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REVIEW ARTICLE

Structure, function, and pathology of Neurexin-3

Rui Zhang [a], HanXiao Jiang [b], YuanJie Liu [a,c,], GuiQiong He [a,c,*]**

[a] *Chongqing Key Laboratory of Neurobiology, Chongqing Medical University, Chongqing 400016, China*
[b] *Department of Neurology, The Second Affiliated Hospital, Chongqing Medical University, Chongqing 400010, China*
[c] *Department of Anatomy, Chongqing Medical University, Chongqing 400016, China*

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Abstract Neurexin-3 is primarily localized in the presynaptic membrane and forms complexes with various ligands located in the postsynaptic membrane. Neurexin-3 has important roles in synapse development and synapse functions. Neurexin-3 mediates excitatory presynaptic differentiation by interacting with leucine-rich-repeat transmembrane neuronal proteins. Meanwhile, neurexin-3 modulates the expression of presynaptic α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors and γ-aminobutyric acid A receptors by interacting with neuroligins at excitatory and inhibitory synapses. Numerous studies have documented the potential contribution of neurexin-3 to neurodegenerative and neuropsychiatric disorders, such as Alzheimer's disease, addiction behaviors, and other diseases, which raises hopes that understanding the mechanisms of neurexin-3 may hold the key to developing new strategies for related illnesses. This review comprehensively covers the literature to provide current knowledge of the structure, function, and clinical role of neurexin-3.

* Corresponding author. No.1 Yixueyuan Road, Yuzhong District, Chongqing 400016, China.
** Corresponding author. No.1 Yixueyuan Road, Yuzhong District, Chongqing 400016, China.
E-mail addresses: liuyuanjie@cqmu.edu.cn (Y. Liu), guiqionghe@cqmu.edu.cn (G. He).
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Introduction

Synapses are key components of information transmission between neurons.[1] Almost all information transmission in the brain depends on synapses through the process of integrating and processing various sensory signals to produce appropriate motor signals. A synapse is mainly comprised of three parts: presynaptic membrane, synaptic cleft and postsynaptic membrane. Signal transduction at synapses is achieved by the release of neurotransmitters and changes in the function of neurotransmitter receptors. Synaptic plasticity, a significant feature of the neural system that refers to the changes in the structure, number and function of synapses under the influence of continuous neuronal activity, is the basis of the cellular biology of cognitive function and includes long-term synaptic plasticity and short-term synaptic plasticity. Long-term synaptic plasticity mainly includes long-term potentiation (LTP) and long-term depression (LTD), while short-term synaptic plasticity mainly consists of facilitation and depression.[2,3] The formation and regulation of synapses rely on signal transduction between presynaptic and postsynaptic membrane proteins, especially neural cell adhesion molecules (NCAMs).[4] NCAMs are cell surface proteins that promote neuronal adhesion, increase axon growth and alter synaptic plasticity.[5]

Neurexin-3 belongs to the neurexin family, which was discovered in the process of studying α-latrotoxin. α-Latrotoxin is a component of the venom of black widow spider that results in the release of neurotransmitters from the presynaptic membrane by binding to a member of the neurexin family.[6] Three neurexin genes (*Nrxn1*, *Nrxn2*, and *Nrxn3*) have been identified in the mammalian genome, and each encodes a longer α-protein and a shorter β-protein from separate promoters. Taken together, the neurexin family contains six main members, including neurexin-1α, neurexin-1β, neurexin-2α, neurexin-2β, neurexin-3α and neurexin-3β.[7] Neurexin-1 and neurexin-2 have been characterized in detail. In this article, we summarize the structure and function and discuss the latest knowledge on the clinical role of neurexin-3.

The structure of neurexin-3

Neurexins have different expression patterns during early development of the human cerebral cortex, in which neurexin-3 is mainly expressed in the cortical plate, and its expression in the ventricular zone increases with age.[8] Early studies suggested that neurexin-3 was specifically expressed in vertebrate brain tissues[9]; however, neurexin-3 expression is also detected in other tissues, including the lung, pancreas, heart, liver, and kidney.[10] Neurexin-3α and neurexin-3β are both type 1 membrane proteins, but they have distinct domain structures. Neurexin-3α consists of a classic N-terminal signal peptide (SP) followed by three copies of a long repeat domain that contains a central epidermal growth factor (EGF)-like domain with bilateral LNS domains (laminin, neurexin, sex-hormone-binding globulin domains), whereas neurexin-3β contains an N-terminal SP followed by a short sequence that is unique to β-neurexins and then splices into the right LNS domain of the third repeat of neurexin-3α (Fig. 1). The LNS domains of neurexin-3α and neurexin-3β are followed by a sequence that consists of large numbers of threonine and serine residues, and the sequence may putatively represent the carbohydrate attachment sequence (CHO). Neurexin-3 is mainly anchored in the presynaptic membrane via a single transmembrane region (TMR) and ends in a short intracellular sequence (Fig. 2).[11,12]

Neurexin-3 is highly homologous to neurexin-1 and neurexin-2; however, neurexin-3α includes four alternative splicing sites (SS1, SS3, SS4, and SS5), while neurexin-3β only includes two alternative splicing sites (SS4 and SS5). Through the analysis of neurexin-3 transcriptional precursors, different alternative splice sites produce a variety of transcripts and protein subtypes. The effective cellular mechanism mediated by alternative splicing constructs involves many different NCAMs and signaling molecules that are located at the synapses and contribute to increasing the specificity and diversity of interactions between cells.[13] At least 12 variations in the C-terminal splice site of neurexin-3 have been detected, where a stop codon can even be inserted to delete the TMR region and tail.[14]

The function of neurexin-3

Neurexin-3 plays important roles in synapse formation, differentiation, maturation and stability, as well as neuromuscular junctions (NMJs). Deletion of neurexin-2α and/or neurexin-3α reduces neurotransmitter release, and adult α-neurexin double-knockout mutant mice die prematurely, although the junction structure of the diaphragm remained normal, indicating that neurexin-3α plays an important role in NMJs.[15] α-Dystroglycan (α-DG) is involved in multiple forms of congenital muscular dystrophy (CMD) associated with improper glycosylation.[16] Interestingly, a recent study showed that neurexin-3 is also the same O-mannosylated glycoprotein as α-DG. Neurexin-3 may be a potential target for the diseases mentioned above; however, compared with neurexin-1, more specific mechanisms of neurexin-3 at NMJs are still unknown.[17,18]

Neurexin-3 and synapse development

Correct synaptic development is indispensable for normal brain functions. Synaptic development includes the whole process from synaptogenesis to the complete establishment of synaptic function and synaptic structure. Numerous studies have been undertaken to reveal the interaction of neurexin-3 and other molecular signaling pathways. Neurexin-3 is expressed in the cerebral cortex of early humans, where the levels of proteins related to synapse formation increase accordingly, such as vesicular GABA transporter and Ca^{2+}/calmodulin-dependent Ser/Thr kinase (CASK).[8] In addition, the levels of postsynaptic proteins, such as neuroligin-2 and LRRTM2, also increase during this period. These proteins have been shown to be related to synapse development, and thus neurexin-3 expression may reflect synapse formation.

Neurexin-3 in the presynaptic membrane binds to leucine-rich-repeat transmembrane neuronal proteins (LRRTMs) and neuroligins to form synaptic complexes that

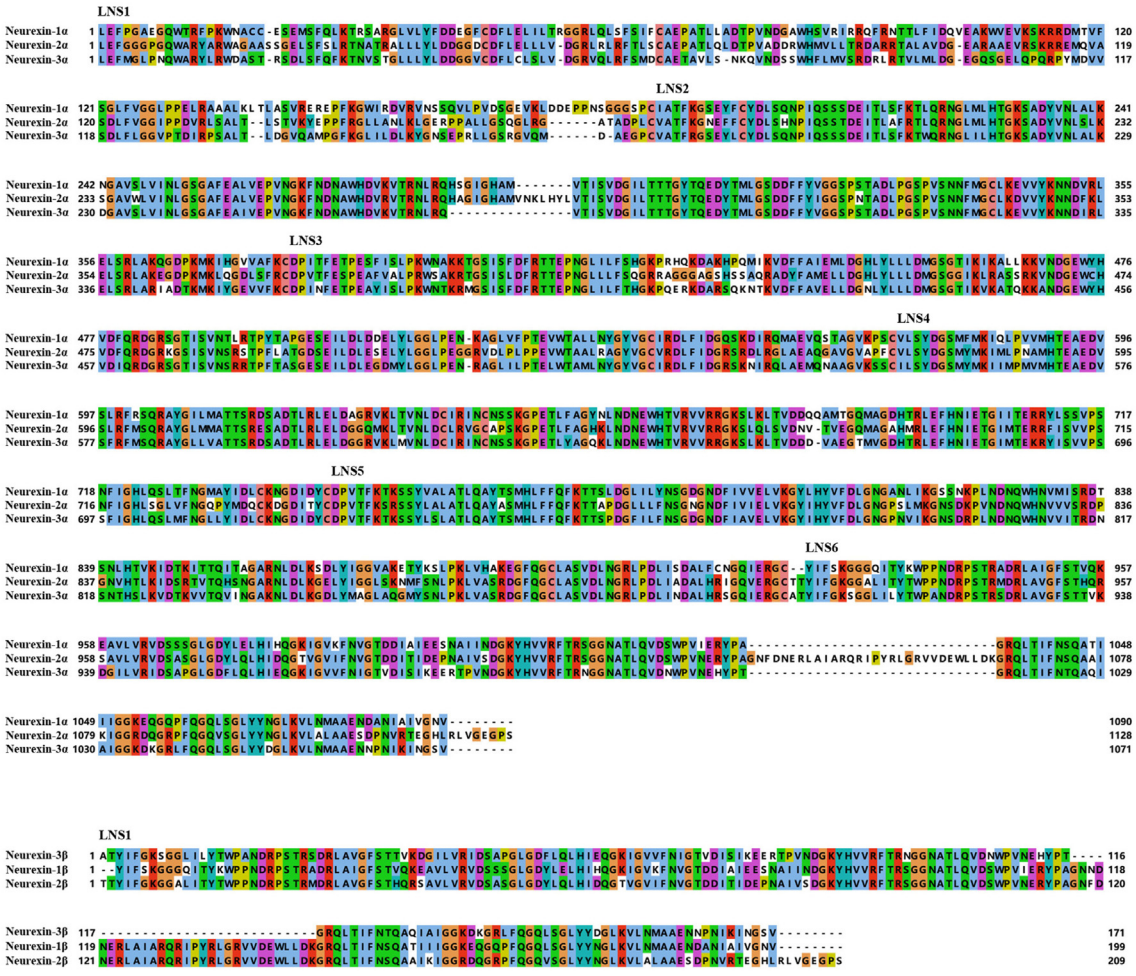

Figure 1 Amino acid sequence alignment of the LNS domains of the neurexin family. The LNS domains of neurexin-3α and neurexin-3β are followed by a sequence that consists of large numbers of threonines and serines, and the sequence may putatively represent the carbohydrate attachment sequence (CHO).

are essential for synapse development.[19–21] The neurexin-3-LRRTMS complexes induce glutamatergic synapse development, while the neurexin-3-neuroligin complexes play a role in both glutamatergic and GABAergic synapse development.[20,22] The specific mechanisms are discussed in the next section.

Neurexin-3 and leucine-rich-repeat transmembrane neuronal proteins

LRRTM is a synapse-organizing protein that is mainly located in the postsynaptic membrane.[23,24] LRRTM genes are exclusively expressed in vertebrates. Four genes that encode LRRTMs have been identified in the human genome, namely *Lrrtm1*, *Lrrtm2*, *Lrrtm3*, and *Lrrtm4*.[25] LRRTM is also a type I membrane protein, including an extracellular sequence, a single TMR and a relatively short cytoplasmic tail. The tail binds to postsynaptic signaling and scaffolding proteins.[20,26] LRRTMs play an important role in synaptic development.[20] LRRTMs promote the development of excitatory synapses without affecting inhibitory synapses.[27,28] LRRTMs stabilize and increase the levels of α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors (AMPARs) expressed in the postsynaptic membrane, which are related to LTP.[29–31] In addition, LRRTMs are associated with a variety of neuropsychiatric diseases, such as bipolar disorder and schizophrenia.[32,33]

LRRTM1, LRRTM2 and LRRTM3 induce presynaptic development by binding to neurexin-3 [20,34]. LRRTMs promote the presynaptic differentiation of glutamatergic neurons, but GABAergic neurons are not affected.[20] When all neurexins are knocked out (KO), LRRTMs do not function properly, indicating that neurexins mediate the function of LRRTMs.[20] In the hippocampus of mice lacking LRRTM1, the distribution of vesicular glutamate transporters is increased.[27] However, the number of synapses is not affected, which may be related to the increased expression of LRRTM2, suggesting that LRRTM1 and LRRTM2 bind to the same neurexin, such as neurexin-3, to perform some similar functions.[27,28] In addition, they have the same ability to induce synapse development.[27] Although triple knockdown (KD) of neurexin-3 and both LRRTMs in neurexin-1 KO mice yield substantial reductions in excitatory synaptic transmission when synapses are forming,[35] LRRTM1 and LRRTM2 KD alone or in combination do not reduce the number of

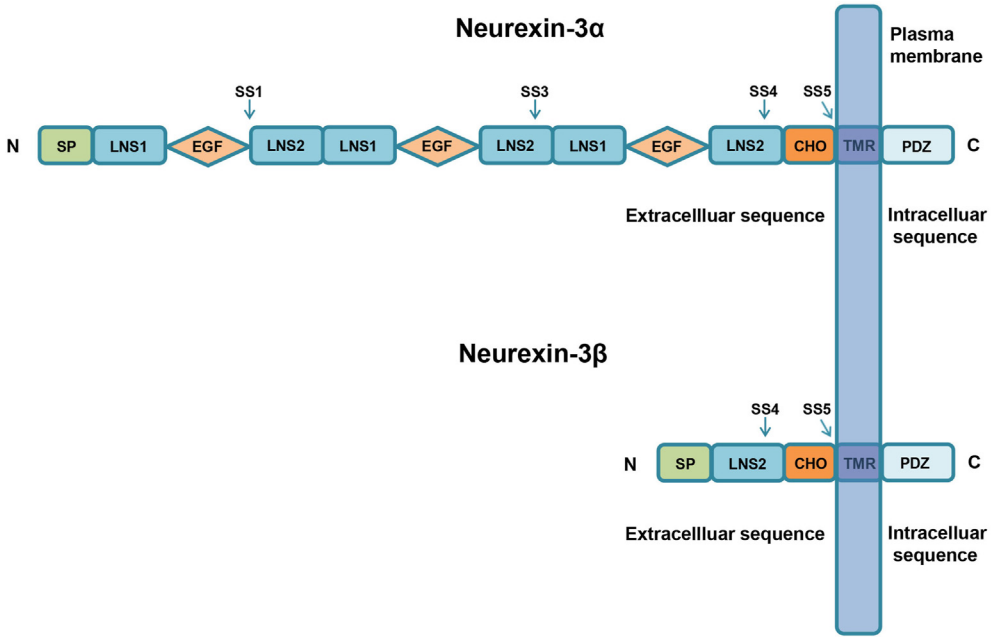

Figure 2 Structures of neurexin-3α and neurexin-3β. The extracellular sequence of neurexin-3α comprises an N-terminal signal peptide (SP) followed by three copies of a long repeat that contains a central epidermal growth factor (EGF)-like domain flanked by LNS domains (laminin, neurexin, sex-hormone-binding globulin domains), and the LNS domain of neurexin-3α is followed by a sequence that consists of large numbers of threonines and serines. This sequence may putatively represent the carbohydrate attachment sequence (CHO). Neurexin-3α is mainly anchored in the presynaptic membrane through a single transmembrane region (TMR) and ends in a short intracellular sequence that includes the PDZ II interaction site. Neurexin-3α contains four alternative splice sites (SS1, SS3, SS4, and SS5). The extracellular region of neurexin-3β contains an SP, an LNS domain and a CHO. Neurexin-3β is also anchored in the presynaptic membrane by a TMR and ends in a short tail. Neurexin-3β consists of only two alternative splice sites (SS4 and SS5).

excitatory synapses.[36] In another study, overexpression of neuroligin1 or LRRTM2 resulted in strong synaptogenic activity during neuronal maturation.[37] This evidence suggests that LRRTMs and neuroligins perform some overlapping functions during the early development of excitatory synapses.

In a fibroblast-neuron coculture experiment, LRRTM2 containing a point mutation in the extracellular domain did not bind to neurexins and did not induce presynaptic differentiation, indicating that LRRTMs promote presynaptic differentiation mediated by neurexins.[28] Meanwhile, LRRTM3 is reported to induce presynaptic recruitment.[27] The number of excitatory synapses is decreased in mice lacking LRRTM3.[31] As mentioned above, neurexin-3 and LRRTMs are involved in synapse development. However, more research is needed to understand the specific mechanisms.

Neurexin-3 and neuroligins

Neurexins and neuroligins are a pair of NCAMs that are very important for synapse development and functions.[38] Different cellular and molecular mechanisms, including alternative splicing and calcium binding, regulate the neurexin-neuroligin interactions to promote differentiation, maturation, stability and plasticity of inhibitory and excitatory synapses.[13] Neuroligin is also a type I membrane protein that is mainly located in the postsynaptic membrane. Neuroligin consists of a short cytoplasmic tail, a TMR and an extracellular sequence.[39] Three genes that encode neuroligins have been detected in mice and rats, namely *Nlgn1*, *Nlgn2* and *Nlgn3*, while five genes that encode neuroligins have been identified in the human genome, namely *Nlgn1*, *Nlgn2*, *Nlgn3*, *Nlgn4X*, and *Nlgn4Y*.[40,41] Neuroligins are expressed in different sites of the central nervous system (CNS). Neuroligin-1 targets excitatory synapses, and neuroligin-2 targets inhibitory, cholinergic and dopaminergic synapses.[42–45] Neuroligin-3 is mainly localized at excitatory and inhibitory synapses,[46] while neuroligin-4 is mainly localized at glycinergic synapses.[47] In addition to the CNS, neuroligins are also expressed in the pancreas, pulmonary endothelial cells, colon and heart.[48,49]

Neuroligins function at excitatory and inhibitory synapses. Overexpressed neuroligins increase synaptic transmission and the synapse density in transfected neurons and the number of synapses in the spine.[50,51] Overexpression of monomeric or dimerized neuroligin-1 increases the number of synapses in neurons, while overexpression of neuroligin-3 increases the synapse number of wild-type neurons.[51] When neuroligin-3 is unable to form dimers with neuroligin-1 or neuroligin-2, neuroligin-3 is unable to promote synapse development, suggesting that neuroligin-1 and neuroligin-2 are very important for synaptic development.[51] In addition to modulating the number of synapses, neuroligins lead to

changes in excitatory and inhibitory receptors. Neuroligin-1 deletion reduces the excitatory synaptic response mediated by AMPARs, while inhibitory synapses are not affected.[50,52] Compared with neuroligin-1, neuroligin-2 deletion reduces inhibitory synaptic responses instead of the responses of excitatory synapses.[53,54]

Neurexin-3 interact with neuroligin-1, neuroligin-2, neuroligin-3 and neuroligin-4X.[34] In distinct classes of neurons, neurexin-3 performs different functions rather than a classical function in all neurons. Two dramatic phenotypes caused by conditional deletion of neurexin-3 are observed in the hippocampal CA1 region. First, the excitatory responses mediated by AMPARs are decreased (~40%) due to a loss of postsynaptic AMPARs. Meanwhile, LTP mediated by postsynaptic NMDARs is blocked completely. However, in the olfactory bulb, conditional deletion of neurexin-3 caused a completely distinct phenotype: the inhibitory responses mediated by γ-aminobutyric acid receptors (GABARs) are decreased (~60%).[55,56] In these processes, neuroligins mediate the function of neurexin-3. Neurexin-3 actively signals through neuroligins to promote postsynaptic differentiation, including the aggregation of excitatory and inhibitory receptors.[19,22,57] These two signaling pathways underscore the role of neurexin-3 in synapse development.

The neurexin-3-neuroligin-1-AMPARs interaction

Glutamatergic neurons are some of the most important excitatory neurons and play a crucial role in the regulation of the CNS. Glutamate receptors include three types of ionic and metabolic receptors. AMPARs are ionic receptors with a substantial regulatory effect on synaptic plasticity. AMPARs that are widely distributed in the CNS are primarily located in the postsynaptic membrane and play an important role in the transmission of postsynaptic excitatory currents. AMPARs bind to glutamate released from the presynaptic membrane and then change its configuration to activate the ion channel. Four genes encoding four subunits have been identified for AMPARs, and these subunits form ion channels with distinct functions by combining in different stoichiometries.[58] The expression of these subunits is highly regulated and is specific to different brain regions.[59] The transport of AMPARs includes insertion, diffusion, immobilization, endocytosis, and cycling or degradation.[60] AMPARs endocytosis is one of the biological bases of LTD.[61] Endocytosis of AMPARs is characterized by a decrease in the number and density of AMPARs in the membrane, which may lead to LTD, memory loss and motor learning deficits.[62,63] AMPARs endocytosis is associated with the progression of several diseases, such as Alzheimer's disease (AD). Inhibition of AMPARs endocytosis slows the speed of forgetting in animal models of AD and strengthens memory in normal animals.[6] Endocytosis of AMPARs and synaptic dysfunction may be common pathophysiological processes in many diseases characterized by cognitive impairment, including AD.[6,64]

All α- and β-neurexins have selective splice site 4 (SS4), which includes or excludes highly conserved 90 bp exons that generate SS4- (exon-included isoforms) and SS4+ (exon-excluded isoforms) variants after splicing.[7] Neurexin-3β (SS4+) interacts with neuroligin-1 to regulate excitatory synapses.[20,65] Neuroligin-1 overexpression increases excitatory postsynaptic currents mediated by N-methyl-D-aspartic acid receptors (NMDARs) and AMPARs.[50] Neuroligin-1 recruits postsynaptic density-95 (PSD-95) and binds to the third PDZ domain of PSD-95, which is an important component of the postsynaptic density.[66] PSD-95 contains three PDZ domains, one SH3 domain and one guanylate kinase (GK) domain, each with its own function.[67] PSD-95 binds to AMPARs through stargazin/TARPs, in addition to recruiting other postsynaptic components.[4,67,68] PSD-95 also binds to guanylate kinase-associated protein (GKAP), which is an adaptor protein that then binds to SHANKs. In cultured neurons, the expression of dominant-negative neuroligin-1, which lacks the last four necessary amino acids of the C-terminal to bind to PSD-95, significantly decreases the number of postsynaptic components, including PSD-95 and AMPARs.[12,57]

Compared with neurexin-1β (SS4+) and neurexin-2β (SS4+), neurexin-3β (SS4+) plays a completely different role in the regulation of excitatory synapses. Neurexin-3β (SS4+) prevents neuroligin-1 pairing, decreases the number of postsynaptic AMPARs and increases AMPARs endocytosis in mouse hippocampal synapses.[13,69] These changes caused by neurexin-3β (SS4+) are reversed by removing SS4 (Fig. 3).[56]

The neurexin-3-neuroligin-2-$GABA_A$ receptors interaction

GABAergic neurons are some of the most important inhibitory neurons and play important roles in the CNS. GABARs mainly consist of three types: $GABA_A$ receptors ($GABA_A$Rs), $GABA_B$ receptors ($GABA_B$Rs) and $GABA_C$ receptors ($GABA_C$Rs).[70] GABARs located in the postsynaptic membrane interact with GABA released from the presynaptic membrane to cause changes in the configuration of GABARs and activate ion channels. The activated ion channels facilitate the hyperpolarization of postsynaptic neurons, which blocks the conduction of nerve impulses. Different types of GABARs have been discovered in different brain regions, but all types exert inhibitory effects on learning and memory through different mechanisms. Antagonists of GABARs ameliorate the inhibition of learning and memory.

Among all types of GABARs, neurexin-3 significantly affects $GABA_A$Rs. $GABA_A$Rs distributed at synaptic and extrasynaptic sites are ligand-gated ion channel receptors that mediate phasic and tonic inhibition.[71] $GABA_A$Rs located at synapses directly control the efficiency of GABA transmission and inhibit the conduction of nerve impulses. $GABA_A$Rs not only inhibit learning and memory but also participate in brain development.[72] Studies have shown that a variety of neuropsychiatric disorders are associated with changes in $GABA_A$Rs. For instance, abnormal $GABA_A$Rs are detected in the brain tissues of patients with schizophrenia.[73] $GABA_A$Rs blockade enhances hippocampal synaptic plasticity in mice with AD.[74]

Neurexin-3α (SS4+) promotes postsynaptic differentiation at inhibitory synapses by binding to neuroligin-2.[12,75] Neuroligin-2 participates in recruiting postsynaptic membrane scaffold proteins, including collybistin and gephyrin. Neuroligin-2 specifically activates collybistin.[46] Activated collybistin is also involved in the recruitment of gephyrin.[76–78] Neuroligin-2 binds to gephyrin through its cytoplasmic motif.[46] Gephyrin is an important component

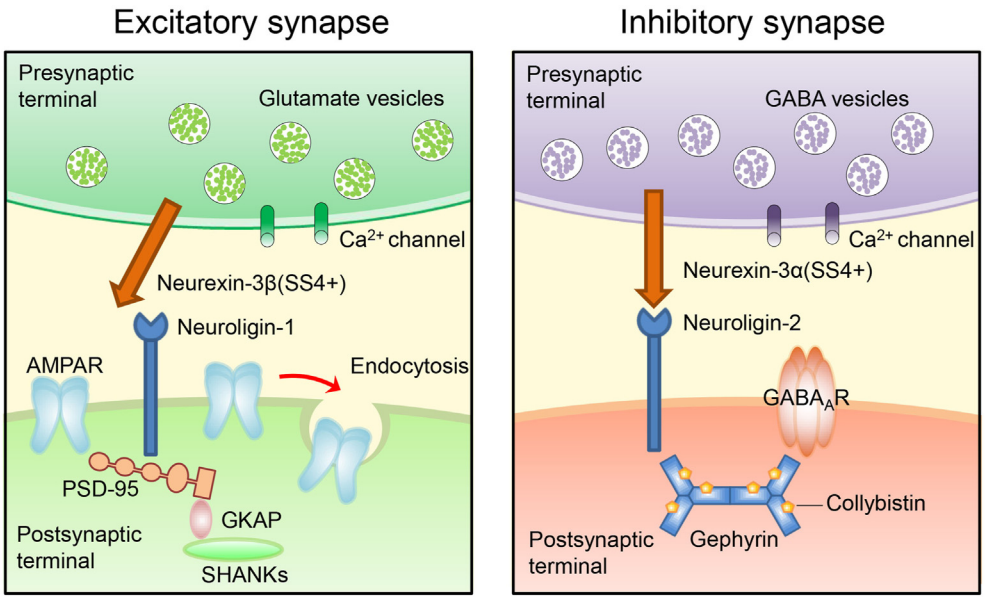

Figure 3 Two signaling pathways reported based on synaptic junctions formed by neurexin-3 that alter synaptic function. To date, the complete signaling pathway responsible for the change in synaptic function in response to the expression of neurexin-3 has not been fully explained; however, the association of neurexin-3 with AMPARs and $GABA_A$Rs signaling pathways has been observed. At excitatory synapses, by blocking neuroligin-1 pairing, neurexin-3β (SS4+) prevents postsynaptic density-95 (PSD-95) from recruiting specific postsynaptic proteins that are present in glutamate synapses, including AMPARs. PSD-95 binds to guanylate kinase-associated protein (GKAP), which is an adaptor protein, and GKAP binds to SHANKs. Moreover, neurexin-3β (SS4+) promotes the endocytosis of AMPARs in the postsynaptic membrane. At inhibitory synapses, neurexin-3α (SS4+) interacts with neuroligin-2 and specifically activates collybistin. Neuroligin-2 and activated collybistin are involved in the recruitment of gephyrin, which then recruits $GABA_A$Rs to the postsynaptic membrane. In addition, neurexin-3α located in the presynaptic membrane acts directly on postsynaptic $GABA_A$Rs.

of GABAergic synapses and recruits $GABA_A$Rs.[79–82] Inhibitory neurotransmitter receptors, including $GABA_A$Rs, are then recruited to the postsynaptic membrane through the action of the complex that comprises neuroligin-2, gephyrin and collybistin (Fig. 3). Animal experiments have revealed that a decrease in the level of neuroligin-2 in mice causes a loss of postsynaptic specificity of inhibitory synapses.[43] In Purkinje cell-specific neuroligin-2 KO mice, the inhibitory postsynaptic current (IPSC) amplitude and the frequency of miniature IPSCs (mIPSCs) are decreased.[54] In transgenic mice overexpressing neuroligin-2, $GABA_A$Rs expression in the frontal cortex is increased, the frequency of mIPSCs is increased, the excitatory inhibition rate is decreased, and anxiety and social phobia are observed.[53] In addition, neurexin-3 located in the presynaptic membrane acts directly on the $GABA_A$Rs located in the postsynaptic membrane and may influence the excitatory/inhibitory synaptic balance.[83] Meanwhile, ectopically expressed neurexin-3α at excitatory synapses interacts with the GABARs complex and recruit $GABA_A$Rs to the postsynaptic membrane.[84] In general, molecules and mechanisms that are involved in regulating $GABA_A$Rs in the CNS may be far more complex than previously known.

Neurexin-3 and synapse functions

Neurexin-3 plays a critical role in regulating synaptic properties. In addition to binding to LRRTMs and NLGNs, neurexin-3 interacts with CASK, cerebellin (Cbln), SHANK2 and Discs-large4 (DLG4).[34] The levels of these proteins increase to varying degrees in the early human cerebral cortex.[8] CASK is mainly located in the presynaptic terminal and is considered a multidomain scaffold protein.[85] Within the presynaptic terminal, CASK binds to neurexin-3β to promote the accumulation of vesicles. In addition, it interacts with VELIs and MINT1 to form the basic framework of the synaptic vesicle fusion mechanism.[7,86–88] Cblns are a family of secreted glycoproteins that are located at excitatory and inhibitory synapses. Cblns simultaneously bind to presynaptic neurexin-3 and postsynaptic ionotropic glutamate delta receptors (GluD) to mediate synapse function.[89] At hippocampal synapses, by activating postsynaptic GluD1, the presynaptic neurexin-3 (SS4+)-Cbln2 complex regulates the activity of AMPARs, but the number of AMPARs is normal.[90] SHANK2 is a scaffold protein that is required for synapse formation and function. In humans, some neuropsychiatric and neurodevelopmental diseases, including autism spectrum disorders (ASD) and schizophrenia, are mediated by different SHANK2 variants.[91] DLG4 (also known as PSD-95) is a scaffold protein that plays a role in the assembly of large signal transduction networks at specific sites within the cell.[92] By interacting directly with neuroligin-1 and indirectly with PSD-95, neurexin-3 regulates postsynaptic AMPARs.[55,67]

In cultured neurons *in vitro* and in the hippocampus *in vivo*, conditional constitutive KOs (cKOs) of neurexin-1β, neurexin-2β and neurexin-3β reduce the release of transmitters at excitatory synapses. A major synaptic phenotype

caused by the deletion of β-neurexins is observed; however, survival is normal.[93] All three α-neurexins are also important for neurotransmitter release. Deletion of all three α-neurexins interferes with synaptic Ca^{2+} channel function, which leads to a decrease in Ca^{2+}-triggered neurotransmitter release, but the number of Ca^{2+} channels remain normal.[94] Neurexin-3 binds to the extracellular junction proteins C1ql2 and C1ql3 and then forms a transsynaptic complex with postsynaptic kainate receptors (Gluk2 and Gluk4) at the synapse of hippocampal mossy fibers.[95] In addition to regulating the expression of postsynaptic receptors, neurexin-3 affects presynaptic receptors. Deletion of neurexin-1, neurexin-2 and neurexin-3 leads to the loss of presynaptic $GABA_B$Rs at calyx synapses.[96] Sufficient evidence suggests the important role of neurexin-3 in synaptic signaling.

The correlation of neurexin-3 with neurodegenerative and neuropsychiatric diseases

Neurexin-3 and AD

AD is one of the most common neurodegenerative diseases and is characterized clinically by progressive cognitive dysfunction and behavioral impairment.[97] The presence of amyloid plaques, tau tangles and neuronal loss are the main pathological features of AD.[98] Amyloid β (Aβ), which is considered to induce the pathogenesis of AD, is a marker protein of AD that causes synaptic dysfunction, neuroinflammation and neurodegeneration.[98,99] Studies have found that synaptic dysfunction often occurs in the first stage of AD and affects multiple brain regions.[100–103] The levels of various proteins are decreased in the frontal cortex of patients with AD.[104,105]

Neurexin-3 may have a critical role in the early stage of AD. The expression levels of neurexin-3α and neurexin-3β are not significantly altered in cerebrospinal fluid (CSF) from patients with AD but are increased in patients with mild cognitive impairment (MCI).[106–108] Neuroligin-1 and PSD-95 levels are decreased in the hippocampus of patients with AD and MCI, but soluble Aβ levels are increased. Importantly, a reduced level of neuroligin-1 is associated with an elevated level of soluble Aβ and disease progression.[109] Aβ deposition inhibits the expression of neuroligin-1 and impairs synaptic function and memory.[110] Therefore, the neurexin-3β-neuroligin-1-AMPARs pathway may lead to synaptic dysfunction to promote the development of AD by blocking the recruitment of AMPARs and increasing the endocytosis of AMPARs.

The neurexin-3α-neuroligin-2-$GABA_A$Rs pathway may be an additional mechanism involved in the pathogenesis of AD. Elevated levels of neurexin-3α and neuroligin-2 have been detected in the CSF of individuals in the preclinical stage of AD,[108] which may further lead to the upregulation of $GABA_A$Rs. The increase in $GABA_A$Rs affects information transmission at inhibitory synapses, synaptic plasticity, learning and memory and promotes the progression of AD.

Neurexin-3 may be associated with susceptibility to AD in males, but the mechanism is unclear.[111] Neurexin-3β is cleaved into an N-terminal extracellular domain and a C-terminal transmembrane fragment (CTF) by α-secretase. The CTF is processed into a smaller intracellular domain (ICD) by γ-secretase. Presenilin1 (PS1) is the catalytic subunit of γ-secretase, and mutations in PS1 will cause γ-secretase inactivation, which lead to an increase in CTF levels and a decrease in ICD levels.[112] The CTF is similar to amyloid precursor protein (APP), which is cleaved to promote the deposition of Aβ. Therefore, researchers have speculated that neurexin-3 may participate in the pathological process of AD through proteolytic processing to generate the CTF in subjects with a PS1 mutation.[113]

Neurexin-3 and addictive behaviors

Addiction is a public health problem of great concern that is related to the euphoria and rewards caused by substance abuse.[114] In fact, addiction is a complex disease affected by heredity and the environment.[115] Many proteins, including neurexin-3, are associated with addictive behaviors, such as opioid abuse, nicotine dependence and alcohol dependence.[116–118] Neurexin-3 is expressed in brain regions related to addictive behaviors, such as the striatum and nucleus accumbens.[119]

Nrxn3 has been considered a potential candidate gene for drug addiction.[120] In European and African American samples, 38 loci containing single nucleotide polymorphisms (SNPs) were strongly associated with amount of opioid abuse, including *Nrxn3*.[116] An animal experiment found that neurexin-3 expression is increased in the globus pallidus of short-term cocaine-addicted mice.[121]

Nrxn3 is a potential factor related to nicotine dependence. A *Nrrxn3* SNP (rs1004212) is associated with the degree of smoking in patients with schizophrenia.[117] Meanwhile, homozygous individuals with the C allele of rs1004212 smoked more often every day than heterozygous individuals.[117] Six SNPs in *Nrxn3* are associated with the maintenance of smoking.[122] Heterozygosity in the C allele of rs1004212 or the G allele of rs11624704 are associated with nicotine use disorder in Turkish individuals.[123] However, some *Nrxn3* SNPs exert the opposite effect. A 16 KB block of *Nrxn3* that includes two SNPs (rs221473 and rs221497) is related to a lower risk of smoking among Spanish Caucasians, suggesting that different SNPs may lead to completely different effects.[124]

Ethanol not only affects ion channels and neurotransmitter receptors but also changes synaptic adhesion.[125] Sufficient evidence suggests that neurexin-3 is associated with alcohol dependence.[118] The SNP (rs8019381) at the SS5 exon 23 donor sites of *Nrxn3* has been shown to be associated with alcohol dependence.[126] Compared with homozygous individuals with the addiction-related rs8019381 SNP, heterozygous individuals express lower levels of two splice variants that encode transmembrane neurexin-3 isoforms in the cerebral cortex,[126] suggesting that alcohol dependence may be associated with alternative splicing of exon 23 and changes in synaptic connections. In men, the SNP rs1004212 is also associated with alcohol problems, but women are not affected, indicating that the association between alcohol use problems and *Nrxn3* SNPs may be related to sex.[127]

Neurexin-3 and autism

Autism is a neurodevelopmental disorder, and impaired social interaction, limited interests, and restricted and repetitive behaviors are all typical features of autism. The disease starts in early childhood, and most of the pediatric patients exhibit varying degrees of mental retardation.[128] The prevalence of autism is approximately 15–20 per 10,000 and four times lower in women than in men.[129] The pathogenesis of autism is associated with genetic factors, but the specific mechanism of autism remains unclear.

Rare genetic or neonatal microdeletions at 14q24.3–31.1, which overlap exons of the α and/or β subtypes of neurexin-3, have been identified in some Canadian patients diagnosed with autism. A clinical study reported that the deletions of neurexin-3 are present in carrier parents who have not been formally diagnosed with autism and a father confirmed to have subclinical autism.[130] In addition, a rare exonic microdeletion of *Nrxn3* was detected in a three-generation Chinese family using a chromosomal microarray analysis (CMA). The proband has a normal karyotype, but the microdeletion that contains an exon of *Nrxn3* at 14q24.3–q31.1 was identified using CMA. Both the mother and the maternal grandfather possess the same microdeletion. Based on clinical observations of the clinical symptoms of members of this family, facial dysmorphism and schizophrenia are potential new clinical characteristics of *Nrxn3* haploinsufficiency.[131]

Other diseases

Accumulating clinical studies have shown that neurexin-3 is associated with other neurological disorders. Neurexin-3 is related to schizophrenia in the Chinese Han population; among seven SNPs, three (rs7157669, rs724373 and rs7154021) are associated with schizophrenia.[132] Anti-neurexin-3 antibody may be related to potential autoimmune encephalitis associated with yellow fever vaccine.[133] Proteomic analysis revealed a decrease in neurexin-3 levels in the CSF of patients with major depressive disorder.[134] Delayed encephalopathy after acute carbon monoxide poisoning is associated with two SNPs (rs2196447 and rs11845632) located in *Nrxn3*.[135] Some *Nrxn3* SNPs may be associated with borderline personality disorder phenotypes. Three SNPs (rs10144398, rs10151731, and rs10083466) are associated with identity disturbance, emotional instability and positive screening.[136] The *Nrxn3* rearrangement may be related to cluster headache (CH) in some cases.[137]

Accumulating evidence from animal models also suggests that neurexin-3 may be involved in the various pathophysiological mechanisms. In an experimental autoimmune encephalopathy (EAE) mouse model that represents multiple sclerosis, an increase in SS4 exon inclusion in *Nrxn3* was detected in the mouse prefrontal cortex, consistent with IL-1β expression, indicating that neuroinflammation may regulate the alternative splicing of *Nrxn3*.[138] Circular RNA-0001367 (circ_0001367) is a differentially expressed circRNA that indirectly regulates the expression and function of neurexin-3 through microRNA-431 (miR-431) to prevent the development of glioma, suggesting that neurexin-3 may be involved in the pathogenesis of glioma.[139] Neurexin-3 may be correlated with empathy-related fear. Selective loss of neurexin-3 in somatostatin-expressing (SST+) interneurons in the anterior cingulate cortex (ACC) damages inhibitory synaptic transmission in SST + neurons and significantly enhances observational fear.[140] The neurexin-3α (SS4+) mRNA level in rat hippocampal CA1 neurons is increased following transient global ischemia.[141]

Conclusions and future perspectives

While the structure of neurexin-3 and its roles in synapse development and functions are well established, the key molecular events involved in neurodegenerative and neuropsychiatric diseases remain unclear. Further studies are needed to determine the roles of neurexin-3 in various diseases and to understand the specific mechanisms, with the exception of some *Nrxn3* SNPs. In particular, we must understand how the neurexin-3β (SS4+)-neuroligin-1-AMPARs and neurexin-3α (SS4+)-neuroligin-2-$GABA_A$Rs pathways dictate or coordinate the function of excitatory and inhibitory synapses in the CNS, respectively. This information may provide opportunities to develop new interventions for AD, autism and other related diseases.

Conflict of interests

The authors have no conflict of interests to declare.

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