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Mechanisms of Synapse Assembly and Disassembly

Review

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The mechanisms that govern synapse formation and elimination are fundamental to our understanding of neural development and plasticity. The wiring of neural circuitry requires that vast numbers of synapses be formed in a relatively short time. The subsequent refinement of neural circuitry involves the formation of additional synapses coincident with the disassembly of previously functional synapses. There is increasing evidence that activity-dependent plasticity also involves the formation and disassembly of synapses. While we are gaining insight into the mechanisms of both synapse assembly and disassembly, we understand very little about how these phenomena are related to each other and how they might be coordinately controlled to achieve the precise patterns of synaptic connectivity in the nervous system. Here, we review our current understanding of both synapse assembly and disassembly in an effort to unravel the relationship between these fundamental developmental processes.

Synapse Formation at the Vertebrate NMJ: A Model of Reciprocal Induction

Studies at the vertebrate NMJ have guided our understanding of synapse formation for several decades. The model that has emerged is one of reciprocal induction (Figure 1). Prior to the arrival of the nerve, there is a rudimentary postsynaptic organization termed prepatterning. The arrival of the nerve then induces the differentiation of the postsynaptic specialization. The induction of the postsynaptic specialization is then necessary for the subsequent induction of the presynaptic terminal including the assembly of the presynaptic active zone and the alignment of this structure with the postsynaptic specialization. Key steps in this process are outlined here (for further review, see Sanes and Lichtman, 1999, 2001; Burden, 2002).

The earliest event in synapse formation at the mammalian NMJ is likely to be the molecular patterning of the postsynaptic membrane prior to the arrival of the nerve. Acetylcholine receptors (AChR) and the synaptic proteoglycan neuregulin are observed to concentrate at the central portion of the muscle fiber at a time that normally coincides with the arrival of the motoneurons. This process has been termed prepatterning because it has been observed to occur in the absence of the presynaptic motoneurons, demonstrating that it is independent of a motoneuron-derived signal (Yang et al., 2000, 2001; Lin et al., 2001; Arber et al., 2002). At present it is unclear how muscle prepattern is established, though it has been demonstrated that prepatterning is independent of Agrin and requires the function of the muscle-specific kinase MuSK (see below) (Yang et al., 2001; Lin et al., 2001). Models for the establishment of muscle prepattern include a unique contribution of founder myoblasts during the formation of the polynucleate muscle cell or intercellular signaling from cells at the muscle insertion site that could be used as information to pattern the muscle cell (Arber et al., 2002). Although prepatterning prefigures the arrival of the nerve, it remains to be determined whether prepatterning is necessary for subsequent synapse formation since synapse formation does occur in vitro where prepatterning is not observed (Sanes and Lichtman, 1999).

The next event in synapse formation at the mammalian NMJ occurs upon the arrival of the motoneuron. Agrin is released from the presynaptic motoneuron and subsequently induces the formation of the postsynaptic specialization (McMahan, 1990; Sanes and Lichtman, 2001). Agrin is a secreted proteoglycan that was initially purified as an activity that induces enhanced clustering of AChRs on myotubes in vitro (Nitkin et al., 1987; McMahan, 1990). Agrin deposited in the basal lamina induces the further clustering and stabilization of prepatterned AChRs at the site of innervation. Although both muscle and nerve synthesize Agrin, the isoform expressed by motoneurons (z-plus Agrin) is reported to be more than 1000-fold better at inducing AChR clustering compared to the isoform expressed by muscle (Burgess et al., 1999; Sanes and Lichtman, 2001). The activity of Agrin requires the presence and activation of the muscle-specific kinase MuSK (Gautam et al., 1996; DeChiara et al., 1996; Herbst et al., 2002). MuSK then acts through the effector protein rapsyn to promote AChR clustering (reviewed by Sanes and Lichtman, 1999). MuSK activation also enhances the concentration of ErbB receptors, which transduce signaling from neuregulin. Neuregulin is a synaptic proteoglycan implicated in AChR synthesis (Jo et al., 1995; Sandrock et al., 1997). Defining evidence in favor of the agrin hypothesis came from analysis of agrin, MuSK, and rapsyn knockout mice in which AChR clusters are absent or severely reduced (Gautam et al., 1995, 1996; DeChiara et al., 1996). There is, however, an important difference between the agrin and MuSK knockout mice. In MuSK knockout animals, AChR clusters are absent at all stages. In the Agrin knockout, however, AChR clusters are initially prepatterned and subsequently disperse upon the arrival of the nerve that lacks agrin (Yang et al., 2001; Lin et al., 2001). These recent data suggest that a factor, perhaps nerve-derived ACh, acts to disperse prepatterned clusters of AChRs in the absence of the stabilizing influence of Agrin.

The phenotype of the agrin and MuSK knockout mice also provided strong evidence that postsynaptic differentiation is necessary for subsequent induction of pre-

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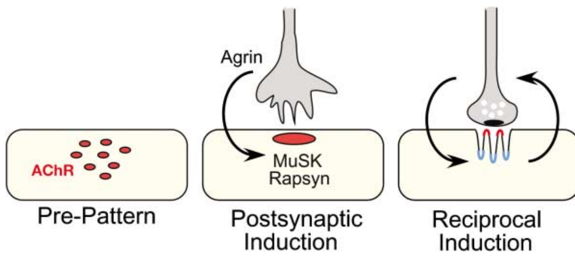


Figure 1. Synaptic Induction at the Mammalian Neuromuscular Junction

Left: Acetyl choline receptors (red) initially concentrate to the central portion of the muscle at a time that normally coincides with the arrival of motoneurons. In the absence of the nerve, this concentration of AChRs is still observed and, as a result, has been termed muscle prepatterning.

Middle: The arrival of the nerve and the presynaptic release of Agrin stimulates the initial events of postsynaptic differentiation including the further clustering and stabilization of AChR via signaling through MuSK and rapsyn.

Right: Subsequent inductive signaling, both anterograde and retrograde, is required for the transformation of the motile growth cone into a stable synaptic structure and for the development of pre- and postsynaptic specialization such as the differentiation and alignment of the presynaptic active zone with the molecularly specialized muscle membrane folds. AChRs are concentrated at the crests of the muscle folds (red), and other synaptic proteins are concentrated at the base of the folds (blue).

synaptic development. In both Agrin and MuSK knockout mice, the motoneuron terminals fail to differentiate, remaining highly dynamic and extending processes along the muscle surface (Gautam et al., 1996; DeChiara et al., 1996). Further evidence that postsynaptic differentiation is necessary for the subsequent induction of the presynaptic nerve terminal comes from muscle transplantation studies in which MuSK knockout muscle are transplanted into wild-type animals, circumventing the early lethality due to paralysis of the MuSK and Agrin knockouts (Nguyen et al., 2000). Nerve terminals contacting transplanted MuSK knockout muscle remain undifferentiated and are observed to remodel continuously over the course of several months. Thus, it appears that the induction of the presynaptic nerve terminal proceeds only after synapse formation is initiated in the postsynaptic muscle cell.

The nature of the muscle-derived signal that is necessary to induce presynaptic differentiation has not been clearly defined (Sanes and Lichtman, 1999). However, signaling via laminins in the synaptic basal lamina are demonstrated to be necessary for several aspects of presynaptic development. Presynaptic differentiation is compromised in the laminin $\beta 2$ knockout (Noakes et al., 1995). More subtle defects are observed in laminin $\alpha 4$ knockouts. In these animals, synaptic differentiation is grossly normal. However, the pre- and postsynaptic specializations are frequently misaligned (Patton et al., 2001). Laminin $\alpha 4$ can be linked biochmically to presynaptic calcium channels, supporting the hypothesis that this laminin isoform participates in the *trans*-synaptic alignment or organization of the synapse (Sunderland et al., 2000). Thus, laminins and possibly other signaling molecules within the synaptic basal lamina are candidates for inducing the presynaptic specialization following the induction of postsynaptic differentiation. One likely scenario, therefore, is that key signaling molecules that induce presynaptic differentiation are deposited in the synaptic basal lamina by the muscle, following the activation of MuSK.

Reciprocal Induction Model Applied to CNS Synaptogenesis

The mechanism of central synapse formation is much less well understood than the formation of the NMJ. The problem is complicated by both the enormous heterogeneity of the neuronal types and the differences in the timing of their development. Yet, in the emerging view, the reciprocal signaling model typified by the developing NMJ may be generalized to describe developing synapses in the CNS (Vaughn, 1989; Verderio et al., 1999b; Davis, 2000; Garner et al., 2002; Tao and Poo, 2001; Craig and Lichtman, 2001). In essence, reciprocal signaling is necessitated by the asymmetric nature of the synaptic junction. That is, a trigger of intercellular junction formation cannot simply induce a series of molecular events that are mirrored on either side of a symmetrical junction. For an asymmetric junction to assemble, each compartment must respond differentially to the initial interaction. In a basic scenario, cell contact mediated by heterophilic cell adhesion molecules could trigger the asymmetric signaling events. Indeed, evidence favors a predominant role for cell adhesion-initiated signaling in CNS synaptogenesis. Additional reciprocal interactions between the presumptive pre- and postsynaptic cells that occur prior to and after the initial cell contact may further shape the specificity of synapse assembly by favoring particular cell combinations.

Reciprocal signaling that occurs before the cell contact requires a diffusible component, whereas after the cell contact it may involve diffusible messengers, *trans*-synaptic adhesion-dependent signals, or both. Signaling during synapse assembly may also make use of a third cell such as a glial cell that does not directly contribute to the synaptic junction per se. We discuss below the types of signaling mechanisms involved in the sequential events of central synapse formation (Figure 2). We largely limit the discussion to excitatory synapse assembly due to space constraints. Comprehensive reviews of inhibitory synapse formation have appeared elsewhere (Grantyn et al., 1995; Moss and Smart, 2001; Meier, 2003).

Priming Synaptogenesis: Filopodia and Early Axo-Dendritic Activity

Filopodial Contacts

The dynamic interaction between filopodial extensions of growth cones or neuronal processes is a central feature of synaptogenesis. Filopodia have been suggested to play an inductive role in synapse formation (Fiala et al., 1998; Jontes and Smith, 2000). Imaging of fluorescently labeled neurons reveals numerous active protrusions from the dendrites in both developing hippocampal slice cultures (Dailey and Smith, 1996; Ziv and Smith, 1996) and the intact spinal cord in zebrafish embryos (Jontes et al., 2000). The number of such motile dendritic filopodia is inversely correlated with the appearance of stable dendritic spines and synapses (Dunaevsky et al.,

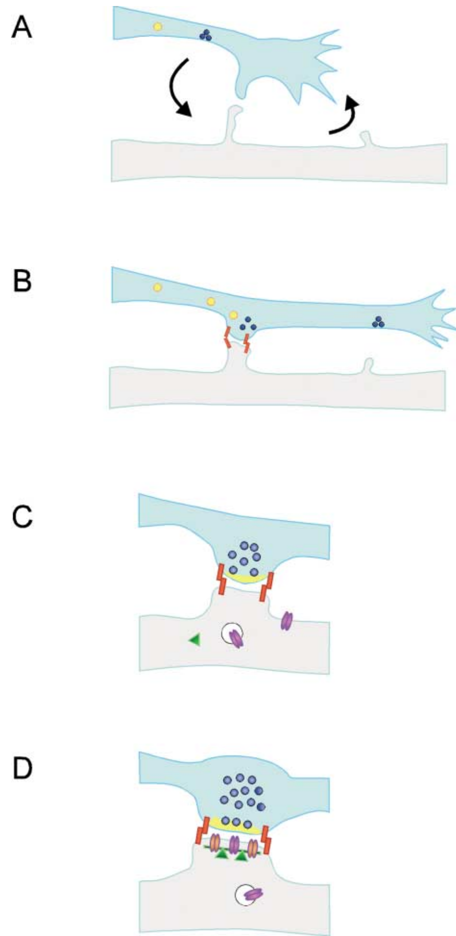


Figure 2. A Model of Excitatory Central Synapse Formation

(A) Early synaptogenic signaling events involving secreted factors precede cell contact, and motile filopodia search for potential partners. Neurotransmitters are released from exocytic hot spots where small clusters of synaptic vesicles are found (blue circles). Transport packets that contain active zone elements traverse along the axon (yellow circle).

(B) Cell adhesion molecules (red rectangles) stabilize select cell contact sites.

(C) Active zone elements (yellow) and synaptic vesicles accumulate at the presynaptic terminal. Postsynaptic terminal assembly follows presynaptic assembly by recruiting neurotransmitter receptors (double ellipses) and postsynaptic scaffolds (green triangle).

(D) In the assembled synapse, the presynaptic terminal has docked, and reserve pool of synaptic vesicles and the postsynaptic terminal show neurotransmitter receptors embedded with the scaffold proteins. See text for details.

1999; Jontes et al., 2000). These observations have led to the proposal that dendritic filopodia initiate synapse formation by reaching out to the axons, with the subsequent stabilization of a subset of the resulting contacts (Ziv and Smith, 1996; Fiala et al., 1998; Jontes and Smith, 2000). This model implies that the action of the dendrite is deterministic for synapse assembly. Compatible with such a proposal, conditions that are thought to culminate in new synapse formation by postsynaptic triggering of long-term synaptic plasticity induce active filopodial formation from dendrite shafts (Maletic-Savatic et al., 1999; Engert and Bonhoeffer, 1999; Harris et al., 2003). Interestingly, NMJ formation in flies and mammals also involves extensions of filopodial-like structures called myopodia from the muscle cells. Myopodia cluster at the site of motor neuron contact and interact with presynaptic filopodia, and these observations have suggested that myopodia play a part in guiding synapse assembly (Ritzenthaler et al., 2000; Misgeld et al., 2002).

An increase in dendritic filopodial outgrowths does not always promote synapse formation, however. For instance, dendritic filopodia formation is enhanced by perturbing the signaling to the actin cytoskeleton by overexpressing either the constitutively active form of the small GTPase Rac, a guanine nucleotide exchange factor for Rac called PIX, or a dominant-negative form of the G protein-coupled receptor kinase interacting protein (GIT)1, which interacts with PIX (Zhang et al., 2003). Despite the increase in motile filopodia, synapse formation is decreased under these conditions. Although the specific mechanisms by which Rac signaling regulates synapse formation require further investigation, this study illustrates that the production of filopodial outgrowth can be uncoupled from the promotion of synapse formation.

Recent studies demonstrate that axons can also modulate synapse formation by regulating their filopodial motility. In cultured hippocampal neurons, for example, motility of filopodia originating from mossy fiber axons decreases with development, and the filopodia that remain in contact with postsynaptic targets become stabilized (Tashiro et al., 2003). This inverse correlation between axonal filopodial motility and the developmental time course of synapse formation is reminiscent of the motile behavior of dendritic filopodia (Dunaevsky et al., 1999). Whether axonal filopodia play an inductive part in synapse formation, however, remains to be investigated.

Early Synaptogenic Signaling Events

If cell-cell contact guided by filopodia plays a central part in inducing synapse formation, what signals promote filopodial formation and how is filopodial motility regulated to enhance the likelihood of synapse formation? Neurotransmitters are capable of playing such a role as an anterograde signal. In addition, several diffusible factors that may provide general synaptogenic signals have been identified. These include secreted signaling proteins such as Wnts, neurotrophins, and CNS agrin.

Growing axons have exocytic "hot spots" that are capable of neurotransmitter release (Sun and Poo, 1987; Kraszewski et al., 1995). Presynaptic electrical activity, by enhancing exocytic glutamate release, stimulates dendritic filopodial motility at the time of synaptogenesis (Dailey and Smith, 1996; Lendvai et al., 2000; Wong et al., 2000). Moreover, like dendritic filopodia, the motility of axonal filopodia is also enhanced by glutamate or electrical stimulation, an effect that is mediated by kainate (Tashiro et al., 2003) or AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid)-type glutamate receptors (De Paola et al., 2003). Coordinate enhancement of the dendritic and axonal filopodial motility by localized release of glutamate from the exocytic hot spots would thus increase the chances of axo-dendritic contact. Confined release of glutamate from axons may also serve to restrict excitatory synapse formation to regions

that have high presynaptic activity and are able to respond to released glutamate. Glutamate release, however, has also been reported to decrease filopodial motility. AMPA or kainate receptor activation blocks movements of both dendritic spines (Fischer et al., 2000) and axonal growth cone filopodia in cultured hippocampal neurons (Chang and De Camilli, 2001). Kainate receptor activation has also been shown to stabilize the motility of mossy fiber filopodia in mature hippocampal slice cultures, opposite to the effect observed in young cultures (Tashiro et al., 2003). Whereas the neurotransmitter-dependent reduction of filopodial motility may function to stabilize and promote the maturation of synaptic contacts (Tashiro et al., 2003), one could also envisage a situation in which stabilization of one partner might increase the chances of an encounter with a motile partner. The overall pattern of synaptic connectivity may therefore be controlled by altering the neurotransmitter-dependent regulation of filopodial motility as development proceeds.

Members of the neurotrophin family of secreted growth factors, including nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and neurotrophin-4/5 (NT-4/5), promote synapse formation, in addition to their originally identified role as neuronal survival and differentiation factors (Bonhoeffer, 1996). BDNF, for example, has been reported to promote dendrite and axon arborization and increase synapse number (McAllister et al., 1999; Alsina et al., 2001), facilitating the development and maturation of excitatory and inhibitory synaptic circuits in cultured neurons (Vicario-Abejon et al., 1998; Bolton et al., 2000; Marty et al., 2000) and inhibitory synapses in the cerebellum (Seil and Drake-Baumann, 2000). Neurotrophins also have roles in synaptogenesis in the peripheral nervous system. BDNF-coated beads were shown to trigger localized neurotransmitter secretion where they contact developing spinal cord axons (Zhang and Poo, 2002). The enhanced neurotransmitter release accompanied a persistent intracellular Ca^{2+} elevation and required presynaptic protein translation, both of which were spatially restricted to the site of contact with the BDNF bead. In another recent study, the ability of NT3 to supplant the activity of BDNF for sensory system development, including synaptogenesis, was examined in mice by replacing the coding region of the BDNF gene with the coding region of NT3 (Agerman et al., 2003). Mutant mice showed a pronounced difference in the ability of NT3 to promote synaptogenesis in different sensory areas. BDNF was required for proper innervation and synaptogenesis in the vestibular system, whereas NT3 was sufficient to promote vestibular ganglion neuron survival. Furthermore, NT3 was unable to replace the actions of BDNF in the gustatory system, possibly due to a temporally regulated expression of the BDNF receptor TrkB in taste neurons. While the mechanism may be indirect, these studies nonetheless demonstrate the importance of the spatial and temporal expression pattern of neurotrophins and the distinct parts played by the particular neurotrophin receptors in guiding synapse formation.

Secreted Wnt proteins act in a wide range of developmental processes, including synaptogenesis. In the cerebellum, for example, Wnt-7a released from granule cells induces the remodeling of mossy fiber axon growth cones via retrograde activation of Frizzled receptors, and the remodeled growth cones accumulate clusters of synapsin I, a synaptic vesicle-associated protein (Hall et al., 2000). The synaptogenic function of Wnt-7a is supported by the observation that synapse formation in the cerebellum is delayed in Wnt-7a-deficient mice, though synaptogenesis ultimately proceeds normally in these mice. Similarly, Wnt-3 secreted by motor neuron dendrites in the spinal cord retrogradely promotes the terminal arborization of sensory neuron axons in the cord (Krylova et al., 2002). Interestingly, Wnt-3 affects a subpopulation of spinal sensory neurons that are responsive to NT-3 but not to NGF. Thus, differential activation of Wnt signaling may contribute to the specificity of synaptic connectivity. That a role for Wnt signaling in synapse formation may be conserved has been suggested by a recent demonstration that Wingless (Wg, the *Drosophila* Wnt homolog) functions during synapse development at the *Drosophila* NMJ (Packard et al., 2002). Wg is normally secreted from presynaptic glutamatergic boutons of motor neurons. When Wg is defective, both pre- and postsynaptic differentiation is abnormal. Whether Wg coordinates synapse assembly by acting on both the pre- and postsynaptic receptors or whether it first activates the muscle receptor, which in turn retrogradely triggers the formation of the presynaptic specializations, remains to be determined.

GSK3-β is a kinase that operates in the Wnt signaling pathway. It is inhibited when Wnt activates a Frizzled receptor. In contrast to the findings that Wnts promote synapse formation, GSK3-β has been reported to positively regulate axon arborization and synapse formation in zebrafish retinal ganglion cells (Tokuoka et al., 2002). Wnt signaling may thus regulate synaptogenesis either positively or negatively in different cell types. Such differences are not surprising given that the signal transduction cascade downstream of Wnt is highly complex (Moon et al., 2002). As with neurotrophin/Trk receptor combinations, temporal and spatial regulation of Wnts and Frizzled receptor expression may provide a versatile mechanism for controlling synapse formation between cell combinations.

Isoforms of agrin, a key synaptogenic molecule at the NMJ, are also present in the brain. As agrin mRNA expression is upregulated during the time of active synaptogenesis and in response to neuronal activity, it has been suggested that agrin may also regulate synapse formation in the brain (Cohen et al., 1997; O'Connor et al., 1995). Consistently, suppression of agrin expression in cultured hippocampal neurons severely inhibits synapse formation (Ferreira, 1999; Böse et al., 2000). Lack of agrin, however, does not completely block synaptogenesis in these studies and, furthermore, synapse formation is normal in primary hippocampal and cortical neurons cultured from agrin-deficient mice (Li et al., 1999; Serpinskaya et al., 1999). Agrin, therefore, has a modulatory role and is apparently not essential for central synapse formation. It remains to be tested, however, whether a recently identified candidate for an agrin receptor that is enriched at CNS synapses is required for synapse formation (Hoover et al., 2003).

Specification of Synaptic Adhesion

As discussed above, the initial contacts between axonal and dendritic outgrowths can be steered by the complementarity of secreted synaptogenic molecules and their receptors, and some aspects of the specificity of synapse formation could be determined by temporal and spatial restriction of these factors. Cell adhesion molecules can also have such a role by triggering the assembly of synaptic specializations (Sanes and Yamagata, 1999; Brose, 1999; Tao and Poo, 2001; Südhof, 2001; Craig and Lichtman, 2001; Benson et al., 2001; Garner et al., 2002; Jin, 2002). The molecular diversity of some of the synaptic adhesion molecules satisfies the requisite specificity of synaptic connections in various regions of the brain, and the *trans*-synaptic link could be used for reciprocally coordinating the differentiation and alignment of pre- and postsynaptic terminals. Several cell adhesion molecules have been implicated in synaptogenesis. These include members of the immunoglobulin (Ig) superfamily such as N-CAM/Fasciclin II, L1, sidekicks, and nectin (Schachner, 1997; Davis et al., 1997; Yamagata et al., 2002; Takai and Nakanishi, 2003), Ca^{2+}-dependent homophilic cell adhesion proteins such as N-cadherins (Shapiro and Colman, 1999; Lee et al., 2001, 2003) and protocadherins (Frank and Kemler, 2002), the heterophilic cell adhesion proteins such as neurexins and neuroligins (Missler and Sudhof, 1998; Rao et al., 2000), and proteoglycans such as syndecans (Yamaguchi, 2002). Here, we highlight some of the recent findings on the role of cell adhesion molecules in contact recognition and synapse assembly.

SynCAM is the latest addition to the brain-specific Ig superfamily enriched at the synaptic junctions (Biederer et al., 2002). The gene encoding the human SynCAM sequence was originally described as a candidate for a tumor suppressor gene called *IGSF4* (Gomyo et al., 1999), also known as *TSLC1* (see for example, Masuda et al., 2002). Biederer et al. (2002) identified SynCAM through a search for vertebrate proteins with extracellular Ig domains and an intracellular PDZ-interaction motif, features that are expected of a potential synapse adhesion protein. SynCAM mediates homophilic binding via its three extracellular Ig domains; the cytoplasmic domain associates with CASK and syntenin, which are PDZ-domain proteins that also interact with intracellular domains of other synaptic cell surface proteins, neurexins (see below) and syndecans. The synaptogenic potency of SynCAM was demonstrated by its ability to promote synapse formation when overexpressed in cultured hippocampal neurons. Importantly, overexpression of SynCAM in nonneuronal cells was sufficient to induce functional presynaptic assembly in contacting axons of hippocampal neurons, which required the extracellular Ig domains of SynCAM. Furthermore, overexpression of a soluble cytoplasmic fragment of SynCAM compromised the presynaptic assembly in axons of transfected neurons. These observations strongly implicate SynCAM-mediated adhesion in instructing presynaptic differentiation. Whether SynCAM also plays a role in triggering postsynaptic differentiation is presently not clear. The ability of a homophilic adhesion protein to induce asymmetric synapse formation implies that other asymmetric cues translate the "symmetrical" association into differential responses on opposite sides of the cell contact.

A potential synaptogenic cell surface interaction that satisfies the necessary asymmetry of pre- and postsynaptic differentiation is provided by the heterophilic adhesion interaction between β-neurexins and neuroligins (Missler and Sudhof, 1998; Rao et al., 2000). Neuroligins were the first cell surface molecule in which ectopic expression in nonneuronal cells was reported to induce presynaptic assembly in contacting axons in vitro (Scheiffele et al., 2000). The synaptogenic activity of neuroligins was blocked by overexpression of exogenous β-neurexins, suggesting that β-neurexins on the axonal plasma membrane mediate the presynaptic differentiation. In a follow-up investigation, Scheiffele and colleagues confirmed that β-neurexins are enriched at presynaptic terminals (Dean et al., 2003). Furthermore, they showed that postsynaptic multimers of neuroligins (at least tetramers) are required to cluster neurexins in the presynaptic membrane, which in turn recruit synaptic vesicles via their cytoplasmic domains. This study is crucially important in several respects. First, it demonstrates the significance of the lateral clustering of synaptic adhesion proteins for nucleating the presynaptic assembly process. That is, a critical density of neurexin cytoplasmic domains must be reached for organizing the presynaptic molecular scaffold, which is likely mediated by proteins such as CASK and syntenin that bind directly to the cytoplasmic domain of neurexins. Second, it underscores the sequential cooperative interactions between the pre- and postsynaptic sides required to assemble the synapse. Signals that activate the oligomerization of neuroligins to initiate the assembly process remain to be identified. A recent study showed that presynaptic α-neurexins, neurexin isoforms that do not bind neuroligin, play a role in calcium-triggered exocytosis (Missler et al., 2003). Deletion of α-neurexins in mice resulted in impaired neurotransmitter release due to a reduction in presynaptic Ca^{2+}-channel activity. However, α-neurexins do not appear to be essential for synaptogenesis since ultrastructurally normal synapses form in the mutant mice.

In addition to homophilic and heterophilic cell adhesion systems between the pre- and postsynaptic cells, synapse assembly can be modulated by adhesion mediated by a third cell, as recently shown in *C. elegans* (Shen and Bargmann, 2003). In the nematode, synapses are formed en passant, similar to most synapses found in the mammalian brain. In the HSNL neuron, which is part of the egg-laying circuit, the formation of stereotypical pattern of synaptic vesicle clusters along the axon is not dependent on the target cells—the VM2 vulval muscles and VC neurons—but rather on signals from the vulval epithelial cells. Sheng and Bargmann identified SYG1, a transmembrane Ig superfamily protein, as the recipient axonal mediator that responds to cues from the vulval epithelium. As expected for an organizer of presynaptic assembly, GFP-tagged SYG1 protein appeared at presumptive synaptic terminals preceding the appearance of synaptic vesicle clusters. SYG-1, however, was not essential for presynaptic assembly per se, as ectopic synaptic vesicle clusters formed both in *syg-1* mutant worms and in the absence of the vulval epithelium under conditions in which SYG-1 was dif-

fusely present. The function of SYG-1 demonstrates the multiple ways in which synapse adhesion molecules can influence synapse assembly. The vulval epithelial cell signal constrains the sites of future synapse assembly by directing SYG-1 cluster formation on the postsynaptic cell to specific loci by acting as a "guidepost," presumably via a mechanism involving cell contact. As SYG-1 mutants are relatively normal—i.e., they are viable, fertile, coordinated, and show no apparent defects in egg laying despite slight abnormalities in the branching of HSN axons—redundant signals may operate to ensure the formation of functional circuits. The guidepost signals from a third cell may be useful if the development of the pre- and postsynaptic cell becomes temporally uncoupled (Shen and Bargmann, 2003): synaptic vesicle clusters held at the correct location by the guidepost cell, for instance, might protect the prospective presynaptic element from responding to competing cues. Alternatively, cues from the guidepost cell might act cooperatively with *trans*-synaptic signaling between the pre- and the postsynaptic neuron, especially when the synaptogenic trigger signals are weak. We next consider the part played by synapse precursors, such as synaptic vesicle clusters, in assembling a synapse.

Assembly of Pre- and Postsynaptic Specializations

Transport Packets of Synaptic Components

Synapse assembly can occur within 1 to 2 hr of initial axo-dendritic contact, and it proceeds at a surprisingly rapid pace (Friedman et al., 2000; Okabe et al., 2001; Antonova et al., 2001; Colicos et al., 2001). Such speed of synapse assembly could be achieved by a rapid recruitment of pre-assembled synaptic components to the sites of cell contact, thereby obviating the need for building a synapse from scratch. Mobile cytoplasmic transport packets containing some synaptic vesicle proteins and active zone components—including piccolo, bassoon, N-cadherin, and a Ca^{2+} channel subunit—have been reported to traverse along the developing axon (Ahmari et al., 2000; Zhai et al., 2001). The transport packets insert into the synaptic plasma membrane to deliver active zone components prior to the appearance of synaptic vesicle proteins required for exocytosis (Friedman et al., 2000; Shapira et al., 2003). The active zone components delivered by such transport packets have been proposed to provide a scaffold for recruiting exocytically competent synaptic vesicles, possibly by either trapping preformed synaptic vesicle clusters (Ahmari et al., 2000; Friedman et al., 2000) or de novo formation of vesicle clusters at the site of active zone (Okabe et al., 2001). In considering this model, it is important to note that the cytoplasmic surface of an active zone transport packet does not appear to recruit synaptic vesicles while in transit. Rather, cues at the prospective synaptic membrane—such as that triggered by the cell adhesion molecules upon dendritic filopodial contact or guidepost signals as discussed above for SYG-1—must act in conjunction with the delivery of the active zone components to make a presynaptic scaffold fully effective.

As noted above, growing axons contain hot spots of synaptic vesicle clusters that are capable of undergoing depolarization-coupled neurotransmitter release (Sun and Poo, 1987; Kraszewski et al., 1995; Zakharenko et al., 1999). What is the function of such exocytic foci? Spontaneous activity in the growing axons may drive the release of neurotransmitters in a confined area to provide trophic synaptogenic signals (see above). Alternatively, the exocytic hot spots may represent the location of future synapses. If the latter case is true, then the following issues require consideration. First, the axonal exocytic machinery is different from the exocytic machinery used for synaptic neurotransmitter release in nerve terminals, as demonstrated by the lack of sensitivity of the axonal machinery to tetanus toxin (Verderio et al., 1999a). Moreover, the exocytic hot spots lack the hallmark features of presynaptic organization such as an active zone and clusters of docked and reserve synaptic vesicle pools (Kraszewski et al., 1995). Conversion of the hot spots into functional presynaptic terminals, therefore, would necessitate, for instance, an alteration in the components of the exocytic machinery and the secondary recruitment of the presynaptic scaffold. Second, if the site of future presynaptic specializations is predetermined, what determines their location? Because the exocytic hot spots exist prior to postsynaptic cell contact, either a third cell such as that described for the vulval epithelium in *C. elegans* (see above; Shen and Bargmann, 2003) or signals from glia may guide the location of the prospective presynaptic terminals. In addition, as yet unidentified axonal cytoskeletal organization may provide the strut for marking the future location of presynaptic terminals.

On the dendrite side, the assembly of the postsynaptic specialization lags behind that of the presynaptic specialization (Friedman et al., 2000; Okabe et al., 2001; Ziv and Garner, 2001). The earliest event in organizing the postsynaptic specialization is the appearance of N-methyl-D-aspartate (NMDA) receptors and PSD95 clusters that can serve as a molecular scaffold opposite the presynaptic specialization (McGee and Bredt, 2003). The accumulation of AMPA receptors follows the PSD95 cluster formation (Friedman et al., 2000). The timing difference of pre- and postsynaptic assembly is thought to depend on reciprocal signaling, which begins with the retrograde activation of the axon by the motile dendritic filopodia, followed by an anterograde signal from the presumptive presynaptic loci that induce postsynaptic differentiation. Accordingly, the motile filopodia that trigger presynaptic assembly are devoid of PSD95 (Okabe et al., 2001). Whether postsynaptic specialization makes use of prefabricated protein assemblies is unclear. Live imaging of fluorescently tagged PSD95 demonstrates that postsynaptic PSD95 clusters originate from a diffuse cytoplasmic pool (Marrs et al., 2001; Bresler et al., 2001). Moreover, postsynaptic AMPA receptors can be recruited from the diffuse plasma membrane pool by lateral migration (Borgdorff and Choquet, 2002). Nevertheless, discrete dendritic transport packets of PSD95 (Prange and Murphy, 2001) and NMDA receptors (Washbourne et al., 2002) have been reported. Furthermore, AMPA receptors are present in a cytoplasmic vesicular pool that participates in rapid modulation of synaptic AMPA receptor number by an exo-endocytic mechanism (Malinow and Malenka, 2002; Barry and Ziff, 2002; Luscher and Frerking, 2001). Analogous to the appearance of presynaptic vesicle clusters, post-

synaptic assembly may involve both the delivery of prefabricated transport packets and de novo clustering of component proteins, including the lateral migration of plasma membrane proteins. When and how these different mechanisms are employed might depend on both the particular synaptogenic inducers involved in different neurons and the particular developmental environment.

Organizers of Synaptic Specializations

In addition to the contact-dependent formation of presynaptic assemblies mediated by synapse adhesion proteins (discussed above), several molecules that are capable of organizing the postsynaptic assemblies at excitatory CNS synapses have been identified. For example, EphB receptor tyrosine kinases bind to and cluster NMDA receptors when activated by their ephrinB ligand in cultured neurons (Dalva et al., 2000). In addition, activated EphB receptors stimulate Src family tyrosine kinases and promote the phosphorylation of NMDA receptors to increase the Ca^{2+}-influx through the receptors (Takasu et al., 2002). NMDA receptor activation plays a key role in activity-dependent formation of synaptic connectivity pattern (Katz and Shatz, 1996; Lüscher et al., 2000). The ability of ephrinB-EphB interactions to organize and modulate synaptic NMDA receptor activity suggests, therefore, that EphB receptors can directly coordinate synapse assembly and subsequent activity-dependent synapse maturation and/or modification (Takasu et al., 2002). EphrinB-EphB interaction has also been implicated in dendritic spine morphogenesis via both synaptic syndecans (Ethell et al., 2001) and modulation of small GTPases (Irie and Yamaguchi, 2002; Penzes et al., 2003).

Another protein that displays postsynaptic receptor clustering activity is Narp, a member of the pentraxin family whose expression is modulated by synaptic activity (Tsui et al., 1996). Narp is a secreted protein that triggers aggregation of AMPA receptors and increases the number of excitatory synapses when overexpressed in cultured spinal neurons (O'Brien et al., 1999). Conversely, dominant-negative Narp suppresses the formation of AMPA receptor clusters when expressed in axons, and to a lesser extent when expressed in dendrites (O'Brien et al., 2002). Alterations in AMPA receptor cluster formation by modulating Narp expression do not accompany changes in the assembly of presynaptic components (O'Brien et al., 2002). This finding suggests that the formation of pre- and postsynaptic assemblies can occur independently, in accord with the presence of prefabricated pre- and posytsynaptic elements in neuronal processes (see above). Additional signals, therefore, must participate in modulating the overall synapse number by Narp. The mechanism by which secreted Narp is confined to the synaptic region is unknown.

It has recently been shown that factors secreted by glial cells can facilitate synapse assembly. Synaptogenesis is highly compromised in purified neurons grown in culture in the absence of glia (Pfrieger and Barres, 1997; Ullian et al., 2001). Characterization of glia-conditioned media has identified cholesterol as one component that enhances synapse assembly and maturation in cultured neurons (Mauch et al., 2001). Cholesterol has many biological functions. It influences the biophysical properties of membranes and is a major constituent of lipid rafts, which are involved in organizing signaling complexes, membrane traffic, and the actin cytoskeleton. Mechanisms by which cholesterol regulates synapse assembly, therefore, are likely to be complex (Pfrieger, 2003). Several points are worth noting. Cholesterol levels have been shown to regulate the availability of steady-state pool of secretory vesicles in PC12 cells (Thiele et al., 2000), and lipid rafts are required for kinesin-dependent axonal transport (Klopfenstein et al., 2002). Cholesterol deficiency may thus limit the availability of presynaptic vesicles for assembling the presynaptic specialization (Pfrieger, 2003). Cholesterol may also influence the formation of the postsynaptic specialization, as several postsynaptic components such as AMPA receptors (Suzuki et al., 2001), GRIP (Bruckner et al., 1999), PSD95 (Perez and Bredt, 1998), and NMDA receptors (Hering et al., 2003) are associated with lipid rafts. In a recent study, Hering et al. (2003) reported that interfering with metabolic synthesis of cholesterol and sphingolipids to deplete lipid rafts following synapse formation in culture results in destabilization of surface AMPA receptors, collapse of dendritic spines, and gradual loss of synapses. Cholesterol levels, therefore, limit the maximal number of synapses that a neuron can form and maintain (Pfrieger, 2003; Hering et al., 2003). Cholesterol likely affects pre- and postsynaptic assemblies independently, and it remains unclear to what extent its effect on synapse formation and maintenance depends on its effect on *trans*-synaptic signaling.

General Considerations for CNS Synaptogenesis

We have seen that CNS synapse formation involves multiple sequential interactions between the pre- and postsynaptic partners. Curiously, none of the identified, individual molecular signals acting at the various steps of excitatory synapse assembly are essential for synapse formation. This may be because CNS synapse formation uses multiple redundant mechanisms. Alternatively, it might be because the components have largely been identified in cell culture systems, which offer many advantages. Cultured neurons are highly accessible to molecular manipulations, and various imaging tools can be used to observe the events of synapse assembly. Despite these advantages, there are inherent limitations of an artificial growth environment. The three-dimensional organization and normal intercellular signaling milieu are lost, and neuronal process outgrowth is not directionally constrained. Moreover, the temporal regulation of the developmental program may be relaxed. Any synaptogenic factors identified under the promiscuous conditions for synapse formation in culture may, therefore, have only a subtle role in vivo, where synapse assembly is likely to be more strictly regulated.

A striking feature of synaptogenesis studied in culture is the ability of contact signaling to induce synapse assembly, as exemplified in the SynCAM and neurexin-neuroligin studies. Additionally, the ease of formation of autaptic connections between the axon and dendrites of the same neuron in culture (Segal and Furshpan, 1990; Bekkers and Stevens, 1991) and the formation of presynaptic elements induced by axon contact with polylysine-coated beads (Burry, 1986) emphasizes the promiscuity

of contact-induced synaptogenesis in cell culture. Is such contact-mediated signaling sufficient for synapse formation in vivo? Apparently not at the vertebrate NMJ, as MuSK knockout mice are unable to form synapses even though motor neuron axons reach the target muscles, implying that contact-induced signaling is insufficient. Nevertheless, promiscuity of synapse formation in vitro must reflect the synaptogenic potential of neurons. Similar promiscuity can be shown in vivo, where axons will form presynaptic specializations where they contact implanted polylysine-coated beads (Burry, 1986). Moreover, errors in synapse formation can arise in vivo: for example, axoglial synapses can form during early phases of synaptogenesis, although they are eliminated in the course of development (Vaughn, 1989). A certain degree of the readiness of synapse formation that is prevalent in culture is thus retained in vivo, and functional synapses are likely to arise from selective retention and maturation of relevant cell contacts involving multiple cooperative signaling events. The ease of synapse formation may be critical during early stages of development, when the synaptic connections are remodeled and tuned to meet the needs of the neural network in an activity-dependent process (see below). By contrast, in the adult brain, inhibitory constraints on synaptogenesis may limit the errors arising from facile rearrangement of network connectivity. Remodeling of synaptic connections inherently requires the loss of particular synaptic contacts and retention of others in addition to new synapse formation. We now turn to the discussion of the mechanisms of synapse disassembly.

Dismantling the Synapse

Throughout the nervous system there is evidence that the refinement and modulation of neural circuitry is driven not only by synapse formation, but also by the regulated disassembly of previously functional synaptic connections. For example, the pruning of initially exuberant synaptic arbors is a common theme during the early activity-dependent refinement of neural circuitry (Katz and Shatz, 1996; Sanes and Lichtman, 1999). It is also increasingly apparent that the mechanisms of regulated synapse disassembly persist in the mature nervous system, although the number of remodeling events declines with age (Gan et al., 2003). For example, live, in vivo observation of synaptic connections over prolonged time periods demonstrate that synaptic structures can be formed and eliminated, even in mature neural networks, implying an ongoing need for both synapse formation and retraction, and an ongoing need for mechanisms that balance these opposing forces (Walsh and Lichtman, 2003; Grutzendler et al., 2002; Trachtenberg et al., 2002; Sin et al., 2002; De Paola et al., 2003; Gan et al., 2003; Eaton and Davis, 2003). The prevalence of synapse disassembly has led to speculation that it could also serve as an important cellular substrate for learning and memory (Bailey and Kandel, 1993; Lichtman and Colman, 2000).

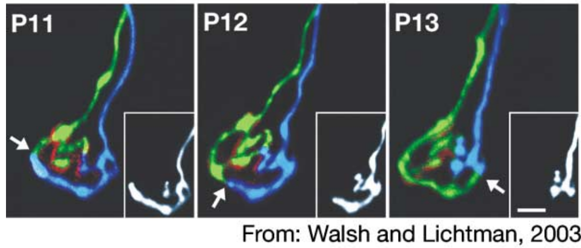
An essential distinction, when considering synapse elimination, is whether the eliminated synaptic structure was previously a functioning synapse. The elimination of previously functional synaptic connections has been clearly demonstrated in the mammalian neuromuscular system, autonomic ganglia, and cerebellum (Purves and Lichtman, 1980; Sanes and Lichtman, 1999). More recently, studies correlating anatomical synapse rearrangement with electrophysiological and ultrastructural analyses support the conclusion that synapse elimination involves the disassembly of previously functional synaptic connections at other vertebrate central synapses as well as at synapses in the invertebrate central and peripheral nervous systems (Chen and Regehr, 2000; Colman et al., 1997; Eaton et al., 2002; Lee et al., 2000; Streichert and Weeks, 1995).

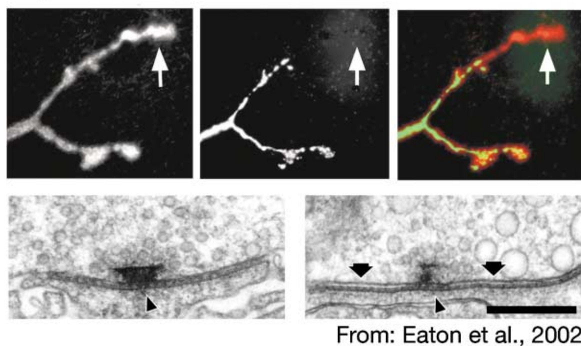
Synapse Disassembly versus Input Elimination

There are two phenomenological extremes that necessitate dismantling previously functional synapses. At one extreme is "input elimination," in which a presynaptic cell loses all synaptic contacts with a postsynaptic target, functionally and anatomically uncoupling from the target (Sanes and Lichtman, 1999). Although synaptic contact to one target is abolished, synaptic contact to other targets persist (Keller-Peck et al., 2001). An input refers to the ensemble of synapses that couple a presynaptic neuron with a target cell. Input elimination, requiring the rapid and complete disassembly of multiple individual synapses, has been studied extensively at the vertebrate NMJ as well as at the cerebellar climbing fiber synapse, but is also observed in many regions of the nervous system including the visual system, auditory system, and autonomic ganglia (Wiesel and Hubel, 1963; Shatz and Stryker, 1978; Jackson and Parks, 1982; Mariani and Changeux, 1980; Sretavan and Shatz, 1986; Sanes and Lichtman, 1999; Hashimoto and Kano, 2003; Purves and Lichtman, 1980).

At the other extreme is "synapse disassembly," which refers to disassembly of an individual synapse, or a small number of synapses, without eliminating connectivity between two cells. Here, we define a synapse as a single intercellular junction composed of a presynaptic active zone and postsynaptic receptor array capable of transducing presynaptically released neurotransmitter. Synapse disassembly could, therefore, represent a mechanism for modulating the strength of connectivity between two cells. Synapse disassembly has been observed centrally and peripherally in invertebrates (Murphey and Lemere, 1984; Streichert and Weeks, 1995; Lee et al., 2000; Eaton et al., 2002). However, synapse disassembly without input elimination has been difficult to conclusively demonstrate in the vertebrate central nervous system. Anatomical studies examining changes in axonal and dendritic arborizations in the visual system strongly suggests that remodeling events, consistent with synapse disassembly, can occur at the same time as the more dramatic process of "input elimination" (Shatz and Stryker, 1978; LeVay et al., 1980; Cline and Constantine-Paton, 1990; Antonini and Stryker, 1993; Katz and Shatz, 1996). Taking these anatomical observations to the level of individual synapses, observed before and after a disassembly event, is a very difficult task but one that is being realized through recent advances in live imaging.

Excitatory axo-dendritic synapses are often formed at dendritic spines, and there is increasing evidence that developmental and activity-dependent changes in synaptic strength are associated with the formation of





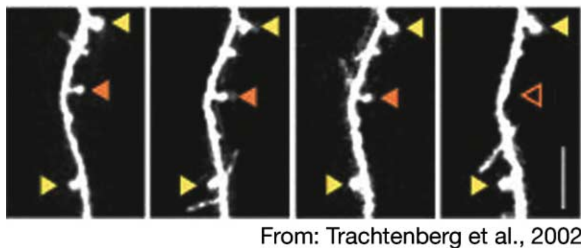


Figure 3. Input Elimination and Synapse Disassembly in the Central and Peripheral Nervous Systems

Top: An example of input elimination at the mammalian NMJ. Three views of the same NMJ imaged at P11, P12, and P13. One motoneuron is labeled with CFP (blue) and the other with YFP (green). The CFP axon is gradually eliminated and the territory formerly occupied by this axon is taken over by the YFP axon. At P15 the CFP axon will have been completely eliminated. Note that the YFP axon initially occupies less territory than the CFP axon, and yet still wins the competition. Scale bar equals 10 μm. See Walsh and Lichtman (2003) for further detail.

Middle: An example of synapse disassembly at the *Drosophila* larval NMJ (from Eaton et al., 2002). The postsynaptic muscle membrane folds are labeled with anti-discs large (red) and the presynaptic terminal is labeled with anti-synapsin (green). The formation of the muscle membrane folds requires the presence of presynaptic terminal. Presynaptic retraction occurs more rapidly than the disassembly of the postsynaptic muscle membrane folds. The retraction is revealed as an area devoid of synapsin where discs-large remains, identifying a site where the presynaptic terminal once resided and has since retracted. Below are ultrastructural images of single synapses that are representative of wild-type (left) and representative of a synapse undergoing disassembly (right). The close apposition of pre- and postsynaptic membranes, and presence of synaptic cleft material, is disrupted at a synapse undergoing disassembly (right, feathered arrowheads). The characteristic T-bar structures are indicated (arrowhead). Note the large vesicular structures present at the disassembling synapse. Scale bar equals 250 nm. For details see Eaton et al. (2002).

Bottom: A section of dendrite labeled with enhanced GFP and imaged repeatedly. Panels 1, 2, and 3 are images taken on days 6, 7, and 8, respectively. The fourth panel is an image taken on day 32. Two large mushroom spines (yellow arrowheads) are observed to be stable with lifetimes of 32 days. A different spine (orange arrowhead) is observed to be stable for 8 days, but is ultimately eliminated by day 32 of imaging (final panel, hollow orange arrowhead). Scale bar equals 5 μm. For details see Trachtenberg et al., 2002.

dendritic spines (Dailey and Smith, 1996; Engert and Bonhoeffer, 1999; Harris and Woolsey, 1981; Maletic-Savatic et al., 1999; Purves and Hadley, 1985; Purves et al., 1986; Sin et al., 2002; Toni et al., 1999; Lendvai et al., 2000; Grutzendler et al., 2002; Trachtenberg et al., 2002). Similar observations have been made examining changes to presynaptic axonal and synaptic arborizations (Antonini et al., 1998; O'Rourke and Fraser, 1990; Darian-Smith and Gilbert, 1994; Lom and Cohen-Cory, 1999). What about synapse disassembly? Recent live imaging studies of developing synapses emphasize the prevalence of synapse remodeling and provide compelling evidence that pre- and postsynaptic dynamics may be associated with the elimination of individual, previously functional, synaptic connections. *Xenopus* tectal dendrites are added and retracted over the course of several days, ultimately reaching a state of dynamic equilibrium, during which the rates of addition and retraction are nearly balanced (Sin et al., 2002). These dynamics are modulated by visual activity, implicating these dynamics in the activity-dependent refinement of functional synaptic circuitry in this system (Sin et al., 2002). In a separate study, two-photon imaging of spine dynamics in the mammalian cortex has been correlated with the formation and elimination of ultrastructurally defined synapses (Figure 3; Trachtenberg et al., 2002). Ultrastructural analysis is required to test whether a spine retraction includes the elimination of a synapse or whether the spine retraction simply translocates a synapse from a spine head to the dendrite shaft. The authors find that the number of spine retractions observed at the light level in a section of dendrite is 2-fold greater than the number of ultrastructurally observed synapses on the same dendritic segment. Thus, a portion of the spine retractions observed at the light level must actually eliminate synapses, since all of the spine retractions cannot be accounted for, ultrastructurally, by a spine synapse being converted into a synapse on the dendrite shaft. These data support the conclusion that a portion of spine elimination events observed at the light level represent the ultrastructural disassembly of individual synapses (though it is not possible to assay whether these were previously functional synapses). Two additional studies provide further evidence for synapse disassembly, in these cases through the visualization of presynaptic terminals over time. Live imaging of individual presynaptic arbors within a mature hippocampal slice demonstrates the continual addition and elimination of varicosities that may represent functional ac-

tive zones (De Paola et al., 2003). A separate presynaptic imaging study examined presynaptic vesicle-associated proteins and correlates their abundance with the imaging of vesicle recycling over prolonged time periods (Hopf et al., 2002). Although the case for the actual disassembly of the synapse is less strong, these data define changes to a population of synapses that include functioning presynaptic active zones.

An important question is whether input elimination is simply an extreme example of synapse disassembly, or whether these processes are fundamentally different in some way. Several phenomenological observations suggest that there will be similarities between these processes. For example, input elimination and synapse disassembly share ultrastructural similarities when comparing input elimination at the mammalian NMJ with synapse disassembly at the *Drosophila* NMJ. In both systems, presynaptic withdrawal includes a decreased caliber of the presynaptic element, detachment of the pre- and postsynaptic elements, and the presence of prevalent large vesicular structures that may reflect a common cellular program to dispose of membrane and protein derived from synapse disassembly (Figure 3; Eaton et al., 2002; Bernstein and Lichtman, 1999).

There is also some evidence for mechanistically separable processes that result in the elimination of synapses. In the *Drosophila* CNS, experiments have identified what appear to be two mechanistically separable phenomena that each result in the elimination of the synapses along a synaptic arbor. In one example, retraction occurs in a distal to proximal fashion along a presynaptic arborization. This retraction is initiated by hormonal cues and involves TGF-β signaling as well as the activation of intracellular signaling via RhoA and its effector dRock (Lee et al., 2000; Billuart et al., 2001). A separate phenomenon of axonal pruning has also been observed in which an entire segment of a presynaptic arborization appears to be dismantled simultaneously. Importantly, this process of pruning is initiated prior to the loss of synaptic antigens, indicating that this process may be initiated and proceed independent of synaptic signaling (Watts et al., 2003). Pruning requires ubiquitin-proteosome signaling and protein degradation, and parallels have been drawn, in this case, to Wallerian degeneration (Watts et al., 2003). It remains to be determined whether these phenomena, retraction and pruning, serve unique purposes during metamorphosis in the *Drosophila* CNS. It also remains to be determined whether these phenomena, which are under hormonal control, are related to the activity-dependent mechanisms of synapse disassembly/input elimination that dominate the vertebrate central and peripheral nervous systems. In this vein, it should be noted that a phenomenon of axonal pruning, involving semaphorin-Plexin A3 signaling, has been observed in the vertebrate CNS, although the relationship of this pruning to synapse function and remodeling awaits further experimentation (Bagri et al., 2003).

Ultimately, a detailed molecular understanding will be necessary to establish the commonality and differences between input elimination and synapse disassembly. It seems logical that there will be a common cell biological mechanism responsible for dismantling the synapse that can be co-opted by different developmental and activity-dependent programs (Eaton and Davis, 2003). However, the data from the *Drosophila* CNS suggest that there may be added complexity. In this context, it is interesting to note that input elimination is prevalent during early development in the vertebrate PNS and CNS, while synapse disassembly persists throughout life. Our current understanding of the phenomenology and underlying mechanisms of synapse disassembly and input elimination are detailed in the following sections.

Input Elimination at the Vertebrate NMJ

The mammalian NMJ is perhaps the most well-characterized synapse in any organism. At birth, each muscle fiber is innervated by multiple motoneurons and all but one motoneuron input are gradually eliminated over the course of several weeks (Figure 3). The most compelling model for input elimination outlines an activity-dependent competition between initially equivalent inputs in which one of the inputs emerges victorious. The involvement of activity was clearly demonstrated in several experiments. For example, if activity is blocked, elimination does not occur (Duxson, 1982; Thompson et al., 1979; however, see Costanzo et al., 2000). The relative synaptic efficacy of two competing axons at a single NMJ also predicts the outcome of synaptic competition. This has been most recently, and elegantly, demonstrated by genetically reducing synaptic efficacy at one of two axons innervating a target. This was achieved by conditional genetic manipulation of choline acetyl transferase, the synthetic enzyme for ACh, in subsets of motoneurons (Buffelli et al., 2003). Several experiments also indicate that this synaptic competition is mediated through the muscle cell. In one experiment it was shown that focal blockage of receptor activation within a small region of the NMJ induced elimination at these sites, suggesting that local differences in receptor activation can drive elimination (Balice-Gordon and Lichtman, 1994). Additional evidence supports the view that postsynaptic activity-dependent mechanisms function locally to drive the elimination of neighboring inputs. For example, if two motoneuron inputs are separated by enough distance on the muscle surface, both can be maintained (Kuffler et al., 1977). These and other observations have led to several theories of activity-dependent competition leading to input elimination at the NMJ. In the first model, presynaptic inputs compete for access to limiting amounts of muscle-derived trophic signals. Less active inputs receive less trophic support and input elimination ensues (Sanes and Lichtman, 1999; however, Callaway et al., 1987). A second type of model invokes signaling mechanisms that actively drive the process of elimination at less active inputs. These putative signals have been termed "synaptotoxins" or "punishment signaling" (Sanes and Lichtman, 1999). Since activity-dependent competition appears to be mediated through the muscle, this model also invokes the idea that less active inputs are somehow more susceptible to the "synaptotoxin" or that inputs with more activity are somehow protected, or both (Sanes and Lichtman, 1999).

These models have provided an important framework for considering the mechanisms of input elimination.

However, recent experiments have provided new insight into the complexity of competition-driven input elimination. The advent of GFP mice has allowed the time-lapse visualization of both the motorneuron terminal and postsynaptic receptors (visualized using subblocking concentrations of α-bungarotoxin). It was demonstrated that an input could initially begin the process of elimination and then subsequently reverse this process by growing to become the single input that is maintained (Walsh and Lichtman, 2003). This observation is important for several reasons. First, it demonstrates that the mechanisms underlying input elimination are reversible. This would suggest that input elimination is not a switch but is a process that is continually driven until an entire input is ultimately eliminated. These data are also important because an ineffective input was observed to overtake a more effective input, indicating that there must be mechanisms in addition to receptor activation that determine the outcome of competition-driven input elimination.

Input Elimination at the Cerebellar Climbing Fiber Synapse

Purkinje cells (PCs) within the cerebellum receive distinct excitatory inputs from parallel fibers (PFs) and climbing fibers (CFs). During early postnatal development in the rodent brain, PCs are multiply innervated by presynaptic CFs, all but one of which are removed over the course of a few weeks, leaving a single CF axon to innervate each PC (Sotelo, 1975; Mason and Gregory, 1984; Ito, 1984). The one-to-one relationship between CF and PC is then maintained throughout the lifetime of the adult.

Work on mutant mice over the last 25 years has suggested that the failure to properly eliminate supernumerary connections in the cerebellum has functional consequences for the animal. Initial studies on the classic mouse mutants *weaver* and *staggerer* found that these mutants with obvious motor ataxia also had multiple CFs innervating single PCs in the adult brain (Sotelo, 1975; Crepel and Mariani, 1976; Crepel et al., 1980). Recent work on mutant mice deficient in PKC, mGluR1, PLC, or $G_{\alpha q}$ has also shown a correlation between locomotor ataxia and the failure to properly eliminate CF innervation on the PCs (Aiba et al., 1994; Conquet et al., 1994; Chen et al., 1995; Kano et al., 1995, 1997; Kim et al., 1997; Offermanns et al., 1997; Ichise et al., 2000). In addition, mutants in mGluR1 are also deficient in Purkinje cell LTD, providing a link between synapse elimination, plasticity, and motor coordination (Ichise et al., 2000).

Since mGluR1 is expressed in other regions of the brain, Ichise and colleagues performed PC-specific rescue in mGluR1$^{-/-}$ mice to conclusively show that mGluR1 is required in the postsynaptic PCs for normal regression of multiple CF innervation (Ichise et al., 2000). Therefore, it is likely that regulation of PLC and PKC via mGluR1 activation is occurring in the postsynaptic cell, which then drives the removal of supernumerary presynaptic connections via the initiation of an unknown elimination program. Although it remains unclear what the mechanisms are leading to the disassembly of synaptic connections between the CFs and PCs, the postsynaptic cell appears to mediate the elimination of presynaptic inputs in the cerebellum, similar to what has been seen at the NMJ.

The Visual System

Anatomical and functional data provide clear evidence of input elimination during the activity-dependent refinement of neural circuitry in the visual system (Sur et al., 1984; Sretavan and Shatz, 1986; Hamos et al., 1987; Cline and Constantine Paton, 1990; Katz and Shatz, 1996). For example, following the occlusion of one eye in early development, most cells in the cortex will lose responsiveness to the occluded eye and respond only to the open eye (Wiesel and Hubel, 1963). Anatomical data demonstrate that the presynaptic arbors of the afferents derived from the occluded eye rapidly shrink in size, consistent with anatomical input elimination (Antonini and Stryker, 1993, 1996). More recently, a detailed electrophysiological analysis provides clear evidence of functional input elimination at the retino-geniculate synapse. It was shown electrophysiologically that geniculate cells initially receive more than 20 functional retinal inputs and all but 1–3 of these inputs are eliminated in a 3 week period spanning eye opening (Chen and Regehr, 2000).

It is now clear that visual plasticity, and by extension input elimination, is driven in part by activity-dependent synaptic competition (Katz and Shatz, 1996). There are several details worth emphasizing. As at the NMJ, activity-dependent competition in the visual system appears to be mediated through the postsynaptic cell (Katz and Shatz, 1996). Again, however, the link between activity and synapse disassembly is complex. For example, anatomical changes associated with input elimination can be driven in opposing directions by changes in correlated activity. Experiments combining visual deprivation with the manipulation of postsynaptic activity demonstrate that identical levels of presynaptic activity can lead to opposite directions of synaptic rearrangement (afferent expansion versus retraction) depending upon whether or not activity in the postsynaptic cell is inhibited (Hata and Stryker, 1994; Hata et al., 1999). Another important point is that the process of input elimination is reversible in the visual system, as it is at the NMJ, and reversibility is driven by changes in activity (Antonini et al., 1998). Since the balance of branch addition and retraction during development of visual neurons can be influenced by visual activity (Cohen-Cory, 1999; Sin et al., 2002), it is interesting to speculate that induction and reversibility of input elimination is achieved not only through the control of disassembly, but through the coordinate control of several processes including synapse disassembly, synapse formation, and cellular growth. The extent to which these processes are separable awaits further experimentation. Taken together, these data underscore the cellular complexity involved in moving from activity to the molecular mechanisms that dismantle a synapse.

There is some consensus regarding the underlying molecular signaling that drives synaptic competition in the visual system. Neurotrophin signaling (Lein and Shatz, 2000; Cohen-Cory, 2002; Huang and Reichardt, 2001; Berardi et al., 2000), NMDA receptor activation

(Cline and Constantine Paton, 1990; Sin et al., 2002; Berardi et al., 2000), calcium signaling via CamKII (Taha et al., 2002; Wu and Cline, 1998; Lisman et al., 2002), and CREB (Pham et al., 2001) appear necessary in various experiments for this morphological and functional plasticity (Lisman et al., 2002; see also Huh et al., 2000). However, the relationship of these signaling systems to the cellular mechanisms of synapse disassembly/input elimination (discussed below) remains unclear. The emerging challenge is to connect the mechanisms that transduce changes in correlated activity to the molecular mechanisms that direct synapse disassembly/input elimination (see also Hensch et al., 1998, in this regard). Experimentally teasing apart these interconnected signaling systems may ultimately require simplified genetic systems such as *Drosophila* and *C. elegans*, where these processes can be studied using forward genetics (Hallam and Jin, 1998; Eaton et al., 2002; Lee et al., 2000).

Synapse Disassembly in *Drosophila* and *C. elegans*

The *Drosophila* larval NMJ, unlike the vertebrate central and peripheral systems described above, is molecularly specified such that each muscle cell receives input from identified motorneurons and these inputs persist throughout development (Keshishian et al., 1996). Though the number of innervating axons does not change during larval development, the size of the synapse increases dramatically. Analysis of an identified synapse demonstrates that it increases in size from ~20 boutons, each bouton containing a single active zone, to ~100 boutons, with each bouton encompassing 7–12 active zones (Schuster et al., 1996). The elaboration of presynaptic morphology and active zone insertion is tightly coupled to the growth of the postsynaptic muscle, indicating that synaptic development is specified by the coupling of pre- and postsynaptic growth (Davis and Bezprozvanny, 2001).

It has been recently shown that synaptic growth at this synapse also includes the rapid disassembly of synapses within the neuromuscular junction (Figure 3; Eaton et al., 2002). Synapse disassembly was assayed using light level, ultrastructural, and electrophysiological assays. Importantly, synapse disassembly is generally restricted to individual branches or even individual synaptic boutons within a single presynaptic arbor, suggesting that these events themselves are locally defined and do not result in the complete elimination of the motoneuron input (Eaton et al., 2002). Developmental analysis demonstrates that synapse disassembly occurs throughout development and is most prevalent during the rapid phases of synaptic growth. These data suggest that growth at the *Drosophila* NMJ is a balance of bouton addition and retraction and that elimination is developmentally regulated. Evidence suggests that these disassembly events are not due to competitive interactions between motoneuron branches innervating a single muscle (G.W.D. and Benjamin A. Eaton, unpublished data). Mechanistically, retrograde synaptic TGF-β signaling and retrograde axonal transport have been implicated in synapse disassembly at the *Drosophila* NMJ. Synaptic TGF-β signaling is necessary for the normal development of the *Drosophila* NMJ (Aberle et al., 2002; Marques et al., 2002; McCabe et al., 2003). Although the absence of this signal has not been directly linked to synapse disassembly, mutations in the dynein/dynactin complex, which are necessary for the retrograde transport of TGF-β signaling in *Drosophila* central neurons (Allan et al., 2003; McCabe et al., 2003), has been shown to increase the rate and frequency of synapse disassembly at the NMJ (Eaton et al., 2002).

Input elimination and synapse disassembly have also been observed in *C. elegans*. In this system, synapse disassembly is necessary for an unusual rewiring event during larval development. Six GABAergic motoneurons send processes to both dorsal and ventral muscles. Initially, synapses are made only with the ventral muscles. However, as development proceeds, this connectivity is reversed. The motoneurons disassemble their synapses at the ventral muscles and form new synapses with the dorsal muscles. There is no change in the architecture of the motoneuron processes despite this rearrangement of synaptic connectivity (Hallam and Jin, 1998; White et al., 1978). This synaptic rearrangement has been observed at both the light and ultrastructural levels (Hallam and Jin, 1998; White et al., 1978). Little is known about the time course of this event, though it can be modulated by mutations in the heterochronic gene *lin-14* (Hallam and Jin, 1998).

Mechanisms of Synapse Disassembly and Input Elimination

Although the molecular mechanisms that initiate synapse elimination are not known, we do have information regarding the sequence of events that occurs during elimination. At the vertebrate NMJ, different synaptic proteins are lost at different rates. The first molecules to disappear include postsynaptic AChRs, utrophin, rapsyn, and phospho-tyrosine-modified AChR (Culican et al., 1998). It remains unclear whether the loss of any of these markers could be a precipitating event, however, since AChRs can be maintained at some types of muscle fibers following nerve degeneration (Pun et al., 2003). A number of other molecules disappear at slower rates including dystrophin and syntrophin, while markers of the extracellular basal lamina are removed at even slower rates (Culican et al., 1998). It has been speculated that the molecular constituents of different molecular scaffolds may be eliminated with different time courses since AChR/rapsyn are located at the top of the junctional fold while dystrophin and syntrophin are located at the base of these folds (Froehner, 1991; Culican et al., 1998).

The majority of data support the conclusion that synapse elimination is specified postsynaptically. For example, many experiments emphasize the importance of signaling from the postsynaptic cell. At both central and peripheral synapses, "input elimination" is driven by activity-dependent competition mediated through the postsynaptic cell (Sanes and Lichtman, 1999; Katz and Shatz, 1996). The role of the postsynaptic cell as intermediary is further strengthened by recent experiments at the mammalian NMJ where visualization of an entire motor unit over time reveals that input elimination occurs asynchronously among branches of a single motoneuron without any apparent regional bias, arguing for local control at each muscle fiber (Keller-Peck et al., 2001).

Despite the relative consensus that the postsynaptic cell acts as intermediary during synaptic competition, it appears that postsynaptic disassembly need not precede presynaptic retraction. Electrophysiological recordings at the mammalian NMJ, correlated with postsynaptic receptor staining, demonstrate that removal of postsynaptic receptors can precede the retraction of the presynaptic element (Colman et al., 1997; Akaaboune et al., 1999). However, live imaging experiments at the mammalian NMJ also provide clear evidence of retracting presynaptic elements at sites where postsynaptic receptors persist (Walsh and Lichtman, 2003). Furthermore, at some muscle fibers types, receptors can persist following complete presynaptic retraction (Pun et al., 2003). It is possible that loss of receptors need not proceed to completion prior to presynaptic elimination at some synapses, and at other synapses postsynaptic disassembly need not occur prior to presynaptic retraction. Thus, while evidence is stacked in favor of the postsynaptic cell mediating the synaptic competition that leads to input elimination or synapse disassembly, the mechanisms that dismantle the synapse may have some degree of autonomy in the pre- versus postsynaptic element.

A similar situation is observed in *Drosophila*. At the *Drosophila* NMJ, signals from the postsynaptic muscle could initiate a presynaptic program of disassembly since synapse disassembly can occur locally at one of several muscles contacted by single motoneurons (Eaton et al., 2002). However, the earliest molecular signatures of retraction occur presynaptically at this synapse, suggesting that the motoneuron may have a deterministic role for synapse disassembly. Examination of fixed preparations suggests that presynaptic elimination of synapsin- and vesicle-associated proteins precede the removal of postsynaptic receptors (Eaton et al., 2002). The loss of these presynaptic antigens has also been implicated as an early event in the elimination of individual synapses in the central nervous system, in vitro (Hopf et al., 2002). Examination of fixed preparations at the fly NMJ also suggests that retraction of the microtubule cytoskeleton may be one of the earliest events during synapse retraction (Eaton et al., 2002). These events appear to be followed by the removal of postsynaptic receptors and the subsequent dissolution of the postsynaptic muscle membrane folds, which occurs in parallel with the retraction of the presynaptic membrane.

There is also increasing evidence for cell-wide processes that can bias a cell, pre- or postsynaptically, toward increased synapse elimination. Perhaps the clearest examples are those demonstrating that hormonal signaling can initiate extensive remodeling and input elimination throughout a cell (Matsumoto et al., 1988; Streichert and Weeks, 1995; Lee et al., 2000). There is also evidence for intrinsic differences between motoneurons (Barry and Ribchester, 1995; Personius and Balice-Gordon, 2001; Buffelli et al., 2002; Kasthuri and Lichtman, 2003) and between different muscle types (Pun et al., 2003) that may influence synaptic competition and input elimination. Finally, time-lapse imaging of Homer 1cGFP in hippocampal cultures was used to assay synapse dynamics (Ebihara et al., 2003). In this analysis it was shown that the dynamics (appearance and disappearance) of Homer-GFP in different regions of a single cell are more highly correlated than the dynamics between different cells (Ebihara et al., 2003). One interpretation is that individual cells have different biases regarding synapse formation and elimination.

Molecularly, it has long been hypothesized that withdrawal of trophic support could initiate synapse disassembly or input elimination (Snider and Lichtman, 1996; Sanes and Lichtman, 1999), and there is recent experimental evidence that trophic support is necessary for synapse maintenance and development (Huang and Reichardt, 2001; McAllister et al., 1999; Cohen-Cory, 2002). Loss of NT4 or TrkB at the NMJ promotes synapse elimination, and loss of TrkB signaling in the cerebellum results in the development of fewer GABAergic synapses assessed at both the light and ultrastructural levels (Gonzalez et al., 1999; Belluardo et al., 2001; Rico et al., 2002). Inhibiting TrkB function during the critical period impairs ocular dominance formation (Cabelli et al., 1997). Conversely, at both central and peripheral synapses, excess neurotrophin signal can prevent competition-based plasticity, presumably because the neurotrophin signal is no longer limiting for synaptic support (Cabelli et al., 1995; Riddle et al., 1995; Nguyen et al., 1998). In the *Drosophila* olfactory system and NMJ, similar mechanisms underlying synapse disassembly may involve TGF-β signaling (Lee et al., 2000; Aberle et al., 2002; Eaton et al., 2002; McCabe et al., 2003).

There are conceptual problems, however, with the hypothesis that synapse disassembly is initiated and driven simply by the withdrawal of trophic support. In instances where only one or a few synapses are disassembled within a presynaptic arbor, it is difficult to imagine how trophic withdrawal could precipitate such a spatially confined event. Furthermore, the speed of synapse disassembly can be substantially faster than the rate of protein turnover at a synapse, indicating that destabilizing mechanisms may be necessary in addition to the removal of the trophic support. For example, the half-life of AMPA receptors at a central synapse has been measured to be 18–23 hr, and the half-life of NR2 is 16 hr (Huh and Wenthold, 1999). Yet live imaging studies have demonstrated that AMPA receptor-containing synapses can be eliminated as quickly as 90 min and dendritic spines have been observed to be eliminated in less than 1 day (Okabe et al., 2001; Grutzendler et al., 2002; Trachtenberg et al., 2002).

Activity-blockade experiments at both central and peripheral synapses highlight the possibility that neurotransmitter release can act to stabilize the synapse. At the vertebrate NMJ, 2 hr of complete blockade of neurotransmission has been observed to enhance the rate of AChR turnover 25-fold (Akaaboune et al., 1999). These data are consistent with genetic studies at the *Drosophila* NMJ examining glutamate receptor clustering in the absence of presynaptic release (Saitoe et al., 2001). In the CNS, spines disappear following glutamate receptor blockade or the addition of botulinum toxin (McKinney et al., 1999). It is conceivable that transmitter release acts through the activity-dependent release of neurotrophins, but it is equally plausible that neurotransmitter could act in concert with trophic support to add necessary specificity.

Another interesting possibility is that the disruption

of specific synaptic scaffolds initiates synapse disassembly. It is clear that scaffolding proteins have an essential function in the organization and integrity of the pre- and postsynaptic protein complexes (Chen et al., 2000; Sheng, 2001; McGee and Bredt, 2003). These scaffolds appear to be more dynamic than once thought, suggesting that there will be cellular signaling responsible for their maintenance and possibly their destruction. There is increasing evidence that modification to synaptic scaffolds can alter synapse formation and stability. For example, overexpression of PSD-95 increases synapse stability and number in hippocampal cell culture and the localization of PSD-95 at the synapse has been recently linked to glutamate receptor activity and visual plasticity in response to eye opening (El-Husseini et al., 2000, 2002; Yoshii et al., 2003). Furthermore, the regulated disassembly of large protein complexes via proteosomal or lysosomal degradation is a common theme throughout cell biology that is recently being linked to synaptic growth and plasticity (Hegde et al., 1997; DiAntonio et al., 2001; Burbea et al., 2002; Sweeney and Davis, 2002; Watts et al., 2003; Eaton and Davis, 2003). Although altered scaffolding has not been linked directly to synapse disassembly (Colledge and Froehner, 1998), ultrastructural visualization of synapse disassembly at the *Drosophila* NMJ suggests that one of the first events in disassembly may be a loss of signaling between the pre- and postsynaptic membranes at the active zone, as evidenced by an increased separation between the synaptic membranes and the clearing of electron-dense material in the synaptic cleft (Figure 3; Eaton et al., 2002). Thus, disruption of the linkages between pre- and postsynaptic scaffolds, including signaling and cell adhesions molecules, could be a precipitating event.

A discussion of synapse disassembly is not complete without considering the involvement of the synaptic cytoskeleton. There is increasing data demonstrating the importance of actin in the maintenance and modulation of the synapse. Actin is enriched both pre- and postsynaptically (Matus, 2000; Luo, 2002) and is highly dynamic (Colicos et al., 2001; Star et al., 2002; Fukazawa et al., 2003), and manipulations that alter actin dynamics, including activity, also alter synapse morphology and stability (Hatada et al., 2000; Lisman, 2003; Finn et al., 2003; Zhang and Benson, 2001; Fukazawa et al., 2003). Furthermore, there are numerous signaling pathways that may lead to modulation of the synaptic actin cytoskeleton that are present at the synapse including intercellular signaling molecules and cytoplasmic actin-regulatory proteins (Murase et al., 2002; Penzes et al., 2003; Finn et al., 2003). Evidence that actin might be directly involved in stabilization and elimination is suggested by pharmacological studies demonstrating that immature, relatively dynamic synapses are susceptible to actin depolymerizing drugs while older, less dynamic synapses appear largely resistant to pharmacological disruption of actin (Zhang and Benson, 2001; Lisman, 2003). It should be noted, however, that there might be core components of the synaptic complex that will persist despite severe disruption of the actin and microtubule cytoskeletons (Allison et al., 2000; Dunaevsky and Connor, 1998).

In conclusion, the subcellular mechanisms that initiate and drive the process of synapse disassembly/input elimination are not known. However, we are learning quite a bit about the criteria that must be met by the underlying signaling systems. Synapse disassembly is controlled both spatially and temporally, affecting specific synapses within a dendritic tree or presynaptic arbor from individual neurons (Keller-Peck et al., 2001; Eaton et al., 2002; Grutzendler et al., 2002; Trachtenberg et al., 2002). Synapse disassembly can be modulated by activity, may require *trans*-synaptic signaling, and may be initiated either pre- or postsynaptically (Sanes and Lichtman, 1999; Eaton et al., 2002; Walsh and Lichtman, 2003; Hopf et al., 2002). Synapse disassembly is also reversible in both the CNS and PNS (Antonini et al., 1998; Walsh and Lichtman, 2003; De Paola et al., 2003). Together, these criteria argue that the process of synapse disassembly, even during "input elimination," is not a switch-like, catastrophic process. Rather, it appears that the underlying mechanisms of disassembly can be turned on and off and that the persistent action of a disassembly program may be necessary for extensive events such as input elimination.

Coordinating Synapse Assembly and Disassembly

It is now well established that the refinement of neural circuitry is achieved by a combination of synapse assembly and disassembly. Remarkably, recent live imaging studies at developing central synapses demonstrate an apparent dynamic equilibrium between synapse formation and synapse disassembly (Sin et al., 2002; De Paola et al., 2003; Trachtenberg et al., 2002; Grutzendler et al., 2002). These studies suggest that the opposing forces of synapse assembly and disassembly are coordinately regulated to attain constant synapse density during the rearrangement of synaptic connectivity. How, then, are the processes of synapse assembly and disassembly balanced? Studies at the NMJ provide insights into potential mechanisms.

One possibility for controlling the extent of synapse assembly and disassembly is to constrain the size of the axonal or dendritic arborization by cell-wide and cell-autonomous growth programs. In addition to the evidence for a cell-wide regulation of synapse disassembly discussed earlier, recent work at the mammalian NMJ lends further support to the generality of a cell-wide, coordinated regulation of synaptic assembly and input elimination. In this study, synaptic competition is examined between two motoneurons, labeled with different GFP variants, at each muscle fiber where the two motoneurons converge (Kasthuri and Lichtman, 2003). The analysis is made possible by visualization of the entire axon arbor of two individual motoneurons among many motoneurons that innervate a set of muscle fibers. It is possible, therefore, to assay the outcome of synaptic competition between two motoneurons at every muscle fiber where they converge, which is a minority of the fibers innervated by each MN (Figure 4). There are two remarkable observations. The first observation is that one motoneuron loses every competition with the second motoneuron (though the loser is able to win at other sites when competing with other motoneurons). Thus, one motoneuron appears to have an intrinsic competitive advantage over the other neuron

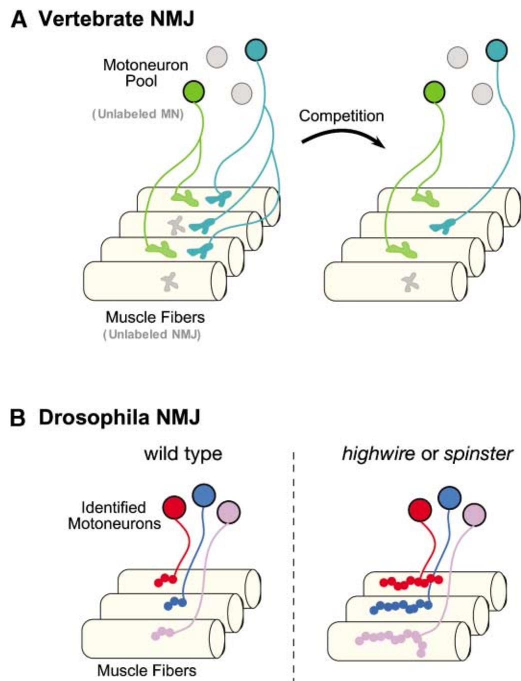


Figure 4. Competition, Elimination, and the Relationship to Total Nerve-Terminal Area

(A) Schematic of synaptic competition at the mammalian NMJ based on results from Kasthuri and Lichtman (2003). When only two motoneurons are labeled (green and blue) from a large pool of unlabeled motoneurons (gray circles with no diagrammed axon), it is possible to study how these two axons compete at each muscle fiber where they converge. Unlabeled motoneurons also project to and form synapses with these muscle fibers (gray NMJs). During competition, the MN with the smaller total synaptic area (green) has a competitive advantage and wins at every site where it converges with the blue neuron. The blue neuron wins competitions at other muscles, competing against unlabeled axons. These and other data define a correlation between competitive vigor, input elimination, and total synaptic arbor area.

(B) Forward genetic screens in *Drosophila* have identified genes that normally function to restrict the total area of the synaptic arborization. Synaptic connectivity is molecularly specified in *Drosophila* and there is no evidence of synaptic competition. At left is diagrammed the stereotyped connectivity of three identified motoneurons at the end of synaptic development. Diagrammed at right is the extraordinary synaptic overgrowth that is observed at the end of synaptic development in two independent mutant backgrounds, *highwire* (Wan et al., 2000) and *spinster* (Sweeney and Davis, 2002). Both mutations are implicated in regulated protein trafficking at the synapse (DiAntonio et al., 2001; Sweeney and Davis, 2002), although it is not understood how these genes normally function to restrict synaptic growth to achieve stereotyped total synaptic arborization sizes in wild-type animals.

throughout its entire presynaptic arborization. It has been recently demonstrated that a more efficacious synapse will likely win a synaptic competition (Buffelli et al., 2003). If synaptic efficacy can be linked to axonal activity, then differences in motoneuron activity could be one means to bias competition throughout the entire arborization of a single motoneuron (Buffelli et al., 2003). The second observation is that motoneurons with larger total arborizations are at a competitive disadvantage when they compete against motoneurons with smaller total arborizations (Kasthuri and Lichtman, 2003). One hypothesis to explain the negative correlation between arbor size and competitive vigor is that a cell-wide finite resource influences the size of an arborization. This resource becomes dilute as a motoneuron gains territories and size and somehow limits the ability of the neuron to engage in synaptic competition (Kasthuri and Lichtman, 2003). Such growth restriction may act to prevent excessive expansion of single arborizations during the refinement of neural circuitry.

Genetic data from *Drosophila* provide some mechanistic insight into this type of synaptic growth regulation (Figure 4). Two recently identified genes, *highwire* (Wan et al., 2000) and *spinster* (Sweeney and Davis, 2002), are required to restrain normal synaptic growth since mutations in these genes result in tremendous synaptic overgrowth (200%–300%). *highwire* encodes a large, multidomain protein that functions in part as an E3 ubiquitin ligase (DiAntonio et al., 2001). *spinster* encodes a multipass transmembrane protein localized to the late endosomal compartment (Sweeney and Davis, 2002). Both proteins appear to be involved in regulating protein traffic. Genetic data indicate that Spinster also regulates synaptic TGF-β signaling (Sweeney and Davis, 2002) that is necessary for synaptic growth at the *Drosophila* NMJ (Marques et al., 2002; Aberle et al., 2002; McCabe et al., 2003). These findings suggest an intimate link between the mechanisms of protein traffic and synaptic growth control, possibly through the regulation of growth factor signaling. Although synaptic competition such as that observed at the mammalian NMJ does not occur in *Drosophila*, it is worth speculating that similar mechanisms may supply the finite resource restriction discussed above and specify synaptic growth and competitive vigor at the mammalian NMJ.

Mechanisms of synapse stabilization or maintenance could alter the effectiveness simultaneously of synapse assembly and disassembly. Increased synapse stabilization could suppress the dynamics of synapse assembly and disassembly, whereas weakening of synapse stabilization could promote synapse assembly and disassembly. In this context it has been demonstrated in both *Aplysia* and *Drosophila* that activity-dependent decreases in a homophillic cell-adhesion molecule that destabilizes the synapse is both necessary and sufficient for increased synaptic growth (Mayford et al., 1992; Schuster et al., 1996). Likewise, modulating synapse stability by neurotrophins affects synapse dynamics. Increased GDNF signaling can block input elimination at the mammalian NMJ, while reduced neurotrophin signaling can be linked to enhanced elimination (Nguyen et al., 1998; Keller-Peck et al., 2001; Gonzalez et al., 1999; Belluardo et al., 2001; Rico et al., 2002). These models and molecules are, however, insufficient to explain how the desired synapse density is recognized and how independent mechanisms of synapse formation and disassembly are coordinated to maintain constant synapse density during the rearrangement of synaptic circuitry. A future challenge, therefore, will be not only to define the molecular mechanisms of synapse assembly, disassembly, and maintenance, but to understand how these mechanisms interact to achieve stereotyped patterns of neural connectivity. The answers are likely to be derived through the intersection of potent

new quantitative live imaging techniques and continued cellular/molecular studies directed at the synapse.

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References

Aberle, H., Haghighi, A.P., Fetter, R.D., McCabe, B.D., Magalhaes, T.R., and Goodman, C.S. (2002). Wishful thinking encodes a BMP type II receptor that regulates synaptic growth in *Drosophila*. Neuron *33*, 545–558.

Agerman, K., Hjerling-Leffler, J., Blanchard, M.P., Scarfone, E., Canlon, B., Nosrat, C., and Ernfors, P. (2003). BDNF gene replacement reveals multiple mechanisms for establishing neurotrophin specificity during sensory nervous system development. Development *130*, 1479–1491.

Ahmari, S.E., Buchanan, J., and Smith, S.J. (2000). Assembly of presynaptic active zones from cytoplasmic transport packets. Nat. Neurosci. *3*, 445–451.

Aiba, A., Kano, M., Chen, C., Stanton, M.E., Fox, G.D., Herrup, K., Zwingman, T.A., and Tonegawa, S. (1994). Deficient cerebellar long-term depression and impaired motor learning in mGluR1 mutant mice. Cell *79*, 377–388.

Akaaboune, M., Culican, S.M., Turney, S.G., and Lichtman, J.W. (1999). Rapid and reversible effects of activity on acetylcholine receptor density at the neuromuscular junction in vivo. Science *286*, 503–507.

Allan, D.W., Pierre, S.E., Miguel-Aliaga, I., and Thor, S. (2003). Specification of neuropeptide cell identity by the integration of retrograde BMP signaling and a combinatorial transcription factor code. Cell *113*, 73–86.

Allison, D.W., Chervin, A.S., Gelfand, V.I., and Craig, A.M. (2000). Postsynaptic scaffolds of excitatory and inhibitory synapses in hippocampal neurons: maintenance of core components independent of actin filaments and microtubules. J. Neurosci. *20*, 4545–4554.

Alsina, B., Vu, T., and Cohen-Cory, S. (2001). Visualizing synapse formation in arborizing optic axons in vivo: dynamics and modulation by BDNF. Nat. Neurosci. *4*, 1093–1101.

Antonini, A., and Stryker, M.P. (1993). Rapid remodeling of axonal arbors in the visual cortex. Science *260*, 1819–1821.

Antonini, A., and Stryker, M.P. (1996). Plasticity of geniculocortical afferents following brief or prolonged monocular occlusion in the cat. J. Comp. Neurol. *369*, 64–82.

Antonini, A., Gillespie, D.C., Crair, M.C., and Stryker, M.P. (1998). Morphology of single geniculocortical afferents and functional recovery of the visual cortex after reverse monocular deprivation in the kitten. J. Neurosci. *18*, 9896–9909.

Antonova, I., Arancio, O., Trillat, A.C., Wang, H.G., Zablow, L., Udo, H., Kandel, E.R., and Hawkins, R.D. (2001). Rapid increase in clusters of presynaptic proteins at onset of long-lasting potentiation. Science *294*, 1547–1550.

Arber, S., Burden, S.J., and Harris, A.J. (2002). Patterning of skeletal muscle. Curr. Opin. Neurobiol. *12*, 100–103.

Bagri, A., Cheng, H.J., Yaron, A., Pleasure, S.J., and Tessier-Lavigne, M. (2003). Stereotyped pruning of long hippocampal axon branches triggered by retraction inducers of the semaphorin family. Cell *113*, 285–299.

Bailey, C.H., and Kandel, E.R. (1993). Structural changes accompanying memory storage. Annu. Rev. Physiol. *55*, 397–426.

Balice-Gordon, R.J., and Lichtman, J.W. (1994). Long-term synapse loss induced by focal blockade of postsynaptic receptors. Nature *372*, 519–524.

Barry, J.A., and Ribchester, R.R. (1995). Persistent polyneuronal innervation in partially denervated rat muscle after reinnervation and recovery from prolonged nerve conduction block. J. Neurosci. *15*, 6327–6339.

Barry, M.F., and Ziff, E.B. (2002). Receptor trafficking and the plasticity of excitatory synapses. Curr. Opin. Neurobiol. *12*, 279–286.

Bekkers, J.M., and Stevens, C.F. (1991). Excitatory and inhibitory autaptic currents in isolated hippocampal neurons maintained in cell culture. Proc. Natl. Acad. Sci. USA *88*, 7834–7838.

Belluardo, N., Westerblad, H., Mudo, G., Casabona, A., Bruton, J., Caniglia, G., Pastoris, O., Grassi, F., and Ibanez, C.F. (2001). Neuromuscular junction disassembly and muscle fatigue in mice lacking neurotrophin-4. Mol. Cell. Neurosci. *18*, 56–67.

Benson, D.L., Colman, D.R., and Huntley, G.W. (2001). Molecules, maps and synapse specificity. Nat. Rev. Neurosci. *2*, 899–909.

Berardi, N., Pizzorusso, T., and Maffei, L. (2000). Critical periods during sensory development. Curr. Opin. Neurobiol. *10*, 138–145.

Bernstein, M., and Lichtman, J.W. (1999). Axonal atrophy: the retraction reaction. Curr. Opin. Neurobiol. *9*, 364–370.

Biederer, T., Sara, Y., Mozhayeva, M., Atasoy, D., Liu, X., Kavalali, E.T., and Sudhof, T.C. (2002). SynCAM, a synaptic adhesion molecule that drives synapse assembly. Science *297*, 1525–1531.

Billuart, P., Winter, C.G., Maresh, A., Zhao, X., and Luo, L. (2001). Regulating axon branch stability: the role of p190 RhoGAP in repressing a retraction signaling pathway. Cell *107*, 195–207.

Bolton, M.M., Lo, D.C., and Sherwood, N.T. (2000). Long-term regulation of excitatory and inhibitory synaptic transmission in hippocampal cultures by brain-derived neurotrophic factor. Prog. Brain Res. *128*, 203–218.

Bonhoeffer, T. (1996). Neurotrophins and activity-dependent development of the neocortex. Curr. Opin. Neurobiol. *6*, 119–126.

Borgdorff, A.J., and Choquet, D. (2002). Regulation of AMPA receptor lateral movements. Nature *417*, 649–653.

Böse, C.M., Qiu, D., Bergamaschi, A., Gravante, B., Bossi, M., Villa, A., Rupp, F., and Malgaroli, A. (2000). Agrin controls synaptic differentiation in hippocampal neurons. J. Neurosci. *20*, 9086–9095.

Bresler, T., Ramati, Y., Zamorano, P.L., Zhai, R., Garner, C.C., and Ziv, N.E. (2001). The dynamics of SAP90/PSD-95 recruitment to new synaptic junctions. Mol. Cell. Neurosci. *18*, 149–167.

Brose, N. (1999). Synaptic cell adhesion proteins and synaptogenesis in the mammalian central nervous system. Naturwissenschaften *86*, 516–524.

Bruckner, K., Pablo Labrador, J., Scheiffele, P., Herb, A., Seeburg, P.H., and Klein, R. (1999). EphrinB ligands recruit GRIP family PDZ adaptor proteins into raft membrane microdomains. Neuron *22*, 511–524.

Buffelli, M., Busetto, G., Cangiano, L., and Cangiano, A. (2002). Perinatal switch from synchronous to asynchronous activity of motoneurons: link with synapse elimination. Proc. Natl. Acad. Sci. USA *99*, 13200–13205.

Buffelli, M., Burgess, R.W., Feng, G., Lobe, C.G., Lichtman, J.W., and Sanes, J.W. (2003). Genetic evidence that relative synaptic efficacy biases the outcome of synaptic competition. Nature *424*, 430–434.

Burbea, M., Dreier, L., Dittman, J.S., Grunwald, M.E., and Kaplan, J.M. (2002). Ubiquitin and AP180 regulate the abundance of GLR-1 glutamate receptors at postsynaptic elements in *C. elegans*. Neuron *35*, 107–120.

Burden, S.J. (2002). Building the vertebrate neuromuscular synapse. J. Neurobiol. *53*, 501–511.

Burgess, R.W., Nguyen, Q.T., Son, Y.J., Lichtman, J.W., and Sanes, J.R. (1999). Alternatively spliced isoforms of nerve- and muscle-derived agrin: their roles at the neuromuscular junction. Neuron *23*, 33–44.

Burry, R.W. (1986). Presynaptic elements on artificial surfaces. A model for the study of development and regeneration of synapses. Neurochem. Pathol. *5*, 345–360.

Cabelli, R.J., Hohn, A., and Shatz, C.J. (1995). Inhibition of ocular

dominance column formation by infusion of NT-4/5 or BDNF. Science *267*, 1662–1666.

Cabelli, R.J., Shelton, D.L., Segal, R.A., and Shatz, C.J. (1997). Blockade of endogenous ligands of trkB inhibits formation of ocular dominance columns. Neuron *19*, 63–76.

Callaway, E.M., Soha, J.M., and Van Essen, D.C. (1987). Competition favouring inactive over active motor neurons during synapse elimination. Nature *328*, 422–426.

Chang, S., and De Camilli, P. (2001). Glutamate regulates actin-based motility in axonal filopodia. Nat. Neurosci. *4*, 787–793.

Chen, C., and Regehr, W.G. (2000). Developmental remodeling of the retinogeniculate synapse. Neuron *28*, 955–966.

Chen, C., Kano, M., Abeliovich, A., Chen, L., Bao, S., Kim, J.J., Hashimoto, K., Thompson, R.F., and Tonegawa, S. (1995). Impaired motor coordination correlates with persistent multiple climbing fiber innervation in PKC gamma mutant mice. Cell *83*, 1233–1242.

Chen, L., Chetkovich, D.M., Petralia, R.S., Sweeney, S.N., Kawasaki, Y., Wenthold, R.J., Bredt, D.S., and Nicoll, R.A. (2000). Stargazin regulates synaptic targeting of AMPA receptors by two distinct mechanisms. Nature *408*, 936–943.

Cline, H.T., and Constantine-Paton, M. (1990). NMDA receptor agonist and antagonists alter retinal ganglion cell arbor structure in the developing frog retinotectal projection. J. Neurosci. *10*, 1197–1216.

Cohen, N.A., Kaufmann, W.E., Worley, P.F., and Rupp, F. (1997). Expression of agrin in the developing and adult rat brain. Neuroscience *76*, 581–596.

Cohen-Cory, S. (1999). BDNF modulates, but does not mediate, activity-dependent branching and remodeling of optic axon arbors in vivo. J. Neurosci. *19*, 9996–10003.

Cohen-Cory, S. (2002). The developing synapse: construction and modulation of synaptic structures and circuits. Science *298*, 770–776.

Colicos, M.A., Collins, B.E., Sailor, M.J., and Goda, Y. (2001). Remodeling of synaptic actin induced by photoconductive stimulation. Cell *107*, 605–616.

Colledge, M., and Froehner, S.C. (1998). Signals mediating ion channel clustering at the neuromuscular junction. Curr. Opin. Neurobiol. *8*, 357–363.

Colman, H., Nabekura, J., and Lichtman, J.W. (1997). Alterations in synaptic strength preceding axon withdrawal. Science *275*, 356–361.

Conquet, F., Bashir, Z.I., Davies, C.H., Daniel, H., Ferraguti, F., Bordi, F., Franz-Bacon, K., Reggiani, A., Matarese, V., Conde, F., et al. (1994). Motor deficit and impairment of synaptic plasticity in mice lacking mGluR1. Nature *372*, 237–243.

Costanzo, E.M., Barry, J.A., and Ribchester, R.R. (2000). Competition at silent synapses in reinnervated skeletal muscle. Nat. Neurosci. *3*, 694–700.

Craig, A.M., and Lichtman, T.C. (2001). Synapse formation and maturation. In Synapses, M.W. Cowan, T.C. Südhof, and C.F. Stevens, eds. (Baltimore, MD: Johns Hopkins University Press), pp. 571–612.

Crepel, F., and Mariani, J. (1976). Multiple innervation of Purkinje cells by climbing fibers in the cerebellum of the Weaver mutant mouse. J. Neurobiol. *7*, 579–582.

Crepel, F., Delhaye-Bouchaud, N., Guastavino, J.M., and Sampaio, I. (1980). Multiple innervation of cerebellar Purkinje cells by climbing fibres in staggerer mutant mouse. Nature *283*, 483–484.

Culican, S.M., Nelson, C.C., and Lichtman, J.W. (1998). Axon withdrawal during synapse elimination at the neuromuscular junction is accompanied by disassembly of the postsynaptic specialization and withdrawal of Schwann cell processes. J. Neurosci. *18*, 4953–4965.

Dailey, M.E., and Smith, S.J. (1996). The dynamics of dendritic structure in developing hippocampal slices. J. Neurosci. *16*, 2983–2994.

Dalva, M.B., Takasu, M.A., Lin, M.Z., Shamah, S.M., Hu, L., Gale, N.W., and Greenberg, M.E. (2000). EphB receptors interact with NMDA receptors and regulate excitatory synapse formation. Cell *103*, 945–956.

Darian-Smith, C., and Gilbert, C.D. (1994). Axonal sprouting accompanies functional reorganization in adult cat striate cortex. Nature *368*, 737–740.

Davis, G.W. (2000). The making of a synapse: target-derived signals and presynaptic differentiation. Neuron *26*, 551–554.

Davis, G.W., and Bezprozvanny, I. (2001). Maintaining the stability of neural function: a homeostatic hypothesis. Annu. Rev. Physiol. *63*, 847–869.

Davis, G.W., Schuster, C.M., and Goodman, C.S. (1997). Genetic analysis of the mechanisms controlling target selection: target-erived Fasciclin II regulates the pattern of synapse formation. Neuron *19*, 561–573.

De Paola, V., Arber, S., and Caroni, P. (2003). AMPA receptors regulate dynamic equilibrium of presynaptic terminals in mature hippocampal networks. Nat. Neurosci. *6*, 491–500.

Dean, C., Scholl, F.G., Choih, J., DeMaria, S., Berger, J., Isacoff, E., and Scheiffele, P. (2003). Neurexin mediates the assembly of presynaptic terminals. Nat. Neurosci. *6*, 708–716.

DeChiara, T.M., Bowen, D.C., Valenzuela, D.M., Simmons, M.V., Poueymirou, W.T., Thomas, S., Kinetz, E., Compton, D.L., Rojas, E., Park, J.S., et al. (1996). The receptor tyrosine kinase MuSK is required for neuromuscular junction formation in vivo. Cell *85*, 501–512.

DiAntonio, A., Haghighi, A.P., Portman, S.L., Lee, J.D., Amaranto, A.M., and Goodman, C.S. (2001). Ubiquitination-dependent mechanisms regulate synaptic growth and function. Nature *26*, 449–452.

Dunaevsky, A., and Connor, E.A. (1998). Stability of frog motor nerve terminals in the absence of target muscle fibers. Dev. Biol. *194*, 61–71.

Dunaevsky, A., Tashiro, A., Majewska, A., Mason, C., and Yuste, R. (1999). Developmental regulation of spine motility in the mammalian central nervous system. Proc. Natl. Acad. Sci. USA *96*, 13438–13443.

Duxson, M.J. (1982). The effect of postsynaptic block on development of the neuromuscular junction in postnatal rats. J. Neurocytol. *11*, 395–408.

Eaton, B.A., and Davis, G.W. (2003). Synapse disassembly. Genes Dev. *17*, 2075–2082.

Eaton, B.A., Fetter, R.D., and Davis, G.W. (2002). Dynactin is necessary for synapse stabilization. Neuron *3*, 729–741.

Ebihara, T., Kawabata, I., Usui, S., Sobue, K., and Okabe, S. (2003). Synchronized formation and remodeling of postsynaptic densities: long-term visualization of hippocampal neurons expressing postsynaptic density proteins tagged with green fluorescent protein. J. Neurosci. *23*, 2170–2181.

El-Husseini, A.E., Schnell, E., Chetkovich, D.M., Nicoll, R.A., and Bredt, D.S. (2000). PSD-95 involvement in maturation of excitatory synapses. Science *290*, 1364–1368.

El-Husseini, Ael-D., Schnell, E., Dakoji, S., Sweeney, N., Zhou, Q., Prange, O., Gauthier-Campbell, C., Aguilera-Moreno, A., Nicoll, R.A., and Bredt, D.S. (2002). Synaptic strength regulated by palmitate cycling on PSD-95. Cell *108*, 849–863.

Engert, F., and Bonhoeffer, T. (1999). Dendritic spine changes associated with hippocampal long-term synaptic plasticity. Nature *399*, 66–70.

Ethell, I.M., Irie, F., Kalo, M.S., Couchman, J.R., Pasquale, E.B., and Yamaguchi, Y. (2001). EphB/syndecan-2 signaling in dendritic spine morphogenesis. Neuron *31*, 1001–1013.

Ferreira, A. (1999). Abnormal synapse formation in agrin-depleted hippocampal neurons. J. Cell Sci. *112*, 4729–4738.

Fiala, J.C., Feinberg, M., Popov, V., and Harris, K.M. (1998). Synaptogenesis via dendritic filopodia in developing hippocampal area CA1. J. Neurosci. *18*, 8900–8911.

Finn, A.J., Feng, G., and Pendergast, A.M. (2003). Postsynaptic requirement for Abl kinases in assembly of the neuromuscular junction. Nat. Neurosci. *6*, 717–723.

Fischer, M., Kaech, S., Wagner, U., Brinkhaus, H., and Matus, A. (2000). Glutamate receptors regulate actin-based plasticity in dendritic spines. Nat. Neurosci. *3*, 887–894.

Frank, M., and Kemler, R. (2002). Protocadherins. Curr. Opin. Cell Biol. *14*, 557–562.

Friedman, H.V., Bresler, T., Garner, C.C., and Ziv, N.E. (2000). Assembly of new individual excitatory synapses: time course and temporal order of synaptic molecule recruitment. Neuron *27*, 57–69.

Froehner, S.C. (1991). The submembrane machinery for nicotinic acetylcholine receptor clustering. J. Cell Biol. *114*, 1–7.

Fukazawa, Y., Saitoh, Y., Ozawa, F., Ohta, Y., Mizuno, K., and Inokuchi, K. (2003). Hippocampal LTP is accompanied by enhanced F-actin content within the dendritic spine that is essential for late LTP maintenance in vivo. Neuron *38*, 447–460.

Gan, W.-B., Kwon, E., Feng, G., Sanes, J.R., and Lichtman, J.W. (2003). Synaptic dynamism measured over minutes to months: age-dependent decline in an autonomic ganglion. Nat. Neurosci. *9*, 956–960.

Garner, C.C., Zhai, R.G., Gundelfinger, E., and Ziv, N.E. (2002). Molecular mechanisms of CNS synaptogenesis. Trends Neurosci. *25*, 243–251.

Gautam, M., Noakes, P.G., Mudd, J., Nichol, M., Chu, G.C., Sanes, J.R., and Merlie, J.P. (1995). Failure of postsynaptic specialization to develop at neuromuscular junctions of rapsyn-deficient mice. Nature *377*, 232–236.

Gautam, M., Noakes, P.G., Moscoso, L., Rupp, F., Scheller, R.H., Merlie, J.P., and Sanes, J.R. (1996). Defective neuromuscular synaptogenesis in agrin-deficient mutant mice. Cell *85*, 525–535.

Gomyo, H., Arai, Y., Tanigami, A., Murakami, Y., Hattori, M., Hosoda, F., Arai, K., Aikawa, Y., Tsuda, H., Hirohashi, S., et al. (1999). A 2-Mb sequence-ready contig map and a novel immunoglobulin superfamily gene IGSF4 in the LOH region of chromosome 11q23.2. Genomics *62*, 139–146.

Gonzalez, M., Ruggiero, F.P., Chang, Q., Shi, Y.J., Rich, M.M., Kraner, S., and Balice-Gordon, R.J. (1999). Disruption of Trkb-mediated signaling induces disassembly of postsynaptic receptor clusters at neuromuscular junctions. Neuron *24*, 567–583.

Grantyn, R., Kraszewski, K., Melnick, I., Taschenberger, H., and Warton, S.S. (1995). In vitro development of vertebrate central synapses. Perspect. Dev. Neurobiol. *2*, 387–397.

Grutzendler, J., Kasthuri, N., and Gan, W.B. (2002). Long-term dendritic spine stability in the adult cortex. Nature *420*, 812–816.

Hall, A.C., Lucas, F.R., and Salinas, P.C. (2000). Axonal remodeling and synaptic differentiation in the cerebellum is regulated by WNT-7a signaling. Cell *100*, 525–535.

Hallam, S.J., and Jin, Y. (1998). *lin-14* regulates the timing of synaptic remodeling in *Caenorhabditis elegans*. Nature *395*, 78–82.

Hamos, J.E., Van Horn, S.C., Raczkowski, D., and Sherman, S.M. (1987). Synaptic circuits involving an individual retinogeniculate axon in the cat. J. Comp. Neurol. *259*, 165–192.

Harris, R.M., and Woolsey, T.A. (1981). Dendritic plasticity in mouse barrel cortex following postnatal vibrissa follicle damage. J. Comp. Neurol. *196*, 357–376.

Harris, K.M., Fiala, J.C., and Ostroff, L. (2003). Structural changes at dendritic spine synapses during long-term potentiation. Philos. Trans. R. Soc. Lond. B Biol. Sci. *358*, 745–748.

Hashimoto, K., and Kano, M. (2003). Functional differentiation of multiple climbing fiber inputs during synapse elimination in the developing cerebellum. Neuron *38*, 785–796.

Hata, Y., and Stryker, M.P. (1994). Control of thalamocortical afferent rearrangement by postsynaptic activity in developing visual cortex. Science *265*, 1732–1735.

Hata, Y., Tsumoto, T., and Stryker, M.P. (1999). Selective pruning of more active afferents when cat visual cortex is pharmacologically inhibited. Neuron *22*, 375–381.

Hatada, Y., Wu, F., Sun, Z.Y., Schacher, S., and Goldberg, D.J. (2000). Presynaptic morphological changes associated with long-term synaptic facilitation are triggered by actin polymerization at preexisting varicositis. J. Neurosci. *20*, RC82.

Hegde, A.N., Inokuchi, K., Pei, W., Casadio, A., Ghirardi, M., Chain, D.G.C., Kandel, E.R., and Schwartz, J.H. (1997). Ubiquitin C-terminal hydrolase is an immediate-early gene essential for long-term facilitation in *Aplysia*. Cell *89*, 115–126.

Hensch, T.K., Fagiolini, M., Mataga, N., Stryker, M.P., Baekkeskov, S., and Kash, S.F. (1998). Local GABA circuit control of experience-dependent plasticity in developing visual cortex. Science *282*, 1504–1508.

Herbst, R., Avetisova, E., and Burden, S.J. (2002). Restoration of synapse formation in Musk mutant mice expressing a Musk/Trk chimeric receptor. Development *129*, 5449–5460.

Hering, H., Lin, C.C., and Sheng, M. (2003). Lipid rafts in the maintenance of synapses, dendritic spines, and surface AMPA receptor stability. J. Neurosci. *23*, 3262–3271.

Hoover, C.L., Hilgenberg, L.G., and Smith, M.A. (2003). The COOH-terminal domain of agrin signals via a synaptic receptor in central nervous system neurons. J. Cell Biol. *161*, 923–932.

Hopf, F.W., Waters, J., Mehta, S., and Smith, S.J. (2002). Stability and plasticity of developing synapses in hippocampal neuronal cultures. J. Neurosci. *22*, 775–781.

Huang, E.J., and Reichardt, L.F. (2001). Neurotrophins: roles in neuronal development and function. Annu. Rev. Neurosci. *24*, 677–736.

Huh, K.H., and Wenthold, R.J. (1999). Turnover analysis of glutamate receptors identifies a rapidly degraded pool of the N-methyl-D-aspartate receptor subunit, NR1, in cultured cerebellar granule cells. J. Biol. Chem. *274*, 151–157.

Huh, G.S., Boulanger, L.M., Du, H., Riquelme, P.A., Brotz, T.M., and Shatz, C.J. (2000). Functional requirement for class I MHC in CNS development and plasticity. Science *290*, 2155–2159.

Ichise, T., Kano, M., Hashimoto, K., Yanagihara, D., Nakao, K., Shigemoto, R., Katsuki, M., and Aiba, A. (2000). mGluR1 in cerebellar Purkinje cells essential for long-term depression, synapse elimination, and motor coordination. Science *288*, 1832–1835.

Irie, F., and Yamaguchi, Y. (2002). EphB receptors regulate dendritic spine development via intersectin, Cdc42 and N-WASP. Nat. Neurosci. *5*, 1117–1118.

Ito, M. (1984). The modifiable neuronal network of the cerebellum. Jpn. J. Physiol. *34*, 781–792.

Jackson, H., and Parks, T.N. (1982). Functional synapse elimination in the developing avian cochlear nucleus with simultaneous reduction in cochlear nerve axon branching. J. Neurosci. *2*, 1736–1743.

Jin, Y. (2002). Synaptogenesis: insights from worm and fly. Curr. Opin. Neurobiol. *12*, 71–79.

Jo, S.A., Zhu, X., Marchionni, M.A., and Burden, S.J. (1995). Neuregulins are concentrated at nerve-muscle synapses and activate ACh-receptor gene expression. Nature *373*, 158–161.

Jontes, J.D., and Smith, S.J. (2000). Filopodia, spines, and the generation of synaptic diversity. Neuron *27*, 11–14.

Jontes, J.D., Buchanan, J., and Smith, S.J. (2000). Growth cone and dendrite dynamics in zebrafish embryos: early events in synaptogenesis imaged in vivo. Nat. Neurosci. *3*, 231–237.

Kano, M., Hashimoto, K., Chen, C., Abeliovich, A., Aiba, A., Kurihara, H., Watanabe, M., Inoue, Y., and Tonegawa, S. (1995). Impaired synapse elimination during cerebellar development in PKC gamma mutant mice. Cell *83*, 1223–1231.

Kano, M., Hashimoto, K., Kurihara, H., Watanabe, M., Inoue, Y., Aiba, A., and Tonegawa, S. (1997). Persistent multiple climbing fiber innervation of cerebellar Purkinje cells in mice lacking mGluR1. Neuron *18*, 71–79.

Kasthuri, N., and Lichtman, J.W. (2003). The role of neuronal identity in synaptic competition. Nature *424*, 426–430.

Katz, L.C., and Shatz, C.J. (1996). Synaptic activity and the construction of cortical circuits. Science *274*, 1133–1138.

Keller-Peck, C.R., Walsh, M.K., Gan, W.B., Feng, G., Sanes, J.R., and Lichtman, J.W. (2001). Asynchronous synapse elimination in neonatal motor units: studies using GFP transgenic mice. Neuron *31*, 381–394.

Keshishian, H., Broadie, K., Chiba, A., and Bate, M. (1996). The *Drosophila* neuromuscular junction: a model system for studying

synaptic development and function. Annu. Rev. Neurosci. *19*, 545–575.

Kim, D., Jun, K.S., Lee, S.B., Kang, N.G., Min, D.S., Kim, Y.H., Ryu, S.H., Suh, P.G., and Shin, H.S. (1997). Phospholipase C isozymes selectively couple to specific neurotransmitter receptors. Nature *389*, 290–293.

Klopfenstein, D.R., Tomishige, M., Stuurman, N., and Vale, R.D. (2002). Role of phosphatidylinositol(4,5)bisphosphate organization in membrane transport by the Unc104 kinesin motor. Cell *109*, 347–358.

Kraszewski, K., Mundigl, O., Daniell, L., Verderio, C., Matteoli, M., and De Camilli, P. (1995). Synaptic vesicle dynamics in living cultured hippocampal neurons visualized with CY3-conjugated antibodies directed against the lumenal domain of synaptotagmin. J. Neurosci. *15*, 4328–4342.

Krylova, O., Herreros, J., Cleverley, K.E., Ehler, E., Henriquez, J.P., Hughes, S.M., and Salinas, P.C. (2002). WNT-3, expressed by motoneurons, regulates terminal arborization of neurotrophin-3-responsive spinal sensory neurons. Neuron *35*, 1043–1056.

Kuffler, D., Thompson, W., and Jansen, J.K. (1977). The elimination of synapses in multiply-innervated skeletal muscle fibres of the rat: dependence on distance between end-plates. Brain Res. *138*, 353–358.

Lee, T., Marticke, S., Sung, C., Robinow, S., and Luo, L. (2000). Cell-autonomous requirement of the USP/EcR-B ecdysone receptor for mushroom body neuronal remodeling in *Drosophila*. Neuron *28*, 807–818.

Lee, C.H., Herman, T., Clandinin, T.R., Lee, R., and Zipursky, S.L. (2001). N-cadherin regulates target specificity in the *Drosophila* visual system. Neuron *30*, 437–450.

Lee, R.C., Clandinin, T.R., Lee, C.H., Chen, P.L., Meinertzhagen, I.A., and Zipursky, S.L. (2003). The protocadherin Flamingo is required for axon target selection in the *Drosophila* visual system. Nat. Neurosci. *6*, 557–563.

Lein, E.S., and Shatz, C.J. (2000). Rapid regulation of brain-derived neurotrophic factor mRNA within eye-specific circuits during ocular dominance column formation. J. Neurosci. *20*, 1470–1483.

Lendvai, B., Stern, E.A., Chen, B., and Svoboda, K. (2000). Experience-dependent plasticity of dendritic spines in the developing rat barrel cortex in vivo. Nature *404*, 876–881.

LeVay, S., Wiesel, T.N., and Hubel, D.H. (1980). The development of ocular dominance columns in normal and visually deprived monkeys. J. Comp. Neurol. *191*, 1–51.

Li, Z., Hilgenberg, L.G., O'Dowd, D.K., and Smith, M.A. (1999). Formation of functional synaptic connections between cultured cortical neurons from agrin-deficient mice. J. Neurobiol. *39*, 547–557.

Lichtman, J.W., and Colman, H. (2000). Synapse elimination and indelible memory. Neuron *25*, 269–278.

Lin, W., Burgess, R.W., Dominguez, B., Pfaff, S.L., Sanes, J.R., and Lee, K.F. (2001). Distinct roles of nerve and muscle in postsynaptic differentiation of the neuromuscular synapse. Nature *410*, 1057–1064.

Lisman, J. (2003). Actin's actions in LTP-induced synapse growth. Neuron *38*, 361–362.

Lisman, J., Schulman, H., and Cline, H. (2002). The molecular basis of CaMKII function in synaptic and behavioural memory. Nat. Rev. Neurosci. *3*, 175–190.

Lom, B., and Cohen-Cory, S. (1999). Brain-derived neurotrophic factor differentially regulates retinal ganglion cell dendritic and axonal arborization in vivo. J. Neurosci. *19*, 9928–9938.

Luo, L. (2002). Actin cytoskeleton regulation in neuronal morphogenesis and structural plasticity. Annu. Rev. Cell Dev. Biol. *18*, 601–635.

Lüscher, C., and Frerking, M. (2001). Restless AMPA receptors: implications for synaptic transmission and plasticity. Trends Neurosci. *24*, 665–670.

Lüscher, C., Nicoll, R.A., Malenka, R.C., and Muller, D. (2000). Synaptic plasticity and dynamic modulation of the postsynaptic membrane. Nat. Neurosci. *3*, 545–550.

Maletic-Savatic, M., Malinow, R., and Svoboda, K. (1999). Rapid dendritic morphogenesis in CA1 hippocampal dendrites induced by synaptic activity. Science *283*, 1923–1927.

Malinow, R., and Malenka, R.C. (2002). AMPA receptor trafficking and synaptic plasticity. Annu. Rev. Neurosci. *25*, 103–126.

Mariani, J., and Changeux, J.P. (1980). Multiple innervation of Purkinje cells by climbing fibers in the cerebellum of the adult staggerer mutant mouse. J. Neurobiol. *11*, 41–50.

Marques, G., Bao, H., Haerry, T.E., Shimell, M.J., Duchek, P., Zhang, B., and O'Connor, M.B. (2002). The *Drosophila* BMP type II receptor Wishful Thinking regulates neuromuscular synapse morphology and function. Neuron *33*, 529–543.

Marrs, G.S., Green, S.H., and Dailey, M.E. (2001). Rapid formation and remodeling of postsynaptic densities in developing dendrites. Nat. Neurosci. *4*, 1006–1013.

Marty, S., Wehrle, R., and Sotelo, C. (2000). Neuronal activity and brain-derived neurotrophic factor regulate the density of inhibitory synapses in organotypic slice cultures of postnatal hippocampus. J. Neurosci. *20*, 8087–8095.

Mason, C.A., and Gregory, E. (1984). Postnatal maturation of cerebellar mossy and climbing fibers: transient expression of dual features on single axons. J. Neurosci. *4*, 1715–1735.

Masuda, M., Yageta, M., Fukuhara, H., Kuramochi, M., Maruyama, T., Nomoto, A., and Murakami, Y. (2002). The tumor suppressor protein TSLC1 is involved in cell-cell adhesion. J. Biol. Chem. *277*, 31014–31019.

Matsumoto, A., Micevych, P.E., and Arnold, A.P. (1988). Androgen regulates synaptic input to motoneurons of the adult rat spinal cord. J. Neurosci. *8*, 4168–4176.

Matus, A. (2000). Actin-based plasticity in dendritic spines. Science *290*, 754–758.

Mauch, D.H., Nagler, K., Schumacher, S., Goritz, C., Muller, E.C., Otto, A., and Pfrieger, F.W. (2001). CNS synaptogenesis promoted by glia-derived cholesterol. Science *294*, 1354–1357.

Mayford, M., Barzilai, A., Keller, F., Schacher, S., and Kandel, E.R. (1992). Modulation of an NCAM-related adhesion molecule with long-term synaptic plasticity in *Aplysia*. Science *256*, 638–644.

McAllister, A.K., Katz, L.C., and Lo, D.C. (1999). Neurotrophins and synaptic plasticity. Annu. Rev. Neurosci. *22*, 295–318.

McCabe, B.D., Marques, G., Haghighi, A.P., Fetter, R.D., Crotty, M.L., Haerry, T.E., Goodman, C.S., and O'Connor, M.B. (2003). The BMP homolog Gbb provides a retrograde signal that regulates synaptic growth at the *Drosophila* neuromuscular junction. Neuron *39*, 241–254.

McGee, A.W., and Bredt, D.S. (2003). Assembly and plasticity of the glutamatergic postsynaptic specialization. Curr. Opin. Neurobiol. *13*, 111–118.

McKinney, R.A., Capogna, M., Durr, R., Gahwiler, B.H., and Thompson, S.M. (1999). Miniature synaptic events maintain dendritic spines via AMPA receptor activation. Nat. Neurosci. *2*, 444–449.

McMahan, U.J. (1990). The agrin hypothesis. Cold Spring Harb. Symp. Quant. Biol. *55*, 407–418.

Meier, J. (2003). The enigma of transmitter-selective receptor accumulation at developing inhibitory synapses. Cell Tissue Res. *311*, 271–276.

Misgeld, T., Burgess, R.W., Lewis, R.M., Cunningham, J.M., Lichtman, J.W., and Sanes, J.R. (2002). Roles of neurotransmitter in synapse formation: development of neuromuscular junctions lacking choline acetyltransferase. Neuron *36*, 635–648.

Missler, M., and Sudhof, T.C. (1998). Neurexins: three genes and 1001 products. Trends Genet. *14*, 20–26.

Missler, M., Zhang, W., Rohlmann, A., Kattenstroth, G., Hammer, R.E., Gottmann, K., and Sudhof, T.C. (2003). Alpha-neurexins couple Ca^{2+} channels to synaptic vesicle exocytosis. Nature *423*, 939–948.

Moon, R.T., Bowerman, B., Boutros, M., and Perrimon, N. (2002). The promise and perils of Wnt signaling through beta-catenin. Science *296*, 1644–1646.

Moss, S.J., and Smart, T.G. (2001). Constructing inhibitory synapses. Nat. Rev. Neurosci. *2*, 240–250.

Murase, S., Mosser, E., and Schuman, E.M. (2002). Depolarization drives beta-catenin into neuronal spines promoting changes in synaptic structure and function. Neuron *35*, 91–105.

Murphey, R.K., and Lemere, C.A. (1984). Competition controls the growth of an identified axonal arborization. Science *224*, 1352–1355.

Nguyen, Q.T., Parsadanian, A.S., Snider, W.D., and Lichtman, J.W. (1998). Hyperinnervation of neuromuscular junctions caused by GDNF overexpression in muscle. Science *279*, 1725–1729.

Nguyen, Q.T., Son, Y.J., Sanes, J.R., and Lichtman, J.W. (2000). Nerve terminals form but fail to mature when postsynaptic differentiation is blocked: in vivo analysis using mammalian nerve-muscle chimeras. J. Neurosci. *20*, 6077–6086.

Nitkin, R.M., Smith, M.A., Magill, C., Fallon, J.R., Yao, Y.M., Wallace, B.G., and McMahan, U.J. (1987). Identification of agrin, a synaptic organizing protein from Torpedo electric organ. J. Cell Biol. *105*, 2471–2478.

Noakes, P.G., Gautam, M., Mudd, J., Sanes, J.R., and Merlie, J.P. (1995). Aberrant differentiation of neuromuscular junctions in mice lacking s-laminin/laminin beta 2. Nature *374*, 258–262.

O'Brien, R.J., Xu, D., Petralia, R.S., Steward, O., Huganir, R.L., and Worley, P. (1999). Synaptic clustering of AMPA receptors by the extracellular immediate-early gene product Narp. Neuron *23*, 309–323.

O'Brien, R., Xu, D., Mi, R., Tang, X., Hopf, C., and Worley, P. (2002). Synaptically targeted narp plays an essential role in the aggregation of AMPA receptors at excitatory synapses in cultured spinal neurons. J. Neurosci. *22*, 4487–4498.

O'Connor, L.T., Lauterborn, J.C., Smith, M.A., and Gall, C.M. (1995). Expression of agrin mRNA is altered following seizures in adult rat brain. Brain Res. Mol. Brain Res. *33*, 277–287.

Offermanns, S., Hashimoto, K., Watanabe, M., Sun, W., Kurihara, H., Thompson, R.F., Inoue, Y., Kano, M., and Simon, M.I. (1997). Impaired motor coordination and persistent multiple climbing fiber innervation of cerebellar Purkinje cells in mice lacking Galphaq. Proc. Natl. Acad. Sci. USA *94*, 14089–14094.

Okabe, S., Miwa, A., and Okado, H. (2001). Spine formation and correlated assembly of presynaptic and postsynaptic molecules. J. Neurosci. *21*, 6105–6114.

O'Rourke, N.A., and Fraser, S.E. (1990). Dynamic changes in optic fiber terminal arbors lead to retinotopic map formation: an in vivo confocal microscopic study. Neuron *5*, 159–171.

Packard, M., Koo, E.S., Gorczyca, M., Sharpe, J., Cumberledge, S., and Budnik, V. (2002). The *Drosophila* Wnt, wingless, provides an essential signal for pre- and postsynaptic differentiation. Cell *111*, 319–330.

Patton, B.L., Cunningham, J.M., Thyboll, J., Kortesmaa, J., Westerblad, H., Edstrom, L., Tryggvason, K., and Sanes, J.R. (2001). Properly formed but improperly localized synaptic specializations in the absence of laminin alpha4. Nat. Neurosci. *4*, 597–604.

Penzes, P., Beeser, A., Chernoff, J., Schiller, M.R., Eipper, B.A., Mains, R.E., and Huganir, R.L. (2003). Rapid induction of dendritic spine morphogenesis by trans-synaptic ephrinB-EphB receptor activation of the Rho-GEF kalirin. Neuron *37*, 263–274.

Perez, A.S., and Bredt, D.S. (1998). The N-terminal PDZ-containing region of postsynaptic density-95 mediates association with caveolar-like lipid domains. Neurosci. Lett. *258*, 121–123.

Personius, K.E., and Balice-Gordon, R.J. (2001). Loss of correlated motor neuron activity during synaptic competition at developing neuromuscular synapses. Neuron *31*, 395–408.

Pfrieger, F.W. (2003). Outsourcing in the brain: do neurons depend on cholesterol delivery by astrocytes? Bioessays *25*, 72–78.

Pfrieger, F.W., and Barres, B.A. (1997). Synaptic efficacy enhanced by glial cells in vitro. Science *277*, 1684–1687.

Pham, T.A., Rubenstein, J.L., Silva, A.J., Storm, D.R., and Stryker, M.P. (2001). The CRE/CREB pathway is transiently expressed in thalamic circuit development and contributes to refinement of retinogeniculate axons. Neuron *31*, 409–420.

Prange, O., and Murphy, T.H. (2001). Modular transport of postsynaptic density-95 clusters and association with stable spine precursors during early development of cortical neurons. J. Neurosci. *21*, 9325–9333.

Pun, S., Sigrist, M., Santos, A.F., Ruegg, M.A., Sanes, J.R., Jessell, T.M., Arber, S., and Caroni, P. (2003). An intrinsic distinction in neuromuscular junction assembly and maintenance in different skeletal muscles. Neuron *34*, 357–370.

Purves, D., and Hadley, R.D. (1985). Changes in the dendritic branching of adult mammalian neurones revealed by repeated imaging in situ. Nature *315*, 404–406.

Purves, D., and Lichtman, J.W. (1980). Elimination of synapses in the developing nervous system. Science *210*, 153–157.

Purves, D., Hadley, R.D., and Voyvodic, J.T. (1986). Dynamic changes in the dendritic geometry of individual neurons visualized over periods of up to three months in the superior cervical ganglion of living mice. J. Neurosci. *6*, 1051–1060.

Rao, A., Harms, K.J., and Craig, A.M. (2000). Neuroligation: building synapses around the neurexin-neuroligin link. Nat. Neurosci. *3*, 747–749.

Rico, B., Xu, B., and Reichardt, L.F. (2002). TrkB receptor signaling is required for establishment of GABAergic synapses in the cerebellum. Nat. Neurosci. *5*, 225–233.

Riddle, D.R., Lo, D.C., and Katz, L.C. (1995). NT-4-mediated rescue of lateral geniculate neurons from effects of monocular deprivation. Nature *378*, 189–191.

Ritzenthaler, S., Suzuki, E., and Chiba, A. (2000). Postsynaptic filopodia in muscle cells interact with innervating motoneuron axons. Nat. Neurosci. *3*, 1012–1017.

Saitoe, M., Schwarz, T.L., Umbach, J.A., Gundersen, C.B., and Kidokoro, Y. (2001). Absence of junctional glutamate receptor clusters in *Drosophila* mutants lacking spontaneous transmitter release. Science *293*, 514–517.

Sandrock, A.W., Jr., Dryer, S.E., Rosen, K.M., Gozani, S.N., Kramer, R., Theill, L.E., and Fischbach, G.D. (1997). Maintenance of acetylcholine receptor number by neuregulins at the neuromuscular junction in vivo. Science *276*, 599–603.

Sanes, J.R., and Lichtman, J.W. (1999). Development of the vertebrate neuromuscular junction. Annu. Rev. Neurosci. *22*, 389–442.

Sanes, J.R., and Lichtman, J.W. (2001). Induction, assembly, maturation and maintenance of a postsynaptic apparatus. Nat. Rev. Neurosci. *2*, 791–805.

Sanes, J.R., and Yamagata, M. (1999). Formation of lamina-specific synaptic connections. Curr. Opin. Neurobiol. *9*, 79–87.

Schachner, M. (1997). Neural recognition molecules and synaptic plasticity. Curr. Opin. Cell Biol. *9*, 627–634.

Scheiffele, P., Fan, J., Choih, J., Fetter, R., and Serafini, T. (2000). Neuroligin expressed in nonneuronal cells triggers presynaptic development in contacting axons. Cell *101*, 657–669.

Schuster, C.M., Davis, G.W., Fetter, R.D., and Goodman, C.S. (1996). Genetic dissection of structural and functional components of synaptic plasticity. I. Fasciclin II controls synaptic stabilization and growth. Neuron *17*, 641–654.

Segal, M.M., and Furshpan, E.J. (1990). Epileptiform activity in microcultures containing small numbers of hippocampal neurons. J. Neurophysiol. *64*, 1390–1399.

Seil, F.J., and Drake-Baumann, R. (2000). Neurotrophins and activity-dependent inhibitory synaptogenesis. Prog. Brain Res. *128*, 219–229.

Serpinskaya, A.S., Feng, G., Sanes, J.R., and Craig, A.M. (1999). Synapse formation by hippocampal neurons from agrin-deficient mice. Dev. Biol. *205*, 65–78.

Shapira, M., Zhai, R.G., Dresbach, T., Bresler, T., Torres, V.I., Gundelfinger, E.D., Ziv, N.E., and Garner, C.C. (2003). Unitary assembly of presynaptic active zones from Piccolo-Bassoon transport vesicles. Neuron *38*, 237–252.

Shapiro, L., and Colman, D.R. (1999). The diversity of cadherins and implications for a synaptic adhesive code in the CNS. Neuron *23*, 427–430.

Shatz, C.J., and Stryker, M.P. (1978). Ocular dominance in layer IV

of the cat's visual cortex and the effects of monocular deprivation. J. Physiol. *281*, 267–283.

Shen, K., and Bargmann, C.I. (2003). The immunoglobulin superfamily protein SYG-1 determines the location of specific synapses in *C. elegans*. Cell *112*, 619–630.

Sheng, M. (2001). Molecular organization of the postsynaptic specialization. Proc. Natl. Acad. Sci. USA *98*, 7058–7061.

Sin, W.C., Haas, K., Ruthazer, E.S., and Cline, H.T. (2002). Dendrite growth increased by visual activity requires NMDA receptor and Rho GTPases. Nature *419*, 475–480.

Snider, W.D., and Lichtman, J.W. (1996). Are neurotrophins synaptotrophins? Mol. Cell. Neurosci. *7*, 433–442.

Sotelo, C. (1975). Anatomical, physiological and biochemical studies of the cerebellum from mutant mice. II. Morphological study of cerebellar cortical neurons and circuits in the weaver mouse. Brain Res. *94*, 19–44.

Sretavan, D.W., and Shatz, C.J. (1986). Prenatal development of retinal ganglion cell axons: segregation into eye-specific layers within the cat's lateral geniculate nucleus. J. Neurosci. *6*, 234–251.

Star, E.N., Kwiatkowski, D.J., and Murthy, V.N. (2002). Rapid turnover of actin in dendritic spines and its regulation by activity. Nat. Neurosci. *5*, 239–246.

Streichert, L.C., and Weeks, J.C. (1995). Decreased monosynaptic sensory input to an identified motoneuron is associated with steroid-mediated dendritic regression during metamorphosis in *Manduca sexta*. J. Neurosci. *15*, 1484–1495.

Südhof, T.C. (2001). The synaptic cleft and synaptic cell adhesion. In Synapses, M.W. Cowan, T.C. Südhof, and C.F. Stevens, eds. (Baltimore, MD: Johns Hopkins University Press), pp. 275–314.

Sun, Y.A., and Poo, M.M. (1987). Evoked release of acetylcholine from the growing embryonic neuron. Proc. Natl. Acad. Sci. USA *84*, 2540–2544.

Sunderland, W.J., Son, Y.J., Miner, J.H., Sanes, J.R., and Carlson, S.S. (2000). The presynaptic calcium channel is part of a transmembrane complex linking a synaptic laminin (alpha4beta2gamma1) with non-erythroid spectrin. J. Neurosci. *20*, 1009–1019.

Sur, M., Weller, R.E., and Sherman, S.M. (1984). Development of X- and Y-cell retinogeniculate terminations in kittens. Nature *310*, 246–249.

Suzuki, T., Ito, J., Takagi, H., Saitoh, F., Nawa, H., and Shimizu, H. (2001). Biochemical evidence for localization of AMPA-type glutamate receptor subunits in the dendritic raft. Brain Res. Mol. Brain Res. *89*, 20–28.

Sweeney, S.T., and Davis, G.W. (2002). Unrestricted synaptic growth in spinster-a late endosomal protein implicated in TGF-beta-mediated synaptic growth regulation. Neuron *36*, 403–416.

Taha, S., Hanover, J.L., Silva, A.J., and Stryker, M.P. (2002). Autophosphorylation of alphaCaMKII is required for ocular dominance plasticity. Neuron *36*, 483–491.

Takai, Y., and Nakanishi, H. (2003). Nectin and afadin: novel organizers of intercellular junctions. J. Cell Sci. *116*, 17–27.

Takasu, M.A., Dalva, M.B., Zigmond, R.E., and Greenberg, M.E. (2002). Modulation of NMDA receptor-dependent calcium influx and gene expression through EphB receptors. Science *295*, 491–495.

Tao, H.W., and Poo, M. (2001). Retrograde signaling at central synapses. Proc. Natl. Acad. Sci. USA *98*, 11009–11015.

Tashiro, A., Dunaevsky, A., Blazeski, R., Mason, C.A., and Yuste, R. (2003). Bidirectional regulation of hippocampal mossy fiber filopodial motility by kainate receptors. A two-step model of synaptogenesis. Neuron *38*, 773–784.

Thiele, C., Hannah, M.J., Fahrenholz, F., and Huttner, W.B. (2000). Cholesterol binds to synaptophysin and is required for biogenesis of synaptic vesicles. Nat. Cell Biol. *2*, 42–49.

Thompson, W., Kuffler, D.P., and Jansen, J.K. (1979). The effect of prolonged, reversible block of nerve impulses on the elimination of polyneuroal innervation of new-born rat skeletal muscle fibers. Neuroscience *4*, 271–281.

Tokuoka, H., Yoshida, T., Matsuda, N., and Mishina, M. (2002). Regulation by glycogen synthase kinase-3beta of the arborization field and maturation of retinotectal projection in zebrafish. J. Neurosci. *22*, 10324–10332.

Toni, N., Buchs, P.A., Nikonenko, I., Bron, C.R., and Muller, D. (1999). LTP promotes formation of multiple spine synapses between a single axon terminal and a dendrite. Nature *402*, 421–425.

Trachtenberg, J.T., Chen, B.E., Knott, G.W., Feng, G., Sanes, J.R., Welker, E., and Svoboda, K. (2002). Long-term in vivo imaging of experience-dependent synaptic plasticity in adult cortex. Nature *420*, 788–794.

Tsui, C.C., Copeland, N.G., Gilbert, D.J., Jenkins, N.A., Barnes, C., and Worley, P.F. (1996). Narp, a novel member of the pentraxin family, promotes neurite outgrowth and is dynamically regulated by neuronal activity. J. Neurosci. *16*, 2463–2478.

Ullian, E.M., Sapperstein, S.K., Christopherson, K.S., and Barres, B.A. (2001). Control of synapse number by glia. Science *291*, 657–661.

Vaughn, J.E. (1989). Fine structure of synaptogenesis in the vertebrate central nervous system. Synapse *3*, 255–285.

Verderio, C., Coco, S., Bacci, A., Rossetto, O., De Camilli, P., Montecucco, C., and Matteoli, M. (1999a). Tetanus toxin blocks the exocytosis of synaptic vesicles clustered at synapses but not of synaptic vesicles in isolated axons. J. Neurosci. *19*, 6723–6732.

Verderio, C., Coco, S., Pravettoni, E., Bacci, A., and Matteoli, M. (1999b). Synaptogenesis in hippocampal cultures. Cell. Mol. Life Sci. *55*, 1448–1462.

Vicario-Abejon, C., Collin, C., McKay, R.D., and Segal, M. (1998). Neurotrophins induce formation of functional excitatory and inhibitory synapses between cultured hippocampal neurons. J. Neurosci. *18*, 7256–7271.

Walsh, M.K., and Lichtman, J.W. (2003). In vivo time-lapse imaging of synaptic takeover associated with naturally occurring synapse elimination. Neuron *37*, 67–73.

Wan, H.I., DiAntonio, A., Fetter, R.D., Bergstrom, K., Strauss, R., and Goodman, C.S. (2000). Highwire regulates synaptic growth in *Drosophila*. Neuron *26*, 313–329.

Washbourne, P., Bennett, J.E., and McAllister, A.K. (2002). Rapid recruitment of NMDA receptor transport packets to nascent synapses. Nat. Neurosci. *5*, 751–759.

Watts, R.J., Hoopfer, E.D., and Luo, L. (2003). Axon pruning during *Drosophial* metamorphosis: evidence for local degeneration and requirement of the ubiquitin-proteosome system. Neuron *38*, 871–885.

White, J.G., Albertson, D.G., and Anness, M.A.R. (1978). Connectivity changes in a class of motorneurons during the development of a nematode. Nature *271*, 764–766.

Wiesel, T.N., and Hubel, D.H. (1963). Single-cell responses in striate cortex of kittens deprived of vision in one eye. J. Neurophysiol. *26*, 1003–1017.

Wong, W.T., Faulkner-Jones, B.E., Sanes, J.R., and Wong, R.O. (2000). Rapid dendritic remodeling in the developing retina: dependence on neurotransmission and reciprocal regulation by Rac and Rho. J. Neurosci. *20*, 5024–5036.

Wu, G.Y., and Cline, H.T. (1998). Stabilization of dendritic arbor structure in vivo by CaMKII. Science *279*, 222–226.

Yamagata, M., Weiner, J.A., and Sanes, J.R. (2002). Sidekicks: synaptic adhesion molecules that promote lamina-specific connectivity in the retina. Cell *110*, 649–660.

Yamaguchi, Y. (2002). Glycobiology of the synapse: the role of glycans in the formation, maturation, and modulation of synapses. Biochim. Biophys. Acta *1573*, 369–376.

Yang, X., Li, W., Prescott, E.D., Burden, S.J., and Wang, J.C. (2000). DNA topoisomerase IIbeta and neural development. Science *287*, 131–134.

Yang, X., Arber, S., William, C., Li, L., Tanabe, Y., Jessell, T.M., Birchmeier, C., and Burden, S.J. (2001). Patterning of muscle acetylcholine receptor gene expression in the absence of motor innervation. Neuron *30*, 399–410.

Yoshii, A., Sheng, M.H., and Constantine-Paton, M. (2003). Eye

opening induces a rapid dendritic localization of PSD-95 in central visual neurons. Proc. Natl. Acad. Sci. USA *100*, 1334–1339.

Zakharenko, S., Chang, S., O'Donoghue, M., and Popov, S.V. (1999). Neurotransmitter secretion along growing nerve processes: comparison with synaptic vesicle exocytosis. J. Cell Biol. *144*, 507–518.

Zhai, R.G., Vardinon-Friedman, H., Cases-Langhoff, C., Becker, B., Gundelfinger, E.D., Ziv, N.E., and Garner, C.C. (2001). Assembling the presynaptic active zone: a characterization of an active one precursor vesicle. Neuron *29*, 131–143.

Zhang, W., and Benson, D.L. (2001). Stages of synapse development defined by dependence on F-actin. J. Neurosci. *21*, 5169–5181.

Zhang, X., and Poo, M.M. (2002). Localized synaptic potentiation by BDNF requires local protein synthesis in the developing axon. Neuron *36*, 675–688.

Zhang, H., Webb, D.J., Asmussen, H., and Horwitz, A.F. (2003). Synapse formation is regulated by the signaling adaptor GIT1. J. Cell Biol. *161*, 131–142.

Ziv, N.E., and Garner, C.C. (2001). Principles of glutamatergic synapse formation: seeing the forest for the trees. Curr. Opin. Neurobiol. *11*, 536–543.

Ziv, N.E., and Smith, S.J. (1996). Evidence for a role of dendritic filopodia in synaptogenesis and spine formation. Neuron *17*, 91–102.