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Neurexins: Molecular Codes for Shaping Neuronal Synapses

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Preface

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 are instructed by molecular codes resulting from transcriptional programs. Recent studies on the Neurexin protein family and its ligands provide fundamental insight into how synapses are assembled and remodeled, how synaptic properties are specified, and how single gene mutations associated with neurodevelopmental and psychiatric disorders might modify the operation of neuronal circuits and behavior. In this review, we first summarize insights into Neurexin function obtained from various model organisms. We then discuss 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- and synapse-specific functional properties.

Introduction

Nervous systems represent remarkable examples of a highly organized tissue with an abundance of specialized cells joint into an intricate structure. During development, neuronal connectivity arises from a series of steps, including cell specification, migration, targeted growth, synapse formation, and remodeling. Spontaneous activity and sensory experience propagated 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 neurotransmission [1-3]. Thus, genetically encoded programs are thought to orchestrate key aspects of the timing and dynamics of neuronal growth and nervous system wiring [4-7]. Cell surface adhesion and signaling 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 is integral to its developmental specification. While signaling 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 specificity [7,8]. 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 Neurexins, one class of cell adhesion

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molecules, to illustrate 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 pre- or postsynaptic assemblies [9-12] . For example, postsynaptic adhesion molecules of the Neuroligin family trigger the assembly of functional presynaptic terminals in axons through interaction with their receptor Neurexin [9,10] (Fig. 1a). Conversely, Neurexin-mediated clustering of Neuroligins triggers the recruitment of NMDA-type glutamate receptors and scaffolding molecules [12,13] . 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 (LRRTM1,2,3,4), and Calsyntenin-3 [14-17] . Recent reviews provided a comprehensive summary of this array of Neurexin ligands [6,18] . 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 release [19,20] . 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 knock-out mice uncovered that inhibitory synapse density in the brainstem is reduced by 50%, whereas the density of excitatory synapses is unchanged at birth [19] . In the nematode *Caenorhabditis elegans*, loss of one particular Neurexin isoform, γ-Neurexin, diminishes synapse numbers in the DA9 motoneuron supporting critical functions for this form in synapse formation [21] . A broader *nrx-1* mutation results in a loss of postsynaptic neurotransmitter receptors from

synapses and loss of spine-like protrusions from the postsynaptic neuron [22,23] . In another class of *C.elegans* neurons that display experience-dependent and sexually dimorphic plasticity, synapse rearrangements are impaired in *nrx-1* mutants [24] . In the fruit fly *Drosophila melanogaster,* mutations in Neurexin and its ligand Neuroligin, result in severe loss of neuromuscular synaptic release sites in larvae [25-27] (Fig.1c). Importantly, impairing Neurexin- Neuroligin adhesion also modifies the growth of axonal and dendritic arbors in some model organisms. Timelapse imaging experiments in developing tadpoles suggest that adhesion through Neurexin and its ligand, Neuroligin, confer transient morphological stabilization of dendritic contacts [28] . Similarly, the growth and arborization of *Drosophila* motoneuron axons during metamorphosis is disrupted in Neurexin-deficient fruit flies (Fig.1d) [29] . While 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. While work in invertebrate model systems has long explored synapse formation between genetically-defined cell types, this approach has only been implemented in mammalian systems this past decade. Second, unlike the vertebrate neuromuscular junction, where synaptic differentiation relies heavily on one primary signaling system [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 *Caenorhabditis elegans nrx-1* severely disrupts AChR clusters in DD GABA neurons but not in muscle [23] . In mice, the conditional ablation of all Neurexin isoforms in somatostatin-versus parvalbumin-positive interneurons exhibit 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 [31] . However, somatostatin-positive interneurons show altered voltage-gated calcium channel function and defects in neurotransmitter release [31] . 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 paralogues. Second, there is an array of additional presynaptic receptors unrelated to Neurexins that contribute to synapse assembly [32,33] . Third, different neuronal cell types express different Neurexin isoforms, generated from alternative promoters (like the α, β, γ forms) and modified through extensive alternative splicing. Notably, such Neurexin isoforms differentially interact with selective synaptic ligands. Thus, deletions 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 posttranslational modifications, impart Neurexins with numerous adhesive motifs that underlie low- 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 Neurexin1, -2, and -3 (1.0, 0.1, 1.6 Mb in mice; 1.1,0.1,1.8 Mb in human). Invertebrates, such as *Caenorhabditis elegans* and *Drosophila melanogaster*, possess a single Neurexin ortholog [34,35] . Each mammalian Neurexin gene contains two promoters that produce a long, α- and a shorter, β-Neurexin pre-mRNA, which encode proteins of approximately 1500 and 450 amino acids (Fig. 2a). For mouse *Nrxn1* an additional, very short γ-isoform is generated from a third, internal promoter [36] , and an orthologous γ-isoform is reported in *Caenorhabditis elegans* [21] . We refer to these transcripts (irrespective of their further modification by alternative splicing as “primary Neurexin transcripts”). Differential usage of α-, β-, γ-promoters in the three mammalian Neurexin paralogues drive highly divergent levels of the primary Neurexin transcripts across neuronal cell types. For example, mouse hippocampal CA3 pyramidal neurons express high levels of *Nrxn1β*, whereas the same transcript is very low in CA1 pyramidal cells [37-39] . In the mouse neocortex, GABAergic SST-interneurons express threefold higher levels of *Nrxn3α* transcripts as compared to layer 4 pyramidal cells [39] .

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 (AS1-6) - 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, singlemolecule PacBio sequencing studies experimentally confirmed the presence of hundreds of Neurexin transcript isoforms in the mouse brain [40,41] . Interestingly, the relative usage and combination of alternative splice insertions are conserved between rodents and humans, evidenced by the analysis of postmortem human brain samples and hIPSC-derived neuronal preparations [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 [40,43] . Advances in targeted proteomics should clarify the accurate relative and absolute quantification of protein abundance, even for peptides derived from specific splice insertions [44] .

Structure of macromolecular assemblies

The defining feature of all Neurexins (except for the non-canonical NRXN1γ) is the presence of extracellular Laminin Neurexin Sex hormone-binding globulin (LNS) domains. α-Neurexins present six LNS domains interspersed by single EGF domains in the extracellular region (Fig.2b). These alternating repeats tether to the cell surface via a rigid

and extensively O-linked glycosylated stalk and a single transmembrane domain [45,46] . The short intracellular tail contains interaction sites for cytoskeletal adaptors (Protein 4.1) and a C-terminal PDZ-binding motif [47] . The smaller β-NRXN proteins have a short, unique N-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 [36] .

Remarkably, despite having low sequence identity (20%) between each other, crystal structures from α-Neurexin1 LNS domains 2-6 reveal high structural homology [48] . The architectural prototype of an LNS domain consists of a β sandwich – two slightly curved β sheets juxtaposed, 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 [49] . A one-quarter turn along the rim of the β-sandwich of LNS2 reveals an additional ligand-binding surface [50] . 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 assorted low- 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 [51] .

Extensive studies of the LNS domain of β-NRXN1 (also NRXN1α LNS6) reveal critical structural elements for binding to Neuroligins [52-56] . The β loops of the Neurexin hypervariable domain clasp a single calcium ion creating an electropositive surface for binding to a complementary electro-negative 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 [57,58] . β-NRXN1,-2, and -3 bind to all Neuroligins with nanomolar affinity in a splice form-dependent manner [58] . Two general conclusions emerged from these in vitro binding assays: (1) the presence of AS4 insertions in β-NRXN1, and -2 diminishes affinity to Neuroligin; (2) 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 Neurexin LNS6 site, whereas Neurexins containing the alternative insertions at AS4 bind to CBLN [15,59] . 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 Neurexins. The juxtamembrane region of α-, β-, and γ-Neurexins contain heparan sulfate carbohydrate structures that provide an additional interaction site for postsynaptic ligands such as LRRTMs and Neuroligins (Fig.2) [60,61] . LRRTM1/2 and Neuroligins require cooperative binding to Neurexin LNS6 and the carbohydrate chains for macromolecular assembly, whereas LRRTM3/4 only requires the carbohydrate structures and thus can act through γ- Neurexin isoforms which 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 [62] . 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 [44] , interact with additional extracellular ligands. Thus, Calsyntenin-3 and α-Dystroglycan, two postsynaptic proteins at GABAergic synapses, interact only with α- but not β-Neurexin isoforms [16,63] . To accommodate the large, α-Neurexin in the narrow synaptic cleft, flexibility in the linker regions connecting the LNS domains bend the large, extracellular domain to fit in the 25nm synaptic cleft [46,48,64] . On the other hand, the short length of β-Neurexin is not constrained by its confirmation by the narrow synaptic cleft. Indeed, β-Neurexin and Neuroligin expressed in heterologous cells form a lattice-like sheet spanning the length of cell-to-cell contacts. By contrast, α-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 [65] . Moreover, while 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 interdomain length (Fig.2d) [40,48] . Shortening or lengthening of these linker regions constrains the configuration of α-Neurexin and modulates the exposure of the ligand-binding domains. Ultimately, adjustments in the ligand-binding interface and changes in interdomain flexibility may govern the high 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 raises 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: (1) the number of distinct recognition tags or "senders" (i.e., Neurexin protein isoforms generated), (2) the number of biochemical interaction partners that detect/distinguish or "read" these protein isoforms, and (3) the spatial logic of how such tags 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 heterogenous brain tissue but a much more narrow isoform complement in a purified neuronal cell population [40] . 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- and CCK-positive interneuron populations [39,66,67] . Moreover, differential usage of combinatorial alternative splicing profiles of *Nrxn1* and *-3* or alternative splicing at AS3, correlate with the developmental origin of interneurons [67] . 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 [37] . A similar trend is observed for alternative splicing at AS4 of *Nrxn3* in somatostatin-positive interneurons [37,68] . Conditional ablation of the *Nrxn1* and *3 AS4* alternative exons in parvalbumin-positive neurons results in elevated hippocampal network activity, and impaired performance in a learning task [37] – 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 *3* splice isoforms [68] . In aggregate, these studies establish that *Nrxn* isoform repertoires link to neuronal cell type identity. This raises the question of how these repertoires are generated, whether they are dynamically regulated, and what specific aspect of neuronal function do such isoforms instruct?

Regulation of Neurexin splice isoforms

Alternative splicing is a highly dynamic process which is guided by cis-acting RNA sequence elements such as donor and acceptor sites, branchpoint and polypyrimidine tract for spliceosome assembly (Fig3a) [69,70] . *Trans*-acting factors such as RNA-binding proteins bind intronic or exonic sequence elements in the pre-mRNAs and bias the choice of splice donor and acceptor sites, resulting in inclusion or skipping of alternative sequence elements [70-72] (Fig.3a). Several classes of RNA binding proteins are implicated in the generation of cell type-specific Neurexin repertoires. In cultured rat neurons, the polypyrimidine tract binding protein PTBP2 modifies exon skipping at *Nrxn2 AS4* [73] . 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 [74-76] , however, 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 5 STAR family proteins, the splicing factors SF1, Quaking, and SAM68, SLM1, and SLM2 – the latter three are closely related paralogues (encoded by the genes *Khdrbs1,2,3*) [77,78] . SAM68, SLM1, SLM2 directly bind RNA recognition motifs in introns flanking the highly conserved alternative exon at AS4 of the Neurexin pre-mRNAs [79-81] . 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 [37,80,81] . Alternative splicing regulation at AS4 is particularly interesting as the 30 amino acids encoded by the alternative exon strongly impact affinities for Neurexin ligands [17,59,82,83] . For example, AS4 containing Neurexins ($NRXN^{AS4+}$) 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 [59,84,85] . SLM1 and SLM2 proteins exhibit highly selective, mutually exclusive expression in neuronal cell types [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- and VIP-positive interneurons. In *SLM2*^{KO} hippocampus, there is a highly selective loss of *Nrxn1,2,3 AS4* insertions [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 [37,80]. 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*^{KO} 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*^{KO} 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 NRXN^{AS4-} splice isoform (which is lost in *Slm2*^{KO} mice) restores normal levels of GluA1, partially restores LTP, and rescues behavioural alterations in the *Slm2*^{KO} mice [89]. A germline mutation in *Nrxn3* that constitutively includes the AS4 exon reduces AMPAR surface expression and impairs long-term potentiation (LTP) in a subset of subicular neurons [90]. Interestingly, the postsynaptic modifications that result from altered presynaptic *Nrxn3* isoforms suggest the disruption of a transsynaptic link. The exact mechanisms resulting in altered postsynaptic properties remain to be worked out. However, the phenotypes in *Slm2*^{KO} and *Nrxn3*^{AS4} mice are consistent with shifted ligand affinities of AS4^{-} versus AS4^{+} splice variants for the interaction with postsynaptic Neuroligins and LRRTMs. Indeed, NRXN^{AS4+} isoforms display reduced affinity for postsynaptic Neuroligins [13,57,58], and Neurexin-Neuroligin1/3 interactions are disrupted in *Slm2*^{KO} hippocampus [89]. In *Nrxn3*$^{AS4+}$ mice, expression of the postsynaptic *Nrxn*$^{AS4-}$ ligand LRRTM2 is reduced [90]. Given that mutations in these postsynaptic Neurexin-ligands likewise disrupt LTP and synaptic transmission [91-93], 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 modify postsynaptic NMDAR- and AMPAR-mediated transmission [94]. Constitutive misexpression of *Nrxn1*$^{AS4+}$ enhances NMDAR-mediated responses at hippocampal CA1-subiculum synapses, whereas *Nrxn3*$^{AS4+}$ misexpression suppress AMPAR-mediated currents [94]. 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* paralogues may exhibit non-overlapping functions [94].

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. While 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 *Nrxn3 AS5*, there are multiple alternative splice acceptor sites in the downstream exon of the segment (sometimes referred to as exon 25a,b,c). The amino acids encoded by *Nrxn3* exon 25b confer binding of the Nrxn 3 isoform to the extracellular linker proteins C1QL2 and C1QL3, which in turn mediate the formation of a tri-partite, trans-synaptic complex with postsynaptic kainate receptors (GluK2 and 4) at hippocampal mossy fiber synapses [95] . Trans-acting factors regulating this splicing event remain to be identified.

While 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 [96,97] . In Neurexins, such paradigms shift alternative exon incorporation at several alternatively spliced segments [81,98] . In the mouse cerebellum, this phenomenon requires calcium influx, calmodulin-dependent kinase IV, and the broadly expressed STAR protein, SAM68 [81] . In granule cells of the mouse dentate gyrus, recall of a contextual fear memory triggers the inclusion of the alternative exon at *Nrxn1*^{AS4} . 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 re-wiring of dentate granule cell synapses to support learning [99] . Yet another level of regulation is the proteolytic cleavage of Neurexins, resulting in shedding of the extracellular domain of the protein [100,101] . 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 genome-wide studies on mRNA transcript isoforms and targets of RNA binding proteins provide insight into the complex logic of neuronal cell type-specific alternative splicing [39,75,76] . Several alternative transcript programs are linked to neuronal cell type, and some alternative splicing regulators increase in expression upon the commitment to a postmitotic fate [102] . 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”, i.e. largely not expressed in non-neuronal cells. These broadly expressed splicing regulators include the widely studied NOVA, 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). Intriguing, 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 [88,89] . Both SAM68 and SLM2 bind the same consensus motif, however, SLM2 but not SAM68 regulates alternative splicing of *Nrxn2* at AS4 *in vivo* [80] . This selective activity is dependent on the abundance of binding sites flanking the alternative exon [79] . Furthermore, the specificity of RNA recognition by SLM2 and SAM68 may be modified by their ability to homo- and heterodimerize [78,80] . Thus, these different

modes of action provide additional flexibility for the STAR family of proteins to generate neuronal cell class-specific synaptic properties (Fig. 3b X8+X12) [88,89] . 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 [75] . 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, selective alternative splicing decisions also arise from histone modifications and alterations in transcriptional kinetics [103] .

Acquiring a specific complement of RNA binding proteins during synaptic terminal differentiation may act much like the terminal selector genes for transcriptional cell type specification. Terminal selector genes have been postulated to be transcription factors which regulate the expression of genes required to give neurons their unique identity [4] . Congruent with this hypothesis, the combinatorial expression of RNA binding proteins in individual neuronal cell classes could ultimately instruct the generation of a cell type-specific complement of Neurexin alternative splice isoforms and their unique functions (Fig.3b). While this review article focuses on the Neurexin gene family, a similar molecular logic likely applies to other neuronal recognition and synaptic proteins [104] . Detailed profiling of transcript isoforms across genetically defined neuronal cell populations uncovered hundreds of differentially regulated alternative splicing events in the mouse neocortex and hippocampus. Furthermore, gene expression analysis of 52 bona fide splicing regulators highlighted broad, overlapping versus highly restricted expression within neuronal cell classes [39] . This supports the possibility that combinatorial expression of RNA binding proteins provides different classes of neurons with unique compositions of synaptic proteins and function (Fig 3b). Studies on additional neuronal recognition systems, such as the receptor protein tyrosine phosphatases, uncovered alternative splice insertions that modify molecular interfaces in trans-synaptic receptor-ligand interactions [33,104,105] . Thus, the principles discussed here for the generation and synaptic function of Neurexins likely extend broadly to the control of neuronal recognition (Fig.3b).

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 [106-108] . Likewise, Neurexin gene mutations are associated with Schizophrenia [109] , Autism [110] , Tourettes Syndrome [111] , Nicotine dependence [112] , developmental delay, dysmorphic features and infantile epileptic encephalopathy [107,113] . Many of these deletions span promoter and initial exons of the *NRXN1* gene (2p16.3). Transcriptomic studies in IPSC-derived neurons carrying such mutations uncover significantly reduced *NRXN*1α transcripts. Moreover, de-novo expression of isoforms divergent from the repertoire in neurotypical controls might occur [42] . Heterozygous exonic deletions do not appear to be fully penetrant as rearrangements in the *NRXN1* gene are frequent in the control population [114] , and *NRXN1* deletions are frequently inherited from

a healthy parent [115] . However, such mono-allelic *NRXN1* deletion carriers may share common alterations in anxiety, intelligence, and impulsivity, which go undiagnosed without an indepth evaluation [116] . Notably, a small number of bi-allelic *NRXN1* mutations result in a severe mental retardation syndrome, which phenotypically overlaps with Pitt-Hopkins syndrome, an autism-like developmental disorder with variable characteristics [107,117,118] .

In some cases, specific *NRXN* 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 which – depending on genetic background and the 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 - in particular 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 in “ESSENCE” (Early Symptomatic Syndromes Eliciting Neurodevelopmental Clinical Examinations) [119] .

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 risk gene mutations [118,120] . In mice, global *Nrxn1α* 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 [121] . Interestingly, some phenotypes are sex-specific: Male homozygous *Nrxn1α* knock-out mice exhibit increased aggressive behaviors. Male heterozygous *Nrxn1α* knock-out mice show increased novelty responses as assessed by locomotor activity in a new environment and enhanced habituation upon repeated exposure to this environment [122] . In rats, non-social deficits, such as hyperactivity and deficits in instrumental and spatial learning tasks, result from *Nrxn1α*-deficiency [123] . *Nrxn2α* homozygous and heterozygous knock-out mice exhibit diminished social approach and social novelty responses in behavioral assays but also impaired recognition of novel objects [124-126] , indicating social and broader cognitive deficits.

Considering that these behavioral observations were made in global, constitutive knock-out mice, linking such phenotypes to selective developmental, circuit, and synaptic functions of the Neurexin proteins is difficult. Reversible overexpression studies using dominant-negative mutant *Nrxn1* isoforms support the notion that behavioral phenotypes may result from dysfunction, rather than irreversible mis-wiring of circuits during development [127] . For *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 [128] . Mice carrying a single nucleotide polymorphism (SNP) in *Nrxn3,* which results in a single amino acid change (R498W), increase observational fear in a behavioral task [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 [130] . Conditional deletion of *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 [129] . The R498W variant maps to the third LNS domain of *Nrxn3α* (see Fig.2c). This region may confer a Ca^{2+}-mediated conformational switch for ligand binding [48,64] . 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, behavior, and neurodevelopmental disorders.

Framework for synaptic action modules

A particular challenge in defining cellular Neurexin functions and predicting the impact of mutations on neuronal circuit function arise 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 paralogues 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 like the type III mGluR – Elfn [131-133] or receptor protein tyrosine phosphatase complexes [134,135] .

We propose that the combinatorial actions of synaptic adhesion and signaling 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 (Fig.4) [136-138] . These trans-synaptic recognition and synapse-organizing systems can be conceptualized as “senders” and “readers” arrayed 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, thereby maintaining cell type-specific properties and circuit function (Fig.5b). Importantly, the messages conveyed by a particular sender (e.g., 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 fiber synapses [84,85] or the Elfn-mGluR6 link in photoreceptor synapses [131] . 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 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 intersect 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.

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TOC Summary

In this review, Scheiffele and colleagues discuss the molecular basis of transsynaptic signaling by Neurexins. Linking cell type-specific alternative splicing to the combinatorial action of Neurexin isoforms, this review synthesizes a mechanistic framework for Neurexins functions in synapse organization.

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Box 1

Non-neuronal and non-synaptic roles for Neurexin complexes

While the vast majority of research focus on the roles for Neurexins at synapses, recent evidence highlights potential functions of these adhesion molecules at other cellular junctions. A starting point for such investigations were transcriptomic and in situ hybridization studies that detected significant Neurexin mRNA expression in nonneuronal cells, including astrocytes, oligodendrocytes, and oligodendrocyte precursor cells [36,144,145] . Moreover, also major Neurexin ligands, such as the Neuroligins were detected in non-neuronal cells. For example, gliomas have been reported to produce significant levels of Neuroligin-3 protein [146-148] . Interestingly, the proliferation of glioma cells is elevated by electrical signalling that may involve synapse-like structures formed by axons onto the Neuroligin-expressing glioma cells as a critical pathological feature. An example for further extrasynaptic roles for Neurexins are interactions between neuronal Neurexins and the Neurexin ligand Neuroligin-2 in astrocytes [149] . These interactions were proposed to contribute to the elaboration of astrocytic morphology in the developing neocortex of mice. Moreover, the extracellular matrix proteins Hevin, SPARC, and Thrombospondin, secreted from astrocytes, have been suggested to directly bind to Neurexins and their postsynaptic ligands [150,151] , thereby reconfiguring their availability for adhesive interactions in the synaptic cleft. Collectively, these recent findings indicate a physiologically relevant “re-purposing” of Neurexins and their ligands to organize cellular interactions beyond synaptic neuron-neuron contacts.

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Glossary

Posttranslational modifications
Enzymatic chemical modifications of specific amino acids in a protein that occur in the cell after or during mRNA translation (e.g. through phosphorylation, glycosylation, acetylation etc.).

Structural motifs
A structurally conserved building block or “supersecondary structure” which appears in a variety of protein molecules that may or may not be functionally related.

Alternative splicing
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 (e.g. resulting from alternative promoters or from alternative splicing).

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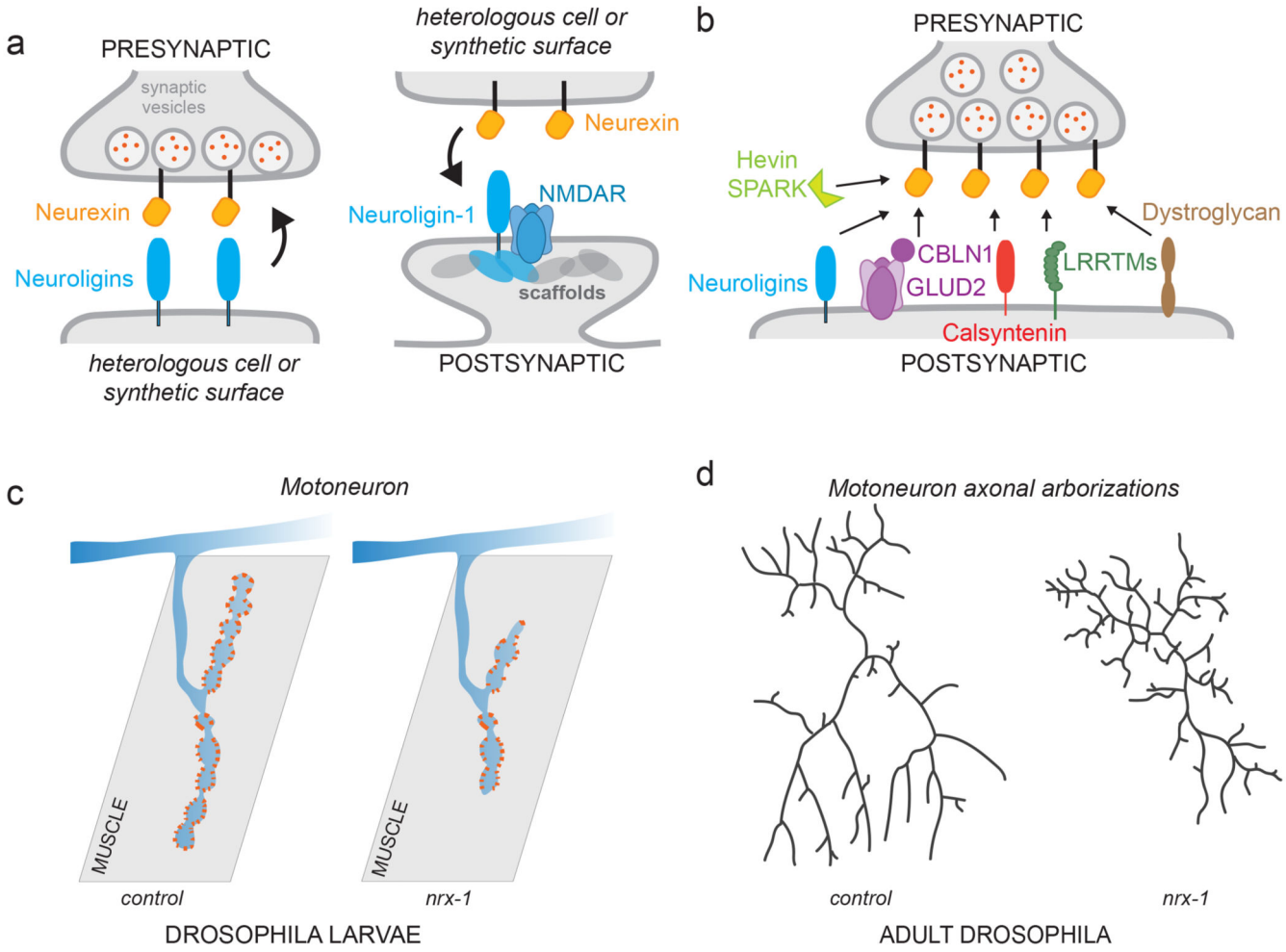

Figure 1. Synaptogenic function of Neurexins.

a) Illustration of bi-directional 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* drives assembly of pre- and postsynaptic structures, respectively.

b) Neurexin isoforms interact with a large array of structurally unrelated extracellular binding partners. Only a selection of ligands is 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 for 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) Illustration of contribution of Neurexin-Neuroligin adhesion system to growth of axonal arborisations of the motoneurons that innervate the abdominal pleural muscles of adult *Drosophila* (adapted from Constance et al. [29]).

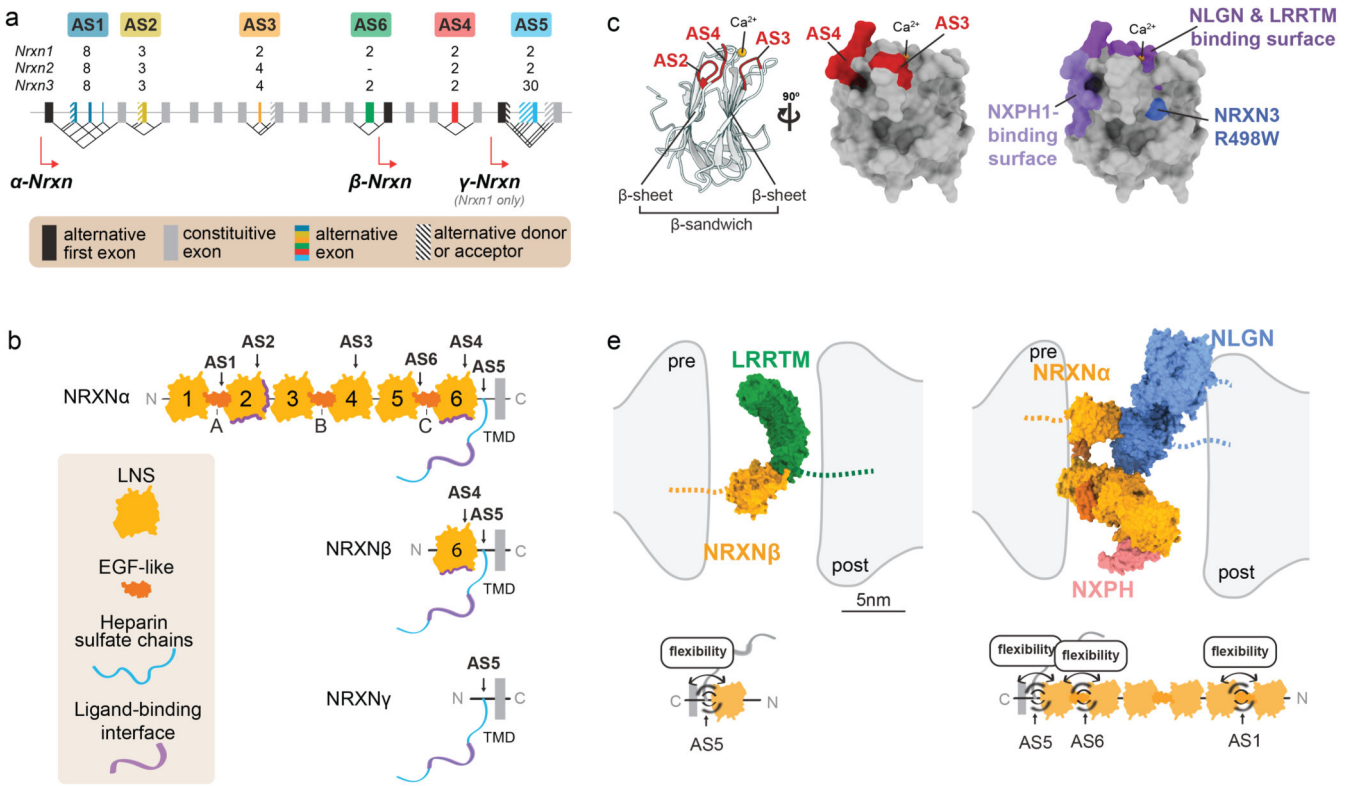

Figure 2. Molecular and structural features of Neurexin isoform diversity.

a) Schematic illustrating the alternatively spliced segments of mouse Neurexin genes. Mouse Neurexin transcript isoforms are generated from three genes (*Nrxn1* = 1.1Mb, *Nrxn2* = 0.1Mb, *Nrxn3* = 1.8Mb; 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 and γ) and exhibiting extensive alternative splicing at six alternatively spliced segments (AS1-6). Individual segments can contain single alternative cassette exons (e.g. AS4, AS6) or consist of complex combinations of alternative splice donor and acceptor sites (e.g. 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 color, constitutive exons in grey, alternative donor or acceptor sites are striped.

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 domains (LNS), epidermal growth factor-like domains (EGF), and attachment sites for heparan sulfates (HS). The largest Neurexin proteins are the NRXNα forms composed of six LNS domains (LNS 1-6), three interposed EGF domains (EGF A-C) and the HS attachment sites. Interaction surfaces for ligands are marked with purple lines. The NRXNβ forms contain a single LNS domain and HS attachment sites whereas the NRXNγ 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

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of alternatively spliced segments, (middle) view of a 90-degree rotation and surface representation LNS domain with mapped alternatively spliced segments, (right) surface representation as in middle panel highlighting the position of ligand-binding domains and the naturally occurring R498W variant in NRXN3α which has been linked to behavioral alterations in mice [129].

d) Illustration of approximate sizes and hypothetical conformation of adhesion molecule complexes in the synaptic cleft: β-Neurexin (orange, left panel) with LRRTM2 (green), and α-Neurexin (orange, right panel) with Neurexophilin (NXPH 1, 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 [48], 3B3Q [53], 6PNP [50], 5Z8Y [139]. The position of stalk, transmembrane and cytoplasmic sequences is indicated as dashed lines. Diagrams at the bottom display positions within these structures where alternative splicing at AS1, AS6 and AS5 in NRXN β (left) and NRXN α (right) modifies the flexibility of the extracellular domains in the synaptic cleft

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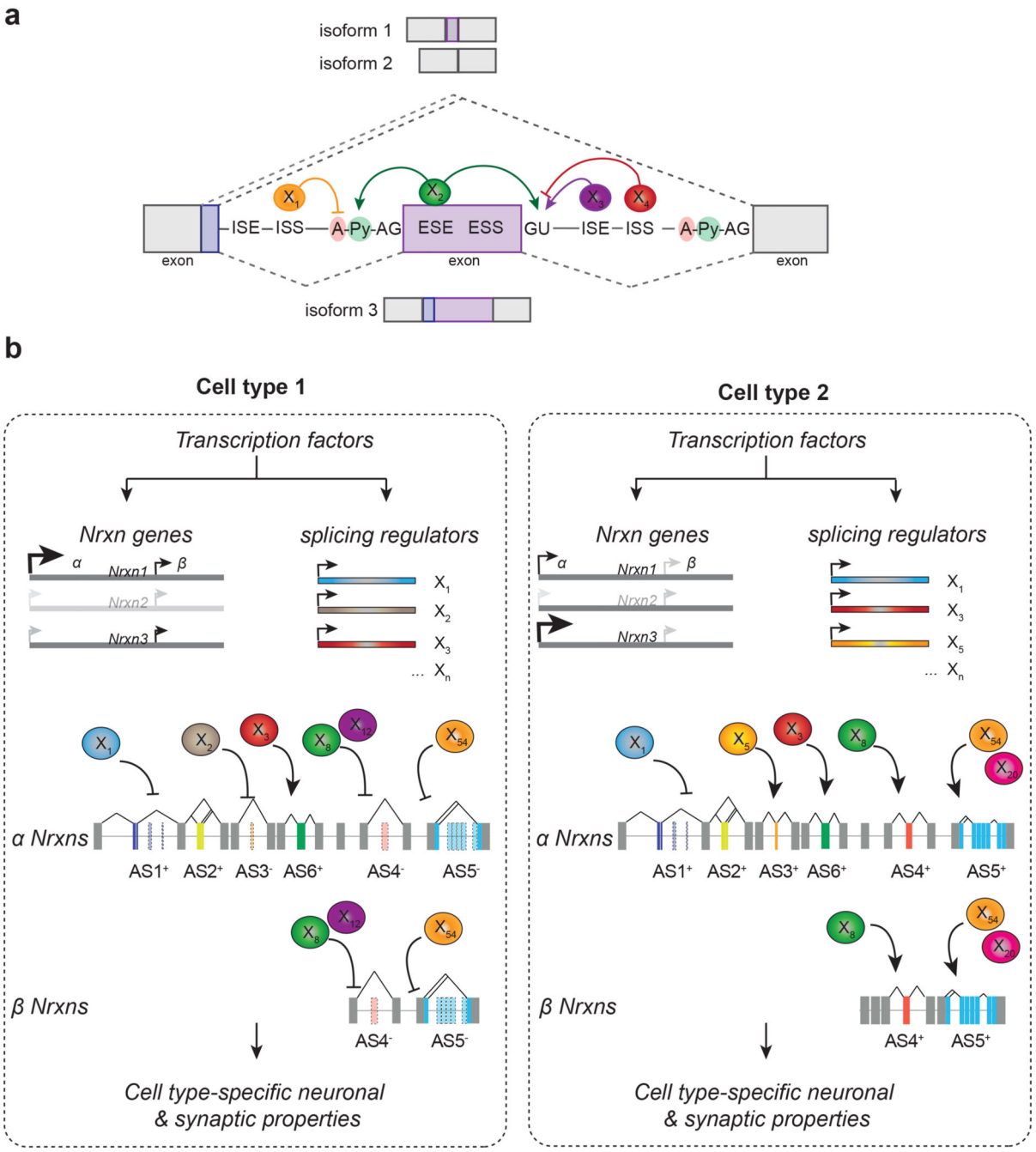

Figure 3. Control of synapse specification by alternative splicing programs.

a) Schematic illustrating the control of alternative splicing by RNA motifs and trans-acting factors (coloured spheres X_1, X_2, X_3…). The displayed alternatively spliced segment contains an alternative splice donor site in the upstream exon (depicted as grey and blue boxes on left), followed by a cassette exon (purple box) and a downstream constitutive exon (grey box, right). Exons can contain exonic splicing enhancers (ESE) and exonic splicing silencers (ESS). The intronic between the exon boxes contain RNA motifs that act as intronic splicing enhancers (ISE) and silencers (ISS). GU marks the 5' splice site, A-Py-AG

marks the branchpoint and Polypyrimidine tract followed by the terminal AG sequence in the intron. Motifs recruit trans-acting RNA-binding proteins (depicted as colored spheres) which either promote or suppress usage of individual splice donor acceptor sites, resulting in inclusion or skipping of the alternative cassette exon (isoform 1 and isoform 2). Usage of the alternative donor site in the first exon, results in a third transcript isoform (isoform3). b) Intersection of *Nrxn* transcriptional programs and the combinatorial action of splicing regulators. The illustration depicts two hypothetical cell types (cell type 1 and cell type 2) that produce different Neurexin transcript repertoires. Cell type-specific transcription from promoters/enhancers (arrowheads) drives the differential transcription of the primary transcripts from the *Nrxn* genes (e.g. Cell type 1 transcribes high levels of *NRXN1*α whereas Cell type 2 transcribes high levels of *NRXN3*α). In addition, each expresses a specific battery of splicing regulators (Cell type 1: X_1,X_2, X_3,…; Cell type 2: X_1, X_3, X_5,…). The intersection of these splicing regulators (colored spheres) with the primary *Nrxn* transcripts then produces the cell type-specific Neurexin isoforms (e.g. β *Nrxn* AS4$^-$AS5$^-$ in cell type 1 and β *Nrxn* AS4$^+$AS5$^+$ in cell type 2) [76,79,89] .

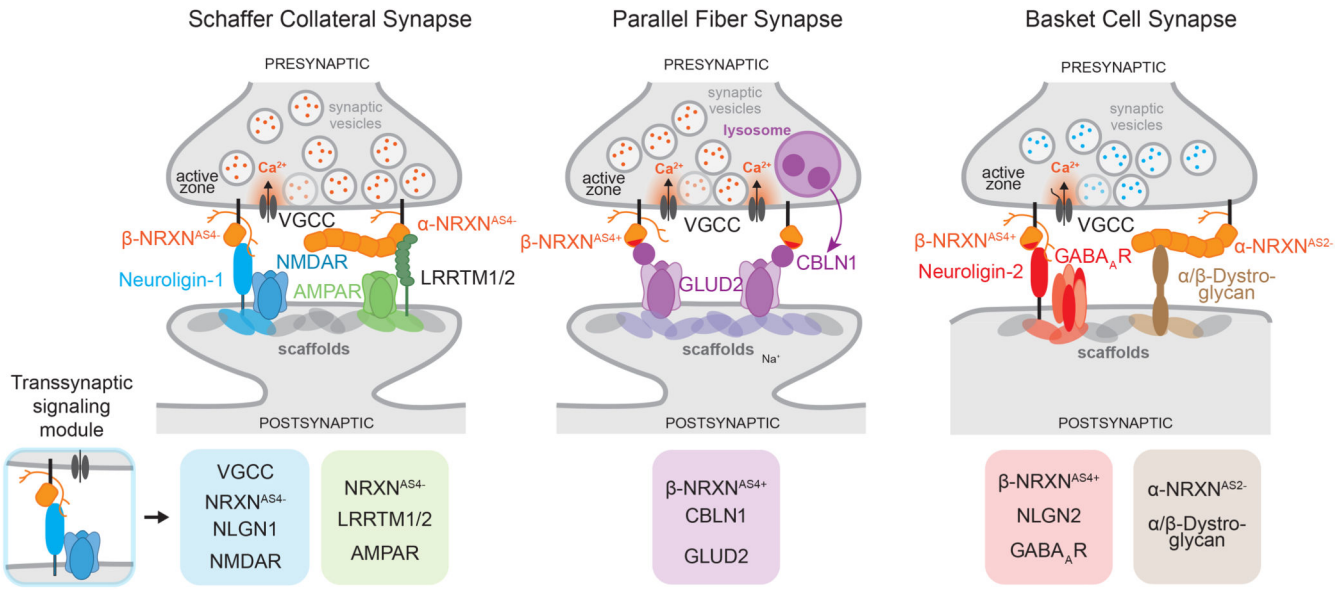

Figure 4. Examples of synaptic interaction modules nucleated by Neurexin proteins.

Simplified model illustrating trans-synaptic interaction modules assembled around presynaptic Neurexin protein isoforms. Amongst other components, CA3-CA1 Schaffer collateral synapses in the mouse hippocampus contain trans-synaptic NRXN^{AS4-}-NLGN1 and NRXN-LRRTM complexes which recruit postsynaptic NMDA and AMPA-type glutamate receptors. Note that LRRTM proteins bind α and β Neurexins – but for simplicity only the interaction with α is depicted here. Cerebellar parallel fiber synapses largely rely on a single trans-synaptic module consisting of NRXN^{AS4+} isoforms, extracellular CBLN1 proteins and the postsynaptic receptor GLUD2. CBLN1 is secreted from lysosome-like carrier vesicles [140] . Note that CBLN1 interacts with α and β Neurexins – but for simplicity only the interaction with β is depicted here. GABAergic basket cell synapses in the mouse hippocampus contain modules of NRXN^{AS4+} and NRXN^{AS2-} isoforms linking to postsynaptic NLGN2 or α/β Dystroglycan proteins, respectively. Red dots within synaptic vesicles indicate the neurotransmitter glutamate, blue dots represent GABA. One major contribution of Neurexins at synapses is the incorporation of functional voltage-gated calcium channels (VGCC) at synapses, facilitating the calcium-dependent release of synaptic vesicles [19,142,143] . The lower row displays illustrations of individual trans-synaptic modules present at the respective synapses.

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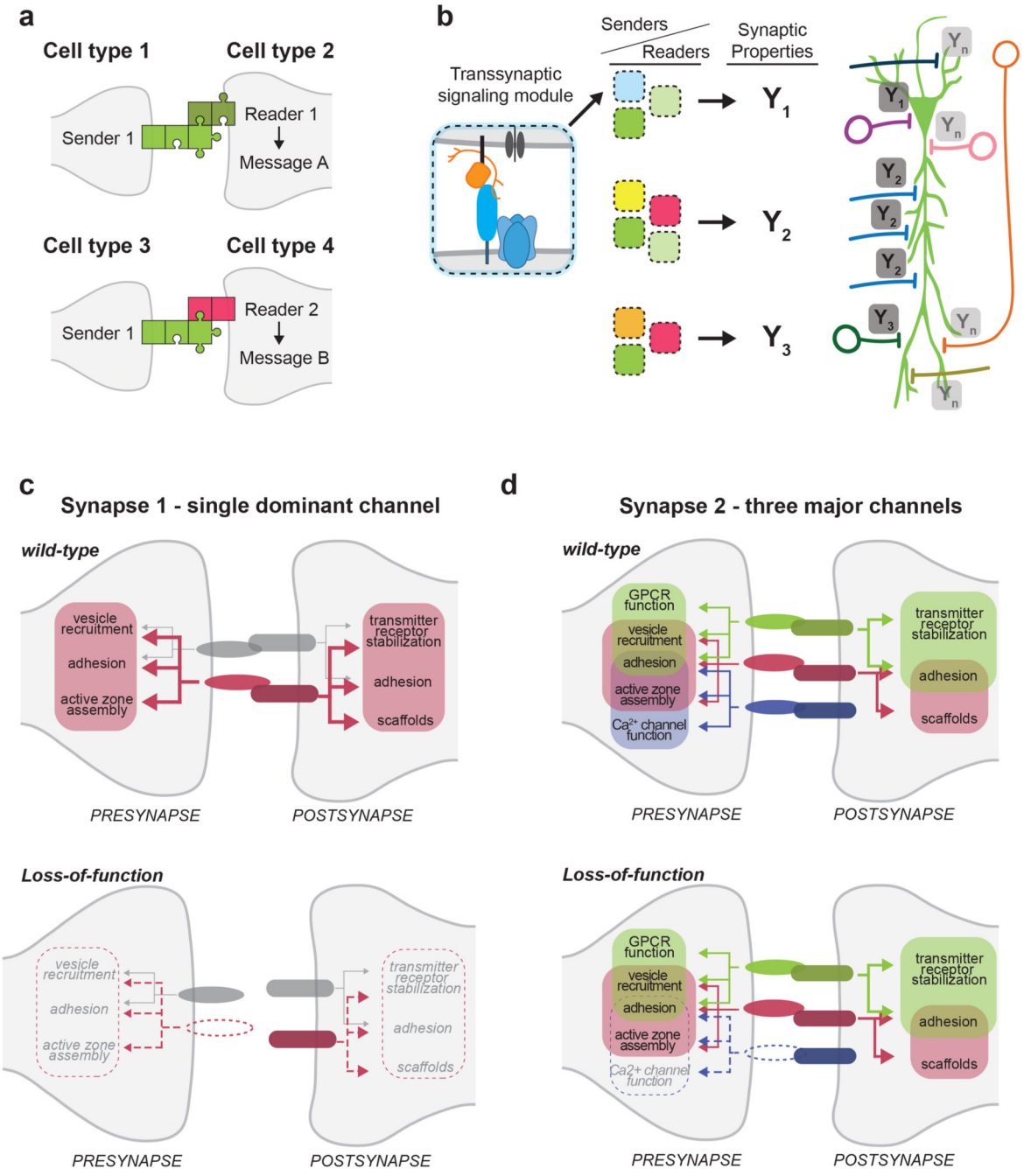

Figure 5. Context-dependent functions of synaptic interaction modules.

a) Model for the action of synaptic interaction modules. Cell types express specific complements of “senders” (light green) and “readers” (dark green and red), such as Neurexin splice isoforms and corresponding binding partners. The impact on synapse assembly and functional specification (“message”) depends on the cell type-specific abundance of corresponding senders and readers (dark green and red). Cell type 1 and cell type 3 both express the same sender but because their downstream synaptic partners cell types 2 and 4 express different readers (reader 1 and 2, respectively), the messages will be

different (message A and message B, respectively). Thus, the same presynaptic sender can convey divergent messages at different synapses.

b) Across the surface of an individual neuron (here an illustration of a pyramidal cell), the combinations of axonal senders and dendritic readers create trans-synaptic modules (depicted as blue, green, light green boxes etc.) which represent a molecular code. This code sets synapse-specific properties (Y_1, Y_2, Y_3, .. Yn; the blue labels represent glutamatergic synapses, the red labels GABAergic synapses) and, thereby, input integration in the postsynaptic cell.

c) Synapses across the central nervous system employ various numbers of trans-synaptic modules that can be viewed as trans-synaptic communication channels (displayed in different colors). 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 fiber synapses in the cerebellum which rely on the NRXN-CBLN1-GLUD2 module [59,85,141]). Loss of a single presynaptic sender (illustrated in the lower panel, e.g. CBLN1) results in disruption of the trans-synaptic module and substantial disruption of synapse formation and function, despite the presence of an additional, minor channel (depicted in grey).

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 bi-directional adhesion, presynaptic vesicle recruitment, presynaptic GPCR function, and post-synaptic 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 a 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 [31,142,143] .