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Short-term forms of presynaptic plasticity

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Synapses exhibit several forms of short-term plasticity that play a multitude of computational roles. Short-term depression suppresses neurotransmitter release for hundreds of milliseconds to tens of seconds; facilitation and post-tetanic potentiation lead to synaptic enhancement lasting hundreds of milliseconds to minutes. Recent advances have provided insight into the mechanisms underlying these forms of plasticity. Vesicle depletion, as well as inactivation of both release sites and calcium channels, contribute to synaptic depression. Mechanisms of short-term enhancement include calcium channel facilitation, local depletion of calcium buffers, increases in the probability of release downstream of calcium influx, altered vesicle pool properties, and increases in quantal size. Moreover, there is a growing appreciation of the heterogeneity of vesicles and release sites and how they can contribute to use-dependent plasticity.

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Introduction

A ubiquitous property of synapses is the ability to keep track of the history of activity. This history is encoded in various forms of activity-dependent plasticity that shape synaptic output and may form the basis of learning and memory. Short-term plasticity lasts from tens of milliseconds to several minutes and is thought to underlie information processing. It can lead to bidirectional changes in synaptic strength, which can be reduced for hundreds of milliseconds to seconds (depression), or it can be enhanced for hundreds of milliseconds to seconds (facilitation), to tens of seconds to minutes (augmentation and post-tetanic potentiation, PTP). Net plasticity at synapses reflects an interaction between multiple forms of plasticity. Here we will discuss recent advances in clarifying the mechanisms underlying these different forms of plasticity.

Synaptic depression

At many synapses, repeated stimuli delivered at short time intervals lead to a transient decrease in synaptic strength. Here, we will focus on presynaptic mechanisms that contribute to a decrease in neurotransmitter release [1]. Several factors can account for reduced release, including but not limited to vesicle depletion, inactivation of release sites, and decreased presynaptic calcium influx (Figure 1a).

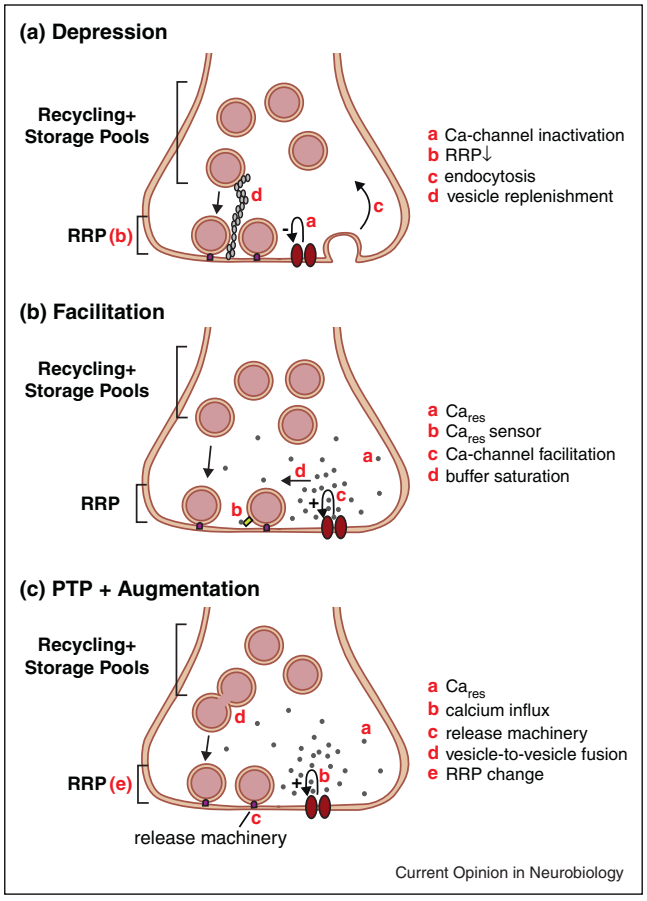
Depletion of the readily releasable pool

There are typically hundreds of vesicles associated with one active zone, but usually fewer than 5% of these vesicles are readily released with repeated stimulation [2]. The number of vesicles released by an action potential depends on the size of this readily releasable pool (RRP) of vesicles, and on the probability of release of these vesicles. Because the number of vesicles in the RRP is limiting, if an action potential releases a large fraction of the RRP, subsequent stimuli delivered before RRP replenishment will release fewer vesicles [1]. This model predicts that depression will increase when the initial release probability and the frequency of activation are increased. These predictions hold true for many synapses such as corticothalamic synapses and synapses in the auditory brainstem [1,3–5]. Recovery from depression occurs within several seconds as vesicles from a recycling pool of vesicles replenish the RRP. Recovery can be significantly accelerated by elevations of presynaptic calcium in a calmodulin-dependent manner [6–11].

Inactivation of release sites

According to a second model of synaptic depression, fusion of a vesicle at a release site can inhibit subsequent fusion events at that site even if the RRP is not depleted [12•,13]. This proposed site inactivation lasts for seconds following exocytosis and could reflect the time it takes to clear vesicular membrane proteins, which get incorporated into the plasma membrane upon vesicle fusion, from the release site [12•]. A recent study suggests a surprising role for endocytosis in limiting the extent of depression by allowing sites to recover from such inactivation. Blocking endocytosis presynaptically reduces the recruitment of readily releasable vesicles and leads to more pronounced depression during trains [14••]. These findings are consistent with endocytosis clearing vesicular membrane proteins from the plasma membrane where they interfere with release, thereby allowing sites to recover from inactivation more rapidly

Figure 1



Presynaptic mechanisms of use-dependent short-term plasticity. Schematic diagrams illustrate proposed mechanisms for depression **(a)**, facilitation **(b)**, and post-tetanic potentiation (PTP) and augmentation **(c)**. RRP: readily releasable pool of vesicles; Ca_{res}: residual calcium.

than if these proteins were removed by diffusion within the membrane.

Reduction in calcium influx

At many synapses including some neocortical synapses, axo-axonic synapses of the Mauthner neuron in the goldfish, and vestibular afferent synapses, the properties of depression are inconsistent with RRP depletion [15–17]. Activity-dependent decreases in calcium influx could account for depression at these synapses. Because of the steep dependence of neurotransmitter release on calcium [12•], even small activity-dependent changes in calcium entry can lead to significant presynaptic plasticity. At the calyx of Held, a synapse in the auditory brainstem, calcium-dependent decreases in calcium influx contribute to synaptic depression [18,19]. Calcium-sensing proteins (CaS), including calmodulin, calcium binding protein 1 (CaBP1), and neuronal calcium sensor 1 (NCS-1), interact with calcium channels and bidirectionally modulate their function [20•]. A recent study in cultures of superior cervical ganglion neurons provides compelling evidence that calcium-dependent inactivation of calcium channels can contribute to synaptic depression: Deleting the calmodulin-binding domain on P-type calcium channels to prevent their inactivation reduces synaptic depression [21••]. Among various synapses, the frequency dependencies of calcium channel inactivation and vesicle depletion are different [4,20•,22], and this could explain differences in the relative contributions of each mechanism for specific experimental conditions [21••].

Molecular determinants of depression and recovery from depression

Pharmacological or genetic manipulation of many proteins can influence depression [1,23–25]. This is not surprising considering that the initial probability of release, presynaptic calcium signaling, endocytosis, the size of vesicle pools, and replenishment of these pools can all influence depression and recovery from depression [4,13,26,27]. Consequently it is often difficult to interpret a change in the extent of depression. This is illustrated by considering the dramatic alleviation of depression when RIM proteins are eliminated [28]. This reduction in depression arises from a decrease in the probability of release [28,29], which is set by RIM via its functions in priming vesicles for release and localizing calcium channels to the active zone [30–32]. Some synapses have molecular specializations that limit the extent of depression. For example, at the cerebellar mossy fiber-to-granule cell synapse genetic deletion of Bassoon, a protein of the active zone, results in more pronounced synaptic depression. Additional results suggest that Bassoon reduces synaptic depression by aiding vesicle replenishment at release sites [33•].

Facilitation

For most synapses with a low initial probability of release, repeated stimulation at short time intervals leads to a transient increase in transmitter release probability [34]. This short-lived synaptic facilitation depends on presynaptic calcium. Several mechanisms have been proposed to account for facilitation (Figure 1b).

Residual calcium

One proposed mechanism for facilitation involves residual calcium (Ca_{res}) that persists in the presynaptic terminal following synaptic activation [1]. At the calyx of Held, linear summation of Ca_{res} (hundreds of nanomolar) with the high local calcium levels at a release site evoked by an action potential (Ca_{local} of tens to hundreds of micromolar) will not lead to sufficient enhancement of synaptic transmission [35]. It has therefore been hypothesized that Ca_{res} increases the probability of release by binding to a sensor distinct from synaptotagmin, the sensor for synchronous release, and activating a site distinct from the low affinity sites on synaptotagmin that are

responsible for vesicle fusion [36,37]. At present no such calcium sensor has been identified.

Saturation of endogenous calcium buffers

Another potential mechanism for facilitation involves calcium-binding proteins within presynaptic terminals that normally intercept calcium ions between calcium channels and release sites, thus reducing the initial probability of release [38,39]. If the first stimulus leads to calcium occupying some of these calcium-binding proteins, then more calcium will reach the release site in response to the second stimulus, and the probability of release will be elevated. This mechanism of facilitation has been demonstrated at some neocortical synapses that contain a high concentration of the calcium binding protein calbindin D-28k [40].

Facilitation of calcium currents

An increase in presynaptic calcium influx could increase the probability of release and contribute to facilitation. It has been known for some time that calcium currents can be enhanced in a use-dependent manner [41,42]. Moreover, calcium-sensitive proteins such as calmodulin have previously been implicated in use-dependent increases in presynaptic calcium entry [20•]. A crucial link among these two sets of observations and facilitation was made when it was found that mutating P-type calcium channels to prevent calcium-dependent facilitation of calcium currents also suppressed synaptic facilitation [21••].

Augmentation and post-tetanic potentiation

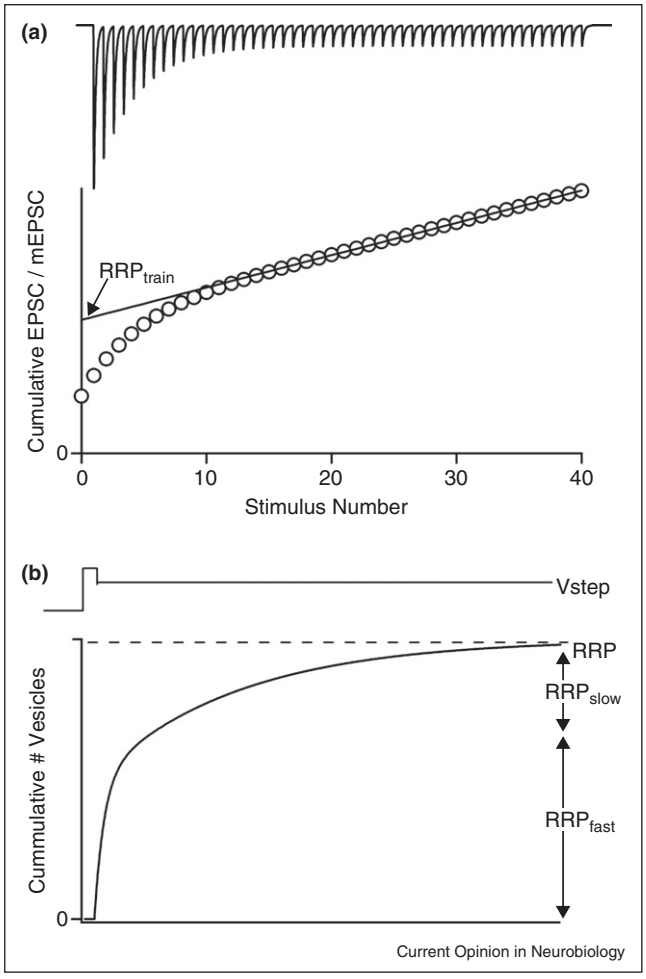
Augmentation and PTP are two closely related forms of enhancement that are observed following sustained, high-frequency synaptic activation [1]. PTP lasts for tens of seconds to minutes, and becomes longer lasting when the stimulus frequency and duration are increased. Augmentation is induced with less prolonged stimulation and lasts for 5–10 s. Different synapses exhibit considerable differences in the frequency and number of stimuli needed to induce augmentation and PTP, and the distinction between the two phenomena is not always clear [1].

Numerous mechanisms have been implicated in PTP (Figure 1c). PTP is accompanied by a decrease in paired-pulse plasticity, suggesting an increase in the probability of release. This increase may result from either an increase in presynaptic calcium entry or changes in the release machinery itself. At the calyx of Held synapse, tetanus-induced increases in action potential-evoked calcium entry could contribute to PTP [43,44], but at superior cervical ganglion cell synapses calcium-induced enhancement of calcium channels does not contribute significantly to PTP even though it accounts for approximately half of augmentation [21••]. The probability of release can also be altered independently of changes in presynaptic calcium entry. For example, protein kinase C (PKC), which has been implicated in PTP [44–46], can decrease the calcium cooperativity such that the same calcium signal can evoke the release of more synaptic vesicles [47•]. Tetanus-induced alterations in the properties of the RRP can also contribute to PTP. At the calyx of Held synapse, it is thought that activation of myosin light chain kinase (MLCK) can produce alterations in the RRP that can account for about 20% of PTP [48]. Tetanic stimulation can also increase the size of miniature synaptic currents that can contribute to PTP [1]. At the calyx of Held synapse, tetanic stimulation can cause some of the vesicles to fuse with each other before fusion with the plasma membrane, and thereby increase the size of miniature synaptic currents [49••].

Calcium signals within the presynaptic bouton play a central role in all of the proposed mechanisms mediating PTP. At the calyceal synapse, tetanic stimulation elevates Ca_{res} to several hundred nanomolar, and this Ca_{res} decays with a time course similar to PTP [44,50], suggesting that the time course of Ca_{res} may dictate the time course of PTP. At the hippocampal synapses, Ca_{res} decays more rapidly than PTP [45,51], suggesting that Ca_{res} activates biochemical cascades with slower kinetics that regulate the duration of PTP. In addition to PKC, possible targets of Ca_{res} include Munc13 [52], calmodulin/CaM kinase II [53] and its downstream effector protein synapsin [24], and the calcium-activated protease calpain [54]. Pharmacological studies support a role for PKC [44,45,55,56], but these studies have been called into question [48] and molecular genetic evidence is unavailable, in part because there are many PKC isoforms [57]. Munc13 proteins, which are required for synaptic transmission, are essential for vesicle priming and can regulate short-term synaptic plasticity [58•,59]. There is still considerable uncertainty about the relative contributions of PKC, Munc13, and other calcium-sensitive proteins to PTP, and it has even been suggested that related forms of synaptic enhancement require both Munc13 and PKC, as well as its downstream target Munc18 [47•,60].

Studies of the contribution of vesicle pool size to PTP at the calyx of Held synapse highlight some of the challenges in interpreting data and drawing mechanistic conclusions. Tetanic stimulation can increase the size of the vesicle pool that is released by a high-frequency train (RRP_{train}, Figure 2a) [48], but paradoxically there is little change in the overall size of the RRP determined by large prolonged presynaptic voltage steps [61•] (Figure 2b). Similarly, PKC activators can produce large increases in RRP_{train} whereas they only produce small increases in the RRP assayed by voltage steps [47•]. The differences in the RRP_{train} and RRP can be explained by non-uniformity in the vesicles that make up the RRP [62,63]. Vesicles that are readily released by action potentials are thought to be near voltage-gated calcium channels, whereas it is difficult for action potentials to liberate vesicles that are far from voltage-gated calcium channels [12•]. In contrast,

Figure 2



Two common approaches of assessing vesicle pools relevant to understanding the mechanisms of short-term plasticity. One approach to assessing the properties of vesicles is to stimulate synapses at high frequencies under conditions where desensitization and saturation of postsynaptic receptors are blocked **(a, top)**. (a, bottom) The amplitudes of the synaptic currents evoked by each stimulus are then measured, and a graph is made of the cumulative excitatory postsynaptic current (EPSC). When these values are divided by the amplitude of miniature EPSCs (mEPSCs) they represent the cumulative number of vesicles. The readily releasable pool liberated by the stimulus train (RRP_{train}) is then determined by fitting over a linear region of this curve and extrapolating back to zero. **(b)** Another approach is to provide a prolonged voltage step that opens presynaptic calcium channels for a long time. The resulting postsynaptic currents then provide a measure of the readily releasable pool (again using the mEPSC size to convert from current to number of vesicles). The total number of vesicles liberated is the readily releasable pool (RRP), which consists of a fast component (RRP_{fast}) and a slow component (RRP_{slow}). As discussed in the text, synaptic plasticity can affect RRP_{train} without influencing RRP. Understanding RRP_{train}, RRP_{fast}, RRP_{slow}, and RRP has important implications for determining the mechanisms underlying short-term plasticity.

vesicles both near and far from voltage-gated calcium channels contribute to the RRP that is determined using protocols that lead to large and prolonged calcium increases [62,64]. Such non-uniformity in vesicles complicates the interpretation of short-term plasticity. For example, an increase in the calcium sensitivity of vesicles might increase RRP_{train} by making some distant vesicles responsive to action potentials.

Conclusions

In the past decade significant advances have been made in clarifying the mechanisms responsible for short-term plasticity. Depletion of readily releasable vesicles, inactivation of release sites, and inactivation of presynaptic calcium channels can all contribute to synaptic depression. Local saturation of calcium buffers, facilitation of presynaptic calcium channels, and Ca_{res}-dependent processes can lead to synaptic facilitation. Increased quantal size, Ca_{res}-dependent increases in the probability of release, facilitation of calcium channels, and alterations in vesicles have all been implicated in PTP. But there are many unresolved questions. Why do some release sites inactivate whereas other do not? Although much is known about the molecular mechanism of calcium channel regulation, much less is known about other mechanisms. Are there specialized calcium sensors that can respond to Ca_{res} to produce facilitation, and if so what are they and how do they work? What are the molecular mechanisms that allow Ca_{res} to produce PTP? How does the heterogeneity of vesicles and release sites influence short-term plasticity? Our current view of synaptic transmission and short-term plasticity is based to a large extent on the calyx of Held, but to what extent can the properties of this synapse be generalized to others? Thus, despite recent progress in the field, many questions remain to be addressed.

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Previous studies showed that tetanic stimulation increases RRP_{train} (Figure 2a), suggesting that pool size increases contribute to PTP. Previously it had not been determined if RRP increases (as in Figure 2b) contribute to PTP, because PTP is not observed during whole cell recordings from presynaptic boutons. Lee *et al.* found that PTP and changes in RRP_{train} were intact when calmodulin was included in the recording pipette. They found that tetanic stimulation did not increase overall RRP size but it did shift the balance between the two components of the RRP, so that vesicles from the slow pool are converted to fast-releasing vesicles. This mechanism, which depends on calmodulin and myosin light chain kinase, can account for 20% of PTP previously observed by this group under control conditions.

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