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Dynamic Aspects of Synapse Formation

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Abstract

The mammalian central nervous system (CNS) requires the proper formation of exquisitely precise circuits to function correctly. These neuronal circuits are assembled during development by the formation of synaptic connections between thousands of differentiating neurons. Proper synapse formation during childhood provides the substrate for cognition while improper formation or function of these synapses leads to neurodevelopmental disorders, including mental retardation and autism. Recent work has begun to identify some of the early cellular events in synapse formation as well as the molecular signals that initiate this process. However, despite the wealth of information published on this topic in the past few years, some of the most fundamental questions about how, whether, and where glutamatergic synapses form in the mammalian CNS remain unanswered. This review focuses on the dynamic aspects of the early cellular and molecular events in the initial assembly of glutamatergic synapses in the mammalian CNS.

Keywords

development; axon; dendrite; vesicle trafficking; glutamate receptors; cell adhesion

INTRODUCTION

Proper synapse formation in the central nervous system (CNS) during childhood provides the substrate for human perception, learning, memory, and cognition. Conversely, improper formation or function of these synapses leads to many disorders of learning and memory, including autism and other neurodevelopmental disorders. Excitatory synapse formation in the CNS requires the coordinated assembly of large numbers of protein complexes and specialized membrane domains required for synaptic transmission. Recent work has identified some of the early cellular events in synapse formation as well as molecular signals that initiate this process (Scheiffele 2003; Waites et al 2005). Despite these advances, a number of fundamental issues, including whether, where, and how glutamatergic synapses form, remain unresolved. This review focuses on the early cellular and molecular events in the initial assembly of glutamatergic synapses in the mammalian CNS. Other important events that affect or involve synaptogenesis, such as the guidance of axons to their correct targets, proper growth of dendrites, determination of the specificity of connections, and activity-dependent modification of excitatory synapses at later stages of postnatal development, are not covered here but have been extensively reviewed elsewhere. In addition, recent advances using non-mammalian animal models as well as the extensive literature covering the mammalian neuromuscular junction are not covered here due to space constraints.

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In the mammalian CNS, most excitatory synapses are highly asymmetric junctions composed of a presynaptic terminal and a postsynaptic density (PSD). The axonal presynaptic terminal is packed with hundreds to thousands of small (~50nm), clear synaptic vesicles (SVs) filled with the neurotransmitter glutamate. The fusion of these vesicles with the plasma membrane in response to an action potential occurs at specific sites of the presynaptic terminal called the active zone (Burns & Augustine 1995; Phillips et al 2001). SV fusion results in the release of glutamate into the synaptic cleft, an ~20–25 nm space between the pre-and postsynaptic cells (Schikorski & Stevens 1997). Once released, glutamate crosses the cleft and binds to glutamate receptors found in the PSD, an electron-dense meshwork of proteins in the dendrite (Kennedy 2000). Opening of the two major classes of ionotropic glutamate receptors, the NMDA and AMPA receptors (NMDARs and AMPARs; Hollmann & Heinemann 1994), then leads to influx of ions, local depolarization, and activation of voltage-gated ion channels as well as a number of signaling cascades. This series of events is called synaptic transmission and forms the basis for most information transfer within the CNS.

Recently, there has been tremendous progress in identifying the molecular components of the CNS glutamatergic synapse (Collins et al 2006; Phillips et al 2001; Sheng & Kim 2002; Zamorano & Garner 2001). The presynaptic terminal and PSD are composed of a remarkably large number of proteins; recent estimates based on mass spectroscopy suggest that there could be upwards of 600 distinct proteins found in the PSD alone (Collins et al 2006). In general, this large number of proteins can be grouped into several protein classes by function. Presynaptically, there are numerous proteins found on SVs that are critical for their proper localization and calcium-dependent fusion (Sudhof 2004). Several scaffolding proteins tether these SVs to the active zone and to the actin cytoskeleton and link them to signaling molecules (Phillips et al 2001). Postsynaptically, glutamate receptors and other ion channels are tethered to the PSD through interactions with a large number of scaffolding proteins, which play critical roles in glutamate receptor trafficking, synaptic transmission, and synaptic plasticity (Kennedy 2000; Kim & Sheng 2004; Montgomery et al 2004). In addition to proteins the mediate synaptic transmission, there are also proteins in the pre-and postsynaptic membranes that bind to each other across the synaptic cleft called trans-synaptic adhesion molecules. Many of these trans-synaptic molecules have been implicated in regulating the initial stages of synapse formation at CNS synapses, as well as in modulating synaptic plasticity (Craig et al 2006; Garner et al 2006; Scheiffele 2003; Waites et al 2005).

From a molecular perspective, synaptogenesis can be defined as the assembly of these hundreds of pre-and postsynaptic proteins into the highly specific structure of the synapse. In order for glutamatergic synapses to form and function properly, these major components of the synapse—SVs, glutamate receptors, active zone proteins, PSD scaffolding proteins, and trans-synaptic adhesion molecules—must each accumulate at sites of physical contact between axons and dendrites with precise timing. Given the importance of synaptogenesis for proper functioning of the nervous system, it is surprising how little we know about the cellular and molecular mechanisms of synapse formation. One of the primary reasons for our lack of understanding of synapse formation at a molecular level comes from the technical difficulty of studying the formation of individual synapses in intact tissue. Synapses are tiny structures of less than a micron in diameter and are packed into the CNS at an incredibly high density (estimates range from an average of 200 million synapses per mm^3 in newborn rat cortex to 4 billion synapses per mm^3 in 5-week-old rat cortex; De Felipe et al 1997), making it almost impossible to study the formation of individual synapses in intact tissue. In addition, synapses assemble in any given area of the CNS over a protracted period of development; at any given time, the age of synapses on a single neuron could span days to weeks, or even months.

The convergence of several technical advances has led to a recent explosion in the number of studies in the field of synaptogenesis. First, many of the molecular components of the presynaptic terminal and PSD were recently identified and cloned (Collins et al 2006; Kennedy 2000; Kim & Huganir 1999; Montgomery et al 2004; Sheng & Kim 2002; Zamorano & Garner 2001). Second, widespread use of primary neuronal cultures, coupled with immunocytochemistry, has readily allowed visualization of the subcellular distribution of synaptic proteins and quantification of synaptic density. Moreover, visualization of GFP-tagged proteins in living, cultured neurons using time-lapse imaging techniques has revealed the distribution and dynamics of these proteins before, during, and after the bulk of synapse formation in these cultures. Finally, several key assays have been developed to allow for screening of molecular signals involved in synapse formation. The convergence of these technical advances has greatly expanded our understanding of the cellular and molecular mechanisms of CNS synapse formation.

CELLULAR MECHANISMS

In order for synapses to form, pre-and postsynaptic proteins must be synthesized and transported to sites of contact between axons and dendrites. In general, pre-and postsynaptic proteins are present in neurons before synapses are formed (Fletcher et al 1991; Rao et al 1998). Recently, many of these synaptic proteins have been localized to small, heterogeneous clusters of proteins, called transport packets, which are mobile within axons and dendrites before most synapses have formed. These mobile transport packets are recruited to sites of axodendritic contact with a rapid time-course but variable hierarchy, depending on the nature of the target and possibly the age of the neurons. The later stage of synaptic maturation is less well-defined and occurs over a more protracted time-course.

Transport of presynaptic proteins

Presynaptic proteins are transported in multi-molecular complexes before and during synaptogenesis (Ziv & Garner 2004). In young neurons, at least two types of presynaptic precursors are present— PTVs and STVs (piccolo transport vesicles and synaptic vesicle (SV) protein transport vesicles, respectively; Sabo et al 2006; Zhai et al 2001). PTVs are 80 nm dense-core vesicles (Zhai et al 2001) that move rapidly within axons of young CNS neurons at rates of greater than 0.35μm/s (Shapira et al 2003). The mobility of PTVs is saltatory; they can move in both directions, split into smaller clusters, or coalesce into larger ones (Shapira et al 2003). PTVs carry the active zone proteins piccolo and bassoon as well as other proteins that mediate SV exocytosis, including Munc13, Munc18, syntaxin, and snap25 (Zhai et al 2001). These precursors are assembled in the trans-Golgi network and are transported via Golgi-derived vesicles (Dresbach et al 2006). In contrast, synaptic vesicle (SV) proteins are transported in heterogeneous organelles called STVs that are biochemically and morphologically distinct from PTVs (Zhai et al 2001). STVs carry many SV-associated proteins and other proteins critical for exo-and endocytosis (Ahmari et al 2000; Zhai et al 2001) and exhibit morphologies ranging from pleiomorphic, tubulo-vesicular organelles (Ahmari et al 2000) to clusters of small SV-like vesicles (Kraszewski et al 1995). STVs are highly mobile within axons of young neurons before synapses form; over half of all VAMP2-EGFP puncta are mobile in young cortical neurons with velocities ranging from 0.1 – 1 μm/sec (Ahmari et al 2000; Dai & Peng 1996; Kaether et al 2000; Kraszewski et al 1995; Nakata et al 1998; Sabo et al 2006). Most reports indicate that STVs can split or coalesce during transport and that their movement is saltatory (Ahmari et al 2000; Bresler et al 2004; Dai & Peng 1996; Kraszewski et al 1995; Nakata et al 1998; Sytnyk et al 2002). Reports on directionality of transport are conflicting with some reporting primarily anterograde transport (Ahmari et al 2000; Lee et al 2003; Nakata et al 1998), while others report frequent reversals in direction (Dai & Peng 1996; Kraszewski et al 1995; Sabo et al 2006; Sytnyk et al 2002). The ability of STVs to change direction implies that they are

transported by multiple motor proteins. To date, three anterograde microtubule motor proteins have been identified for SV proteins: conventional kinesin-1 (Leopold et al 1992; Sato-Yoshitake et al 1992), KIF1a (Okada et al 1995; Yonekawa et al 1998) and KIF1Bβ2 (Nakamura et al 2002). In addition, the dynein complex is critical for retrograde transport of STVs in *C. elegans* (Koushika et al 2004) and the actin motor protein myosinVa is transported with STVs along microtubules (Ohyama et al 2001; Prekeris & Terrian 1997).

Interestingly, many laboratories have reported that STVs undergo depolarization-dependent cycling before contact with postsynaptic cells (Dai & Peng 1996; Kraszewski et al 1995; Krueger et al 2003; Matteoli et al 1992; Nakata et al 1998; Sabo et al 2006; Zakharenko et al 1999, but see Ahmari et al 2000). This depolarization-dependent STV cycling is distinct from SV cycling at mature synapses in its calcium dependence (Coco et al 1998), increased sensitivity to brefeldin-A (Zakharenko et al 1999), and reduced sensitivity to tetanus toxin (Verderio et al 1999). Surprisingly, STV cycling occurs within filopodia of axonal growth cones (Sabo & McAllister 2003), as well as along the axon shaft, suggesting that all areas of the axon are capable of releasing the contents of STVs before synapses form. Because these cycling STVs contain the vesicular glutamate transport, vGlut1 (Sabo et al 2006), it is likely that they are capable of releasing glutamate, and possibly other diffusible molecules, along the growing axon before synapses are formed.

Transport of postsynaptic proteins

One of the most critical events in synaptogenesis of glutamatergic synapses is the recruitment of ionotropic glutamate receptors. Although there are many fewer studies of formation of the PSD, it is clear that glutamate receptors and scaffolding proteins are present in dendrites before synapses are formed (Craig et al 1993; Gerrow et al 2006; Washbourne et al 2002; Washbourne et al 2004). Similar to presynaptic STVs, NMDARs are transported in discrete transport packets that move within dendrites bidirectionally with an average velocity of 6–8 μm/min in young cortical neurons (Washbourne et al 2002; Washbourne et al 2004). Although the composition of these NMDAR transport packets has not yet been determined biochemically, retrospective immunostaining suggests that they also transport a scaffolding molecule called SAP-102, but not PSD-95, and an exocyst protein called Sec 8 (Sans et al 2003; Washbourne et al 2004), and that a subset of NMDAR transport packets also carries AMPA receptors (AMPARs; Washbourne et al 2002). It has been suggested that these NMDAR transport packets are transported by the anterograde microtubule motor KIF17 through interactions with CASK and mlin-10 (Guillaud et al 2003; Setou et al 2000). However, the rates of transport of KIF17 are an order of magnitude faster than those for NMDAR transport packets in young cortical neurons (Guillaud et al 2003; Washbourne et al 2002), indicating that additional and/or alternative motor proteins must be involved in the rapid, bidirectional transport of NMDARs. Surprisingly, NMDAR transport packets undergo a novel form of transport during synapse formation—cycling with the plasma membrane during pauses in their trafficking in intracellular vesicles along microtubules (Washbourne et al 2004). This cycling of NMDAR transport packets suggests that they may be capable of sensing glutamate during their transport.

The conclusion that glutamate receptors are trafficked in discrete transport packets has recently been questioned (Bresler et al 2004) and evidence has been provided that NMDARs are recruited to synapses from a diffuse pool (Bresler et al 2004). These authors suggest that the mode of NMDAR transport might depend on the age or type of neuron analyzed. However, discrete mobile NMDAR transport packets are clearly present in both young and older cortical and hippocampal neurons when visualized using five separate constructs (NR1-EGFP; NR1-DsRed, EGFP-NR1; EGFP-NR2B; EGFP-NR2A) and two different transfection protocols (Washbourne et al 2004; E. Clark and P. Washbourne, unpublished data). In addition, discrete NMDAR transport packets can be observed by surface labeling

endogenous NMDARs (Washbourne et al 2004), indicating that these mobile clusters are not an artifact of overexpression. Finally, these discrete, mobile transport packets are recruited to axodendritic contacts as one of the first events during synapse formation (Washbourne et al 2002).

This debate over whether postsynaptic proteins are transported in discrete packets, or rather coalesce from diffuse cytoplasmic pools, also clouds our understanding of the trafficking of postsynaptic scaffolding proteins. Initially, a number of reports described the presence of discrete, but mostly immobile clusters of PSD-95 in neurons before synapses had formed (Bresler et al 2001; Friedman et al 2000; Marrs et al 2001; Okabe et al 1999; Okabe et al 2001; Rao et al 1998; Sans et al 2000; Washbourne et al 2002). However, others have shown evidence for modular transport of PSD-95 (Prange & Murphy 2001) and preformed scaffold complexes containing PSD-95, GKAP, and Shank (Gerrow et al 2006). Thus, similar to STVs and PTVs in the axon, postsynaptic proteins appear to be trafficked in multi-molecular transport packets that are mobile within dendrites of young neurons and can accumulate at synapses during their formation (see below). In contrast to presynaptic development, however, these postsynaptic transport packets are less mobile than STVs or PTVs and may transport more overlapping cargo. In addition, gradual accumulation of proteins at glutamatergic synapses may play a greater role in the assembly of the PSD as compared to the presynaptic terminal. In sum, despite the assertion that postsynaptic assembly is fundamentally different from presynaptic zone assembly (Bresler et al 2004), there seem to be as many similarities as differences in the rules for trafficking of pre-and postsynaptic proteins. Increasing the number of PSD proteins examined, determining which of these proteins are trafficked together using imaging and biochemical approaches, and using alternative methods for visualizing endogenous PSD proteins may clarify these issues in the future.

Contact and recruitment of synaptic proteins

For a synapse to form, contact must first be made between the presynaptic axon and the postsynaptic dendrite. A number of possible types of contact have been described. Axodendritic contacts can be initiated by either filopodia from axonal growth cones (Meyer & Smith 2006; Washbourne et al 2002) or from dendritic growth cones (Sabo et al 2006). In addition, synaptogenesis can be initiated by filopodia from axons or dendrites to form en passant synapses (Ahmari et al 2000; Dailey & Smith 1996; Fiala et al 1998; Gerrow et al 2006; Jontes et al 2000; Niell et al 2004; Portera-Cailliau et al 2003; Saito et al 1992; Washbourne et al 2002; Ziv & Smith 1996). Finally, synapses can form from contact between axon and dendrite shafts (Friedman et al 2000; Gerrow et al 2006; Washbourne et al 2002). Both axonal and dendritic filopodia contact their targets in a seemingly random manner; most of these contacts are fleeting and transitory, resulting in retraction of the filopodium (Niell et al 2004; Okabe et al 2001; Sabo et al 2006; Ziv & Smith 1996). However, a small subset of these filopodia become stabilized and nascent synapses subsequently form at those sites. The signals that lead to the stabilization of such filopodia, thought to be cell adhesion molecules, are likely to be some of the first signals that lead to synapse formation.

One of the most elusive questions in the field of synaptogenesis has been whether synapses can form at any site along axons and dendrites where filopodia are stabilized, or whether there are specified sites on these neuronal processes that are predisposed for synaptogenesis. Although current models for synapse formation imply that synapses can form anywhere along the axon or dendrite, there is little experimental evidence supporting this hypothesis. Two recent reports provide the first evidence to date that axons and dendrites may, in fact, contain intrinsic sites for synapse formation. The first report (Sabo et al 2006) shows that presynaptic terminals in young cortical neurons are formed selectively at predefined sites

along the axon. These sites are not determined by contact with neighboring neurons or glial cells. Remarkably, dendritic filopodia are exclusively stabilized at these predefined axonal sites and not at other sites along the axon. Although the composition of these sites has not yet been determined, they coincide with sites where STVs pause and cycle with the axonal membrane, suggesting that release of glutamate and/or other diffusible molecules might selectively attract dendritic filopodia to those sites and stabilize them there (Lohmann et al 2005; Tashiro et al 2003). The second report (Gerrow et al 2006) shows that presynaptic terminals can form at stable sites of accumulation of scaffolding complexes in postsynaptic dendrites. Stable complexes of neuroligin, PSD-95, GKAP and Shank appear to be located at predefined sites in dendrites that can induce the formation of a presynaptic terminal specifically at those sites (Gerrow et al 2006). Taken together, these results suggest that filopodial stabilization may not be a completely stochastic event and that there may well be predefined sites within axons and dendrites that determine where synapses can form along those processes.

Ultimately, the transport of pre-and postsynaptic proteins must be altered by signals at sites of axodendritic contact to cause their accumulation at those sites. The time-course of synaptogenesis is usually measured by determining the time-course of stable accumulation of both “core components” of the glutamatergic synapse—presynaptic vesicles and postsynaptic glutamate receptors. Initial studies of the time-course of recruitment of proteins to new synapses using immunocytochemistry indicated that synapse formation occurs over a protracted time-course of days to weeks (Rao et al 1998). In contrast, recent time-lapse imaging studies have shown that synapse formation occurs on a much shorter timescale of minutes. The first of these reports imaged the recruitment of synaptic proteins to sites of new, stable accumulation of FM4-64 (which labels cycling SVs) and showed that new synapses can form within 1–2 hours after FM4–64 accumulation (Friedman et al 2000). In these experiments, bassoon was recruited to sites of FM4-64 shortly after their stabilization. Although it is often proposed that active zone proteins are recruited to new synapses before SVs (Ziv & Garner 2004), these results instead indicate that recruitment of STVs either occurs simultaneously or is followed quickly by the recruitment of PTVs. Interestingly, accumulation of 2–3 PTVs can account for the total amount of piccolo or bassoon protein at mature synapses (Shapira et al 2003), suggesting that presynaptic sites might be formed by the quantal recruitment of a small number of precursor organelles. However, this rule is unlikely to be the case for STVs as their composition is much more heterogeneous than PTVs (Sabo et al 2006). Moreover, the synaptic content of other proteins critical for cycling, such as SNAP-25, syntaxin, and rab 3, is difficult to model by quantal insertion since these proteins are found on both PTVs and STVs (Zhai et al 2001). Finally, there appear to be at least two distinct mechanisms for accumulation of presynaptic protein at new synapses. In immature neurons, de novo accumulation of presynaptic proteins occurs via rapid recruitment of STVs and PTVs to axo-dendritic contacts (Ahmari et al 2000; Friedman et al 2000; Shapira et al 2003; Washbourne et al 2002). In more mature neurons, portions of existing terminals can split off and become mobilized to form new presynaptic terminals (Krueger et al 2003). These “orphan release sites” are functional presynaptic units containing both cycling SVs and an active zone.

In the initial report of the time-course of synaptogenesis, presynaptic differentiation occurred well before postsynaptic development (Friedman et al 2000). PSD-95 was recruited to sites of FM4-64 after about 30min and accumulated there in a gradual manner (Bresler et al 2001; Friedman et al 2000; Okabe et al 2001); clusters of AMPA and NMDARs were recruited after PSD-95 (Friedman et al 2000). In contrast to this initial report, two recent studies demonstrate different mechanisms of synapse formation with different temporal orders of pre-and postsynaptic protein recruitment. Using the timing of the first stabilized contact between axonal growth cone filopodia and dendrites as a reference, pre-and

postsynaptic proteins were found to be recruited simultaneously to those contacts with a time-course of just under 10 min. In fact, NMDAR transport packets were recruited to these contacts just prior to the recruitment of STVs (Washbourne et al 2002). PSD-95 was found at these nascent synapses with a variable time-course; sometimes coincident with NMDARs and sometimes not present even after 1 hour following NMDAR recruitment. AMPA receptors were not found at these sites within 1 hour of NMDAR recruitment, but were always present after 1 hour, suggesting that young cortical synapses are “silent” for only a brief period shortly after their formation (Washbourne et al 2002). In addition, a recent study suggested yet a third temporal order of recruitment of proteins to new synapses. In this study, the authors focused on determining if postsynaptic proteins might precede the recruitment of presynaptic proteins to new synapses. To that end, the stable accumulation of mobile postsynaptic scaffolding complexes (including PSD-95, GKAP, Shank, and neuroligin) was used as a reference (Gerrow et al 2006). Although these mobile scaffolding complexes can also be recruited to sites of presynaptic protein, a significant proportion of these complexes are present at sites where STVs will accumulate within 2 hours of their stabilization (Gerrow et al 2006).

Taken together, there appear to be multiple mechanisms for the recruitment and stabilization of pre-and postsynaptic proteins to new sites of axo-dendritic contact. The differing time-courses and hierarchies of recruitment of synaptic proteins may by dictated by the different types of synapse formation observed (shaft/shaft versus filopodial-initiated synapse formation) or by the differing ages of cultured neurons examined. Because this field is relatively new, these kinds of discrepancies are to be expected. However, these issues must be clarified in the future by additional studies using multiple imaging approaches accompanied by direct comparisons of the cellular mechanisms of synaptogenesis at multiple stages of neuronal maturation. It is also important to note that most studies of the time-course of synaptogenesis to date have focused on cultured neurons; in the future, it will be essential to develop methods to study the time-course of synaptogenesis at identified, single synapses in vivo.

Synapse maturation

Although the initial assembly of a synapse can be quite rapid (occurring within minutes of contact), the development of a mature synapse is generally prolonged as evidenced by the delay in formation of its mature ultrastructure (Ahmari & Smith 2002) and in its development of mature electrophysiological properties (Bolshakov & Siegelbaum 1995; Chavis & Westbrook 2001; Liu & Tsien 1995; Mohrmann et al 2003; Tovar & Westbrook 1999). One of the most dramatic events in the maturation of glutamatergic synapses on CNS excitatory neurons is the change in their physical location. Synapses are initially formed on dendritic filopodia and dendrite shafts, but later these synapses are primarily located on dendritic spines. Despite the ongoing controversy about whether filopodial synapses transform directly into spine synapses (Fiala et al 1998; Okabe et al 2001; Ziv & Smith 1996), dendritic spine morphogenesis is clearly a critical event in the maturation of glutamatergic synapses (see Yuste & Bonhoeffer 2004 and Tada & Sheng 2006 for recent reviews).

In general, synaptic maturation consists of synapses growing larger and the amount of pre- and postsynaptic protein increasing considerably. So far, only the core components of the synapse—its SVs, presynaptic active zone, postsynaptic ionotropic glutamate receptors, and directly associated scaffolding proteins—have been examined for the time-course of their recruitment to nascent synapses. Recruitment of the remaining, extremely large number of proteins to nascent synapses remains a complete mystery. Some of these proteins might be added to the synapse only at later stages of synaptic maturation. For example, AMPARs and their associated scaffolding proteins seem to be part of a second wave of protein recruitment

to nascent synapses that may serve to stabilize the nascent synapse and mediate synaptic plasticity (Malenka 2003; Song & Huganir 2002). By studying the time-course and physiological impact of recruitment of each synaptic protein, it is possible that we may eventually be able to define each stage in the lifetime of a synapse by its molecular composition. However, we are currently far from understanding the precise molecular differences between a nascent synapse, a stabilized synapse, a destabilized synapse, a mature synapse, and an aging synapse.

MOLECULAR MECHANISMS

In order for synapses to form so quickly, specific contacts between axons and dendrites must initiate signals that lead to rapid accumulation of pre-and postsynaptic proteins. In the past five years, an increasingly large number of molecular signals have been identified that play a role in synapse formation. The large majority of these molecules are called synaptogenic since they contribute to the formation, or genesis, of synapses.

Synaptogenic molecules

Trans-synaptic signaling molecules—The initial formation of contacts between axons and dendrites appears to be mediated by trans-synaptic adhesion molecules. This class of synaptogenic molecules includes the cadherins, integrins and members of the immunoglobulin (Ig) superfamilies, including sidekicks, NCAM, nectins, neuroligins, SynCAMs, SALMs, neuronal pentraxins, and ephrins (Akins & Biederer 2006; Scheiffele 2003; Waites et al 2005). Many of these molecules are found in neurons at mature synapses in both the presynaptic terminal and PSD (Li & Sheng 2003; Scheiffele 2003; Sytnyk et al 2004; Ziv & Garner 2004). Trans-synaptic molecules are attractive candidates to regulate synapse formation since they can initiate simultaneous bidirectional signaling in the axon and dendrite; this kind of signaling could be critical for the rapid and simultaneous recruitment of STVs and NMDARs to new axodendritic contacts (Washbourne et al 2002). Functional studies of these molecules indicate that they are involved in maintaining synapses and dendritic spines and many are involved in synaptic plasticity (Li & Sheng 2003). Each of these molecules has also been implicated in the initial formation of synapses.

The first class of molecules implicated in promoting axo-dendritic adhesion during synapse formation is the cadherins. Cadherins signal through binding to α-and β-catenins which in turn bind to the actin cytoskeleton (Daniels et al 2001; Ivanov et al 2001). Several studies support the notion that cadherins mediate selective adhesion of specific pre-and postsynaptic cells (Benson et al 2001; Shapiro & Colman 1999). In addition, a role for cadherins in the initial stages of synapse formation is supported by their rapid appearance at developing synapses (Benson & Tanaka 1998) and by the decrease in excitatory synapse number caused by expression of a dominant-negative form of N-cadherin in young neurons (Bozdagi et al 2004; Togashi et al 2002). This effect of cadherins in promoting synapse assembly appears to depend on interactions with p120catenin, which enhances cadherin stability and mediates cadherin signaling to the Rho-family of GTPases to regulate cytoskeletal changes (Elia et al 2006). This role for cadherin has lead to the hypothesis that initial cadherin-based adhesion stabilizes transient, dynamic axodendritic contacts long enough to allow other classes of synaptogenic molecules to interact and activate intracellular cascades that recruit synaptic proteins (Togashi et al 2002; Ziv & Garner 2004). However, the roles for cadherins/catenins may extend beyond simply promoting adhesion. Altering N-cadherin function in young hippocampal cultures causes a dispersal of synapsin (Togashi et al 2002; Ziv & Garner 2004) and perturbing β-catenin alters the clustering of SV proteins (Bamji et al 2003), suggesting that cadherin/β-catenin signaling may be critical for the initial recruitment of SVs to new synapses.

Members of the Nectin family have also been proposed to mediate adhesion between axons and dendrites during the initial contact that leads to synapse formation. Interestingly, different nectin isoforms interact heterophilically; specifically nectin-1 on axons interacts with nectin-3 on dendrites to promote synapse formation (Mizoguchi et al 2002; Togashi et al 2006). These initial heterophilic nectin connections have been proposed to be one of the very first steps in axo-dendritic contact, preceding and promoting the formation of homophilic cadherin connections at those synapses (Mizoguchi et al 2002; Togashi et al 2006).

Like the cadherins and nectins, NCAM has also been proposed to act as a classic cell adhesion molecule in target recognition during synapse formation (Dityatev & Schachner 2006). However, recent reports suggest that NCAM may play a more instructive role in synapse formation by influencing the trafficking and synaptic targeting of trans-Golgi network (TGN) organelles. In young neurons before the bulk of synapse formation, NCAM is found in mobile clusters in the plasma membrane that are transported together with intracellular TGN organelles through a spectrin linker (Sytnyk et al 2002). Importantly, NCAM and TGN organelles accumulate at sites of contact with neighboring neurites (Sytnyk et al 2002), possibly through interactions of NCAM with its binding partners in the target cell, including heparan sulfate proteoglycans and the FGF receptor (Dityatev et al 2004). Taken together with the observation that NCAM-deficient neurons form fewer synapses (Dityatev et al 2000), these data suggest that NCAM may play an instructive role in the recruitment of TGN organelles to nascent synapses.

In addition to classic adhesion molecules, two trans-synaptic molecules have recently been identified that directly induce the recruitment of pre-and postsynaptic proteins to new synapses (Li & Sheng 2003; Scheiffele 2003; Sytnyk et al 2004; Ziv & Garner 2004). The first and most compelling of these molecular families as instructive signals for the induction of synapse formation are the neuroligins and neurexins (reviewed in Dean & Dresbach 2006). Neuroligins are postsynaptic transmembrane proteins that bind across the synaptic cleft to members of the large family of alternatively spliced presynaptic cell-surface receptors, the β-neurexins (Brose 1999; Ichtchenko et al 1996; Missler & Sudhof 1998; Song et al 1999). A large number of recent reports demonstrate that the β-neurexin-neuroligin complex is necessary and sufficient for pre-and postsynaptic differentiation (Chih et al 2005; Chubykin et al 2005; Dean et al 2003; Fu et al 2003; Graf et al 2004; Levinson & El-Husseini 2005; Nam & Chen 2005; Prange et al 2004; Sara et al 2005; Scheiffele et al 2000). Different isoforms of neuroligin are expressed at specific types of synapses: neuroligin-1 is specifically found at glutamatergic synapses (Song et al 1999, but see Levinson & El-Husseini 2005) whereas neuroligin-2 is specific for GABAergic synapses (Graf et al 2004; Varoqueaux et al 2004), suggesting that neurexins can regulate postsynaptic differentiation by recruiting the proper neuroligin isoforms to nascent contact sites (Graf et al 2004). Indeed splice variants of neuroligin and β-neurexin appear to play instructive roles in excitatory and inhibitory synapse formation (Chih et al 2006; Graf et al 2006). Moreover, interactions between neuroligin and PSD-95 can alter the functional balance of excitatory and inhibitory synaptic inputs onto CNS excitatory neurons (Chih et al 2005; Graf et al 2004; Levinson & El-Husseini 2005; Prange et al 2004).

The second class of molecules sufficient to induce pre-and postsynaptic differentiation are the SynCAMs (Biederer et al 2002). SynCAMs and nectins are closely related and are present at multiple kinds of cell-cell junctions, including synapses (Biederer et al 2002; Irie et al 2004). SynCAM (or IGSF4) is a homophilic cell adhesion molecule that, like neuroligin, binds intracellularly to CASK. Also like neuroligin, SynCAM can induce the formation of presynaptic terminals capable of glutamate release when expressed in non-neuronal cells (Biederer et al 2002). However, SynCAM overexpression in neurons indicates

that SynCAM enhances presynaptic function at existing synapses and not synapse number. In contrast, neuroligin overexpression in neurons increases synapse number, but those new synapses are non-functional (Sara et al 2005). Taken together, these data suggest a model in which SynCAM and neuroligin act together to induce new, functional excitatory synapses (Sara et al 2005).

Additional molecules that can induce the clustering of glutamate receptors have been found, although their effects on presynaptic components are unclear. The neuronal activity-regulated secreted pentraxin, Narp, induces clustering of AMPARs in spinal neurons (O'Brien et al 1999). Another pentraxin, NP1, can interact with Narp, leading to super-additive effects on synapse formation (Xu et al 2003). Localization of Narp suggests that it regulates glutamatergic synaptogenesis on inhibitory neurons and not on pyramidal neurons (Mi et al 2002). The ephrin axon guidance molecule family has also been implicated in altering the synaptic localization of NMDARs. The ephrin-B family seems to be especially important for postsynaptic differentiation since knockout of all three ephrin-B receptors dramatically decreases excitatory synapse number in the hippocampus (Henkemeyer et al 2003). In addition, EphB receptor clustering leads to clustering of NMDARs during synapse formation through a direct extracellular interaction between these transmembrane proteins (Dalva et al 2000). The ephrin-A family has also been implicated in synapse formation or maintenance; disruption of ephrin-A/EphA signaling decreases synapse density in various regions of the hippocampus (Martinez et al 2005). Similar to their diversity of function in axon guidance, the roles for the ephrins in synapse formation may also be quite complicated. For example, specific ephrin family members may play distinct roles in synapse formation since ephrin B3 knockout mice show an increased number of excitatory synapses, implicating ephrin B3 in the negative regulation of excitatory synapse number (Rodenas-Ruano et al 2006) in addition to its synaptogenic role.

Recently, a new synaptic cell adhesion molecule family, called SALMs (for synaptic adhesion-like molecules), has been discovered that is found specifically in excitatory CNS synapses and influences postsynaptic differentiation (Ko et al 2006; Wang et al 2006). SALM1 selectively interacts with NMDARs via their extracellular domain and overexpression of SALM1 enhances NMDAR and PSD-95 clustering at excitatory synapses (Wang et al 2006). SALM2 interacts biochemically with PSD-95 and appears to preferentially influence and induce AMPAR synaptic localization. SALM2 overexpression increases the number of excitatory synapses and dendritic spines, while knockdown decreases synapse and spine number. Interestingly, SALM2 does not appear to induce presynaptic differentiation (Ko et al 2006). Future studies of this exciting new family of CAMs should further identify the mechanism by which different SALM family members affect the initial formation of excitatory synapses.

Secreted molecules—Although most attention has focused on trans-synaptic adhesion molecules as instructive signals for excitatory synapse formation, evidence is rapidly accumulating that secreted factors are also critical for CNS synaptogenesis (Scheiffele 2003; Waites et al 2005; Ziv & Garner 2004). Based on the localization of cycling SV precursors in axonal filopodia (Dai & Peng 1996; Kraszewski et al 1995; Krueger et al 2003; Matteoli et al 1992; Nakata et al 1998; Sabo et al 2006; Zakharenko et al 1999) and NMDARs in the plasma membrane of dendrites before synapse formation (Washbourne et al 2004), it would seem likely that glutamate release from axons could influence excitatory synaptogenesis. However, a number of reports have clearly demonstrated that vesicular glutamate release is not necessary for excitatory synapse formation (Craig et al 1994; Harms & Craig 2005; Varoqueaux et al 2002; Verhage et al 2000). Yet, the number of synapses formed in the absence of neurotransmitter release is dramatically decreased (Bouwman et al 2004), suggesting the possibility that glutamate release plays a more subtle role in determining

whether or where excitatory synapses will form between CNS neurons. Consistent with this idea, STV cycling at predefined sites along the axon appears to selectively attract dendritic filopodia to those sites and initiate synapse formation there (Sabo et al 2006).

Perhaps most evidence has accumulated to support a role for the secreted neurotrophin, brain-derived neurotrophic factor (BDNF), in CNS synapse formation. BDNF treatment increases numbers of excitatory synapses (Vicario-Abejon et al 1998), TrkB knockout mice have decreased numbers of excitatory CNS synapses (Martinez et al 1998), and conditional TrkB knockouts suggest that BDNF acts both pre-and postsynaptically to regulate glutamatergic synapse formation (Luikart et al 2005). However, a recent report suggests that BDNF influences glutamatergic synapse formation only indirectly through homeostatic mechanisms (Elmariah et al 2004). Recently, the BDNF receptor, TrkB, has been found in structures in pyramidal neurons that actively participate in synapse formation—axonal growth cones and dendritic filopodia—as well as in axon and dendrite shafts. TrkB is specifically localized to transport packets that are highly mobile within axons and dendrites of developing cortical neurons before the bulk of synapse formation. Surprisingly, these transmembrane receptors are trafficked in axons in conjunction with SV precursor transport packets (Gomes et al 2006). Over time, surface TrkB becomes enriched at glutamatergic synapses. Taken together, these results suggest that TrkB is in the right place at the right time to play a direct role in the formation of glutamatergic synapses between cortical neurons, possibly by directly influencing the trafficking of co-transported intracellular presynaptic precursors.

Members of the Wnt, FGF, and TGFβ families also induce presynaptic differentiation. First, Wnt-7a and Wnt-3 enhance clustering of synapsin 1 in cerebellar and spinal neurons, respectively (Hall et al 2000; Krylova et al 2002), while *wnt7a* mutant mice display delayed accumulation of synapsin 1 at synapses (Hall et al 2000). Moreover, one of the Wnt receptors, Dishevelled, is necessary for proper formation of presynaptic terminals (Ahmad-Annuar et al 2006). Second, FGF22, and related family members FGF 7 and –10, are secreted by target neurons and promote clustering of SVs in axons of young neurons; knockout of the FGF22 receptor, FGFR2, inhibits presynaptic differentiation (Umemori et al 2004). Finally, mutations in *wishful thinking*, a gene that encodes a receptor for a ligand of the BMP/TGFβ family, also cause defects in presynaptic development and smaller synapses in *Drosophila* (Aberle et al 2002; Marques et al 2002); however, a role for TGFβ family members in mammalian CSN synapse formation has yet to be examined.

Finally, secreted molecules from neighboring glial cells also appear to play a role in glutamatergic synapse formation. This idea was first demonstrated by the potent effects of astrocyte-conditioned media in promoting synapse formation (Nagler et al 2001; Pfrieger & Barres 1997; Ullian et al 2004; Ullian et al 2001). The first glial-derived molecule to be discovered was cholesterol bound to apolipoprotein E (Mauch et al 2001). More recently, thrombospondins (TSPs) have been added to the list of glial-derived synaptogenic factors. TSPs are extracellular matrix proteins that are secreted by astrocytes and promote excitatory synapse formation between CNS neurons (Christopherson et al 2005). However, because these TSP-induced synapses are postsynaptically silent, it is likely that there are other as yet undiscovered glial-derived signals that modulate synapse formation and function (Allen & Barres 2005).

Synapse limiting molecules

One of the most exciting recent developments in the field of synaptogenesis has been the discovery of molecules that act to limit the ability of CNS neurons to form excitatory synapses. Although these molecules appear to mediate synapse elimination at later stages of postnatal development, they may also act to limit the initial formation of synapses during

early postnatal development. The first of these molecules are transcription factors of the myocyte enhancer factor 2 (MEF2) family. In young hippocampal neurons that have just started to form synapses, knockdown of MEF2A and MEF2D dramatically enhances synapse number and overexpression decreases synapse number (Flavell et al 2006). These results suggest that young neurons have the capacity to form many more synapses than they normally do and that activation of MEF2 transcription factors normally limits this capacity. A second class of molecules, major histocompatibility complex I molecules (MHCI), have also recently been shown to limit synapse number in very young neurons (Wampler and McAllister 2005 Soc Neurosci Abs 25.5). Similar to MEF2, knockdown of MHCI expression on the surface of neurons results in a dramatic increase in synapse number while overexpression of MHCI decreases synapse number. Interestingly, the increase in synapse number resulting from either MEF2 or MHCI knockdown is activity-dependent (Flavell et al 2006; Wampler and McAllister 2004 Soc Neurosci Abs 614.5). Although the mechanism by which these molecules normally limit synapse formation remains unknown, it is possible that these molecules are just two examples of a larger number of synapse limiting molecules. Surprisingly, these results suggest that the initial number of synapses that a young neuron forms may be as highly regulated as synapse number is during later postnatal stages of activity-dependent synaptic competition.

BRIDGING CELLULAR AND MOLECULAR MECHANISMS

Although much progress is being made in identifying synaptogenic molecules, surprisingly little is known about the mechanisms used by any of these molecules to signal the accumulation of synaptic proteins at new axodendritic contacts. It is generally assumed that contact between axons and dendrites recruits synaptogenic molecules that initiate intracellular signals that lead to rapid accumulation of pre-and postsynaptic proteins at those sites. Because of the large number of synaptogenic molecules, it is likely that many of these intracellular signaling pathways will converge within the cell to alter the cytoskeleton at nascent synapses or the mobility and directionality of trafficking of pre-and postsynaptic protein precursors.

One of the most attractive points of convergence for synaptogenic signaling within neurons is the actin cytoskeleton (Zito et al 2004). Of the molecules discussed above, NCAM, cadherins, integrins, nectins and neuroligins have in common the capacity to anchor themselves to the actin cytoskeleton and most of the other molecular signals can alter actin dynamics, which can, in turn, modulate transport of intracellular organelles. As synapses are formed, F-actin concentrates at sites of contact (Dai & Peng 1996; Zhang & Benson 2002). This accumulation of F-actin is essential for the development and maintenance of synapses since actin depolymerization in young neurons results in an almost complete loss of synapses (Zhang & Benson 2001). This requirement of actin dynamics for synapse formation in young neurons may be mediated by a GIT1/PIX/Rac/PAK signaling complex involved in Rho GTPase signaling (Zhang et al 2003). Despite the requirement for actin polymerization in synapse formation and pre-and postsynaptic function, little is known about how actin dynamics regulate the transport of proteins in axons and dendrites. The only report on this issue to date is a recent paper showing that altering actin dynamics by latrunculin treatment decreases mobility of STVs in young axons by increasing the duration of their pausing during transport (Sabo et al 2006), suggesting that actin may promote transfer of STVs from pause sites to microtubules for long-range transport.

Another likely point of convergence for signaling of synaptogenic molecules is activation of kinases and phosphatases since many of the synaptogenic molecules discovered to date initiate signal transduction cascades after binding to transmembrane receptors. Phosphorylation potently alters the function of motor proteins (Hollenbeck 1993; Morfini et

al 2004) and the binding of motors to cargo (Karcher et al 2002; Lee & Hollenbeck 1995; Sato-Yoshitake et al 1992), and thus can influence the transport of intracellular vesicles. However, the specific kinase/phosphatase pathways that are initiated by synaptogenic molecules and can alter presynaptic vesicle transport are diverse, complex, context-dependent and in many cases unknown. Recently, JNKs (c-Jun kinases) have been implicated in regulating SV precursor transport. The JNK signaling complex is associated with STVs in *C. elegans* through JIP-3, a JNK-interacting protein (Byrd et al 2001). Mutations in the *C. elegans* JIP-3 homolog, unc-16, and in JNK1, cause mislocalization of SVs and glutamate receptors (Byrd et al 2001). JIP proteins organize components of JNK signaling pathways into functional modules (Davis 2000; Kelkar et al 2000) and bind these JNK signaling complexes to vesicular cargo (Byrd et al 2001; Goldstein 2001; Verhey et al 2001). Association of the JNK signaling complex with STVs suggests that JNK signaling could alter transport of the vesicles to which the complex is attached. However, the association of activated JNK with STVs in mammalian neurons is unknown, as are the effects of blocking JNK signaling. Identifying the intracellular signaling cascades initiated by activation of synaptogenic molecules and determining how they alter trafficking of pre- and postsynaptic precursors is one of the major future challenges for the field of synaptogenesis.

Although it is generally assumed that activation of synaptogenic molecules is linked to accumulation of synaptic protein precursors through intracellular signaling that alters the velocity and/or directionality of transport packets, it is also possible that the accumulation of these synaptic protein packets could be directly altered through co-trafficking with synaptogenic molecules. In support of this idea, NCAM in the plasma membrane is trafficked with intracellular TGN organelles through a spectrin linker (Sytnyk et al 2004). Decreasing NCAM levels decreases the number of excitatory synapses, possibly because accumulation of TGN organelles at synapses is dependent on co-transport with NCAM. Similarly, the BDNF receptor, TrkB, is co-transported with STVs in young axons before the bulk of synapse formation (Gomes et al 2006). Because other synaptogenic molecules might interact with intracellular proteins through associated scaffolding proteins such as CASK, it is possible that they could also transport synaptic protein precursors to new synapses through a direct association. In order to test this highly speculative idea in the future, it will be critical to determine whether synaptogenic molecules are transported alone and are recruited to new axodendritic contacts prior to accumulation of synaptic protein precursors or whether they can be transported together with synaptic proteins and directly alter their accumulation at new synapses.

Hierarchical signaling or parallel pathways for synaptogenesis?

Historically, the field of synaptogenesis has focused on finding a single molecule, or family of molecules, that is essential for excitatory synapse formation. The assumption has been that there is a hierarchy of synaptogenic molecules that leads to the construction of the synapse. However, mutant mice for most of these synaptogenic molecules support a role, but not an absolute requirement, for them in synapse formation. It is possible that the key synaptogenic protein is yet to be discovered, but an equally plausible idea is that there are multiple, redundant pathways for excitatory synapse formation. In support of this idea, many synaptogenic molecules are colocalized at excitatory synapses where they appear to perform the same basic functions in enhancing the assembly of presynaptic terminals or PSDs (Craig et al 2006; Scheiffele 2003). In addition, the range of time-courses for synapse formation coupled with the diversity in hierarchy of recruitment of pre-or postsynaptic proteins to new synapses suggests that synapses may be formed by several mechanisms. An important question is whether these parallel pathways are linked into large signaling complexes that together influence synapse formation or whether they are physically parallel pathways that

converge intracellularly to initiate accumulation of synaptic proteins. Imaging the recruitment of each synaptogenic molecule to new synapses and determining their interaction in recruiting synaptic protein precursors to new synapses should elucidate this issue in the future.

Models for excitatory synapse formation in the mammalian CNS

It is currently believed that synaptogenesis consists of a series of steps from stabilization of initial axodendritic contacts, to recruitment of pre-and postsynaptic protein precursors, to maturation of the synapse and activity-dependent regulation of its molecular composition and function. In recent reviews, it has been proposed that secreted molecules first act as "priming factors" that are secreted by target neurons and glia that promote axonal and dendritic growth and make them competent to form synapses (Craig et al 2006; Waites et al 2005). As axons and dendrites find their targets, classic adhesion molecules act to transiently stabilize those initial contacts and trans-synaptic adhesion molecules then act to induce the formation of presynaptic terminals and PSDs by initiating signaling cascades that lead to the recruitment of pre-and postsynaptic protein transport packets.

Although this current model is appealing and is likely correct in a number of its assumptions, there are several new pieces of data that do not fit with this model. First, the idea that secreted synaptogenic molecules simply act to prime neurons for synapse formation (Craig et al 2006; Waites et al 2005) may be over-simplistic. This classification is based on the assumption that secreted molecules are likely to act at a distance since they are diffusible and the observation that many of these factors also influence axonal and/or dendritic growth and could therefore influence the likelihood of contact between neurons. In addition to these "priming" roles, however, it is entirely possible that these diffusible molecules also act locally to instruct the formation of synapses at specific sites of accumulation of their receptors. For example, the observation that STVs pause and cycle at predefined sites within axons that selectively attract and stabilize dendritic filopodia during synapse formation supports the idea that secreted molecules could instruct whether and where en passant synapses are formed (Sabo et al 2006). Second, the current model for synapse formation does not consider the possibility raised above that cell-surface synaptogenic molecules may be transported together with intracellular packets that transport synaptic proteins. Finally, there is little evidence for the assumption that axodendritic contacts actively recruit synaptic protein precursors. It is equally plausible that synaptogenic molecules simply alter the cytoskeleton beneath nascent contacts, leading to passive accumulation of mobile synaptic protein precursors that are transported through those sites. In support of this alternative, manipulating actin dynamics, the synapsins, and calcium does not alter the velocity or directionality of STV transport, but rather alters the pausing of these precursors during their transport in young cortical neurons (Sabo et al 2006). These data suggest that perhaps regulation of pausing of synaptic protein precursors, rather than actively altering the velocity or directionality of their transport, might be most important for accumulation of these proteins at nascent synapses.

CONCLUSIONS

In sum, there has been a remarkable recent expansion in our understanding of the cellular and molecular mechanisms of the formation of glutamatergic synapses in the mammalian CNS. However, fundamental aspects of whether, where, and how these synapses form remain unknown. In particular, the field now faces the daunting task of bridging the cellular and molecular mechanisms of synapse formation by addressing the important questions of the time-course and mechanism of action of synaptogenic molecules. Imaging studies of the distribution and dynamics of multiple synaptogenic signals relative to pre-and postsynaptic transport packets should help to clarify this issue in the future. In addition, determining the

localization and dynamics of receptors for secreted synaptogenic molecules, as well as the effects of focal perfusion of these molecules on the mobility of synaptic proteins, should address whether diffusible molecules can act in an instructive manner or whether they are simply permissive "priming" factors. It will also be important to determine the role for the new family of synapse limiting molecules in the initial formation of synapses, and how these synapse-limiting molecules interact with synaptogenic molecules to regulate the number of synapses a young neuron can form. Finally, the cellular and molecular mechanisms of glutamatergic synaptogenesis identified using cell culture models, as described throughout this review, must be tested for their relevance to synaptogenesis in vivo.

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Acronyms

AMPA	α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid type glutamate receptor
AMPAR	AMPA receptor
CNS	central nervous system
GFP	green fluorescent protein
KIF	kinesin superfamily
NMDA	N-methyl-D-aspartate type of glutamate receptor
NMDAR	NMDA receptor
PSD	postsynaptic density
PSD-95	postsynaptic density protein-95
PTV	piccolo transport vesicle
STV	synaptic vesicle protein transport vesicle
SV	synaptic vesicle

Terms/Definitions List

Synapse	A junction between neurons that allows for electrochemical signaling. Synapses can be defined morphologically, molecularly, or electrophysiologically. In this review, a synapse is defined as the stable colocalization of pre-and postsynaptic proteins

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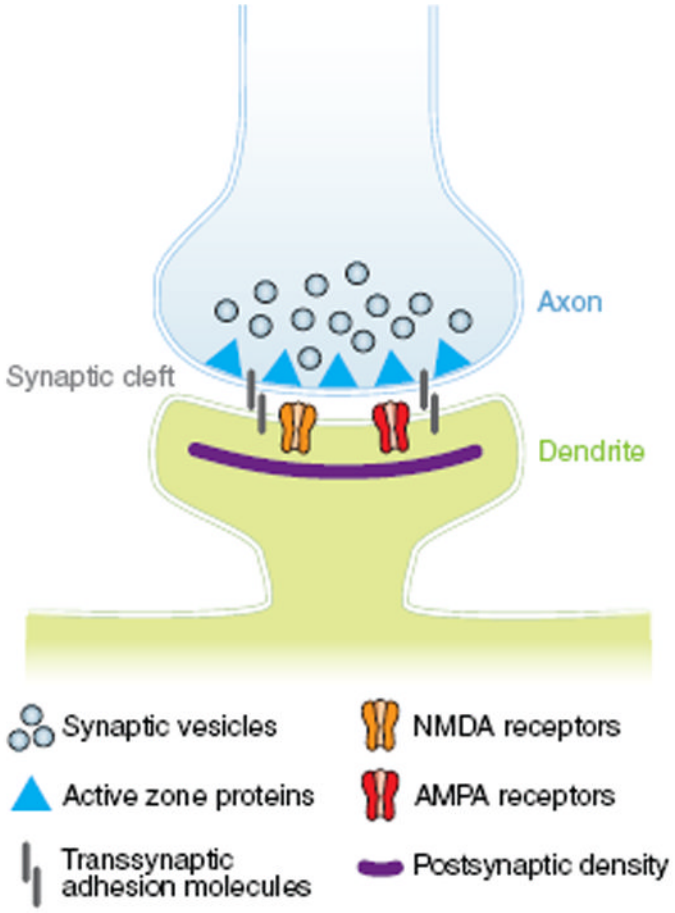

Figure 1.
CNS glutamarergic synapses are comprised of several major protein classes. Synaptic vesicles containing the neurotransmitter glutamate cycle at the active zone, which is composed of many kinds of proteins including presynaptic scaffolding proteins. The presynaptic terminal is separated from the postsynaptic dendrite by the synaptic cleft; a number of trans-synaptic adhesion molecules span this cleft, providing a molecular connection between the pre-and postsynaptic membranes. Glutamate receptors, including AMPA and NMDA receptors, are found in rhe postsynaptic membrane, where they are associated with a large number of scaffolding and signaling proteins that together comprise the postsynaptic density. Although glutamatergic synapses are usually located on dendritic spines in the adult, these synapses are more often found on dendritic shafts and filopodia in the CNS during the initial stages of synaptogenesis.

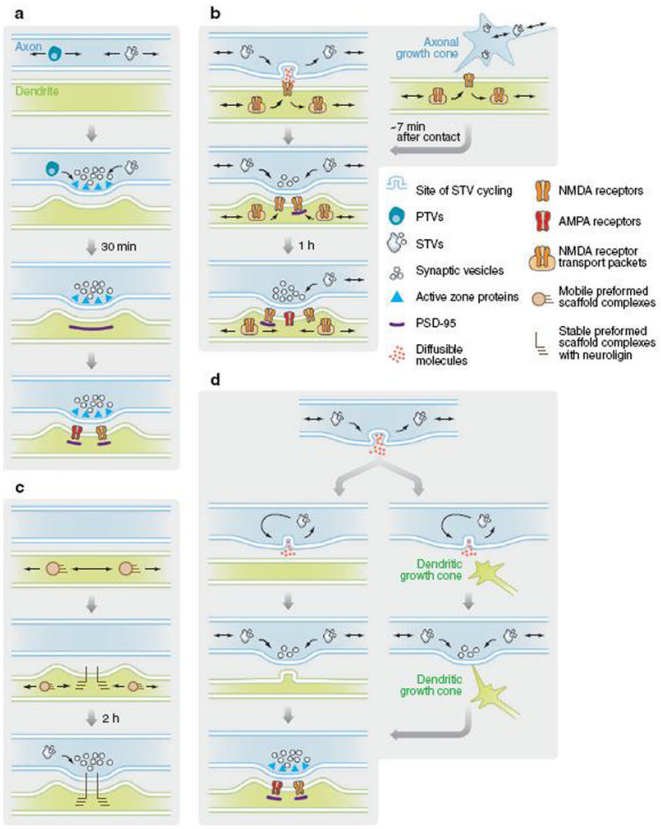

Figure 2.

Multiple cellular mechanisms for CNS synaptogenesis. There appear to be multiple mechanisms for the recruitment and stabilization of pre-and postsynaptic proteins to new sites of axo-dendritic contact. (*a*) Glutamatergic synapses between axon and dendrite shafts of hippocampal neurons can form in about an hour of the initial accumulation of presynaptic vesicles. Presynaptic proteins, including synaptic vesicle precursors (STVs) and piccolo-transport vesicles (PTVs), are mobile in axons before synapses are formed (*upper axon/dendrite pair*). These precursors are the first proteins recruited to nascent synapses (*second axon/dendrite pair*). After ~30 min, PSD-95 accumulates at these sites (*third pair*) followed by glutamate receptors (*fourth pair*, Friedman et al. 2000). (*b*) In young cortical neurons, glutamatergic synapses can form even faster, on a timescale of several minutes. In these cells, STVs and NMDARs are both found in transport packers that are highly mobile in the axons and dendrites, respectively, before synapse formation (*upper axon/dendrite pair*). Both STVs and NMDAR transport packets cycle with the membrane during their transport (Washbourne et al. 2004, Sabo et al. 2006). Contact between an axonal growth cone filopodium and a dendrite (*right*), or between axon and dendrite shafts (*left*), leads to the rapid and simultaneous recruitment of STVs and NMDARs at nascent synapses within ~7 min of contact (*second pair*). PSD-95 is recruited to these sites with a variable time course, and AMPARs are recruited an hour following initial recruitment of NMDARs (*third pair;* Washbourne et al. 2002). (*c*) Glutamatergic synapses can also form at prespecified sites along the dendritic shaft of hippocampal neurons, defined by stable preformed scaffold complexes associated with neuroligin. In this scenario, complexes of scaffolding proteins (including PSD-95, Shank, and GKAP) are mobile within dendrites before synapses are formed (*upper axon/dendrite pair*). When these complexes associate with neuroligin, they often become stabilized in the dendritic membrane (*second pair).* A significant proportion of

these complexes then recruit STVs to form synapses within 2 h of their stabilizarion (*third pair;* Gerrow et al. 2006). (*d*) There are also predefined sites along the axon shaft of cortical neurons where en passant synapses selectively form. These predefined sites are stable locations along the axon where STVs cycle with the plasma membrane (*first axon/dendrite pair*) and presumably release diffusible molecules before synapses are formed (*second pair*). Filopodia from dendritic growth cones (*right*) and presumably also dendritic shafts (*lift*) are selectively attracted to, and stabilized, at these sites (*third pair*). Following stabilization of this contact at this predefined site, the presynaptic terminal is farmed and additional pre-and postsynaptic proteins are recruited to form a nascent synapse (*fourth pair;* Sabo et al. 2006).

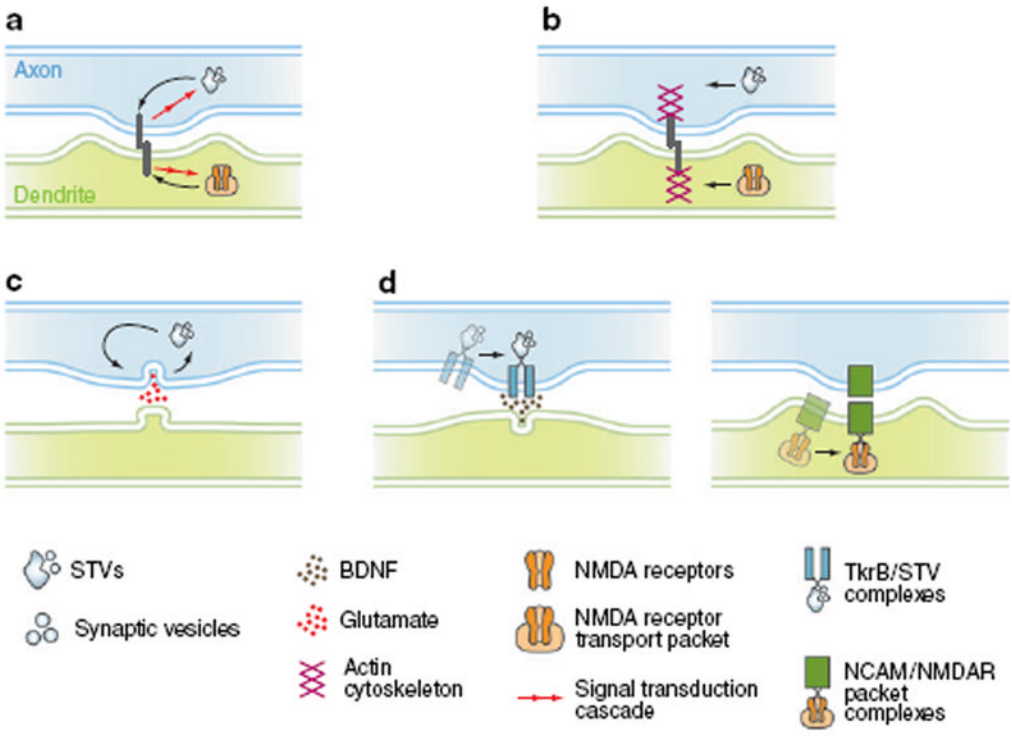

Figure 3.

Possible mechanisms for recruitment of proteins to nascent synapses. (*a*) Current models, for CNS synaptogenesis suggest that synaptogenesis is initiated by the binding of trans-synaptic adhesion molecules, such as neuroligin and β-neurexin, across the synaptic cleft. Binding of these molecules then leads to the activation of intracellular signal transduction cascades, which somehow alter the trafficking of STVs and NMDAR transport packets, causing them no rapidly accumulate at nascent contact sites. This model implies that synaptic proteins are actively recruited to nascent synapses through intracellular signaling, (*b*) It is also possible that activation of synaptogenic molecules locally alters the cytoskeleton, leading to the passive capture of mobile precursors that get stuck at these sires, (*c*) In addition to adhesion molecules, diffusible molecules may also play instructive roles in glutamatergic synaptogenesis. Diffusible molecules released from cycling STVs, such as glutamate, may selectively attract d dendritic filopodia to form synapses at sites of their release (Sabo et al. 2006). (*d*) More speculative is the idea that activation of synaptogenic molecules could alter STV or NMDAR transport packer accumulation at nascent synapses through direct interactions between adhesion molecules in the membrane and intracellular transport packers. For example, TrkB is trafficked with STVs (Gomes et al. 2006) and NCAM may move with NMDA receptors (Sytnyk et al 2006). Dendritic release of BDNF may alter and recruit STVs to its source through a direct interaction with TrkB. Similarly, homophilic NCAM interactions across the synaptic cleft could lead to the accumulation of NMDARs at those sites owing to a direct interaction with the NMDA receptor transport packers.