Remodeling the Plasticity Debate: The Presynaptic Locus Revisited

The cellular mechanisms contributing to long-term potentiation and activity-induced formation of glutamatergic synapses have been intensely debated. Recent studies have sparked renewed interest in the role of presynaptic components in these processes. Based on the present evidence, it appears likely that long-term plasticity utilizes both pre- and postsynaptic expression mechanisms.

Presynaptic Expression of Long-Term Potentiation

The cellular mechanisms underlying use-dependent, long-lasting changes in the efficacy of neurotransmission, learning, and memory have captured the fascination of researchers for several decades. In the heated controversy that peaked in the 1990s over the locus of expression of long-term potentiation (LTP), observations of changes in presynaptic probability of neurotransmitter release seemed inconsistent with other data, suggesting postsynaptic changes in sensitivity. For “classical” NMDA receptor-dependent LTP (e.g., at the Schaffer collateral-CA1 synapse), the silent synapse hypothesis quelled much of the rancor (28, 31). The hypothesis proposed that a subset of synapses containing NMDA receptors but lacking functional AMPA receptors did not participate in basal synaptic transmission. On induction of LTP, AMPAR receptors would be inserted at these synapses, thus effectively “unsilencing” them. In electrophysiological experiments assessing the efficacy of transmission at ensembles of synapses, this postsynaptic activation of additional synapses would have easily been mistaken as a change in release probability. The silent synapse hypothesis thus reconciled seemingly contradictory evidence pointing toward changes in release probability with an entirely postsynaptic mechanism (31). Only the expression of a second NMDA receptor-independent form of LTP at hippocampal mossy fiber and cerebellar parallel fiber synapses was generally accepted to be mediated through changes in presynaptic release probability.

The ability to study the efficacy of transmission at single synapses, using a combination of traditional electrophysiological approaches and imaging techniques, has recently allowed a critical reexamination of these conclusions. At individual mossy fiber synapses in the hippocampus, optical quantal analysis of evoked postsynaptic Ca\(^{2+}\) transients has demonstrated that induction of NMDA receptor-independent LTP resulted in an enhanced probability of transmitter release at existing functional release sites and increases in the number of active release sites (33), consistent with the findings of earlier electrophysiological studies of a presynaptic expression mechanism. For NMDA receptor-dependent LTP, recent studies using such methods that allow high spatial resolution challenge the notion of an entirely postsynaptic locus of expression. By directly visualizing presynaptic release at individual CA1 synapses using FM1-43, Zakharenko et al. (51) observed enhanced rates of synaptic vesicle recycling following induction of a form of LTP that depends on both NMDA receptor and L-type Ca\(^{2+}\) channel activation. Within the same study, however, a more standard LTP induction protocol requiring only NMDA receptor activation was not associated with any change in the rate of FM1-43 destaining. Postsynaptic calcium imaging, a technique offering better temporal resolution and, therefore, arguably better detection of changes in the probability of neurotransmitter release in response to stimulation at low frequency, has yielded more unequivocal results. Optical quantal analysis of evoked postsynaptic Ca\(^{2+}\) transients at Schaffer collateral CA1 and associational CA3 synapses revealed an increase in release probability with the induction of LTP (14), suggesting that LTP induced by tetanic stimulation at this synapse is in part due to changes in neurotransmitter release.

The debate about a possible contribution of presynaptic mechanisms in the maintenance of NMDA receptor-dependent LTP is thus ongoing, and it is even spilling over from the hippocampus into other brain regions. A new battleground in this dispute is the amygdala, the computational hub for circuits mediating fear conditioning. LTP induction at synapses from cortical afferents onto neurons in the lateral nucleus has been shown to result in increases in the neurotransmitter release probability, suggesting a presynaptic potentiation of synaptic efficacy (18, 45). On the other hand, LTP at thalamo-amygdala synapses leads to augmented surface-expression of GluRI-containing AMPA receptors and is blocked through interference with AMPA receptor trafficking, suggesting a postsynaptic increase in neurotransmitter sensitivity as LTP expression mechanism (36). These data could possibly be reconciled by assuming that cortical and thalamic afferents to the amygdala display different forms of LTP with distinct induction requirements and loci of expression. In line with such a model, a postsynaptic form of LTP can be induced in the amygdala by pairing presynaptic stimu-
lation with backpropagating action potentials at thalamic, but not cortical, afferents converging onto adjacent spines of the same projection neurons in the lateral amygdala (19). Conversely, a presynaptically induced and expressed form of NMDA receptor-dependent LTP is elicited at cortical, but not at thalamic, synapses by coincident stimulation of converging thalamic and cortical afferents (20). These findings offer the intriguing possibility that different pre- and postsynaptically maintained forms of LTP can coexist at neighboring synapses onto the same postsynaptic neuron. Moreover, they indicate that mechanisms of LTP induction and expression, both pre- and postsynaptic, may be more diverse than previously anticipated.

**Cellular Mechanisms of Sustained Alterations in Neurotransmitter Release**

Neurotransmitter release from synaptic vesicles is contingent on a complex series of processes, such as synaptic vesicle docking, priming, calcium influx through voltage-gated channels tightly coupled to the synaptic vesicle release machinery, and the fusion of synaptic vesicles with the plasma membrane. Regulatory processes leading to persistent changes in any of these steps could potentially cause the alterations in neurotransmitter release, as observed during some forms of long-term synaptic plasticity. Two presynaptic proteins, the synaptic vesicle-associated monomeric G-protein Rab3A (5) and the active zone cytomatrix protein RIM1α (6, 25), have been implicated in the expression of LTP at hippocampal mossy fiber and cerebellar parallel fiber synapses. However, their precise function in neurotransmitter release is not known, making it difficult to pinpoint from a molecular perspective the processes that lead to a persistent enhancement of neurotransmitter release probability at these synapses. From electrophysiological and cellular imaging studies, two main models for sustained increases in neurotransmitter release during long-term plasticity have emerged (FIGURE 1). The first hypothesis builds on evidence of a “kiss-and-run” mechanism of gradual, regulated release of synaptic vesicle contents through a fusion pore (3, 17, 22). At glutamatergic synapses, incomplete kiss-and-run-type release of glutamate through a particularly small fusion pore may lead to glutamate concentrations of the neurotransmitter sufficient to activate NMDA receptors (dark blue), while being insufficient to activate AMPA receptors (light blue). A change in the release mechanism leading to full-collapse fusion events would recruit this synapse for AMPA receptor-mediated transmission. Variations of this mechanism are possible; for example, classical full-fusion and “kiss-and-run” release may coexist at a synapse, and induction of plasticity may change the relative frequency of these mechanisms. Also, glutamate released through a transient fusion pore might activate, but not saturate, AMPA receptors. In this case, plasticity induction would increase the number of AMPA receptors recruited. B: structural alterations at release sites, such as changes in synaptic vesicle pool or active zone size, could lead to sustained changes of neurotransmitter release probability. B1: in Aplysia, long-term facilitation leads to synaptic vesicle accumulation at previously “empty” varicosities. B2: recruitment of mobile modules of active zone cytomatrix and associated synaptic vesicles to an existing synaptic release site enlarges this release site, possibly leading to an increase in release probability.

**FIGURE 1. Possible mechanisms leading to sustained alterations of neurotransmitter release**

A: incomplete glutamate release through a transient fusion pore (top) may lead to cleft concentrations of the neurotransmitter sufficient to activate NMDA receptors (dark blue), while being insufficient to activate AMPA receptors (light blue). A change in the release mechanism leading to full-collapse fusion events would recruit this synapse for AMPA receptor-mediated transmission. Variations of this mechanism are possible; for example, classical full-fusion and “kiss-and-run” release may coexist at a synapse, and induction of plasticity may change the relative frequency of these mechanisms. Also, glutamate released through a transient fusion pore might activate, but not saturate, AMPA receptors. In this case, plasticity induction would increase the number of AMPA receptors recruited. B: structural alterations at release sites, such as changes in synaptic vesicle pool or active zone size, could lead to sustained changes of neurotransmitter release probability. B1: in Aplysia, long-term facilitation leads to synaptic vesicle accumulation at previously “empty” varicosities. B2: recruitment of mobile modules of active zone cytomatrix and associated synaptic vesicles to an existing synaptic release site enlarges this release site, possibly leading to an increase in release probability.
and the dimensions of the active zone (39). Thus structural alterations of these presynaptic components should lead to sustained changes in release probability. Intriguingly, recent studies have demonstrated that synaptic vesicle pools and active zones are subject to structural remodeling and suggested that these processes represent an important presynaptic mechanism to achieve activity-dependent changes in the efficacy of neurotransmission.

Evidence for an activity-dependent structural remodeling of release sites has come from studies addressing the mechanisms of synaptic scaling, a homeostatic form of plasticity that leads to strengthening or weakening, respectively, of all synapses in response to global decreases or increases in neuronal activity to maintain a constant level of synaptic input (46). In cultured hippocampal neurons, long-term blockade of activity through inhibition of voltage-gated sodium channels resulted in an overall increase in synaptic vesicle pool sizes, active zone areas, and bouton volumes (30). These authors observed a parallel increase in neurotransmitter release probability, suggesting that the enlargement of synaptic vesicle pool and active zone sizes induced by activity blockade results in an increase in the number of readily releasable synaptic vesicles, which in turn leads to increases in neurotransmitter release probability. A recent study (43) has likewise found increases in synaptic vesicle pool size and release probability in response to prolonged synaptic inactivity but also pointed out that the enhancement of neurotransmitter release observed can only partially be explained by an increase in vesicle pool size. As an added complica-

![FIGURE 1A](image1.png)

**FIGURE 1A.** Release of neurotransmitter from release sites, either “orphan” or synaptic, may lead to localized extension of dendritic filopodia from an adjacent dendrite. Axonal filopodia establish terminal boutons upon contact with a postsynaptic target.

![FIGURE 1B](image2.png)

**FIGURE 1B.** Mediated through glutamate receptors (blue), repetitive neuronal activation induces the extension of filopodia and short branches from axons.

![FIGURE 2](image3.png)

**FIGURE 2.** Role of presynaptic specializations in activity-induced synapse formation

A: mediated through glutamate receptors (blue), repetitive neuronal activation may induce the extension of filopodia and short branches from axons. Axonal filopodia establish terminaus boutons on contact with a postsynaptic target. B: release of neurotransmitter from release sites, either “orphan” or synaptic, may lead to localized extension of dendritic filopodia from an adjacent dendrite. Dendritic filopodia are converted into spines upon formation of a contact with the presynaptic varicosity.
tion, the very notion that chronic changes in neuronal activity levels lead to persistent changes in neurotransmitter release (4, 30, 43, 44) is not universally accepted. Thus other reports have found synaptic scaling at glutamatergic synapses in the cortex to be an exclusive function of postsynaptic changes in neurotransmitter sensitivity (46, 48). Further studies are clearly needed to address a possible contribution of changes in release probability and structural presynaptic remodeling in homeostatic plasticity.

Structural presynaptic alterations have also been implicated in synapse-specific long-term plasticity. A rapid and sustained increase in the number of synaptic vesicle clusters, for example, has been reported following the induction of NMDA receptor-dependent LTP by application of glutamate to hippocampal neurons in culture (2). These new synaptic vesicle clusters were co-localized with GluR1-positive postsynaptic specializations. However, it was unclear from this study whether the appearance of new synaptic vesicle clusters corresponded to the de novo formation of synapses as a consequence of LTP induction or whether they represented the “unsilencing” of preexisting presynaptic varicosities lacking synaptic vesicles. Evidence for the latter possibility has come from a recent study in Aplysia sensory neurons (21) describing the existence of presynaptic varicosities lacking markers for synaptic vesicles and consequently not exhibiting any synaptic vesicle recycling. Induction of long-term facilitation led to two distinct types of presynaptic structural and functional changes with different time courses. In the short term (within 3—6 h of stimulation), a dynamic redistribution of synaptic vesicles enabled the functional activation of the preexisting but silent varicosities. In the long term (after 24 h), the total number of sensory neuron varicosities significantly increased, suggesting the de novo formation of synapses.

To provide for a mechanism that can account for rapid changes in synaptic efficacy as seen during synapse-specific plasticity, structural presynaptic alterations would have to occur rapidly on a minute time scale. Recent time-lapse imaging studies have suggested that this is indeed the case. Packets of synaptic vesicles are rapidly delivered to synapses in developing (1) and mature neurons (9, 24). Many of the synaptic vesicle packages undergoing fast axonal transport actually constitute recycled vesicles from nearby synapses (9, 24). This demonstrates that recycling vesicles can be shared between synapses and suggests a mechanism by which pools of recycling vesicles at individual synapses can undergo rapid, dynamic adjustments. Remodeling of the presynaptic actin cytoskeleton, which is modulated by neuronal activity (8, 37), may be instrumental in regulating these processes (9, 29). Likewise, components of the active zone cytomatrix are transported in preassembled packages (52), allowing for active zone assembly in a rapid, quantal fashion (40). Some evidence from our laboratory’s work supports the existence of highly motile, yet fully functional, modules of preassembled synaptic vesicles and active zones without postsynaptic apposition (24). Dynamic exchange of these “orphan” release site modules between synapses could provide a rapid and sustained mechanism for the dynamic modulation of the size of their active zone and synaptic vesicle pools, allowing for immediate and persistent changes of neurotransmitter release, as observed following the induction of synapse-specific plasticity.

An Active Role of Presynaptic Elements in Activity-Induced Synaptogenesis

An expression mechanism of input-specific plasticity that has received considerable interest in the past years is activity-induced synapse formation. Although initial synaptogenesis during development has been shown to proceed in the absence of neurotransmission (47), repetitive neuronal activity can likely lead to the formation of new synapses during later development and in the mature nervous system. In seminal studies, stimulation protocols that induce LTP have been shown to enhance outgrowth of filopodial protrusions from dendrites of CA1 pyramidal neurons in the hippocampus (15, 27). These filopodia frequently develop into dendritic spines (53), the postsynaptic structures receiving most of the excitatory input in these neurons, and are thus thought to contribute to the formation of new glutamatergic synapses. Since then, the finding that spinogenesis is augmented by neuronal activity and, in many brain areas, continues to occur in mature neurons has been replicated in vitro and in vivo (for references, see Refs. 49, 50).

The view of activity-induced spinogenesis has been a mainly postsynaptic one in which presynaptic specializations are merely passive recipients in the axodendritic contact formation leading up to the generation of new synapses. However, presynaptic elements likely play a far more active role in activity-dependent synapse formation (FIGURE 2). First and foremost, presynaptic specializations themselves are dynamic and appear to be continuously added and eliminated throughout the lifetime of a neuron. In the mature neocortex in vivo, for example, filopodia and short axonal branches are frequently extended and retracted to form terminaux boutons onto postsynaptic structures, and en-passant boutons are added and eliminated along the axonal shaft (11, 41). Overall, presynaptic structures are less frequently subject to turnover than postsynaptic spines (12, 26). However, the ability of axons to add and eliminate presynaptic specializations varies widely among neuronal cell types, and some afferents, such as those from layer 6 neurons projecting to layer 1 in the mature somatosensory cortex, show a
degree of structural plasticity far exceeding that of postsynaptic spines (11). Another argument in favor of an active role of presynaptic elements in activity-dependent synaptogenesis comes from several in vitro studies suggesting that presynaptic structural alterations are subject to regulation by neuronal activity. Thus tetanic stimulation leads to an enhancement in the formation of en-passant boutons in axons of hippocampal neurons in dissociated culture (8) and in the generation of axonal filopodia and en-passant boutons in Schaffer collateral and mossy fiber axons in hippocampal slice cultures (10, 32). The activity-dependent regulation of axonal dynamics is mediated through glutamate receptors and calcium signaling pathways (8, 10, 32, 42). Finally, filopodia and axon branches generated spontaneously or in response to high-frequency stimulation have been shown to engage in the formation of synaptic contacts onto dendritic shafts and spines (11, 32). In summary, the available experimental evidence indicates that activity-dependent synaptogenesis can be initiated either through post- or presynaptic structural modifications, depending on the type of the synaptically connected neurons. Even in instances where synapse formation is initiated by dendritic filopodia, neurotransmitter released from presynaptic structures likely play an instructive role in triggering the initial outgrowth of the dendritic filopodium in a highly localized fashion, directing its growth to the presynaptic target (35), and attenuating spine mobility after synapse formation (16, 23). Together, these mechanisms may provide for input specificity during activity-induced synaptogenesis initiated through spinogenesis.

Bridging the Synaptic Cleft

Two major roles for presynaptic mechanisms in synaptic plasticity have emerged. First, sustained changes in neurotransmitter release, through mechanisms yet to be fully determined, contribute to LTP at many synapses. Second, presynaptic specializations can initiate and direct activity-dependent synaptogenesis. It appears likely that long-lasting, use-dependent synaptic plasticity, both input-specific and homeostatic, will employ a number of pre- and postsynaptic expression mechanisms, varying with type, developmental and use-dependent state of the synapse, and eliciting stimulus. The challenge in the future is to determine which pre- and postsynaptic mechanisms are utilized under which circumstances, how they are integrated and balanced, and how they serve specialized functions in neuronal computation.

References


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