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T*rans*-synaptic interactions of ionotropic glutamate receptors

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Abstract

Trans-synaptic interactions organize the multiple steps of synaptic development and are critical to generate fully functional neuronal circuits. While *trans*-synaptic interactions are primarily mediated by cell adhesion molecules (CAMs), some directly involve ionotropic glutamate receptors (iGluRs). Here, we review the expanding extracellular and *trans*-synaptic proteome of iGluRs. We discuss the role of these molecular networks in specifying the formation of excitatory and inhibitory circuits and in the input-specific recruitment of iGluRs at synapses in various cell types and brain regions. We also shed light on human-specific mutations affecting iGluR-mediated *trans*-synaptic interactions that may provide unique features to the human brain and contribute to its susceptibility to neurodevelopmental disorders. Together, these data support a view in which iGluR function goes far beyond fast excitatory synaptic transmission by shaping the wiring and the functional properties of neural circuits.

Introduction

Synapses ensure and shape the transfer of information throughout the brain. They are sub-micrometric multimolecular machineries connecting an axon terminal from a pre-synaptic neuron to specialized membrane domains of a post-synaptic neuron. Synapse formation, maturation and maintenance require physical and functional *trans*-synaptic interactions. This is mainly mediated by cell adhesion molecules (CAMs), which bring together pre- and postsynaptic membranes and convey signals that organize the multiple stages of synapse development, from initial contact to synaptic maturation and plasticity (reviewed in [1–3]). CAMs can operate through homophilic or heterophilic interactions with other CAMs. They can also interact with proteins secreted by neurons or glial cells, which form a dense matrix in the synaptic cleft and provide extracellular scaffolds that link pre- and postsynaptic CAMs [4]. The large repertoire of CAMs greatly contributes to the diversity of synapses and to the specification of neuronal circuits by matching the appropriate presynaptic and postsynaptic neurons and directing synaptogenesis to certain membrane compartments (e.g. soma, proximal dendrites, distal dendrite, axon initial segment) [5]. Recently, it has emerged that conventional CAMs do not hold the monopoly of synaptic adhesion and that some ionotropic glutamate receptors (iGluRs) also engage in *trans*-synaptic interactions.

iGluRs are the main excitatory neurotransmitter receptors in the central nervous system. They comprise four subfamilies: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), *N*-methyl-D-aspartate (NMDA), kainate (KA) and delta (GluD) receptors. All iGluRs form tetramers and possess the same domain modular architecture. Each subunit has a large extracellular domain that includes the N-terminal domain (NTD), which dictates the organization of iGluRs subunits within the tetramers, and the agonist binding domain (ABD), which determines ligand binding specificity. The transmembrane domain (TMD) defines the pore of the ion channel, while the cytosolic C-terminal domain (CTD) mediates intracellular interactions, along the secretory pathway or at the synapse, and exhibit the highest degree of diversity in length and sequence. In this review, we discuss recent progress in identifying and understanding the implications of iGluRs in *trans*-synaptic interactions. The NTD of GluD, AMPA and KA receptors projects into the synaptic cleft with a vertical height and structure permitting extracellular interactions [6]. The extracellular domain of NMDA receptors is more compact and differently organized [6]. NMDA receptors are the only iGluRs for which no *bona fide trans*-synaptic interactions have been reported and won't be discussed here.

The glutamate receptor delta-2: synapse specification and plasticity in the cerebellum

Trans-synaptic functions of iGluRs are best characterized for GluD2, a receptor predominantly expressed in the cerebellum whose mutations cause ataxia [7]. GluD2 is a postsynaptic receptor enriched at excitatory synapses between parallel fibers (PFs) and Purkinje cells (PCs). GluD2 interacts with presynaptic Neurexins (Nrxns) containing the splice segment (SS)4 via Cerebellin 1 (Cbln1), an extracellular scaffolding protein belonging to the C1q family of the classical complement pathway (Figure 1) [4]. Cbln1 is secreted by PF lysosomes in an activity- and Ca^{2+}-dependent manner. It is thought to first interact with Nrxn on the axonal membrane and then recruit GluD2 postsynaptically [8–10]. The *trans*-

synaptic triad Nrxn^{SS4+}-Cbln1-GluD2 is synaptogenic in artificial synapse formation assays [8,9]. It induces axonal remodeling and accumulation of presynaptic vesicles *in vitro*, and it is required for the alignment of the PSD and the active zone *in vivo* [8,9,11–13]. As a consequence, PC deficient for *grid2* (the gene encoding GluD2) harbor dendritic spines that are either not opposed to a presynaptic terminal ("free" spines), or whose PSD is misaligned with the presynaptic active zone ("mismatched" synapses), leading to a ~50% loss of PF-PC synapses. The mechanisms underlying the formation of dendritic spines and the assembly of PSDs in PC distal dendrites are largely intrinsic and independent of synapse formation, which differs from the neocortex and hippocampus [14]. Hence, despite the loss of synapses, the density of dendritic spines is not altered by *grid2* deletion. At the molecular level, the C-terminal PDZ binding motif is dispensable for synapse formation and it remains to be determined how GluD2 regulates pre-post alignment on the postsynaptic side. Interestingly, in *grid2* knockout mice, the remaining PF-PC synapses show increased content of AMPA receptors (AMPARs) [15]. This is consistent with the role of GluD2 in AMPAR endocytosis and long-term depression (LTD) [15,16] and suggests that GluD2 could organize the formation and maintenance of silent synapses, which represent around 90% of PF-PC synapses in the adult cerebellum [17]. It is also remarkable that *grid2* deficiency leads to the abnormal innervation of PCs by climbing fibers (CFs), which invade PF territory in distal dendrites, and by molecular layer interneurons (INs) [12, 18]. Conversely, the synaptic accumulation of GluD2 in proximal dendrites after blockade of synaptic activity results in the innervation of proximal dendritic spines by PFs instead of CFs [18,19]. This indicates that GluD2 not only instructs the formation of PF-PC synapses, but also plays a key role in PF versus CF competition for PC innervation. A recent study suggests that another level of competition exists between neighboring PCs for the formation of synapses with PFs and contributes to sculpt PC dendritic tree. Accordingly, the sparse (but not the global) knockout of *grid2* leads to severe alteration of PC dendritic morphology [20].

The mechanism underlying GluD2 function is best characterized in LTD, a fundamental mechanism behind motor learning, which is completely abolished in *grid2*-deficient mice. LTD at PF-PC synapses requires GluD2-mediated *trans*-synaptic interaction, GluD2 binding to its agonist D-serine (released by Bergmann glia), and postsynaptic signaling cascades involving GluD2 C-terminal PDZ binding motif, which ultimately regulate the phosphorylation of GluA2-containing AMPARs and their surface expression [7]. Recent crystallographic structures and functional tests have demonstrated that constraining the relative mobility of the NTD and ABD of GluD2 with a glycan linker occludes cerebellar LTD, supporting a model in which Cbln1 and agonist binding (at the NTD and ABD of GluD2, respectively) operate in synergy to propagate a postsynaptic signaling that modulates AMPAR trafficking [13]. Remarkably, neither GluD2 function in synapse formation and maintenance nor its role in synaptic plasticity requires ion flux through its channel, which underlines the atypical nature of this receptor.

The glutamate receptor delta-1: region-specific formation of excitatory and inhibitory circuits.

GluD1, the other member of the GluD subfamily, has also recently been implicated in *trans*-synaptic interactions. Like GluD2, GluD1 forms a *trans*-synaptic complex with Cbln and Nrxn^{SS4+} and induces

artificial synapses in co-culture assays (Figure 1) [21,22]. Yet, its function is just starting to be understood. GluD1 is highly expressed in several brain regions, including the cerebral cortex, the cerebellum, the striatum and the hippocampus [23,24]. Its expression peaks postnatally during synaptogenesis and remains high in adults [23,25]. Mice deficient for *grid1*, the gene encoding GluD1, show social and cognitive deficits consistent with the association between *GRID1* mutations and neurodevelopmental and psychiatric diseases such as autism and schizophrenia [7]. In the cerebellum, GluD1 is detected at excitatory synapses between PFs and molecular layer INs, where it controls synaptic density and IN survival [23]. In CA1 pyramidal neurons (PNs) of the hippocampus, GluD1 localizes to dendritic spines [25] and enhances excitatory synaptogenesis through Cbln2 [26]. In the somatosensory cortex however, GluD1 is dispensable for excitatory synapse formation but necessary to establish specific inhibitory synaptic connections. Accordingly, GluD1 is detected in ~50% of inhibitory synapses in oblique apical dendrites of layer 2/3 CPNs, and even in a higher proportion in the apical tuft [27]. In layer 2/3 CPNs, GluD1 mediates *trans*-synaptic interaction by binding to Cbln4 (Figure 1), a Cbln isoform specifically expressed by a subpopulation of somatostatin-positive (SST^+) INs [28]. Hence, Cbln4 and GluD1 specify the formation of inhibitory synapses between SST^+-INs and layer 2/3 CPNs [27,29]. In addition to being engaged in *trans*-synaptic interaction, GluD1 needs to bind to its agonist (glycine or D-serine) to mediate inhibitory synaptogenesis. The joint action of Cbln and agonist binding has been proposed to regulate intracellular interactions in the C-terminal tail and signaling pathways inducing inhibitory postsynaptic assembly [27]. Many postsynaptic partners of GluD1 have been identified in a proteomic screen. At least two of them, the GTPase exchange factor ARHGEF12 and PPP1R12A, a regulatory subunit of the phosphatase PP1 are required for GluD1 function in the formation and specification of inhibitory synapses [27]. Major partners of GluD1 (including SRGAPs and ARHGAP32) have also been shown to either directly bind to gephyrin, the core component of inhibitory postsynaptic scaffolds, or to regulate the trafficking of $GABA_A$ receptors [30,31], indicating that GluD1 serves as a signaling hub during inhibitory synaptogenesis. Further work is needed to determine (1) how structural changes in the uniquely arranged extracellular domains of GluD1 [32] are transmitted to the intracellular C-terminal tail of the receptor for signal transduction; (2) whether GluD1 requires tonic activation or activation during critical periods, and whether this activation depends on neuronal or synaptic activity; and (3) how GluD1 signaling organizes postsynaptic assembly.

Other functions of GluD1 have been suggested in various brain regions. They include contribution to mGuR signaling in the midbrain and to α1-adrenergic receptor signaling in dorsal raphe nucleus through ionotropic mechanisms [33,34], and regulation of spine pruning and GluN2A/GluN2B ratio [35]. So far, it is not known if these functions involve *trans*-synaptic signaling. Furthermore, in some individual neurons of cortical and other extracerebellar regions, GluD1 is co-expressed with GluD2 [25]. The two subunits can be found in complex and they have been detected together in PSDs [27,36]. It remains to be investigated if GluD1 and GluD2 have overlapping, independent or new functions when colocalized, and maybe when co-assembled as heterotetramers. Overall, the implication of GluD1 in excitatory or inhibitory synaptogenesis depending on brain regions (and maybe neuron subtypes) raises fundamental questions about the molecular determinants of excitatory and inhibitory synaptogenesis (e.g. do they

involve the same signaling cascades downstream of GluD1?). It also questions our understanding of synaptic defects associated with neurodevelopmental and psychiatric disorders because *GRID1* mutations are expected to disrupt excitatory and/or inhibitory circuits depending on brain areas, leading to complex dysfunctions at the level of the brain.

AMPA and Kainate receptors: input-specific trapping

AMPARs mediate fast excitatory neurotransmission. Their number at synapses determines synaptic strength and is regulated during synaptic plasticity [37]. KA receptors (KARs) exhibit slower channel kinetics than AMPARs and their functions are carried out via ionotropic and non-canonical modes of action, including metabotropic-like signaling [38]. The trafficking of neurotransmitter receptors in neurons involves exocytosis and endocytosis in the secretory and recycling pathways, lateral diffusion in the neuronal membrane and trapping at synapses. The surface expression of AMPARs and KARs critically depends on their C-terminal domain and auxiliary transmembrane regulatory proteins (TARPs for AMPARS and Neto1/2 for KARs) [37,39,40]. However, their trapping at synapses also requires extracellular and *trans*-synaptic interactions. Hence, AMPARs lacking their NTD are normally trafficked to the cell surface but poorly trapped at synapses (with subunit-specific differences), which dramatically decreases synaptic transmission and prevents long-term potentiation [41,42].

The first identified extracellular ligands of AMPARs were neuronal pentraxins (Nptx) [4]. Nptx1 and the immediate early gene Nptx2 (or Narp) are secreted proteins anchored to the axon terminal via the Nptx receptor (NptxR), a transmembrane protein that can be cleaved for LTD. Nptx stabilize AMPARs at synapses in a receptor activity-dependent manner (Figure 1) [43]. They have been shown to promote the conversion of silent synapses in the visual system and in parvalbumin-positive cortical and hippocampal INs. In *Nptx2*$^{-/-}$/*NptxR*$^{-/-}$ deficient mice, the maturation of neuronal circuits is delayed and critical periods are extended [44]. For extensive details on the role of neuronal pentraxins, we refer the reader to this excellent recent review [4]. The NTD of AMPARs also binds to N-Cadherins in *cis* or *trans* (Figure 1), which induces dendritic spine formation, stabilizes AMPARs at synapses [45], regulates mGluR-dependent LTD [46] and might contribute to activity-dependent spine maturation or pruning via inter-spine competition [47]. Multi-epitope proteomic analyses of native AMPAR complexes have identified other components that engage or may engage in *trans*-synaptic interactions [48,49]. They include the leucine-rich repeat transmembrane neuronal protein 4 (LRRTM4) and the extracellular proteins noelins/olfactomedins, brorin and brorin-2l (Figure 1). LRRTM4 promotes the formation of dendritic spines and the activity-dependent synaptic recruitment of AMPARs through several *trans*-synaptic interactions involving heparan sulfate (HS) proteoglycans [50,51]: (1) LRRTM4 binds to Glypican-4 (GPC4), which in turn interacts with presynaptic RPTPσ and RPTPδ [50–54]; (2) LRRTM4 can also directly interact with neurexins through their HS modification [54,55] (Figure 1). Importantly, GPC4, which is highly expressed by astrocytes at the beginning of synaptogenesis, induces the formation of active synapses by recruiting GluA1-containing receptors in retinal ganglion cells and throughout the visual system [52,56]. Its interaction with RPTPδ induces the presynaptic release of Nptx1, which amplifies the

synaptic recruitment of AMPARs [52]. In contrast to LRRTM4, the role of Brorins and Noelins/ Olfactomedins is less characterized. While Brorins have not been implicated at synapses yet, Chordin-like 1, a protein secreted by astrocytes which shares functional domains with brorins, has also been suggested to directly interact with AMPARs. Chordin-like 1 is highly expressed in layer 2/3 of the visual cortex, slightly later than GPC4 during synaptic development. It drives synaptic maturation by recruiting GluA2-containing AMPARs, which promotes the switch between calcium-permeable and GluA2-containing calcium-impermeable AMPARs at excitatory synapses and limits synaptic plasticity [57]. Noelin1/Olfactomedin1 has been shown to restrict the lateral mobility of AMPARs [49]. Furthermore, an Olfactomedin (OLF) domain, the main functional domain of Noelins/Olfactomedins, is found in the extracellular region of the postsynaptic adhesion proteins Latrophilins. This domain mediates the interaction with presynaptic Teneurins [58] and critically contributes to specify the formation of synaptic connections in the hippocampus [59]. This raises the possibility that Noelins/Olfactomedins contribute to synapse-specific trapping. Together, this body of work highlights the involvement of glial cells in *trans*-synaptic interactions and the cooperativity between *trans*-synaptic signaling pathways for the recruitment and stabilization of AMPARs at synapses. It remains to be determined which combinations operate at which synapses *in vivo* (i.e. the molecular code) to orchestrate input-specific trapping of AMPARs during synaptic development and plasticity.

KARs are best characterized in the hippocampus, where they are confined at excitatory synapses formed by Mossy fiber (MF) on the proximal dendrites of CA3 synapses. Input-specific localization of KARs at MF-CA3 synapses is achieved via *trans*-synaptic interactions [40,60]. The NTD of GluK2 and GluK4 interacts with the extracellular scaffolding proteins C1ql2 and C1ql3, which belong the C1q complex of the complement pathway [4]. C1ql2/3 are secreted by dentate gyrus (DG) granule cells and interact with presynaptic Nrxn3 containing exon 25b in the splice site 5 ($SS5^{25b}$) (Figure 1) [60]. KAR recruitment to ectopic synapses formed by MF onto DG granule cells play a major role in temporal lobe epilepsy and inactivation of C1ql2/3 partially prevents pathological recurrent excitatory burst activities in a mouse model of chronic epilepsy [61][60]. The NTD of KARs is also required for subunit-specific interaction with their auxiliary proteins Neto1 and Neto2 (Figure 1), which regulate their surface and synaptic delivery [39,62]. Neto1/2 are single pass transmembrane proteins that harbor two extracellular CUB (for complement C1r/C1s, Uegf, Bmp1) domains typically mediating protein-protein interactions. At the neuromuscular junction in *C. elegans* the CUB domain-containing protein lev-10, an auxiliary protein of the acetylcholine receptor, forms an extracellular scaffold by interacting with secreted proteins [63]. This raises the possibility that Neto1/2 extracellular interactome may contribute the synaptic stabilization of KARs. Further investigations are needed to identify the extracellular interactome of Neto1/2 and better understand the trapping of KARs only at selective synapses in the brain. Recent and future innovations in mass spectrometry and proteomic approaches, such as *in vivo* cross-linking, ecto-Fc affinity purification mass spectrometry (AP-MS), *in vivo* proximity-dependent biotin identification (iBioID), multi-epitope proteomics of native molecular complexes or cell surface proteomic profiling of genetically identified cells

in intact brain tissues [8,48,64–66] will be of invaluable help to provide an integrated picture of iGluR *trans*-synaptic complexes and their role in the brain.

Human-specific regulations of iGluR *trans*-synaptic interactions

Remarkably, several mutations that appeared during human evolution affect iGluR *trans*-synaptic complexes. These mutations include human-accelerated regions (HARs) [67–69], which represent conserved genomic loci with elevated divergence in humans and often operate as enhancers. *GRID1* is one of the genes that contain the highest number of human-specific motifs in its intronic regions, suggesting unique transcriptional regulations [67]. During synaptic development, GluD1 expression at the cell surface is also regulated by *SRGAP2* [27], one of the few genes specifically duplicated in humans [70,71], which could contribute to delay inhibitory synaptogenesis in human cortical pyramidal neurons and support the extremely prolonged period of maturation that characterizes the human brain [30,70]. Besides, the GluD extracellular scaffolding protein Cbln2 shows distinct patterns of expression in the mouse and human neocortex [72]. Together, this strongly suggests specific regulations of GluD-Cbln *trans*-synaptic interactions in humans, with potential consequences on the wiring of the brain. Similar to *GRID1*, genomic sequences within the genes encoding GPC4 and RPTPδ, which bind AMPARs through LRTTM4, contain HARs, and mutations in HARs causing a decrease in GPC4 expression have been identified in two cases of ASD with ID comorbidity [68]. Other HARs affecting glutamatergic synaptic transmission have been associated with schizophrenia. It remains to be understood how these HARs impact iGluR trapping at specific synapses in neuronal circuits.

Conclusion

In recent years, the growing repertoire of *trans*-synaptic interactions involving iGluRs has shed a new light on the molecular code that specifies the identity and the functional properties of synaptic connections. iGluR-dependent *trans*-synaptic interactions organize the formation of synaptic connections between specific types of neurons and enable input-specific recruitment of AMPARs or KARs during synaptic maturation, maintenance and plasticity. They are regulated by neuronal, synaptic or receptor activity. Several *trans*-synaptic interactions cooperate to trap AMPARs at certain synapses and promote their maturation. Others can instruct the formation of different circuits, including inhibitory circuits, depending of brain areas. New questions arise on the structure/function of these interactions: How do *trans*-synaptic interactions impact the conformation of the receptors? How do they transduce signaling pathways? Do they modify the ionotropic properties of the iGluRs? Further work is also needed to (1) better characterize the cell-surface proteome of iGluRs and map their spatial, temporal and functional diversity at the subcellular, cellular and circuit levels; (2) determine the contribution of iGluR *trans*-synaptic interactions to the nano-organization of synapses and the formation of *trans*-synaptic nano-columns between the pre- and postsynaptic compartments [73]; and (3) understand how *trans*-synaptic interactions cooperate or compete during the assembly of neural circuits. In this context, the convergence of numerous iGluR- and CAM-mediated *trans*-synaptic interactions on shared ligands such as Nrxns is especially puzzling and

underlines the importance of extensive alternative splicing and post-translational modifications such as glycosylation in synapse specification. Importantly, human-specific mutations affect several genes in iGluR *trans*-synaptic interactions. Investigating the functional consequences of these mutations on synaptic connections in neural circuits will be essential to better understand the uniqueness of the human brain and the pathophysiology of neurodevelopmental disorders.

CONFLICT OF INTEREST

The authors declare no competing interests.

AUTHOR CONTRIBUTIONS

M.F. and C.C. conceived and wrote the manuscript.

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Figure and legend

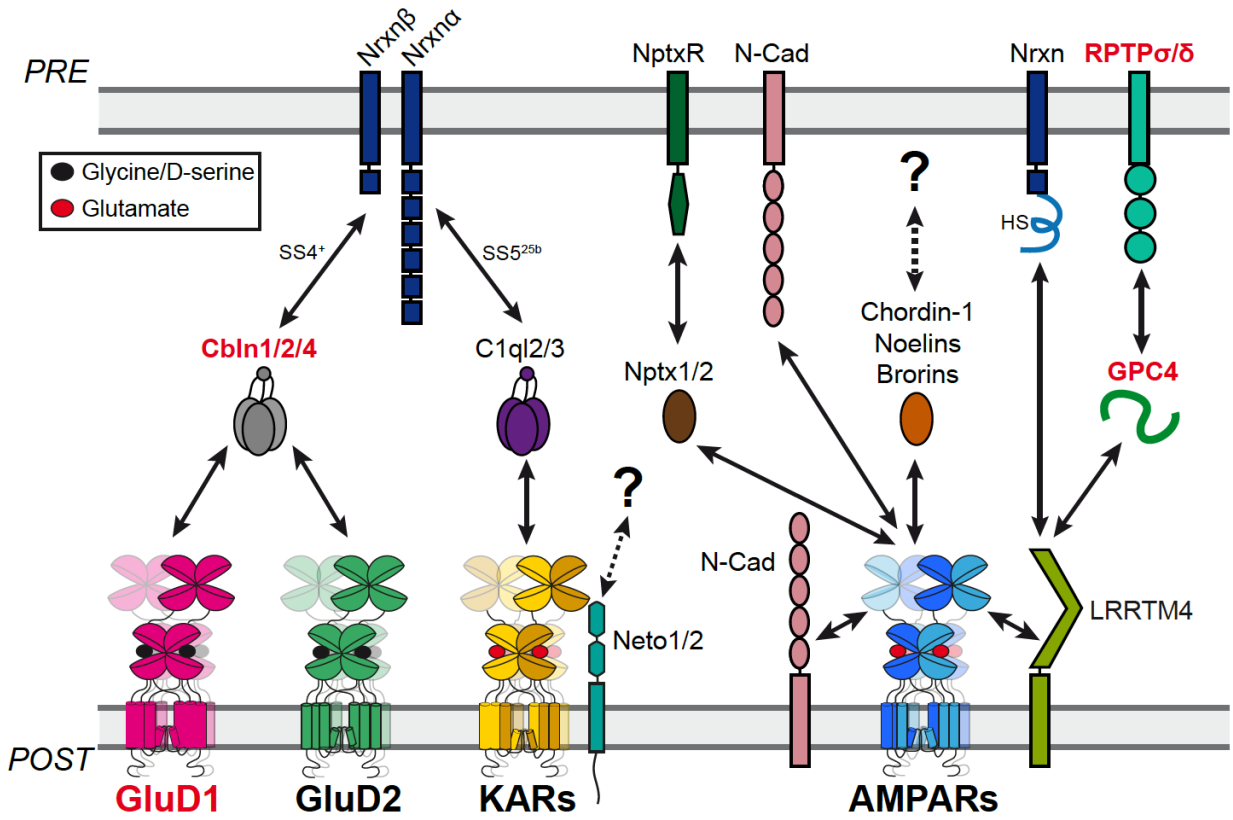

Figure 1. Overview of iGluR extracellular and *trans*-synaptic interactions. AMPARs and KARs use glutamate as ligand while GluDs use glycine and D-serine. Proteins shown in red are or may be differently expressed in humans due to genomic mutations in their regulatory sequences or regulations by human-specific proteins. PRE, presynaptic; POST, postsynaptic; Nrxn, neurexin; NptxR, neuronal pentraxin receptor; Nptx1/2, neuronal pentraxin 1/2; N-Cad, N-cadherin; RPTPσ/δ, receptor protein tyrosine phosphatase σ/δ; HS, heparan sulfate modification; Cbln1/2/4, cerebellin 1/2/4; C1ql2/3, C1q-like 2/3; GPC4, glypican 4; LRRTM4, leucine-rich repeat transmembrane 4; GluD1/2, glutamate receptor delta 1/2; KARs, kainate receptors; AMPARs, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid. Question marks indicate other potential extracellular or presynaptic interactions.