## Synapse development in health and disease

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Synapse development in health and disease

Abbreviated title: Disorders of Synapse Development

Authors:

Jan Elizabeth Melom

Department of Biology

The Picower Institute for Learning and Memory

Massachusetts Institute of Technology

43 Vassar St. 46-3251

Cambridge, MA 02139

jmelom@mit.edu

J. Troy Littleton (corresponding author)

Department of Biology

Department of Brain and Cognitive Sciences

The Picower Institute for Learning and Memory

Massachusetts Institute of Technology

43 Vassar St. 46-3251

Cambridge, MA 02139

troy@mit.edu
Summary

Recent insights into the genetic basis of neurological disease have led to the hypothesis that molecular pathways involved in synaptic growth, development, and stability are perturbed in a variety of mental disorders. Formation of a functional synapse is a complex process requiring stabilization of initial synaptic contacts by adhesive protein interactions, organization of pre- and post-synaptic specializations by scaffolding proteins, regulation of growth by intercellular signaling pathways, reorganization of the actin cytoskeleton, and proper endosomal trafficking of synaptic growth signaling complexes. Many neuropsychiatric disorders, including autism, schizophrenia, and intellectual disability, have been linked to inherited mutations which perturb these processes. Our understanding of the basic biology of synaptogenesis is therefore critical to unraveling the pathogenesis of neuropsychiatric disorders.

Introduction

The formation of a functional, mature neuronal synapse requires a host of molecular players to mediate coordinated pre- and post-synaptic growth. In recent years, clinically diverse disorders such as autism spectrum disorders (ASDs), schizophrenia, epilepsy, and intellectual disability (ID) have been linked to dysfunction of a number of proteins implicated in synaptic development [1,2]. For example, deletions of neurexin-1α, a synaptic cell adhesion protein, were initially identified by large-scale genetic screens in patients with autism and schizophrenia, and subsequently found in patients with severe ID and epilepsy [3]. Mutations in genes encoding the SHANK postsynaptic scaffolding protein family were first identified in a patient with ID, and later associated with autism
and schizophrenia [4,5]. As the number of genes contributing to neuropsychiatric disorders has grown, it has become increasingly clear that pathways contributing to synaptic development and activity-dependent growth are important in their etiology.

Here, we examine genes implicated in synaptogenesis which have been associated with neurological disease. In particular, we highlight the role of these genes in synaptic cell adhesion, organization of pre- and post-synaptic specializations, growth signaling pathways, and endosomal function. Insights into the function of these genes at the *Drosophila* neuromuscular junction (NMJ) are emphasized, as this model glutamatergic synapse is well studied within the context of activity-dependent synaptic development.

**Specification and stabilization of the synapse is mediated by cell adhesion proteins**

One of the earliest steps in synaptogenesis is the induction and adhesion of precisely opposed pre- and post-synaptic domains. Multiple transsynaptic cell adhesion complexes operate in parallel to ensure proper synaptic alignment (Figure 1). Mutation of synaptic adhesion proteins has been linked to various neuropsychiatric disorders, indicating that abnormal formation or maintenance of synaptic contacts may be impaired in several neurological diseases [6]. Members of the cadherin and immunoglobulin protein superfamilies play an important role in mediating synaptic adhesion. Synaptic cell adhesion molecule 1 (SynCAM1) is an immunoglobulin domain containing protein found on axonal growth cones, and is one of the first transsynaptic adhesion molecules to assemble at developing synapses [7]. Homophilic binding of SynCAM1 across the synaptic cleft promotes synapse formation in cultured hippocampal neurons [7]. Recently, missense mutations in SynCAM1 have been identified in patients with ASDs, and a
SynCAM1 knock-out mouse was found to have impaired social behaviors [8,9]. Mutations in a Drosophila SynCAM-like protein, FasII, disrupt synaptic growth at the NMJ due to synaptic destabilization [10]. Several members of the cadherin protein family, including protocadherins, also mediate transsynaptic adhesion and have been linked to ASDs in genome-wide association studies [11,12]. The role of cadherins and protocadherins in synaptogenesis is poorly understood, although they likely participate in synapse formation and refinement.

One well studied transsynaptic adhesion complex is neurexin-neuroligin. Mutations in neurexins and neuroligins have been identified in several families with heritable neurological disorders, including ASDs, schizophrenia, and ID [13]. The binding of a presynaptic neurexin to a postsynaptic neuroligin is required for synapse function, but whether neurexin-neuroligin binding affects synapse formation is unclear [13]. In Drosophila, mutations in the homolog of neurexin, dnrx, cause reduced synaptic growth and defective presynaptic active zone formation [14]. Likewise, mutations in neuroligin, dnlg1, decrease synaptic growth due to deficits in bouton addition and postsynaptic differentiation [15]. In mammals, a synaptogenic role for neurexins is suggested by the observation that neurexins bind the postsynaptic glutamate receptor δ2 via an adaptor protein, which is essential for excitatory synaptogenesis in the cerebellum [16].

Another family of postsynaptic cell adhesion proteins, the leucine-rich repeat transmembrane proteins (LRRTMs), were identified in a screen for synaptogenic proteins and subsequently found to bind neurexins [17-19]. Similar to neurexins and neuroligins, mutations in LRRTMs have been associated with both autism and schizophrenia, and are
thought to promote synapse formation [20,21]. The netrin-G ligand (NGL) proteins form a subfamily of LRRTM synaptic adhesion molecules implicated in the etiology of schizophrenia and bipolar disorder [22,23]. NGLs promote excitatory synaptogenesis by binding to presynaptic netrin G and LAR receptors, as well as postsynaptic proteins [23]. The combinatorial action of neurexins, neuroligins, LRRTMs, and NGLs is not well understood, and these complexes may act redundantly at the synapse.

Several other cell adhesion proteins whose synaptic role is unclear have also been associated with neurological disease. Contactin-associated protein-2 (CNTNAP2) is a single-pass transmembrane protein distantly related to neurexins, and is associated with ASDs, schizophrenia, ID, and epilepsy by genome-wide linkage analysis [24]. A synaptic role for CNTNAP2 was recently demonstrated in Drosophila, in which neuronal knockdown of CNTNAP2 (NrxIV) resulted in aberrant synaptic morphology and misregulation of a critical active zone component [3]. Another recently recognized synaptic cell adhesion molecule is amyloid precursor protein (APP), a type I membrane protein involved in Alzheimer’s disease pathogenesis. APP was found to promote synaptogenesis in culture and is required both pre- and post-synaptically to regulate mammalian NMJ structure and function, presumably by forming a transsynaptic adhesion complex [25]. Similarly, regulation of the Drosophila APP homolog critically controls synaptic growth at the NMJ [26]. The involvement of various synaptic cell adhesion proteins in neuropsychiatric diseases indicates that abnormal synapse formation or specification may be a common risk factor for mental disorders.

**Organization of pre- and post-synaptic domains by scaffolding proteins**
Formation of a functional synapse requires assembly of synaptic proteins into domains specialized for neurotransmitter release and reception. Scaffolding proteins are critical mediators of this process on both sides of the synapse. Scaffolds are composed of multiple protein-protein interaction domains, and form a physical link between adhesion proteins, ion channels, neurotransmitter receptors, intracellular signaling cascades, and the actin cytoskeleton.

One of the strongest links between synaptic organization and neurological disease is the SHANK family of scaffolding proteins. SHANK proteins localize to the excitatory postsynaptic density and contain multiple protein-protein interaction domains. Mutations in SHANK2 and SHANK3 have been identified in patients with autism, mental retardation, and schizophrenia [4,5]. In conjunction with HOMER, SHANKs form a mesh-like matrix at the postsynaptic density which regulates the morphology of dendritic spines and recruits postsynaptic density proteins [27]. The connection between SHANK scaffolds and mental dysfunction was recently strengthened by the finding that FMRP, the RNA binding protein mutated in Fragile X syndrome, inhibits the translation of SHANK1 [28]. Fragile X syndrome is the most common inherited form of mental retardation and is associated with abnormal dendritic morphology. Deregulated synthesis of SHANK scaffolds may contribute to this abnormal morphology.

The membrane-associated guanylate kinase (MAGUK) scaffolding protein family has multiple members that are present pre- and post-synaptically. One MAGUK family member, CASK, is a causative gene in X-linked mental retardation [29]. CASK is an essential vertebrate protein involved in assembly and functional maturation of the synapse [30]. Interestingly, CASK directly interacts with neurexin-1, which it
phosphorylates in a developmentally regulated manner [29], although the functional significance of neurexin phosphorylation is unclear. CASK also interacts with the adhesion protein SynCAM1, which recruits CASK from cytosol to the membrane [31]. The strong association of mutations in presynaptic CASK and postsynaptic SHANKs with neurological disease highlights an important role for synaptic scaffolding proteins.

**Activity-dependent synaptic development depends on intercellular signaling pathways**

Once a synapse has formed, neuronal activity plays an essential role in shaping and maintaining synaptic connections [32]. Anterograde and retrograde secreted signaling molecules, including the Wnts and BMPs, regulate synaptic growth (Figure 1) and may also contribute to the development of neurological disease. The Wnt family of signaling molecules participates in a broad range of processes, from neurogenesis to synaptic plasticity. Several Wnt pathway components have been implicated in the pathogenesis of schizophrenia and Alzheimer’s, raising the question of how this pathway functions at the synapse [33,34]. At the *Drosophila* NMJ, the Wnt pathway signals bidirectionally to mediate synaptic growth in response to patterned neuronal stimulation [35]. Presynaptically, Wnt regulates cytoskeletal dynamics through inhibition of glycogen synthase kinase 3β (GSK-3β), a cytoplasmic pathway component which interacts with the microtubule and actin cytoskeletons. Single nucleotide polymorphisms in GSK-3β are associated with schizophrenia, and GSK-3β was recently found to interact with DISC1, a major schizophrenia susceptibility gene [33,36]. Postsynaptically, Wnt signaling causes translocation of the cleaved receptor Frizzled into the nucleus, which promotes growth of
the postsynaptic membrane [35,37]. Polymorphisms in Wnt signaling components have been linked to neurodegenerative diseases such as Alzheimer’s, though whether this late-onset disease is related to Wnt’s synaptogenic function is unclear [38].

A second synaptic growth signaling pathway is the bone morphogenetic protein (BMP) pathway, dysregulation of which is associated with multiple neurodegenerative diseases [39]. Retrograde BMP signaling at the Drosophila NMJ is initiated by release of the BMP ligand Gbb by the muscle, which triggers a signaling cascade in the presynaptic neuron [40]. BMP signaling activates a transcription factor which targets multiple genes, including a recently described Rac GTPase activator [40]. Rac GTPases regulate the actin cytoskeleton and are important for growth of dendritic spines, which mechanistically links BMP signaling to structural synaptic plasticity [41]. Recently, synaptic Rac1 GTPase activity was shown to be regulated by FMRP and DISC1, raising the possibility that both Fragile X and schizophrenia may have impaired BMP mediated activity-dependent synaptic growth [42,43].

**Synaptic growth signaling requires proper endosomal trafficking**

Regulation of endosomal traffic is a critical component of synaptic growth and development. Growth signals released during activity, such as the Wnts and BMPs described above, bind to synaptic transmembrane receptors and are internalized as receptor-ligand signaling complexes. These signaling complexes are transported within the endosomal system, in which receptors signal from an early endosome population before signal inactivation in the recycling endosome or lysosome (Figure 2). Many of the synaptic growth mutants identified in Drosophila alter endocytic trafficking, with
mutants that disrupt formation of signaling endosomes causing reduced synaptic growth, and mutants altering traffic to the recycling endosome or lysosome causing synaptic overgrowth due to enhanced signaling [44,45]. Retrograde transport of signaling endosomes containing neurotrophins is also important for neuronal survival and may be disrupted in neurodegenerative diseases such as Alzheimer’s and Down’s syndrome [46].

The hereditary spastic paralegias (HSPs) are a group of neurological disorders caused by mutations in several genes which regulate the endosomal trafficking of BMP receptors [39,47]. Mutation of the early endosomal HSP gene NIPA1 results in less efficient sorting of the type II BMP receptor to the lysosome, suggesting that HSP etiology may involve upregulation of BMP signaling due to altered endocytic trafficking [47]. Disruption of endosomal function is also implicated in neurodevelopmental disorders, including autism and attention-deficient/hyperactivity disorder, for which recent genome-wide association and copy number variation studies have implicated the Na\(^+\)/H\(^+\) exchangers 6 and 9 (NHE6, 9) [48,49]. NHE6 and 9 are thought to fine-tune the pH of early endosomes, disruption of which may alter ligand-receptor dissociation and lead to abnormal synaptic growth signaling [50]. Endosomal regulation of signaling pathways involved in synaptic growth and development appears critical for activity-dependent circuit refinement.

**Conclusion**

Abnormal synaptic development is thought to underlie multiple neurodevelopmental disorders which present with clinically distinct phenotypes. Altered synaptic structure or function may also lead to changes in neuronal connectivity which
predisposes an individual to neurodegenerative disease. We have highlighted several
genes implicated in neuropsychiatric disease which have defined roles in synaptic
development. Mutations in different genes or gene combinations may lead to a similar
disease phenotype; thus understanding common molecular pathways in which these genes
function is important for unraveling disease pathogenesis. Recent advances in genome
sequencing and large scale genetic screening has identified numerous susceptibility loci
which converge on pathways involved in synaptogenesis. Our understanding of the basic
biology of synaptic growth and development is critical to furthering our understanding of
synaptic dysfunction in brain disorders.

**Figures Legends**

**Fig. 1.** Proteins implicated in neuropsychiatric disorders that participate in synaptic
growth and development. Formation of a functional synapse requires transsynaptic
interaction of cell adhesive proteins, organization of synaptic domains by scaffolding
proteins, intercellular growth signaling pathways, actin cytoskeletal remodeling, and
endosomal trafficking of receptor-ligand signaling complexes. Many of the proteins
associated with brain disorders have been implicated in these processes.

**Fig. 2.** Endosomal regulation of synaptic growth signaling pathways. Growth signals
released during neuronal activity, including Wnts and BMPs, bind to presynaptic
receptors and are internalized as receptor-ligand signaling complexes. These complexes
signal from early endosomes, and are recycled to the plasma membrane through the recycling endosome or degraded in the lysosome.

References


The authors report that SynCAM1 is required on developing neuronal growth cones to regulate neurite morphology. This study is the first to identify a role for SynCAM1 in events prior to synapse formation.


This group identified Drosophila neuroligin, dnlg1, in an unbiased genetic screen for mutations that disrupt synaptic growth. Dnlg promotes postsynaptic differentiation and addition of new synaptic boutons at the NMJ, but is not required for initial synaptic formation.


This study indicates that a transsynaptic interaction between the postsynaptic glutamate receptor 2δ subunit, Cbln1, and the presynaptic transmembrane protein Neurexin mediates synaptogenesis in vivo.


This study, along with [19], identifies the LRRTM protein family as novel Neurexin ligands which promote excitatory synapse formation. Neurexin binding to LRRTM was found to depend on alternative splicing of Neurexin, which may contribute to specificity of synapse formation.


This study also describes LRRTM-Neurexin interaction in excitatory synaptogenesis independent of [18]. This group found that LRRTM alters synaptic function by regulating surface expression of AMPA receptors.


This study identifies a transsynaptic interaction between presynaptic LAR and postsynaptic NLG-3 which appears to function in excitatory synaptogenesis.


By studying mice lacking APP either in neurons or muscles, the authors found that APP is required in both compartments to regulate structure and function of the developing neuromuscular junction. The authors also found that the intracellular domain of SynCAM1 can functionally replace the APP intracellular domain in a synaptogenesis culture assay, indicating that common intracellular interactions may occur downstream of both synaptic cell adhesion molecules.


The authors of this study found that the postsynaptic proteins Homer and Shank interact to form a mesh-like network which may serve as a structural framework for the postsynaptic density. Disruption of the ability of Homer to multimerize reduces the postsynaptic localization of PSD-95 and affects spine structure and function.


The authors demonstrate a novel role for DISC1 in neural progenitor cell proliferation that depends on DISC1 regulation of GSK3β, a downstream mediator of the Wnt signaling pathway. These findings suggest that loss of DISC1 may affect neuronal development, which could lead to enhanced susceptibility to psychiatric disorders.


This study shows that retrograde BMP signaling at the NMJ regulates synaptic growth through transcriptional activation of the Rac GEF Trio, a cytoskeletal regulator. This finding is an important first step in elucidating the mechanism by which the BMP pathway controls synaptic remodeling.


