Dynamic Aspects of CNS Synapse Formation

A. Kimberley McAllister

Center for Neuroscience, University of California, Davis, California 95616; email: kmcallister@ucdavis.edu

Key Words
development, axon, dendrite, vesicle trafficking, glutamate receptors, cell adhesion

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, whereas 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.
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 nonmammalian animal models as well as the extensive literature covering the mammalian neuromuscular junction are not covered here owing to space constraints.

In the mammalian CNS, most excitatory synapses are highly asymmetric junctions composed of a presynaptic terminal and a postsynaptic density (PSD; Figure 1). The axonal presynaptic terminal is packed with hundreds of small (∼50 nm), 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, a ∼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 an 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, tremendous progress has been made in identifying the molecular components of the CNS glutamatergic synapse (Figure 1) (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, numerous proteins found on SVs are critical for their proper localization and calcium-dependent fusion (Sudhof 2004). Several different 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 that 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. 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; DeFelipe 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

**Figure 1**

CNS glutamatergic 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 the 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.
a protracted period of development; at any
given time, the age of synapses on a single neu-
ron could span days to weeks, or even months.

The convergence of several technical ad-
vances has led to a recent explosion in the
number of studies in the field of synaptoge-
nesis. First, many of the molecular compo-
nents 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). Sec-
ond, widespread use of primary neuronal cul-
tures, coupled with immunocytochemistry,
has readily allowed visualization of the sub-
cellular distribution of synaptic proteins and
quantification of synaptic density. Moreover,
visualization of GFP-tagged proteins in liv-
ing, cultured neurons using time-lapse imag-
ing techniques has revealed the distribution
and dynamics of these proteins before, dur-
ing, 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 forma-
tion. The convergence of these technical ad-
vances has greatly expanded our understand-
ing of the cellular and molecular mechanisms
of CNS synapse formation.

CELLULAR MECHANISMS

For synapses to form, pre- and postsynaptic
proteins must be synthesized and transported
to sites of contact between axons and den-
drites. In general, pre- and postsynaptic pro-
tein are present in neurons before synapses
are formed (Fletcher et al. 1991, Rao et al.
1998). Recently, many of these synaptic pro-
teins have been localized to small, hetero-
geous 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, depend-
ing on the nature of the target and possibly
the age of the neurons (Figure 2). Subsequent
maturation of synapses 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
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
0.35 μm/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, SV proteins are transported in
heterogeneous organelles called STVs, which
are biochemically and morphologically dis-

tinct 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 ex-
hibit morphologies ranging from pleiomor-
phic, tubulo-vesicular organelles (Ahmari
et al. 2000) to clusters of small SV-like vesi-
cles (Kraszewski et al. 1995). STVs are highly
mobile within axons of young neurons be-
fore synapses form; over half of all VAMP2-
EGFP puncta are mobile in young cortical
neurons with velocities ranging from 0.1–1
μm/s (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 coa-
lesce during transport and that their move-
ment 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; some researchers have reported primarily anterograde transport (Ahmari et al. 2000, Lee et al. 2003, Nakata et al. 1998), whereas 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-Y oshitake et al. 1992), KIF1a (Okada et al. 1995, Yonekawa et al. 1998), and KIF1Bβ (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 (Ohymama et al. 2001, Prekeris & Terrian 1997).

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 owing to 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). STV cycling surprisingly occurs within filopodia of axonal growth cones (Sabo & McAllister 2003), as well as along the axon shaft, which suggests 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, vGlut (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 ionotrophic glutamate receptors. Although there are many fewer studies of formation of the PSD, glutamate receptors and scaffolding proteins are clearly present in dendrites before synapses are formed (Craig et al. 1993; Gerrow et al. 2006; Washbourne et al. 2002, 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, 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 rate of transport of KIF17 is an order of magnitude faster than that 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
Axon
Dendrite
30 min
1 h
~7 min after contact

NMDA receptors
AMPA receptors
NMDA receptor transport packets
Mobile preformed scaffold complexes
Stable preformed scaffold complexes with neuroligin

Synaptic vesicles
PTVs
STVs
Active zone proteins
PSD-95
Diffusible molecules

Site of STV cycling

NMDA receptor transport packets

AMPA receptors

Stable preformed scaffold complexes with neuroligin

McAllister
recently been questioned, 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 axo-dendritic 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, 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 with 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 with the presynaptic terminal. In sum, despite the assertion that postsynaptic assembly is fundamentally different from presynaptic 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 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, little experimental evidence supports 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 (Figure 2d).

These sites are not determined by contact with neighboring neurons or glial cells. 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 complex in postsynaptic dendrites (Figure 2). 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 predefined sites within axons and dendrites may 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 protein recruitment 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 (Figure 2a) (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. 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 because 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, syntxin, and rab 3, is difficult to model by quantal insertion because 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 30 min and accumulated there in a gradual manner (Figure 2a) (Bresler et al. 2001, Friedman et al. 2000, Okabe et al. 2001); clusters of AMPARs and NMDARs were recruited after PSD-95 (Friedman et al. 2000). In contrast with this initial report, two recent studies demonstrated different mechanisms of synapse formation with different temporal orders of pre- and postsynaptic protein recruitment. Using as a reference the timing of the first stabilized contact between axonal growth cone filopodia and dendrites, pre- and postsynaptic proteins were found to be recruited simultaneously to those contacts with a time course of just under 10 min (Figure 2b). 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 h following NMDAR recruitment. AMPARs were not found at these sites within 1 h of NMDAR recruitment but were always present after 1 h, which suggests 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 h of their stabilization (Figure 2c) (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 be 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. In addition, 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 located primarily 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 proteins, 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

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 because 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, neuropilins, 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 the presynaptic terminal or the PSD (Li & Sheng 2003, Scheiffele 2003, Sytnyk et al. 2004, Ziv & Garner 2004). Trans-synaptic molecules are attractive candidates to regulate synapse formation because 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 led 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. Different nectin isoforms interact heterophilically; specifically nectin-1 on axons interacts with nectin-3 on dendrites to promote synapse formation (Mizoguchi et al. 2002, Tagashi et al. 2006). These initial heterophilic nectin connections have been proposed as 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 plays 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). 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 plays an instructive role in the recruitment of TGN organelles to nascent synapses.

In addition to classic adhesion molecules, investigators have identified two trans-synaptic molecules that directly induce the recruitment of pre- and postsynaptic proteins to new synapses (Li & Sheng 2003, Scheiffele et al. 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 likely 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 nonneuronal 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 nonfunctional (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. 2003).
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 superadditive 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 because 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 because 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 influences and induces AMPAR synaptic localization preferentially. SALM2 overexpression increases the number of excitatory synapses and dendritic spines, whereas knockdown decreases synapse and spine number. SALM2 does not 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 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 attract dendritic filopodia selectively 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 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, another 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 localized specifically to transport packets that are highly mobile within axons and dendrites of developing cortical neurons before the bulk of synapse formation. 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 cotransported 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 I in cerebellar and spinal neurons, respectively (Hall et al. 2000, Krylova et al. 2002), whereas 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-Annar 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 CNS synapse formation has yet to be examined.

Finally, secreted molecules from neighboring glial cells also likely 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. 2001, 2004). 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 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 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 can 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), also limit synapse number in very young neurons (Wampler & McAllister 2005). Similar to MEF2, knockdown of MHCI expression on the surface of neurons results in a dramatic increase in synapse number, whereas overexpression of MHCI decreases synapse number. The increase in synapse number resulting from either MEF2 or MHCI knockdown is activity-dependent (Flavell et al. 2006, Wampler & McAllister 2004). 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. 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. Investigators generally assume 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 (Figure 3a). Because of the large number of synaptogenic molecules, many of these intracellular signaling pathways will likely 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 (Figure 3b) (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 because 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 because 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 it 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 cotrafficking with synaptogenic molecules (Figure 3d). 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 cotransported 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, they may also transport synaptic protein precursors to new synapses through a direct association. 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, results from experiments using mutant mice for most of these synaptogenic molecules support a role, but not an absolute requirement, for these molecules in synapse formation. The key synaptogenic protein may not yet 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.
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

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

---

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 to 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 those sites. (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 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 packet accumulation at nascent synapses through direct interactions between adhesion molecules in the membrane and intracellular transport packets. For example, TrkB is trafficked with STVs (Gomes et al. 2006) and NCAM may move with NMDA receptors (Symyk 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 packets.
recent reviews, investigators have proposed that secreted molecules first act as “priming factors” that are secreted by target neurons and glia and promote axonal and dendritic growth to make neurons competent to form synapses (Craig et al. 2006; Waites et al. 2005). As axons and dendrites find their targets, classic adhesion molecules act to stabilize those initial contacts transiently, 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 presynaptic protein transport packets (Figure 3a). Although this current model is appealing and is likely correct in a number of its assumptions, several new pieces of data 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 oversimplistic. This classification is based on the assumption that secreted molecules are likely to act at a distance because 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, these diffusible molecules may also act locally to instruct the formation of synapses at specific sites of accumulation of their receptors (Figure 3c). 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 (Figure 3d). 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 (Figure 3b). In support of this alternative, manipulating molecules that regulate synaptogenesis—such as actin, 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, our understanding of the cellular and molecular mechanisms of the formation of glutamatergic synapses in the mammalian CNS has remarkably expanded over the past few years. 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 presynaptic 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.

ACKNOWLEDGMENTS

I thank all past and present members of my laboratory for stimulating discussions on this topic, especially S.L. Sabo and P. Washbourne, whose work was critical to the development of many of the ideas presented in this review. Thanks also to E. Diaz for critical reading of this manuscript. Research in my laboratory has been funded by the Alfred P. Sloan Foundation, the PEW Charitable Trusts, the March of Dimes, the National Eye Institute, the John F. Merck Fund, NARSAD, and Cure Autism Now.

LITERATURE CITED


ANRV314-NE30-17 ARI 10 May 2007 20:8


Contents

Information Processing in the Primate Retina: Circuitry and Coding  
G.D. Field and E.J. Chichilnisky ................................................................. 1

Orbitofrontal Cortex and Its Contribution to Decision-Making  
Jonathan D. Wallis ....................................................................................... 31

Fundamental Components of Attention  
Eric I. Knudsen .......................................................................................... 57

Anatomical and Physiological Plasticity of Dendritic Spines  
Veronica A. Alvarez and Bernardo L. Sabatini ............................................ 79

Visual Perception and Memory: A New View of Medial Temporal Lobe Function in Primates and Rodents  
Elisabeth A. Murray, Timothy J. Busey, and Lisa M. Saksida ......................... 99

The Medial Temporal Lobe and Recognition Memory  
H. Eichenbaum, A.P. Yonelinas, and C. Ranganath .................................... 123

Why Is Wallerian Degeneration in the CNS So Slow?  
Mauricio E. Vargas and Ben A. Barres ...................................................... 153

The Head Direction Signal: Origins and Sensory-Motor Integration  
Jeffrey S. Taube ............................................................................................ 181

Peripheral Regeneration  
Zu-Lin Chen, Wei-Ming Yu, and Sidney Strickland .................................... 209

Neuron-Glial Interactions in Blood-Brain Barrier Formation  
Swati Banerjee and Manzoor A. Bhat ......................................................... 235

Multiple Dopamine Functions at Different Time Courses  
Wolfram Schultz .......................................................................................... 259

Ventral Tegmental Area Neurons in Learned Appetitive Behavior and Positive Reinforcement  
Howard L. Fields, Gregory O. Hjelmstad, Elyssa B. Margolis, and Saleem M. Nicola ................................................................. 289
Copper and Iron Disorders of the Brain
Erik Madsen and Jonathan D. Gitlin .................................................. 317

The Micromachinery of Mechanotransduction in Hair Cells
Melissa A. Vollrath, Kelvin Y. Kwan, and David P. Corey ..................... 339

Neurobiology of Feeding and Energy Expenditure
Qian Gao and Tamas L. Horvath ............................................................ 367

Mechanisms that Regulate Establishment, Maintenance, and
Remodeling of Dendritic Fields
Jay Z. Parrish, Kazuo Emoto, Michael D. Kim, and Yuh Nung Jan .......... 399

Dynamic Aspects of CNS Synapse Formation
A. Kimberley McAllister ........................................................................ 425

Adhesion Molecules in the Nervous System: Structural Insights into
Function and Diversity
Lawrence Shapiro, James Love, and David R. Colman ................. 451

Development of Neural Systems for Reading
Bradley L. Schlaggar and Bruce D. McCandliss ................................ 475

Molecular Architecture of Smell and Taste in Drosophila
Leslie B. Vosshall and Reinhard F. Stocker ....................................... 505

The Neural Basis of Decision Making
Joshua I. Gold and Michael N. Shadlen .......................................... 535

Trinucleotide Repeat Disorders
Harry T. Orr and Huda Y. Zoghbi ......................................................... 575

Indexes

Cumulative Index of Contributing Authors, Volumes 21–30 ............ 623
Cumulative Index of Chapter Titles, Volumes 21–30 ....................... 627

Errata

An online log of corrections to Annual Review of Neuroscience chapters (if any, 1997
to the present) may be found at http://neuro.annualreviews.org/