

The Mechanisms and Functions of Synaptic Facilitation

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<http://dx.doi.org/10.1016/j.neuron.2017.02.047>

The ability of the brain to store and process information relies on changing the strength of connections between neurons. Synaptic facilitation is a form of short-term plasticity that enhances synaptic transmission for less than a second. Facilitation is a ubiquitous phenomenon thought to play critical roles in information transfer and neural processing. Yet our understanding of the function of facilitation remains largely theoretical. Here we review proposed roles for facilitation and discuss how recent progress in uncovering the underlying molecular mechanisms could enable experiments that elucidate how facilitation, and short-term plasticity in general, contributes to circuit function and animal behavior.

Introduction

It is well established that synapses are regulated by their past activity on time scales of milliseconds to days and longer. Most attention has focused on long-term changes in synaptic strength. Even before Sherrington coined the term “synapse,” Ramón y Cajal suggested that the brain might store memories by modifying the connections between neurons (Sotelo, 2003). More than 70 years later, this hypothesis gained experimental support with the discovery of long-term potentiation (LTP) of synapses that lasts for hours or much longer. In subsequent years, a growing consensus has been reached regarding the mechanisms that produce LTP (Nicoll, 2017). In contrast, even though short-term synaptic facilitation was discovered long before LTP, clarifying the mechanisms and determining the functions of facilitation has been elusive.

Synaptic transmission was first studied at the frog neuromuscular junction (NMJ), where one of the initial observations was that synaptic responses become much larger when presynaptic cells are activated by closely spaced stimuli (Eccles et al., 1941; Feng, 1940). When pairs of stimuli are used to study this phenomenon, it is termed paired-pulse facilitation (Figures 1A and 1B), and facilitation generally leads to synaptic enhancement for more complex presynaptic activation (Figures 1C and 1D), provided stimuli are separated by tens to hundreds of milliseconds. Facilitation runs counter to the natural tendency of synapses to weaken during repeated activation, a phenomena known as depression that can result from depletion of the readily releasable pool (RRP) of synaptic vesicles and a decrease in neurotransmitter release (Zucker and Regehr, 2002). This suggests that facilitating synapses possess specialized mechanisms that boost neurotransmitter release even as the pool of available synaptic vesicles decreases.

Synaptic facilitation has subsequently been observed throughout the nervous system, yet clarifying the mechanisms and functional roles of facilitation has been remarkably difficult. Recently, however, progress has been made in identifying proteins that mediate synaptic facilitation. This opens up the exciting possibility that manipulating these proteins could finally enable experimental tests of the effects of facilitation on circuit

activity, behavior, and neuropsychiatric diseases. In this Review, we discuss the hypothesized roles of facilitation and highlight the newly discovered mechanisms for facilitation that could allow the first direct tests of the functional importance of any form of short-term plasticity.

Theorized Roles of Facilitation

The NMJ, in addition to being the synapse where facilitation was discovered, also provided early insights into the functional importance of facilitation. The crayfish was a particularly useful model, because crayfish muscles with known roles in behavior are innervated by NMJs with distinct forms of synaptic plasticity (Atwood and Karunanithi, 2002) (Figure 2A). In the crayfish abdomen, a deep layer of “phasic” muscles generates the fast powerful contractions required for swimming and escape responses, while a superficial layer of “tonic” muscles produces sustained and finely graded contractions required for postural control (Atwood, 2008). Phasic NMJs produce large initial responses that generate immediate, powerful contractions, but synaptic responses and muscle contractions both fatigue rapidly with repeated activation (Bruner and Kennedy, 1970; Kennedy and Takeda, 1965a). In contrast, at the onset of activity, tonic NMJs produce small responses and do not elicit contractions, but during sustained activation, these synapses undergo profound frequency-dependent facilitation and produce graded tension (Kennedy and Takeda, 1965b). The use of parallel pathways with different synaptic plasticity appears to be a common strategy for muscle control in crustaceans (Bradacs et al., 1997; Sherman and Atwood, 1972) and mammals, where motor neurons that innervate fast and slow muscles exhibit differing firing properties (Hennig and Lomo, 1985) and short-term plasticity (Reid et al., 1999).

Facilitation Counteracts Depression

Depletion of the RRP becomes increasingly prominent when synapses are activated at high frequencies for prolonged periods, and consequently, many synapses exhibit frequency-dependent depression. At some synapses, however, facilitation counteracts depression by increasing the probability of release (p) in a frequency-dependent manner. This is thought to be the

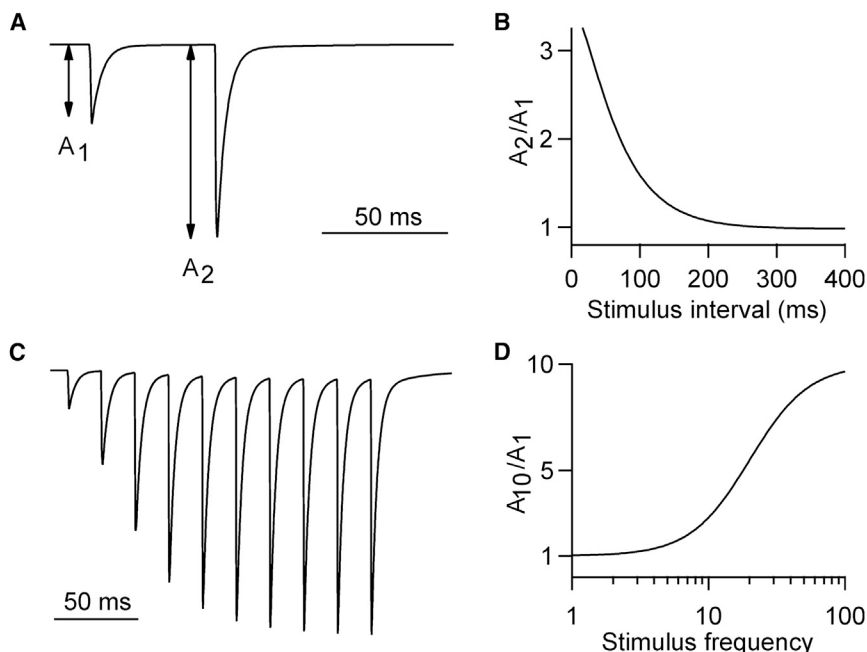


Figure 1. Synaptic Facilitation

(A) Illustrated excitatory postsynaptic currents evoked by a pair of pulses delivered at a 50 ms interval. At many synapses, facilitation increases the amplitude of the second response more than 2-fold.

(B) Typical time course of paired-pulse facilitation. (C) Illustrated facilitation produced by a 50 Hz train of 10 stimuli.

(D) Facilitation as a function of stimulus frequency.

case in the avian auditory system, where synapses that convey intensity information maintain their response amplitudes over a wide range of firing frequencies (MacLeod et al., 2007) and are thereby able to encode the absolute firing rate of auditory inputs (Sullivan and Konishi, 1984; Takahashi et al., 1984). A similar role for facilitation was observed in mice at cerebellar output synapses between Purkinje cells (PCs) and the deep cerebellar nuclei (DCN) (Turecek et al., 2016). Purkinje cells typically fire action potentials continuously at high rates in vivo and can encode information by modulating their firing rates (Heck et al., 2013). At many synapses, depletion is more prominent at high frequencies, and steady-state transmission decreases with firing frequency. But, at PC to DCN synapses, facilitation increases with firing frequency, thereby offsetting depletion. This leads to steady-state synaptic strength that is independent of frequency (Figure 2B).

Temporal Filtering

Synapses preferentially transmit certain patterns of presynaptic activity as a consequence of their short-term plasticity (Abbott and Regehr, 2004). Synapses with a low initial p transmit single action potentials unreliably, often failing to elicit any response in the postsynaptic cell. However, facilitation can raise p dramatically during bursts of activity to allow synapses to strongly influence postsynaptic cells. In this way, facilitating synapses act as high-pass filters (Atluri and Regehr, 1996) (Figure 1D) and play a special role in encoding burst activity that is a common feature of neural circuits (Lisman, 1997). In contrast to facilitating synapses, depressing synapses are low-pass filters that preferentially signal the onset of activity after periods of quiescence (Abbott et al., 1997; Rose and Fortune, 1999). Synapses where facilitation and depression are both present can act as band-pass filters (Dittman et al., 2000). In addition, a single neuron can contact multiple postsynaptic partners using synapses with different

forms of short-term plasticity, suggesting that short-term plasticity is tuned in a target-dependent manner (Bao et al., 2010; Maccaferri et al., 1998; Markram et al., 1998; Reyes et al., 1998; Rozov et al., 2001; Scanziani et al., 1998; Sherman and Atwood, 1972). Short-term plasticity could also be important for the induction of long-term forms of plasticity. For facilitating synapses, increased neurotransmitter release during bursts could account for the observation that presynaptic bursts are more effective than low-frequency firing at inducing long-term potentiation (Dunwiddie and Lynch, 1978; Huerta and Lisman, 1995).

An example of high-pass filtering imposed by facilitation is provided by medial olivocochlear (MOC) efferents that inhibit cochlear outer hair cells (OHCs). OHCs increase acoustic sensitivity by mechanically amplifying vibrations produced by sound (Ashmore, 2008; Brownell et al., 1985). To maintain acoustic sensitivity over a wide dynamic range, and to avoid cochlear damage caused by runaway amplification, MOC afferents increase their firing rate with sound intensity (Brown et al., 1998). MOC synapses have a very low initial p but gradually facilitate in a frequency-dependent manner during sustained firing (Balestero et al., 2011; Goutman et al., 2005). This allows OHCs to ignore spontaneous or low-frequency MOC activity while scaling inhibition of OHCs as a function sound intensity.

Information Transfer at Synapses

Facilitation is widely believed to play an important role in determining how synapses transmit information (Abbott and Regehr, 2004; Deng and Klyachko, 2011). Information theory provides a framework for assessing how reliably information encoded by presynaptic firing is conveyed to postsynaptic neurons (Zador, 1998). Because short-term plasticity regulates the strength of inputs as a function of past activity, dynamic synapses convey information about both the mean firing rate and the temporal history of presynaptic spikes (Fuhrmann et al., 2002). Information theory has been applied to understand the role of synaptic depression in circuits (Abbott et al., 1997; Cook et al., 2003), but the role of facilitation is less clear. In general, facilitating synapses transmit information most effectively at high frequencies (9–70 Hz) (Fuhrmann et al., 2002; Rotman et al., 2011). Facilitation appears to be crucial to information transfer in the hippocampus during high-frequency burst firing, as it has been shown that information transfer remains intact during

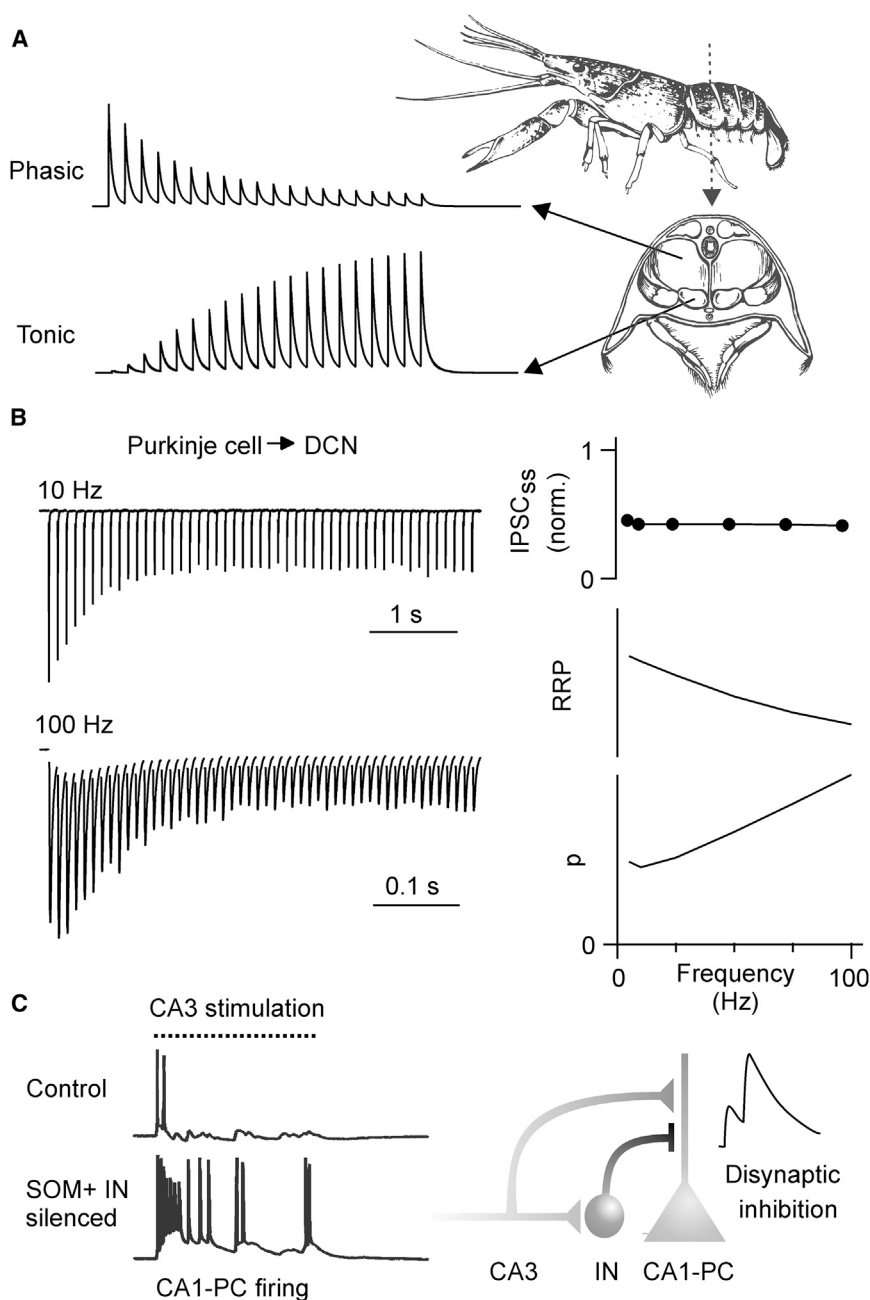


Figure 2. Functional Consequences of Facilitation

(A) Illustration of differential short-term plasticity of NMJs in crayfish abdominal muscles. Phasic NMJs produce large initial responses but depress rapidly. Initial responses from tonic NMJs are small but facilitate markedly as a function of firing frequency.

(B) Left: inhibitory postsynaptic currents (IPSCs) recorded from a cerebellar nuclear neuron evoked by stimulating Purkinje cell axons at different frequencies. Right: average steady-state responses (top) are maintained at high frequencies because depletion of the readily releasable pool of vesicles (RRP, middle) is offset by frequency-dependent facilitation of p (bottom). The sizes of the RRP and p were predicted by a model fit to data. Adapted from Turecek et al. (2016).

(C) Left: prolonged stimulation of Schaffer collaterals normally elicits only a short burst of activity with place field characteristics, but when interneurons are pharmacogenetically silenced, CA3 cells fire for the entire stimulus duration. Adapted from Lovett-Barron et al. (2012). Right: excitatory synapses from CA3 impinge on CA1 pyramidal cells (CA1-PC), as well as on SOM⁺ interneurons (IN) that inhibit CA1-PC dendrites. As CA3 inputs facilitate during the course of stimulation, IN firing gradually increases. This leads to a net facilitation of disynaptic inhibition that allows interneurons to shut down pyramidal cell activity late in the train. Adapted from Bartley and Dobrunz (2015).

tion are important. The interaction between facilitation and feed-forward inhibition may play a fundamental role in encoding spatial information in the hippocampus. Place cells in the CA3 and CA1 regions signal an animal's location within its environment by firing high-frequency spike bursts when the animal enters a given location, known as a place field (O'Keefe and Dostrovsky, 1971). CA3 pyramidal cells send excitatory synapses to CA1 pyramidal neurons and also to local SOM⁺ interneurons that provide feedforward inhibition (Alger and Nicoll, 1982; Losonczy et al., 2002; Lovett-Barron et al., 2012). Thus, CA3 inputs drive a sequence of excitation followed by inhibition (Pouille and Scanziani, 2001). The two inputs

bursts even when synaptotagmin 1 (sytl) is removed and fast synchronous release is replaced by a slow form of facilitating release (Xu et al., 2012). However, information will be lost if facilitation drives the postsynaptic cell to fire at its maximum firing rate. Thus, it has been proposed that circuits match the properties of presynaptic facilitation to the dynamic range of postsynaptic neurons in order to maintain optimal coding (Scott et al., 2012).

Circuit Dynamics

The contributions of short-term plasticity become more complex in neuronal circuits, where both excitation and disynaptic inhibi-

exhibit opposing plasticity: excitation facilitates while the feed-forward inhibition depresses (Bartley and Dobrunz, 2015). In principle, the opposing plasticity of excitation and inhibition would act as a simple high-pass filter. However, CA3 inputs must fire multiple times before they facilitate enough to drive action potentials in the interneurons that inhibit the CA1 cell dendrites and terminate spike bursts (Figure 2C) (Bartley and Dobrunz, 2015; Lovett-Barron et al., 2012; Pouille and Scanziani, 2004; Royer et al., 2012). The dynamic properties of the excitation/inhibition balance allows the circuit to act in a switch-like manner to amplify short bursts of high-frequency activity and selectively transmit

signals with place-field-like characteristics (Klyachko and Stevens, 2006; Moreno et al., 2016).

Facilitation in Working Memory

Facilitation is also proposed to play a critical role in working memory, which involves the short-term storage of information for subsequent manipulation and decision making. Working memory has long been attributed to persistent activity in recurrent networks in areas like the prefrontal cortex (PFC). Early electrophysiological recordings in non-human primates engaged in delayed-response tasks identified cells in the PFC that increase their firing during the delay period but return to baseline activity after a response is executed (Fuster and Alexander, 1971). This elevated firing was initially hypothesized to be the neural basis of working memory (Goldman-Rakic, 1995). However, the idea that working memory is stored by elevated firing has recently been challenged. Many prefrontal cortex neurons do not fire persistently during the delay period but instead exhibit dynamic patterns of activity along with periods of inactivity (Sreenivasan et al., 2014; Stokes, 2015; Stokes et al., 2013). This inspired models of working memory where information is stored not by absolute firing frequency, but by temporarily restructuring functional connectivity (Barak and Tsodyks, 2007; Deco et al., 2010; Mongillo et al., 2008; Sugase-Miyamoto et al., 2008). According to these models, information about the initial stimulus is stored by facilitating synapses between reciprocally connected prefrontal cortex cells. The information can be retrieved by a subsequent sweep of activity through the network (Itskov et al., 2011; Mongillo et al., 2008).

It has been difficult to experimentally test the theorized functions of facilitation described above. To evaluate how facilitation influences neural circuits and behavior, it is desirable to have a means of selectively eliminating facilitation, preferably at a defined subset of synapses. This may become possible in the near future as clarification of the molecular mechanisms of facilitation provides targets for manipulation (Jackman et al., 2016; Nanou et al., 2016). Discovering these mechanisms could finally make it possible to test theories regarding the functional roles of facilitation that will help to clarify how neural circuits perform computations.

Proposed Mechanisms of Facilitation

Facilitation Is an Increase in the Number of Vesicles Released

Facilitation is observed at many synapses with a low initial p , whereas synapses with a high initial p usually exhibit use-dependent depression. This is thought to be a consequence of having a finite number of vesicles in a RRP. If initial p is high and a large fraction of vesicles fuse during the first action potential, the RRP becomes depleted and synaptic responses are depressed until the RRP is replenished. The importance of the initial p was first demonstrated at the NMJ, which is transformed from depressing in high external calcium (Ca_e) when p is high to facilitating in low external calcium when p is low. The dependence of short-term plasticity on the initial p has made the measurement of facilitation a widely used approach to detect changes in the initial p . However, this dependence of facilitation on the initial p makes it difficult to distinguish between an actual change in the mechanism of facilitation and a change in initial p .

The first mechanistic question that was addressed was whether facilitation was a consequence of an increase in the number of vesicles released or an increase in quantal responses (the response to an individual vesicle). Intracellular recordings were used to detect quantal events under conditions where p was reduced by raising external magnesium and lowering external calcium (Del Castillo and Katz, 1954). Paired-pulse facilitation and facilitation during stimulus trains were found to reflect increased vesicle fusion, because there was a decrease in failures (trials in which presynaptic action potentials did not produce a postsynaptic response), an increase in the number of quanta released per stimulus, and quantal size did not change. However, these experiments did not determine whether the increase in vesicle fusion was caused by an increase in p for vesicles in the RRP or an increase in the size of the RRP.

Although facilitation is a presynaptic phenomenon, postsynaptic contributions must also be considered. In rare circumstances, short-term enhancement of synaptic strength can arise from the relief of polyamine block of calcium permeable AMPA receptors (see Rozov and Burnashev, 1999). For many synapses, when p is high, multivesicular release occurs at a single active zone and leads to high occupancy of postsynaptic receptors and a sublinear relationship between the number of vesicles that fuse and the postsynaptic response (Wadiche and Jahr, 2001). This can decrease the amplitude of facilitation because p is higher for facilitated responses (Christie and Jahr, 2006; Foster et al., 2005).

Presynaptic Calcium Signaling and Facilitation

Understanding calcium signaling within presynaptic boutons has been vital to evaluating the role of calcium in facilitation. It is now known that calcium can act on different spatial and temporal domains. When an action potential invades a presynaptic bouton calcium channels open, which leads to a large calcium signal of tens to hundreds of micromolar that lasts as long as the calcium channels are open (<1 ms) (Augustine et al., 1991; Simon and Llinás, 1985; Yamada and Zucker, 1992). Calcium signals drop off significantly with distance from an open channel due to diffusion and capture by calcium buffers (Neher, 1998). If docked vesicles are located in the immediate vicinity of open calcium channels, this large, brief calcium signal (Ca_{local}) activates fast, low-affinity calcium sensors such as synaptotagmin 1. The resulting vesicle fusion is primarily short latency and short lived because Ca_{local} is so brief and synaptotagmin 1 has such rapid kinetics. But calcium also rapidly diffuses and equilibrates with calcium-binding proteins within presynaptic boutons, which gives rise to a residual calcium signal (Ca_{res}) that is initially tens to hundreds of nanomolar and persists for tens to hundreds of milliseconds until the calcium is extruded from the presynaptic bouton.

It is exceedingly difficult to measure Ca_{local} because it is so short lived and localized. As a result, much of what we know about Ca_{local} is based on models, simulations, and ultrastructure (Chen et al., 2015; Fogelson and Zucker, 1985; Simon and Llinás, 1985). The calcium dependence of release has often been studied using caged calcium to rapidly elevate calcium throughout presynaptic terminals (Heidelberger et al., 1994). This approach suggested that at the calyx of Held vesicle fusion is triggered by a calcium increase of 25 μ M lasting several hundred microseconds (Schneggenburger and Neher, 2000). Ca_{res} is readily measured

with fluorometric calcium indicators, and it is typically tens to a few hundred nanomolar (Atluri and Regehr, 1996; Brenowitz and Regehr, 2007; Scott and Rusakov, 2006).

One of the leading ways of studying the role of calcium in synaptic transmission is to introduce exogenous buffers into presynaptic boutons (Augustine et al., 2003). The introduction of calcium buffers into presynaptic terminals can affect Ca_{local} and Ca_{res} . A simple equation can be used to estimate how an exogenous buffer will affect the steady-state Ca_{local} signal that develops near the mouth of an open Ca^{2+} channel within microseconds of the channel opening (Naraghi and Neher, 1997; Neher, 1998).

$$Ca_{local} = \frac{I_{Ca}}{4\pi FDr} e^{(-r/\lambda)} \quad \text{Equation 1}$$

where I_{Ca} is the calcium current through an open channel, F is the faraday constant, D_{Ca} is the diffusion coefficient of free calcium, r is the distance from an open channel, and λ is the length constant defined by

$$\lambda = \sqrt{D_{Ca}/(k_{on}[B])} \quad \text{Equation 2}$$

where k_{on} is the buffer's on rate and $[B]$ is the concentration of free buffer. In general, high concentrations of fast calcium buffers are most effective at reducing Ca_{local} . This is why fast buffers, such as BAPTA, are generally effective at reducing Ca_{local} and decreasing fast synaptic transmission. Even slow buffers, such as EGTA, can reduce Ca_{local} , with EGTA most effective if the release site is some distance away from open calcium channels. The kinetics of calcium buffers also influences their effect on residual calcium. Fast buffers, such as BAPTA, reduce the amplitude of Ca_{res} and slow the decay of Ca_{res} (Neher and Augustine, 1992; Tank et al., 1995). The effects of slow buffers, such as EGTA, are more complex. During the time in which EGTA slowly binds calcium, Ca_{res} decays rapidly (Atluri and Regehr, 1996). After the initial rapid decay, calcium equilibrates with EGTA, and there is a slow decay phase of Ca_{res} .

Manipulation of presynaptic calcium levels with calcium buffers provided the first indication that facilitation was produced by Ca_{res} . At the crayfish neuromuscular junction, facilitation produced by a burst of presynaptic activity was strongly attenuated when Ca_{res} was rapidly lowered by optically uncaging the caged calcium chelator diazo-2 (Kamiya and Zucker, 1994). At the granule cell to Purkinje cell synapse, the introduction of EGTA into granule cell presynaptic terminals accelerated the decay of Ca_{res} and paired-pulse facilitation (Atluri and Regehr, 1996). The acceleration in the decay of facilitation occurred even when the EGTA introduced into the presynaptic terminal did not affect the initial p and thus did not significantly reduce Ca_{local} . These studies suggested that Ca_{res} produces facilitation at these synapses, but the target of Ca_{res} and the mechanism of facilitation remained unclear.

The Residual Calcium Hypothesis

Katz and Miledi (1968) proposed that facilitation could be a consequence of a larger calcium signal available for the second of two closely spaced stimuli (Figure 3A). In the late 1960s, little was known about the time course of calcium entry evoked by an action potential or the amplitude and duration of calcium signals. More-

over, concepts, such as local and global calcium signals, were not fully developed. Despite these limitations, Katz and Miledi hypothesized that calcium could persist in nerve terminals following an initial stimulus and could lead to a larger calcium signal and more release by a second action potential. The observation that small increases in calcium entry could produce large changes in synaptic strength (Dodge and Rahamimoff, 1967) was crucial to the formulation of the residual calcium hypothesis of facilitation. Subsequent studies quantified the extent of facilitation under a variety of experimental conditions and modeled facilitation while taking into account the known calcium dependence of release (Barrett and Stevens, 1972; Magleby, 1973; Zengel and Magleby, 1982). These studies suggested that the observed facilitation was consistent with the residual calcium hypothesis, but critical evaluation of the residual calcium hypothesis was limited by a lack of information regarding presynaptic calcium signals.

According to the simplest form of the residual calcium hypothesis, for the first stimulus, release is proportional to $(Ca_{local})^4$ and, for the second, release is proportional to $(Ca_{local} + Ca_{res})^4$.

$$PPR = \frac{(Ca_{local} + Ca_{res})^4}{Ca_{local}^4} \quad \text{Equation 3}$$

This formulation of the residual calcium hypothesis is based on a single calcium sensor, such as syt1 or syt2, acting as the sensor for both rapid transmission and facilitation. It is possible to evaluate the residual calcium hypothesis model of facilitation by determining the relative amplitudes of Ca_{local} and Ca_{res} and by measuring PPR. Ca_{res} , as measured with fluorometric calcium indicators, is typically tens to a few hundred nanomolar, while Ca_{local} is 25–100 μ M (Zucker, 1996). Thus, Ca_{res}/Ca_{local} is ~ 0.01 at most synapses and would only produce a PPR of approximately 1.04, which could not account for significant facilitation. However, syt1 and syt2 possess multiple calcium-binding sites, and it is possible that one of these sites has slow kinetics and a high affinity that contributes to facilitation. Although it is difficult to exclude a contribution from such a mechanism because so little is known about Ca_{local} for most synapses, it seems unlikely that such a mechanism could account for large facilitation.

The Search for a Second Calcium Sensor for Facilitation

The great disparity between the calcium concentrations that trigger release and those that produce facilitation is inconsistent with the simplest form of the residual calcium hypothesis in which a single calcium sensor triggers release and produces facilitation (Yamada and Zucker, 1992). This prompted the hypothesis that facilitation is mediated by a second calcium sensor distinct from the one that triggers fusion (Figure 3B). Experimental findings constrain the properties of such a sensor. First, it must respond to the submicromolar calcium signals that drive facilitation. This is most readily accomplished if the sensor has a high affinity for calcium. Second, it must not be saturated by the high local calcium that triggers release. Saturation is avoided if the sensor has slow kinetics or is located at a distance from calcium channels. Finally, it must be capable of enhancing p .

It is a daunting task to identify the calcium sensor for facilitation, because there are many candidate calcium-binding proteins (CaBPs) present in presynaptic terminals (Burgoyne,

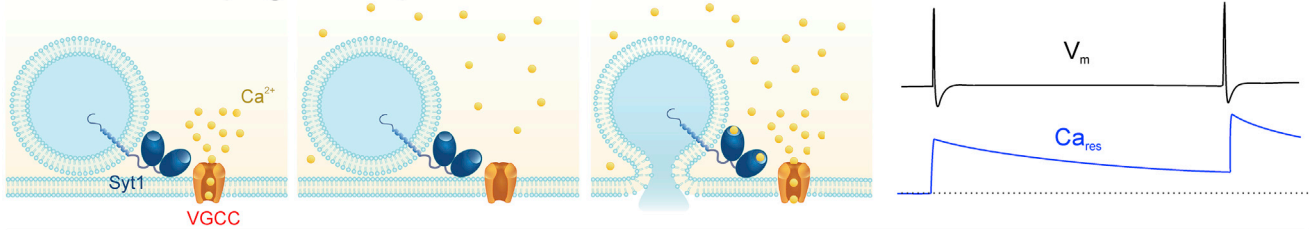
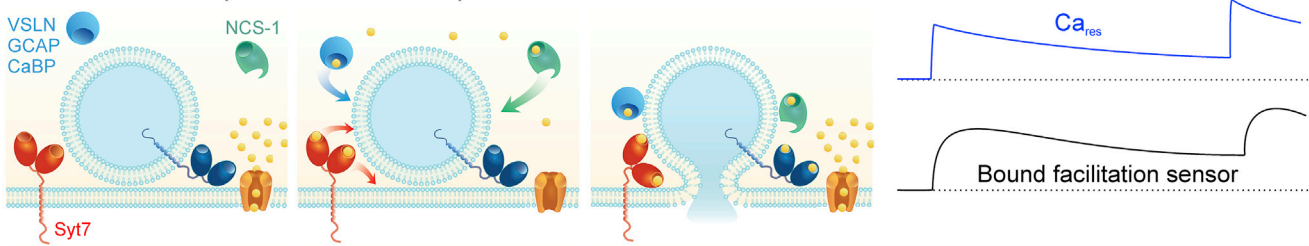
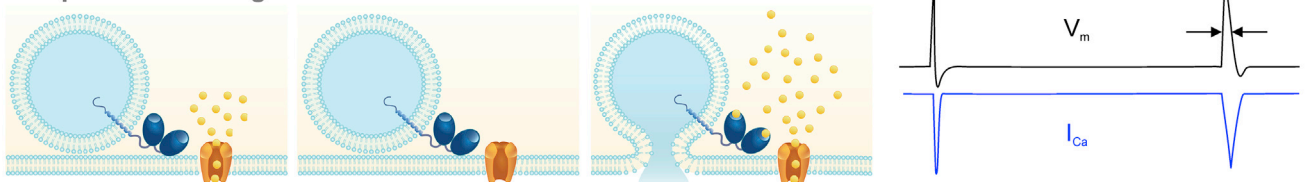
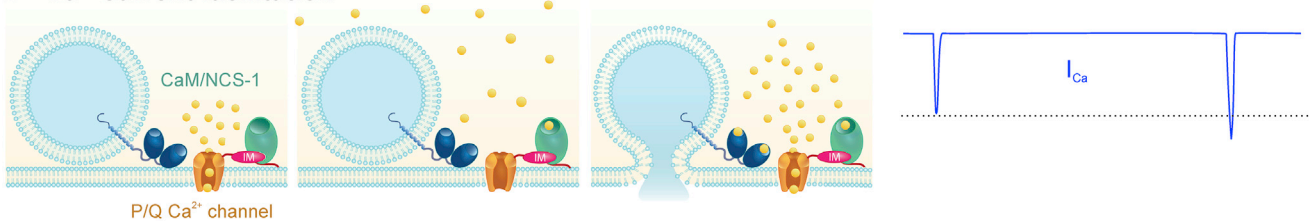
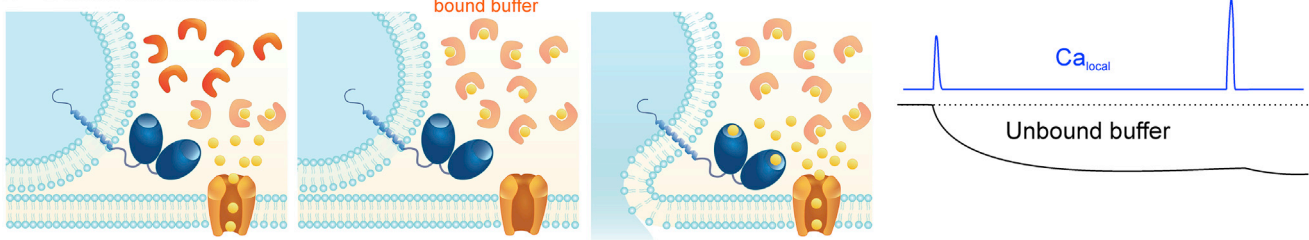
A Residual Ca^{2+} (single sensor)**B Residual Ca^{2+} (facilitation sensor)****C Spike broadening****D Ca^{2+} current facilitation****E Buffer saturation****Figure 3. Proposed Mechanisms for Facilitation**

Illustration of the proposed mechanisms for facilitation. Voltage-gated Ca^{2+} channels (VGCCs) open during the first action potential (AP) and allow Ca^{2+} influx (I_{Ca}). During the first action potential, Ca^{2+} influx fails to fully activate Syt1 and trigger fusion.

(A) The residual Ca^{2+} hypothesis: Ca_{res} from the first AP adds to Ca_{local} during the second AP. Because the fusion sensor binds Ca^{2+} in a supralinear manner, a small increase in the Ca^{2+} signal drives a large increase in p .

(B) Residual Ca^{2+} binds to a facilitation sensor: a high-affinity sensor, distinct from Syt1, binds to Ca_{res} in between APs and raises p by interacting with the fusion machinery.

(C) Spike broadening: inactivation of K^{+} channels during the first AP broadens the waveform of the second AP, prolonging the time course and total amount of I_{Ca} . (D) Calcium current facilitation: Ca^{2+} from the first AP binds to NCS-1 or CaM, which interact with the IQ-like motif (IM) on the intracellular C terminus of P/Q channels to increase I_{Ca} during the second AP.

(E) Buffer saturation: during the first AP, there is enough unbound Ca^{2+} buffer to capture incoming ions and decrease the Ca^{2+} signal sensed by Syt1 (Ca_{local}). During the second AP, the buffer becomes saturated and cannot constrain Ca_{local} .

2007; Craxton, 2010). Most of these proteins bind calcium with one or more E/F hand or C2 domains. The E/F hand domain proteins include the ubiquitously expressed CaM; NCS-1; visinin-like proteins (VSNLs) that include hippocalcin, neurocalcin d, and VILIPs1–3; recoverin; guanylyl-cyclase activating proteins (GCAPs); KCHIP proteins that regulate potassium channels; and finally a much less studied family of proteins known as calcium-binding proteins (CBPs). Many of these proteins have been implicated in the calcium-dependent regulation of calcium entry through voltage-gated calcium channels (see below), but they could also regulate other targets and increase p independent of changes in calcium entry. For most of these E/F hand proteins, their possible role in regulating synaptic facilitation independent of regulating calcium entry has not been examined. NCS-1 was the first protein suggested to be a calcium sensor for facilitation that acts independently of regulating calcium signaling or the initial p . Expressing NCS-1 in cultured hippocampal cells transformed depressing synapses into facilitating synapses without altering calcium entry or initial p (Sippy et al., 2003). However, this facilitation decayed with a time constant of 14 ms, which is about ten times faster than facilitation in wild-type (WT) animals, and they did not establish that facilitation in wild-type animals relies on NCS-1.

There are also many C2-domain-containing proteins within presynaptic terminals, many of which are known to be important for synaptic transmission (Corbalan-Garcia and Gómez-Fernández, 2014). Most notable are the synaptotagmin isoforms syt1 and syt2, which act as the calcium sensors for synchronous vesicle fusion at most central synapses (Südhof, 2013). Other C2-domain-containing proteins include classical PKCs, ferlins, DOC2, rabphilin, RIM1 and RIM2, Munc13, perforin, and copine. Further studies are required to determine whether any of these proteins act as calcium sensors for facilitation, although, at present, there is no evidence to support such a role.

Synaptotagmins are a large family of membrane-associated proteins that include eight calcium-sensitive isoforms (1–3, 5–7, 9, and 10) that are candidate calcium sensors for facilitation (Craxton, 2010; Südhof, 2002). A tandem pair of C2 domains confers these isoforms with the ability to bind calcium, membranes, and SNARE complexes. The isoforms bind to phospholipids with different calcium affinities (Li et al., 1995) and different kinetics (Davis et al., 1999; Hui et al., 2005; Tucker et al., 2003). Syts 1, 2, and 9 act as low-affinity calcium sensors for rapid neurotransmitter release and consequently are not promising candidates to mediate facilitation (Xu et al., 2007). Syts 3 and 5–7 are widely expressed in the brain (Mittelstaedt et al., 2009) but do not rescue fast transmission in syt1-deficient hippocampal cultures (Xu et al., 2007), suggesting that they do not mediate fast synaptic transmission and instead perform some other function. Of all isoforms, syt 7 has the highest affinity for calcium (Sugita et al., 2002). In vitro assays found that the speed of disassembly of syt-liposome complexes was fast for syts 1–3, moderate for syts 5, 6, 9, and 10, and far slower for syt7 (Brandt et al., 2012; Hui et al., 2005).

Syt7's high calcium affinity, slow binding kinetics, and widespread distribution in the brain led to the hypothesis that syt7 could be a sensor for facilitation (Zucker, 1996; Zucker and Regehr, 2002). It was also suggested that syt7 could mediate a de-

layed form of synaptic transmission known as asynchronous release (Hui et al., 2005), where exocytosis persists at a low rate for tens to hundreds of milliseconds after an action potential (Atluri and Regehr, 1998; Best and Regehr, 2009; Goda and Stevens, 1994). However, initial tests found no role for syt7 in synaptic transmission. At the *Drosophila* NMJ, knockdown of syt7 affected neither synchronous release nor facilitation (Saraswati et al., 2007), and cultured cortical neurons from syt7 knockout (KO) mice also exhibited normal synaptic transmission (Maximov et al., 2008) (although facilitation was not explicitly examined).

The role of syt7 in facilitation was recently examined in brain slices at four synapses where syt7 is expressed and facilitation is prominent (Jackman et al., 2016). Paired-pulse facilitation was eliminated in syt7 KO mice at Schaffer collateral synapses, hippocampal mossy fiber synapses, corticothalamic synapses, and lateral perforant path synapses onto dentate granule cells (Figures 4A and 4B). Synaptic enhancement during brief trains was also eliminated at all synapses with the notable exception of mossy fiber synapses, where spike broadening and local buffer saturation have been shown to contribute to synaptic enhancement. At Schaffer collateral synapses, loss of syt7 did not affect the calcium dependence of release, the initial p , calcium entry, or residual calcium signals. In addition, viral expression of wild-type syt7 in presynaptic cells rescued facilitation in syt7 knockout animals, but expression of mutant syt7 with a calcium-insensitive C2A domain did not. Together, these findings indicate syt7 is a calcium sensor for facilitation for many synapses in the brain.

It is surprising that syt7's role in facilitation was not revealed sooner. Syt7 was proposed as a candidate sensor for facilitation based on the high affinity for calcium (Zucker, 1996; Zucker and Regehr, 2002) and syt7 KO mice became available in 2003 (Chakrabarti et al., 2003). However, previous studies focused on the roles of syt7's other aspects of synaptic transmission and saw no effect on facilitation. Initially, it was questioned whether conflicting observations were the result of differences between the two syt7 knockout mouse lines used in various studies: syt7 mRNA has been detected in one line (Chakrabarti et al., 2003), but not the other (Maximov et al., 2008). However, neither line expresses syt7 protein, and direct comparisons have detected no significant differences in synaptic properties between neurons from the two lines (Bacaj et al., 2013, 2015).

In addition to its role in facilitation, syt7 is a multifunctional protein that responds to submicromolar Ca_{res} signals to control multiple aspects of synaptic transmission and release. Syt7 mediates asynchronous release during prolonged high-frequency stimulation (Wen et al., 2010). Similarly, cultured hippocampal neurons from syt1 KO mice display prominent asynchronous release that is mediated by syt7 (Bacaj et al., 2013, 2015; Li et al., 2017). Syt7 interacts with calmodulin to accelerate recovery from depression in cultured hippocampal neurons (Liu et al., 2014). Syt7 also acts as a high-affinity calcium sensor for exocytosis from non-neuronal cells (Gustavsson et al., 2008; Martinez et al., 2000; Schonn et al., 2008; Sugita et al., 2001). Thus, syt7 functions as a high-affinity calcium sensor to regulate release in multiple ways in both neurons and non-neuronal cells.

There are still many unresolved questions regarding syt7. Syt7 has three alternative splice variants in mice and humans (Fukuda

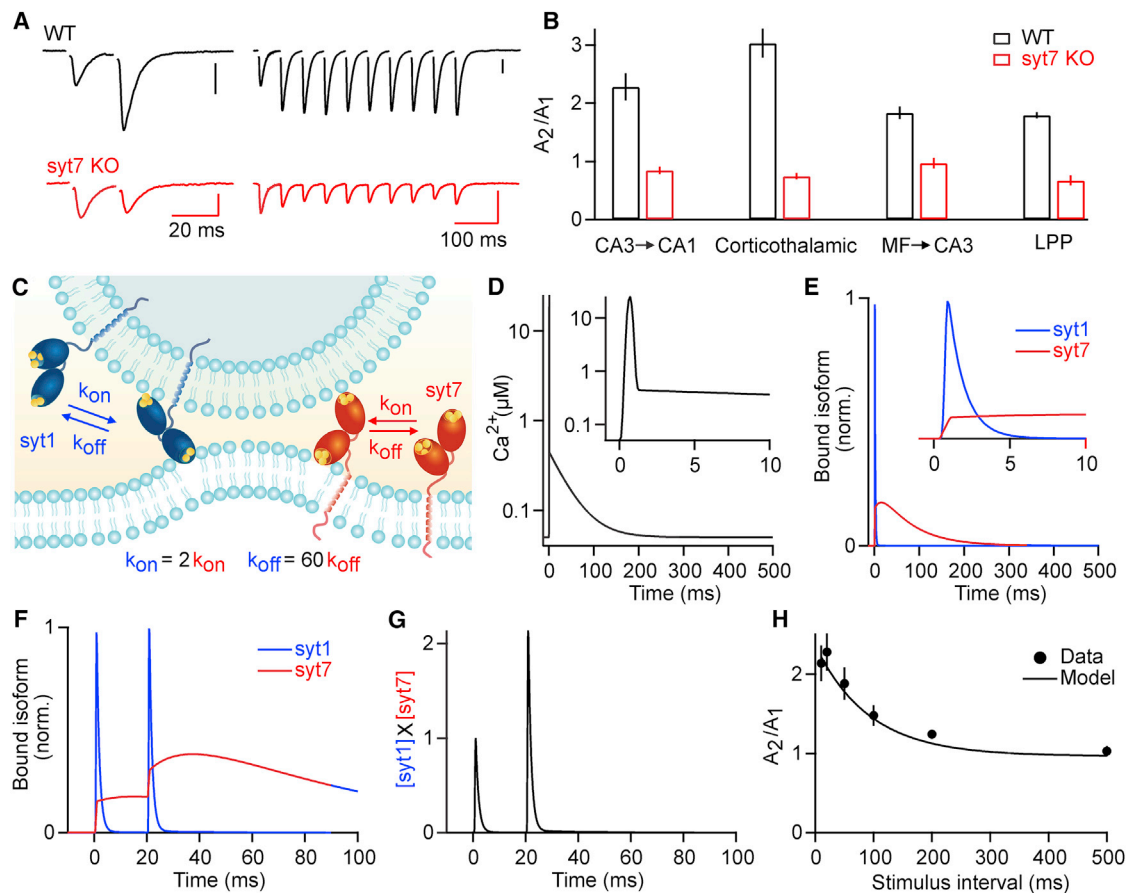


Figure 4. Facilitation Mediated by syt7

(A) EPSCs recorded from CA1 pyramidal cells in WT and syt7 KO mice. Vertical scale bars, 100 pA. Adapted from Jackman et al. (2016).

(B) Paired-pulse ratios at 20 ms intervals from hippocampal Schaffer collaterals (CA3 → CA1), corticothalamic synapses, hippocampal mossy fibers (MF → CA3), and hippocampal lateral perforant path synapses (LPPs) from WT and syt7 KO mice.

(C) In the presence of Ca^{2+} , the C2A domain of syt1 binds to phospholipids twice as fast as the C2A domain syt7 and unbinds ~60-fold faster than syt7 (Brandt et al., 2012).

(D) Simulated Ca^{2+} signal at a release site following an action potential. During the action potential, local Ca^{2+} rises to 25 μM briefly (FWHM = 0.34 ms; Sabatini and Regehr, 1998). After the action potential, a residual Ca^{2+} signal of 400 nM decays back to resting Ca^{2+} (50 nM) with a 40 ms time constant (Brenowitz and Regehr, 2007).

(E) Simulated phospholipid binding of both syt1 and syt7 during a single action potential normalized to the maximal binding produced by a train of ten action potentials at 100 Hz.

(F) Phospholipid binding in response to a pair of action potentials.

(G) The multiplied fraction of bound syt1 and bound syt7 ($[\text{syt1}] \times [\text{syt7}]$) normalized to the peak initial response.

(H) Paired-pulse ratios at different stimulus intervals recorded from Schaffer collateral synapses (adapted from Jackman et al., 2016), along with the facilitation predicted by $[\text{syt1}] \times [\text{syt7}]$.

In (E)–(H), phospholipid binding was simulated using parameters from stopped-flow experiments of syt association with PIP2-containing target membrane liposomes (Brandt et al., 2012). K_d for Ca^{2+} was 41 μM and 1.5 μM , with a cooperativity of 1.9 and 2.8 for syt1 and syt7, respectively. For comparison to physiological recordings, binding kinetics were adjusted for temperature (34°C) (Hui et al., 2005). K_{off} 900 s^{-1} and 15 s^{-1} and K_{on} 415 s^{-1} and 190 s^{-1} for syt1 and syt7, respectively.

et al., 2002) and more in rats (Sugita et al., 2001). Rescue experiments have been performed using syt7 α , the dominant splice variant in the brain (Bacaj et al., 2013; Jackman et al., 2016; Liu et al., 2014), and it is not known whether splice variants make different contributions to facilitation and other processes. Finally, syt7 is expressed by neurons that exhibit net depression, such as Purkinje cells, and it will be interesting to determine the role of syt7 in transmission at other such synapses. At high p synapses, facilitation may still be present but obscured by prominent depression (Dittman et al., 2000). Facilitation can still make

important contributions during repetitive activation at depressing synapses (Turecek et al., 2016).

How Does Syt7 Produce Facilitation?

The structural similarity between syt7 and the fast isoforms syt1 and syt2 suggests that they may have related functions in vesicle fusion. It is therefore useful to review current models of how syt1 acts as a calcium sensor for fusion, with the caveat that the mechanism by which syt1 triggers fusion has yet to be resolved (Jahn and Fasshauer, 2012). The core machinery for vesicle fusion is composed of syntaxin-1, synaptobrevin, and SNAP-25, which

together form SNARE complexes that link vesicles to the plasma membrane (Südhof and Rothman, 2009). The two C2 domains of syt1, C2A and C2B, coordinate calcium binding (Ubach et al., 1998) and confer calcium sensitivity to SNARE-mediated fusion through calcium-dependent interactions with membranes and SNAREs (Chapman, 2008; Südhof, 2013). Calcium binding induces a charge switch in the C2 domains that leads to fusion through mechanisms that likely involve SNARE and membrane binding.

Although both C2 domains are structurally similar, mutations of the calcium-binding pocket of the C2B cause greater deficits in neurotransmitter release than similar mutations in C2A (Fernández-Chacón et al., 2002; Gaffaney et al., 2008; Mackler et al., 2002; Nishiki and Augustine, 2004; Robinson et al., 2002; Stevens and Sullivan, 2003; Xue et al., 2008). The C2B domain also contains basic regions that support calcium-independent binding to SNAREs and the acidic phospholipid phosphatidylinositol-4,5-bisphosphate (PIP2) (Bai et al., 2004; Wang et al., 2016; Zhou et al., 2015). C2B binding to PIP2 may target vesicles to release sites that are enriched in PIP2 (Honigsmann et al., 2013), coordinate SNAREs with the opposing membrane to stabilize vesicles in a pre-fusion state (Chicka et al., 2008; de Wit et al., 2009; Mohrmann et al., 2013; van den Bogaart et al., 2011; Zhou et al., 2015), and raise p by increasing the calcium affinity of syt1 (Bai et al., 2004; Li et al., 2006; Mackler and Reist, 2001; Radhakrishnan et al., 2009; van den Bogaart et al., 2012).

A prominent model posits that, upon binding calcium, the top loops of C2B insert into the plasma membrane while the basic regions remain bound to SNAREs and PIP2 (Wang et al., 2016; Zhou et al., 2015). This calcium-dependent membrane insertion appears to be essential for vesicle fusion, as neurotransmitter release is abolished by a point mutation that blocks membrane insertion (Bai et al., 2002; Herrick et al., 2006; Paddock et al., 2011). It is thought that by penetrating the plasma membrane while remaining bound to SNAREs, syt1 induces inward curvature in the plasma membrane (Bharat et al., 2014; Martens et al., 2007; Wang et al., 2016). Curvature may lower the energy barrier for fusion by placing elastic stress on the membrane and bringing the vesicle and membrane closer together (Kozlovsky and Kozlov, 2002; Kuzmin et al., 2001).

It is likely that syt7 produces facilitation by increasing the probability of syt1-mediated release rather than by triggering fusion on its own. Although syt7 may mediate calcium-dependent release from non-neuronal cells (Martinez et al., 2000; Sugita et al., 2001), syt7 is only capable of driving slow asynchronous release from neurons in the absence of syt1 (Bacaj et al., 2013; Xu et al., 2007). Syt7 has been shown to bind membranes and SNAREs in a calcium-dependent manner (Osborne et al., 2007; Sugita et al., 2002), but syt7 has not been biochemically characterized to the same degree as syt1 and many questions must be answered to understand how syt7 raises p during facilitation. Several membrane-binding properties of syt7 could help to explain its role in facilitation. Syt7 exhibits calcium-dependent phospholipid binding with ~10-fold higher affinity than syt1 and is activated by submicromolar calcium concentrations (Sugita et al., 2002). This makes syt7 capable of sensing the Ca_{res} signals that drive facilitation. Phospholipid binding is also much slower for syt7 than for syt1

(Hui et al., 2005), with binding and unbinding proceeding 2-fold and 60-fold slower, respectively (Brandt et al., 2012) (Figure 4C).

If phospholipid binding is a determinant for triggering fusion, syt7's slow kinetics are optimal for a facilitation sensor. This is illustrated by simulating how syt1 and syt7 bind dynamically to membranes in response to the calcium signals produced by action potentials using the measured calcium affinity, cooperativity, and kinetics of phospholipid binding (Brandt et al., 2012). Consider an action potential that produces Ca_{local} of 25 μ M for 0.5 ms, followed by Ca_{res} of 400 nM that decays with a time constant of 40 ms back to a resting calcium of 50 nM (Figure 4D). Ca_{local} causes syt1 to transiently bind to membranes, but Ca_{res} is not effective at activating syt1 because of its low affinity for calcium (Figure 4E). In contrast, Ca_{local} is so brief that it does not lead to much syt7 binding to the membrane, but the longer-lasting Ca_{res} activates syt7 due to its high calcium affinity. Syt7 will continue to bind to membranes tens of milliseconds after an initial action potential. Even after Ca_{res} returns to resting levels, slow dissociation keeps syt7 bound to membranes, where it could facilitate release during subsequent action potentials. The slow dissociation of syt7 could allow facilitation to outlast Ca_{res} increases (Atluri and Regehr, 1996).

Remarkably, simply multiplying the amount of membrane-bound syt1 and syt7 can account for facilitation (Figures 4F–4H). This is consistent with the idea that syt1 and syt7 membrane binding lowers the energy barrier for membrane fusion by E_{syt1} and E_{syt7} , respectively. The rate of fusion (k^+) predicted by the Arrhenius equation is then given by:

$$k^+ \propto e^{-\frac{(E_A - E_{syt1} - E_{syt7})}{kt}} = e^{-\frac{E_A}{kt}} e^{\frac{E_{syt1}}{kt}} e^{\frac{E_{syt7}}{kt}} \quad \text{Equation 4}$$

where E_A is the activation energy in the absence of syt binding, k is the Boltzmann constant, and T is the temperature. This predicts that the fusion rate depends on the multiplied contributions of the two bound isoforms. Theoretical studies of membrane fusion suggest that E_A is ~40 kT (Kozlovsky and Kozlov, 2002; Kuzmin et al., 2001), for saturating concentrations of membrane-bound syt1 E_{syt1} is ~20 kT (Martens et al., 2007), and E_{syt7} is not known. Using these parameters, simulated rates of fusion with realistic amounts of facilitation were obtained when membrane-bound syt7 lowered the energy barrier only modestly ($E_{syt7} = \sim 2\text{--}5$ kT).

The prediction that syt7 contributes far less than syt1 (or syt2) to lowering the energy barrier may help to explain why syt7 produces facilitation in the presence of syt1 and supports asynchronous release in the absence of syt1. When syt1 is present, a small contribution from syt7 could dramatically increase the probability of release for syt1-triggered fusion because the fusion rate depends exponentially on the energy barrier. In the absence of syt1, syt7 could drive fusion on its own but would do so at much lower rates, and syt7-triggered fusion would persist for tens to hundreds of milliseconds. This is consistent with the syt7-triggered asynchronous release observed in neurons lacking syt1 (Bacaj et al., 2013, 2015). Syt7-triggered asynchronous release rates are orders of magnitude lower than syt1-triggered synchronous release rates, but release continues for hundreds of milliseconds. Interestingly, asynchronous

release and facilitation have been suggested to rely on the same mechanism (Rahamimoff and Yaari, 1973; Zucker, 1996). While the time course and magnitude of facilitation predicted by this model support membrane binding as a critical determinant of syt7-mediated facilitation, more work is required to understand how syt7 and other syt isoforms interact with vesicles and fusion machinery to promote exocytosis.

Use-Dependent Increases in Calcium Entry

It has been hypothesized that use-dependent increases in presynaptic calcium entry could underlie facilitation. This could occur either as a consequence of a change in the presynaptic waveform that leads to the opening of more presynaptic calcium channels, or as a result of calcium channel modulation that enhances calcium entry. Based on the steep calcium dependence of release, even a small change in calcium entry could produce large facilitation.

Spike Broadening

Studies of peptide release from presynaptic terminals in the neurohypophysis provided the first indication that changes in presynaptic waveform in presynaptic terminals could lead to use-dependent enhancement of release. Peptide release from pituitary nerve terminals is highly frequency dependent. Optical measurements of presynaptic waveform revealed that frequency-dependent spike broadening occurs and likely contributes to the frequency dependence of peptide release (Dreifuss et al., 1971; Gainer et al., 1986; Salzberg et al., 1986). Similarly, whole-cell recordings from pituitary nerve terminals confirmed that use-dependent spike broadening occurs, likely as a consequence of potassium channel inactivation, and that spike broadening increases presynaptic calcium entry (Jackson et al., 1991).

Use-dependent spike broadening (Figure 3C) was subsequently shown to occur at some fast glutamatergic synapses in the mammalian brain. Mossy fiber synapses between hippocampal granule cells and CA3 pyramidal cells have the unusual property that they undergo profound use-dependent enhancement during sustained activation for a broad range of stimulus frequencies (Regehr et al., 1994; Salin et al., 1996). Whole-cell patch-clamp recordings from mossy fiber boutons revealed activity-dependent spike broadening and increases in calcium entry during high-frequency stimulation as a result of inactivation of a DTX-sensitive potassium channel (Geiger and Jonas, 2000). This spike broadening is sufficient to make a major contribution to synaptic enhancement during prolonged stimulation, which could explain the remaining facilitation observed during train stimulation at mossy fiber synapses in syt7 KO animals (~30% compared to WT animals; Jackman et al., 2016). However, spike broadening was not prominent for pairs of closely spaced stimuli, suggesting that it is unlikely to contribute significantly to paired-pulse facilitation at this synapse. In presynaptic boutons of layer 5 pyramidal cells, a slowly inactivating K channel, possibly Kv1.2, can also influence presynaptic waveform during prolonged somatic depolarization (Shu et al., 2007), but waveform changes are small and unlikely to contribute to facilitation during brief trains (Shu et al., 2006).

It appears that spike broadening does not contribute to facilitation at most synapses. The contribution of waveform changes to facilitation was first tested at the squid giant synapse, where

direct measurements from presynaptic terminals were possible. Facilitation is observed at the squid giant synapse in the presence of low external calcium. Use-dependent changes in the presynaptic action potentials were small and insufficient to account for this facilitation (Charlton and Bittner, 1978). At synapses made by cerebellar granule cells, presynaptic waveforms were monitored by measuring the presynaptic volley, which reflects the current flow associated with a presynaptic action potential, and by using voltage-sensitive dyes (Sabatini and Regehr, 1997). Granule cell to Purkinje cell synapses exhibit robust facilitation (Atluri and Regehr, 1996) but do not exhibit use-dependent spike broadening (Kreitzer and Regehr, 2000; Sabatini and Regehr, 1997). Similarly, for hippocampal CA3 to CA1 synapses, presynaptic volleys and presynaptic waveforms measured with voltage-sensitive dyes do not show use-dependent waveform changes (Qian and Saggau, 1999). At the calyx of Held, whole-cell recordings indicated that presynaptic waveforms are essentially unchanged for pairs of stimuli that produce almost a doubling of synaptic strength (Müller et al., 2008). High-frequency (40 Hz) stimulation does not lead to spike broadening in presynaptic boutons of cerebellar interneurons (Pouzat and Hestrin, 1997; Rowan et al., 2014). Synapses made between cultured Purkinje cells facilitate, but waveform changes are not responsible for facilitation (Díaz-Rojas et al., 2015).

Thus, use-dependent waveform changes only occur at specialized synapses, such as mossy fiber synapses, and even at those synapses, spike broadening is not responsible for the bulk of synaptic facilitation. It is known that specific complements of ion channels are present in presynaptic boutons (Trimmer, 2015), and the finding that most synapses allow reliable action potential propagation and stereotyped presynaptic waveforms during repetitive activation suggests that the density and complement of ion channels in presynaptic terminals allow presynaptic terminals at most types of synapses to avoid waveform changes.

Calcium Current Facilitation

It has also been proposed that calcium-dependent facilitation of calcium entry (CDF) that is independent of presynaptic waveform changes could contribute to synaptic facilitation (Figure 3D). It is predicted that if CDF contributes to synaptic facilitation, there should be use-dependent increases in presynaptic calcium influx that are not a consequence of changes in presynaptic waveform. This could result in an increase in presynaptic calcium current or an increase in stimulus-induced presynaptic calcium increases. At the squid giant synapse, measurements of presynaptic calcium with arsenazo III, an early calcium indicator, found that each of two closely spaced action potentials produced the same incremental increase in Ca_{res} (Charlton et al., 1982). These studies suggested that at the squid giant synapse, facilitation is not a consequence of use-dependent increases in calcium entry. Similarly, Ca_{res} measurements suggest that there is no use-dependent increase in calcium entry at other facilitating synapses, including the granule cell to Purkinje cell synapse and hippocampal synapses (Atluri and Regehr, 1996; Jackman et al., 2016). It is difficult, however, to exclude this mechanism because Ca_{res} reflects influx through all calcium channels in a presynaptic bouton, and, in theory, CDF could selectively occur at those calcium channels near release sites. Elevation of intracellular

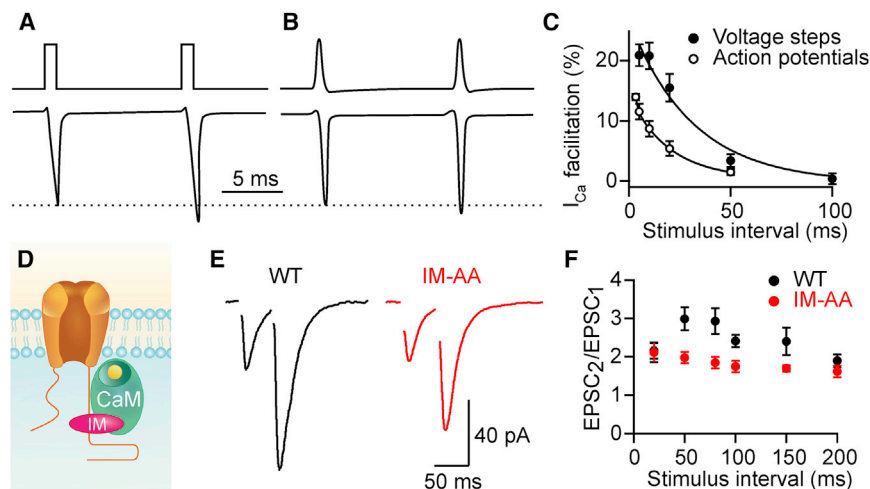


Figure 5. Calcium Current Facilitation

(A and B) Ca^{2+} influx at the Calyx of Held evoked by a pair of 1 ms depolarizing steps (adapted from Cuttle et al., 1998) (A) and action potentials (adapted from Di Guilmi et al., 2014) (B). (C) Paired-pulse facilitation of Ca^{2+} influx at different stimulus intervals produced by voltage steps at the Calyx of Held steps (adapted from Cuttle et al., 1998) and action potentials between cultured Purkinje cell synapses. Adapted from Díaz-Rojas et al. (2015).

(D) The Ca^{2+} sensors NCS-1 and CaM bind to the IQ-like motif (IM) of P/Q-type Ca^{2+} channels to produce facilitation of Ca^{2+} entry. Substituting alanine for a pair of isoleucine and methionine residues in IM (IM-AA) disrupts this interaction. (E) EPSCs recorded from CA1 pyramidal cells in WT and IM-AA knockin mice. Recordings were made in the presence of ω -conotoxin GVIA to block N-type Ca^{2+} channels.

(F) Paired-pulse facilitation in WT and IM-AA mice. (E) and (F) adapted from Nanou et al. (2016).

calcium can either inhibit or facilitate calcium entry (Ben-Johny and Yue, 2014; Christel and Lee, 2012). Use-dependent increases in presynaptic calcium currents were first described at the calyx of Held (Borst and Sakmann, 1998; Cuttle et al., 1998). Calcium currents generated by a pair of 1 ms voltage steps facilitated by approximately 20% for a 10 ms interstimulus interval (ISI) (Cuttle et al., 1998) (Figures 5A and 5C). High-frequency trains of these voltage steps transiently facilitated calcium currents by almost a factor of 2. This facilitation was strongly attenuated in low external calcium, eliminated when external calcium was replaced with either sodium or barium, and was reduced by about 50% when 10 mM EGTA was included in the recording pipette (Cuttle et al., 1998).

Comparing the degree of facilitation of presynaptic calcium current and synaptic responses is particularly illuminating. For action potential-driven release at the calyx of Held, synaptic facilitation more than doubled synaptic strength, and the facilitation of calcium channels accounted for less than half of the synaptic facilitation (Müller et al., 2008). At synapses between cultured Purkinje cells, there is a 16% enhancement of calcium current and a doubling of synaptic strength for stimuli separated by 10 ms. Calcium current enhancement is sufficient to account for synaptic enhancement, provided release is steeply dependent on calcium influx (4.5 power; Díaz-Rojas et al., 2015). In cultured superior cervical ganglion neurons that express P-type calcium channels, both calcium entry and synaptic strength show facilitation, but quantitative comparisons were complicated because calcium currents were evoked by 5 ms voltage steps and synaptic responses were evoked by action potentials (Yan et al., 2014).

Facilitation of calcium current is short lived. A recent study quantified the time course of calcium-dependent facilitation of P-type calcium channels in Purkinje cells using optical manipulations of intracellular calcium levels (Lee et al., 2015). They found that the onset of facilitation is rapid ($\tau_{\text{on}} = 2$ ms) and, following a return to resting calcium levels, enhancement is short lived ($\tau_{\text{off}} = 31$ ms). The time course of paired-pulse facilitation of calcium current had a similar time course at the calyx of Held (Cuttle et al., 1998) and in Purkinje cell presynaptic boutons (Díaz-Rojas

et al., 2015) (Figure 5C). These experiments were performed at room temperature, so it is likely that the onset and decay of facilitation of calcium entry is even more rapid at physiological temperatures. Maximal CDF of P-type calcium channels in Purkinje cells (Lee et al., 2015) was approximately a doubling of the current, and it occurred at a half maximal value of approximately 500 nM with a Hill coefficient of 1.8. These properties suggest that facilitation of calcium currents can occur in response to high Ca_{local} increases that only last for about a millisecond, but that Ca_{res} could also be sufficiently large to enhance presynaptic calcium currents.

A number of factors influence the magnitude of CDF. First, the voltage command used to induce CDF has a major influence on the extent of facilitation. At the calyx of Held, 1 ms voltage steps facilitated calcium current by 21% (Cuttle et al., 1998), but for action potentials, there was only a 7% enhancement (Di Guilmi et al., 2014) (Figure 5B). 100 Hz trains of 1 ms steps enhanced calcium influx by 100%, whereas action potential trains enhanced influx by 20% (Cuttle et al., 1998; Di Guilmi et al., 2014). This suggests that studies that used voltage steps of 5 ms and longer significantly overestimate the extent of enhancement that occurs under physiological conditions. The waveform of the presynaptic action potential, which is synapse specific, will also influence the extent of CDF. Second, alternative splicing of P-type calcium has a major influence on CDF. Multiple splice variants of P-type channels have been identified and can be grouped into two classes: EFa, which supports CDF, and EFb, which does not (Chaudhuri et al., 2004). Alternative splicing can also regulate whether CDF is driven by high local calcium (insensitive to calcium buffers) or by global calcium (highly sensitive to calcium buffers). Splice variants are developmentally regulated. Early in development, EFb is the predominantly expressed splice variant, but EFa becomes equally prevalent in the adult brain. Moreover, in the adult brain, the relative expression of each variant is regionally specific, with EFa being highly enriched in the thalamus, amygdala, and cerebellar Purkinje cells (Bourinet et al., 1999; Chaudhuri et al., 2004). The developmental and regional specificity of the different splice variants suggest that they could act as a molecular switch to

set the degree of CDF and determine the properties of synaptic plasticity.

Calcium Sensors for CDF

In considering how calcium current facilitation could occur, and how it could contribute to synaptic facilitation, it is most relevant to consider the three types of calcium channels responsible for most synaptic transmission: P-type (CaV 2.1), N-type (CaV 2.2), and R-type (CaV 2.3) calcium channels. All of these channels can undergo calcium/calmodulin (CaM)-dependent inhibition, but only P-type calcium channels exhibit calcium-dependent facilitation (Ben-Johny and Yue, 2014; Christel and Lee, 2012; Lee et al., 1999). In support of the importance of P-type calcium channels, it was found that in P-type calcium channel knockout animals in which N-type calcium channels take the place of P-type channels at the calyx of Held, presynaptic calcium current facilitation was absent and synaptic facilitation was strongly attenuated (Inchauspe et al., 2004). Calcium-dependent facilitation of P-type calcium currents is impaired by mutating CaM or by mutating an “IQ-like” motif (IM) on the carboxy tail of P-type calcium channels (DeMaria et al., 2001; Lee et al., 1999). These observations suggest that calcium binds to CaM, which in turn binds to the IM domain of P-type calcium channels (Mori et al., 2008) to produce CDF.

Calmodulin was the first calcium sensor to be implicated in calcium channel modulation. Expression of a calcium insensitive form of CaM suppresses CDF of P-type calcium channels (DeMaria et al., 2001; Lee et al., 2003), and mutagenesis of P-type calcium channels has established the importance of the IQ domain for CaM binding and CDF (DeMaria et al., 2001; Kim et al., 2008; Lee et al., 2003; Mori et al., 2008). Remarkably, the C lobe of CaM triggers rapid CDF of P-type calcium channels, but the N lobe triggers slow CDI (Ben-Johny and Yue, 2014; DeMaria et al., 2001; Lee et al., 2003). An important feature of CaM-mediated modulation is that CaM pre-associates with P-type calcium channels by binding to the IQ domain (Erickson et al., 2001).

The calcium-binding protein NCS-1 is also proposed to play a role in synaptic facilitation, but there is considerable controversy over the mechanism by which NCS-1 influences facilitation. NCS-1 binds calcium with high affinity and is present in presynaptic boutons (Dason et al., 2012). At the *Drosophila* NMJ (Pongs et al., 1993), overexpression of the NCS-1 homolog frequenin enhanced frequency-dependent facilitation. However, a subsequent study at the fly NMJ found that frequenin overexpression increased basal synaptic strength, increased depression in high Ca_e , but did not alter paired-pulse plasticity in low Ca_e . In frequenin null mutants, basal synaptic strength decreased, depression in high Ca_e decreased, and paired-pulse plasticity in low Ca_e was unaltered (Dason et al., 2009). These findings are reminiscent of the calyx of Held, where introducing NCS-1 into the presynaptic terminal enhances presynaptic calcium currents and occludes activity-dependent increases in calcium entry, and a peptide that blocks NCS-1 interactions with its targets prevents CDF of calcium current (Tsujimoto et al., 2002). NCS-1 binds to the IQ-like domain and the CaM binding domain of P-type calcium channels to reduce calcium-dependent inactivation of calcium channels, thereby allowing calcium/CaM-dependent facilitation to dominate (Yan et al., 2014). These findings

suggest that calcium binding to NCS-1 leads to synaptic facilitation by contributing to CDF.

Numerous other calcium sensors could potentially detect calcium and modulate calcium channels (Burgoyne and Haynes, 2015). Visinin-like protein 2 (VILIP-2) is highly expressed in the neocortex and hippocampus, blocks inactivation of calcium currents, reduces depression, and increases facilitation (Lautermilch et al., 2005; Leal et al., 2012).

The contribution of CDF to synaptic facilitation was assessed using IM-AA mice in which native P-type calcium channels were replaced by calcium channels that cannot undergo CDF because they have a mutated IM domain (Nanou et al., 2016) (Figures 5D–5F). They studied facilitation at hippocampal synapses that normally rely on both N-type and P-type calcium channels. To isolate the contribution of P-type calcium channels to transmission, experiments were performed in the presence of antagonists of N-type calcium channels. The IM-AA mutation did not alter the basal properties of synaptic transmission, but it eliminated the small synaptic facilitation (~20% enhancement) observed in cultured hippocampal cells. Facilitation was also assessed in hippocampal slices. Previous studies found that facilitation is still present at these synapses when P-type calcium channels are blocked and transmission is mediated by N-type calcium channels (Scheuber et al., 2004), which indicates that CDF of P-type calcium channels does not account for all facilitation at this synapse. In hippocampal slices, facilitation was still prominent in IM-AA mice (PPR~2) (Nanou et al., 2016), but it was reduced in magnitude compared to wild-type mice. This suggests that CDF can contribute to facilitation at this synapse, although syt7 appears to be the dominant mechanism of facilitation (Jackman et al., 2016). It will be important to determine the contribution of CDF to facilitation in IM-AA mice in control conditions when both P-type and N-type calcium channels mediate transmission.

Buffer Saturation Model

The properties of calcium regulation in presynaptic terminals can lead to elevated Ca_{local} during repetitive activation, even if successive action potentials evoke the same calcium entry. This is a consequence of the properties of calcium binding, which Neher and his colleagues realized could potentially contribute to synaptic facilitation (Klingauf and Neher, 1997; Neher, 1998; Rozov et al., 2001). In order to understand this mechanism, it is necessary to appreciate several aspects of presynaptic calcium signaling. First, the vast majority of calcium inside terminals is bound to calcium-binding proteins (Matthews and Dietrich, 2015; Neher, 1995; Smith and Zucker, 1980). Second, vesicle fusion is triggered when Ca_{local} reaches tens of micromolar, which only occurs within tens of nanometers of open calcium channels. Third, calcium-binding proteins can intercept some of the calcium that enters the presynaptic terminal before it reaches the calcium sensor for release, which can reduce p for the initial excitatory postsynaptic current (EPSC). Calcium-binding proteins decrease the initial p most effectively when: (1) they are present at high concentrations, (2) calcium sensors for release are distant from calcium channels, and (3) the buffers are fast. Fourth, if the calcium buffer has a high affinity for calcium, less free calcium buffer is available to bind calcium during repetitive activation. This could lead to progressively

larger Ca_{local} signals during high-frequency firing and could produce facilitation (Figure 3E).

Experiments at the layer 2/3 pyramidal cell to bitufted interneurons were the first to implicate buffer saturation in facilitation (Rozov et al., 2001). At this and many other synapses, paired-pulse facilitation gets smaller when Ca_e is increased. This is thought to be a secondary consequence of increased Ca_e elevating p , which increases depletion and reduces the extent of facilitation. However, introducing the fast calcium-chelator BAPTA into the presynaptic cell led to facilitation that increased when Ca_e was elevated. This is compatible with the partial buffer saturation model that predicts that as Ca_e increases, buffer saturation becomes more prominent, which results in more facilitation. Rozov et al. (2001) concluded that introducing a fast, high-affinity calcium buffer into presynaptic terminals led to artificial facilitation that relied on partial buffer saturation.

It was subsequently found that partial buffer saturation contributed to facilitation at native synapses that express high concentrations of the endogenous calcium-binding protein calbindin-D28k (Blatow et al., 2003). The mossy fiber synapse between hippocampal dentate granule cells and CA3 pyramidal cells is unusual in that facilitation gets larger when Ca_e is increased. In calbindin knockout animals, facilitation gets smaller as Ca_e is increased. Thus, it was suggested that in control conditions, facilitation is a consequence of saturation of the fast, high-affinity calcium buffer calbindin. Calbindin is likely effective at lowering initial p and producing facilitation at mossy fiber synapses because of its calcium-binding properties (fast on rate of $8 \times 10^7 M^{-1}s^{-1}$, and high affinity of 290 nM; Eggermann et al., 2011; Nägerl et al., 2000) and the loose coupling between calcium channels and release sensors at this synapse (Vyleta and Jonas, 2014). However, when calbindin was eliminated, a form of facilitation remained that was similar to that observed at other synapses, such as the CA3 to CA1 synapse (Blatow et al., 2003).

Simulations suggest that partial buffer saturation can be a consequence of either global saturation of a mobile buffer or local saturation of an immobile buffer (Matveev et al., 2004). If global saturation of a mobile buffer occurs within the presynaptic bouton, optical measurements using calcium indicators should detect use-dependent increases in action potential-evoked calcium signals. If, instead, local saturation of an immobile buffer occurs, then calcium signals would only be elevated very locally, which would be difficult to detect with standard fluorescence approaches. Although it is difficult to exclude the possibility that local buffer saturation occurs, there is no evidence that it contributes to facilitation at most synapses.

Conclusion

In the 70 years since its initial description by T.P. Feng and others (Eccles et al., 1941; Feng, 1940), substantial progress has been made in clarifying the mechanism of facilitation, while modeling studies have provided insight into possible functional roles of facilitation. It appears that a primary mechanism of facilitation is that the Ca_{res} activates syt7 to increase the probability of release. Calcium modulation of P-type calcium channels can also contribute to short-lived synaptic facilitation at many synapses. Other mechanisms, such as spike broadening and local cal-

cium buffer saturation, contribute to facilitation at some synapses, but they appear to be less prevalent. Further studies will determine whether other presynaptic calcium sensors or additional mechanisms also contribute to facilitation and whether multiple mechanisms interact to produce facilitation.

A concrete understanding of how facilitation affects neural processing and behavior is lacking because no study has addressed the impact of eliminating facilitation on behavioral phenotypes or in vivo circuit performance. Discovering the underlying mechanisms is an important first step in determining the functional importance of facilitation. In the case of syt7, only global constitutive knockout lines are currently available, making it difficult to evaluate the impact of removing facilitation from specific circuits. Synapse-specific elimination of facilitation using genetic approaches will make it possible to test theories of the function of facilitation and provide insight into its role in neural processing, neuropsychiatric diseases, and animal behavior.

ACKNOWLEDGMENTS

We thank Jasmine Vazquez for illustrations and Pascal Kaeser, Stephanie Rudolph, Josef Turecek, Chong Guo, and Chris Chen for comments on the manuscript. This work was supported by grants from the National Institutes of Health (R01NS032405 and R35NS097284) to W.G.R. and a Nancy Lurie Marks Fellowship to S.L.J.

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