Neurexins: molecular codes for shaping neuronal synapses

Andrea M. Gomez1, Lisa Traunmüller2 and Peter Scheiffele1✉

Abstract | The function of neuronal circuits relies on the properties of individual neuronal cells and their synapses. We propose that a substantial degree of synapse formation and function is instructed by molecular codes resulting from transcriptional programmes. Recent studies on the Neurexin protein family and its ligands provide fundamental insight into how synapses are assembled and remodelled, how synaptic properties are specified and how single gene mutations associated with neurodevelopmental and psychiatric disorders might modify the operation of neuronal circuits and behaviour. In this Review, we first summarize insights into Neurexin function obtained from various model organisms. We then discuss the mechanisms and logic of the cell type-specific regulation of Neurexin isoforms, in particular at the level of alternative mRNA splicing. Finally, we propose a conceptual framework for how combinations of synaptic protein isoforms act as ‘senders’ and ‘readers’ to instruct synapse formation and the acquisition of cell type-specific and synapse-specific functional properties.

Alternative splicing

A process in which exons of an mRNA are assembled in multiple different (alternative) ways to yield multiple different versions of a final mRNA molecule that may contain different RNA regulatory motifs or encode alternative protein forms.

Isoforms

Variants of an mRNA transcript or protein generated from a single gene but differing in sequence (for example, resulting from alternative promoters or from alternative splicing).

Nervous systems represent remarkable examples of a highly organized tissue with an abundance of specialized cells in an intricate structure. During development, neuronal connectivity arises from a series of steps, including cell specification, migration, targeted growth, synapse formation and remodelling. Spontaneous activity and sensory experience propagate through the developing networks play a significant role in organizing aspects of neuronal wiring. However, many fundamental steps of neuronal morphogenesis and synapse formation proceed normally even in the absence of neurotransmission1–3. Thus, genetically encoded programmes are thought to orchestrate key aspects of the timing and dynamics of neuronal growth and nervous system wiring4–7. Cell surface adhesion and signalling molecules significantly contribute to all of these developmental steps. Thus, each neuronal cell type carries an array of cues linked to cellular origin and cell fate that are integral to its developmental specification. Although signalling processes, neuronal activity and disease states may shift these codes, there are constraints that restrict this plasticity, thereby maintaining cell type-specific properties. One critical and extensively studied process in nervous system development is the selective growth and targeting of neurites, which encompasses axon guidance and synaptic specificity8–14. The present Review aims to discuss a second key aspect of neuronal wiring: the molecular principles of neuronal synapse formation and the specification of synapse function. We will use the Neurexin family, one class of cell adhesion molecules, to illustrate the fundamental principles of this process that likely apply to many other adhesion systems operating at neuronal synapses.

Adhesive modules for synapse assembly. Synaptic differentiation relies on a large number of synaptic adhesion and signalling molecules with so-called synaptogenic properties, that is, the ability of an isolated factor to trigger a substantial degree of the synaptic differentiation process. When presented in non-neuronal cells or on synthetic surfaces, synaptogenic proteins nucleate the formation of functional presynaptic or postsynaptic assemblies10–12. For example, postsynaptic adhesion molecules of the Neuroligin family trigger the assembly of functional presynaptic terminals in axons through interaction with their receptor Neurexin9,10 (Fig. 1a). Conversely, Neurexin-mediated clustering of Neuroligins triggers the recruitment of NMDA-type glutamate receptors and scaffolding molecules12,13. This early cell biological analysis uncovered fundamental activities of Neurexin proteins and their ligands. Subsequent genetic studies then probed the functional consequences of inactivating Neurexin genes in various model organisms (see below). What makes the roles for Neurexins in this process so fascinating is twofold. First, the Neurexin gene family encodes a vast array of distinct transcript isoforms generated from multiple genes (Nrxn1, Nrxn2, Nrxn3), alternative promoters (α, β, γ) and extensive alternative splicing, with individual isoforms linked to specific neuronal cell types. Second, Neurexins serve as presynaptic receptors for several structurally unrelated extracellular binding partners, indicating that they
represent a hub for presynaptic organization (Fig. 1b). For example, Neurexins are presynaptic receptors for the secreted protein Cerebellin 1 (CBLN1), the transmembrane proteins Neuroligin 1–4, α-Dystroglycan, Leucine-rich repeat transmembrane proteins (LRRTM1, LRRTM2, LRRTM3, LRRTM4) and Calsyntenin 3 [REFS 14–17]. Recent reviews provide a comprehensive summary of this array of Neurexin ligands6,18. In the present article, we focus on the contribution of alternative splicing of Neurexins in controlling such interactions and on the interplay of multiple synaptic recognition systems at neuronal synapses. In the following, we will first discuss genetic studies in various model organisms where many or most Neurexin isoforms are ablated.

Neurexin and neuronal connectivity

Genetic loss-of-function studies highlight critical roles for Neurexin proteins at synapses in vivo. Initial work emphasized functional alterations in synaptic transmission, in particular calcium-dependent neurotransmitter release19,20. Consistent with the synaptogenic activity of Neurexins, a large body of genetic studies also support an evolutionary conserved role for Neurexin proteins in structural synapse assembly. In vivo models present with a wide array of phenotypes, depending on the cellular context and the Neurexin isoforms and/or genes ablated. Early studies in Neurexin 1,2,3α triple-knockout mice discovered that inhibitory synapse density in the brainstem is reduced by 50%, whereas the density of excitatory synapses is unchanged at birth19. In the nematode Caenorhabditis elegans, loss of one particular Neurexin isoform, γ-Neurexin, diminishes synapse numbers in the DA9 motor neuron supporting critical functions for this form in synapse formation21. A broader nrx-1 mutation results in a loss of postsynaptic neurotransmitter receptors from synapses and loss of spine-like protrusions from the postsynaptic neuron22,23. In another class of C. elegans neurons that display experience-dependent and sexually dimorphic plasticity, synapse rearrangements are impaired in nrx-1 mutants24. In the fruit fly Drosophila melanogaster, mutations in Neurexin and its ligand, Neuroligin, result in severe loss of neuromuscular

Fig. 1 | Synaptogenic function of Neurexins. a | Bidirectional synapse-organizing activity of Neurexins. Presentation of Neurexin proteins or their ligands on synthetic surfaces in vitro and overexpression of Neurexin ligands in vitro and in vivo drive assembly of presynaptic and postsynaptic structures, respectively. Certain specific Neurexins only interact with certain receptors, for example, Neurexin 1 interacts with the NMDA receptor (right). b | Neurexin isoforms interact with a large array of structurally unrelated extracellular binding partners. Only a selection of ligands are displayed in this simplified schematic. Depending on cellular context, several ligands can be co-expressed at single synapses or can be differentially expressed across neuronal cell populations. c | Example of a loss-of-function phenotype resulting from loss of Neurexin (nrx-1 mutant) at the neuromuscular junction of Drosophila larvae (adapted from Li et al.27); synaptic release sites are marked in orange. d | Contribution of the Neurexin–Neuroligin adhesion system to growth of axonal arborizations of the motor neurons that innervate the abdominal pleural muscles of adult Drosophila (adapted from Constance et al.29). CBLN1, Cerebellin 1; LRRTM, Leucine-rich repeat transmembrane protein.
synaptic release sites in larvae\textsuperscript{28–27} (FIG. 1c). Importantly, impairing Neurexin–Neuroligin adhesion also modifies the growth of axonal and dendritic arbors in some model organisms. Time-lapse imaging experiments in developing tadpoles suggest that adhesion through Neurexin and its ligand, Neuroligin, confer transient morphological stabilization of dendritic contacts\textsuperscript{28}. Similarly, the growth and arborization of Drosophila motor neuron axons during metamorphosis is disrupted in Neurexin-deficient fruit flies\textsuperscript{29} (FIG. 1d). Although such macroscopic alterations in neuronal arborizations have not been reported in mice, the roles for Neurexins in synapse assembly are conserved from invertebrates to mammals.

Two aspects have significantly delayed the emergence of the present picture for Neurexin functions: first, in the mammalian system, the phenotypic space explored in in vivo studies was quite limited. Although work in invertebrate model systems has long explored synapse formation between genetically defined cell types, this approach has only been implemented in mammalian systems during this past decade. Second, unlike the vertebrate neuromuscular junction, where synaptic differentiation relies heavily on one primary signalling system\textsuperscript{30}, central synapses engage a complex combination of signals. This cooperation between multiple trans-synaptic signals greatly complicates generalizing conclusions from individual genetic experiments. The same Neurexin mutation can result in very different phenotypes when analysed in different cell types. For example, mutation of C. elegans nrx-1 severely disrupts AChR clusters in DD GABA neurons but not muscle\textsuperscript{31}. In mice, the conditional ablation of all Neurexin isoforms in somatostatin-positive versus parvalbumin-positive interneurons exhibits very different phenotypes. Mutant parvalbumin-positive interneurons severely reduce synapse formation on principal neurons in the medial prefrontal cortex. By contrast, the number of synapses formed by Neurexin-deficient somatostatin-positive interneurons in the same region is unchanged\textsuperscript{32}. However, somatostatin-positive interneurons show altered voltage-gated calcium channel function and defects in neurotransmitter release\textsuperscript{33}. The reasons for such disparate observations are likely manifold. First, many studies examine mutations in cells without knowing the expression of the disrupted Nrxn gene, its transcript isoforms and paralogs. Second, there is an array of additional presynaptic receptors unrelated to Neurexins that contribute to synapse assembly\textsuperscript{34,35}. Last, different neuronal cell types express different Neurexin isoforms, generated from alternative promoters (such as the \(\alpha\), \(\beta\) and \(\gamma\) forms) and modified through extensive alternative splicing. Notably, such Neurexin isoforms differentially interact with selective synaptic ligands. Thus, deletion of individual Neurexin genes precipitates impairment of different receptor–ligand modules in different cell types. This complexity most likely underlies the diversity of phenotypes reported in previous studies.

**Molecular diversity of Neurexins.** Combinations of genomic and proteomic features, including post-translational modifications, impart Neurexins with numerous adhesive motifs that underlie low-affinity and high-affinity interactions. These structural motifs—acting individually or cooperatively—recruit macromolecular complexes that span the synaptic cleft and coordinate bidirectional signalling and organization.

Three very large genes encode mammalian Neurexin 1, Neurexin 2 and Neurexin 3 (1.0 Mb, 0.1 Mb and 1.6 Mb in mouse; 1.1 Mb, 0.1 Mb and 1.8 Mb in human). Invertebrates, such as C. elegans and D. melanogaster, possess a single Neurexin orthologue\textsuperscript{36,37}. Each mammalian Neurexin gene contains two promoters that produce a long, \(\alpha\)-Neurexin pre-mRNA and a shorter, \(\beta\)-Neurexin pre-mRNA, which encode proteins of approximately 1,500 and 450 amino acids (FIG. 2a). For mouse Nrxn1, an additional, very short \(\gamma\)-isoform is generated from a third, internal promoter\textsuperscript{38}, and an orthologous \(\gamma\)-isoform is reported in C. elegans\textsuperscript{39}. We refer to these transcripts (irrespective of their further modification by alternative splicing) as ‘primary Neurexin transcripts’. Differential usage of \(\alpha\), \(\beta\) and \(\gamma\)-promoters in the three mammalian Neurexin paralogs drives highly divergent levels of the primary Neurexin transcripts across neuronal cell types. For example, mouse hippocampal CA3 pyramidal neurons express high levels of Nrxn1\(\beta\), whereas the same transcript is very low in CA1 pyramidal cells\textsuperscript{32–39}. In the mouse neocortex, GABAergic SST interneurons express threefold higher levels of Nrxn3\(\gamma\) transcripts as compared with layer 4 pyramidal cells\textsuperscript{38}.

Besides the use of these alternative promoters and corresponding transcription start sites, extensive diversification of Neurexin transcripts is further driven by alternative splicing. Thus far, up to six alternatively spliced segments (AS1–AS6) — some containing multiple alternative splice acceptor and donor splice sites — exist in primary Neurexin transcripts (FIG. 2b). The combinatorial usage of alternative promoters and alternative splice sites has the potential to yield >12,000 Neurexin transcript isoforms in mice. Long-read, single-molecule PacBio sequencing studies experimentally confirmed the presence of hundreds of Neurexin transcript isoforms in the mouse brain\textsuperscript{40,41}. Interestingly, the relative usage and combination of alternative splice insertions are conserved between rodents and humans, evidenced by the analysis of post-mortem human brain samples and human induced pluripotent stem cell-derived neuronal preparations\textsuperscript{42}. These transcriptomic studies provide a basis for interpreting the function of Neurexin diversity. However, one caveat is that transcript levels are not sufficiently informative regarding Neurexin protein isoform expression as multiple Neurexin gene products undergo further control at the level of mRNA translation\textsuperscript{40,41}. Advances in targeted proteomics should clarify the accurate relative and absolute quantification of protein abundance, even for peptides derived from specific splice insertions\textsuperscript{42}.

**Structure of macromolecular assemblies.** The defining feature of all Neurexins (except for the non-canonical NRXN1y) is the presence of extracellular Laminin–Neurexin–Sex hormone-binding globulin (LNS) domains. \(\alpha\)-Neurexins present six LNS domains interspersed by single epidermal growth factor (EGF) domains in the extracellular region (FIG. 2b).
Fig. 2 | Molecular and structural features of Neurexin isoform diversity. a | Schematic of the alternatively spliced segments of mouse Neurexin genes. Mouse Neurexin transcript isoforms are generated from three genes (Nrxn1, 1.1 Mb; Nrxn2, 0.1 Mb; Nrxn3, 1.8 Mb; note that given the big differences in gene sizes, the exons and introns are not drawn to scale), each containing up to three alternative promoters (α, β, γ) and exhibiting extensive alternative splicing at six alternatively spliced segments (AS1–AS6). Individual segments can contain single alternative cassette exons (for example, AS4, AS6) or consist of complex combinations of alternative splice donor and acceptor sites (for example, AS1, AS2, AS3, AS5). Numbers (2, 3, 4, 8, 30) depict counts of potential splice variations generated at each segment. Alternative exons are illustrated in colour, constitutive exons in grey and alternative donor or acceptor sites are white. b | Alternative promoters and alternative mRNA splicing result in Neurexin protein isoforms that share transmembrane domain (TMD) and cytoplasmic sequences but differ in their extracellular protein sequences. The extracellular sequences are composed of three major elements: Laminin–Neurexin–Sex hormone-binding globulin (LNS) domains, epidermal growth factor (EGF)-like domains and attachment sites for heparan sulfates. The largest Neurexin proteins are the α-NRXN forms composed of six LNS domains (LNS1–LNS6), three interposed EGF domains (EGFα–EGFβ) and the heparan sulfate attachment sites. Interaction surfaces for ligands are marked with purple lines. β-NRXN contains a single LNS domain and heparan sulfate attachment sites, whereas γ-NRXN is the smallest form lacking LNS and EGF domains.

c | Mapping of alternatively spliced segments (AS2, AS3, AS4), ligand-binding domains and sequence variants on a prototypical LNS domain: (left) ribbon diagram and positions of alternatively spliced segments; (right) view of a 90° rotation and surface representation of an LNS domain with mapped alternatively spliced segments; (bottom) surface representation, as in right panel, highlighting the position of ligand-binding domains and the naturally occurring R498W variant in NRXN3a that has been linked to behavioural alterations in mice.

d | Illustration of approximate sizes and hypothetical conformation of adhesion molecule complexes in the synaptic cleft: β-Neurexin (orange, left panel) with Leucine-rich repeat transmembrane protein 2 (LRRTM2; green); and α-Neurexin (orange, right panel) with Neurexophilin (NXPH1; pink) and Neuroligin (NLGN; blue). Structural models of the extracellular domains were drawn with ChimeraX 1.0 from the following Protein Data Bank IDs: 3POY (REF. 19), 3B3Q (REF. 19), 6PNP (REF. 19), 5Z8Y (REF. 19). The position of stalk, transmembrane and cytoplasmic sequences indicated as dashed lines. Diagrams at the bottom display positions within these structures where alternative splicing at AS1, AS6 and AS5 in β-NRXN (left) and α-NRXN (right) modifies the flexibility of the extracellular domains in the synaptic cleft. Part d (left) is adapted from REF. 19, CC BY 4.0 (https://creativecommons.org/licenses/by/4.0/). Part d (right) adapted with permission from REF. 19, Elsevier. Part 2d (right) is adapted from REF. 19, CC BY 4.0 (https://creativecommons.org/licenses/by/4.0/). Parts c, d (right) adapted from REF. 19, Springer Nature Limited.

These alternating repeats tether to the cell surface via a rigid and extensively O-linked glycosylated stalk and a single transmembrane domain. The short intracellular tail contains interaction sites for cytoskeletal adaptors (Protein 4.1) and a carboxy-terminal PDZ-binding motif. The smaller β-NRXN proteins have a short, unique amino-terminal sequence but are otherwise identical to α-NRXN beginning at the sixth LNS domain. γ-Nrxn transcripts encode a truncated isoform that lacks the extracellular LNS and EGF structured domains, yet retains a transmembrane and intracellular tail.

Remarkably, despite having low sequence identity (20%) between each other, crystal structures from the α-NRXN1 LNS2–LNS6 domains reveal high structural homology. The architectural prototype of an LNS domain consists of a β-sandwich — two juxtaposed slightly curved β-sheets, forming a ‘lens-like’ structure (Fig. 2c). Importantly, at the rim of this β-sandwich are calcium and ligand-binding sites. A ligand-binding surface emerges from the folds that connect the two β-sheets and is subject to alteration by AS2, AS3 and AS4, and accordingly is referred to as the hypervariable domain. A one-quarter turn along the rim of the β-sandwich of LNS2 reveals an additional ligand-binding surface that has been linked to behavioural alterations in mice.

Exon inclusion or exclusion of Neurexin pre-mRNA encoding the hypervariable domain alters the length of the folds at the rim, modifies the binding surface topography and regulates associated low-affinity and high-affinity interactions of Neurexins. Interestingly, an analogous surface of LNS-containing proteins agrin and laminin also confers ligand-binding specificity at the mammalian neuromuscular junction.

Extensive studies of the LNS domain of β-NRXN1 (also Nrxn1α LNS6) reveal critical structural elements for binding to Neuroligins (REF. 32–34). The β-loops of the Neurexin hypervariable domain clasp a single calcium ion, creating an electropositive surface for binding to a complementary electronegative surface on Neuroligin. Neurexin ligand selectivity also relies on the accessibility of ligand-binding surfaces, ligand concentration and identity of the splice isoform. This dynamic balance is best documented in the interaction of β-NRXN and Neuroligin (REF. 35). The β-NRXN1, β-NRXN2 and β-NRXN3 bind to all Neuroligins with nanomolar affinity in a splice form-dependent manner. Two general conclusions emerged from these in vitro binding assays: the presence of AS4 insertions in β-NRXN1 and β-NRXN2 diminishes affinity to Neuroligin; and, by contrast, the presence of AS4 insertions in β-NRXN3 increases affinity to Neuroligin. LRRTM1/2 also exhibits AS4 isoform-dependent binding at the same Neuroligin LNS6 site, whereas Neurexins containing the alternative insertions at AS4 bind to CBLN1 (REF. 36,37). These examples highlight the combinatorial and competitive activities of Neurexin–ligand interactions.

In addition to these protein–protein interaction sites, some interactions of Neurexins with ligands involve interactions with carbohydrate moieties on the Neuromuscular. The juxtamembrane region of α, β and γ-Neurexins contains heparan sulfate carbohydrate structures that provide an additional interaction site for postsynaptic ligands such as LRRTMs and Neuroligins (REF. 38,39). LRRTM1/2 and Neuroligins require cooperative binding to Neuroligin LNS6 and the carbohydrate chains for macromolecular complexes, whereas LRRTM3/4 only requires the carbohydrate structures and, thus, can act through γ-Neurexin isoforms that lack LNS domains. Interestingly, the identity of heparan sulfate proteoglycan structures is controlled by a series of cellular enzymes that produce cell-type-specific carbohydrate modifications. Individual glycosyltransferases, sulfotransferases and epimerases have emerged as critical regulators for neuronal development and wiring. Thus, the molecular diversity of Neurexins generated at the level of alternative splicing may be complemented by a ‘glycan code’ generated by differential heparan sulfate modifications.

The larger α-Neurexin isoforms, which are more abundant at the protein level, interact with additional extracellular ligands. Thus, Calcentin 3 and α-Dystroglycan, two postsynaptic proteins at GABAergic synapses, interact only with α-Neurexin but not β-Neurexin isoforms (REF. 40,41). To accommodate the large, α-Neurexin in the narrow synaptic cleft, flexibility in the linker regions connecting the LNS domains bends the large, extracellular domain to fit into the
25-nm synaptic cleft. On the other hand, the short length of \( \beta \)-Neurexin is not constrained by its conformation in the narrow synaptic cleft. Indeed, \( \beta \)-Neurexin and Neuroligin expressed in heterologous cells form a lattice-like sheet spanning the length of cell-to-cell contacts. By contrast, \( \alpha \)-Neurexin–Neuroligin interactions fail to recruit widespread lateral assemblies in this assay. Thus, there might be an isoform-specific constraint on the macromolecular assembly of adhesion complexes within the limits of the synaptic cleft. Moreover, whereas alternative splicing of Neurexin AS2, AS3 or AS4 tunes the affinity of Neurexin to ligands, the splice insertions at AS1, AS5 and AS6 — regions that encode for the linker regions — adjust the inter-domain length (Fig. 2d). Shortening or lengthening of these linker regions constrains the configuration of \( \alpha \)-Neurexin and modulates the exposure of the ligand-binding domains. Ultimately, adjustments in the ligand-binding interface and changes in inter-domain flexibility may govern the high-affinity and low-affinity interactions of Neurexins with their ligands.

**Cell type-specific Neurexin isoforms.** The molecular diversification of the Neurexin transcripts and the selective biochemical interactions of the resulting proteins raise the question of whether these proteins contribute to some form of molecular code that specifies aspects of neuronal wiring. The key elements to consider the coding power of such a system are: the number of distinct recognition tags or ‘senders’ (that is, Neurexin protein isoforms generated); the number of biochemical interaction partners that detect/distinguish or ‘read’ these protein isoforms; and the spatial logic of how such ‘senders’ and ‘readers’ array over neuronal cell types. Quantitative single-molecule sequencing of full-length \( Nrxn1 \) transcripts uncovered a large number of highly represented transcript isoforms in heterogeneous brain tissue but a much more narrow isoform complement in a purified neuronal cell population. Similarly, alternative exon and splice site choices at individual alternatively spliced segments display cell type-specific regulation of individual splicing decisions. For example, the relative abundance of exon usage at several alternatively spliced segments differs between parvalbumin-positive and CCK-positive interneuron populations. Quantitative assessments of the absolute usage of alternative exons uncovered that hippocampal CA3/CA1 pyramidal neurons and parvalbumin-positive interneurons contain different pools of \( Nrxn1 \) AS6 and \( Nrxn2 \) AS2. Hippocampal excitatory neurons contain substantially higher amounts of AS4 (exon lacking) than AS4+ (exon containing) transcript isoforms for all three \( Nrxn \) genes, whereas the higher AS4 inclusion rates can be observed in parvalbumin-positive interneurons. A similar trend is observed for alternative splicing at AS4 of \( Nrxn1 \) in somatostatin-positive interneurons. Conditional ablation of the \( Nrxn1 \) and \( Nrxn3 \) AS4 alternative exons in parvalbumin-positive neurons results in elevated hippocampal network activity and impaired performance of mice in a learning task — a first demonstration that this cell type-specific isoform regulation is indeed essential for circuit function. A recent single-cell study on somatostatin-positive interneurons residing in the stratum oriens of the hippocampus further revealed that neurons with similar electrophysiological properties exhibit similar expression of \( Nrxn1 \) and \( Nrxn3 \) splice isoforms. In aggregate, these studies establish that Neurexin isoform repertoires link to neuronal cell type identity. This raises the questions of how these repertoires are generated, whether they are dynamically regulated and which specific aspect of neuronal function such isoforms instruct.

**Regulation of Neurexin splice isoforms.** Alternative splicing is a highly dynamic process that is guided by cis-acting RNA sequence elements such as donor and acceptor sites, the branch-point sequence and the polypyrimidine tract for spliceosome assembly. Genome-wide screens for transcript isoform alterations in mouse mutants for the RNA-binding proteins NOVA2, PTBP2 and RBFOX1 uncover modifications in \( Nrxn \) transcripts at several segments, but the functional consequences of these alterations are unknown. Probably the best-characterized regulators of Neurexin alternative splicing are members of the STAR (signal transduction activators of RNA) protein family. These proteins are defined by an evolutionarily conserved RNA-binding domain of approximately 200 amino acids. In mice, there are five STAR family proteins: the splicing factors SF1, Quaking and SAM68, and SLM1 and SLM2 — the latter three are closely related paralogs (encoded by the genes \( Khdrbs1 \), \( Khdrbs2 \) and \( Khdrbs3 \)). SAM68, SLM1 and SLM2 directly bind RNA recognition motifs in introns flanking the highly conserved alternative exon at AS4 of the Neurexin pre-mRNAs. This binding promotes skipping of the alternative exon at AS4. Indeed, a close correlation exists between AS4 (exon containing) and AS4+ Neurexin isoforms and the absence and presence, respectively, of SLM1 or SLM2 in neuronal cell types. Alternative splicing regulation at AS4 is particularly interesting as the 30 amino acids encoded by the alternative exon strongly impact affinities for Neurexin ligands. For example, AS4-containing Neurexins (NRXN4AS4+) in cerebellar granule cell axons form tripartite complexes with the extracellular scaffolding protein CBLN1 and the postsynaptic receptor GLUD2 expressed in Purkinje cells. Mutation of any of the three components of this tripartite complex impairs presynaptic differentiation and results in a severe reduction in synapse assembly and density. SLM1 and SLM2 proteins
exhibit highly selective, mutually exclusive expression in neuronal cell types\textsuperscript{80,86,87}. In the mouse hippocampus, SLM2 protein expression is primarily restricted to principal cells of the pyramidal cell layer (CA1, CA2, CA3) and subsets of somatostatin-positive and VIP-positive interneurons. In the SLM2\textsuperscript{KO} hippocampus, there is a highly selective loss of Nrxn1, Nrxn2 and Nrxn3\textsuperscript{AS4–} splice isoforms\textsuperscript{87–89}. Conversely, ectopic expression...
of SLM1 or SLM2 in cells that do not endogenously express either of these proteins results in the generation of AS4 isoforms. Thus, single, cell type-specific RNA-binding proteins selectively instruct alternative splicing at one of the six alternatively spliced segments of Neurexin genes.

Detailed functional analysis of Slm2<sup>−/−</sup> mice, as well as conditional mutations of AS4, uncovered that Neurexin splice variants in hippocampal CA3 pyramidal cells control postsynaptic properties of Schaffer collateral synapses. In Slm2<sup>−/−</sup> hippocampi, macroscopic neuronal morphology and density of Schaffer collateral synapses is normal. However, SLM2-deficiency selectively elevates AMPA-receptor (GluA1) surface expression, leading to increased evoked glutamatergic transmission and impaired Schaffer collateral long-term potentiation (LTP). Selective genetic restoration of the NRXN3<sup>−/−</sup>-splice isoform (which is lost in Slm2<sup>−/−</sup> mice) restores normal levels of GluA1, partially restores LTP and rescues behavioural alterations in Slm2<sup>−/−</sup> mice<sup>97</sup>. A germline mutation in Nrxn3 that constitutively includes the AS4 exon reduces AMPA receptor (AMPA) surface expression and impairs LTP in a subset of subicular neurons<sup>98</sup>. Interestingly, the postsynaptic modifications that result from altered presynaptic Nrxn3 isoforms suggest the disruption of a trans-synaptic link. The exact mechanisms resulting in altered postsynaptic properties remain to be worked out. However, the phenotypes in Slm2<sup>−/−</sup> and Nrxn3<sup>−/−</sup> mice are consistent with shifted ligand affinities of AS4 versus AS4′ splice variants for the interaction with postsynaptic Neuroligins and LRRTMs. Indeed, NRXN3<sup>−/−</sup> isoforms display reduced affinity for postsynaptic Neuroligins<sup>98,99</sup> and Neurexin–Neuroligin1/3 interactions are disrupted in Slm2<sup>−/−</sup> hippocampus<sup>100</sup>. In Nrxn3<sup>−/−</sup> mice, expression of the postsynaptic Nrxn3<sup>−/−</sup>-ligand LRRTM2 is reduced<sup>98</sup>. Given that mutations in these postsynaptic Neurexin ligands likewise disrupt LTP and synaptic transmission<sup>101,102</sup>, their altered interactions with presynaptic Neurexin isoforms may be responsible for aspects of synaptic dysfunction.

Interestingly, selective manipulation of AS4 alternative splice insertions in Nrxn1 and Nrxn3 differentially modifies postsynaptic NMDAR-mediated and AMPAR-mediated transmission<sup>103</sup>. Constitutive misexpression of Nrxn1<sup>−/−</sup> enhances NMDAR-mediated responses at hippocampal CA1–subiculum synapses, whereas Nrxn3<sup>−/−</sup> misexpression suppresses AMPAR-mediated currents<sup>104</sup>. Whether these differential phenotypes are a consequence of different Nrxn1 and Nrxn3 expression levels in CA pyramidal cells or unique properties of the proteins derived from either gene is unknown. However, these findings raise the possibility that AS4 isoforms derived from two Nrxn paralogs may exhibit non-overlapping functions<sup>105</sup>.

In aggregate, these studies demonstrate that cell type-specific RNA-binding proteins drive highly selective regulation of Neurexin alternative splicing. This regulation establishes cell type-specific molecular Neurexin isoform repertoires that engage in trans-synaptic interactions with dedicated receptors in the postsynaptic cell, thereby shaping fundamental synaptic properties. Although this concept is best analysed for the alternative splicing factor SLM2 and the AS4 alternative exons, it may extend to the other Neurexin alternatively spliced segments. At Nrxn1 AS5, there are multiple alternative splice acceptor sites in the downstream exon of the segment (sometimes referred to as exons 25a, 25b and 25c). The amino acids encoded by Nrxn1 exon 25b confer binding of the NRXN3 isoform and, ultimately, contributing to rewiring of the AS4 alternative exons<sup>53</sup>. Trans-acting factors regulating this splicing event may remain to be identified.

Although splicing choices link to cell identity, they also underlie dynamic regulation in response to neuronal signalling. Previous studies demonstrate shifts in alternative splicing regulation by strong pharmacological or electrical stimulation<sup>106,107</sup>. In Neurexins, such paradigms shift alternative exon incorporation at several alternatively spliced segments<sup>106,108</sup>. In the mouse cerebellum, this phenomenon requires calcium influx, calmodulin-dependent kinase IV and the broadly expressed STAR protein, SAM68<sup>109</sup>. In granule cells of the mouse dentate gyrus, recall of a contextual fear memory triggers the inclusion of the alternative exon at Nrxn1<sup>110</sup>. Interestingly, this shift in alternative splicing requires HDAC2 and is controlled by a selective histone modification (H3K9me3) in the Nrxn1 gene. This modification is thought to control memory stability, by temporarily shifting Nrxn1 alternative splicing at AS4 and, ultimately, contributing to rewiring of dentate granule cell synapses to support learning<sup>99</sup>. Yet another level of regulation is the proteolytic cleavage of Neurexins, resulting in shedding of the extracellular domain of the protein<sup>105,107,108</sup>. The physiological contexts and functional relevance of these modes of regulation remain to be explored. However, these mechanistically diverse modes of transcriptional, post-transcriptional and proteolytic regulation highlight the perplexing complexity of Neurexin cell biology and pose challenges...
for linking cell type-specific repertoires to synaptic function.

**Cell type logic of splicing regulators.** Recent genomewide studies on mRNA transcript isoforms and targets of RNA-binding proteins provide insight into the complex logic of neuronal cell type-specific alternative splicing. Several alternative transcript programmes are linked to neuronal cell type, and some alternative splicing regulators increase in expression upon the commitment to a postmitotic fate. Examining RNA-binding protein expression across neuronal cell types highlights a remarkable range of cell type selectivity: some splicing regulators are expressed in essentially all neurons, and many of them are ‘neuron-specific’ — that is, largely not expressed in non-neuronal cells. These broadly expressed splicing regulators include the widely studied NOVA proteins, PTBPs or RBFOX proteins. Other splicing regulators such as SLM1 and SLM2 exhibit a much more selective expression in a subset of neuronal cell types (Fig. 3b). Intriguingly, the selectively expressed RNA-binding protein SLM2 controls alternative splicing choices of only a few genes, acting as a highly targeted regulator of a small number of synaptic proteins. Both SAM68 and SLM2 bind the same consensus motif; however, SLM2 but not SAM68 regulates alternative splicing of Nrxn2 at AS4 in vivo. This selective activity is dependent on the abundance of binding sites flanking the alternative exon. Furthermore, the specificity of RNA recognition by SLM2 and SAM68 may be modified by their ability to homodimerize and heterodimerize. Thus, these different modes of action provide additional flexibility for the STAR family of proteins to generate neuronal cell class–specific synaptic properties (X1 + X3 in Fig. 3b). By contrast, broadly expressed RNA-binding proteins tend to regulate alternative splicing events in mRNAs from hundreds of genes, possibly generating cell type-specific outcomes by coordinate and/or competitive action of multiple trans-acting factors on a single RNA segment. For example, NOVA2-dependent intron retention in hundreds of transcripts sequesters the trans-acting splicing factor PTBP2 (Ref. 79). Remarkably, NOVA2 regulates diverse target transcripts in different cell populations, demonstrating that selectivity can emerge from a complex, cell type-specific interplay of splicing regulators. Moreover,
Presynaptic neuron

Postsynaptic neuron

Trans-synaptic signalling modules

Cell type 1

Cell type 2

Cell type 3

Cell type 4

Senders and readers

Synaptic properties

Y1

Y2

Y3

Yn

Pyramidal cell

Synapse 1

Single dominant channel

Wild type

Loss of function

Synapse 2

Three major channels

Wild type

Loss of function

Adhesion

Vesicle recruitment

Ca\(^{2+}\) channel function

Transmitter receptor stabilization

Adhesion

Scaffolds

GPCR function

Adhesion

Vesicle recruitment

Ca\(^{2+}\) channel function

Transmitter receptor stabilization

Adhesion

Scaffolds
NRXN–Cerebellin 1 (CBLN1)–GLUD2 module example for this would be parallel fibre synapses in the cerebellum that rely on the contribution to synapse assembly, stability and functional properties (‘Synapse 1’ — an example for this would be parallel fibre synapses in the cerebellum that rely on the NRXN–Cerebellin 1 (CBLN1)–GLUD2 module).

- **c** | Synapses across the CNS employ various numbers of trans-synaptic modules that can be viewed as trans-synaptic communication channels (displayed in different colours). Some synapses rely on a single dominant channel (here depicted in red), which has a major contribution to synapse assembly, stability and functional properties (‘Synapse 1’ — an example for this would be parallel fibre synapses in the cerebellum that rely on the NRXN–Cerebellin 1 (CBLN1)–GLUD2 module).

- **d** | Other synapses (‘Synapse 2’) contain multiple prominent trans-synaptic modules (here depicted in red, green, and purple) — likely to afford a larger dynamic range of plasticity. These modules drive overlapping elements of synaptic differentiation. For example, the green module drives bidirectional adhesion, presynaptic vesicle recruitment, presynaptic G protein-coupled receptor (GPCR) function and postsynaptic stabilization of neurotransmitter receptors, whereas the purple module controls adhesion, active zone assembly and calcium channel function. Loss of a single presynaptic sender (the purple module) results in loss of presynaptic calcium channel function, but active zone assembly and adhesion are maintained by the overlapping red and green modules at this synapse. See references for examples on Ca\(^{2+}\) channel function.

**Circuits and disorders.** From human genetic studies, the Neurexins, and predominantly NRXN1, emerge as significant risk genes for a wide range of neurodevelopmental, psychiatric, neurological and neuropsychological phenotypes. Likewise, Neurexin gene mutations are associated with schizophrenia, autism, Tourette syndrome, nicotine dependence, developmental delay, dysmorphic features and infantile epileptic encephalopathy. Many of these deletions span promoters and initial exons of the NRXN1 gene (2p16.3). Transcriptomic studies in induced pluripotent stem cell-derived neurons carrying such mutations uncover significantly reduced NRXN1a transcripts. Moreover, de novo expression of isoforms divergent from the repertoire in neurotypical controls might occur.

Heterozygous exonic deletions do not appear to be fully penetrant as rearrangements in the NRXN1 gene are frequent in the control population, and NRXN1 deletions are frequently inherited from a healthy parent. However, such mono-allelic NRXN1 deletion carriers may share common alterations in anxiety, intelligence and impulsivity, which go undiagnosed without an in-depth evaluation. Notably, a small number of biallelic NRXN1 mutations result in a severe mental retardation syndrome, which phenotypically overlaps with Pitt–Hopkins syndrome, an autism-like developmental disorder with variable characteristics.

In some cases, specific NRXN1 sequence variants may elevate risk to certain disorders. However, considering the wide range of neurodevelopmental conditions observed in individuals with NRXN mutations, it is more likely that alterations in NRXN gene expression alter neurodevelopmental trajectories that — depending on the genetic background and environmental conditions — precipitate diverse phenotypes. It is widely appreciated that many symptoms are comorbid with multiple neurodevelopmental disorders, such as attention-deficit/hyperactivity, tic disorder, developmental coordination disorder and autism. For clinical evaluations, it is encouraged — particularly for young children — to focus on impairments in specific domains, such as communication and language, motor coordination, attention, mood and sleep, rather than to separate patients into discrete disorders. This is conceptualized as ‘ESSENCE’ (early symptomatic syndromes eliciting neurodevelopmental clinical examinations).

The significant disease association with the human NRXN genes has spurred efforts to obtain insights into how Neurexin mutations impact neuronal circuits and behaviour. It should be noted that some components of the Neurexin adhesion systems are also expressed in non-neuronal cells (Box 1). However, studies modelling the impact of disease-associated NRXN mutations have largely focused on synaptic phenotypes. Invertebrate systems have provided important opportunities for probing common cellular nodes modified by various selective alternative splicing decisions also arise from histone modifications and alterations in transcriptional kinetics.
risk gene mutations\textsuperscript{118,120}. In mice, global \textit{Nrxn1\alpha} knock-out results in multiple behavioural alterations. These include impaired nest building, decreases in prepulse inhibition of startle responses and an improvement in motor learning\textsuperscript{121}. Interestingly, some phenotypes are sex-specific: male homozygous \textit{Nrxn1\alpha} knockout mice exhibit increased aggressive behaviours. Male heterozygous \textit{Nrxn1\alpha} knockout mice show increased novelty responses as assessed by locomotor activity in a new environment and enhanced habituation upon repeated exposure to this environment\textsuperscript{122}. In rats, non-social deficits, such as hyperactivity and deficits in instrumental and spatial learning tasks, result from \textit{Nrxn1\alpha} deficiency\textsuperscript{123}. \textit{Nrxn2\alpha} homozygous and heterozygous knockout mice exhibit diminished social approach and social novelty responses in behavioural assays but also impaired recognition of novel objects\textsuperscript{124-126}, indicating social and broader cognitive deficits.

Considering that these behavioural observations were made in global, constitutive knockout mice, linking such phenotypes to selective developmental, circuit and synaptic functions of the Neurexin proteins is difficult. Reversible overexpression studies using dominant-negative mutant \textit{Nrxn1} support the notion that behavioural phenotypes may result from dysfunction, rather than irreversible mis-wiring of circuits during development\textsuperscript{127}. For \textit{Nrxn3}, a requirement for Neurexin function in somatostatin interneurons in the anterior cingulate cortex affects empathy in conditional mutant mice. Empathy is a key element of social interactions, and the loss of empathy is an important feature of autism spectrum disorders and psychiatric conditions\textsuperscript{128}. Mice carrying a single-nucleotide polymorphism in \textit{Nrxn3}, which results in a single amino acid change (R498W), increase observational fear in a behavioural task\textsuperscript{129}. In this task, an observer mouse adopts a conditioned context-dependent freezing response after observing a second mouse receiving repetitive foot shocks. Given that human performance in a similar paradigm correlates with metrics of empathy, this task is thought to assess an evolutionarily conserved aspect of empathy\textsuperscript{130}. Conditional deletion of \textit{Nrxn3} in somatostatin-positive interneurons of the anterior cingulate cortex impairs synaptic transmission from these GABAergic neurons and elevates freezing responses, whereas activation of the same neuronal population suppresses them\textsuperscript{131}. The R498W variant maps to the third LNS domain of \textit{Nrxn3\alpha} (see Fig. 2c). This region may confer a Ca\textsuperscript{2+}-mediated conformational switch for ligand binding\textsuperscript{46,64}. However, ligands that contribute to the differential function of the NRXN3 R498W protein remain to be uncovered. Nevertheless, these studies illustrate that Neurexin functions — and likely, specifically, the synaptic recognition codes controlled by the Neurexins — are not a cell biological detail but are fundamental for nervous system operation, behaviour and neurodevelopmental disorders.

\textit{Framework for synaptic action modules.} A particular challenge in defining cellular Neurexin functions and predicting the impact of mutations on neuronal circuit function arises from extensive multiplexing at the biochemical level. A single presynaptic Neurexin isoform can recruit fundamentally different postsynaptic ligands. At a single synapse, multiple ligands compete for interaction with a limited pool of Neurexin molecules. Moreover, numerous Neurexin paralogs cooperate with various additional, independent trans-synaptic systems localized at the same synaptic contact. This complexity demands significant caution when interpreting loss-of-function studies. As discussed above, the same Neurexin loss-of-function manipulation applied in different cellular contexts results in widely differing phenotypes, ranging from a substantial loss of synaptic structures and entire axonal branches to the comparably subtle impairment of one or multiple synaptic ion channels. Such context-dependent synaptic phenotypes are not unique to the Neurexin gene family and have been reported for other trans-synaptic adhesion systems such as the type III mGluR–Elfn\textsuperscript{135-138} or receptor protein tyrosine phosphatase complexes\textsuperscript{134,135}.

We propose that the combinatorial actions of synaptic adhesion and signalling proteins (Neurexins and other protein families) can be rationalized as modules for nucleating synaptic structures, scaffolding proteins and ion channels. Multiple modules can be present at single synapses and contain overlapping components\textsuperscript{136-138} (Fig. 4). These trans-synaptic recognition and synapse-organizing systems can be conceptualized as senders and readers across neuronal populations (Fig. 5a). Upon fate specification, each neuronal cell type contains a set of cues — or a molecular code — that is integral to its neuronal identity. We postulate that this code instructs, but also constrains, cellular interactions, and thereby directs aspects of neuronal wiring and plasticity, thus maintaining cell type-specific properties and circuit function (Fig. 5b). Importantly, the messages conveyed by a particular sender (for example, a specific Neurexin isoform) are strongly context-dependent. Thus, the nucleation of a trans-synaptic module largely depends on the molecular repertoire of readers available (Fig. 5a) and may even rely on certain extracellular proteins being absent from a particular synaptic site. A second critical parameter is the number of trans-synaptic communication channels. Some synapses with little demand for plasticity and extensive neuromodulation may heavily rely on a few trans-synaptic channels, or even just a single dominant sender–reader pair (Fig. 5c). In such cases, loss of any of the core components results in a substantial dissociation of synaptic contacts, for example loss of the Neurexin–CBLN1 link at cerebellar parallel fibre synapses\textsuperscript{46,485} or the Elfn–mGluR6 link in photoreceptor synapses\textsuperscript{137}. At synapses with multiple prominent trans-synaptic channels, the same mutation may modestly destabilize a particular neurotransmitter receptor recruited by the sender or reader — however, a second trans-synaptic channel would take over additional functions and maintain the overall structural integrity of the synapse (Fig. 5d).

Such complex systems likely evolved for CNS synaptogenesis as they render a synaptic contact more tunable, providing a high degree of freedom to control plasticity of individual synaptic sites — but, at the same time, they provide constraints for wiring in highly complex circuits.
By integrating the observations made in reductionist biochemical and in vitro systems, across vertebrate and invertebrate systems, and from loss-of-function studies in multiple cell types, we can define action modules for synaptic adhesion molecules. A key question for the future will be to explore how molecular codes and activity-dependent mechanisms interseck to shape circuitry during development. There is mounting evidence that synaptic transmission per se is not required for a significant degree of neuronal wiring and cell type-specific connectivity. Thus, activity-independent mechanisms generate an initial blueprint of neuronal circuits. However, within one molecularly and anatomically recognizable cell type, subpopulations of cells are recruited to represent or encode unique aspects of the external world, such as direction-selective cells in the visual cortex, ‘reward’ cells in the cerebellum or place cells in the hippocampus. Neuronal activity and synaptic plasticity mechanisms play a major role in establishing such neuronal ensembles — and future work may elucidate how molecular recognition systems constrain and execute such key steps of circuit assembly.

Published online 8 January 2021
The molecular diversity of glycosaminoglycans shapes animal development. Annu. Rev. Cell Dev. Biol. 22, 575–607 (2006).

118. Sugita, S. et al. A stomatocystic complex of Neurexins and dystroglycan in brain. J. Cell Biol. 156, 445–454 (2001).

119. Chen, F., Venugopal, V., Murray, B. & Rudenko, G. The carbohydrate modifications to the assembly of postsynaptic receptor GLUD2. Nucleic Acids Res. 42, 7816–7829 (2014).

120. Roppongi, R. T. et al. LRRTMs organize synapses cell type-specific alternative splicing is regulated by highly dedicated alternative splicing program. Science 332, 982–986 (2011).

121. Kornblihtt, A. R. Transcriptional control of alternative splicing of Neurexin II results in behaviors relevant to autism spectrum disorders. Mol. Cell. Neurosci. 752–767 (2016).

122. T raunmüller, L., Bornmann, C. & Scheiffele, P. Control of neuronal synapse specification by highly dedicated alternative splicing program. Science 352, 982–986 (2011).

123. Xie, J. & Black, D. L. A CaMK IV responsive RNA element mediates depolarization-induced alternative splicing of ion channels. Nature 410, 936–939 (2001).

124. Rozo-Kotlirov, G. & Zisapel, N. Ca2+ influx triggers alternative splicing of presynaptic Neuroplaxin. Nature Neurosci. 7, 521–531 (2004).

125. Chen, M. & dimerization by the STAR proteins T-STAR and TPRM2. Neuron 70, 145–161 (2011).

126. Xue, F., Vaugnet, D., Lecarpentier, Y., Stoss, O. et al. p59fyn- mediated phosphorylation regulates the activity of the tissue-specific splicing factor rSLM-1. Mol. Cell. Neurosci. 27, 3–12 (2004).

127. Traummler, L., Bornmann, C. & Scheiffele, P. Alternative splicing coupled nonsense-mediated decay generates neuronal cell type-specific expression of SL1 proteins. Neuron 34, 1675–1676 (2001).

128. Ehrmann, I. et al. The tissue-specific RNA binding protein TSTAR controls regional splicing patterns of Neuroplaxin in the brain. PLoS Genet. 9, e1003474 (2013).

129. Traummler, L., Bornmann, C. & Scheiffele, P. Presynaptic Neuroplaxin-3 alternative splicing trans-synaptically controls postsynaptic AMPA receptor trafficking. Cell 154, 75–88 (2013).

130. Together with Traummler et al. (2016) this study demonstrates a major impact of Neuroplaxin alternative splicing on glutamatergic synaptic function.

131. Chubyrkina, A. A. A functional validation of excitatory versus inhibitory synapses by Neuroplaxin-1 versus Neuroplaxin-2. J. Neurochem. 54, 919–931 (2000).

132. Chubyrkina, A. A. and dimerization by the STAR proteins T-STAR and TPRM2. Neuron 70, 145–161 (2011).

133. Xue, F., Vaugnet, D., Lecarpentier, Y., Stoss, O. et al. p59fyn- mediated phosphorylation regulates the activity of the tissue-specific splicing factor rSLM-1. Mol. Cell. Neurosci. 27, 3–12 (2004).

134. Traummler, L., Bornmann, C. & Scheiffele, P. Alternative splicing coupled nonsense-mediated decay generates neuronal cell type-specific expression of SL1 proteins. Neuron 34, 1675–1676 (2001).

135. Ehrmann, I. et al. The tissue-specific RNA binding protein TSTAR controls regional splicing patterns of Neuroplaxin in the brain. PLoS Genet. 9, e1003474 (2013).

136. Traummler, L., Bornmann, C. & Scheiffele, P. Presynaptic Neuroplaxin-3 alternative splicing trans-synaptically controls postsynaptic AMPA receptor trafficking. Cell 154, 75–88 (2013).

137. Together with Traummler et al. (2016) this study demonstrates a major impact of Neuroplaxin alternative splicing on glutamatergic synaptic function.

138. Chubyrkina, A. A. A functional validation of excitatory versus inhibitory synapses by Neuroplaxin-1 versus Neuroplaxin-2. J. Neurochem. 54, 919–931 (2000).

139. Chubyrkina, A. A. and dimerization by the STAR proteins T-STAR and TPRM2. Neuron 70, 145–161 (2011).

140. Xue, F., Vaugnet, D., Lecarpentier, Y., Stoss, O. et al. p59fyn- mediated phosphorylation regulates the activity of the tissue-specific splicing factor rSLM-1. Mol. Cell. Neurosci. 27, 3–12 (2004).

141. Traummler, L., Bornmann, C. & Scheiffele, P. Alternative splicing coupled nonsense-mediated decay generates neuronal cell type-specific expression of SL1 proteins. Neuron 34, 1675–1676 (2001).

142. Ehrmann, I. et al. The tissue-specific RNA binding protein TSTAR controls regional splicing patterns of Neuroplaxin in the brain. PLoS Genet. 9, e1003474 (2013).

143. Traummler, L., Bornmann, C. & Scheiffele, P. Presynaptic Neuroplaxin-3 alternative splicing trans-synaptically controls postsynaptic AMPA receptor trafficking. Cell 154, 75–88 (2013).

144. Together with Traummler et al. (2016) this study demonstrates a major impact of Neuroplaxin alternative splicing on glutamatergic synaptic function.

145. Chubyrkina, A. A. A functional validation of excitatory versus inhibitory synapses by Neuroplaxin-1 versus Neuroplaxin-2. J. Neurochem. 54, 919–931 (2000).

146. Chubyrkina, A. A. and dimerization by the STAR proteins T-STAR and TPRM2. Neuron 70, 145–161 (2011).

147. Xue, F., Vaugnet, D., Lecarpentier, Y., Stoss, O. et al. p59fyn- mediated phosphorylation regulates the activity of the tissue-specific splicing factor rSLM-1. Mol. Cell. Neurosci. 27, 3–12 (2004).

148. Traummler, L., Bornmann, C. & Scheiffele, P. Alternative splicing coupled nonsense-mediated decay generates neuronal cell type-specific expression of SL1 proteins. Neuron 34, 1675–1676 (2001).

149. Ehrmann, I. et al. The tissue-specific RNA binding protein TSTAR controls regional splicing patterns of Neuroplaxin in the brain. PLoS Genet. 9, e1003474 (2013).

150. Traummler, L., Bornmann, C. & Scheiffele, P. Presynaptic Neuroplaxin-3 alternative splicing trans-synaptically controls postsynaptic AMPA receptor trafficking. Cell 154, 75–88 (2013).

151. Together with Traummler et al. (2016) this study demonstrates a major impact of Neuroplaxin alternative splicing on glutamatergic synaptic function.

152. Chubyrkina, A. A. A functional validation of excitatory versus inhibitory synapses by Neuroplaxin-1 versus Neuroplaxin-2. J. Neurochem. 54, 919–931 (2000).

153. Chubyrkina, A. A. and dimerization by the STAR proteins T-STAR and TPRM2. Neuron 70, 145–161 (2011).

154. Xue, F., Vaugnet, D., Lecarpentier, Y., Stoss, O. et al. p59fyn- mediated phosphorylation regulates the activity of the tissue-specific splicing factor rSLM-1. Mol. Cell. Neurosci. 27, 3–12 (2004).

155. Traummler, L., Bornmann, C. & Scheiffele, P. Alternative splicing coupled nonsense-mediated decay generates neuronal cell type-specific expression of SL1 proteins. Neuron 34, 1675–1676 (2001).

156. Ehrmann, I. et al. The tissue-specific RNA binding protein TSTAR controls regional splicing patterns of Neuroplaxin in the brain. PLoS Genet. 9, e1003474 (2013).

157. Traummler, L., Bornmann, C. & Scheiffele, P. Presynaptic Neuroplaxin-3 alternative splicing trans-synaptically controls postsynaptic AMPA receptor trafficking. Cell 154, 75–88 (2013).

158. Together with Traummler et al. (2016) this study demonstrates a major impact of Neuroplaxin alternative splicing on glutamatergic synaptic function.
130. Panksepp, J. & Panksepp, J. B. Toward a cross-species understanding of empathy. Trends Neurosci. 36, 489–496 (2013).
131. Cao, Y. et al. Mechanism for selective synaptic wiring of rod photoreceptors into the retinal circuitry and its role in vision. Neuron 87, 1244–1260 (2015).
132. Sylwestrak, E. L. & Ghosh, A. Elfn1 regulates target-specific release probability at CA1–interneuron synapses. Science 358, 556–560 (2012).
133. Tomioka, N. H. et al. Elfn1 recruits presynaptic mGlur7 in trans and its loss results in seizures. Nat. Commun. 5, 4501 (2014).
134. Park, H. et al. Splice-dependent trans-synaptic PTPα–IL1RAPL1 interaction regulates synapse formation and non-REM sleep. EMBO J. 39, e104150 (2020). This work uncovers a key role for an alternative exon in the regulation of trans-synaptic interactions, synaptic transmission and mouse behaviour.
135. Kim, K. et al. Presynaptic PTPα regulates postsynaptic NMDA receptor function through direct adhesion-independent mechanisms. eLife 9, e54224 (2020).
136. Shen, K. & Scheiffele, P. Genetics and cell biology of building specific synaptic connectivity. Annu. Rev. Neurosci. 33, 473–507 (2010).
137. Shen, K. & Scheiffele, P. Multiple conserved cell adhesion protein interactions mediate neural wiring of a sensory circuit in C. elegans. eLife 6, e29257 (2017).
138. Schroeder, A. et al. A modular organization of LRR protein-mediated synaptic adhesion defines synapse identity. Neuron 99, 529–546.e7 (2018).
139. Yamagata, A. et al. Structural insights into modulation and selectivity of transsynaptic Neurexin–LRRTM interaction. Nat. Commun. 9, 5964 (2018).
140. Ibata, K. et al. Activity-dependent secretion of synaptic organizer Cbln1 from lysosomes in granule cell axons. Neuron 102, 1184–1198.10 (2019).
141. Ito-Ishida, A. et al. Presynaptically released Cbln1 induces dynamic axonal structural changes by interacting with GuD2 during cerebellar synapse formation. Dev. Cell 23, 923–924 (2012). Together with Matsuda et al. (2010), this study demonstrates critical in vivo functions of the Neurexin–Cerebellin–GLUD2 complex in cerebellar synapse formation and maintenance.
142. Brockhaus, J. et al. a-Neurexins together with a26-1 auxiliary subunits regulate Ca2+ influx through Ca,2.1 channels. J. Neurosci. 38, 8377–8394 (2018).
143. Tong, X. J. et al. Retrograde synaptic inhibition is mediated by α-Neurexin binding to the α5β2 subunits of N-type calcium channels. Neuron 95, 526–540.e5 (2017).
144. Hrvatin, S. et al. Single-cell analysis of experience-dependent transcriptomic states in the mouse visual cortex. Nat. Neurosci. 21, 120–128 (2018).
145. Uchigashima, M., Cheung, A., Suh, J., Watanabe, M. & Futah, K. Differential expression of Neurexin genes in the mouse brain. J. Comp. Neurol. 527, 1940–1965 (2019).
146. Venkatesh, H. S. et al. Targeting neuronal activity-regulated neuroligin-3 dependency in high-grade glioma. Nature 549, 535–537 (2017).
147. Venkataramani, V. et al. Glutamatergic synaptic input to glioma cells drives brain tumour progression. Nature 573, 552–558 (2019).
148. Zeng, Q. et al. Synaptic proximity enables NMDAR signalling to promote brain metastasis. Nature 573, 526–531 (2019).
149. Stogsdill, J. A. et al. Astrocytic neuroligins control astrocyte morphology and synaptogenesis. Nature 551, 192–197 (2017).
150. Singh, S. K. et al. Astrocytes assemble thalamocortical synapses by bridging NR1α and NLI via Hevin. Cell 164, 185–196 (2016).

151. Xu, J., Xiao, N. & Xia, J. Thrombospondin 1 accelerates synaptogenesis in hippocampal neurons through neurolgin 1. Nat. Neurosci. 13, 22–24 (2009).

Acknowledgements
A.M.G. was financially supported by an advanced European Molecular Biology Organization (EMBO) long-term fellowship. L.T. was supported by the Boehringer Ingelheim Fonds and the Doris Dietzsch and Denise Dietzsch-Frick-Stiftung. Work in the laboratory of P.S. is supported by the Swiss National Science Foundation, a European Research Council Advanced Grant (SPLICECODE), and EU-AIMS and AIMS-2-TRIALS supported by the Innovative Medicines Initiatives from the European Commission. This joint undertaking receives support from the European Union’s Horizon 2020 research and innovation programme, the European Federation of Pharmaceutical Industries and Associates (EFPIA), Autism Speaks, Autistica and the Simons Foundation Autism Research Initiative (SFARI).

Author contributions
All authors contributed equally to the manuscript.

Competing interests
The authors declare no competing interests.

Peer review information
Nature Reviews Neuroscience thanks Michael Hart, who co-reviewed with Mara Cowen, Robin Hiesinger and the other anonymous reviewer for their contribution to the peer review of this work.

Publisher’s note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

© Springer Nature Limited 2021