracellularly, hence potentially involving the ATD, are Neuropilin-2/PlexinA3 (Wang et al., 2017) and LRRTM2 (de Wit et al., 2009). The GluA2 ATD has also been shown to participate in synaptic targeting via N-cadherin interaction (Saglietti et al., 2007).

Other important roles for the AMPAR ATD are emerging. Recently, the ATD has emerged as a receptor region involved in allosteric modulation, previously attributed mainly to the LBD (Jin et al., 2005; Lee et al., 2019). It has been proposed that the ATD contributes to AMPAR/TARP interaction in a subunit-composition dependent fashion (Cais et al., 2014) and that this interaction might participate in the TARP mediated control of AMPAR gating. The non-psychoactive cannabinoid cannabidiol (CBD) can bind to the GluA1 and GluA2 ATD and reduce evoked AMPAR EPSC size and mini EPSC amplitude and frequency (Yu et al., 2020). CBD is currently being used in combination with antiepileptic drugs in some forms of epilepsy such as Dravet syndrome and Lennox–Gastaut syndrome (Devinsky et al., 2017, 2018; Thiele et al., 2018). Part of its anti-seizure actions might be mediated by an ATD-dependent AMPAR allosteric inhibition (Yu et al., 2020).

Role of TARP/MAGUK interactions in AMPAR synaptic clustering and LTP

Native AMPARs at excitatory synapses exist tightly associated to auxiliary subunits, together forming AMPAR complexes. These auxiliary subunits modulate assembly, trafficking, gating and pharmacology (Greger et al., 2017; Jackson and Nicoll, 2011; Schwenk et al., 2012; Straub and Tomita, 2012). Some of the best characterized AMPAR

auxiliary subunits are TARPs, cornichons, CKAMPs and GSG1L (Chen and Gouaux, 2019; Jacobi and von Engelhardt, 2021; Kamalova and Nakagawa, 2021; Schwenk et al., 2014; Schwenk et al., 2012). TARPs are the most widely expressed AMPAR auxiliary proteins and play an essential role in AMPAR function (Chen et al., 2000; Jackson and Nicoll, 2011; Tomita et al., 2003). Recent advances in single-particle cryo-electron microscopy have allowed the elucidation of the interactions, stoichiometry and functional implications of TARP/AMPAR complexes (Herguedas et al., 2019; Twomey et al., 2016; Zhao et al., 2019) (Fig. 2).

The membrane-associated guanylyl kinase (MAGUK) family of scaffolding proteins plays an essential role in ionotropic glutamate receptor localization. Simultaneous knockdown of PSD-93, PSD-95, and SAP102 results in severely reduced AMPAR and NMDAR-mediated EPSCs (Levy et al., 2015). PSD-95 is the key postsynaptic organizer and plays a fundamental role in clustering AMPARs at the synapse. Overexpression of PSD-95 results in a robust and specific enhancement of AMPAR-mediated EPSCs (El-Husseini et al., 2000; Schnell et al., 2002), which mimics and occludes LTP (Ehrlich and Malinow, 2004; Stein et al., 2003), and PSD-95 loss of function results in decreased AMPAR EPSCs (Beique et al., 2006; Elias et al., 2006). TARP-PSD-95 interaction stabilizes AMPAR complexes at the PSD (Bats et al., 2007; Schnell et al., 2002). These interactions, which involve PDZ domains in PSD-95 and PBMs in TARPs (Fig. 2), constitute the most plausible “AMPAR slots” in the PSD and their availability regulates access of AMPAR to the PSD both in basal synaptic transmission and during LTP (Figs. 2 and 3). One study found that the PBM in TARP γ -8 is critical for constitutive synaptic transmission but not for LTP using a -PBM TARP γ -8 knock-in mouse (Sumioka et al., 2011). However, other TARPs expressed in CA1 neurons might influence this finding. In order to study the contribution of different protein domains in a particular TARP to AMPAR synaptic clustering without the confounding presence of other TARPs, endogenous AMPARs were replaced by AMPAR-TARP tethered constructs in an AMPAR null background (Shi et al., 2009). Tethering virtually excludes the possibility of endogenous TARPs binding to the expressed AMPARs (Fig. 2). In these experimental conditions, the TARP C-terminal PBM is essential for baseline AMPAR transmission and LTP (Sheng et al., 2018; Zeng et al., 2019), in agreement with previous data (Bats et al., 2007; Chen et al., 2000; Schnell et al., 2002). However, surprisingly, isothermal titration calorimetry (ITC) measurements revealed that the isolated TARP PBM and MAGUKs interact with low affinity, insufficient to explain the physiological relevance of this interaction, whereas PSD-95 interacts with the entire TARP C-tail with an affinity almost 2 orders of magnitude higher, sufficient to drive spontaneous condensation via liquid-liquid phase transition with other PSD proteins (Zeng et al., 2019). This work also identified other motifs in the TARP C-tail which make a substantial contribution to MAGUK binding and AMPAR synaptic clustering, including a stretch of arginine- and serine-rich sequences and a small number of aromatic residue-containing hydrophobic sites. Mutation of these motifs results in impaired AMPAR trafficking and (at least in the case of the arginine residues) LTP (Fig. 2 (Zeng et al., 2019)). Thus, a multivalent binding model was proposed in which the TARP PBM binds to PDZ2 of PSD-95 and the more proximal region of the CTD of TARPs binds to PDZ1.

Therapeutic targeting of AMPAR complexes

Given its central role in excitatory synaptic transmission throughout the CNS, altered AMPAR trafficking and function can have devastating consequences and result in psychiatric and neurological conditions as well as cognitive dysfunction (Henley and Wilkinson, 2016). Therefore, AMPARs are highly sought-after drug targets for therapies. However, AMPAR subunits expression is widespread, consistent with their essential role in mediating glutamatergic synaptic transmission. Hence, although there are pharmacological tools directly acting on AMPAR subunits currently used in the clinic, such as perampanel for epilepsy, their blockade, not unexpectantly, has several associated side effects, such as dizziness and ataxia (Zwart et al., 2014).

TARPs, on the other hand, have redundant functions (Menuz et al., 2008), but relatively non-overlapping expression domains, with TARP γ -8 highly enriched in the hippocampus and cerebral cortex and TARP γ -2 in the cerebellum, for example (Fukaya et al., 2005; Schwenk et al., 2014; Tomita et al., 2003). Hence, TARPs are promising targets for region-specific AMPAR modulation, since drugs can be designed to affect specifically the pool of AMPARs associated with a particular TARP. For instance, LY3130481, a drug which targets specifically forebrain TARP γ -8 associated AMPARs shows promising results as an antiepileptic drug in preclinical studies without the motor side effects associated with AMPAR antagonists (Kato et al., 2016; Maher et al., 2016).

Concluding remarks

In conclusion, synaptic clustering of AMPARs is a fundamental process directly determining synaptic strength and is central to LTP and other synaptic plasticity phenomena. Recent evidence discussed in this review points to mechanisms involving the AMPAR ATD and the AMPAR auxiliary proteins TARPs as the essential orchestrators of this process. This body of research supports an updated slot model for synaptic AMPAR trafficking which considers both intracellular as well as extracellular interactions, which in concert precisely direct and hold AMPARs at the synapse. Numerous questions remain to be elucidated regarding the *trans*-synaptic positioning of AMPARs, the coordination of intracellular and extracellular mechanisms and how these phenomena respond to synaptic plasticity. Answering these questions will expand our understanding of the molecular neurobiology of learning and memory and other brain functions. Furthermore, it will provide insights potentially relevant to efforts towards the efficient targeting of AMPAR in multiple disease states.

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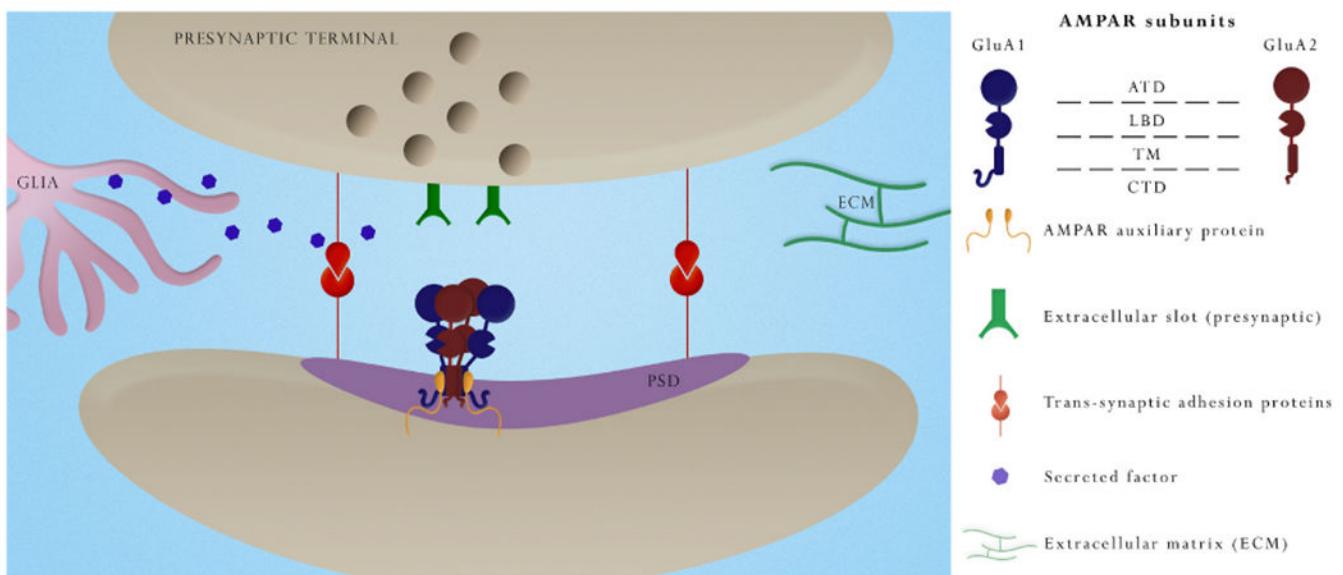
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Box 1**Simplifying the experimental LTP problem.**

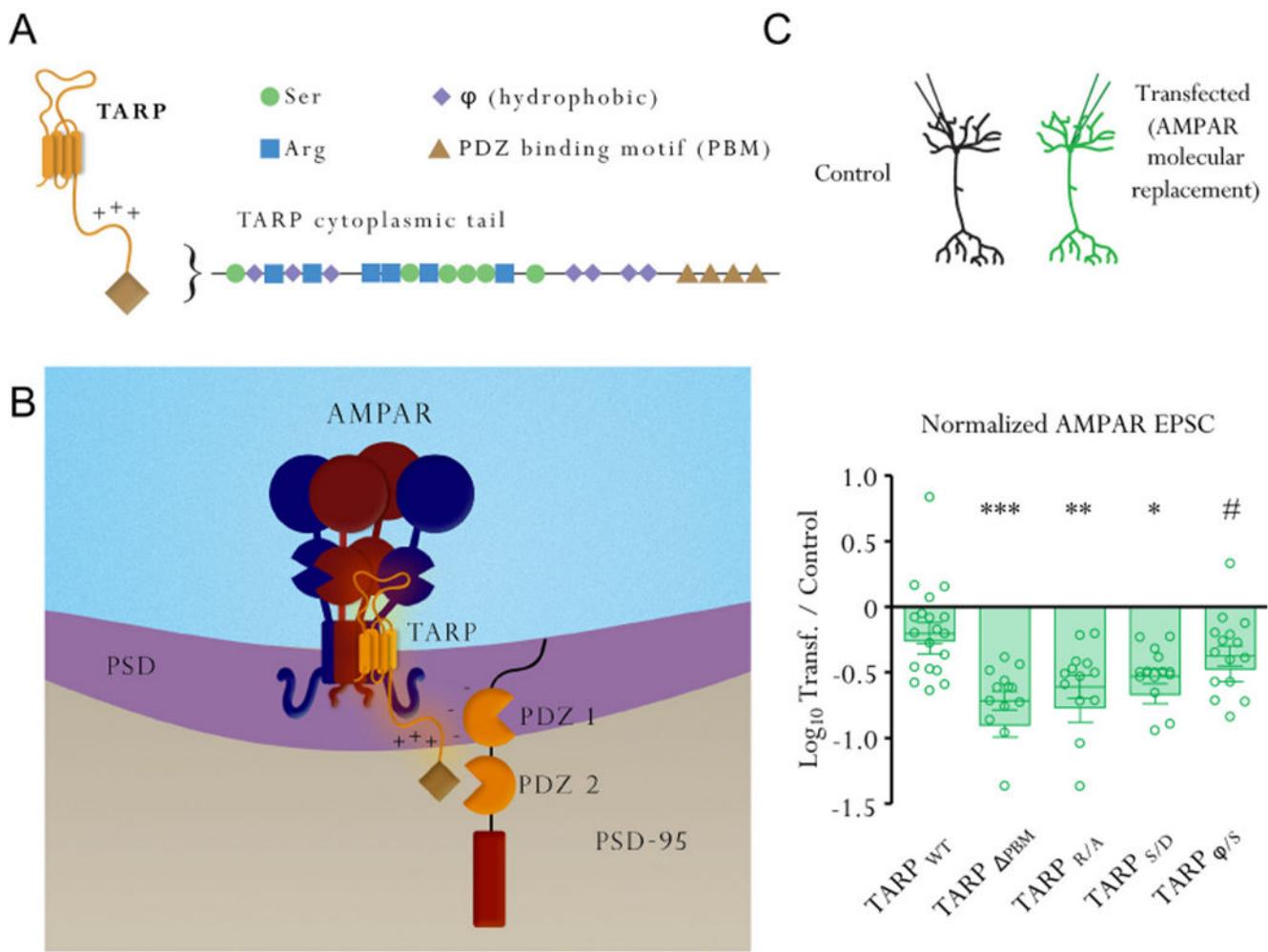
To the outsider, the LTP literature seems extremely complex, verging on off putting. One of the problems is that the field has never explicitly settled on a precise definition for this phenomenon and it is clear that fundamentally distinct forms can exist at different synapses, e.g., hippocampal CA1 LTP and hippocampal mossy fiber LTP (Nicoll and Malenka, 1995; Nicoll and Schmitz, 2005). Most research on LTP comes from studies on the excitatory synapses onto CA1 hippocampal pyramidal cells. Given the possibility that differences exist at different synapses in the brain, it would seem prudent to focus one's attention on the CA1 excitatory synapse where most of our knowledge already exists. Furthermore, it is generally agreed that it is the unique properties of the NMDAR, which mediates CA1 LTP, that make LTP such a compelling model of learning and memory (Martin et al., 2000; Nicoll, 2017). However, even at the CA1 synapse, multiple forms of NMDAR LTP have been postulated that depend on both the frequency and pattern of stimulation (e.g., 100 versus 200 Hz, theta burst stimulation etc.), the stimulus strength, the age of the animal, and the time after induction (Nicoll, 2017).

What strategies can be used in an attempt to simplify the problem? Part of the confusion in the study of LTP is the failure to appreciate that there are two separate questions regarding LTP. The first question is *What controls the activation of the NMDAR?* and the second question is *What happens after activation of the NMDAR?* It is well established that there are only two requirements for the induction of LTP: glutamate binding to the NMDAR and membrane depolarization. Most studies have used various forms of tetanic stimulation to cause the depolarization of the postsynaptic membrane. However, the effectiveness of the tetanus in depolarizing the neuron is influenced by a large number of variables. For instance, altering the level of GABAergic inhibition will have a profound effect on the degree to which a tetanus will depolarize the postsynaptic neuron and therefore contribute to NMDAR activation. In fact, any manipulation that affects the level of depolarization (e.g., postsynaptic excitability, number of synapses activated, presynaptic transmitter release, etc.) and therefore the degree of NMDAR activation will all affect LTP, but this has nothing to do with the central mechanisms underlying LTP. This confusion may well explain the long list of proteins postulated to mediate LTP (Sanes and Lichtman, 1999). To determine the essential requirements for LTP, the important question is *What happens after NMDAR activation.* Thus, in order to study LTP in a controlled fashion, one must be able to precisely control the depolarization. This is accomplished by a “pairing” protocol. Specifically, one uses cesium in the recording pipette to block potassium channels in the postsynaptic neuron, so the neuron can be held at a given membrane potential (e.g., 0 mV) during synaptic stimulation. If a manipulation blocks LTP, there are only two possible explanations. If it blocks NMDAR activation it tells us nothing new about LTP, but if the NMDAR is spared, the manipulation has identified a critical component of LTP.

**Fig. 1.**

Synaptic clustering mechanisms involving the extracellular AMPAR ATD.

AMPAR ATDs are essential for AMPAR mediated synaptic transmission. The existence and identity of extracellular AMPAR “slots” is yet to be demonstrated, but multiple lines of evidence point to that possibility, including the necessary role played by this domain in synaptic trafficking and LTP. Several elements of the synaptic cleft, which can potentially modulate AMPAR synaptic clustering by interacting with the ATD, are shown, such as: i) *trans*-synaptic adhesion proteins, including synaptic cell adhesion molecules (CAMs), neuroligin/neurexin, LRRTM/neurexin, etc.; ii) the extracellular matrix (ECM); iii) neuronal or glial-derived secreted factors, such as pentraxins, glypicans, etc. and iv) pre- and postsynaptic AMPAR ATD-interacting membrane-associated proteins spanning into the synaptic cleft.

**Fig. 2.**

Synaptic clustering mechanisms involving multiple TARP C-tail domains.

A; diagram showing highly conserved and functionally relevant domains in the TARP cytoplasmic tail involved in the interaction with MAGUKs, based on TARP- γ -2, including a series of serine, arginine and hydrophobic amino acids, and a PDZ-binding motif. B; cartoon showing the recently proposed simplified TARP-MAGUK binding mechanism, which involves, in addition to the well documented PBM-PDZ 2 interactions, strong ionic interactions between a series of positively charged arginine residues in the TARP c-tail and negative charges on the surface of the PDZ 1 domain opposite to the binding pocket (Zeng et al., 2019). C; Functional consequences of TARP mutations that impact its binding to the PSD scaffolding protein PSD-95. Top, diagram illustrating simultaneous patch clamp recording from a transfected cell (green) and a neighboring control cell (black). Bottom, summary bar graph showing that removing the PBM (Δ), replacing the arginines with alanine (R/A), replacing the serines with aspartate (S/D) and replacing the hydrophobic residues ϕ123 with serine (ϕ/S), all decreased AMPAR mediated EPSCs in transfected vs control cells. These physiological results parallel the effects of these mutations on the binding of TARP to PSD-95. *, p < 0.05; **, p < 0.01 and ***, p < 0.001 versus control

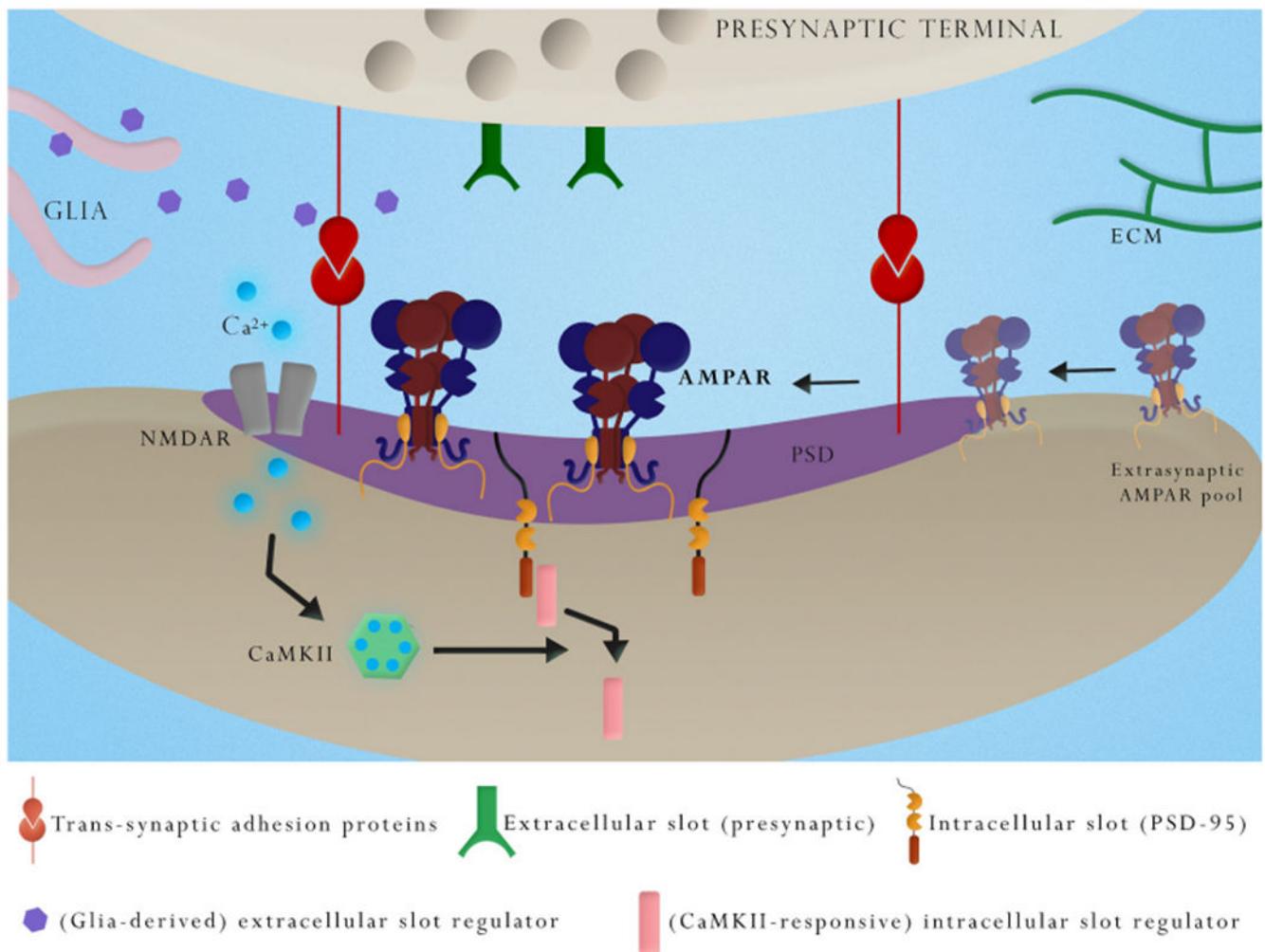
condition. #, $p < 0.05$ versus GluA1- γ -8 $_-$ 4 condition. Image partially reproduced from Zeng et al. (2019).

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**Fig. 3.**

A simplified model for AMPAR accumulation during LTP mediated by increased intra- and extracellular slot availability.

Postsynaptic rise in Ca^{2+} upon NMDAR activation and subsequent CaMKII activity triggers LTP. Of the many targets of CaMKII identified over the past decades, the essential components mediating LTP are yet to be fully discerned. Ultimately, the stabilization of laterally diffusing AMPARs involves the concerted interactions between elements of the AMPAR complex such as the ATD and TARPs with extracellular and intracellular slots. The availability of extracellular slots might be regulated by pre-, post- or peri-synaptic ATD-interacting protein(s), *trans*-synaptic adhesion proteins, including synaptic cell adhesion molecules (CAMs), neuronal or glial-derived secreted proteins and extracellular matrix (ECM) elements. Availability of extracellular slots may be regulated by synaptic activity. Increased availability of intracellular AMPAR slots can also occur in an activity-regulated fashion. An attractive hypothetic mechanism involves the detachment of “masking” PDZ-binding proteins from PSD-95 by the action of CaMKII, thereby exposing TARP binding sites.