The neuroligins and their neurexin ligands: from structure to function at the synapse

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Abstract The neuroligins are cell adhesion proteins whose extracellular domain belongs to the α/β-hydrolase fold family of proteins, mainly containing enzymes and exemplified by acetylcholinesterase. The ectodomain of postsynaptic neuroligins interacts through a calcium ion with the ectodomain of presynaptic neurexins to form flexible trans-synaptic associations characterized by selectivity for neuroligin or neurexin subtypes. This heterophilic interaction, essential for synaptic differentiation, maturation, and maintenance, is regulated by gene selection, alternative mRNA splicing, and posttranslational modifications. Mutations leading to deficiencies in the expression, folding, maturation, and binding properties of either partner are associated with autism spectrum disorders. The currently available structural and functional data illustrate how these two families of cell adhesion molecules bridge the synaptic cleft to participate in synapse plasticity and support its dynamic nature. Neuroligin partners distinct from the neurexins, and which may undergo either *trans* or *cis* interaction, have also been described, and tridimensional structures of some of them are available. Our study emphasizes the partnership versatility of the neuroligin ectodomain associated with molecular flexibility and alternative binding sites, proposes homology models of the structurally non-characterized neuroligin partners, and exemplifies the large structural variability at the surface of the α/β-hydrolase fold subunit. This study also provides new insights into possible surface binding sites associated with non-catalytic properties of the acetylcholinesterase subunit.

Keywords Acetylcholinesterase · Adhesion · α/β-hydrolase · Autism · Complex · Ectodomain · Enzyme · Model · Neurexin · Neuroligin · Partnership · Structure · Synapse

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Abbreviations

AChE	Acetylcholinesterase
EGF	Epidermal growth factor
FnIII	Fibronectin type III
GPI	Glycophospholipid inositol
Ig	Immunoglobulin
LNS	Laminin/neurexin/sex hormone-binding globulin
MAM	Meprin/A5 protein/receptor protein tyrosine phosphatase mu
MDGA	MAM domain-containing glycosylphosphatidylinositol anchor
NLGN	Neuroligin
NRXN	Neurexin
NMDA	*N*-Methyl-*D*-aspartate
NTD	N-terminal domain
PDZ	Postsynaptic density protein (PSD95)/*Drosophila* disc large tumor suppressor (Dlg1)/zonula occludens-1 protein (zo-1)
RPTP	Receptor protein tyrosine phosphatase
SS	Splice site
SSM	Secondary-structure matching
TSP	Thrombospondin
TSR	Thrombospondin structural repeat

Introduction

Synapse formation, function, and specification are intricately linked to the actions of synaptic cell adhesion molecules (Südhof 2008). The neuroligins (NLGNs) are postsynaptic cell adhesion molecules discovered as ligands for the presynaptic cell adhesion molecules, neurexins (NRXs), and are composed of a single N-terminal extracellular domain, a type I transmembrane region, and a short cytoplasmic C-terminal tail containing several binding/scaffold motifs including a PDZ domain-binding motif, a WW-binding domain, and a gephyrin-binding site (Ichtchenko et al. 1995; Ichtchenko et al. 1996; Irie et al. 1997; Iida et al. 2004; Poulopoulos

et al. 2009). In mammals, there are four *NLGN1-4* genes, of which those encoding NLGN3 and NLGN4 are localized on the X chromosome. In humans, a fifth *NLGN5* or *NLGN4Y* gene located on the Y chromosome encodes a similar NLGN4 protein. All NLGN mRNAs except for the NLGN4 mRNA can be alternatively spliced at a canonical position referred as splice site A (SSA), while the NLGN1 mRNA also harbors a second splice site, B (SSB). Presence versus absence of the splice inserts confers partnership variability to the mature NLGNs (Siddiqui et al. 2010). NLGN1 and NLGN2 are exclusively localized to glutamatergic excitatory and GABAergic/glycinergic inhibitory synapses, respectively, while NLGN3 appears to be present in both synapses, and NLGN4 is preferentially localized to glycinergic synapses (Song et al. 1999; Varoqueaux et al. 2004; Budreck and Scheiffele 2007; Hoon et al. 2011). Sequence comparison indicates that the various NLGNs are highly similar in vertebrates but are more distantly related in invertebrates.

The NRXNs form a large family of neuronal cell adhesion molecules first identified as receptors for the large presynaptic neurotoxin from black widow venom, α-latrotoxin (Ushkaryov et al. 1992; Reissner et al. 2013). Mammals contain three *NRXN1-3* genes, each encoding long NRXNαs and short NRXNβs via independent promoters and alternative mRNA splicing at five (α) and one (β) canonical positions, to generate thousands of possible isoforms. Like the NLGNs, the NRXNs are type I transmembrane proteins, with an extracellular N-terminal adhesion domain and an intracellular C-terminal PDZ-binding motif. The NRXNα ectodomain encompasses six laminin/neurexin/sex hormone-binding globulin (LNS) domains intercalated with three epidermal growth factor (EGF)-like domains. In contrast, the NRXNβ ectodomain consists of a single LNS domain corresponding to the sixth LNS domain of NRXNα. The LNS domains are made of two β-sheets stacked as a β-sandwich related to the jellyroll fold also found in L-type lectins. A functional region, called the hypervariable surface and encompassing a calcium-binding site, is located at one edge of the β-sandwich, a suitable position for recruiting binding partners.

NLGNs bind to both NRXNs α and β in a calcium-dependent manner, and with nanomolar to submicromolar affinities whose differences are dictated by subtle sequence variations and presence versus absence of splice inserts (Nguyen and Südhof 1997). Mutations in the *NLGN3*, *NLGN4,* and *NRXNα1/β1* genes have been associated with familial forms of autism spectrum disorders and schizophrenia (Jamain et al. 2003; Kim et al. 2008; reviewed in Südhof 2008).

The NLGN extracellular domain displays high homology in sequence (~35 % identity) and structure (rmsd value of ~1.35 Å for at least 330 Cα atoms) with acetylcholinesterase (AChE), but it lacks a functional catalytic site (Leone et al. 2010). Indeed, the AChE subunit is characterized by a buried active site, located at the bottom of a deep and narrow gorge (Sussman et al. 1991). The face of the subunit where the active site gorge opens also bears the peripheral anionic site, which binds positively charged non-competitive inhibitors of AChE such as the peptidic toxin from snake venom, fasciculin; inhibitory antibodies such as Elec403 and Elec410; and small organic inhibitors such as propidium and gallamine. AChE is also believed to play "nonclassical" roles, e.g., as an adhesion protein, in addition to its "classical" role in terminating synaptic transmission (Silman and Sussman 2005).

Compared to AChE sequences, the NLGN sequences reveal absence of a catalytic Ser residue, a distinct linkage for the third disulfide bridge, distinct positions for *N*-glycosylation, and the surface positions for splicing inserts SSA and SSB. Crystal structures of the NLGN1, NLGN2, and NLGN4 ectodomains confirmed the high structural similarity with AChE, including conservation of the dimeric assembly of subunits through a four-helix bundle (Araç et al. 2007; Fabrichny et al. 2007; Koehnke et al. 2008). Most importantly, these data illustrated the structural variability within the α/β-hydrolase fold to trigger catalytic versus cell adhesion functions (Lenfant et al. 2014). Beyond NLGNs, other non-catalytic members of the α/β-hydrolase fold superfamily include the neurotactin, glutactin, and gliotactin proteins in insects and the C-terminal domain of thyroglobulin in mammals. Existence of yet uncharacterized potential non-catalytic members found mainly in invertebrates and nematodes illustrates the biological importance of the large "bloc C non-catalytic" subfamily of proteins, as referred to within the ESTHER database (Lenfant et al. 2013).

Small-angle x-ray scattering (SAXS) and crystallographic analyses of soluble NLGN1 and NLGN4 complexes with NRXNβ1 revealed the location and orientation of NRXNβ1 binding to the face of the NLGN subunit opposite to the gorge entry in AChE (Comoletti et al. 2007; Araç et al. 2007; Fabrichny et al. 2007; Chen et al. 2008; Leone et al. 2010). The respective orientation of the two partners in the complex positions their C-terminal tails oppositely, consistent with the functional organization of transsynaptic NRXN-NLGN complexes forming across the 20-nm-wide synaptic cleft. More recent crystallographic analyses of the NRXNα1 ectodomain unveiled the overall architecture of this flexible molecule, which adopts an elongated L-shaped assembly of three main segments: a flexible repeat I (LNS1-EGF1-LNS2), a rigid horseshoe-shaped repeat II (LNS3-EGF2-LNS4) reminiscent of the renin repeats, and an extended repeat III (LNS5-EGF3-LNS6) with controlled flexibility (Miller et al. 2011; Chen et al. 2011). This flexible organization associated with the existence of many NRXN α and β isoforms may dictate variations in the intermembrane distance (15–25 nm in rodents) and in the architectural organization of the synaptic machinery when cell-associated NRXNs recognize and bind cell-associated NLGN1 (Savtchenko and Rusakov 2007; Tanaka et al. 2012).

However, the NRXN and NLGN ectodomains do not bind exclusively to each other, but they also recognize and interact

with other synaptic partners. Indeed, NRXNs α or β (or both) also interact with dystroglycans (in a calcium-dependent manner) (Sugita et al. 2001); with neurexophilins (in a calcium-independent manner) (Missler et al. 1998); with one of the four members of the leucine-rich repeat transmembrane family of proteins, LRRTM2 (in a calcium-dependent manner and competitively with NLGN1) (Linhoff et al. 2009; de Wit et al. 2009; Ko et al. 2009); and with the ligand of the postsynaptic glutamate receptor (GluR) 2, cerebellin-1 (in a calcium-independent manner, and competitively with NLGN1, but not LRRTM2) (Uemura et al. 2010; Matsuda et al. 2011). Interestingly, alternative splicing of NRXNs at splice site SS4 on the LNS6 domain influences whether they bind to either cerebellin-1 or to NLGNs and LRRTM2. Calsyntenin-3 (Clstn3), also known as alcadein-β, has been recently evidenced as a calcium-dependent binding partner of NRXNα1-3 but not NRXNβ1 (Pettem et al. 2013). In turn, NLGNs interact with four non-NRXN partners through *multiple and selective* binding interfaces. Co-immunoprecipitation experiments in heterologous cells indicated that both NLGNs and NRXNαs bind the ectodomain of the receptor-type protein tyrosine phosphatase (PTPRT) (calcium dependency is not documented) although with varying intensities (no specific effects of PTPRT when co-expressed with NLGN2, as reported by Lee et al. 2013). This interaction appears to regulate synapse formation in neuronal synapses, a feature consistent with the reported role of several PTPs in the control of axonal outgrowth guidance and synapse formation in the central nervous system (Lim et al. 2009). Co-immunoprecipitation in heterologous cells also suggested that NLGN1, NLGN2, and NLGN3 interact with thrombospondin-1 (TSP1) (calcium dependency is not documented) to mediate its synaptogenic effect on neuron development (Xu et al. 2010). The effect of TSP1 on synapse formation is similar to that of NRXNs, which induce formation of synapses lacking AMPA receptors via NLGNs. In contrast, NRXNβ1 does not bind to TSP1, indicating that the interactions between NLGNs and TSP1 are specific. NLGN2, but not the other NLGNs, interacts selectively and in a calcium-dependent manner with the MAM domain-containing GPI anchor protein MDGA1, as shown by quantitative cell-surface binding assays (Lee et al. 2013). Yet, MDGA1 may disrupt the NLGN-NRXN interactions in competing for the same calcium-binding site on the LSN6 domain of NRXNs, as do NLGN and LRRTM, to regulate inhibitory synapse formation, a function consistent with its unique localization at inhibitory synapses. MDGA1 and its MDGA2 homolog are mainly expressed in selected neuronal populations in the central and peripheral nervous systems (Litwack et al. 2004). More recent co-immunoprecipitation in heterologous cells and proximity ligation assays indicated that NLGN1, but not NLGN2 or NLGN3, uniquely plays an instructive role for controlling synaptic abundance of NMDA receptors at glutamatergic synapses through binding to the GluN1 subunit (calcium dependency is not documented) (Budreck and Scheiffele 2013). This finding contrasts with earlier observations of NLGN1 clusters associated with GluN1 via PDZ-containing adapter proteins during early synaptogenesis (Barrow et al. 2009). The requirement for NMDA receptor coupling, which drastically differs in term of molecular architecture from the other synaptic cell adhesion molecules, provides an interesting parallel to the requirement of the NLGN1 ectodomain in hippocampus long-term potentiation for synaptic plasticity (Shipman and Nicoll 2012). This unique function depends on inclusion of a SSB insert, whose presence also affects the specificity of NRXN binding. Specifically, NLGN1 containing an insert at SSB binds preferentially to NRXNβs lacking an insert at SS4 and does not bind the NRXNαs (Boucard et al. 2005). Herein, we review and picture the main architecture and structural features of these multimodular NLGN partners acting as key synaptic organizers. Given the high structural homology between the NLGNs and AChEs, this study also provides unprecedented insights into existence of alternative binding sites on AChEs to support its suspected non-catalytic functions.

Materials and Methods

Structural comparisons used the secondary-structure matching (SSM) module in COOT (Emsley and Cowtan 2004) and structures of human NLGN4 (3BE8, 2.2 Å (Fabrichny et al. 2007)); human NLGN4 bound with rat NRXNβ1 (2XB6, 2.6 Å (Leone et al. 2010)); mouse AChE (1J06, 2.35 Å (Bourne et al. 2003)); the bovine NRXNα1 LNS2-6 ectodomain (3POY, 3.02 Å (Miller et al. 2011)); and the laminin α1 LG4-5 domain (2JD4, 1.9 Å (Harrison et al. 2007)). Models of the MDGA1 ectodomain were built using MODELLER (Sali et al. 1995) and, as templates, the structures of the titin Ig6 and Ig3 fragments (3B43, 3.3 Å and 2RIK, 1.6 Å, 20-22 % identity (von Castelmur et al. 2008)) selected using the TM-score from the HHpred server (Söding et al. 2005). Models of the PTPRT ectodomain and NMDAR GluN1 subunit were generated using a similar approach and, as respective templates, the structures of the RPTPmu ectodomain (2V5Y, 3.1 Å (Aricescu et al. 2007), 63 % identity) and the glutamate receptor 2 (GluA2) (3KG2, 3.6 Å (Sobolevsky et al. 2009), 25 % identity). Models of the TSP1 ectodomain was only partially generated using available structures of the N-terminal LNS domain (1ZA4, 1.9 Å (Tan et al. 2006), the 3 EGF-like modules, 13 aspartate-rich repeats, and the C-terminal L-type lectin domain (1YO8, 2.6 Å (Carlson et al. 2005). The TSP1 region 236–301 preceding the TSR motifs remains unresolved. Figures 1 and 3 were generated with PyMoL (Schrödinger 2010).

Results and Discussion

Functional architecture of the NRXNα1 ectodomain

While peptidic and organic non-competitive inhibitors of AChE bind the peripheral anionic site, a surface site that surrounds the active site gorge entrance (Taylor and Radic 1994), NRXNβ1 binds the NLGN1 or NLGN4 subunit on the face corresponding to the AChE face opposite to the gorge entrance, a difference exemplifying the structural diversity of the α/β-hydrolase fold to recruit distinctive partners. Compared to the single LNS domain in NRXNβ1, the remarkable L shape of the crystalline NRXNα ectodomain positions the spatially remote LNS6 domain as to preserve the primary NLGN binding site observed in the NLGN-NRXNβ1 complex, while the intercalated EGF3 domain functions as a spacer to ideally position the linear LNS2-LNS5 assembly along the NLGN subunit (Fig. 1). Conservation of this overall arrangement in 2D electronic microscopy images of unbound NRXNα1 confirms the high flexibility of the linker between repeats I and II (Comoletti et al. 2010). In fact, this remarkable arrangement exposes the hypervariable surfaces of each of the five LNS domains on one side of the molecule and reveals possible additional direct interaction from the LNS3 and/or LNS4 domain of NRXNα1 with NLGN (Fig. 1). The overlay shows the bound NLGN to be adjacent to the LNS4 domain of NRXNα1, and the buried interface contains a flexible loop that includes the position for splice insert SSA. In this orientation, insert SSA in NLGN can cooperate with insert SS3 in LNS4 a feature explaining why it modulates NRXNα binding, but not NRXNβ binding. SSB in NLGN is close to SS4 in LNS6, consistent with the splice code that governs NRXNα binding but leaves NRXNβ1 largely unaffected to trigger selective functions at glutamergic and GABAergic synapses (Südhof 2008). Hence, changes in the orientation of the linear LNS2-LNS5 portion of the NRXNα ectodomain, e.g., dictated by presence or absence of each splice insert, may regulate NLGN binding and likely influence binding of additional partners. Indeed, the structure of NRXNβ1 with a splice insert at SS4 reveals major rearrangements of the hypervariable surface involved in NLGN binding (Shen 2008; Koehnke 2010). Most importantly, the primary and putative secondary NRXN binding sites leave the NLGN face corresponding to the gorge entry in AChE

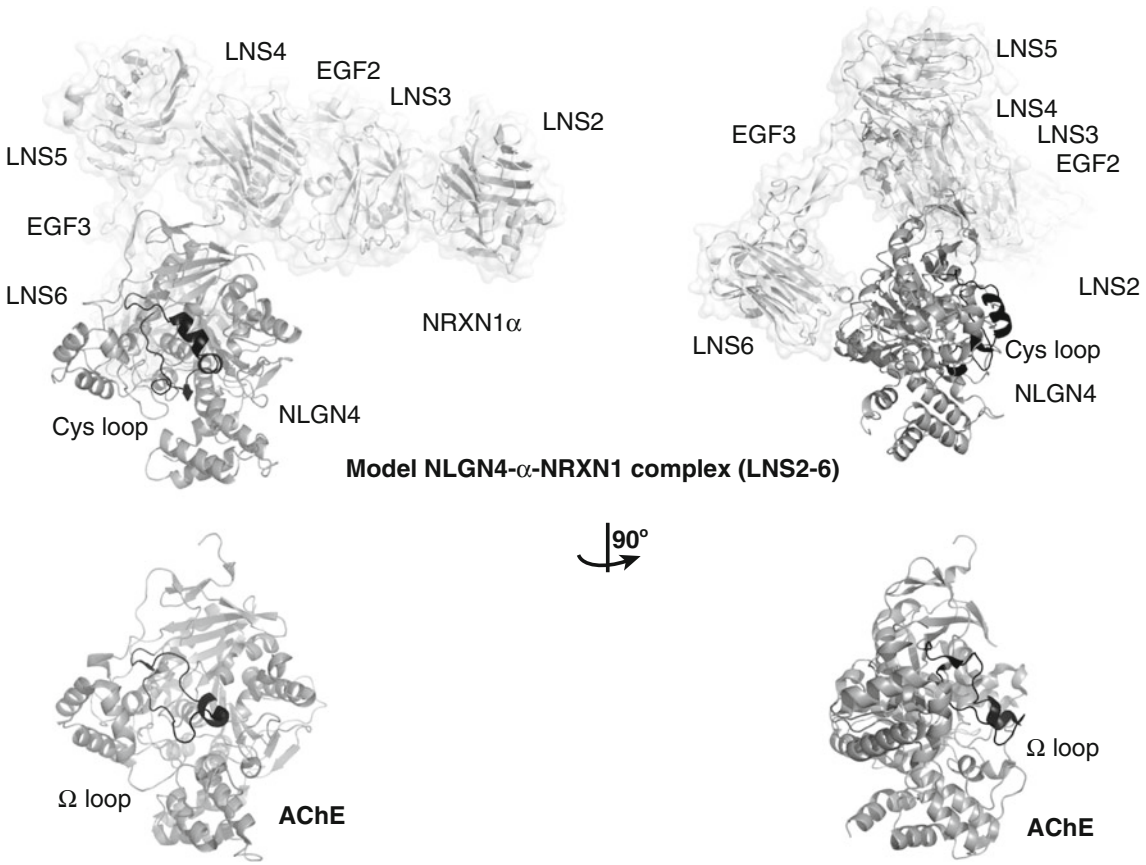

Fig. 1 Model of the NRXNα1 LNS2-6 ectodomain bound to NLGN4 (accession codes, 3POY, 2XB6) and implication for binding interactions with other NLGN partners. The complex is displayed top-to-bottom with unbound AChE (1J06) displayed in similar 90° rotated orientations. NLGN4 and AChE are displayed in gray and NRXNα1 is in white. The long Ω loop at the entrance of the active center gorge of AChE (Cys69-Cys96) and the corresponding Cys-loop in NLGN4 (Cys110-Cys146) are displayed darker than the rest of the molecules

accessible for binding to additional partners (Fig. 1). Compared to AChE, this surface shows large conformational variability associated with deletions and insertions in the various loops, which are dominated by the functionally important Cys-loop in AChE, as to construct a novel surface topography whose conservation within the NLGNs makes compatible as a binding interface (Fabrichny et al. 2007). However, whether this surface region in NLGNs encompasses a binding interface for a specific partner remains to be ascertained.

Organization and 3D Modeling of the Ectodomains of the Non-neurexin Neuroligin Partners

Receptor-like protein tyrosine phosphatases (PTPRT or RPTP) are transmembrane proteins usually composed of a variable N-terminal extracellular domain, a single transmembrane pass and one or two highly conserved cytoplasmic PTP catalytic domains (Fig. 2). The resemblance of the extracellular domains of some RPTPs with those of other synaptic cell adhesion molecules has led to identify potential binding partners and determine whether these interactions can regulate the intracellular PTP activity. In fact, the extracellular domain of PTPRT, which belongs to the same class I cysteine-based subfamily as PTPRκ, PTPRμ, and PTPRU, consists of a meprin/A5 protein/receptor protein tyrosine phosphatase mu (MAM) domain, an immunoglobulin (Ig)-like domain and four consecutive fibronectin (FN)-type III-like domains as also found in MDGA1 (Alonso et al. 2004). Hence, brain-specific PTPRT localized specifically to excitatory synapses could interact with NLGN and NRXNα in a *cis* manner and consolidate, through a clustering mechanism, the interaction between NLGN and NRXNα in addition to its homophilic *trans* interaction (Lim et al. 2009). Our model of the PTPRT ectodomain, based on its high structural homology with that of PTPRTμ, shows an elongated and rigid structure with very short linkers and extensive interdomain interactions (Fig. 3). This class of PTPRTs consists of homophilic cell adhesion molecules that form high-affinity antiparallel dimers in solution involving conserved hydrophilic residues from the MAM-Ig-FN1-FN2 domains (Aricescu et al. 2007). Unlike the flexible NRXNα ectodomain, the rigid ruler-like architecture of the PTPRT ectodomain may act as a sensor of intercellular distances, while the *trans* interaction would serve as a spacer clamp to lock the intracellular phosphatase domain to its appropriate functional location.

Thrombospondins (TSPs) are secreted macromolecules that act as regulators of cell interactions in vertebrates (Adams and Lawler 2011). Members of the TSP gene family share a similar overall architecture consisting of a variable N-terminal domain (NTD) and an invariant C-terminal "signature" domain. This family is divided into two subfamilies, A and B, depending on the molecular organization of each domain. Hence, the NTD of TSP1 consists of a laminin

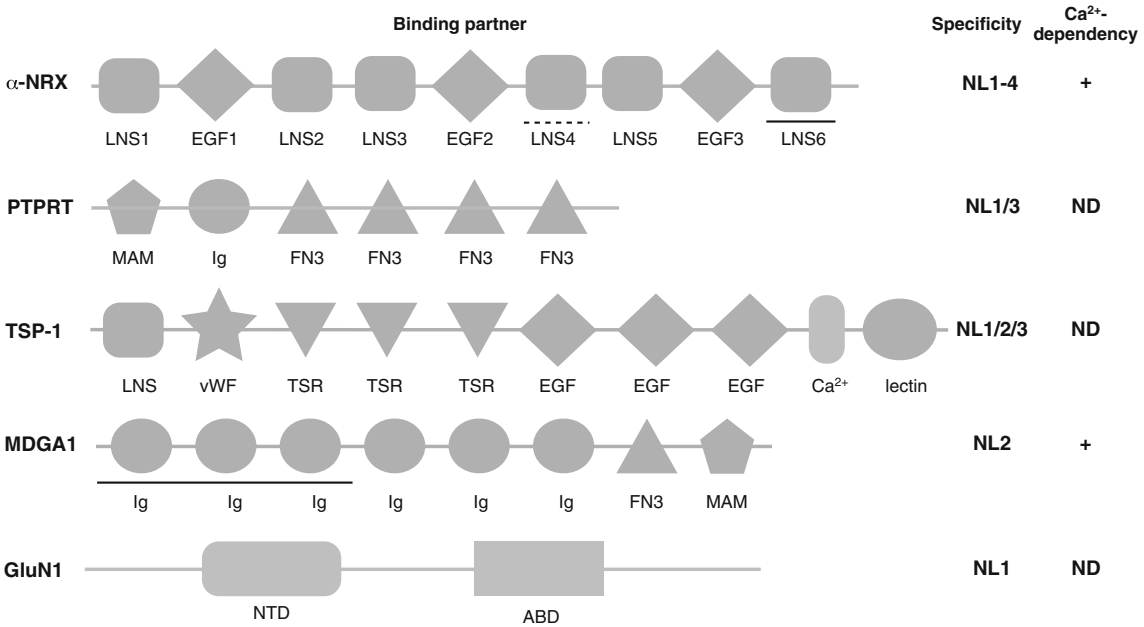

Fig. 2 Molecular architecture, binding selectivity, and calcium dependency of the ectodomains of the identified and characterized NLGN partners. The *plain* and *dashed lines* respectively denote regions or domains with primary and secondary binding interfaces with NLGNs. *Rounded squares* are for LNS domains, *lozenges* for EGF-like domains, *up triangles* for FN type III-like domains, *pentagons* for MAM domains, *circles* for Ig-like domains, *stars* for vWF type C domains, *down triangles* for TSR domains, *rounded bars* for calcium-binding type 3 repeats, *ovals* for L-lectin domains, *rounded rectangles* for NTD domains, and *squared rectangles* for agonist-binding domains. (Molecules and domains not drawn to scale.)

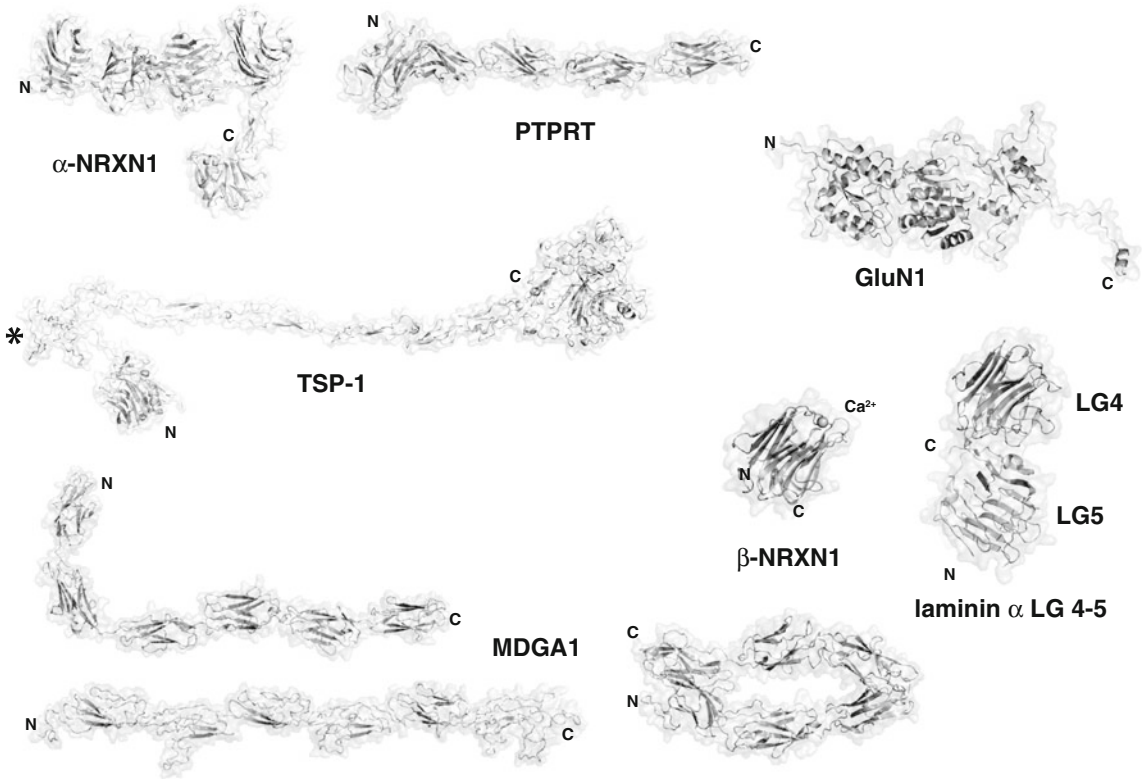

Fig. 3 Molecular and structural comparison of the various identified NLGN partners. Shown are the experimental crystal structures of the NRXNα1 LNS2-6 ectodomain, the NRXNβ1 LNS6 domain, and the laminin α LG4-5 domain (accession codes, 3POY, 3BOD, 2JD4), and the theoretical models of the MDGA1 (with 3 different conformations), TSP1, PTPRT ectodomains and of the GluN1 subunit (this study). The *asterisk* in TSP1 denotes the region of unknown structure

Lam-G/LNS domain as found in the NRXNs, followed by a procollagen region folded into a disulfide-bonded α-helical coiled-coil domain that stabilizes the cotranslational trimeric assembly found for subfamily A (Tan et al. 2006; Misenheimer et al. 2000) (Fig. **2**). A von Willebrand factor type C (vWF C) domain, homologous to that of collagen IIA, intercalates between the procollagen region and each of the three thrombospondin structural repeat (TSR) motifs composed of three β-strands with alternating orientation and stabilized by disulfide bonds at the NTD extremity. In TSP1, the conserved CTD comprises a series of EGF-like domains, 13 aspartate-rich calcium-binding type 3 repeats, homologous to calmodulin EF-hand repeats, and a domain homologous to the L-type lectin domain. A second hallmark of the TSPs is their ability to bind large numbers of calcium ions through the type 3 repeats to induce major conformational changes. TEM images suggest that the type 3 repeats and C-terminal domain fold together in a calcium-bound state to form a C-terminal globular entity (Lawler et al. 1985). The available structures of the isolated TSP1 domain and of the first two TSR domain of TSP1 suggest a flexible ectodomain architecture, with the typical three TSR modules that can play a similar spacer role as the isolated EGF domains in NRXNα, in addition to the three following EGF-like domains. Models of the structurally related properdin, which consists of six TSP1 domains, built using a combination of x-ray scattering, analytical ultracentrifugation, and homology modeling approaches, reveal different arrangements of the TSR domains reflecting a high degree of flexibility between adjacent domains (Sun et al. 2004) (Fig. **3**). Compared to NRXNα, the single LNS domain in TSP1 bears a major heparin-binding site at its base and displays large differences in the hypervariable loop region at the top forming a calcium-binding site and involved in NLGN binding. Molecular dissection of TSP1 interaction with NLGN should further delineate the molecular determinants for NLGN binding in key functional domains and repeats.

The ectodomain of NLGN2 weakly binds that of NRXNβ1, but it selectively binds MDGA1 with a nanomolar equilibrium dissociation constant, and in a *cis* configuration (Lee et al. 2013). In fact, in NLGN2 compared to the other NLGNs, a single side chain substitution, of Gly500 to Gln in the NRXN LNS6 binding site, is responsible for the very weak NRXNβ1 binding affinity (Leone et al. 2010). At the molecular level, MDGA1 and MDGA2, which share ~40 % sequence identity (up to 80 % for the first Ig-like domain, cf. below), are GPI-anchored proteins and display a unique structural organization consisting of six Ig-like domains, a fibronectin type III (FnIII) repeat followed by a single MAM domain that is known to mediate protein

dimerization (Fig. 2). Cells expressing only the Ig domains of MDGA1 or MDGA2 at their surface, but not their fibronectin and MAM domains, were found to bind strongly to NLGN2, and the three N-terminal Ig domains of MDGA1 were shown to be sufficient to mediate binding (Lee et al. 2013). A model of the Ig1-6 portion of the of MDGA1 ectodomain reveals a linear and horseshoe shape associated with flexibility in the hinge connecting the second to the third Ig domain, a feature reminiscent of the two flexible hinge regions found in NRXNα between LNS5 and EGF-3 and between EGF-1 and LNS2, respectively (Fig. 3).

The ectodomain of NLGN1 specifically mediates *cis* synaptic interactions with the NMDAR GluN1 subunit (Budreck and Scheiffele 2013). In contrast to the other cell adhesion molecules, NMDARs assemble as tetramers or di-heteromers composed of GluN1 and GluN2 subunits, and alternative mRNA splicing generates additional complexity through protein isoforms. The typical modular organization of the extracellular domain of the GluN1 subunit, which is devoid of those LNS, EGF-like, or Ig-like domains typically found in most other cell adhesion molecules, consists of a tandem of large bi-lobed clamshell-like domains, the NTD involved in subunit assembly, and the agonist-binding domain that binds glycine (Paoletti 2011) (Figs. 2 and 3). The extracellular domain of GluN1 for which solely the structure of the NTD domain is known is homologous in sequence (25 % identity) to that of the Glutamate receptor 2 (GluA2) (Sobolevsky et al. 2009; Karakas et al. 2011). Such atypical interaction further extends our knowledge on the architectural diversity of partners of cell adhesion molecules.

What About Putative AChE Binding Partners?

Given the high structural similarity between NLGNs and AChEs, would binding partners of these two members of the α/β-hydrolase fold family of proteins share similar domain architectures and binding sites, supporting existence of non-catalytic AChE functions? The ectodomain of NLGN1, but not NLGN2, was proposed to bind the amyloid β peptide and enhance its polymerization into amyloid deposits in vitro (Dinamarca et al. 2011). However, whether the neurexin binding surface, which is functional on NLGN1, but not NLGN2 (Comoletti et al. 2006; Leone et al. 2010), is involved in this event was not documented. The AChE subunit had also been reported to accelerate in vitro amyloid-β fibril formation, an event triggered by its peripheral anionic site, as assessed from inhibition of the pro-aggregating effect by peripheral site, but not active site, AChE ligands (for a review see Inestrosa et al. 2008). Alternatively, inverse effects of the "T" and "R" splice variants of AChE on amyloid fibril formation would also argue for a contribution from the subunit C-terminus (Berson et al. 2008). Hence, molecular determinants or surfaces sharing the charge or hydrophobicity properties required for amyloid peptide binding might be present at distinctive places of the NLGN/AChE subunit.

Among the various peptidic ligands that bind the peripheral anionic site of AChE, laminin-1 that contains LNS domains was shown to interact with residues from the long Ω loop that constitutes part of the peripheral site, an interaction that could modify the catalytic activity of the enzyme (Johnson et al. 2004). More recently, observation that laminin-1 binding on AChE affects neurite outgrowth argued for a non-catalytic role of AChE in fiber growth in producing a signal that could enhance the laminin-1 affinity for the α6β1 integrin receptor (Sperling et al. 2012). The α1 chain provides most of the unique characteristics of laminin-1, which also contains β1 and γ1 chains. The N-terminal (LN) domains of each chain contribute to the polymerization process of laminin-1 while the C-terminal (LG) domains LG1-3 bind to the α6β1 integrin and domains LG4-5 bind to heparin, sulfated glycolipids, and α-dystroglycan. Of the five C-terminal LNS domains that characterize the laminin α1 chain, domain LNS4, which shares 17 % sequence identity with NRXNβ1 and part of the cysteine-rich LNS3 domain, binds AChE (Johnson 2008). Other studies showed that the binding site on laminin-1 is located on the N-terminal region of the β-chain and includes the G4 domain and part of the cysteine-rich domain G3 (Paraoanu and Layer 2004). Structural analysis of the LNG4-LNG5 tandem on the laminin α1 chain suggests that the interdomain angle may dictate or regulate AChE binding (Harrison et al. 2007) (Fig. 3). Such interdomain motions reflect the molecular architecture of the NRXNα1 ectodomain with the separate spatial position of the LNS6 domain. Moreover, the marked electropositive character at the surface of the LG4 domain near the bivalent ion and heparin binding sites would be in favor of its binding at the peripheral anionic site of AChE, consistent with the observed decrease of AChE activity upon laminin-1 binding (Sperling et al. 2012). Such an interaction is consistent with a diversity of functional sites within the LNS domains while maintaining a conserved structural scaffold (Rudenko et al. 2001).

In summary, the fact that most of the identified or characterized NLGN partners are synaptic organizers emphasizes the structural diversity of synaptogenic cell adhesion molecules that promotes *cis* interactions compatible with the architecture of the dominant transsynaptic NLGN-NRXN complex. Would *trans* adhesion events at early neuronal synapses trigger recruitment of neighboring adhesion molecules in *cis* to induce synaptic maturation? In fact, lateral *cis* oligomerization following *trans* binding of, e.g., cadherins, ephrins, and T-cell receptors, appears to be a mechanism widely used by this family of cell adhesion molecules. Moreover, it is puzzling that two families of molecules with no sequence similarity, the NLGNs and LRRTMs, not only bind the same presynaptic NRXN partner but also compete for the same calcium-binding site on NRXN LSN6. This feature argues for a master organizer role of

NRXNs in binding to (either competitively as for NLGN1 and LRRTM2 or not competitively as for NLGN1 and dystroglycan) and acting together with multiple synaptic partners, as cadherin does with its multiple partnering neighbors.

Alternative splicing events, isoform-specific interactions, and cofactors add another level of complexity in the regulation of binding selectivity of these synaptic complexes to create a dynamic cell adhesion network. Moreover, a ruler-like architecture of the ectodomain or intercellular spacing can be associated with a clamping action to regulate intercellular multimodular assemblies.

Comparative structural analysis of the NLGN subunit relative to its cell adhesion partners and of the enzyme controlling neurotransmitter concentration, AChE, relative to its non-competitive, surface binding inhibitors, led to exemplify how a common α/β-hydrolase fold varies to confer, or perhaps emphasize, adhesion properties to a non-catalytic form of the subunit. Other binding sites for yet undefined partners of either the NLGNs or the AChEs could also be present at the surface of the "generic" subunit and associated with non-catalytic functions of AChE. Further work is needed to assess the functional binding regions of these modular cell adhesion proteins and document further the functional diversity of the structural α/β hydrolase fold.

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