REVIEW

Synapse disassembly

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The transfer of information between cells in the nervous system is mediated, primarily, through synaptic connections. A synapse is an intercellular junction defined ultrastructurally as a postsynaptic electron density (PSD) in direct opposition to a presynaptic density associated with clustered synaptic vesicles that is referred to as an active zone. The synaptic cleft is a tight, stable intercellular junction that is resistant to biochemical disruption. The intervening space at the synapse is termed the synaptic cleft and includes extrasynaptic material that has been best defined at the mammalian neuromuscular junction (NMJ) where it is continuous with the basal lamina. Synaptic vesicles released presynaptically are in direct opposition to the postsynaptic neurotransmitter receptors that are activated upon binding ligand. A synapse, thus, is defined as a single active zone and associated postsynaptic specialization. In many cases, multiple synapses connect two cells as at the neuromuscular junction where the ensemble of synapses can be referred to as a synaptic input or junction.

We are rapidly moving toward a complete biochemical description of the proteins that constitute the mammalian glutamatergic synapse that predominates the central nervous system and the vertebrate NMJ that uses acetylcholine as a neurotransmitter (Sanes and Lichtman 1999; Sheng and Lee 2000; Walikonis et al. 2000). The emerging picture of the synapse is of a large, macromolecular complex that spans the cleft between the two connected neurons (or neuron and muscle). This large macromolecular complex is capable of coupling the influx of calcium driven by a nerve impulse (action potential) to the probabilistic release of single vesicles of neurotransmitter. Though only a portion of the proteins at the active zone are essential for the release of synaptic vesicles, many scaffolding and signaling proteins that localize to the active zone are implicated in the modulation or plasticity of the active zone (Sheng and Kim 2002).

A remarkable feature of synaptic connections is that they are dynamic. Individual synapses can be stable for prolonged periods of time, which is presumably necessary for the stable function of neural circuits, but syn-

apses also retain the capacity to be rapidly disassembled and reformed. The formation of new synaptic connections and the elimination of previously functional synaptic connections are particularly prominent during early development, but these processes also continue throughout the lifetime of an animal (Purves and Lichtman 1980). The dynamic restructuring of connections between neurons is essential for the initial wiring of specific neural circuits and may be an essential process during the continued modification of neural circuits in response to a changing environment or during the cellular processes that underlie learning and memory. A particularly well-studied example is the remodeling of highly ramified retinal projections within the visual cortex resulting in the formation of eye-specific dominance columns.

Considerable progress is being made examining the dynamic nature of synaptic connections. Recent advances in live-imaging technologies have allowed time-lapse imaging of synapse morphology and, in some cases, correlated changes in synaptic function (Yuste and Bonhoeffer 2001; Cohen-Cory 2002). At the molecular level, we are gaining an increasingly detailed view of the mechanisms of synapse assembly. Synaptogenesis requires inductive, intercellular signaling events between the pre- and postsynaptic partners that lead to the clustering of synaptic molecules and their precise apposition at the pre- and postsynaptic membranes (Sanes and Lichtman 1999; Luo 2000; Scheiffele 2003).

Although considerable attention has been focused on the mechanisms of synaptogenesis, relatively little is understood about the mechanisms that control the regulated disassembly of a previously functional synaptic connection. When considering the mechanisms of synaptic remodeling, the elimination of a synaptic connection between two cells is a dramatic event and is one that must also be highly regulated. The significance of regulated synapse disassembly is suggested by recent, *in vivo*, live-imaging studies. Postsynaptic dendritic spines, which may reflect functional synaptic connections, turnover continually with 5%–30% of spines in an individual neuron within the brain of a mouse being remodeled over the course of 3–4 wk (Grutzendler et al. 2002; Trachtenberg et al. 2002). Extrapolated over the lifetime of the animal, this suggests that potentially ~50% of all spines might be remodeled at some point during a lifetime. If changes in spine morphology reflect the forma-

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tion and elimination of individual active zones, then the regulated disassembly of synapses is a prominent process and one that must be regulated and balanced with the mechanisms of synaptogenesis. Here we review the literature, phenomenology, and mechanisms of synapse disassembly.

Synapse elimination during neural development

Synapse disassembly can modify the strength of the connectivity between two cells and may participate in the mechanisms of synaptic plasticity (Bailey and Kandel 1993; Lichtman and Colman 2000). In the more extreme examples, synapse disassembly can lead to the complete elimination of synaptic connectivity between two cells, which is referred to as "input elimination." Although all input elimination events necessarily involve synapse disassembly, not all synaptic disassembly events lead to the elimination of an input. It should also be noted that synapse disassembly has been observed in neurons during periods in which input elimination is known to occur, suggesting that these processes can coexist within the same cell (Cline 2001). Despite this intimate relationship between synapse disassembly and input elimination, it is unclear at this point whether these processes are governed by similar molecular processes or are fundamentally different.

Input elimination has been observed in many parts of the nervous system, including the visual system, auditory system, cerebellum, autonomic ganglia, and skeletal muscle, where the number of inputs declines during critical periods early in development (Purves and Lichtman 1980). Even in cases where mature target cells are innervated by more than one presynaptic partner, such as the sympathetic ganglion, the number of innervating axons declines significantly during development (Lichtman and Purves 1980). In addition, input elimination also occurs during the extensive neuronal remodeling observed during metamorphosis in many invertebrate systems (Lee et al. 2000). Thus, the reduction of inputs via elimination appears to be an essential aspect of the normal development of many different neural circuits in both vertebrates and invertebrates and may indicate a conserved process of synaptic disassembly.

In many of the well-studied examples of input elimination, synapse disassembly appears to be a consequence of activity-dependent competition between multiple inputs contacting a single target. However, synaptic competition is not necessary to drive input elimination or synapse disassembly. For example, the elimination of inputs during metamorphosis in insects appears to be governed by hormones rather than activity-dependent competition (Lee et al. 2000). In addition, synapse disassembly appears to be a normal process associated with the growth of the *Drosophila* NMJ where synaptic competition does not occur. Thus, the mechanisms of synapse disassembly may be separable from the process of synaptic competition. In this scenario, diverse processes ranging from growth to activity-dependent competition and synaptic plasticity could govern a conserved mecha-

nism of synapse disassembly. A goal for the future may be to define the common mechanisms of synapse disassembly and elucidate how growth, competition, and plasticity regulate this mechanism.

The mammalian NMJ

The mammalian NMJ is perhaps the most well-characterized synapse in mammals. At birth, the muscle fiber is innervated by multiple motoneurons, which are gradually eliminated over the course of several weeks (Sanes and Lichtman 1999). Concurrently, contacts not being eliminated are expanded and differentiated into mature endplates. This brings up an interesting problem of how the correct input is selected for maintenance at the same time that others are selected for synapse disassembly and elimination.

The classic model of selection involves an activity-dependent competition between equivalent inputs in which one of the inputs emerges victorious at the expense of the others, which are subsequently eliminated. The involvement of activity was clearly demonstrated in several experiments. If activity is blocked, selection does not occur (Balice-Gordon and Lichtman 1994; but see Costanzo et al. 2000). Several classic experiments also indicate that selection is mediated through the muscle cell. If two motoneuron inputs are separated by enough distance on the muscle surface, both can be maintained (Kuffler et al. 1977). Experiments using focal blockade of postsynaptic receptors further support this view. In these experiments it was shown that focal blockade 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). These observations have led to the following theories of competition at the NMJ where input elimination is driven by 1) the production of limiting amounts of muscle-derived trophic signals which are more effective at sustaining active inputs; 2) the secretion of muscle-derived "synaptotoxins," which promote the withdrawal of less active inputs; or 3) the generation of a "punishing signal" at the most active contact that would attack competing axons leading to their withdrawal (Sanes and Lichtman 1999).

Recent experiments suggest that there is added complexity to mechanisms that select which input will be disassembled versus maintained. The advent of GFP mice has allowed the time-lapse visualization of the motoneuron terminal in combination with receptor visualization using sub-blocking concentrations of labeled α -bungarotoxin. In recent experiments, 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 synapse disassembly are reversible. This suggests that elimination is not a switch but is continually driven until the input is ultimately eliminated. Second, this result also indicates that selection for

elimination involves more than simply receptor activation. Focal blockade experiments demonstrated that reduction in receptor activation led to elimination (Balice-Gordon and Lichtman 1994). But in the situation in which elimination is reversed, only a small portion of the receptor field is being activated prior to the reversal event. Thus, there may be other contributing processes that can, in some cases, overcome the muscle-mediated mechanisms of synapse elimination. For example, there may be intrinsic difference between motoneurons that converge on a single muscle that contribute to selection. Mechanisms such as cell-specific surface recognition molecules or neuron-specific programs of activity could act to specify motoneurons. Although specification of cellular identity via protein expression has not been demonstrated at the vertebrate NMJ, two recent reports have demonstrated that pools of motoneurons have diverse activity patterns during periods of synapse elimination (Personius and Balice-Gordon 2001; Buffelli et al. 2002). These authors show that the activity patterns of motoneurons are highly correlated early in development but become uncorrelated by the onset of synapse elimination, suggesting a role for asynchronous firing patterns in synapse elimination. Earlier studies also support the idea that differences in activity can affect the outcome of competition (Duxson and Vrbova 1985; Thompson 1985; Barry and Ribchester 1995). Therefore, intrinsic differences in firing patterns between motoneurons could play an important role in specifying synapse formation and elimination. In addition, it has been reported that there exist intrinsic differences between muscle fibers that affect both assembly and disassembly of NMJs in young adult mice (Pun et al. 2002). These data demonstrate that intrinsic differences may exist between motoneurons and between muscles, which could directly regulate the process of elimination.

Although the molecular identification of trophic factors, synaptotoxins, or punishing signals has remained elusive, it was recently shown that neurotrophin-4 (NT-4) is produced by slow muscle fibers in an activity-dependent manner and that production of NT-4 promotes growth and remodeling of the adult NMJ (Belluardo et al. 2001). This raises the interesting possibility that intrinsic changes in presynaptic activity could initiate muscle-mediated elimination programs such as the release of trophic factors.

The cerebellum

Purkinje cells (PCs) within the cerebellum receive distinct excitatory inputs from parallel inputs (PFs) and climbing fibers (CFs). During early postnatal development in the rodent brain, PCs are multiply innervated by three to four CFs, which are removed over the course of a few weeks until only one CF axon innervates a single PC (Sotelo 1975; Ito 1984; Mason and Gregory 1984; Mason et al. 1990). The one-to-one relationship between CF and PC is then maintained throughout the lifetime of the adult.

The cerebellum has long been considered a site for

motor control and is thought to facilitate the type of discrete motor learning associated with the application of simple movements to more coordinated acts (Thach et al. 1992). 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 (Crepel and Mariani 1976; Crepel et al. 1980). Recent work on mutant mice deficient in PKC, mGluR1, PLC, or $G_{\alpha q}$ have 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 long-term depression (LTD), providing a link between synapse elimination, plasticity, and motor coordination (Ichise et al. 2000).

Because mGluR1 is expressed in other regions of the brain, Ichise and colleagues (2000) 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. 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.

Elimination in the absence of competition

Unlike the mammalian cerebellum and NMJ, the *Drosophila* larval NMJ is molecularly specified such that each muscle cell receives input from identified motoneurons and these inputs persist throughout development (Keshishian et al. 1996). Though the number of innervating axons doesn't change during larval development, the size of the synapse increases dramatically as the animal increases in size nearly 20-fold. This is due to a tight coupling between synaptic growth and muscle growth (Davis and Bezprozvanny 2001). In vivo imaging of the *Drosophila* NMJ with a postsynaptic GFP-tagged Shaker-CD8 chimera has demonstrated that the addition of synaptic boutons at the NMJ is an active process occurring throughout larval development (Zito et al. 1999). Retraction of synaptic area and the removal of boutons were not documented in these experiments, although the rate at which this postsynaptic marker protein disassembles might mask rapid presynaptic disassembly events.

It has been recently shown that rapid disassembly of axonal branches occurs at the *Drosophila* NMJ during

development (Eaton et al. 2002). Based on the earlier observations at the frog NMJ that local presynaptic branch retraction led to a loss of opposition of pre- and postsynaptic markers (synaptic footprints; Wernig et al. 1980), an assay was developed to identify synaptic footprints in *Drosophila* using antibodies to the presynaptic marker synapsin and the PSD95 homolog discs-large. Using this assay, it was shown that footprints occur throughout development and that the percentage of synapses with footprints was increased during the most rapid phases of synaptic growth. This suggests two things, first, that growth at the *Drosophila* NMJ is a balance of bouton addition and retraction and, second, that the amount of elimination that occurs at the NMJ is regulated developmentally. Importantly, footprints often were restricted to individual branches or regions of branches suggesting that the events themselves are local and presumably do not result in the complete elimination of the input. Therefore, models of elimination from the mammalian NMJ using asynchronous activity probably do not apply to the elimination of a single branch within a single *Drosophila* NMJ. It is possible, though, that the synapse as a whole is supported by a limited amount of muscle-supplied trophic support and that when synaptic growth exceeds this level, a program of elimination is activated to bring the size of the NMJ back in line with the level of support. It is also possible that competition exists between branches of the same junction, although the observation that multiple branches retract within the same NMJ does not support this interpretation.

How do synapses disassemble?

Local versus cell-wide synapse disassembly

The local remodeling of synaptic connections (i.e., synapses) without the complete removal of an input is a prominent feature of plasticity and may be associated with the rapid formation or elimination of dendritic spines (Dailey and Smith 1996; Engert and Bonhoeffer 1999; Maletic-Savatic et al. 1999; Toni et al. 1999; Lendvai et al. 2000; Grutzendler et al. 2002; Trachtenberg et al. 2002; Dunaevsky and Mason 2003), as well as changes in axonal or dendritic arborization (Harris and Woolsey 1981; Greenough et al. 1985; Purves and Hadley 1985; Purves et al. 1986; Petit et al. 1988; O'Rourke and Fraser 1990; Darian-Smith and Gilbert 1994; Baker and Van Pelt 1997; Rajan and Cline 1998; Lom and Cohen-Cory 1999; Rajan et al. 1999; Sin et al. 2002). Despite the progress made recently in understanding the dynamics and driving forces behind these events, there is still little known about the downstream signals that instruct a synapse where and when to assemble or disassemble.

Recent in vivo imaging studies have provided strong evidence that synaptic elimination events are localized both spatially and temporally. In vivo imaging of dendritic segments within both the barrel cortex and the visual cortex of mice have shown spines disappearing at different places and at different times within the same region of dendrite (Lendvai et al. 2000; Grutzendler et al.

2002; Trachtenberg et al. 2002). A similar in vivo imaging technique of the axonal arborization of retinal ganglion cells within the tadpole visual system has also demonstrated that asynchrony of branch elimination can also occur presynaptically (Cohen-Cory 1999; Sin et al. 2002). At present, although there are strong data correlating new spine formation with enhanced efficacy, it remains unclear whether the loss of a spine or branch is associated with the disassembly of a previously functional active zone. At the vertebrate NMJ the evidence for local elimination of functional active zones is clearer. In vivo imaging of an entire motor unit has revealed asynchrony and spatial heterogeneity in the elimination of functional endplates within a single motor unit (Keller-Peck et al. 2001). Thus, because synapse disassembly can vary both spatially and temporally at the terminals of a single cell in a variety of different systems, these data indicate that synaptic elimination can be regulated locally at the site of disassembly.

Data suggesting the existence of cell-wide programs that influence synapse assembly and disassembly have also been obtained from in vivo imaging of Homer 1c-GFP (PSDZip45-GFP) clusters in hippocampal neuronal cultures (Ebihara et al. 2003) and synaptophysin-GFP puncta in presynaptic arbors in hippocampal slice cultures (De Paola et al. 2003). Ebihara and colleagues (2003) imaged PSDZip45-GFP clusters in four distinct dendritic regions within neurons every 24 h over the course of a week and show both assembly and disassembly of clusters. Analysis of five sets of culture preparations showed that correlation of cluster changes within the same cell was significantly higher than the correlation of changes between different cells. In vivo imaging of synaptophysin-GFP puncta in hippocampal slice cultures have also demonstrated that both cAMP and receptor activation can elevate numbers of puncta forming and disappearing within 1 d (De Paola et al. 2003). Interestingly, the gains equal the losses of synaptophysin-GFP puncta suggesting that in these mature cultures the increase in dynamics does not lead to a net increase or decrease of synaptic size. These data support the existence of cell-wide programs that are able to bias the cell towards synaptogenesis or disassembly. Although the mechanisms that bias a cell toward enhanced remodeling are unclear, they may relate to developmental phenomena such as the existence of critical periods for synaptic rearrangement.

Sequence of synapse disassembly

Data from the mammalian NMJ and from the cerebellum support the model that elimination is mediated via the postsynaptic cell. This does not necessarily mean that the disassembly process itself begins in the postsynaptic cell. In fact, most data supports presynaptic disassembly preceding postsynaptic disassembly. At the mammalian NMJ, recent live-imaging data has clearly demonstrated that retraction of presynaptic membrane can precede the loss of postsynaptic receptors (Walsh and Lichtman 2003). It should be noted though that previous work from the same laboratory supported the hypothesis

that disassembly of the postsynaptic apparatus preceded the withdrawal of the axon terminal (Balice-Gordon et al. 1993; Colman et al. 1997), suggesting that at the vertebrate NMJ perhaps elimination can initiate either pre- or postsynaptically. At the *Drosophila* NMJ, experimental disruption of the dynactin/dynein motor complex by transgenic expression presynaptically of a dominant-negative P150/Glued (DN-Glued) increases the frequency of elimination events (Eaton et al. 2002). Subsequent immunolocalization studies in these animals suggest that the dissolution of the postsynaptic specialization follows presynaptic disassembly, based on the observations that the retraction of the presynaptic markers synapsin and futsch, a synaptic microtubule-binding protein, precedes the elimination of Discs-large immunoreactivity and GluR clusters. In addition, there is no observed change in quantal size in animals expressing DN-Glued despite severely impaired release supporting the existence of normal receptor clusters at poisoned synapses. These results present an initial blueprint for the stepwise disassembly of synaptic contacts, which begins with the disassembly of the presynaptic cytoskeleton, removal of synaptic vesicle release machinery, retraction of presynaptic membrane, disassembly of postsynaptic receptor clusters, and finally dissolution of postsynaptic scaffolding structures. Because presynaptic innervation is thought to provide instructive and maintenance functions for postsynaptic structures (Saitoe et al. 2001; Sanes and Lichtman 2001), it is still unclear from these results whether postsynaptic disassembly is a directed process or the result of the loss of some maintenance factor(s) normally provided by the motoneuron.

Mechanisms of synapse disassembly

Theoretically, synapse disassembly could be achieved by loss of a subcellular mechanism that maintains the synapse or could be due to mechanisms that actively drive the disassembly of the synapse. There is ample evidence at both central and peripheral synapses that trophic factors participate in synapse maintenance (McAllister et al. 1999; Huang and Reichardt 2001). Specifically, impairment of either TrkB signaling to the muscle or NT-4 production within the muscle promotes synapse disassembly at the vertebrate NMJ (Gonzalez et al. 1999; Bel-luado et al. 2001). At the *Drosophila* NMJ, impaired TGF- β signaling caused by receptor mutations or impaired retrograde trafficking of the TGF- β signal enhances the incidence of synaptic retraction events (Eaton et al. 2002; B. Eaton and G. Davis, unpubl.).

These data might suggest that loss of trophic signaling could account for synapse elimination, perhaps because of a loss of synaptic maintenance. However, the speed at which synapse disassembly occurs can be more rapid, in many cases, than the normal rates of protein turnover at the synapse indicating that passive disassembly cannot account for all synapse disassembly. For example, the half-life of AMPA receptors at a central synapse has been measured to be 18–23 h, and the half-life of NR2 is 16 h (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 <1 d (Okabe et al. 2001; Grutzendler et al. 2002; Trachtenberg et al. 2002). At both central and peripheral synapses, the rates of transmitter receptor trafficking are influenced by pre- and postsynaptic activity and an increasingly well-understood array of signaling molecules (Akaaboune et al. 1999; Chen et al. 2000; Esteban et al. 2003). These recent data point to the existence of mechanisms that can regulate the assembly and disassembly of synaptic connections. At the vertebrate NMJ, the existence of a “punishment” signal or a “synaptotoxin” has long been hypothesized to participate in the induction of synaptic input elimination (Nguyen and Lichtman 1996). Although these putative factors that would drive synaptic disassembly remain to be identified, they may be related to the factors that modulate the rate of protein turnover at the synapse.

One potential mechanism of synapse disassembly could involve the targeted degradation of synaptic components via ubiquitin-mediated degradation, a process that has been demonstrated to be involved in the degradation of proteins and protein complexes in many systems (von Arnim 2001; Stahl and Barbieri 2002). For example, evidence from *Caenorhabditis elegans* suggests that transgenic elevation of ubiquitin levels postsynaptically results in a reduction in glutamate receptor density at the synapse (Burbea et al. 2002). Also, recent studies of input elimination during metamorphosis in *Drosophila* show that disruption of the ubiquitin–proteasome system blocks axon pruning (Watts et al. 2003). These data support the possibility that altering the rates of degradation or trafficking of synaptic components could modulate synapse stability or maintenance.

The disassembly of large protein complexes is common throughout biology. Perhaps insight into the molecular mechanisms that drive synapse disassembly can be gleaned from examples of junctional disassembly in other cell biological contexts. A potentially relevant process is the disassembly of adherens junctions (AJs) in epithelial cells. AJs are specialized forms of cadherin-based adhesive contacts that are important for tissue organization including within the nervous system. Cadherins are type I transmembrane proteins that form a complex with cytosolic catenins, which, in turn, provide anchorage for the actin cytoskeleton resulting in the formation of stable sites of contact between cells (Yap et al. 1997). In epithelial cells, AJs are disassembled in response to hepatocyte growth factor/scatter factor (HGF/SF) via the activation of MAP kinase and PI 3-kinase (Potempa and Ridley 1998). The process of AJ disassembly is thought to include the internalization of E-cadherin via an ARF6-regulated membrane trafficking event resulting in a decrease in cell-to-cell adhesion and the loss of AJ integrity (Palacios et al. 2001). Perhaps the local activation of membrane trafficking events leading to the down-regulation of cell adhesion will also play an important role in synaptic disassembly as well. It is intriguing that EM analysis of retraction events in *Dro-*

Figure 1. The disassembly of functional active zones. (A) Synaptic elimination is a local event involving the directed disassembly of complex macromolecular machines. Disassembly would necessarily involve the removal of release machinery, receptor complexes, synapse-spanning protein complexes, and pre- and postsynaptic scaffolding proteins. Synapse stability is maintained by trophic factors such as neurotrophins released from both the pre- and postsynaptic cell and may be regulated by additional factors including muscle activity. (B) Ultrastructural analysis of a wild-type bouton at the *Drosophila* NMJ. (B') Close-up of wild-type active zone demonstrating the close opposition of the pre- and postsynaptic membranes within the EM dense region surrounding the T-bar, a morphological marker of active zones in *Drosophila*. (C) Ultrastructural analysis of a bouton undergoing disassembly at the *Drosophila* NMJ (Eaton et al. 2002). Note the increase in vacuolarization, potentially indicating the internalization of plasma membrane. (C') Close-up of an active zone at a synapse undergoing disassembly. Arrows indicate the “unzipping” of pre- and postsynaptic membranes suggesting a loss of adhesion at the active zone.

sophila demonstrate a loss of adhesion between pre- and postsynaptic membranes within the active zone, a specialized site of cell-to-cell contact within the bouton (Fig. 1B',C').

As discussed above, a prominent feature of synapse elimination in all systems is the localized disassembly of synaptic complexes. Whether the removal of trophic support or the active disassembly of the synapse or both drives synapse elimination, it remains to be determined how these mechanisms are locally specified. It is interesting to speculate that precise regulation could be achieved by requiring that multiple signaling systems simultaneously converge to eliminate a synaptic connection.

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