Calcium- and activity-dependent synaptic plasticity
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Calcium ions play crucial signaling roles in many forms of activity-dependent synaptic plasticity. Recent presynaptic \([\text{Ca}^{2+}]_{\text{P}}\), measurements and manipulation of presynaptic exogenous buffers reveal roles for residual \([\text{Ca}^{2+}]_{\text{P}}\), following conditioning stimulation in all phases of short-term synaptic enhancement. Pharmacological manipulations implicate mitochondria in post-tetanic potentiation. New evidence supports an influence of \(\text{Ca}^{2+}\) in replacing depleted vesicles after synaptic depression. In addition, high-resolution measurements of \([\text{Ca}^{2+}]_{\text{P}}\), showed dendritic spines show how \(\text{Ca}^{2+}\) can encode the precise relative timing of presynaptic input and postsynaptic activity and generate long-term synaptic modifications of opposite polarity.

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Abbreviations
AMPA \(\alpha\)-amino-3-methyl-5-isoxazolepropionic acid
AP action potential
BAPTA 1,2-bis(2-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid
\([\text{Ca}^{2+}]_{\text{P}}\) intracellular concentration of \(\text{Ca}^{2+}\)
CaMKII \(\text{Ca}^{2+}\)/calmodulin-dependent protein kinase type II
EGTA ethyleneglycol-bis-(\(\beta\)-amino-ethyl)-tetraacetic acid
EPSP excitatory PSP
GTP guanosine triphosphate
IP_{3} inositol 1,4,5-trisphosphate
K_{D} dissociation constant
LTD long-term depression
LTP long-term potentiation
mGluR metabotropic glutamate receptor
NMDA \(N\)-methyl-D-aspartate
NMJ neuromuscular junction
NT-3 neurotrophin-3
PKC protein kinase C
PSP postsynaptic potential
PTP post-tetanic potentiation

Introduction
Long-term synaptic plasticity has received a great deal of attention, because of its probable role both in memory and learning and in forming and selecting connections in the developing nervous system. Recently, short-term plasticity has also become the focus of much research, probably in recognition of its essential roles in shaping the information-processing characteristics of neurons. \(\text{Ca}^{2+}\) is involved in the induction of, or recovery from, all forms of short- and long-term synaptic plasticity. This review will focus on the roles played by \(\text{Ca}^{2+}\) in activity-dependent synaptic plasticity and will emphasize results reported in the past two years. Several recent reviews of synaptic plasticity may be consulted for summaries of work published before this period [1–4].

Short-term synaptic enhancement
During repetitive presynaptic activity, successive post-synaptic potentials (PSPs) often increase in three phases — with time constants of less than one second (facilitation), several seconds (augmentation), and a few minutes (potentiation) — and the effects decay after activity with the same three phases. The late decay of potentiation after a tetanus — after the other phases and any concurrent depression have worn off — is called post-tetanic potentiation (PTP). All phases of enhancement are, with few exceptions, attributable to a presynaptic increase in the number of transmitter quanta released, and all phases require \(\text{Ca}^{2+}\) entry during the conditioning stimulation for their induction.

In most instances, enhanced release occurs in response to unaltered presynaptic \(\text{Ca}^{2+}\) influxes and \([\text{Ca}^{2+}]_{\text{P}}\), transients per action potential (AP) [5,6]. Originally, it was thought that residual \(\text{Ca}^{2+}\) left over from prior activity simply added to the increments in \([\text{Ca}^{2+}]_{\text{P}}\), at active zones — the clusters of presynaptic vesicles near \(\text{Ca}^{2+}\) channels where transmitter release takes place — to yield higher peaks of \([\text{Ca}^{2+}]_{\text{P}}\), to conditioned APs, and therefore more transmitter release. However, the measured levels of residual \([\text{Ca}^{2+}]_{\text{P}}\), after stimulation, during phases of facilitation, augmentation, or PTP, are often 1 \(\mu\text{M}\) or less, whereas the peak \([\text{Ca}^{2+}]_{\text{P}}\), triggering release of docked vesicles to a single AP is on the order of 100 \(\mu\text{M}\). It is difficult to see how this minute increase in peak \([\text{Ca}^{2+}]_{\text{P}}\), acting on the low-affinity \(\text{Ca}^{2+}\)-binding site(s) triggering exocytosis can enhance transmission by 10-fold or more. Thus, it is assumed that \(\text{Ca}^{2+}\) acts at targets other than the secretory trigger to enhance release.

How does \(\text{Ca}^{2+}\) activate synaptic enhancement?
Which \(\text{Ca}^{2+}\) triggers enhancement? Is it bound \(\text{Ca}^{2+}\) that enters during an AP and acts on a site to trigger processes with their own slow kinetics (a fraction of a second, several seconds, and a few minutes)? Or is it the residual \(\text{Ca}^{2+}\) left over from previous APs continuing to act in rapid equilibrium with its targets for as long as it is present?

Bound \(\text{Ca}^{2+}\), or a residual effect of local high \([\text{Ca}^{2+}]_{\text{P}}\), transients, may cause enhancement
Studies showing that presynaptic introduction of the \(\text{Ca}^{2+}\) chelators EGTA or BAPTA does not always block synaptic enhancement [5,7,8] support the view that enhancement results from the slow unbinding kinetics of \(\text{Ca}^{2+}\) from its targets [9–11] or slow secondary reactions that outlast the action of \(\text{Ca}^{2+}\). But in these experiments, an exogenous high-affinity buffer can introduce an artificial local buffer saturation and an abnormal growth of local \([\text{Ca}^{2+}]_{\text{P}}\) transients [12], which could substitute for the normal processes of enhancement.
Fast acting residual Ca\(^{2+}\) may cause enhancement

Studies showing that the various phases of synaptic enhancement correspond to phases in the decay of residual [Ca\(^{2+}\)], — as shown originally at the crayfish neuromuscular junction (NMJ) [13,14] and more recently at cerebellar, autonomic, and retinocortical synapses [15–20] — support the view that residual Ca\(^{2+}\) underlies enhancement. Moreover, we [21] found that rapid reduction of residual [Ca\(^{2+}\)], by flash photolysis of a photolabile Ca\(^{2+}\) buffer during decay of synaptic enhancement results in erasure of that process within a few milliseconds (facilitation) or a few hundred milliseconds (augmentation or PTP). In Aplysia central neurons, similar experiments by Fischer et al. [22] show that all phases of enhanced release are dependent on the continuing action of residual Ca\(^{2+}\). Consistent with a role for residual Ca\(^{2+}\), many early studies and several recent ones [5,14,17,23,24] show that presynaptic introduction of slowly equilibrating Ca\(^{2+}\) chelators reduces synaptic enhancement but not unconditioned release. Transfection of hippocampal pyramidal neurons with the Ca\(^{2+}\)-binding protein calbindin also reduces PTP [25]. In their elegant experiments on cerebellar synapses, Aturiri and Regehr [16] found that presynaptic EGTA reduces facilitation substantially and that what is left decays unusually quickly (i.e. in about 40 ms). They propose that a moderately fast process normally tracks the rise and fall of residual [Ca\(^{2+}\)] and that it is only modestly activated by the phasic rise in [Ca\(^{2+}\)] accompanying APs.

One problem with the rapidly equilibrating high-affinity target implicated in facilitation is that it would be saturated within the [Ca\(^{2+}\)] microdomains accompanying APs and then all PSPs would be fully facilitated. However, using simulations, my colleagues and I (Y Tang, T Schlumberger, RS Zucker, Soc Neurosci Abstr 1998, 24:S68) have shown that moving the facilitation target 50–100 nm from the nearest Ca\(^{2+}\) channel, perhaps hiding it behind part of the vesicle docking complex, shields it from local saturating [Ca\(^{2+}\)] and enables facilitation to accumulate in a train.

A variant of the residual Ca\(^{2+}\) hypothesis requires saturation of the facilitation target. The idea is that the facilitation target is a high-affinity and easily saturated cytoplasmic Ca\(^{2+}\) buffer that normally acts to blunt the peak Ca\(^{2+}\) transients to unconditioned stimuli [26]. Perfusion of chromaffin cells with exogenous saturable buffer sometimes results in enhanced facilitation of secretory responses. Computer simulations [26] suggest this arises from both enhancement of [Ca\(^{2+}\)]; transients and summation of residual and transient Ca\(^{2+}\) at vesicles that are not docked near Ca\(^{2+}\) channels, which provide most of the Ca\(^{2+}\) release in chromaffin cells, unlike in neurons [12]. Additional simulations [27] indicate that saturation of a rapid high-affinity (K\(_D\) of 100 nM) Ca\(^{2+}\) buffer by submicromolar [Ca\(^{2+}\)] levels could amplify peak [Ca\(^{2+}\)]; transients several-fold at vesicles docked near Ca\(^{2+}\) channels, as in neuronal synapses. But careful measurements of Ca\(^{2+}\) buffering characteristics of presynaptic axoplasm [6], and of the cytoplasm of excitable cells [28], reveal no such highly saturable buffer.

Ca\(^{2+}\) dependence of enhanced phasic and delayed release

What is the relationship between synaptic enhancement and residual [Ca\(^{2+}\)]? Recent experiments [29,30] indicate that facilitation of release to constant test pulses depends linearly on the Ca\(^{2+}\) influx in conditioning pulses of variable magnitude (presumably reflecting the dependence of facilitation on residual [Ca\(^{2+}\)]. This is in contrast to the highly nonlinear dependence of evoked release on the Ca\(^{2+}\) influx during the pulse (reflecting the dependence of the secretory trigger on [Ca\(^{2+}\)].)

A temporal correlate of enhanced AP-evoked transmission is an increase in rate of spontaneous release of quanta [31], often termed ‘delayed release’. This release also facilitates to repeated APs and is blocked by presynaptic EGTA [32]. Recent studies suggest that the increase in miniature PSP frequency is triggered by Ca\(^{2+}\) acting at one or more fairly high-affinity (K\(_D\) of about 2 μM) binding sites distinct from the low-affinity secretory trigger [14,33], and this may reflect action at the same sites that condition enhancement of phasic release. However, differences in the kinetics and Ca\(^{2+}\) dependence of facilitation and delayed release [30,33,34] suggest that these processes may not be identical.

Why are there different temporal phases of enhanced release?

The many phases of synaptic enhancement result from the action of Ca\(^{2+}\) on at least two different targets and from the different phases of residual Ca\(^{2+}\) removal, which depend on the pattern of stimulation [13–20]. The two components of facilitation (time constants of tens and hundreds of milliseconds) arise from Ca\(^{2+}\) acting at a site with fast kinetics, and they probably reflect rapid diffusion of recently entered residual Ca\(^{2+}\) away from active zones, followed by fairly swift removal of small Ca\(^{2+}\) loads and diffusion of Ca\(^{2+}\) out of boutons into pre-terminal regions. The longer-lasting augmentation seems to correspond to the slowest phase of Ca\(^{2+}\) extrusion to tetani of moderate duration. It is attributable to Ca\(^{2+}\) acting at both the facilitation site and another target with slower kinetics (about a third of a second) that is unaffected by brief Ca\(^{2+}\) rises in short trains [21]. As Ca\(^{2+}\) loads increase further, the extrusion rate begins to depend nonlinearly on the Ca\(^{2+}\) level, reminiscent of mitochondrial extrusion processes [35]. Even larger Ca\(^{2+}\) loads following long tetani result in a post-tetanic plateau of residual Ca\(^{2+}\) that is attributable to tetanic loading of mitochondria with Ca\(^{2+}\) that slowly leaks out afterwards [20,36**]. Thus, block of mitochondrial Ca\(^{2+}\) fluxes abolishes PTP [36**]. Uptake and release of Ca\(^{2+}\) from the endoplasmic reticulum plays little or no role in presynaptic Ca\(^{2+}\) regulation at some synapses [19,20,36**], whereas at other synapses, both the endoplasmic reticulum and mitochondria regulate presynaptic residual Ca\(^{2+}\) following repetitive stimulation [37,38]. Only one study,
However, has described effects on synaptic plasticity of Ca\(^{2+}\) released from, or sequestered in, these stores [36**].

**How are more vesicles released during synaptic enhancement?**

An increase in quantal content during synaptic enhancement can be attributed to either of two processes: more vesicles become docked at release sites or the probability of releasing docked vesicles increases. At hippocampal synapses, although there are about five docked and readily releasable vesicles at each active zone [39*,40], an active zone only releases one or no vesicles (i.e. zero or one quantum) to an AP, and facilitation increases the probability of release from active zones [41]. Thus, there is an increase in reliability, rather than potency, of release from each synaptic contact. Larger terminals have larger active zones that have more docked vesicles and a higher initial probability of release, but less facilitation [39*,40,42*]. Small active zones display even more facilitation than would be expected given that they start out farther from saturation (which occurs when probability of release equals 1) [39*]. Facilitation takes place whether or not a conditioning stimulus releases a quantum, and it is more likely to take place if the conditioning stimulus is successful [43,44]. Perhaps fluctuations in release probability and facilitation both reflect fluctuations in Ca\(^{2+}\) influx during the conditioning stimulus. These fascinating observations on details of the private lives of active zones do not exclude either an increase in number of docked vesicles per active zone or an increase in release probability per vesicle as the proximate cause of facilitation (Figure 1).

Many studies have reported an increase in the probability parameter \(p\) in binomial models of the statistics of release (see [31] for a review), but this could result from either an increase in release efficiency per docked vesicle or an increase in docking or priming efficiency. Similarly, reported increases in the binomial parameter \(n\) could reflect activation of dormant active zones or their new occupation by docked vesicles. Evidence for an increase in release efficiency comes from a recent study by Stevens and Wesseling [45**], showing no increase in the size of the readily releasable pool (dumped by osmotic shock) during augmentation, but a greater sensitivity of release of that pool to the stimulus. Presynaptic EGTA blocks augmentation and the increased rate of release to osmotic shock. Another indication of increased release efficiency comes from the finding of Vyshedskiy and Lin [46] that facilitation at crayfish NMJs is accompanied by an increase in the early rate of release, or decrease in release latency, with no increase in maximum rate of release. Facilitation at *Aphidial synapses shows a form of metaplasticity, whereby repeated paired stimulation results in a loss of facilitation [24*]. These results might reflect the recruitment of release from previously dormant active zones, as has been suggested to underlie part of the enhancement of release observed when extracellular [Ca\(^{2+}\)] is increased [47].

![Figure 1](https://example.com/figure1.png)

Schematic of presynaptic Ca\(^{2+}\)-dependent processes involved in short-term enhancement of synaptic transmission. (a) Phasic exocytosis to an AP. (b) Increased probability of releasing docked vesicles by residual Ca\(^{2+}\) acting rapidly in facilitation and more slowly in augmentation/PTP. (c) Ca\(^{2+}\)-dependent replenishment of readily releasable store after depletion. (d) Tetanic loading of mitochondria with Ca\(^{2+}\) and (e) subsequent post-tetanic release of mitochondrial Ca\(^{2+}\) ([Ca\(^{2+}\)]\(_{m}\)).

Chromaffin cells exhibit a phase of secretory enhancement [48] that resembles neural augmentation or potentiation and that is attributable to an increase in the number of readily releasable vesicles [49]. As this enhancement follows changes in residual [Ca\(^{2+}\)], much more slowly than in neurons, it may not reflect the same process as neural augmentation or potentiation. Nevertheless, recent simulations [50] of a possible Ca\(^{2+}\)-dependent mobilization of vesicles to docking sites make this a viable hypothesis for synaptic facilitation.

**Molecular Ca\(^{2+}\) targets during short-term synaptic enhancement**

The Holy Grail of synaptic plasticity is the identification of molecular targets of Ca\(^{2+}\) action, but this goal remains discouragingly elusive. Initial attention focused on Ca\(^{2+}\)/calmodulin-dependent protein kinase type II (CaMKII)-dependent phosphorylation of synapsin I as a mechanism for increasing the releasable vesicle pool during PTP, but CaMKII and synapsin I knockout mice retain PTP, as well as facilitation [51]. CaMKII inhibitors also spare synaptic enhancement at crayfish NMJs [21], *Aphidial central synapses* [24*], hippocampal CA1 afferent [21], and hippocampal CA3 association/commissural synapses [23]; however, they reduce facilitation at mossy fiber synapses.
[23] and block potentiation at synapses in the lateral line lobe of an electric fish brain [52]. In addition, presynaptic CaMKII expression can reduce depression and activate facilitation at Drosophila synapses [53]. In chromaffin cells, the slowest phase of secretory enhancement (perhaps analogous to PTP) is blocked by protein kinase C (PKC) inhibitors [49]. Therefore, protein phosphorylation by these Ca<sup>2+</sup>-dependent kinases may mediate enhancement of release in some endocrine and neuronal cells.

A variety of other genetic manipulations have been reported to influence the expression of short-term synaptic enhancement. The Leonardo Drosophila mutant [54], the Rab3A mouse knockout [55], overexpression of frequenin [56], exposure to aminergic and peptide neuromodulators [57], presynaptic injection of GTP-activated Rab3 [58] or of the synaptotagmin peptide blocking Ca<sup>2+</sup>-channel–syntaxin binding [59], and overexpression of synaptotagmin I or II [60] all exhibit enhanced facilitation. Leonardo mutants also display a reduction in PTP [54]. Conversely, ataxin-1 [61] and metabotropic glutamate receptor type 4 (mGluR4) [62] knockouts, and cells exposed to brain-derived neurotrophic factor (BDNF) [63] and neurotrophin-3 (NT-3) [64] all exhibit reduced facilitation. PTP was reduced in cells exposed to NT-3 [64], but increased in the mGluR4 knockouts [62]. Unfortunately, in many instances where there was a change in facilitation, it took place in the presence of an opposite change in unconditioned release. As facilitation is inversely proportional to baseline release, attributing the results to an effect on synaptic enhancement is problematic. Also, reductions in PTP were accompanied by an increase in fatigue of responses to prolonged stimulation, and it is possible these two effects were confounded. Even if the effects on facilitation and PTP are sometimes genuine, it is unclear whether the manipulated factor mediates, or merely modulates, synaptic plasticity.

Other mechanisms
Recently, facilitation of presynaptic Ca<sup>2+</sup> currents has been observed at brainstem synapses (i.e. calyx of Held) that could possibly contribute to synaptic facilitation [65,66]. At pituitary terminals, facilitation is partly attributable to increasing Ca<sup>2+</sup> influx during broadening APs [67]. At hippocampal synapses, a postsynaptic rise in [Ca<sup>2+</sup>]<sub>i</sub>, coming partly from endoplasmic reticulum and acting via calmodulin, CaMKII, and PKC, has been shown to mediate not only the expression of long-term potentiation (LTP), but also a net reduction in synaptic facilitation. But this apparent reduction in facilitation may actually reflect the activation of a countervailing desensitization of postsynaptic AMPA receptors [68,69]. AMPA receptors from cortical neurons have recently been shown to display a use-dependent relief of intracellular polyamine block [70]. It remains to be shown whether this can produce a postsynaptic form of facilitation. A rise in postsynaptic [Ca<sup>2+</sup>]<sub>i</sub> has been reported to play a role in a form of synaptic enhancement called PTP by the authors [71], but its long duration (30 min) suggests that this is a form of short-term potentiation related to LTP.

Short-term depression
At many synapses, transmitter release declines during repeated stimulation, and recovers slowly afterwards. This phase of synaptic depression may occur in isolation or in combination with one or more of the phases of enhancement discussed above. Depression is usually relieved by procedures that reduce initial transmitter release and is often attributed to depletion of a readily releasable vesicle pool.

Ca<sup>2+</sup>-dependent recovery
It has long been thought (see [31]) that Ca<sup>2+</sup> may mobilize vesicles into a releasable pool because of the fact that depression to repeated stimulation is not as great as expected from predictions of depletion models. Only in the past year, however, has evidence been obtained that recovery from depression proceeds more quickly after repetitive stimulation, and that this process requires accumulation of residual Ca<sup>2+</sup> during the conditioning stimulation [72**–74**] (Figure 1). A Ca<sup>2+</sup>-dependent mobilization of reserve vesicles into a readily releasable pool has also been shown to speed recovery from depression in chromaffin cells [75], and a part of this mobilization involves PKC activity.

Other mechanisms
Other Ca<sup>2+</sup>-related processes recently found to contribute to depression include inactivation of presynaptic Ca<sup>2+</sup> channels involved in a slow phase of depression at the calyx of Held [76] and a slow (seconds-long) component of Ca<sup>2+</sup>-dependent postsynaptic desensitization of NMDA receptors in synapses and autapses of cultured hippocampal neurons [77]. Whether these are widespread or isolated phenomena need to be determined. At many synapses [31], including one described recently in Aplysia ganglia [78*], depression takes place without alteration of presynaptic APs or Ca<sup>2+</sup> transients, or changes in postsynaptic receptivity, and residual [Ca<sup>2+</sup>], also seem to play no role.

Long-term plasticity
Ca<sup>2+</sup> requirements for inducing LTP and LTD
The role of a postsynaptic rise in [Ca<sup>2+</sup>]<sub>i</sub>, in inducing LTP and long-term depression (LTD) in vertebrate cortex is well established. A persistent dilemma is how one postsynaptic signal can induce opposite synaptic modifications. The most popular hypothesis is that LTD has a lower Ca<sup>2+</sup> induction threshold than LTP [79–81]. However, photolysis of a small amount of postsynaptic caged Ca<sup>2+</sup> chelator, which should briefly elevate [Ca<sup>2+</sup>]<sub>i</sub>, by less than 1 μM, induces LTD and LTD with equal probability, even in the absence of presynaptic activity [82,83]. Recently, we [84*] have used a more powerful caged Ca<sup>2+</sup> chelator to elevate postsynaptic [Ca<sup>2+</sup>]<sub>i</sub> to higher levels for a few seconds, mimicking the effect of the usual LTP-inducing stimuli [85], or to modest levels for about 1 min, mimicking the effect of typical LTD-inducing stimuli. These manipulations selectively induced only LTP.
Figure 2

Coincidence detection in cortical dendritic spines. Back-propagated APs triggered by large EPSPs, or by other inputs, enhance Ca\(^{2+}\) influx through NMDA receptors by voltage-dependent relief of Mg\(^{2+}\) block. The large \([\text{Ca}^{2+}]_i\) elevation induces LTP. APs preceding EPSPs may induce Ca\(^{2+}\)-dependent NMDA receptor inactivation, and prolonged reduced Ca\(^{2+}\) influx through NMDA receptors induces LTD.

or LTD, respectively, suggesting that the amplitude and duration of \([\text{Ca}^{2+}]_i\); elevation are critical in selecting both the enzymatic machinery that is activated and the synaptic modification that is induced.

Other examples of long-lasting synaptic modifications dependent on postsynaptic \([\text{Ca}^{2+}]_i\); elevations have been described recently. A persistent depression of transmitter release from nerve endings at developing chick NMJs is caused by cholinergic activation of the receptors responding to the depressed neuron. Simultaneous presynaptic activation protects the terminals from depression. This induction of depression was found to be accompanied by, and could be mimicked by, a local postsynaptic elevation of \([\text{Ca}^{2+}]_i\) at the depressed synapses [86]. In addition, the retrograde effect on the neuron spread to synapses onto neighboring myocytes, but could only be initiated by \([\text{Ca}^{2+}]_i\); elevation in a myocyte synaptically coupled to the nerve cell [87]. An electrical component of junctional potentials from goldfish auditory afferents synapsing onto Mauthner cells displays a form of LTP that, like the chemical component, appears to involve a postsynaptic rise in \([\text{Ca}^{2+}]_i\); acting on CaMKII [88].

The source of \(\text{Ca}^{2+}\) in cortical LTP and LTD induction is thought to be mainly via \(\text{Ca}^{2+}\)-permeable NMDA receptors. However, recent experiments indicate that \(\text{Ca}^{2+}\) entering through L-type \(\text{Ca}^{2+}\) channels may be involved in induction of one form of mossy fiber LTP in hippocampal CA3 neurons [89], in LTD induction in CA1 pyramidal cells [90,91], and in LTP induction in adult CA1 cells [92]. LTD of parallel fiber inputs to cerebellar Purkinje neurons requires a postsynaptic \([\text{Ca}^{2+}]_i\); elevation triggered by depolarization normally induced by climbing fiber activation, which apparently involves a \(\text{Ca}^{2+}\)-induced \(\text{Ca}^{2+}\) release from stores regulated by inositol 1,4,5-trisphosphate (IP\(_3\)) [93]. Parallel fiber inputs to Purkinje cells also elicit localized postsynaptic \([\text{Ca}^{2+}]_i\); elevations, which arise, in part, from AMPA-receptor-dependent dendritic depolarizations and, in part, from mGluR-dependent \(\text{Ca}^{2+}\) release from IP\(_3\)-regulated stores [94]. Ca\(^{2+}\) released from IP\(_3\)-regulated stores can also induce a localized form of parallel fiber LTD [95].

**Coincidence detection within dendritic spines**

An important recent advance in understanding synaptic plasticity is the discovery that repeated pairing of postsynaptic APs with afferent stimulation to pyramidal cells can induce LTP or LTD in cortical pyramidal synapses [96,97,98], depending on the precise timing of the stimuli. When LTP is triggered by afferent stimulation 0–200 ms before antidromic stimulation, supralinear summation of dendritic spine \([\text{Ca}^{2+}]_i\); transients with back-propagated AP \([\text{Ca}^{2+}]_i\); transients generates large \(\text{Ca}^{2+}\) signals in dendritic spines. This results from the voltage-dependent unblocking of NMDA receptors and enhanced \(\text{Ca}^{2+}\) entry through these channels [99,100,101,102]. If the order of stimulation is reversed, the AP- and EPSP-associated \([\text{Ca}^{2+}]_i\); transients sum sublinearly, perhaps because of a \(\text{Ca}^{2+}\)-dependent inactivation of NMDA
receptors, and the resulting small Ca\textsuperscript{2+} signal generates LTD (Figure 2). A similar coincidence detector is present at *Xenopus* retinotectal synapses [103]. High-frequency afferent stimulation of inhibitory junctions between Purkinje cells and deep cerebellar nucleus cells induces a form of LTP that also involves back-propagating APs [104\textdagger]. Large postsynaptic [Ca\textsuperscript{2+}] elevations at the latter junctions accompany APs induced by rebound depolarization after inhibition. Postsynaptic hyperpolarization blocks the APs and attenuates the [Ca\textsuperscript{2+}] rise, and then usually LTD results.

**Conclusions**

Calcium ions continue to assume more responsibilities in the regulation of synaptic transmission. Long recognized as triggering phasic exocytosis in response to APs, presynaptic Ca\textsuperscript{2+} remaining after conditioning stimulation is now perceived as acting on at least two additional targets to generate facilitation, augmentation and PTP (see Figure 1). The latter process arises from a post-tetanic plateau of residual [Ca\textsuperscript{2+}], that results from the leakage of mitochondrial Ca\textsuperscript{2+} and subsequent extrusion from cytoplasm. During augmentation of hippocampal synapses, Ca\textsuperscript{2+} increases the probability of release of docked vesicles at active zones, without increasing the size of the readily releasable store. On the contrary, in chromaffin cells, Ca\textsuperscript{2+} recruits additional vesicles into an enlarged readily releasable pool. Many more synapses will have to be investigated before generalizations can be made from these few examples. At depressed synapses, Ca\textsuperscript{2+} also acts to hasten the replenishment of readily releasable vesicles. It is unclear what relationship this process has, if any, to phases of activity-dependent synaptic enhancement. The molecular targets of these Ca\textsuperscript{2+} actions remain largely unknown and identifying them will be a major focus of future research.

Postsynaptic elevation of [Ca\textsuperscript{2+}], triggers a variety of long-lasting synaptic modifications. In addition to LTP and LTD at cortical and cerebellar excitatory synapses, recent studies have revealed similar modifications at cerebellar inhibitory synapses, central electrical synapses, and developing NMJs. At hippocampal synapses, we now know that sufficiently strong inputs, or other temporally associated inputs, can generate postsynaptic APs that boost the Ca\textsuperscript{2+} influx through NMDA receptors into dendritic spines and elevate [Ca\textsuperscript{2+}], to sufficient levels to reliably induce LTD (see Figure 2). On the other hand, repeated EPSPs that follow APs or occur in isolation result in a persistent modest [Ca\textsuperscript{2+}] elevation that reliably induces LTD. The microcomputational possibilities of Ca\textsuperscript{2+} signaling are likely to receive much more attention in the near future.

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**References and recommended reading**

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest


22. Fischer TM, Zucker RS, Carew TJ: Activity-dependent potentiation of synaptic transmission from L30 inhibitory interneurons of *Aplysia* depends on residual presynaptic Ca\textsuperscript{2+} but not on postsynaptic Ca\textsuperscript{2+}. J Neurophysiol 1997, 78:2061-2071.

The caged Ca\textsuperscript{2+} chelator diazo-2 was injected into a presynaptic neuron regulating the siphon withdrawal circuit in *Aplysia*. Reduction of residual Ca\textsuperscript{2+} by photolysis demonstrated its role in the production of facilitation.
amplification and PTP. Postsynaptic chelation of Ca\(^{2+}\) with BAPTA had no effect on synaptic plasticity.


Repeated paired stimulation of sensorimotor synapses caused a persistent decrement in facilitation that occurred independently of long-lasting depression. Neither facilitation nor its decrement was blocked by a variety of protein kinase inhibitors. Facilitation was dependent on residual Ca\(^{2+}\) and was blocked by presynaptic EGTA injection, but not by postsynaptic Ca\(^{2+}\) chelation. The authors suggest that facilitation involves recruitment of previously dormant release sites.


Transmitter release was evoked by paired depolarizing pulses under presynaptic voltage clamp at crayfish NMJ. Variation of conditioning pulse amplitude showed that release during that pulse varied with the third power of integrated Ca\(^{2+}\) current, whereas facilitation expressed by the test pulse varied approximately linearly with the Ca\(^{2+}\) entering during the conditioning pulse. The results are consistent with the classic model of facilitation based on simple summation of residual [Ca\(^{2+}\)], with peak [Ca\(^{2+}\)] transients during APs.


Facilitation was studied with paired depolarizations of the inhibitory motor nerve terminals at crayfish NMJ. As the conditioning stimulus was increased, facilitation of the test pulse increased with the cube root of the increase in transmission to the conditioning pulse, implying an approximately linear dependence of facilitation on residual Ca\(^{2+}\) from the conditioning pulse.


The authors report that inhibitors of mitochondrial Ca\(^{2+}\) transport abolish PTP. Postsynaptic Ca\(^{2+}\) accumulates to a higher level during tetanic stimulation, and transmission increases more than normal, when mitochondrial Ca\(^{2+}\) buffering is blocked. However, the slowly decaying post-tetanic plateau of [Ca\(^{2+}\)]\(_{s}\) and synaptic enhancement disappear, as Ca\(^{2+}\) no longer accumulates in mitochondria during stimulation or leaks out afterwards.


Synapses of CA3 neurons onto CA1 pyramidal cells usually contain a single active zone [40]. A detailed electrophysiological study of the properties of these single synaptic contacts reveals a surprising heterogeneity. The authors report that facilitation is attributable to an increase in release probability that is inversely correlated to the initial release probability and the size of the readily releasable pool. The latter was measured by high-frequency depolarization of the pool. Depression was found to be attributable to a reduced release probability as the pool was depleted.


52. FM1-43, a fluorescent dye, was used to study synaptic transmission as revealed by vesicle recycling at single autaptic contacts in isolated cultured hippocampal neurons. Facilitation was inversely correlated with initial release probability, which, in turn, was correlated to the size of the recyclable pool of vesicles (reserve and immediately releasable stores combined), which was measured by quantifying the amount of FM1-43 endocytosed.


Augmentation was induced by repetitive stimulation in isolated cultured hippocampal cell autapses and synapses between cell pairs. Hypercromic stimuli were used to measure the size of the immediately releasable transmitter pool. The authors found that augmentation was accompanied by an increase in the rate of release by hypertonic shock, without any change in the size of the readily releasable pool. The effect was blocked by intracellular EGTA.


This is the first report to show an accelerated recovery from depression following 300 Hz stimulation for 100 ms or broadening of APs at 200 Hz with tetraethylammonium. Control experiments showed that postsynaptic receptorter desensitization and presynaptic Ca2+ channel inactivation or AP waveform changes were not involved in depression at these calyxes of Held synapses. Reduction of presynaptic Ca2+ influx, or added presynaptic EGTA, blocked the fast phase of recovery, which had a time constant of about 40-85 ms.


74. Levels of Ca2+ in Purkinje cells were low (72 nM), while in cerebellar synapses also show a fast phase of recovery (100 ms time constant) from paired-pulse depression and a slow phase of depression during brief trains (20 Hz for 100 ms) that are enhanced in high extracellular concentrations of Ca2+ but reduced in low concentration medium or by addition of presynaptic EGTA. Cyclodiazidate eliminated possible effects of postsynaptic desensitization.


The readily releasable pool of synaptic vesicles in isolated cultured hippocampal neurons was probed using hyperosmotic stimuli. Electrical stimulation for 1-3 s at 10 Hz could nearly double the rate of refilling this pool, and the effect decayed within 20 s. This procedure used a more direct probe of the releasable pool size that, unlike depletion induced by AP trains, is not confounded by effects of stimulation on probability of release of vesicles in the pool by single APs, which can occur during short-term enhancement of release [45*].


Nitrophenyl-EGTA was used to elevate [Ca2+] in pyramidal cells in CA1 hippocampal slices. A brief intense photolysis elevating [Ca2+] to over 10 μM produced only LTD; LTD was never induced. Repeated modest photolysis elevated [Ca2+], to submicromolar levels for 1 min and induced LTD, but never LTD. Brief modest [Ca2+] elevations could induce either LTD or LTD. Apparently, both the amplitude and duration of the postsynaptic Ca2+ signal are important in determining the nature of the alteration in synaptic transmission.


The first demonstration of bidirectional regulation of synaptic strength dependent on the temporal separation between repeated paired afferent and antidromic stimulation.


Extending the pioneering observations of Yuste and Denk [99], the authors show a correlation between induction of LTP and the supralinear summation of dendritic [Ca2+] transients associated with EPSPs and back-propagating APs when the stimuli are paired.


Using two-photon fluorescence to detect [Ca2+] changes in single spines associated with EPSPs and spikes, the authors observed supralinear and sublinear [Ca2+] summations depending on whether the EPSP preceded the back-propagating AP or vice versa. They found that most of the postsynaptic Ca2+ influx induced by afferent stimulation is through NMDA receptor channels.


Using caged glutamate photolysis to stimulate postsynaptic receptors without presynaptic APs, the authors compared the [Ca2+] transients measured with two-photon fluorescence in response to isolated or paired receptor activation and antidromic APs. They found concurrent AP amplification of Ca2+ entry through NMDA receptor channels, whereas unpaired receptor activation by caged glutamate photolysis admitted Ca2+ mainly through N-, P/Q-, and T-type voltage-dependent Ca2+ channels.


Shows that LTD and LTP of inhibitory synaptic transmission are associated with different levels of postsynaptic [Ca2+] elevation. Afferent stimulation, with and without postsynaptic hyperpolarization, induces small and large degrees of postsynaptic rebound depolarization, which, respectively, admit small amounts of Ca2+ directly to induce LTD or activates antidromic APs to admit large amounts of Ca2+ and induce LTP.