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Regulation of Long-Term Plasticity Induction by the Channel and C-Terminal Domains of GluN2 Subunits

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Abstract Conventional long-term potentiation (LTP) and long-term depression (LTD) are induced by different patterns of synaptic stimulation, but both forms of synaptic modification require calcium influx through NMDA receptors (NMDARs). A prevailing model (the "calcium hypothesis") suggests that high postsynaptic calcium elevation results in LTP, whereas moderate elevations give rise to LTD. Recently, additional evidence has come to suggest that differential activation of NMDAR subunits also factors in determining which type of plasticity is induced. While the growing amount of data suggest that activation of NMDARs containing specific GluN2 subunits plays an important role in the induction of plasticity, it remains less clear which subunit is tied to which form of plasticity. Additionally, it remains to be determined which properties of the subunits confer upon them the ability to differentially induce long-term plasticity. This review highlights recent studies suggesting differential roles for the subunits, as well as findings that begin to shed light on how two similar subunits may be linked to the induction of opposing forms of plasticity.

Keywords NMDA receptor · GluN2A · GluN2B · Long-term potentiation · Long-term depression · Synaptic plasticity

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Introduction

NMDA receptors (NMDARs) are tetramers, with each subunit consisting of four domains. The extracellular N-terminal domain is thought to play a role in subunit assembly [1] and, in the case of GluN2A and GluN2B (formerly NR2A and NR2B; for revised nomenclature, see [2]) subunits, binding of allosteric modulators [3]. The membrane-associated domain consists of three transmembrane domains and a reentrant loop which lines the pore and consequently controls ion permeability and Mg^{2+} affinity. The ligand-binding domain is formed by the extracellular loop between the third and fourth membrane domains and the extracellular domain situated just N-terminally to the first membrane domain. Finally, a C-terminal domain interacts with signaling and scaffolding proteins intracellularly [4–6]. This intracellular tail varies in size among the subunits, and in the case of the GluN2 subunits, it comprises nearly half of the protein.

Each NMDAR tetramer is composed of two obligatory GluN1 subunits. The other two subunits may be GluN2, of which there are four kinds encoded by distinct genes—GluN2A, B, C, and D [7], or in some cases GluN3 [8]. While the GluN1 transcript is present ubiquitously throughout development and across nearly all regions of the brain, the various GluN2 transcripts are more variable [9]. GluN2B and GluN2D transcripts are present in a large number of brain structures embryonically. Postnatally, expression of GluN2A and GluN2C increases, while expression of GluN2D decreases in forebrain structures [9, 10].

In mature hippocampal CA1 pyramidal cells, NMDARs contain mainly GluN2A and/or GluN2B subunits such that the complete NMDAR is composed of either two GluN1 and two GluN2A (GluN1/GluN2A), two GluN1 and two

GluN2B (GluN1/GluN2B), or two GluN1 plus one GluN2A and one GluN2B (GluN1/GluN2A/GluN2B) [10]. The GluN1/GluN2A and GluN1/GluN2B receptors are often referred to as diheteromeric, while the GluN1/GluN2A/GluN2B receptors are often referred to as triheteromeric. Neonatally, little GluN2A is present in the NMDARs of CA1 pyramidal cells, and the GluN2B subunit predominates synaptically. Over the first few weeks of development, GluN2A subunits accumulate such that synapses contain both GluN2A and GluN2B [10, 11]. In hippocampal membrane fractions from adult rat brain, which presumably include both synaptic and extrasynaptic receptor populations, it has been estimated that 15–40% of GluN2A and GluN2B subunits are found in GluN1/GluN2A/GluN2B receptors, with the remainder in diheteromeric receptors [4]. On the other hand, electrophysiological experiments targeting mainly synaptic receptors suggest that the majority of GluN2B in adult hippocampus is incorporated into triheteromeric receptors with only small population of receptors being comprised of diheteromeric GluN1/GluN2B [12].

Synaptic Versus Extrasynaptic Distribution of GluN2 Subunits

There is some debate as to the relative synaptic location of the GluN2A and GluN2B containing NMDARs in adult synapses. Historically, GluN2A and GluN2B subunits have been postulated to be somewhat segregated, with GluN2A-containing NMDARs predominating synaptically and GluN2B-containing NMDARs becoming extrasynaptically localized in mature synapses. In support of this idea are studies suggesting that spontaneous transmitter release preferentially activates GluN2A receptors. For example, spontaneous NMDA excitatory postsynaptic currents (EPSCs) display faster kinetics than evoked NMDA EPSCs in dentate granule cells [13], consistent with spontaneously released glutamate preferentially activating GluN2A-containing NMDARs. Additionally, evoked but not spontaneous transmitter release elicits an NMDA-mediated EPSC in GluN2A knockout mice [14]. Furthermore, whole cell currents, which contain both synaptic and extrasynaptic receptors, have been demonstrated to be much more sensitive to GluN2B-specific antagonists than synaptic currents [15]. Overall, these results suggest a higher concentration of GluN2A subunits synaptically, with GluN2B subunits predominating extrasynaptically.

Some recent findings contradict this model, however, suggesting that GluN2A and GluN2B subunits are more homogenously distributed. Recently, Harris and Pettit have used glutamate uncaging to demonstrate that extrasynaptic receptor populations are not enriched for GluN2B [16]. Similarly, autaptic synapses of hippocampal neurons have been shown to contain GluN2A NMDARs extrasynaptically [17]. Electron microscopic studies of the CA1 region of hippocampus as well as the lateral amygdala reveal that both GluN2A and GluN2B are preferentially associated with synaptic (defined as the area associated with the postsynaptic density) as opposed to extrasynaptic regions [18]. Further studies will be required to definitively establish the relative synaptic locations of the GluN2A and GluN2B subunits.

Pharmacological Experiments Addressing the Roles of GluN2 Subunits in Plasticity

It has long been evident that NMDARs are critical for both long-term potentiation (LTP) and long-term depression (LTD) inductions as established through pharmacological experiments and experiments involving transgenic animals [19]. Experiments demonstrating reduced hippocampal LTP in GluN2A knockout mice [20] and enhanced LTP in mice overexpressing GluN2B [21] were consistent with an important role for both subunits in LTP induction. However, these studies were not able to fully address the issue of the individual and potentially dichotomous roles of the subunits. More recently, experiments employing subunit-specific antagonists have given rise to the hypothesis that the two subunits are differentially linked to LTP and LTD. However, considerable controversy surrounds the seemingly contradictory results of these pharmacological experiments.

Early experiments investigating the roles of distinct GluN2 subunits in plasticity employed antagonists with differential selectivity for GluN2A/B versus GluN2C/D and revealed that antagonists preferential for GluN2A/B more potently inhibited induction of LTP than LTD [22, 23]. The development of GluN2B-specific antagonists such as ifenprodil and Ro25-6981 [24, 25] and more recently a GluN2A preferring antagonist (NVP-AAM077) [26] has allowed for experiments probing the specific roles of GluN2A- and GluN2B-containing NMDARs in LTP and LTD. Initial results in hippocampus showed that ifenprodil and Ro25-6981 block LTD but not LTP, whereas NVP-AAM077 blocks LTP but not LTD [27]. These findings were subsequently duplicated in perirhinal cortex [28] and supported the hypothesis that activation of GluN2A-containing NMDARs underlies LTP induction, while GluN2B-containg NMDAR activation supports LTD induction.

Similar experiments conducted by other groups have yielded varying results, leading to controversy regarding the roles of the individual subunits in plasticity induction. While several studies indicate that LTP is unaffected by GluN2B antagonists in various brain regions [27–32],

others have found that LTP is blocked or partially blocked by GluN2B antagonists in cultured hippocampal slices [33], acute hippocampal slices [34–36], or slices from cingulate cortex [37]. Still others have found that LTP at CA3-CA1 synapses [38, 39] and the nucleus accumbens [40] is affected by neither GluN2A nor GluN2B antagonists, while in the lateral amygdala both antagonists partially block LTP [41]. Other findings suggest that the GluN2B dependence of LTP induction varies with respect to age [42] or induction protocol [43].

Similar disparities exist in pharmacological findings associated with LTD. Contrary to findings that LTD is blocked by Ro25-6981 [27, 28, 44], other studies find that LTD is impervious to [34, 35, 45] or even enhanced by GluN2B antagonists [46]. Kollen et al. have shown that LTD in hippocampus is blocked by NVP-AAM077 but not Ro25-6981 [47]. Studies in adult anterior cingulate cortex [48] and lateral amygdala [41] reveal that LTD can be blocked by either Ro25-6981 or NVP-AAM077, suggesting both subunits play a role in LTD induction in these regions. Studies employing a model of LTD based on AMPA receptor internalization have more consistently supported a role for GluN2B in LTD induction. Pre-application of ifenprodil but not NVP-AAM077 decreased NMDA-induced internalization of GluA1 [49] and GluA2 [50] in dissociated hippocampal cultures. Similarly, at thalamic synapses in the lateral amygdala, GluA1 and GluA2 internalization resulting from a low-frequency stimulation paradigm that induces LTD is blocked by Ro25-6981 but not NVP-AAM077 [51]. In aggregate, however, results from pharmacological experiments designed to determine the respective roles of GluN2A and GluN2B in LTD have been variable.

One reason for these mixed results could be variability in preparing slices or dissociated neuronal cultures. In vivo experiments eliminate the potential variation in experimental preparation. Indeed, results from in vivo preparations have more consistently supported a role for GluN2A in LTP and GluN2B in LTD. Intraperitoneal injection of Ro25-6981 into either anesthetized [29, 52] or freely moving [53] rats prevents LTD but not LTP. Conversely, injection of NVP-AAM077 prevents LTP but not LTD [29, 53].

Another potential problem facing the interpretation of pharmacological results is certain kinetic properties of the antagonists. NVP-AAM077 was initially reported to have a 100-fold preference for human GluN2A-NMDARs over GluN2B-NMDARs [26]. However, its selectivity drops to 10-fold for rodent GluN2A versus GluN2B NMDARs [54]. As such, NVP-AAM077 was shown to inhibit GluN2B NMDARs, as well as block LTP, in GluN2A knockout mice [55]. GluN2B antagonists also demonstrate some complications. Ifenprodil has been shown to block triheteromeric receptors much less effectively than GluN2B diheteromeric receptors [56], and the GluN2B antagonist CP-101,606 may not block triheteromeric receptors at all [57]. Thus, these drugs leave a large population of GluN2B-containing NMDARs presumably unblocked. Additionally, in low glutamate concentrations, ifenprodil potentiates the response of GluN2B NMDARs to glutamate rather than antagonizing it [58]. The problems associated with the available GluN2 selective antagonists have no doubt contributed to some of the difficulties in interpreting the outcomes from the many experiments that have utilized them. Development of novel, more selective, antagonists [59] will aid in determining the contributions of channels containing GluN2A subunits versus GluN2B subunits to plasticity induction.

Experiments Addressing the Role of Entire GluN2 Subunits in Plasticity

In addition to limitations in the ability of the antagonists to differentiate between subunits, pharmacological approaches also do not address the physical and/or structural aspect of the subunits' contributions to signaling in plasticity induction. Studies that analyze changes in surface expression of the entire subunit obviate the specificity problem of the pharmacologic approach. Additionally, they can provide information about the subunit as a whole, including the intracellular C-terminal domain, as opposed to only the ion-conducting channel portion. Below, we address experiments in which the GluN2 content of synapses has been altered in one of two ways: either indirectly through activity or other signaling pathways, or directly in transgenic animals.

Indirect Modulation of GluN2 Surface Expression

Several lines of evidence support a correlation between the synaptic GluN2A/GluN2B ratio and the threshold for LTP induction. Activity tends to increase the GluN2A/GluN2B ratio at synapses and is in many cases correlated with an increase in the LTP induction threshold (i.e., an increase in the strength of the induction protocol that is required to induce LTP). In visual cortex, as the GluN2A/GluN2B ratio increases, so does the threshold for LTP induction [60]. Dark rearing delays the normal activity-induced increase in the GluN2A/GluN2B ratio [61, 62] and also lowers the threshold for LTP induction [63]. Similarly, suppressing activity in hippocampal neurons increases the GluN2B content of synapses and lowers the LTP induction threshold [64, 65]. In hippocampus, priming with a high-frequency train increases the GluN2A/GluN2B ratio at synapses and facilitates subsequent LTD induction. Conversely, priming with a low-frequency train decreases the GluN2A/GluN2B ratio and facilitates subsequent LTP induction [66].

GluN2 surface expression may also be altered through secondary signaling pathways, which in some cases yields results consistent with the activity-dependent experiments described above. Specifically, an increase in the GluN2A/GluN2B ratio is correlated with an increased threshold for LTP induction. For example, D_1/D_5 receptors interact with and modulate NMDAR current [67] and cause a decrease in the GluN2A/GluN2B ratio [68] as well as enhanced LTP [69]. Cdk5 also appears to play a role in the regulation of GluN2B current, as knockout mice display increased GluN2B surface expression and current as well as enhanced GluN2B-dependent LTP [70]. Src mediates phosphorylation of NR2B at Tyr1472 [71], which is enhanced following LTP in the hippocampus [71–73].

Conversely, other groups have found that manipulations leading to an increase in the GluN2A/GluN2B ratio cause the LTP threshold to be lowered. For example, Zhao et al. find that following block of synaptic receptors with MK-801, the EPSC recovers with mainly GluN2B-containing NMDARs, which correlates with an increase in the LTP induction threshold [74]. p75 neurotrophin receptor ($p75^{NTR}$) knockout mice display decreased expression of GluN2B as well as decreased LTD [75]. Recently, the transmembrane protein neuropilin tolloid-like 1 (Neto1) has been recognized to associate with NMDARs and has been shown necessary for proper GluN2A incorporation into synapses. Neto1 knockouts exhibit reduced GluN2A current and reduced LTP [76]. Interestingly, polysialylated NCAM has been implicated in the regulation of current through GluN2B diheteromeric receptors [77, 78]. NCAM-deficient mice have increased GluN2B signaling and decreased LTP that is restored by application of Ro25-6981 [78].

Direct Modulation of GluN2 Surface Expression

In addition to experiments in which the subunit ratio is altered indirectly, either through activity or signaling pathways, a number of experiments have addressed the roles of the subunits by directly manipulating their expression. Synapses from mice lacking expression of the GluN2A subunit retain some ability to undergo LTP, though the amplitude of LTP may or may not be impaired depending on the brain region in question and perhaps the induction protocol. The amplitude of LTP is greatly diminished in hippocampus [20] and eliminated in superior colliculus [79], while it is unaffected in dorsolateral portion of the bed nucleus of the stria terminalis [55]. Mice lacking the GluN2B subunit die perinatally [80], impeding study of plasticity in these animals for some time. However, the recent development of conditional knockouts which lack expression of GluN2B only in the forebrain or hippocampus circumvented this problem and revealed a dichotomous effect depending upon the induction protocol. LTP induced by high-frequency stimulation was unaffected in the knockout animal while LTP induced by a low-frequency pairing protocol was reduced [81, 82]. LTD induced by low-frequency stimulation was eliminated [82]. Conversely, both mice [21] and rats [83] overexpressing GluN2B exhibit enhanced LTP but no change in LTD. Overall then, results from transgenic animals would seem to suggest that both subunits are important for plasticity induction.

While experiments utilizing transgenic mice eliminate the specificity problem of pharmacology experiments, they introduce the potential problem of confounding developmental effects or genetic compensation of GluN2A and GluN2B when one of them is deleted in the germline and absent throughout ontogeny. Experiments employing more acute methods of molecular disruption have yielded results consistent with a role for GluN2B in LTP. In hippocampal organotypic slices, RNAi or overexpression of constructs can be used to change subunit expression over the course of a few days. These types of experiments indicate that synapses expressing predominantly GluN2A as opposed to GluN2B cannot support LTP [33, 42]. Conversely, synapses containing predominantly GluN2B NMDAR are able to undergo LTP [42]. Gardoni et al. [84] performed a still more acute disruption of GluN2B localization to hippocampal synapses with a peptide designed to disrupt GluN2B interaction with PSD-95. Synapses acutely depleted of GluN2B in this way were still able to undergo LTD, while LTP induction was blocked. Thus, experiments in which GluN2 expression is more acutely manipulated are consistent with the idea that the physical presence of GluN2B is necessary for LTP induction while the presence of GluN2A cannot support LTP induction.

Mechanisms of Differential GluN2 Subunit Involvement in Plasticity

Through what mechanism could GluN2A and GluN2B be differentially linked to LTP and LTD induction? As noted above, the GluN2 subunits consist of three major domains: the N-terminal extracellular domain, the “middle” channel forming portion in the membrane, and the C-terminal intracellular scaffolding portion. One may imagine that differences in either the channel portion, the intracellular portion, or both could confer upon the subunits their distinct linkage to either LTP or LTD. The channel portion of the subunits may bestow different kinetics, open probability, or calcium permeability upon the NMDAR. These differences could result in distinct intracellular

calcium profiles upon stimulation and thus different forms of plasticity induction in keeping with the calcium hypothesis. Alternatively, differences in the C-terminal tails of the subunits may cause them to interact with different proteins, thereby leading to preferential activation of these proteins during calcium influx through the channel. We examine both possibilities below.

Kinetic Differences Between GluN2A- and GluN2B-Containing NMDARs

Studies of the differences in the biophysical and kinetic properties between the GluN2A and GluN2B subunits have yielded somewhat conflicting results. Experiments in heterologous systems indicate that GluN2A-containing NMDARs display both faster kinetics of deactivation and a higher open probability than GluN2B-containing NMDARs [9, 85, 86]. Owing to these differences, stimulation of GluN2B-containing NMDARs has been suggested to result in greater charge transfer than stimulation of GluN2A-containing NMDARs at lower frequencies, while stimulation at higher frequencies tends to produce greater charge transfer through GluN2A-containing NMDARs [85]. This has led to the hypothesis that channel kinetics support a propensity for GluN2B-containing NMDARs to induce LTD and GluN2A-containing receptors to induce LTP.

Alternatively, studies in cerebellar granule cells have indicated that the peak open probability of GluN2A- and GluN2B-containing NMDARs are similar (while the kinetics of GluN2A-containing receptors are still faster than GluN2B-containing receptors) [87]. While these experiments are under more physiological conditions than experiments in heterologous systems, they are less direct. Additionally, GluN2B subunits may be more conductive to Ca^{2+} than GluN2A subunits [88], in which case, it becomes difficult to predict which subunit would be more likely to produce a Ca^{2+} transient favoring LTP versus LTD.

Another factor complicating the prediction of Ca^{2+} influx through NMDARs during LTP induction is the number of different experimental stimulus paradigms used for inducing LTP. These include various protocols employing either high-frequency stimulation alone or lower-frequency stimulation paired with postsynaptic depolarization. One study indicates that for high-frequency field LTP, Ca^{2+} influx through both receptor subtypes is required, while for lower-frequency pairing protocols influx through either subtype will suffice [89]. Additional work is needed to determine the specific biophysical properties of the subunits under physiological conditions in neurons, as well as their contribution to calcium influx during specific protocols.

Differential Protein Interactions of the GluN2A and GluN2B C-Terminal Tails

An additional possibility, that the C-terminal tail plays a role in determining which type of plasticity a subunit is linked to, relies on the fact that a large number of intracellular signaling proteins have been identified that interact with the GluN2 subunits (Fig. 1). Additionally, these proteins are often differentially associated with the tails of GluN2A and GluN2B (Table 1). In this way, the C terminus may act as a scaffold to bring specific signaling proteins into proximity with the channel, and calcium influx through channels containing different subunits will lead to activation of distinct proteins and signaling cascades.

Direct C-Terminal Protein Interactions: GluN2A Versus GluN2B

One of the most significant proteins involved in LTP induction that preferentially interacts with GluN2B as opposed to GluN2A is CamKII. Activation of CamKII following Ca^{2+} binding increases its affinity for the C-terminal tail of GluN2B [90, 91]. GluN2B binding can cause the enzyme to become persistently active independent of autophosphorylation [92]. Both PKC and CamKII phosphorylate GluN2B at Ser1303 [93, 94], which inhibits the interaction of GluN2B and autophosphorylated CamKII [95] and has been demonstrated to enhance desensitization of GluN2B containing NMDARs [96]. Given the intimate association between GluN2B and CamKII, it has been speculated that their interaction may be critical for LTP induction, and indeed, when their binding is disrupted, LTP is blocked [33]. Furthermore, increasing the affinity of the GluN2A subunit for CamKII allowed this subunit to support LTP when it had formerly been unable to do so [33]. Thus, it appears that the direct association between CamKII and the GluN2B subunit is necessary for the enzyme to participate in LTP induction.

In addition to CamKII, Ras-GRF1 and α-actinin also directly and preferentially interact with the tail of GluN2B. α-Actinin binds directly to the tails of the GluN2B and GluN1 subunits, though it displays no interaction with the tail of GluN2A [97], thereby providing a potentially unique way in which GluN2B can anchor the NMDAR to the actin cytoskeleton. Ras-guanine nucleotide-releasing factor 1 (Ras-GRF1) is a Ras-specific GDP/GTP exchange factor that activates Ras in a Ca^{2+}/calmodulin-dependent manner and also interacts preferentially with GluN2B as opposed to GluN2A [98]. The interaction between GluN2B and Ras-GRF1 has been linked both to ERK activation and LTP induction in one study [98] and to the p38 MAPK pathway and LTD induction in another [99].

Table 1 Proteins that interact either directly or indirectly with GluN2A or GluN2B

Protein interactor	GluN2 subunit preference	Interaction	Reference
CaMKII	GluN2B	Direct	94, 95
α-Actinin	GluN2B	Direct	101
Ras-GRF1	GluN2B	Direct	102
SAP102	GluN2A, GluN2B	Direct	8, 109
PSD-95	GluN2A, GluN2B	Direct	8, 109
SAP97	GluN2A	Direct	108
SynGAP	GluN2B	Indirect	53, 115
nNOS	GluN2A	Indirect	8, 119
Homer	GluN2A	Indirect	8, 120
β-Catenin	GluN2A	Indirect	8
CRMP2	GluN2B	Indirect	8
TANC	GluN2B	Indirect	116
Densin-180	GluN2B	Indirect	118
Flotillin-1	GluN2A, GluN2B	Direct	127
Flotillin-2	GluN2B	Direct	127

This table highlights a subset of proteins known to interact differentially with the GluN2A and GluN2B subunits. Owing to space limitations, this is not a comprehensive list of all GluN2 protein interactors

CaMKII Ca2+/calmodulin-dependent protein kinase II, *CRMP2* collapsin response mediator protein 2, *nNOS* neuronal nitric oxide synthase, *PSD-95* postsynaptic density protein 95, *Ras-GRF1* Ras-guanine nucleotide-releasing factor 1, *SAP102* synapse-associated protein 102, *SAP97* synapse-associated protein 97, *SynGAP* synaptic Ras GTPase-activating protein

Both GluN2 subunits interact directly with the PSD-95 subfamily of MAGUKs, which includes PSD-95, SAP102. SAP97, and PSD-93, via their extreme C-terminal 4 amino acids [5]. This interaction seems to play an important role in localizing NMDARs to the synapse [100–102] and provides a scaffold to link proteins within the postsynaptic density [103]. Several studies have addressed the question of which MAGUKs associate with which GluN2 subunits. Overexpression of SAP97 in hippocampus results in an acceleration of NMDA receptor currents, consistent with a preferential association of SAP97 with GluN2A [104]. PSD-95 binding to NMDARs is decreased in GluN2A knockout mice relative to controls [14]. This result coupled with immunoprecipitation data [105] suggested that GluN2B preferentially interacts with SAP102 whereas GluN2A preferentially binds PSD-95. More recently, however, Al-Hallaq et al. demonstrated SAP102 and PSD-95 interact with diheteromeric GluN1/GluN2A and GluN1/GluN2B receptors at comparable levels [4]. Therefore, the details of potentially differential associations between certain MAGUKs and the GluN2 subunits remain unclear. Additionally, posttranslational modification of individual GluN2 subunits may affect their interaction with MAGUKs, creating specific interactions under certain circumstances. For example, CK2 phosphorylates GluN2B at Ser1480, thereby disrupting its interaction with PSD-95 and SAP102 and decreasing its surface expression [106, 107].

Indirect C-Terminal Protein Interactions: GluN2A Versus GluN2B

In addition to proteins that directly interact with GluN2 C termini, several proteins involved in plasticity signaling also indirectly interact with GluN2B and GluN2A while showing preference for one or the other subunit. SynGAP regulates synaptic strength and plasticity, with overexpression causing a marked decrease in surface AMPA receptor expression [108] and LTP induction [109, 110] consistent with its role as a RasGap [111]. SynGAP is indirectly associated with NMDARs through PSD-95 and SAP102 [111] and interestingly shows preferential association with complexes containing the GluN2B subunit as opposed to the GluN2A subunit [49]. CRMP2, a member of the collapsin response mediator protein family and potentially involved in intracellular trafficking, is also preferentially associating with GluN2B, presumably through a secondary interaction [4]. The scaffolding protein TANC coimmunoprecipitates with GluN2B but not GluN2A from solubilized postsynaptic densities [112], and mice deficient in TANC1 expression exhibit reduced spine density in CA3 neurons as well as impaired spatial memory [113]. Densin-180, a member of a family of leucine-rich repeat proteins that mediate protein interactions within the postsynaptic density (PSD), coimmunoprecipitates with GluN2B from PSD-enriched fractions [114]. It also interacts with CamKII and

α-actinin and in that way serves to bring these molecules into a complex [114].

Several signaling proteins display a preferential indirect association with GluN2A including neuronal oxide synthase (nNOS) synthase, β-catenin, and Homer [4]. The interaction between nNOS and NMDARs is mediated by PSD-95 [115]. Homer also interacts with PSD-95, though via a more indirect route involving Shank and GKAP [116]. β-Catenin may interact with NMDARs via S-SCAM and PSD-95 [117, 118].

It is intriguing that a large number of signaling proteins show preferential association with one of the GluN2 subunits in spite of the fact that their interaction with the subunit is known to be indirect. These signaling molecules often associate with GluN2A and GluN2B via MAGUKs, very often PSD-95. However, as mentioned above, many of the MAGUKs, including PSD-95, do not necessarily display a preferential association for one subunit over the other. How then is specific association of signaling molecules with either GluN2A or GluN2B achieved? One possibility is that posttranslational modification of GluN2 subunits [106, 107] or the MAGUKs themselves [119] could change the affinity of a given MAGUK for one of the subunits under certain conditions.

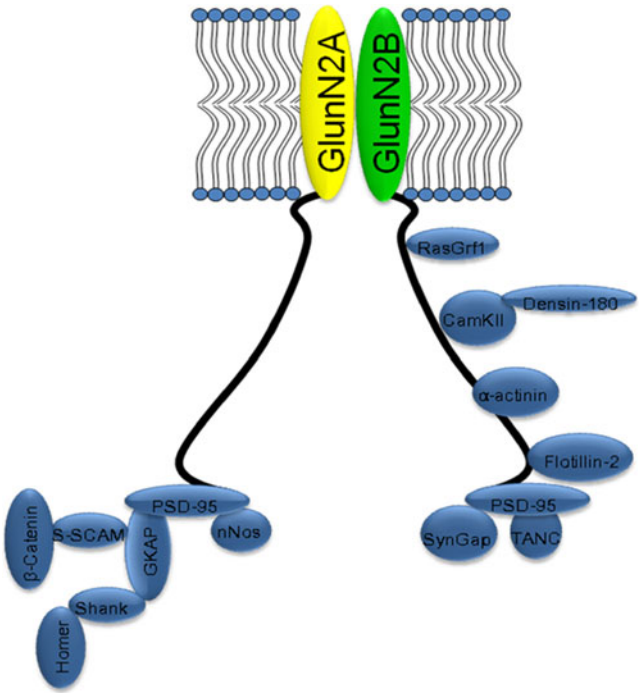

Fig. 1 Schematic summarizing experimental evidence for preferential protein interactions of the C-terminal tails of the GluN2A subunit (*yellow*) and the GluN2B subunit (*green*). Proteins are shown proximal to the subunit with which they have been experimentally determined to have a preferred interaction. Relative binding to individual subunits may vary depending on environmental factors such as posttranslational modifications. For references, see Table 1. *CaMKII* Ca2+/calmodulin-dependent protein kinase II, *nNOS* neuronal nitric oxide synthase, *PSD-95* postsynaptic density protein 95, *Ras-GRF1* Ras-guanine nucleotide-releasing factor 1, *SynGAP* synaptic Ras GTPase-activating protein

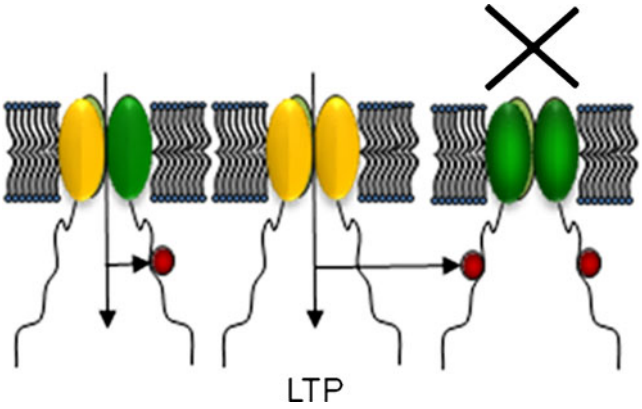

Fig. 2 Potential activation of molecules associated with GluN2B subunits (*green*) by Ca^{2+} entering through GluN2A-containing (*yellow*) channels. Ca^{2+} influx through NMDARs leads to activation of intracellular proteins that regulate LTP induction. In the case of CaMKII, the physical association of this signaling protein with the intracellular tails of GluN2B subunits is critical for its activation by Ca^{2+} flowing through these NMDARs [33]. Pharmacologic antagonists such as Ro25-6981 block Ca^{2+} influx through GluN2B-containing NMDARs (represented by the *X*). However, Ca^{2+} entering through unblocked GluN2A-containing NMDARs in close proximity may allow activation of CaMKII associated with the C-terminal tail of blocked GluN2B-containing NMDARs. Thus, although proteins may be associated with one particular subunit, they may still be activated by Ca^{2+} entering through channels containing the other subunit

Another possible reason for the differential association of proteins with GluN2A and GluN2B is that they may be differentially located in the synapse. If GluN2A and GluN2B are largely restricted to different subdomains, they may have access to different populations of intracellular proteins. This could occur through relative separation of GluN2A into synaptic space and GluN2B into extrasynaptic space. Indeed, some studies have shown LTP is preferentially induced by synaptic stimulation while LTD is induced by extrasynaptic stimulation [28, 120]. Additionally, activation of synaptic receptors activates CREB [121] and ERK [122] while activation of extrasynaptic receptors deactivates CREB [121], ERK [122], and Ras [49]. These results are consistent with a physical subdivision of signaling pathways.

Alternatively, the different GluN2 subunits could be segregated via interaction with lipid rafts. Consistent with this idea are data showing that both GluN2A and GluN2B interact with the lipid raft associated protein flotillin-1, but GluN2B also interacts with flotillin-2 while GluN2A does not [123]. Additionally, GluN2A is divided evenly between the postsynaptic density and rafts, whereas GluN2B is relatively depleted from rafts [124]. Lack of PSD-95 allows

GluN2A to enter rafts, suggesting that PSD-95 restricts GluN2A from rafts [124].

Experiments Addressing the Role of the Channel Versus the Role of the C-Terminal Tail of GluN2A and GluN2B

As noted above, pharmacological experiments address the role of the channel portion of GluN2 subunits in plasticity induction. Complementing these pharmacological experiments, a number of transgenic and knockout animals have addressed the role of the subunit as a whole, including both the channel and the C-terminal tail. Importantly, several experiments have recently addressed the role of the intracellular portion of the subunit independently from the channel portion.

Highlighting the importance of the intracellular C-terminal tail alone, mice engineered to express subunits that lack this portion of the subunit have been shown to phenocopy mice lacking the entire subunit [102]. As such, mice expressing only the N-terminal half of GluN2B die perinatally and mice expressing a truncated GluN2A subunit exhibit reduced LTP [6]. More recent evidence suggests that this reduction in LTP is highly dependent on the induction paradigm, with only LTP induced at frequencies of less than 20 Hz being affected [125].

Further evidence for a specific role of the C-terminal tail of GluN2 subunits comes from experiments in organotypic hippocampal cultures. In this preparation, synapses expressing predominantly GluN2A as opposed to GluN2B cannot support LTP [33, 42], while synapses containing predominantly GluN2B NMDARs are able to undergo LTP [42]. Interestingly, synapses expressing chimeric constructs only underwent LTP when subunits contained the C terminus of GluN2B rather than GluN2A, while the identity of the channel portion of the chimeric subunit did not affect its ability to evoke LTP [42]. Experiments were also conducted on synapses containing GluN2A subunits with enhanced binding to CamKII and GluN2B subunits reduced binding to CamKII [33]. In contrast to their wild-type counterparts, the mutated GluN2A supported LTP while altered GluN2B subunits could not. Taken together, these data suggest a very important role for the C terminus of the GluN2 subunits, particularly the GluN2B subunit, in supporting LTP [33].

In addition to purely molecular experiments, pharmacology has been combined with molecular experiments to further differentiate between the roles of the channel and intracellular portions of the subunits. In more mature organotypic slices, LTP is not blocked by GluN2B antagonists even though molecular removal of GluN2B (and replacement with GluN2A) does block LTP [42]. This result suggests that Ca^{2+} influx through GluN2A-containing channels may support LTP, though structurally GluN2A lacks the ability to support LTP. This difference may arise because GluN2A lacks the appropriate C-terminal interactions to recruit key LTP signaling molecules into the vicinity of Ca^{2+} entering the synapse (Fig. 2). Similarly, a role for the C terminus of GluN1 subunits has been identified in spine stability. Molecular removal of the C-terminal C2 cassette within the GluN1 subunit destabilizes spines, though pharmacological blockade of NMDARs does not [126]. These experiments suggest that intracellular interactions of NMDAR subunits play a crucial role in the maintenance of synaptic function and stability that is independent from that of the channel portion.

Conclusion and Future Perspectives

The GluN2A and GluN2B subunits each confer a number of distinct properties upon the NMDAR, including different biophysical properties and protein interactions. It is becoming increasingly clear that these differences cause the subunits to couple to different signaling pathways within the cell. However, controversy remains as to exactly which subunit is linked to which pathways, particularly in the case of LTP versus LTD induction. Also, it remains unclear exactly which properties are important for associating each subunit with a specific pathway. Additional work will undoubtedly continue to shed light on how the various unique properties of the two subunits connect them to different intracellular signaling pathways.

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