# Specification of synaptic connectivity by cell surface interactions

*Joris de Wit[1] and Anirvan Ghosh[2]*

Abstract | The molecular diversification of cell surface molecules has long been postulated to impart specific surface identities on neuronal cell types. The existence of unique cell surface identities would allow neurons to distinguish one another and connect with their appropriate target cells. Although progress has been made in identifying cell type-specific surface molecule repertoires and in characterizing their extracellular interactions, determining how this molecular diversity contributes to the precise wiring of neural circuitry has proven challenging. Here, we review the role of the cadherin, neurexin, immunoglobulin and leucine-rich repeat protein superfamilies in the specification of connectivity. The emerging evidence suggests that the concerted actions of these proteins may critically contribute to the assembly of neural circuits.

Axon initial segment
A specialized subcellular compartment in the first part of the axon that contains high concentrations of channels, scaffolding proteins and adhesion molecules and that initiates action potentials.

[1]*VIB Center for the Biology of Disease and Center for Human Genetics, KU Leuven, Herestraat 49, 3000 Leuven, Belgium.*
[2]*Neuroscience Discovery, Roche Innovation Center Basel, F. Hoffman-La Roche, Grenzacherstrasse 124, 4070 Basel, Switzerland.*
*Correspondence to J.d.W.*
*joris.dewit@cme.vib-kuleuven.be*

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A hallmark of CNS organization is the highly precise pattern of connectivity between neurons. The location of a synapse on a target cell and its distinctive structural and functional properties are key factors in determining the flow of information in a neural circuit.

The nervous system uses many mechanisms to accomplish the formidable task of connecting neurons with their appropriate target cells (reviewed in REF. 1). The organization of many brain regions into distinct anatomical layers (laminae) is one key structural feature that aids wiring specificity. The targeting of processes to specific laminae, which we refer to as laminar specificity, limits the range of target cells or surfaces available for synapse formation. Within a particular lamina, processes can synapse with a specific cell type that resides in that layer, such as a principal cell or an interneuron (cellular specificity). Neurons can also form synapses onto a specific subcellular domain of a target cell, such as the axon initial segment or dendritic compartment (subcellular specificity). In the final step of assembling functionally connected circuits, synapses between different types of neurons are differentiated into structurally and functionally distinct synapse types (synaptic diversity). These different levels of specificity are closely related. For example, dendrites from principal neurons in brain regions such as the hippocampus or the cortex span multiple laminae and form a different type of synapse with a distinct presynaptic partner in each lamina. Depending on the viewpoint, this could be considered as an example of laminar specificity, subcellular specificity or synaptic diversity.

The molecular mechanisms that regulate wiring specificity and synaptic diversity in the vertebrate brain are only beginning to be understood. Classic work from Langley[2] and Sperry[3] on the regeneration of nerve fibres in the mature nervous system indicated that neurons can rewire with remarkable specificity. Their work suggested the presence of "individual identification tags" on cells and fibres that would allow neurons to distinguish one another and selectively connect to target cells by "specific chemical affinities" (REF. 3). In recent years, substantial progress has been made in identifying and characterizing molecularly diverse cell surface protein families, which may to some extent act as the surface tags envisioned by Langley and Sperry. Advances in genomics and proteomics are enabling the analysis of cell type-specific repertoires of surface proteins and the systematic, large-scale mapping of their extracellular interaction networks. Despite these advances, determining how this molecular diversity contributes to the encoding of wiring specificity and synaptic diversity remains a considerable challenge.

Several requirements can be envisioned for cell surface proteins that regulate wiring specificity and synaptic diversity. Such proteins should be expressed in distinct populations of neurons, be capable of interacting in *trans* with their binding partners, and provide enough molecular diversity to confer cell type- and synapse type-specific identities. In this Review, we start by introducing several superfamilies of cell surface proteins that meet these requirements: the cadherins, neurexins, leucine-rich repeat (LRR) proteins and

immunoglobulin (Ig) proteins. The molecular diversity of these protein families arises either through the large size of the underlying gene family or through the alternative splicing of a more-limited number of genes. We review the evidence for cell type-specific expression of these proteins and discuss to what extent current *in vivo* evidence supports a role for them in wiring specificity and synaptic diversity. Other cell surface proteins exhibiting cell type-specific expression patterns have been implicated in the specification of connectivity, and a brief, albeit incomplete, overview of additional molecular players in this process is provided in BOX 1. Note that, owing to space limitations, we do not discuss the cellular and molecular mechanisms that guide axons towards their target cells, nor do we provide an overview of all the cellular and molecular mechanisms that are known to contribute to synaptic specificity, as these have been extensively reviewed elsewhere[1,4–6]. Finally, this Review focuses on the rodent CNS, and we refer the reader to several excellent recent reviews that discuss synaptic specificity in invertebrate systems[7–9].

## Cell surface protein families

***Classic cadherins and protocadherins.*** The cadherin superfamily comprises more than 100 transmembrane glycoproteins that can be grouped into several subfamilies, of which the classic cadherins and protocadherins have been the most studied in relation to connectivity[10–13]. Classic cadherins contain five extracellular cadherin (EC) repeats and a conserved intracellular domain that contains catenin-binding sites (FIG. 1A). Approximately 20 classic cadherins exist, and these are further classified as type I or type II cadherins based on amino acid sequence identity in their first EC repeat[11]. Classic cadherin *trans* interactions are mediated by the EC1 domain (FIG. 1A) and are preferentially homophilic, but they can also be heterophilic within each class; thus, type I cadherins can interact with other type I cadherins, and type II cadherins can interact with other type II cadherins[14–18].

The protocadherins were identified based on their structural similarity to cadherins[19] and are subdivided into clustered and non-clustered protocadherins. The role of non-clustered protocadherins in connectivity is not well understood[20] and is not discussed here. The clustered protocadherins comprise the α-, β- and γ-clusters, which are arranged in three tandem arrays on a single chromosome that encodes a total of 58 isoforms in mice[21–23]. The α- and γ-protocadherin clusters contain variable exons encoding complete extracellular, transmembrane and proximal intracellular domains. Each exon contains its own promoter and is spliced to three constant exons that encode the common remainder of the cytoplasmic domain[24,25]. The β-cluster lacks constant exons and instead contains exons that seem to encode complete proteins. Five phylogenetically divergent members from the α- and γ-clusters, which differ from their respective cluster members and are more similar to each other, have been grouped into a separate C-cluster[23].

Although structurally similar, clustered protocadherins differ from classic cadherins in several aspects, with direct implications for their role in connectivity. Protocadherins contain six EC repeats instead of five, and their cytoplasmic tails lack canonical catenin-binding sites (FIG. 1B). Protocadherin isoforms from the α-, β- and γ-gene clusters engage in highly specific homophilic *trans* interactions that are mediated by the EC2 and EC3 domains, instead of by the EC1 domain[26]. Protocadherins can associate in *cis* and form multimers (FIG. 1B), which have different *trans* binding specificities than individual isoforms alone and display remarkable recognition specificity[26,27]. For example, cells expressing a combination of five different protocadherin isoforms will co-aggregate with a second set of cells expressing the same combination of isoforms. However, the inclusion of a single mismatched isoform in the second set of cells is sufficient to prevent co-aggregation[27]. Thus, co-expression of multiple protocadherin isoforms greatly diversifies recognition specificity, and protocadherin multimers consisting of random isoform combinations would have enormous potential for unique adhesive interactions. However, it should be noted that these aggregation experiments have been performed in heterologous cells and that much remains uncertain about the exact nature of protocadherin recognition[27], as well as about the composition of putative protocadherin multimers in neurons. In addition, protocadherins can form *cis* complexes with other surface proteins, including classic cadherins[28] and the $GABA_A$ receptor ($GABA_AR$)[29], which may affect protocadherin *trans* interactions.

### Box 1 | Other cell surface proteins that contribute to wiring specificity

Many cell surface proteins other than those focused on in this Review have been implicated in the specification of connectivity in the vertebrate nervous system, and we highlight a few of them here. The semaphorins (SEMAs) and their plexin signalling receptors are best known for their roles as repulsive axon-guidance cues[132], but they also contribute to laminar and cellular specificity. In the retina, the transmembrane protein SEMA6A and its receptor plexin A4 are localized in specific sublaminae of the inner plexiform layer (IPL). Loss of SEMA6A or plexin A4 severely disrupts lamina-specific arborization of amacrine and retinal ganglion cells[133]. A complex interplay between SEMA6s and plexin receptors also contributes to lamina-specific targeting of hippocampal mossy fibres[134,135]. Thus, repulsive interactions are an important addition to the homo- and heterophilic cell surface interactions that specify laminar connectivity described in this Review.

Examples of additional homophilic adhesion molecules that are involved in wiring specificity are the Teneurins, which instruct synaptic partner matching in *Drosophila melanogaster*[136] and may have a role in specific laminar targeting of a subset of retinal ganglion cell dendrites in the IPL[137]. Moreover, cell adhesion molecule 1 (CADM1; also known as SYNCAM) is a homophilic immunoglobulin superfamily synaptic adhesion molecule that induces presynaptic differentiation[138], is expressed in regional-specific patterns[139] and regulates synapse number[140]. In addition to transmembrane cues, secreted factors are also important regulators of precise connectivity. For example, expression of the secreted molecule Sonic Hedgehog (SHH) in specific postsynaptic cortical neurons guides the formation of synapses by presynaptic neurons expressing the SHH receptor Brother of CDO (BOC)[141]. Moreover, restricted expression of the secreted molecule complement component 1, q subcomponent-like 1 (C1ql1) in climbing fibres contributes to specific connectivity on cerebellar Purkinje cells[142,143], and bone morphogenetic protein 4 (BMP4), which is secreted by Purkinje cells, acts as a secreted cue that prevents aberrant innervation by mossy fibres[144]. Finally, expression of the secreted SEMA3E in a subset of spinal cord motor neurons prevents monosynaptic innervation by plexin D1-expressing afferent fibres[145]. Thus, a balance of positive and negative secreted cues contributes to the specification of connectivity.

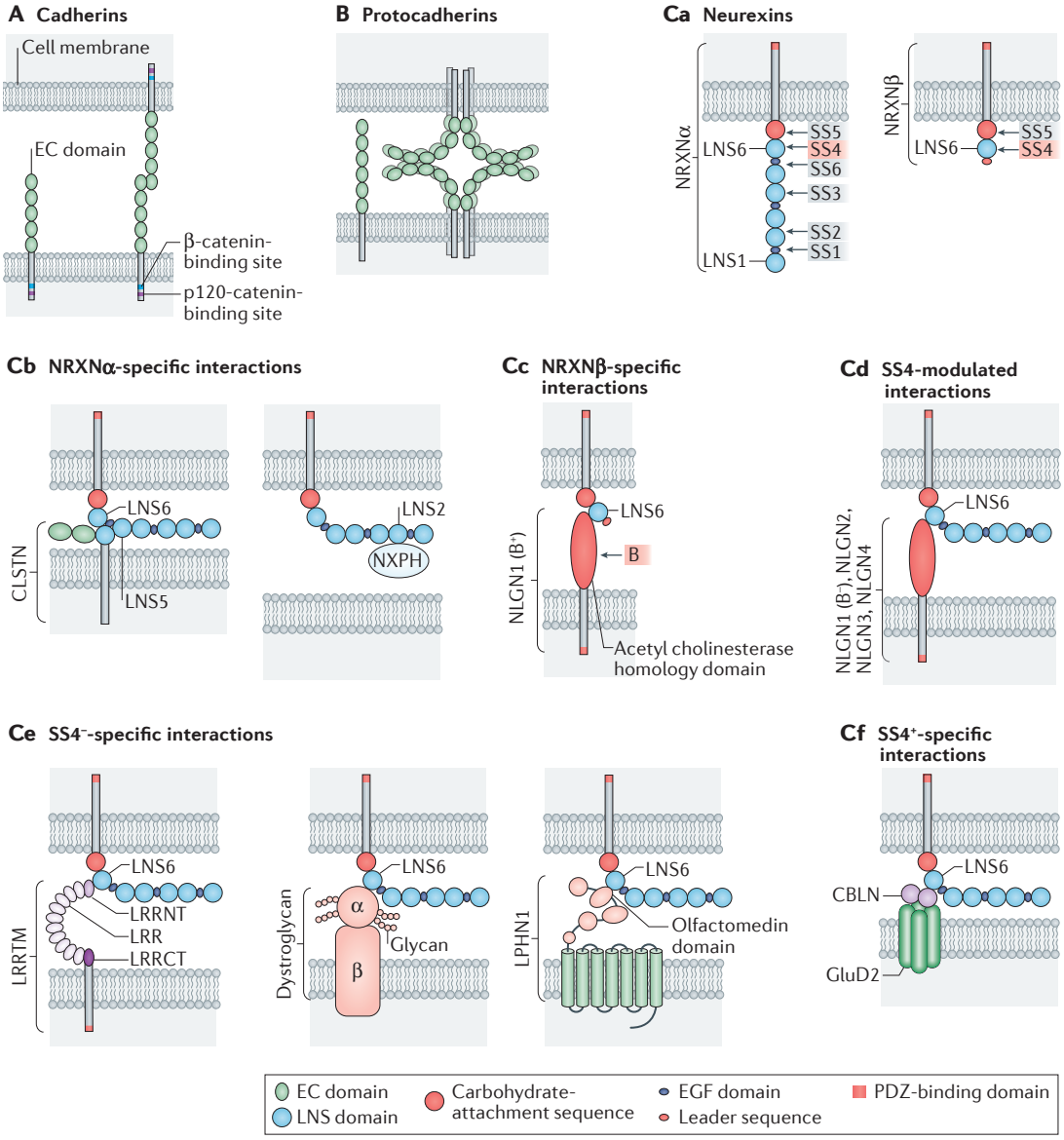



Figure 1 | **Domain organization and interactions of classic cadherins, protocadherins and neurexins. A** | Classic cadherins contain five extracellular cadherin (EC) repeats and a largely constant intracellular domain containing p120-catenin- and β-catenin-binding sites. The EC1 domains of *trans*-interacting cadherins mediate recognition specificity. **B** | Protocadherins contain six EC repeats and their cytoplasmic tails lack canonical catenin-binding sites. Studies in non-neuronal cells suggest that protocadherin *trans* interactions are homophilic and mediated by their EC2 and EC3 domains. The protocadherin recognition unit is a multimer, possibly a tetramer, as shown here. **Ca** | Neurexins can exist as long (α) and short (β) forms. The α-neurexin (NRXNα) proteins contain six extracellular laminin–neurexin–sex-hormone-binding globulin (LNS) domains, interspersed with three epidermal growth factor (EGF)-like domains, and contain a carbohydrate-attachment sequence near the plasma membrane. The NRXNα extracellular domain contains up to six splice sites (SS1–SS6). β-neurexin (NRXNβ) proteins contain a short amino-terminal NRXNβ-specific leader sequence, a single LNS domain and a carbohydrate-attachment sequence. Their extracellular domain contains two splice sites (SS4 and SS5). The carboxyl terminus of all neurexins contains a PDZ (postsynaptic density 95, Discs large 1 and zonula occludens 1)-binding domain. **Cb**–**Cf** | Presynaptic neurexins interact heterophilically in *trans* with multiple (postsynaptic) binding partners, which can interact specifically with NRXNα or NRXNβ (**Cb**,**Cc**), or with both NRXNα and NRXNβ (note that only the NRXNα interactions are shown) (**Cd**–**Cf**). Calsyntenin (CLSTN) and neurexophilin (NXPH) are NRXNα-specific binding partners. CLSTN interacts with the NRXNα LNS5–EGF3–LNS6 domains (**Cb**). NXPH interacts with the LNS2 domain of NRXNα (**Cb**). A splice variant of neuroligin 1 that contains a 'B' splice insert (NLGN1 ($B^+$)) is a NRXNβ-specific binding partner (**Cc**). Neurexin splicing at SS4 in LNS6, which is common to both NRXNα and NRXNβ, regulates neurexin–ligand interactions. The presence of SS4 ($SS4^+$) modulates the interaction with NLGN1 ($B^-$), NLGN2, NLGN3 and NLGN4 (**Cd**). Neurexins lacking an SS4 insert ($SS4^-$) interact with leucine-rich repeat transmembrane neuronal protein (LRRTM), dystroglycan (which can also interact with the LNS2 domain of NRXNα) and latrophilin 1 (LPHN1) (**Ce**). $SS4^+$ neurexins interact with the secreted ligand cerebellin (CBLN), which forms a complex with the δ2 glutamate receptor (GluD2) (**Cf**). LRRCT, LRR C-terminal domain; LRRNT, LRR N-terminal domain.

***Neurexins.*** In contrast to the classic cadherins and protocadherins, the neurexin family consists of only three genes (*Nrxn1–Nrxn3*), each of which has two alternative promoters that allow the generation of long (α) and short (β) forms of neurexins. The α-neurexins contain six extracellular laminin–neurexin–sex-hormone-binding globulin (LNS) domains, interspersed with three epidermal growth factor (EGF)-like domains[30] (FIG. 1C). The shorter β-neurexins contain a single LNS domain. Neurexins all share the same short intracellular domain but contain a varying number of splice sites in their ectodomains (six for NRXN1α and NRXN3α, five for NRXN2α and two for β-neurexins). Extensive alternative splicing of neurexins was noted soon after their discovery[31,32]. The theoretical total number of possible neurexin variants ranges in the thousands[33,34], but the true extent of neurexin alternative splicing has become clearer with recent deep-sequencing approaches[35,36]. Comprehensive quantitative analysis of neurexin diversity in the adult mouse cortex has indicated near-exhaustive combinatorial alternative exon use in *Nrxn1a* and *Nrxn2a* transcripts, resulting in hundreds of observed variants[36]. However, *Nrxn3a* transcript diversity is much lower than the theoretical number of such transcripts, resulting in hundreds, rather than the theoretical thousands, of observed NRXN3α variants.

The alternative splicing of neurexins regulates neurexin–ligand interactions. Neurexins are thought to be predominantly presynaptically localized and to engage in heterophilic *trans* interactions with multiple, highly diverse binding partners. The first neurexin ligand to be identified was the postsynaptic adhesion molecule neuroligin[37]. Neuroligin binding to neurexin induces presynaptic differentiation, and neurexin binding to neuroligin induces postsynaptic differentiation[38–41]. The neurexin–neuroligin interaction plays a major part in the formation, maturation and function of synapses[42,43]. Additional neurexin ligands, transmembrane and secreted, have since been identified. Neurexins interact with various transmembrane proteins, namely LRR transmembrane neuronal proteins (LRRTMs)[44–46], calsyntenin[47], dystroglycan[48], latrophilin (LPHN)[49] and the $GABA_AR$[50], as well as with the secreted molecules neurexophilin[51,52] and cerebellin[53] (FIG. 1C). Of these, LRRTMs, calsyntenins and cerebellins have a capacity to induce presynaptic differentiation similar to that of the neuroligins. Most neurexin ligands interact with the last LNS domain, which is common to both α- and β-neurexins. This LNS domain contains alternative splice site 4 (SS4), which is the best-characterized splice site in terms of regulating neurexin–ligand interactions. For some neurexin ligands, SS4 acts as a molecular switch in determining binding. LRRTM2, LPHN1 and dystroglycan only bind to neurexin that lacks SS4 (REFS 45,46,48,49), whereas cerebellin 1 exclusively binds to neurexin containing a small SS4 insert[53] (FIG. 1C). By contrast, for neuroligin, the presence of SS4 in neurexin acts as a modulator that decreases the affinity of the neurexin–neuroligin interaction[54]. The remaining, largely uncharacterized, splice sites probably also modulate neurexin interactions with postsynaptic ligands. Consistent with this possibility, a recent study using proteomic neurexin isoform profiling showed that LRRTM2 preferentially associates with NRXN1α and NRXN2α over NRXN1β and NRXN2β, and that the presence of the SS6 insert negatively modulates the NRXN1α–LRRTM2 interaction[55].

***LRR and Ig protein superfamilies.*** The LRR and Ig domains are major protein–protein interaction motifs that are often found in cell surface molecules. The LRR protein superfamily is considerably larger in vertebrates than in *Drosophila melanogaster*[56]. The LRR domain is a versatile protein-interaction domain[57], and recent studies indicate that neuronal LRR proteins engage in molecularly diverse *trans* interactions[58] (FIG. 2A). In contrast to LRRTM2, LRRTM4 preferentially interacts with presynaptic heparan sulphate proteoglycans[59,60]. Fibronectin-like domain-containing leucine-rich transmembrane protein 3 (FLRT3), another postsynaptic LRR protein, interacts with the presynaptic G protein-coupled adhesion receptor LPHN[61]. The postsynaptic LRR protein netrin-G2 ligand (NGL2; also known as LRRC4) *trans*-synaptically interacts with the glycosylphosphatidylinositol-anchored protein netrin-G2 (REF. 62).

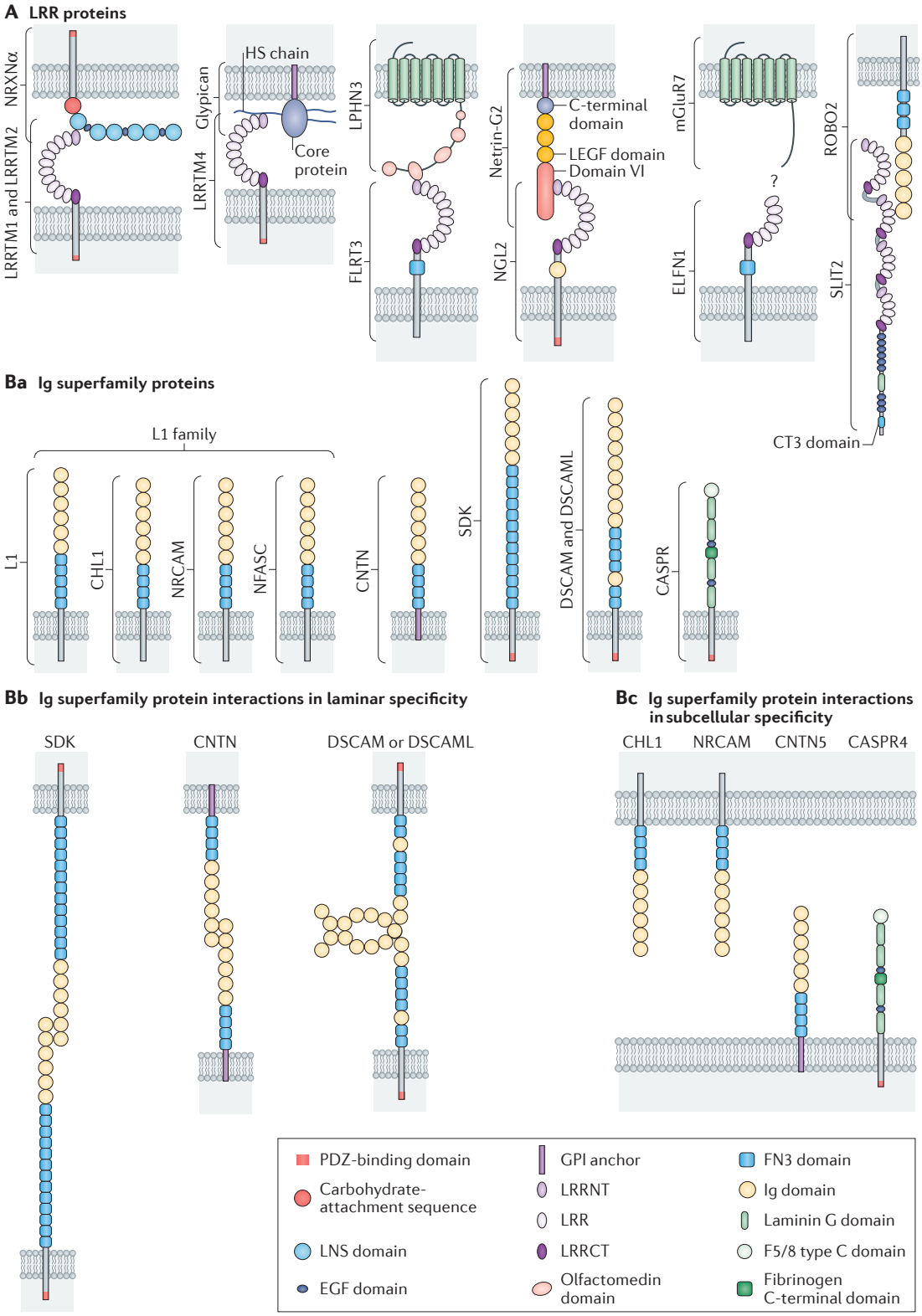
The Ig domain is among the most-common protein domains in the human genome[63]. A prominent family of Ig superfamily proteins that has been implicated in connectivity is the L1 (also known as the neuron–glia cell adhesion molecule (NgCAM)) family, consisting of L1, close homologue of L1 (CHL1), neuronal CAM (NRCAM) and neurofascin (FIG. 2B). These proteins can engage in homophilic and heterophilic interactions and have a domain organization consisting of Ig and fibronectin type III (FN3) domains. The L1 family proteins are closely related to the contactin (CNTN), sidekick (SDK), Down syndrome CAM (DSCAM) and DSCAM-like (DSCAML) proteins (FIG. 2B), which bind homophilically but do not seem to interact heterophilically with each other[64].

## Cell type-specific expression

Before discussing how molecularly diverse cell surface proteins contribute to wiring specificity and synaptic diversity, we first consider evidence for cell type-specific expression of cadherins, neurexins, and LRR and Ig superfamily proteins in the nervous system.

Type I classic cadherins are widely expressed in the nervous system, but type II cadherins are expressed in discrete patterns. Tracing studies showed that a dye injected into specific cortical regions expressing either of the type II cadherins cadherin 6 (*Cdh6*) or *Cdh8* retrogradely labelled, respectively, *Cdh6*- or *Cdh8*-expressing thalamic nuclei projecting to these regions, indicating that type II cadherins are expressed in projection-specific patterns[65,66]. Mapping studies of the expression patterns of multiple classic cadherins in various brain regions corroborated these initial findings and showed that cadherins are expressed in complex, partially overlapping patterns, labelling specific neuronal populations and fibre tracts[67–71]. Together with the enriched localization of several classic cadherins at synapses[72–76], these observations gave rise to the idea that cadherin-based interactions might contribute to cell–cell recognition and wiring specificity[76–78].

Proteoglycans
Heavily glycosylated proteins consisting of a core protein and covalently attached polysaccharide, highly sulphated glycosaminoglycan (GAG) chains. Heparan sulphate is a major type of GAG.




Purkinje cells
Large GABAergic output neurons of the cerebellum. Purkinje cells form highly complex and elaborate dendritic arbors.

Clustered protocadherins are broadly expressed in the CNS, but single-cell reverse transcription PCR (RT-PCR) analysis has shown that individual Purkinje cells express different combinations of clustered protocadherins[79–85]. Their variable single-cell expression and extraordinary potential for encoding unique adhesive interactions suggest that clustered protocadherins might be involved in generating distinct cell surface identities. One role for such identities might be in the recognition of self versus non-self, similarly to the part played by Dscam1 diversity in *D. melanogaster*[86,87]. Protocadherins have been detected in synaptic and extrasynaptic membranes, but

Figure 2 | **Domain organization and interactions of LRR and Ig superfamily proteins.** **A** | The leucine-rich repeat (LRR) domain is made up of individual LRRs, which are usually flanked by an LRR amino-terminal domain (LRRNT) and an LRR carboxy-terminal domain (LRRCT). LRR proteins interact heterophilically in *trans* with diverse ligands. The LRR domain of the postsynaptic proteins LRR transmembrane neuronal protein 1 (LRRTM1) and LRRTM2 interacts with the sixth laminin–neurexin–sex-hormone-binding globulin (LNS6) domain of presynaptic α-neurexin (NRNXα) or β-neurexin (not shown) that lacks splice site 4 (SS4), whereas LRRTM4 preferentially interacts with the heparan sulphate (HS) chains of presynaptic HS proteoglycans (HSPG), such as glycosylphosphatidylinositol (GPI)-anchored glypican. The postsynaptic protein fibronectin-like domain-containing leucine-rich transmembrane 3 (FLRT3) contains a fibronectin type III (FN3) domain in addition to its LRR domain, which interacts with the olfactomedin domain of presynaptic latrophilin 3 (LPHN3). The LRR domain of postsynaptic netrin-G2 ligand (NGL2) interacts with domain VI (laminin N-terminal domain) of the GPI-anchored presynaptic protein netrin-G2[62]. The extracellular LRR and FN3 domain-containing 1 (ELFN1) protein interacts with presynaptic metabotropic glutamate receptor 7 (mGluR7), although the binding site remains unclear (as indicated by the question mark). The secreted LRR protein Slit homologue 2 (SLIT2) interacts via its second LRR domain with the first immunoglobulin (Ig) domain of its receptor Roundabout homologue 2 (ROBO2). **Ba** | Ig superfamily proteins of the L1 (also known as neuron–glia cell adhesion molecule (NgCAM)) family, consisting of L1, close homologue of L1 (CHL1), neuronal CAM (NRCAM) and neurofascin (NFASC) have a domain organization consisting of Ig and FN3 domains. The contactin (CNTN), sidekick (SDK), Down syndrome CAM (DSCAM) and DSCAM-like (DSCAML) proteins are closely related to the L1 family proteins and share a similar domain organization. GPI-anchored CNTN signals via CNTN-associated protein (CASPR). **Bb** | Homophilic interactions of SDKs, CNTNs and DSCAMs have been implicated in the control of laminar specificity in the retina. **Bc** | A complex consisting of CHL1 and NRCAM interacting in *trans* with CNTN5 and CASPR4 has been implicated in control of subcellular specificity the spinal cord. CT3, C-terminal cysteine knot domain; LEGF, laminin EGF-like domain.

they are also found in intracellular compartments and it is still unclear to what extent they are present on the neuronal surface[22,23,88]. Protocadherins remain enigmatic surface molecules and much remains to be discovered about their roles in circuit formation.

The α- and β-forms of *Nrxn1–Nrxn3* transcripts are differentially expressed in the nervous system, in discrete but overlapping neuronal populations[32]. A quantitative comparison of neurexin alternative splicing in different brain regions showed that a complex structure such as the cortex contains a more-varied neurexin repertoire than the relatively less complex cerebellum[36]. Purified cerebellar granule cells displayed further reduced complexity, indicating that neurexin molecular diversity is linked to cellular diversity[36]. Alternative splicing of neurexins is regulated in a cell type-specific manner[89,90], supporting the idea that different populations of neurons might express distinct repertoires of neurexins. A recent study profiling neurexin isoform expression in single cells (BOX 2) that were isolated from neural tissue confirmed that neurexin repertoires are indeed highly cell type-specific and are reproducible for a specific neuron type[91]. For example, different populations of interneurons located in the same hippocampal lamina and projecting to the same pyramidal target cell population displayed divergent neurexin expression profiles. Different populations of long-range projection neurons converging on the same target cell also displayed distinct neurexin profiles[91]. Together with the predominantly presynaptic localization of neurexins and the absence of major axon-pathfinding defects in knockout mice deficient in all three α-neurexins[92], these findings suggest that cell type-specific neurexin repertoires do not convey target cell specificity, but are more likely to modulate the range of postsynaptic ligands with which these cells interact and, as a result, may contribute to the specification of synaptic properties.

LRR proteins are often expressed in discrete neuronal populations and are generally localized to the postsynaptic surface. For example, *Lrrtm4* expression in the hippocampus is restricted to dentate gyrus (DG) granule cells. Postsynaptic LRRTM4 interacts with presynaptic heparan sulphate proteoglycans to regulate excitatory synapse development in these neurons[59,60]. FLRT3 is expressed in hippocampal granule cells and CA3 pyramidal neurons and regulates granule cell excitatory synapse development via its interaction with presynaptic LPHN3 (REF. 61). Their discrete expression patterns and diversity in *trans* interactions suggest that LRR proteins may contribute to the specification of cell type-specific synaptic properties.

A good example of cell type-specific expression of Ig superfamily proteins is found in the retina, in which SDK1 and SDK2, DSCAM, DSCAML and their close relatives CNTN1–CNTN5, are differentially expressed in largely non-overlapping subsets of presynaptic amacrine cells and postsynaptic retinal ganglion cells[64,93,94]. Different classes of amacrine and retinal ganglion cells extend their processes in distinct synaptic laminae of the inner plexiform layer (IPL), where they form synapses. The discrete expression patterns of SDKs, DSCAMs and CNTNs in matching populations of partner cells, combined with their homophilic binding properties, suggests that these Ig family proteins could regulate lamina-specific targeting of retinal neuron processes.

## From surface protein to interactome

As it will become increasingly feasible to elucidate the surface molecule repertoire of specific cell types as technology develops (BOX 2), a next step would be to determine the complete cell surface interaction networks, or extracellular interactomes, of these cells. Technical challenges have impeded the identification of extracellular interactions[95], but recent advances in genomics, bioinformatics, assay technology and proteomics are enabling novel extracellular interactions to be discovered at a much higher rate than before. Advances in genomics and bioinformatics now allow for systematic surveys of all cell surface proteins encoded in the genomes of model organisms. These analyses have revealed that the expansion of genes encoding CAMs correlates with the increasing cellular complexity of organisms[96]. For example, the LRR and Ig superfamilies have expanded in the genome of vertebrate organisms compared with invertebrates[56,63,96], suggesting that an expanded repertoire of cell surface proteins allows for an increased complexity of connectivity.

Large-scale, systematic analysis of the extracellular interactions between surface proteins has been accelerated by the development of sensitive, high-throughput binding assays. One study developed an enzyme-linked immunosorbent assay (ELISA)-based assay to quantitatively assess thousands of interactions of Dscam1

Amacrine cells
Retinal interneurons that project their dendrites to the inner plexiform layer, where they can connect to retinal ganglion cells and bipolar cells. There are many subtypes of amacrine neurons.

Retinal ganglion cells
Output neurons of the retina.

Inner plexiform layer
(IPL). A dense synaptic layer in the retina, spanning only tens of microns, that can be subdivided in at least ten anatomical sublaminae. Each of these is innervated by processes of amacrine, bipolar and retinal ganglion cell subtypes.

Interactomes
Complete sets of interactions, in this context between surface molecules.

Box 2 | **New methodologies for understanding wiring specificity**

Recent technological advances are facilitating cell type-specific molecular profiling and the identification of cell surface receptor interaction networks. Transcriptome analysis of purified neuronal populations has been a powerful approach to gain insight into the gene expression profiles of specific cell types. An increasing ability to label distinct neuronal cell types in highly heterogeneous CNS tissues — through the use of transgenically expressed fluorescent reporters[146–149], a combination of genetic labelling and specific surface markers[150] or fluorescent tracers retrogradely transported from distal projections[146,151] — has been a key element of this approach. Labelled cell types can be isolated from the tissue by fluorescent sorting or laser-capture methods and then used in transcriptome analysis. Comparisons of gene expression profiles of different cell types from brain regions such as the hippocampus, retina, striatum and cortex have revealed an enormous heterogeneity in gene transcription between cell types[152,153]. Gene ontology (GO) analysis of the gene expression profiles of 12 different cortical cell types identified 'axon', 'extracellular matrix' and 'synapse' as significantly over-represented cellular components, and 'cell–cell communication' as an overrepresented biological process[146]. This finding suggests that differentially expressed genes are especially important for regulating connectivity. Affinity-capturing green fluorescent protein (GFP)-tagged polyribosomes expressed in genetically defined neuronal populations eliminates the need for isolating cells from tissue[154]. GO analysis of the translated mRNAs from 24 distinct cell types isolated using this method also identified 'extracellular matrix' as an overrepresented cellular component and 'cell–cell signalling' as an overrepresented biological process, and indicated that cell type diversity in the CNS is mainly driven by the differential expression of cell surface proteins[155]. A recent brain proteome analysis similarly concluded that cell surface proteins contribute the most to cell type and regional diversity[156]. Projection-based profiling of neurons is enabled by a strategy that involves the tagging of ribosomal proteins with nanobodies against GFP, which can be immunoprecipitated in the presence of GFP, allowing the selective purification of nanobody-tagged polyribosomes from GFP-labelled cells[157]. This technique can be further modified to allow projection-based profiling of specific cell types by injecting two viral vectors in a cell type-specific Cre line: one retrogradely transported viral vector encoding GFP in distal projection areas to retrogradely label neurons, and another encoding Cre recombinase-dependent nanobody-tagged ribosomal proteins in a genetically defined cell type. Rapid advances in single-cell sequencing are enabling the identification of novel cell types as well as a much-deeper characterization of their gene expression profiles[158]. All of these methods still require disruption of tissue to access cell type-specific profiles. Novel gene expression profiling methods are starting to enable *in situ* sequencing of RNA fragments in tissue[159], which may eventually eliminate the need for disrupting tissue context to profile specific cell types. Advances in proteomics enable the molecular characterization of large receptor complexes[160,161] and the identification of novel extracellular interactions by combining affinity chromatography with recombinant 'bait' proteins on brain extract and proteomics analysis[162]. Such approaches can be scaled up to include larger arrays of bait proteins, and refined to include smaller brain regions.

isoforms in flies[97]. Similar approaches have since been adopted for LRR and Ig superfamily proteins. These assays make use of secreted 'bait' and 'prey' proteins obtained from the media of transfected cells, and they rely on the clustering of bait proteins, which are immobilized on plates, and the oligomerization of prey proteins to enhance the avidity of low-affinity interactions. Binding is then assessed by an enzymatic reaction[98–101]. Large-scale binding assays for nearly all members of zebrafish and *D. melanogaster* Ig and LRR superfamilies that tested tens of thousands of unique interactions between hundreds of recombinant proteins have yielded close to a hundred novel interactions[99,101]. Technical problems such as incorrect folding of recombinant bait and prey proteins aside, the outcomes of these experiments are currently limited by the number and type of proteins assayed, suggesting that much of the extracellular interactome remains undiscovered. Another limitation is that the use of recombinant ectodomains in these binding assays does not reflect the complex nature of assemblies of adhesion proteins on the cell surface *in vivo*. However, scaling-up these assays to eventually test millions of interactions between thousands of proteins could deliver complete extracellular interactomes of specific cell types. These and other technological advances (BOX 2) will enable the combination of expression profiles of specific neuronal populations with their extracellular interactomes and connectivity maps[102], allowing researchers to start generating models of connectivity, which can then be experimentally tested in model systems.

## Surface proteins and wiring specificity

Despite rapid progress in elucidating cell type-specific gene expression profiles and extracellular interactomes, testing how cell surface molecules contribute to specifying connectivity in the vertebrate nervous system is still largely limited to the study of single genes. Screening large numbers of cell surface genes for their roles in connectivity is more feasible in simpler organisms, such as *D. melanogaster*[103]. However, improved genetic access to specific cell types increasingly allows testing the consequences of the gain or loss of function of surface proteins in neural connectivity with single-cell resolution *in vivo* in the vertebrate CNS. Below, we highlight several examples of surface proteins that regulate different aspects of wiring specificity.

***Self-avoidance.*** To achieve the correct wiring specificity, neurons must recognize not only the processes of other neurons but also their own neurites. The propensity of neurites from the same neuron to repel each other, a process termed self-avoidance, is indispensable for proper patterning of the *D. melanogaster* nervous system[87]. In *D. melanogaster*, the extraordinary diversity of the Dscam1 family of Ig superfamily cell surface proteins regulates self-avoidance[104].

The diversity of clustered protocadherins and their potential for encoding unique adhesive interactions suggested that protocadherins might have a similar role in vertebrates, which lack DSCAM diversity. Deletion of the γ-protocadherin cluster in retinal starburst amacrine cells (SACs), which have radially symmetric dendrites that rarely cross, resulted in frequent crossing and bundling of dendrites, without affecting their laminar targeting[105]. This process was cell-autonomous, and replacement of a single γ-protocadherin isoform was sufficient to rescue self-avoidance in SACs (FIG. 3a). However, protocadherin diversity is needed to allow these neurons to distinguish their own dendrites from the dendrites of other neurons, so that dendrites from the same SAC avoid each other but dendrites from different SACs interact freely. A similar role for γ-protocadherins in self-avoidance was found in Purkinje cells[105]. These results indicate that clustered protocadherins define unique cell surface identities that are important for self-recognition in vertebrates, much like Dscam1 diversity does in flies. However, it remains to be determined

Gene ontology
(GO). A unified representation of genes and gene products across species covering three domains: cellular component, molecular function and biological process.

Polyribosomes
Complexes of mRNA molecules and attached ribosomes in the process of translation.

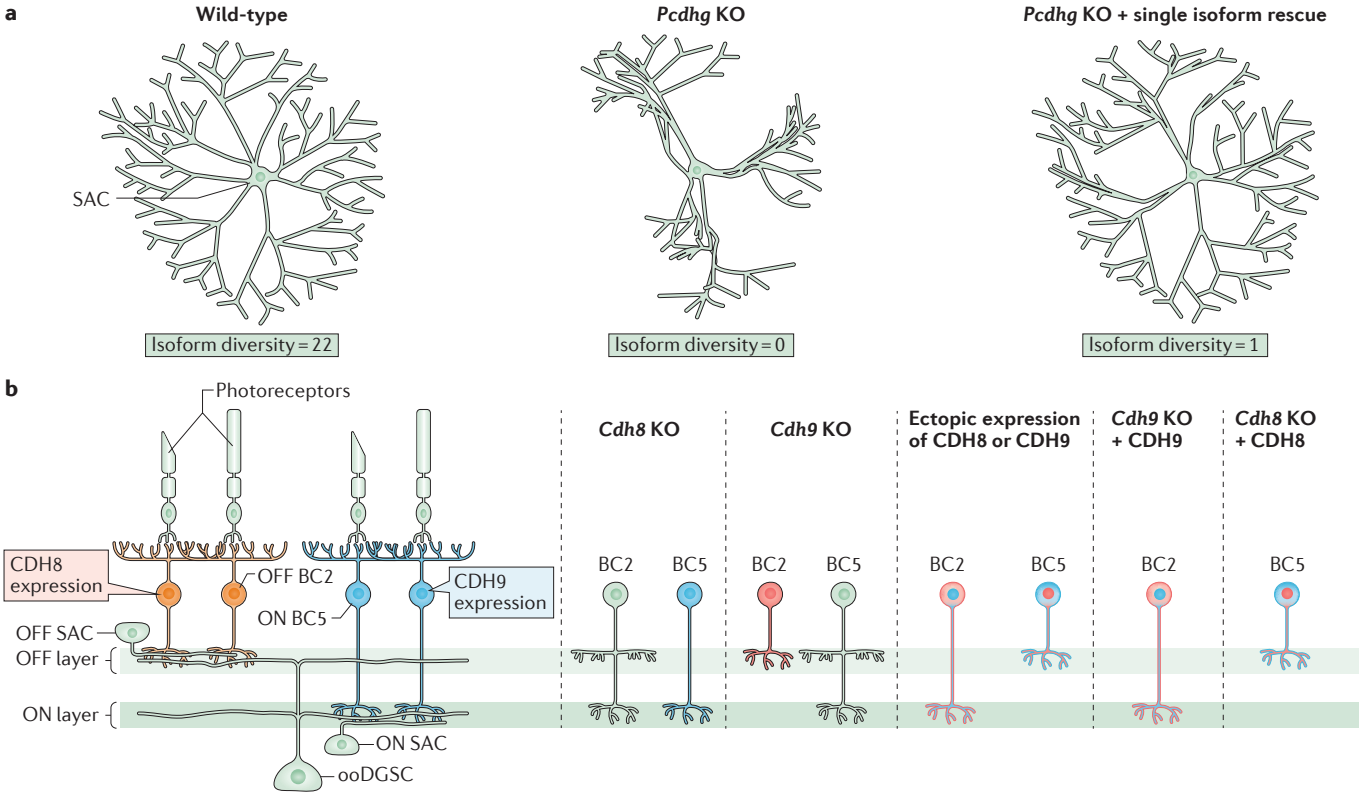



Figure 3 | **Protocadherins and classic cadherins in retinal circuit assembly. a** | Protocadherins regulate self-avoidance. Retinal starburst amacrine cells (SACs) have radially symmetric dendrites that rarely cross (left-hand panel). Deletion of the entire γ-protocadherin (*Pcdhg*) gene cluster (encoding 22 isoforms) in SACs results in frequent crossing and even bundling of dendrites (centre panel). Replacement of a single γ-protocadherin isoform in *Pcdhg*-knockout (KO) SACs is sufficient to rescue self-avoidance in SACs (right-hand panel). **b** | Type II classic cadherins regulate laminar targeting in the retina. Two distinct types of bipolar cells (BCs), each expressing a different type II cadherin, target different sublaminae in the retinal inner plexiform layer (IPL). Cadherin 8 (CDH8)-expressing 'OFF' type 2 BCs (BC2s; orange) target the outer IPL (the OFF layer), where they synapse onto the dendrites of OFF SACs and the upper branch of the bistratified dendrites of 'ON–OFF' direction-selective ganglion cells (ooDSGCs). CDH9-expressing 'ON' BC5s (blue) target the inner IPL (ON layer), where they synapse onto the dendrites of ON SACs and the lower dendritic branch of ooDSGCs. Loss of *Cdh8* in BC2s results in mistargeting of their axonal arbor to the inner IPL, but loss of *Cdh8* in BC5s does not affect their axonal targeting. Conversely, loss of *Cdh9* in BC5s results in the mistargeting of their axons to the outer IPL, but loss of *Cdh9* in BC2s does not affect their axonal targeting. Ectopic expression of CDH9 in BC2s leads to displacement of their axons to the inner IPL, and ectopic expression of CDH8 in BC5s displaces their arbors to the outer IPL. Ectopic expression of CDH9 in BC2s in a *Cdh9*-KO background still displaces their axons to the inner IPL, and introduction of CDH8 in BC5s in a *Cdh8*-KO background still displaces their axons to the outer IPL, indicating that type II cadherins instruct laminar targeting via a heterophilic mechanism.

to what extent protocadherin-mediated self-avoidance is a general principle, as cortical pyramidal neurons lacking the γ-protocadherin cluster displayed impaired arborization but not bundling or crossing of dendrites[106]. Furthermore, protocadherins are not the only class of surface proteins mediating self-avoidance in vertebrates. Recent studies have identified a role for multiple EGF-like domain (MEGF) receptors, as well as the transmembrane protein semaphorin 6A and its receptor plexin A2 in SAC self-avoidance[107,108], and the secreted LRR protein Slit homologue 2 (SLIT2) and its receptor Roundabout homologue 2 (ROBO2) in Purkinje cell self-avoidance[109]. Taken together, current *in vivo* evidence supports a role for protocadherins in self-avoidance, but given the many questions that remain about protocadherin interactions, localization and function, additional roles for the proteins in, for example, synapse development[110] can presently not be ruled out.

***Laminar specificity.*** Laminar targeting is a key step in establishing specific wiring patterns. Classic experiments using co-cultures demonstrated that type I neural cadherin (N-cadherin) contributes to laminar specificity[111,112]. Exploiting advances in genetic targeting of specific cell types (BOX 2), a recent study demonstrated that type II cadherins regulate laminar targeting in the retina[113]. Two distinct types of retinal bipolar cells (BCs), each expressing a different type II cadherin, target different synaptic laminae in the retinal IPL. Axons of *Cdh8*-expressing type 2 BCs (BC2s) target the outer IPL (FIG. 3b), whereas *Cdh9*-expressing BC5s target the inner

Bipolar cells
(BCs). Retinal neurons that transmit signals from photoreceptors to retinal ganglion cells.

IPL. Loss of *Cdh8* in BC2s resulted in mistargeting of part of their axonal arbor to the inner IPL. Conversely, loss of *Cdh9* in BC5s resulted in partial mistargeting of BC5 axons to the outer IPL (FIG. 3b), suggesting that CDH8 and CDH9 function to bias BC axon choice between inner and outer IPL laminae. Ectopic expression of CDH9 in BC2s redirected their axons to the inner IPL, whereas expression of CDH8 in BC5s redirected their arbors to the outer IPL (FIG. 3b), indicating that CDH8 and CDH9 instruct laminar targeting. Remarkably, introduction of CDH9 in BC2s on a *Cdh9*$^{-/-}$ background still redirected BC2 axons to the inner IPL (FIG. 3b). These results show that differentially expressed type II cadherins act heterophilically with laminar ligands in instructing the laminar choice of BC axons. Functional synaptic connectivity between BCs and ganglion cells in *Cdh8*$^{-/-}$ or *Cdh9*$^{-/-}$ mutants was more-strongly reduced than might be expected from the partial mistargeting defects[114], suggesting that, in addition to instructing BC axon laminar choice, CDH8 and CDH9 may promote synapse formation with their target cells.

In addition to the classic cadherins, many other proteins have been implicated in laminar targeting in the IPL. The Ig superfamily proteins SDK1, SDK2, DSCAM and DSCAML, as well as CNTN1–CNTN5, are expressed in discrete, largely non-overlapping subsets of pre- and postsynaptic retinal neurons and are concentrated in specific IPL synaptic laminae[64,93,94]. Homophilic interactions between such proteins on neurites from matching pre- and postsynaptic partner cells that project to the same IPL lamina have been proposed to promote lamina-specific connectivity. Gain- and loss-of-function studies lend some support to this idea. For example, knockdown of *CNTN2* in broad regions of the chick retina results in a more diffuse distribution of presumptive *CNTN2*-expressing neurites, identified by SDK1, which is co-expressed in a subset of *CNTN2*-positive neurons. Conversely, overexpression of *CNTN2* in groups of retinal cells tends to bias neurites towards CNTN2-positive laminae[64]. Different Ig superfamily proteins may thus act together to form an adhesive code for laminar specificity, but whether these proteins instruct laminar targeting or rather act to promote connectivity between matching synaptic partners within specific laminae is not yet clear. Homophilic interactions between matching pre- and postsynaptic partners alone seem insufficient to specify in which lamina such interactions occur, especially in the crowded environment of the IPL. Many of these Ig proteins can also interact with heterophilic ligands, which may contribute to encoding laminar specificity. In addition to heterophilic type II cadherin and homophilic Ig superfamily interactions, repulsive interactions also contribute to laminar specificity in the IPL (BOX 1), indicating that the concerted action of many cues controls precise laminar connectivity in this system.

***Subcellular specificity.*** The subcellular precise targeting of synapses is a key aspect of functional connectivity, as the location of a synapse on a target cell can have a profound influence on the output of that cell. The identity of the cell surface molecules that regulate subcellular synaptic specificity is still largely unknown, although a few examples involving type II cadherins and Ig superfamily proteins have now been described.

In the hippocampus, the proximal dendrites of CA3 pyramidal neurons in the stratum lucidum have complex spines, which are contacted by elaborate presynaptic boutons that originate from DG granule cells. The more-distal segments of CA3 dendrites have regular spines and receive inputs that do not originate from the DG. Both CA3 and granule cells express CDH9. In dissociated hippocampal cultures, knockdown of *Cdh9* in CA3 neurons specifically decreases the number of DG-CA3 synapses, without affecting non-DG synapses onto CA3 neurons[115]. *In vivo*, knockdown of *Cdh9* in granule cells reduces the number and size of DG synapses in the stratum lucidum, but laminar targeting of DG axons does not seem to be affected. *Cdh9* knockdown in CA3 cells disrupts spine formation and results in the formation of long filopodia on the proximal region of CA3 dendrites. Because ectopic expression of CDH9 in cultured CA1 hippocampal neurons, which normally lack *Cdh9*, is not sufficient to increase the number of DG synapses onto those cells[115], these findings are most-consistent with a permissive role for a homophilic CDH9 interaction in regulating the proper development of DG–CA3 synapses. In contrast to disruption of CDH9, disruption of CDH8, which is also expressed in granule cells and CA3 neurons, through treatment of organotypic slices with a peptide that interferes with CDH8 function does result in DG axon mistargeting[116], suggesting that CDH8 and CDH9 might regulate different aspects of DG–CA3 connectivity.

The L1 family of Ig proteins has been implicated in subcellular-specific targeting in several circuits. A classic example is neurofascin, which contributes to the specific subcellular targeting of GABAergic basket cell axons to the axon initial segment of Purkinje cells[117]. A recent study provided evidence for a role of L1 family proteins and CNTNs in mediating subcellular specificity in the spinal cord, where a class of GABAergic interneurons called 'GABApre' neurons selectively target excitatory proprioceptive sensory terminals on motor neurons (FIG. 4). The remarkably precise subcellular targeting of GABApre axo-axonic synapses is mediated by a receptor complex of NRCAM and CHL1 on GABApre axons interacting heterophilically with a complex of CNTN5 and CNTN-associated protein 4 (CASPR4; also known as CNTNAP4) on sensory terminals[118] (FIG. 2B). Loss of these Ig superfamily proteins, alone or in combination, results in a similar reduction in the density of GABApre boutons on sensory terminals (FIG. 4) but not in mistargeting of GABApre axons. These findings suggest that an Ig superfamily adhesive code contributes to determining the density of GABApre boutons on sensory terminals, and they imply that cells maintain subcellular compartment-specific distributions of surface proteins to guide the specificity of connectivity. Little is known about how the surface protein composition of subcellular compartments is organized and maintained. An example of a surface receptor with a subcellular-specific distribution is the LRR protein NGL2, which localizes to

Stratum lucidum
A thin layer in the hippocampal CA3 area. Axons originating from granule cells in the dentate gyrus course through this layer and synapse onto CA3 proximal dendrites.

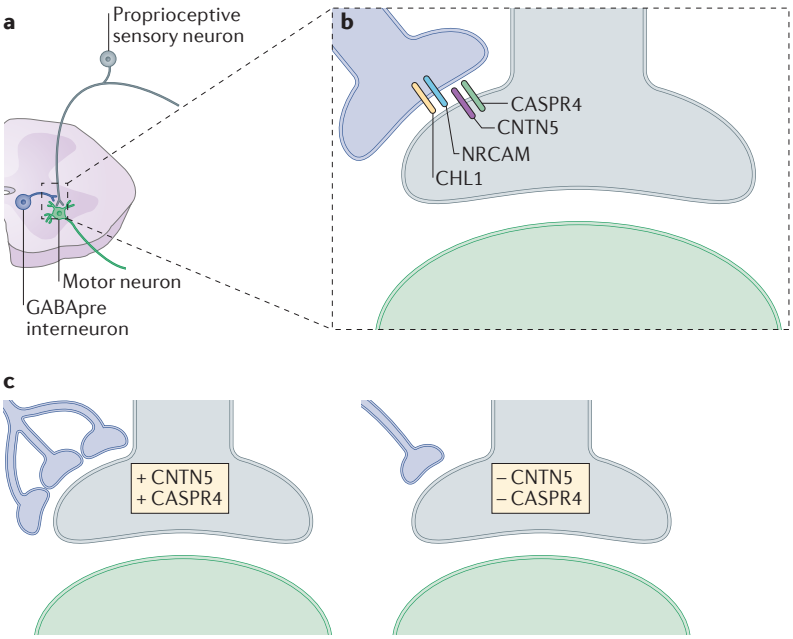



Figure 4 | **An Ig superfamily combinatorial code regulates subcellular specificity in the spinal cord. a** | Proprioceptive sensory neurons in the dorsal root ganglion form excitatory synapses on motor neurons in the spinal cord. These sensory nerve terminals represent the sole synaptic target of 'GABApre' interneurons located in the spinal cord. **b** | The remarkably precise subcellular targeting of these axo-axonic synapses is mediated in part by a complex of neuronal cell adhesion molecule (NRCAM) and close homologue of L1 (CHL1) on GABApre axons, and a receptor complex of the immunoglobulin (Ig) superfamily proteins contactin 5 (CNTN5) and CNTN-associated protein 4 (CASPR4) on sensory neuron terminals. **c** | Loss of these Ig superfamily proteins, alone or in combination, results in a similar reduction in the density of GABApre boutons on sensory terminals.

proximal hippocampal CA1 pyramidal cell dendrites via a *trans*-synaptic interaction with netrin-G2 expressed on Schaffer collateral axons innervating this dendritic segment[119]. Loss of *Ngl2* reduces the density and plasticity of excitatory inputs on the proximal segment of the CA1 dendrite without affecting Schaffer collateral targeting or excitatory synapse density on the distal dendrite[120,121].

## Surface molecules and synaptic diversity

In addition to a highly specific wiring pattern, the properties of synaptic transmission critically contribute to information processing in the CNS. Presynaptic release probabilities, postsynaptic receptor compositions and plasticity vary widely across CNS synapse types. Molecular diversity could also contribute to generating unique synaptic properties, but little is known about the mechanisms by which surface proteins regulate synaptic diversity. Below, we highlight several examples of surface proteins regulating synaptic properties in the vertebrate CNS.

***Neurexins regulate postsynaptic receptor composition.*** Alternative splicing of neurexins regulates their interactions with multiple synaptic ligands, which may contribute to the specification of synaptic properties. To directly test the *in vivo* significance of neurexin alternative splicing at SS4, a major regulator of neurexin–ligand interactions, one study genetically engineered mice in which SS4 was constitutively included in *Nrxn3a* and *Nrxn3b* transcripts, and could be excised by expression of Cre recombinase[122]. Constitutive inclusion of SS4 in *Nrxn3* (leading to expression of NRXN3-SS4$^+$) does not affect synapse number but selectively alters postsynaptic trafficking of the AMPA glutamate receptor (AMPAR), without affecting NMDA receptor trafficking. In neurons expressing NRXN3-SS4$^+$, synaptic levels of AMPARs are decreased and AMPAR endocytosis is increased (FIG. 5a), resulting in a decrease in synaptic strength and impaired long-term potentiation (LTP). These effects are non-cell-autonomous and can be reversed by Cre recombinase-mediated excision of SS4, indicating that presynaptic SS4-lacking neurexins regulate postsynaptic AMPAR stabilization. The impaired AMPAR trafficking in neurons expressing NRXN3-SS4$^+$ is associated with decreased surface expression of LRRTM2 (REF. 122), which only binds to SS4-lacking neurexins[45,46] and also interacts with AMPARs[44]. This study indicates that presynaptic neurexins *trans*-synaptically control postsynaptic AMPAR stabilization through an SS4-dependent interaction with LRRTM2 and possibly other ligands, such as neuroligins. The picture that emerges suggests that alternative splicing of neurexins modulates a cell surface interaction network with various ligands, thereby affecting AMPAR trafficking and changing postsynaptic receptor composition and the functional properties of synapses.

***LRR proteins control presynaptic release properties.*** The above example illustrates how cell surface interactions between pre- and postsynaptic neurons regulate postsynaptic properties. Presynaptic release properties are also well known to depend on interactions between pre- and postsynaptic cells[123,124]. A striking example is found in the hippocampus, where CA1 pyramidal cell axons contact two types of interneurons: somatostatin-positive interneurons in the stratum oriens (oriens lacunosum-moleculare (OLM) cells) and parvalbumin-positive interneurons (PV$^+$ cells) (FIG. 5b). The synapses onto OLM cells are facilitating (with a low release probability), whereas the synapses of the same CA1 axon onto PV$^+$ cells are depressing (with a high release probability), indicating that the identity of the postsynaptic target cell defines these presynaptic properties (FIG. 5b). The postsynaptic LRR protein extracellular LRR and FN3 domain-containing 1 (ELFN1), which is expressed in OLM cells but not in PV$^+$ cells, has a key role in regulating the presynaptic release probability of CA1 axons. Knockdown of *Elfn1* in OLM cells decreases the facilitation of CA1–OLM synapses, whereas ectopic expression of ELFN1 in PV$^+$ cells moderately increases the facilitation of CA1–PV$^+$ synapses[114], indicating that postsynaptic ELFN1 decreases the presynaptic release probability. The molecular mechanism by which ELFN1 regulates release probability is not completely understood. ELFN1 *trans*-synaptically recruits metabotropic glutamate receptor 7 (mGluR7)[125], which displays a striking presynaptic enrichment at pyramidal cell–OLM synapses but not at synapses of pyramidal cells with other pyramidal

Release probabilities
The cumulative chance that at least one synaptic vesicle will be released on stimulation.

Facilitation
A presynaptic form of short-term plasticity in which a series of stimuli produces an increase in neurotransmitter release due to a build-up of $Ca^{2+}$ concentration in the nerve terminal.

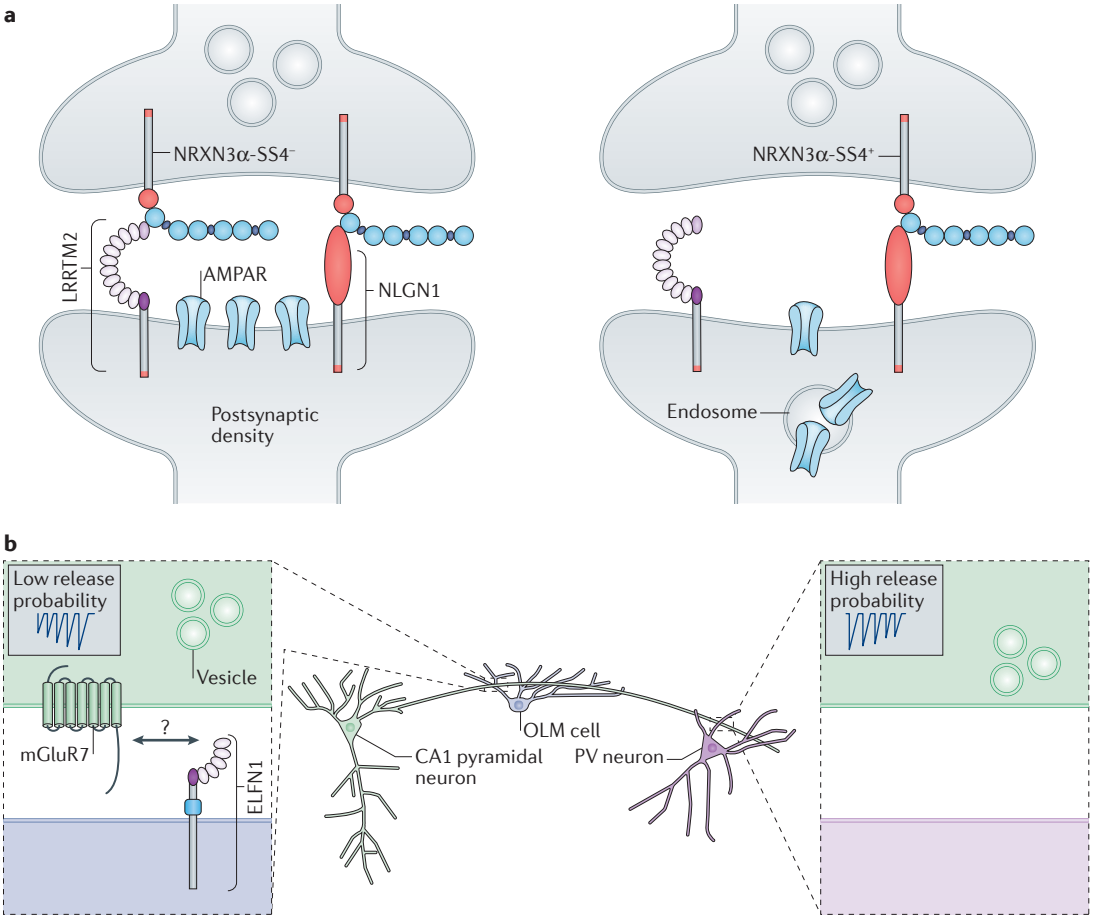



Figure 5 | **Neurexins and LRR proteins regulate the properties of synaptic transmission. a** | Presynaptic neurexin 3 (NRXN3) *trans*-synaptically controls postsynaptic AMPA glutamate receptor (AMPAR) stabilization through a splice site 4 (SS4)-dependent interaction with leucine-rich repeat transmembrane neuronal protein 2 (LRRTM2) and possibly other ligands, such as neuroligin 1 (NLGN1). LRRTM2 only binds to SS4-lacking NRXN3 (NRXN3-SS4$^-$; only the α-isoform is shown) and also interacts with AMPARs (not shown). Constitutive inclusion of SS4 in NRXN3 (NRXN3-SS4$^+$; only the α-isoform is shown) disrupts the interaction with LRRTM2 and selectively alters the postsynaptic trafficking of AMPARs without affecting NMDA receptor trafficking. In neurons expressing NRXN3-SS4$^+$, synaptic levels of AMPARs are decreased and AMPAR endocytosis is increased, resulting in a decrease in synaptic strength and impaired long-term potentiation. **b** | The postsynaptic LRR protein extracellular LRR and fibronectin type III domain-containing 1 (ELFN1) regulates presynaptic release probability. CA1 axons form facilitating synapses with a low release probability on oriens lacunosum-moleculare (OLM) cells, and depressing synapses with a high release probability on parvalbumin-positive (PV$^+$) cells. ELFN1 is expressed in OLM cells but not in PV$^+$ cells. Knockdown of *Elfn1* (not shown) in OLM cells decreases facilitation of CA1–OLM synapses, whereas ectopic expression of ELFN1 in PV$^+$ cells moderately increases facilitation of CA1–PV$^+$ synapses. These findings indicate that postsynaptic ELFN1 decreases presynaptic release probability, possibly via a *trans*-synaptic interaction with metabotropic glutamate receptor 7 (mGluR7; as indicated by the question mark).

cells or interneurons[126]. A high concentration of presynaptic mGluR7 could act to reduce neurotransmitter release at facilitating CA1–OLM synapses. Together, these studies illustrate how cell type-specific expression of a postsynaptic surface molecule instructs presynaptic molecular composition and functional properties.

## Conclusions and perspectives

Molecular diversity of surface molecules has long been envisioned to mediate the specificity of connectivity in neural circuits. In recent years, substantial progress has been made in identifying cell type-specific expression patterns of molecularly diverse families of surface molecules and characterizing the complexity of their extracellular interactions. Despite this progress, we are still a long way from understanding how, and to what extent, molecular diversity contributes to the specific wiring of vertebrate neural circuits. Recent advances in single-cell profiling are beginning to support the existence of cell type-specific surface protein repertoires. For at least some cell types, *in vivo* evidence indicates that protocadherin diversity defines unique cell surface identities that are important for self-recognition. Other surface proteins contribute to this process as well, preventing the inadvertent formation of contacts with a neuron's own processes and thus indirectly contributing to wiring specificity. Whether unique repertoires of cell surface molecules also encode laminar,

cellular and subcellular specificity, as well as synaptic diversity of individual neuron types is only beginning to be explored. The *in vivo* evidence for a role in specifying connectivity is probably strongest for the type II cadherins CDH8 and CDH9, whose cell type-specific expression instructs the laminar choice of BC axons in the retina. Even here, type II cadherins interact heterophilically with unknown ligands localized in the lamina, rather than in a 'lock-and-key' fashion with target cells, as envisioned by Sperry. The main role of type II cadherins in assembling this circuit may be to bias the choice of lamina, with additional surface molecules probably mediating the correct partner choice within that lamina. Much remains to be learned about the identity of the cues that govern lamina-specific innervation and how pre-patterned laminar environments develop[127,128]. Although cadherins, Ig and LRR superfamily proteins have now been shown to contribute to cellular and subcellular specificity in a few instances, we still know very little about the identity of the other cues regulating wiring specificity at these levels. Neurexin diversity does not seem to encode cellular specificity, although a role in subcellular specificity cannot yet be ruled out. The identity of the molecular cues instructing the final step of circuit assembly, synaptic diversity, is still largely unknown, although a few examples involving neurexins and LRR proteins have now been described.

The emerging picture is that no single cue regulates specificity, and that the same cue can be reused in multiple steps of circuit assembly. This suggests that many different surface molecules act in various combinations at each of the steps that lead to establishing precise connectivity. Combinatorial coding enhances robustness, increases information-coding capacity and, at the same time, limits the need for a vast number of different cues to encode specific connectivity[127]. In addition to requiring cell type-specific repertoires of surface molecules, such a combinatorial coding model implies that distinct subcellular compartments and synaptic subtypes will have different combinations of surface molecules. Unravelling how different combinations of surface molecules collaborate to build a functional nervous system constitutes a major future challenge that will need to be addressed in intact circuits and at specific synapse types. Determining how neurons dynamically alter their surface repertoires in response to activity, and how activity modulates protein interaction networks at synapses *in vivo*, will be another challenge. Bridging the gap between systems-level analyses of cell type-specific expression profiles or extracellular interactomes and the functional testing of candidate genes in connectivity will also be essential to arrive at a more-complete understanding of the molecular specification of synaptic connectivity.

Finally, many of the genes encoding cell surface molecules described in this Review have been linked to neurodevelopmental and psychiatric disorders[30,58,129–131]. Their cell surface localization makes these proteins valuable targets for interference with small molecules or drugs. A deeper understanding of the molecular mechanisms determining precise synaptic connectivity in neural circuits will therefore be essential to develop and realize future treatments for cognitive disorders.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**Competing interests statement**
The authors declare no competing interests.