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Review

Calcium Channel Regulation and Presynaptic Plasticity

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DOI 10.1016/j.neuron.2008.09.005

Voltage-gated calcium (Ca^{2+}) channels initiate release of neurotransmitters at synapses, and regulation of presynaptic Ca^{2+} channels has a powerful influence on synaptic strength. Presynaptic Ca^{2+} channels form a large signaling complex, which targets synaptic vesicles to Ca^{2+} channels for efficient release and mediates Ca^{2+} channel regulation. Presynaptic plasticity regulates synaptic function on the timescale of milliseconds to minutes in response to neurotransmitters and the frequency of action potentials. This article reviews the regulation of presynaptic Ca^{2+} channels by effectors and regulators of Ca^{2+} signaling and describes the emerging evidence for a critical role of Ca^{2+} channel regulation in control of neurotransmission and in presynaptic plasticity. Failure of function and regulation of presynaptic Ca^{2+} channels leads to migraine, ataxia, and potentially other forms of neurological disease. We propose that presynaptic Ca^{2+} channels serve as the regulatory node in a dynamic, multilayered signaling network that exerts short-term control of neurotransmission in response to synaptic activity.

Introduction

Ca^{2+} entry through presynaptic voltage-gated Ca^{2+} (Ca_v) channels initiates release of neurotransmitters. Multiple mechanisms directly or indirectly modulate the function of these presynaptic Ca^{2+} channels and thereby regulate synaptic transmission (Catterall, 2000; Dunlap et al., 1995; Snutch and Reiner, 1992; Tedford and Zamponi, 2006). Neuromodulation affects the ability of Ca_v channels to open, close, or inactivate in response to membrane depolarization and alters their response to repetitive stimuli in an activity-dependent manner. These forms of channel regulation have an important impact on neurotransmission (Catterall, 2000; Tedford and Zamponi, 2006). Following brief overviews of Ca^{2+} channel structure/function and presynaptic plasticity, this article reviews progress toward understanding the cellular and molecular mechanisms that modulate the activity of presynaptic Ca^{2+} channels, regulate synaptic transmission, and induce short-term synaptic plasticity. We focus here on activity-dependent mechanisms that have been shown to regulate synaptic transmission in functional synapses, including regulation by G protein-coupled receptors, SNARE proteins, and residual intracellular Ca^{2+}. Broader reviews of Ca^{2+} channel regulation in transfected cells and in the cell bodies of a wide range of native cell types have been presented elsewhere (Catterall, 2000; Jarvis and Zamponi, 2005; Striessnig et al., 2006; Tedford and Zamponi, 2006).

Calcium Channels

Ca^{2+} currents in different cell types have diverse physiological roles and pharmacological properties, and an alphabetical nomenclature has evolved for the distinct classes of Ca^{2+} currents (Tsien et al., 1988). N-type, P/Q-type, and R-type Ca^{2+} currents require strong depolarization for activation (Tsien et al., 1991) and are blocked by specific polypeptide toxins from snail and spider venoms (Miljanich and Ramachandran, 1995). N-type and P/Q-type Ca^{2+} currents are observed primarily in neurons, where they initiate neurotransmission at most fast conventional synapses (Catterall, 2000; Dunlap et al., 1995; Olivera et al., 1994). The Ca^{2+} channels that have been characterized biochemically are composed of four or five distinct subunits (Figure 1A) (Catterall, 2000; Takahashi et al., 1987). The α_1 subunit of 190–250 kDa is the largest subunit, and it incorporates the conduction pore, the voltage sensors and gating apparatus, and most of the known sites of channel regulation by second messengers, drugs, and toxins. The α_1 subunit is composed of about 2000 amino acid residues organized in four homologous domains (I–IV). Each domain of the α_1 subunit consists of six transmembrane α helices (S1 through S6) and a membrane-associated P loop between S5 and S6. Intensive studies of the structure and function of the related pore-forming subunits of Na^+, Ca^{2+}, and K^+ channels have led to identification of their principal functional domains (Yu et al., 2005). The S1 through S4 segments serve as the voltage sensor module (Figure 1B, yellow), whereas transmembrane segments S5 and S6 in each domain and the P loop between them form the pore module (Figure 1B, green). The large intracellular segments of Ca^{2+} channels serve as a signaling platform for Ca^{2+}-dependent regulation of neurotransmission, as discussed below.

The α_1 subunits are associated with four distinct auxiliary protein subunits (Catterall, 2000) (Figures 1A and 1B). The intracellular β subunit is a hydrophilic protein of 50–65 kDa. The transmembrane, disulfide-linked $\alpha_2\delta$ subunit complex is encoded by a single gene, but the resulting prepolypeptide is posttranslationally cleaved and disulfide-bonded to yield the mature α_2 and δ subunits. A γ subunit having four transmembrane segments is a component of skeletal muscle Ca^{2+} channels, and related subunits are expressed in heart and brain. The auxiliary subunits of Ca^{2+} channels have an important influence on their function (Dolphin, 2003; Hofmann et al., 1999). $Ca_V\beta$ subunits greatly enhance cell surface expression of the α_1 subunits and

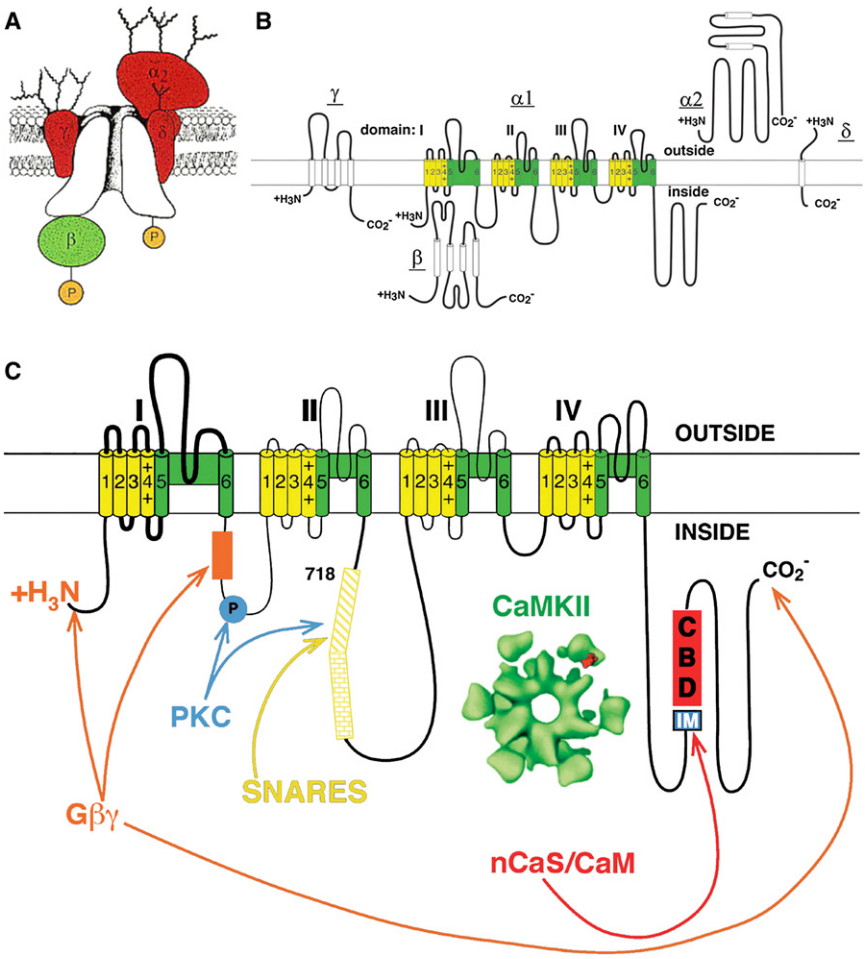

Figure 1. Subunit Structure of Ca_V Channels
(A and B) The subunit composition and structure of high-voltage-activated Ca^{2+} channels are illustrated. (B) Predicted helices are depicted as cylinders. The lengths of lines correspond approximately to the lengths of the polypeptide segments represented. The voltage-sensing module is illustrated in yellow and the pore-forming module in green. (C) The sites of interaction of different regulatory proteins on the intracellular surface of the α_1 subunit of Ca_V2 channels are illustrated.

shift their kinetics and voltage dependence of activation and inactivation. The $\alpha_2\delta$ subunits also enhance cell surface expression of α_1 subunits, but have smaller and less consistent effects on the kinetics and voltage dependence of gating (Davies et al., 2007). The γ subunits do not increase cell surface expression of Ca_V channels and, in some cases, reduce it substantially. The functional role of the γ subunits of Ca^{2+} channels is the least well-defined. Although these four auxiliary subunits modulate the functional properties of the Ca^{2+} channel complex, the pharmacological and physiological diversity of Ca^{2+} channels arises primarily from the existence of multiple α_1 subunits.

Ca^{2+} channel α_1 subunits are encoded by ten distinct genes in mammals, which are divided into three subfamilies by sequence similarity (Catterall, 2000; Ertel et al., 2000; Snutch and Reiner, 1992). Division of Ca^{2+} channels into these three subfamilies is phylogenetically ancient, as single representatives of each are found in the *C. elegans* genome. The Ca_V2 subfamily members ($Ca_V2.1$, $Ca_V2.2$, and $Ca_V2.3$) conduct P/Q-type, N-type, and R-type Ca^{2+} currents, respectively (Catterall, 2000; Ertel et al., 2000; Olivera et al., 1994; Snutch and Reiner, 1992).

The Presynaptic Ca^{2+} Current and Neurotransmission

Ca^{2+} entering neurons through $Ca_V2.1$ and $Ca_V2.2$ channels is primarily responsible for initiating synaptic transmission at fast conventional synapses (Dunlap et al., 1995; Olivera et al., 1994). $Ca_V2.2$ channels, which conduct N-type Ca^{2+} current, are most important at synapses formed by neurons of the peripheral nervous system. In contrast, $Ca_V2.1$ channels, which conduct P/Q-type Ca^{2+} currents, play the major role at most synapses formed by neurons of the mammalian central nervous system. However, in some central synapses, including a subset of inhibitory interneurons of the hippocampus (Poncer et al., 1997), $Ca_V2.2$ channels are predominant in neurotransmitter release.

Ca^{2+} entry through a single Ca^{2+} channel can trigger vesicular release (Stanley, 1993), and Ca^{2+}-triggered synaptic vesicle exocytosis depends on the assembly of the SNARE complex, in which the vesicle-associated v-SNARE protein synaptobrevin (VAMP) interacts with two plasma-membrane-associated t-SNARE proteins, SNAP-25 and syntaxin-1 (Bajjalieh and Scheller, 1995; Sollner et al., 1993; Sudhof, 1995, 2004). Maturation into a release-ready SNARE complex requires synaptotagmin, an integral Ca^{2+}-binding protein of the synaptic vesicle membrane that provides Ca^{2+}-dependent regulation of the fusion machinery. Ca^{2+} influx into the presynaptic terminal binds to the Ca^{2+} sensor, synaptotagmin, and the SNARE complex changes conformation from a *trans* to a *cis* state, resulting in the fusion of apposing membranes and the release of neurotransmitter.

Neurotransmitter release occurs in two phases: a fast synchronous (phasic) component and a slow asynchronous (tonic) component (Atluri and Regehr, 1998; Barrett and Stevens, 1972; Goda and Stevens, 1994; Hubbard, 1963; Rahamimoff and Yaari, 1973). Both forms of transmission are Ca^{2+} dependent. Synchronous release driven by the precisely timed presynaptic Ca^{2+} current results in a large, fast postsynaptic response (Llinas et al., 1981; Sabatini and Regehr, 1996). The slower asynchronous component, resulting from residual Ca^{2+} remaining in the terminal after an action potential, provides a basal or tonic level of neurotransmitter release at many synapses (Atluri and Regehr, 1998; Hagler and Goda, 2001; Lu and Trussell, 2000). Neurotransmitter release is proportional to the third or fourth power of Ca^{2+} entry (Augustine et al., 1987; Dodge and Rahamimoff, 1967; Katz and Miledi, 1970; Zucker and Regehr,

2002). Thus, regulation of presynaptic Ca^{2+} channels provides a sensitive and efficient means to regulate neurotransmitter release, as a 2-fold change in the presynaptic Ca^{2+} current results in an 8- to 16-fold change in exocytosis.

Synaptic Plasticity

Neurons fire at frequencies ranging from less than once per second (1 Hz) to several hundred Hz. Changes in firing rate induce different forms of synaptic plasticity that alter the amplitude of both synchronous and asynchronous components of the postsynaptic response (Hagler and Goda, 2001; Lu and Trussell, 2000; Zucker and Regehr, 2002). Short-term synaptic plasticity, which occurs on a timescale of milliseconds to minutes, regulates the activity of neural networks and information processing in the nervous system (Abbott and Regehr, 2004; Katz and Miledi, 1968; Zucker and Regehr, 2002). Short-term plasticity typically reflects a presynaptic change in neurotransmitter release (Del Castillo and Katz, 1954; Katz and Miledi, 1968; Zucker and Regehr, 2002). Short-term plasticity can result in synaptic enhancement through three processes—facilitation, augmentation, and posttetanic potentiation (PTP)—that vary in duration (Figure 2A) (Zucker and Regehr, 2002). It can also reduce neurotransmission, resulting in synaptic depression (Figure 2A) (Zucker and Regehr, 2002). The molecular mechanisms mediating the various forms of short-term plasticity are still a topic of debate, but all of these forms of short-term plasticity are Ca^{2+} dependent (Katz and Miledi, 1968; Zucker and Regehr, 2002).

Katz and Miledi proposed that residual Ca^{2+} remaining in the synapse after an action potential acts to enhance synaptic transmission (Katz and Miledi, 1968; Zucker and Regehr, 2002). This model is supported by more recent experiments in which introduction of the slow Ca^{2+} chelator ethylene glycol-bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid (EGTA) into the presynaptic terminal reduces synaptic enhancement (Atluri and Regehr, 1996; Habets and Borst, 2005; Hochner et al., 1991; Korogod et al., 2005; Regehr et al., 1994; Van der Kloot and Molgo, 1993). The simplest explanation is that residual Ca^{2+} binds to the Ca^{2+} sensor for exocytosis and increases neurotransmitter release (Katz and Miledi, 1968). However, more recent data indicate that residual Ca^{2+} acts on Ca^{2+}-binding protein(s) other than the sensor for neurotransmitter release to enhance synaptic transmission (Blatow et al., 2003; Felmy et al., 2003; Muller et al., 2007; Sippy et al., 2003; Tsujimoto et al., 2002).

Short-term synaptic plasticity has two mechanistic elements: (1) the source and regulation of the residual Ca^{2+} that initiates the process and (2) the effector mechanism(s) that respond to residual Ca^{2+} and enhance neurotransmitter release. Two major effector mechanisms that may contribute to synaptic facilitation have been proposed. In one mechanism, high-affinity presynaptic Ca^{2+} buffers, such as calbindin-D28K and parvalbumin, are partially saturated by residual Ca^{2+} remaining after an action potential. Thus, when another action potential follows in close succession, more of the entering Ca^{2+} remains free and available to act on the normal Ca^{2+} sensor(s) for neurotransmitter release, presumably the synaptotagmins (Blatow et al., 2003; Felmy et al., 2003; Muller et al., 2007). In this case, the effector mechanism of residual Ca^{2+} is occupancy of high-affinity Ca^{2+} buffers. In the second type of mechanism, residual Ca^{2+} binds to a Ca^{2+} sensor other than that for neurotransmitter release to increase the probability of release. Activation of this "facilitation sensor" may increase Ca^{2+} entry (Mochida et al., 2008; Tsujimoto et al., 2002) and thereby enhance neurotransmitter release according to the power law or may directly modulate the vesicular release machinery to enhance neurotransmitter release (Dittman et al., 2000; Sippy et al., 2003).

Augmentation and PTP require longer trains of stimuli than facilitation and are defined by their longer decay time constants (augmentation τ = 5–10 s; PTP τ = 30 s to minutes) (Figure 2A) (Zucker and Regehr, 2002), but they may represent overlapping

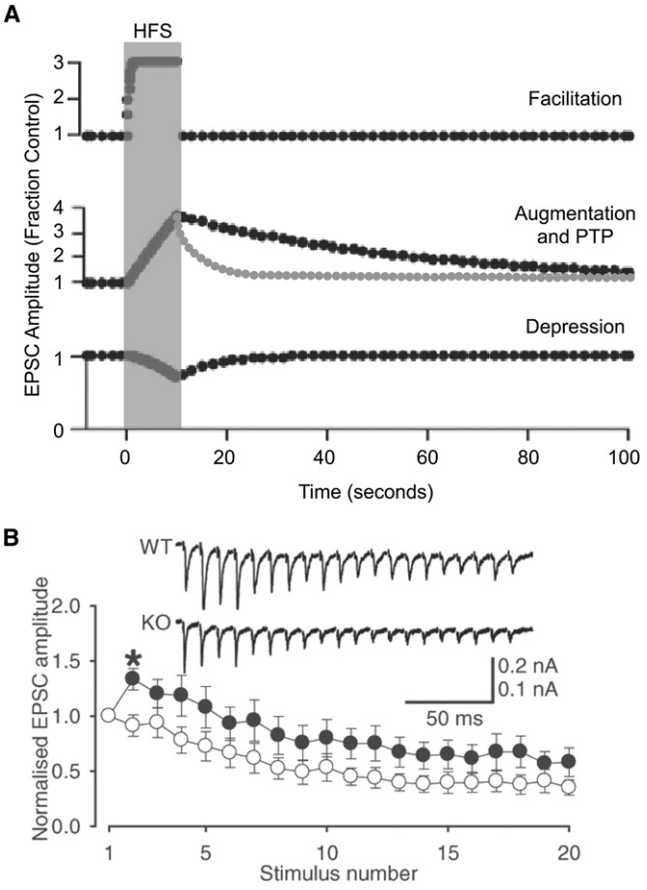

Figure 2. Multiple Forms of Short-Term Synaptic Plasticity

(A) Simulated experiment showing the relative rise and decay times for multiple forms of short-term synaptic plasticity. Excitatory postsynaptic currents were evoked at 0.5 Hz versus time with tetanic stimulation (HFS, 10 Hz for 10 s) beginning at time 0. Adapted from Zucker and Regehr (2002). Tetanic stimulation required to induce short-term plasticity varies from synapse to synapse. Augmentation (gray) decays more rapidly than PTP (black).

(B) Facilitation and depression of synaptic transmission at the calyx of Held. EPSCs recorded from principal neurons in an auditory nucleus in the brainstem receiving input from the calyx of Held in a solution containing 1 mM Ca^{2+} and 2 mM Mg^{2+} were evoked by a train of 20 stimuli at 100 Hz. EPSCs were recorded from slices of either wild-type (●) mice or mice lacking P/Q channels (○). EPSCs in wild-type neurons show facilitation that is absent in neurons lacking P/Q channels, suggesting that facilitation is caused by P/Q channels. Adapted from Ishikawa et al. (2005).

physiological processes. Augmentation is caused by an increase in the probability of vesicle release rather than by an increase in the size of the readily releasable pool of vesicles (Stevens and Wesseling, 1999). The rate of Ca^{2+} clearance from the synapse can determine whether augmentation or PTP occurs (Korogod et al., 2005; Zucker and Regehr, 2002). Residual Ca^{2+} that accumulates during the long stimuli that induce augmentation and PTP is eliminated from the synapse by the Ca^{2+}-ATPase and the Na^+/Ca^{2+} exchanger. Long trains of action potentials increase intracellular Ca^{2+} and Na^+ concentrations and slow the rate of Ca^{2+} clearance by Na^+/Ca^{2+} exchange or even drive it in reverse. Residual Ca^{2+} driving PTP can also result from the slow efflux of mitochondrial or endoplasmic reticulum Ca^{2+} that accumulates during tetanic stimulation (Lin et al., 1998; Narita et al., 2000; Tang and Zucker, 1997).

Synaptic depression reduces the strength of synaptic transmission during repeated stimuli, whether delivered as closely paired stimuli (paired-pulse depression) or as trains of stimuli. It is thought that depression primarily results from depletion of the pool of readily releasable vesicles (Zucker and Regehr, 2002). Electron microscopic studies directly demonstrate depletion of the total pool of synaptic vesicles, but only after long (several minutes) trains of stimuli (Dickinson-Nelson and Reese, 1983). Depletion and recovery of the readily releasable pool of synaptic vesicles, as defined by high-sucrose treatment or long depolarization, is correlated with depression and recovery of synaptic responses following trains of action potentials (Rosenmund and Stevens, 1996; Wu and Borst, 1999). However, physiological studies show that vesicle depletion does not fully account for rapid synaptic depression at some synapses (Sullivan, 2007; Xu and Wu, 2005). Decreased release probability caused by decreased Ca^{2+} entry (Forsythe et al., 1998; Xu and Wu, 2005), or changes downstream of Ca^{2+} entry (Wu and Borst, 1999) have been proposed. Therefore, like facilitation, augmentation, and PTP, multiple mechanisms contribute to synaptic depression, and their relative roles remain under debate.

Presynaptic Ca^{2+} Channel Signaling Complexes

Ca^{2+} entering neurons through Ca^{2+} channels forms a transient microdomain of high Ca^{2+} concentration in the presynaptic nerve terminal (Llinás et al., 1992; Smith et al., 1993; Stanley, 1997). Neurotransmitter release is initiated within 200 μs after the arrival of the action potential. Exocytosis of synaptic vesicles requires high Ca^{2+} concentration, with a threshold of 10 μM and near-maximal activation at 50 μM (Schneggenburger and Neher, 2005). SNARE proteins and other intracellular proteins that bind Ca^{2+} to initiate and regulate synaptic transmission must be located near Ca^{2+} channels in order to receive the Ca^{2+} signal. In many cases, this close localization is achieved by direct interaction with the intracellular domains of Ca^{2+} channels, which serve as signal transduction platforms for cytosolic Ca^{2+} signaling (Catterall, 2000). The signaling complexes of presynaptic Ca^{2+} channels contain SNARE proteins involved in exocytosis, G proteins involved in feedback regulation of Ca^{2+} channels, and many Ca^{2+}-binding proteins involved in regulation of channel activity and initiation of Ca^{2+}-dependent responses, including short-term synaptic plasticity.

Interactions of Presynaptic Ca^{2+} Channels with SNARE Proteins

Both $Ca_V2.1$ and $Ca_V2.2$ channels colocalize densely with syntaxin-1 at the presynaptic nerve terminals (Cohen et al., 1991; Westenbroek et al., 1992, 1995). These channels can be isolated as a complex with SNARE proteins (Bennett et al., 1992; Leveque et al., 1994; Yoshida et al., 1992). The plasma membrane SNARE proteins syntaxin-1A and SNAP-25, but not the synaptic vesicle SNARE synaptobrevin, specifically interact with the $Ca_v2.2$ channel by binding to the intracellular loop between domains II and III (L_{II-III}) of the $\alpha_1 2.2$ subunit (Figure 1C) (Sheng et al., 1994) at the *syn*aptic *p*rotein *int*eraction (*synprint*) site. This interaction is Ca^{2+} dependent, with maximal binding at 20 μM Ca^{2+} and reduced binding at lower or higher Ca^{2+} concentrations (Sheng et al., 1996), suggesting sequential steps of association and dissociation of SNARE proteins with Ca_V2 channels as a function of Ca^{2+} concentration. Two peptide segments separated by a flexible linker within the *synprint* site independently bind both syntaxin-1A and SNAP-25 (Yokoyama et al., 2005). $Ca_V2.1$ channels have an analogous *synprint* site, and different channel isoforms have distinct interactions with syntaxin and SNAP-25 (Kim and Catterall, 1997; Rettig et al., 1996), which may confer specialized regulatory properties that contribute to synaptic modulation. The molecular interaction between syntaxin and presynaptic $Ca_V2.2$ channels has been observed in intact nerve terminals by molecular imaging and correlation analysis (Li et al., 2004b).

Synaptotagmin-1, -2, -3, and -9 serve as the Ca^{2+} sensors for the fast, synchronous neurotransmitter release (Geppert et al., 1994; Sudhof, 2004; Xu et al., 2007). Synaptotagmin-1 contains two homologous C2 domains, which bind Ca^{2+} to initiate synchronous transmitter release (Sudhof, 2004). The C2B domain of synaptotagmin-1 binds to the *synprint* sites of both $Ca_V2.1$ and $Ca_V2.2$ channels (Sheng et al., 1997). Moreover, syntaxin interacts competitively with either *synprint* or synaptotagmin in a Ca^{2+}-dependent manner, such that at low Ca^{2+} concentrations syntaxin-1 binds *synprint*, whereas at higher concentrations (>30 μM) its association with synaptotagmin increases. The sequential Ca^{2+}-dependent binding of syntaxin to the *synprint* site and then to synaptotagmin in vitro may reflect stepwise protein interactions that occur during exocytosis (Sheng et al., 1996).

Several protein kinases are localized in presynaptic terminals and phosphorylate Ca^{2+} channels and SNARE proteins. Phosphorylation of the *synprint* peptide by protein kinase C (PKC) and Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) in vitro strongly inhibits its binding to syntaxin-1A and SNAP-25 (Yokoyama et al., 1997). The two separate segments of the *synprint* site that each bind syntaxin-1 and SNAP-25 in vitro are regulated by PKC phosphorylation at serines 774 and 898 and by CaMKII phosphorylation at serines 784 and 896, respectively (Yokoyama et al., 2005). Each phosphorylation site controls syntaxin-1 and SNAP-25 binding to half of the *synprint* site (Yokoyama et al., 2005). These studies suggest that phosphorylation of the *synprint* site by PKC or CaMKII may serve as a biochemical switch controlling the SNARE-*synprint* interaction. This mechanism provides a potential functional link between neurotransmitter-activated protein phosphorylation and tethering docked synaptic vesicles in an optimal position to respond to the Ca^{2+} signal from presynaptic Ca^{2+} channels.

Regulation of Ca^{2+} Channels by SNARE Proteins

In addition to linking presynaptic Ca^{2+} channels to the vesicle release machinery, syntaxin-1A and SNAP-25 also regulate channel function. Coexpression of syntaxin-1A and/or SNAP-25 with $Ca_V2.1$ or $Ca_V2.2$ channels reduces the availability of the channels to open and shifts the voltage dependence of inactivation toward more negative membrane potentials (Bezprozvanny et al., 1995; Wiser et al., 1996; Zhong et al., 1999). The inhibitory effects of syntaxin on $Ca_V2.2$ channels can be reversed by coexpressing SNAP-25 (Jarvis and Zamponi, 2001; Wiser et al., 1996), and the inhibitory effects of SNAP-25 on $Ca_V2.1$ channels can be relieved by coexpressing synaptotagmin-1 (Wiser et al., 1997; Zhong et al., 1999). Relief of inhibition of Ca^{2+} channels by formation of a complete synaptotagmin/SNARE complex favors Ca^{2+} influx through Ca_V2 channels having docked synaptic vesicles nearby that are ready for release, thus providing a potential mechanism to increase the release probability of synaptic vesicles that are docked close to Ca_V2 channels.

The *synprint* site binds to the entire H3 helix in the cytoplasmic domain of syntaxin-1A (Bezprozvanny et al., 2000; Sheng et al., 1994, 1996). However, the transmembrane region and only a short segment within the H3 helix are critical for channel modulation (Bezprozvanny et al., 2000). Deletion of the *synprint* site weakened the modulation of the channels by syntaxin-1A, but did not abolish it, arguing that the *synprint* site acts as an anchor in facilitating channel modulation but is not required for modulatory action.

SNARE protein modulation of Cav2 channels is also regulated by protein phosphorylation. PKC phosphorylation blocks the negative shift of steady-state inactivation of $Ca_V2.2$ channels caused by syntaxin, possibly by altering the interaction of syntaxin-1 with the *synprint* site (Jarvis and Zamponi, 2001). PKC activation in transfected tsA-201 cells does not completely dissociate syntaxin-1A from the channels but does completely reverse the negative shift of the voltage dependence of inactivation caused by syntaxin-1A (Yokoyama et al., 2005).

Many presynaptic proteins regulate the synaptic vesicle cycle via interactions with the SNARE proteins, and these interactions have potential impacts on the modulation of the SNARE-Ca_V2 channel complex. Rab-interacting molecule (RIM), an active zone protein that is required for synaptic transmission and is implicated in synaptic plasticity, interacts with the *synprint* region in vitro (Coppola et al., 2001; Hibino et al., 2002). In addition, RIM interacts with $Ca_V\beta$ subunits and shifts the voltage dependence of inactivation to more positive membrane potentials, increasing Ca^{2+} channel activity (Kiyonaka et al., 2007). In the neuroendocrine cell line PC12, interaction of RIM with $Ca_V2.2$ channels increases docking of neurotransmitter-containing vesicles (Kiyonaka et al., 2007). Regulation of presynaptic Ca^{2+} channel function and vesicle docking by RIM provides an additional potential pathway to increase the release probability of synaptic vesicles docked close to Ca_V2 channels.

Regulation of Presynaptic Ca^{2+} Channels by Interaction with G Proteins

G protein-coupled receptors in presynaptic nerve terminals bind released neurotransmitters and provide negative feedback to inhibit presynaptic N-type and P/Q-type Ca^{2+} currents and thereby reduce neurotransmitter release (Hille, 1994; Ikeda and Dunlap, 1999). Autoreceptors in one nerve terminal bind neurotransmitter(s) released from that terminal, whereas other G protein-coupled receptors in the same nerve terminal may respond to neurotransmitters released by nearby nerve terminals from other neurons. Most neurotransmitters inhibit Ca^{2+} currents in this manner, including acetylcholine, glutamate, GABA, biogenic amines, and many neuropeptides. Negative regulation of neurotransmitter release through inhibition of Ca^{2+} currents is very potent because of the power law of synaptic transmission. The most prominent form of G protein-induced inhibition causes a positive shift in the voltage dependence of activation of the Ca^{2+} current, which can be reversed by strong positive depolarization (Figure 3A) (Bean, 1989; Marchetti et al., 1986; Tsunoo et al., 1986). $G\beta\gamma$ subunits released from heterotrimeric G proteins of the G_i/G_o class, which are sensitive to inhibition by

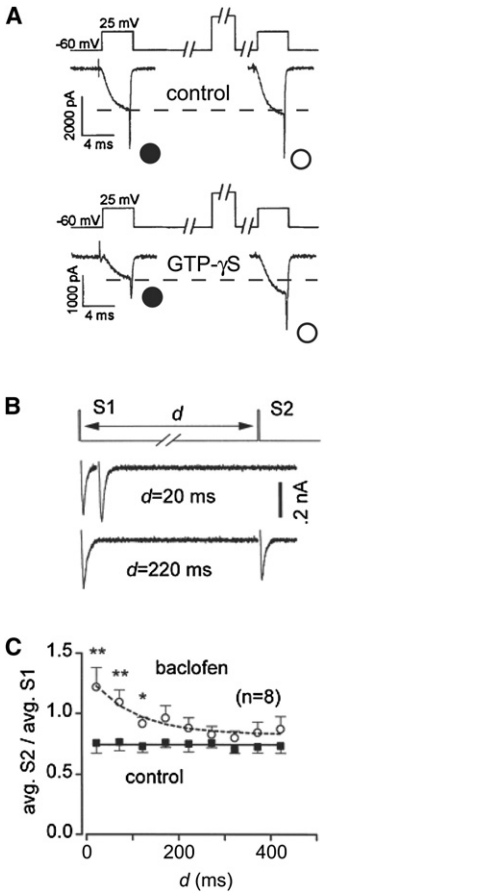

Figure 3. G Protein Regulation of Ca^{2+} Channels and Synaptic Transmission

(A) Activation of G proteins by intracellular GTP-γS induced facilitation of Ba^{2+} currents conducted by $Ca_V2.1$ channels expressed in tsA-201 cells following a conditioning prepulse to 100 mV for 10 s. Adapted from Herlitze et al. (1996). (B and C) Relief of synaptic depression and resulting synaptic enhancement induced by activation of G proteins with extracellular baclofen, a GABA-B receptor agonist, in microisland cultures of hippocampal neurons. Adapted from Brody and Yue (2000). (B) Stimulus protocol (top) and example traces recorded in the presence of baclofen (bottom). (C) Averaged data showing synaptic depression in control cells at all intervals measured and initial facilitation in the presence of baclofen.

pertussis toxin, are usually responsible for this form of Ca^{2+} channel inhibition (Hille, 1994; Ikeda and Dunlap, 1999). Reversal of this inhibition by depolarization provides a point of intersection between chemical and electrical signal transduction at the synapse and can potentially provide novel forms of short-term synaptic plasticity that do not rely on residual Ca^{2+}.

Studies in transfected nonneuronal cells and in neurons revealed that this form of Ca^{2+} channel regulation is caused by binding of G protein $\beta\gamma$ subunits directly to the Ca^{2+} channel (Herlitze et al., 1996; Ikeda, 1996). Extensive studies have implicated three sites of interaction with Ca^{2+} channel α_1 subunits (Figure 1C): the N terminus (Canti et al., 1999), the intracellular loop connecting domains I and II ($L_{I\text{-}II}$ [Herlitze et al., 1997; Zamponi et al., 1997]), and the C terminus (Delmas et al., 2005; Furukawa et al., 1998; Li et al., 2004a; Qin et al., 1997). The sites in the N terminus and $L_{I\text{-}II}$ exert the most potent effects.

In addition to this widespread, voltage-dependent inhibition of Ca_V2 channels by direct interaction with G proteins, many neurons also exhibit voltage-independent inhibition of Ca_V2 channels that is dependent on intracellular signaling pathways and involves multiple protein kinases (Dunlap et al., 1995; Hille, 1994; Strock and Diverse-Pierluissi, 2004). Voltage-independent regulation by G proteins often involves the G_q family of G proteins, which regulate the levels of phosphatidylinositide lipids by inducing hydrolysis of phosphatidylinositol bisphosphate via activation of phospholipase C enzymes (Delmas et al., 2005). Regulation of Ca^{2+} channels by this pathway has not yet been shown to modulate synaptic transmission, but it is likely that new information on this subject will appear in the near future.

Regulation of the $Ca_v2.2$ channels also involves interplay between *synprint* site interactions and second messenger modulation of Ca^{2+} channels by G proteins. Syntaxin-1A is required for G protein inhibition of presynaptic Ca^{2+} channels in intact neuronal terminals (Stanley and Mirotznik, 1997), as cleavage of syntaxin-1A by botulinum toxin prevents G protein modulation of presynaptic Ca^{2+} channels in chick calyx synapses. Further studies (Jarvis et al., 2000) showed that the *synprint* site and $G\beta$ subunit bind to distinct portions of syntaxin-1A and that expression of syntaxin-1A is a prerequisite for tonic G protein inhibition of the $Ca_V2.2$ channels.

Regulation of Presynaptic Ca^{2+} Channels by Ca^{2+} and Calmodulin

L-type Ca^{2+} currents in cardiac myocytes have long been known to be regulated by Ca^{2+}-dependent inactivation (Yue et al., 1990), but N-type and P/Q-type Ca^{2+} currents in neuronal cell bodies typically do not show this form of Ca^{2+}-dependent regulation (Chaudhuri et al., 2005; Mochida et al., 2008). In contrast, Ca^{2+}-dependent inactivation of presynaptic P/Q-type currents is observed at the calyx of Held, a giant synapse in an auditory nucleus in the brainstem (Forsythe et al., 1998). Similarly, with a level of internal buffering (0.5 mM EGTA) similar to the cytosol, Ca^{2+}-dependent inactivation is also observed for $Ca_v2.1$ channels transfected in nonneuronal cells (Lee et al., 1999, 2000) (Figure 4A). These results suggest that the high density of Ca^{2+} channels in the active zones of nerve terminals and in transfected cells is necessary to support Ca^{2+}-dependent inactivation of $Ca_V2.1$ channels, whereas the lower density in neuronal cell bodies is not. Thus, it seems that Ca^{2+} entry through a single $Ca_V2.1$ channel is not sufficient to cause its inactivation and therefore that global increases in Ca^{2+} mediated by multiple nearby Ca^{2+} channels are required for Ca^{2+}-dependent inactivation of these channels. With low concentrations of EGTA (0.5 mM), Ca^{2+}-dependent inactivation is also observed for $Ca_v2.2$ and $Ca_v2.3$ channels (Liang et al., 2003). Therefore, all three Ca_v2 family channels show Ca^{2+}-dependent inactivation in response to global increases in Ca^{2+}.

During trains of depolarizations, P/Q-type Ca^{2+} currents increase in size during the first pulses due to facilitation and then inactivate in a pulsewise manner (Cuttle et al., 1998; Lee et al., 2000) (Figures 2B and 4A). Both facilitation and inactivation are prevented when Ba^{2+} is the permeant ion (Figure 4A) and when Ca^{2+} is rapidly chelated by BAPTA. However, inactivation, but not facilitation, is prevented by a high intracellular concentration of EGTA (10 mM) (Lee et al., 2000). These results indicate that the facilitation process has higher affinity and/or more rapid binding of Ca^{2+} than the inactivation process. This dual feedback regulation may permit activity-dependent sharpening of presynaptic Ca^{2+} signals by enhancing the Ca^{2+} transients in response to the early action potentials in a train and reducing the Ca^{2+} transients in response to the later action potentials. This would have the effect of increasing release probability during short trains of impulses followed by reduction of release probability during long trains.

Both Ca^{2+}-dependent facilitation and inactivation of $Ca_V2.1$ channels are dependent on calmodulin (CaM) (DeMaria et al., 2001; Lee et al., 1999, 2000). In the C-terminal domain of the full-length $\alpha_1 2.1$ subunit, CaM interacts with a modified IQ-like motif, which begins with the sequence isoleucine-methionine (IM) rather than isoleucine-glutamine (IQ), and with a second nearby downstream site (the CaM binding domain, CBD), both of which are involved in Ca^{2+}-dependent feedback regulation of full-length $Ca_v2.1$ channels (Figure 1C) (DeMaria et al., 2001; Lee et al., 1999, 2003). Ca^{2+}-dependent facilitation is impaired by mutations in CaM that prevent binding of Ca^{2+} at the C-terminal EF-hands (DeMaria et al., 2001; Lee et al., 2003). In contrast, Ca^{2+}-dependent inactivation is preferentially inhibited by mutations of the Ca^{2+}-binding sites in the N-terminal lobe of CaM (DeMaria et al., 2001; Lee et al., 2003).

Recent studies using multiphoton microscopy and a microfluidic mixer have revealed two sequential, rapid conformation changes of CaM upon binding Ca^{2+}, which may be the molecular basis for its biphasic regulation of Ca^{2+} channel function (Park et al., 2008). The first transition in the C-terminal lobe proceeds with a time constant of ~0.5 ms. The second transition in the N-terminal lobe proceeds with a time constant of ~20 ms. These absolute rate constants are faster than facilitation and inactivation of $Ca_V2.1$ channels, but these lobe-specific conformational transitions in CaM would be expected to be slowed by its binding to a regulatory target in which it must induce additional conformational changes as part of its regulatory mechanism. The 40-fold difference in the rates of the two conformational changes in CaM approximates the difference in rates of facilitation and inactivation, supporting the idea that they may indeed represent the molecular mechanism for biphasic regulation of $Ca_V2.1$ channels.

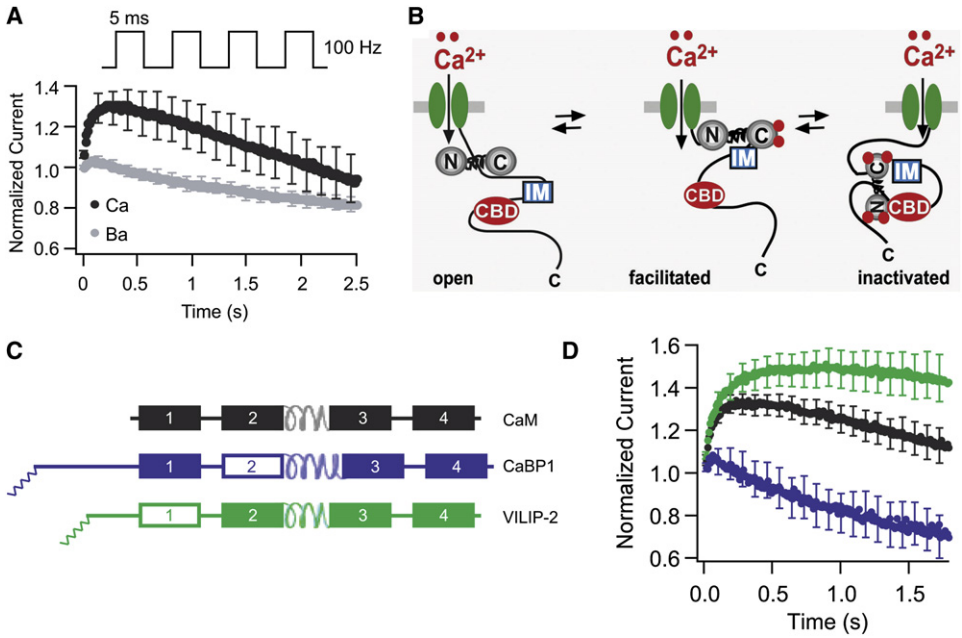

Figure 4. Regulation of $Ca_V2.1$ Channels by CaM and CaM-like nCaS Proteins

(A) Ca^{2+}/CaM-dependent regulation of $Ca_V2.1$ channels expressed in tsA-201 cells. Currents were evoked by 5 ms depolarizations from −80 to +20 mV (Ca^{2+}) or +10 mV (Ba^{2+}) at 100 Hz. Ca^{2+} currents (black) show initial Ca^{2+}-dependent facilitation followed by Ca^{2+}-dependent and voltage-dependent inactivation. The regulation remaining in Ba^{2+} currents is voltage dependent.

(B) Model for sequential Ca^{2+}/CaM-dependent facilitation and inactivation. Local rises in intracellular Ca^{2+} activate the two C-terminal Ca^{2+}-binding EF-hands of CaM, which strengthens or initiates an interaction with the IQ-like motif causing facilitation. Following prolonged Ca^{2+} entry, global rises in intracellular Ca^{2+} allow CaM to become fully liganded where it interacts with both the IQ-like motif and CaM-binding domain (CBD) to produce inactivation. Adapted from Lee et al. (2003).

(C) Schematic representation of CaM and two CaM-like nCaS proteins, CaBP1 and VILIP-2, showing N-terminal myristoyl group (CaBP1 and VILIP-2), EF-hands, and central helical linker. Filled boxes represent Ca^{2+}-binding EF-hands. Open boxes represent EF-hands that are inactive in binding Ca^{2+}.

(D) Averaged normalized Ca^{2+} currents elicited as in panel (A) in tsA-201 cells expressing $Ca_V2.1$ channels modulated by endogenous CaM (black) or overexpressed CaBP1 (blue) or VILIP-2 (green).

(C and D) Adapted from Lautermilch et al. (2005).

The two lobes of CaM interact differentially with the two CaM-binding subsites in the C-terminal domain of $Ca_V2.1$ channels (Lee et al., 2003). Mutations of the IQ-like domain primarily impair facilitation, indicating that they interact primarily with the C-terminal lobe of CaM (DeMaria et al., 2001; Lee et al., 2003). In contrast, mutations of the CBD primarily impair Ca^{2+}-dependent inactivation (Lee et al., 2003), suggesting that they interact primarily with the lower affinity N-terminal lobe of CaM. These results lead to a model in which rapid, high-affinity binding of Ca^{2+} to the C-terminal lobe of CaM and interaction with the IQ-like motif of $Ca_V2.1$ channels cause facilitation, whereas subsequent slower and/or lower-affinity binding of Ca^{2+} to the N-terminal lobe of CaM and interaction with the CBD of $Ca_V2.1$ channels cause inactivation (Figure 4B).

It is interesting to compare this biphasic regulation of $Ca_V2.1$ channels in synapses and transfected cells to Ca^{2+}/CaM-dependent regulation of other Ca_V1 and Ca_V2 channels. Both $Ca_V2.2$ and $Ca_V2.3$ channels have Ca^{2+}-dependent inactivation that depends on global Ca^{2+} and the N-terminal lobe of CaM, but neither of these channels shows marked Ca^{2+}/CaM-dependent facilitation of Ca^{2+} channel activity (Liang et al., 2003). $Ca_V1.2$ channels, which conduct L-type Ca^{2+} currents in cardiac myocytes, endocrine cells, and neurons, have striking Ca^{2+}/CaM-dependent inactivation (Peterson et al., 1999; Zühlke et al., 1999). This form of Ca^{2+}-dependent inactivation of $Ca_V1.2$ channels relies on local Ca^{2+} and involves high-affinity interaction of the C-terminal lobe of CaM with a classical IQ domain in the C-terminal domain (Peterson et al., 1999). However, a second novel CaM-interacting domain in the N-terminal domain of $Ca_V1.2$ channels can transform the regulation by the N-terminal lobe of CaM such that it also mediates Ca^{2+}-dependent inactivation in response to local rather than global Ca^{2+} (Dick et al., 2008; Zhou et al., 2005). $Ca_V1.3$ channels, which conduct L-type Ca^{2+} currents in endocrine cells and in the nerve terminals of specialized ribbon synapses in auditory hair cells, also have rapid Ca^{2+}/CaM-dependent inactivation when expressed in nonneuronal cells (Cui et al., 2007), and this rapid inactivation is controlled by an alternatively spliced autoregulatory domain in the distal C terminus of these channels (Singh et al., 2008). In contrast, $Ca_V1.4$ channels, which conduct L-type Ca^{2+} currents in the synaptic terminals of photoreceptors, have no Ca^{2+}/CaM-dependent inactivation, but deletion of the distal C-terminal autoregulatory domain reveals latent Ca^{2+}/CaM-dependent inactivation (Singh et al., 2006). Evidently, the form of Ca^{2+}/CaM-dependent regulation of Ca_V1 channels is tailored to their specific functional roles in local Ca^{2+}-dependent signal transduction by interactions of the C-terminal IQ domain with other regulatory domains in the N and C termini.

Regulation of Ca_V2 Channels by Ca^{2+} Regulatory Proteins

CaM is the primordial member of a large family of related Ca^{2+} sensors (CaS), some of which are expressed specifically in neurons (nCaS) (Burgoyne and Weiss, 2001; Haeseleer et al., 2002). Like CaM, these nCaS proteins possess four EF-hand Ca^{2+}-binding motifs organized in two lobes connected by a central α helix (Figure 4C). At least one of the two N-terminal EF-hands of nCaS is nonfunctional in Ca^{2+} binding due to changes in amino acid sequence, and the nCaS proteins are myristolyated at their N terminus (Figure 4C) (Burgoyne and Weiss, 2001). nCaS are similar enough to displace CaM from shared binding sites in the α_1 subunits of Ca_V channels, but different enough to confer distinct forms of regulation.

CaBP1 is a member of a subfamily of nCaS highly expressed in the brain and retina (Haeseleer et al., 2000) and is colocalized with presynaptic $Ca_V2.1$ channels in some synapses (Lee et al., 2002). Like CaM, CaBP1 binds to the CBD of $\alpha_1 2.1$, but its binding is Ca^{2+} independent (Lee et al., 2002). It causes rapid inactivation that is independent of Ca^{2+}, and it does not support Ca^{2+}-dependent facilitation (Lee et al., 2002) (Figure 4D). A second nCaS that modulates $Ca_V2.1$ channels is visinin-like protein-2 (VILIP-2), which is highly expressed in the neocortex and hippocampus (Burgoyne and Weiss, 2001). When cotransfected with $Ca_V2.1$ channels in mammalian cells, VILIP-2 increases Ca^{2+}-dependent facilitation, but inhibits Ca^{2+}-dependent inactivation (Lautermilch et al., 2005) (Figure 4D). These effects of VILIP-2 may involve displacement of CaM from the CBD, because both the CBD and IQ-like motifs of $\alpha_1 2.1$ are required for binding of VILIP-2. Thus, CaBP-1 and VILIP-2 bind to the same site as CaM but have opposite effects on $Ca_V2.1$ channel activity (Figure 4D). In a presynaptic terminal, these differential effects on facilitation and inactivation of the P/Q-type Ca^{2+} current would substantially change the encoding properties of the synapse in response to trains of action potentials (Abbott and Regehr, 2004).

How can VILIP-2 and CaBP1 have such opposing effects on $Ca_V2.1$ function? The mechanism for these effects is not yet clear, but both proteins must be myristolyated on the N termini to have their distinctive regulatory effects (Few et al., 2005). Because CaM is not myristoylated at its N terminus and does not have an inactive N-terminal EF-hand, it is tempting to propose that differential interactions of the inactive EF-hands, which differ in their positions in the N-terminal domains of these Ca^{2+}-binding proteins (Figure 4C), with the IQ-like domain and CBD of $Ca_V2.1$ channels are responsible for their differences in action. The divergent actions of nCaS proteins on $Ca_V2.1$ channels may fine-tune the function and regulatory properties of presynaptic P/Q-type Ca^{2+} currents, allowing a greater range of input-output relationships and short-term plasticity at different synapses.

Ca^{2+}/CaM-dependent protein kinase II (CaMKII) is the most prominent Ca^{2+}/CaM-dependent regulator of the postsynaptic response, including long-term potentiation (Kennedy et al., 1990; Luscher et al., 2000; Schulman and Greengard, 1978; Shepherd and Huganir, 2007). CaMKII also regulates presynaptic function (Llinas et al., 1985, 1991), including effects on synaptic plasticity (Chapman et al., 1995; Lu and Hawkins, 2006). Recent studies show that CaMKII binds to a specific site in the C-terminal domain of cardiac $Ca_V1.2$ channels (Hudmon et al., 2005). CaMKII also binds to $Ca_V2.1$ channels and enhances their activity by slowing inactivation and positively shifting the voltage dependence of inactivation (Jiang et al., 2008). Surprisingly, these effects on the function of $Ca_V2.1$ channels require binding of an activated form of CaMKII, but do not require the catalytic activity of the enzyme (Jiang et al., 2008). It was proposed that noncatalytic regulation of $Ca_V2.1$ channels by bound CaMKII serves to enhance the activity of those channels that have the effector of the Ca^{2+} signal (i.e., CaMKII) in position to bind entering Ca^{2+} and respond to it (Jiang et al., 2008). This form of regulation is similar to regulation by SNARE proteins and RIM, as described above; that is, the activity of the $Ca_V2.1$ channels is increased by formation of a complete SNARE complex with synaptotagmin and RIM bound (Kiyonaka et al., 2007; Zhong et al., 1999), which serves as the effector of the Ca^{2+} signal for initiation of synaptic transmission. This "*effector checkpoint*" mechanism serves to focus the Ca^{2+} entry through those Ca^{2+} channels whose effectors (i.e., a complete SNARE complex and CaMKII) are bound and ready to respond (Jiang et al., 2008).

In addition to the wide range of protein interactions that regulate Ca_V2 channels, emerging evidence suggests that alternative splicing of their mRNAs also has an important impact on the diversity of their regulation. Alternative splicing of exons encoding the C-terminal domain of $Ca_V2.1$ channels alters regulation by Ca^{2+} and CaM (Chaudhuri et al., 2004), and alternative splicing of exons encoding the intracellular linker between domains I and II of $Ca_V2.2$ channels controls their regulation by opiates and tyrosine phosphorylation (Altier et al., 2007; Raingo et al., 2007). It is likely that these studies reveal only the tip of the iceberg of the enormous regulatory diversity and complexity introduced by alternative splicing of Ca_V2 channels.

Calcium Channel Signaling Complexes and Synaptic Transmission

Analysis of the functional effects of presynaptic Ca^{2+} channel regulation in synaptic transmission is an important step toward understanding these regulatory processes in their physiological context, but these experiments are made challenging by the difficulty of specifically manipulating Ca^{2+} channel interactions in the presynaptic terminal. Two approaches have been successful: injection of specific peptide antagonists of protein interactions into the presynaptic cell and expression of specific peptide antagonists or mutant Ca_V2 channels from cDNA injected into the presynaptic cell.

SNARE-*Synprint* Interactions in Synaptic Transmission

Peptides derived from the *synprint* site competitively inhibit interactions between SNARE proteins and Ca_V2 channels in vitro. Injection of *synprint* peptides from $Ca_V2.2$ channels into presynaptic superior cervical ganglion neurons (SCGNs) in culture significantly reduced the excitatory postsynaptic response by competitive uncoupling of the endogenous Ca^{2+} channel-SNARE interaction at nerve terminals (Mochida et al., 1996). Rapid, synchronous synaptic transmission was selectively inhibited following the injection, while late asynchronous release and paired-pulse facilitation were increased (Figure 5). Similarly, injection of the *synprint* peptides into embryonic

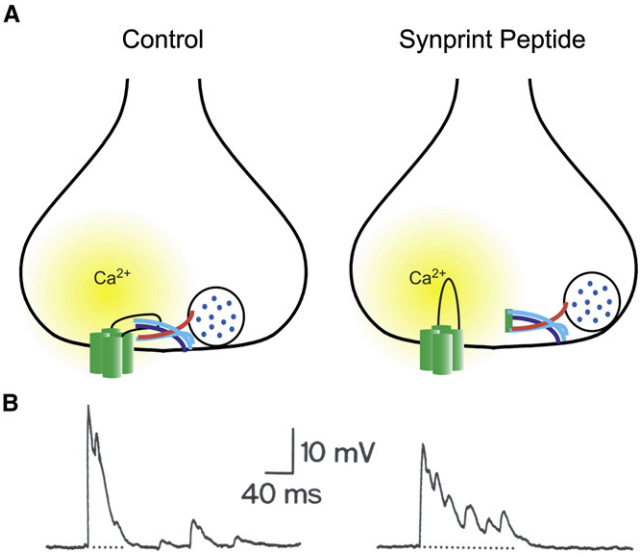

Figure 5. Asynchronous Release Is Increased in the Presence of Synprint Peptides

(A) Model showing SNARE complex: syntaxin (dark blue), SNAP-25 (light blue), and synaptobrevin (red) on docked vesicles interacting with presynaptic Ca^{2+} channels (left) or synprint peptide (right).

(B) Synaptic transmission recorded from pairs of cultured SCG neurons is synchronous in the absence of synprint peptides (left), and asynchronous release is increased after synprint peptides were dialyzed into the presynaptic neuron for 20 min. EPSPs were evoked by three presynaptic current pulses at 100 Hz. Adapted from Mochida et al. (1996).

Xenopus spinal neurons reduced transmitter release substantially when cells were stimulated in an extracellular solution containing physiological Ca^{2+} concentration (Rettig et al., 1997). Increasing the external Ca^{2+} concentrations effectively rescued this inhibition, implying that the Ca^{2+} channels are competitively displaced away from docked synaptic vesicles by the injected *synprint* peptides, and this effect can be overcome by flooding the presynaptic terminal with Ca^{2+} from the extracellular pool (Rettig et al., 1997).

A requirement for close coupling of $Ca_V2.1$ channels to synaptic vesicles for efficient release of neurotransmitters also emerged from studies at the calyx of Held. P/Q-type Ca^{2+} currents are more effective than N-type Ca^{2+} currents and R-type Ca^{2+} currents in eliciting neurotransmitter release at this synapse in postnatal day 7 rats where all three channels are expressed (Inchauspe et al., 2007; Iwasaki et al., 2000; Wu et al., 1999). The high efficiency of P/Q-type Ca^{2+} currents in initiating neurotransmitter release is correlated with the close localization of docked vesicles near $Ca_V2.1$ channels, as assessed by immunocytochemistry (Wu et al., 1999).

At first glance, it seems that interactions of Ca_V2 channels with SNARE proteins have two opposing effects: tethering synaptic vesicles near the point of Ca^{2+} entry would increase synaptic transmission, whereas enhancing Ca_V2 channel inactivation would reduce synaptic transmission. These effects were dissected by use of competing *synprint* peptides and mutant syntaxin in *Xenopus* neuromuscular junctions in vivo (Keith et al., 2007). Injection of competing *synprint* peptides into developing neuromuscular junctions reduced the basal efficiency of synaptic transmission, as reflected in increased paired-pulse facilitation and reduced quantal content of synaptic transmission. Evidently, the effect of the *synprint* peptide to reduce linkage of docked synaptic vesicles to Ca_V2 channels is predominant, because its potentially opposing effect to relieve inhibition of Ca^{2+} channels by SNARE proteins would be occluded by SNAP-25 and synaptotagmin for the subset of channels interacting with a complete SNARE complex that could participate in vesicle release. In contrast, overexpression of a syntaxin mutant that is unable to regulate $Ca_V2.2$ channels, but still binds to them (Bezprozvanny et al., 2000), increased the efficiency of synaptic transmission, as reflected in reduced paired-pulse facilitation and increased quantal content (Keith et al., 2007). In this case, the syntaxin mutant likely relieves enhanced inactivation of $Ca_V2.2$ channels caused by endogenous syntaxin, thereby increasing Ca^{2+} entry and synaptic transmission, but does not alter linkage of docked synaptic vesicles to $Ca_V2.2$ channels. These results demonstrate a bidirectional regulation of synaptic transmission in vivo by interactions of SNARE proteins with $Ca_V2.2$ channels.

Regulation of Synaptic Transmission by G Protein Modulation of Ca_V2 Channels

Classical work has provided many examples of potent negative regulation of neurotransmission by receptor activation (Hille, 1992). This form of modulation by GABA acting at GABA-B receptors and glutamate acting at metabotropic glutamate receptors has been directly demonstrated at the calyx of Held with parallel measurements of Ca^{2+} currents and synaptic transmission (Kajikawa et al., 2001; Takago et al., 2005; Takahashi et al., 1996). Similar modulation by cannabinoids acting at CB1 receptors has been demonstrated by optical measurements of Ca^{2+} transients together with electrophysiological recordings of synaptic transmission at the nerve terminals of the parallel fibers of cerebellar granule cells innervating Purkinje neurons (Brown et al., 2004). Depolarization relieves this form of inhibition of Ca^{2+} channels (Figure 3A), leading to the prediction that trains of action potentials would reverse receptor/G protein inhibition of synaptic transmission. This prediction has been tested in microisland cultures of hippocampal neurons in which autapses are formed by single hippocampal pyramidal neurons (Brody and Yue, 2000). In this preparation, trains of action potential-like stimuli relieve the inhibition of synaptic transmission caused by activation of GABA-B receptors with baclofen or adenosine A1 receptors with 2-Cl^-adenosine. This relief of inhibition resulted in facilitation of synaptic transmission in the range of 1.5-fold, which was blocked by inhibition of $Ca_V2.1$ channels, but not $Ca_V2.2$ channels, with peptide neurotoxins. Regulator of G protein signaling-2 (RGS-2) relieves G protein inhibition of presynaptic Ca^{2+} channels, resulting in a higher basal probability of release and consequently a reduction in paired-pulse facilitation ratio (Han et al., 2006). These results demonstrate that voltage-dependent relief of G protein inhibition of Ca_V2 channels in paired pulses and trains can cause synaptic facilitation. However, this form of facilitation does not contribute to short-term synaptic plasticity at the synapse of parallel fibers onto Purkinje neurons (Kreitzer and Regehr, 2000). Moreover, classical paired-pulse facilitation of synaptic transmission and facilitation by

trains of depolarizations is induced by increases in residual intracellular Ca^{2+} (Katz and Miledi, 1968; Zucker and Regehr, 2002); therefore, relief of G protein-induced inhibition of $Ca_V2.1$ channels is unlikely to contribute substantially to these forms of short-term synaptic plasticity.

Regulation of Presynaptic Ca^{2+} Channels and Short-Term Synaptic Plasticity

Short-term synaptic plasticity of neurotransmitter release from presynaptic terminals shapes the response of postsynaptic neurons to bursts of impulses and is crucial for fine-grained encoding of information in the nervous system (Abbott and Regehr, 2004; Zucker and Regehr, 2002). Regulation of presynaptic Ca^{2+} channels by Ca^{2+}, CaM, and nCaS proteins causes facilitation and inactivation of the Ca^{2+} current (Figure 4). The steep dependence of neurotransmitter release on the presynaptic Ca^{2+} current predicts that these types of regulation should profoundly alter short-term synaptic plasticity. Recent studies have shown that this form of regulation of presynaptic Ca^{2+} channels plays a crucial role in short-term synaptic plasticity. Differential expression of these Ca^{2+}-dependent regulatory proteins may provide a means of cell-type-specific regulation of presynaptic Ca^{2+} channels and short-term synaptic plasticity.

The residual Ca^{2+} that controls short-term synaptic enhancement is not thought to act directly on the Ca^{2+} sensor for neurotransmitter release (Blatow et al., 2003; Dittman et al., 2000; Felmy et al., 2003; Muller et al., 2007; Sippy et al., 2003; Tsujimoto et al., 2002). The presynaptic Ca^{2+} current was not detectably altered during synaptic facilitation at the squid giant synapse, as studied with three-microelectrode voltage-clamp methods (Augustine et al., 1987; Charlton et al., 1982). However, several lines of evidence indicate that residual Ca^{2+} regulates the presynaptic Ca^{2+} current in vertebrate synapses. At the calyx of Held, the presynaptic Ca^{2+} current can be recorded directly by voltage-clamp methods. In synapses from young mice, a combination of P/Q- and N-type currents shows activity-dependent facilitation that predicts the amount of synaptic facilitation according to the power law (Inchauspe et al., 2004; Ishikawa et al., 2005; Figure 2B). In contrast, both facilitation of the presynaptic Ca^{2+} current and synaptic facilitation are lost in $Ca_V2.1$ knockout mice (Inchauspe et al., 2004, 2007; Ishikawa et al., 2005). The N-type Ca^{2+} currents conducted by $Ca_V2.2$ channels that remain in the calyx of Held of these $Ca_V2.1$ knockout mice are less efficient in mediating synaptic transmission, do not show facilitation, and do not support facilitation of synaptic transmission, but they are more sensitive to modulation by G protein-coupled receptors (Inchauspe et al., 2007). Together, these results suggest that activity-dependent increases in presynaptic $Ca_V2.1$ channel currents cause synaptic facilitation and that $Ca_V2.2$ channel currents are not increased by facilitation but have strong G protein regulation.

Augmentation and PTP also rely on residual Ca^{2+}. The relationship between presynaptic Ca^{2+} transients and PTP was measured at the calyx of Held using fluorescent Ca^{2+} indicators. After induction of PTP, the presynaptic Ca^{2+} influx increased to an extent that predicted PTP when the power law of neurotransmission was applied (Habets and Borst, 2006). Furthermore, the presynaptic Ca^{2+} transient decayed with a time course that paralleled the decay of PTP (Habets and Borst, 2006). These results are consistent with a role for regulation of presynaptic Ca^{2+} channels in PTP at the calyx of Held.

In order to critically test the role of activity-dependent regulation of presynaptic Ca^{2+} channels in short-term synaptic plasticity, it is necessary to compare synaptic transmission initiated by wild-type $Ca_V2.1$ channels and by mutant channels with impaired Ca^{2+}-dependent regulation. This is a challenging experiment because endogenous Ca^{2+} channels are present in high concentration in presynaptic active zones, and replacement of them with exogenously expressed mutant Ca^{2+} channels is difficult. Cultured superior cervical ganglion (SCG) neurons have no endogenous P/Q-type Ca^{2+} currents. They can be transfected successfully with cDNA encoding $Ca_V2.1$ channels by microinjection, and these transfected neurons then have P/Q-type Ca^{2+} currents in their cell bodies and synapses (Mochida et al., 2003a). Whole-cell voltage-clamp recordings of transfected $Ca_V2.1$ channels at the cell body show that they undergo Ca^{2+}-dependent facilitation (Mochida et al., 2008). The contribution of these transfected $Ca_V2.1$ channels to initiation of synaptic transmission can be isolated by blocking the endogenous N-type Ca^{2+} current specifically with ω-conotoxin GVIA. EPSPs recorded in the postsynaptic neurons in response to action potentials elicited in the presynaptic neuron are 30%–40% of the size of those initiated by endogenous N-type Ca^{2+} currents (Mochida et al., 2003a).

In these transfected SCG neurons, mutations in the IQ-like motif of $Ca_V2.1$ channels that prevent Ca^{2+}-dependent facilitation of Ca^{2+} currents recorded from the cell bodies of SCG neurons also reduced paired-pulse facilitation of EPSPs at SCG synapses (Figure 6) (Mochida et al., 2008). Thus, CaM and other CaS proteins may respond to residual Ca^{2+} as "facilitation sensors" by binding to the IQ-like motif in the C terminus of $Ca_V2.1$ channels and causing Ca^{2+}-dependent facilitation of the presynaptic Ca^{2+} current.

Transfected SCG neurons have a form of synaptic enhancement of intermediate duration, which is similar to augmentation. This enhancement of synaptic strength is also reduced by mutations that prevent Ca^{2+}-dependent facilitation of $Ca_V2.1$ channels by blocking association of CaS proteins with the IQ-like motif (Mochida et al., 2008). In contrast, PTP induced by longer trains of stimuli was not significantly affected (Mochida et al., 2008). Thus, synaptic facilitation and augmentation in transfected SCG neurons share a common mechanism: activation of Ca^{2+} sensor proteins by residual Ca^{2+} increases "instantaneous" Ca^{2+} entry via $Ca_V2.1$ channels in an activity-dependent manner, which in turn increases neurotransmitter release according to the power law of neurotransmission.

What do these findings mean in terms of the residual Ca^{2+} hypothesis? These results argue that residual Ca^{2+} acts on a facilitation sensor, a CaM-like CaS protein, which causes Ca^{2+}-dependent facilitation of presynaptic Ca^{2+} currents by binding to the IQ-like motif of $Ca_V2.1$ channels and thereby increases Ca^{2+} entry. In this model, Ca^{2+}-dependent facilitation of $Ca_V2.1$ channels is the effector mechanism for residual Ca^{2+} in short-term plasticity. This increase in Ca^{2+} entry via $Ca_V2.1$ channels directly mediates multiple forms of synaptic enhancement—facilitation, augmentation, and perhaps PTP in some synapses—by

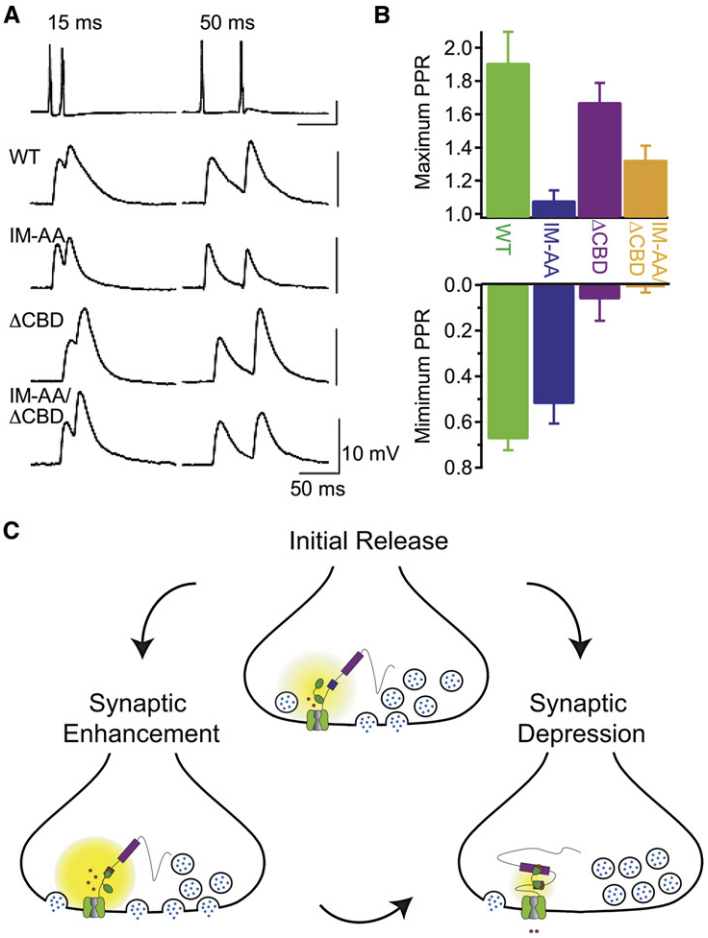

Figure 6. PPF and PPD Mediated by $Ca_V2.1$ Channel Facilitation and Inactivation

(A) Representative EPSPs in which $Ca_V2.1$ channels are the only active channels in the presynaptic terminal were evoked by paired action potentials with 15 ms or 50 ms interstimulus interval. The amplitude of the second EPSP was measured from the intercept of the decay of the first EPSP with the rise of the second EPSP.

(B) Paired-pulse ratio (PPR, P2/P1) at the interstimulus interval (tested range 10–200 ms) of maximum facilitation (top) and maximum depression (bottom). Adapted from Mochida et al. (2008).

(C) Model illustrating $Ca_V2.1$-mediated mechanisms of synaptic enhancement (facilitation and augmentation) and synaptic depression. In synaptic facilitation and augmentation, facilitation of the Ca^{2+} current caused by residual Ca^{2+} binding to CaS proteins interacting with the IQ-like motif increases Ca^{2+} entry and subsequently neurotransmitter release. In synaptic depression, CaS proteins interact with the CBD to cause channel inactivation and reduce Ca^{2+} entry, thus reducing neurotransmitter release.

increasing neurotransmitter release according to the power law. While facilitation of presynaptic Ca^{2+} channels may contribute to all three forms of synaptic enhancement at some synapses (Ishikawa et al., 2005; Mochida et al., 2008), augmentation and PTP likely represent overlapping processes that are caused by different combinations of mechanisms at different synapses (Zucker and Regehr, 2002), and residual Ca^{2+} may engage multiple effector mechanisms for these slower forms of synaptic plasticity. A good candidate for an additional effector mechanism for PTP is the Ca^{2+}/phospholipid-dependent kinase protein kinase C (PKC), which has been shown to play a role in PTP at synapses between the CA3-CA1 neurons of the hippocampus (Brager et al., 2003) and at the calyx of Held (Korogod et al., 2007).

Synaptic depression is generally thought to be a result of vesicle depletion during trains of action potentials (Zucker and Regehr, 2002). At the calyx of Held, synaptic depression caused by a decrease in release probability is a prominent feature of transmission (Wu and Borst, 1999). Physiological studies indicate that Ca^{2+}-dependent inactivation of the presynaptic Ca^{2+} current, rather than vesicle depletion, causes rapid synaptic depression for stimuli ranging from 2 to 30 Hz (Forsythe et al., 1998; Xu and Wu, 2005). Introduction of peptides that disrupt CaM interactions reduced both Ca^{2+}-dependent inactivation of the P/Q-type Ca^{2+} current and paired-pulse depression of synaptic transmission (Xu and Wu, 2005). In contrast, stimulation at 100 Hz induced more robust synaptic depression that was likely caused by vesicle depletion (Xu and Wu, 2005). The rate of Ca/CaM-dependent inactivation and its role in synaptic depression is greatest at immature calyx of Held synapses and diminishes in mature synapses (Inchauspe et al., 2007; Nakamura et al., 2008). Synaptic vesicles remaining after complete depression of synaptic transmission at the calyx of Held are resistant to release by action potentials (Moulder and Mennerick, 2005). However, this remaining pool of vesicles can be released with normal Ca^{2+} sensitivity by uncaging Ca^{2+} throughout the nerve terminal, suggesting that these remaining synaptic vesicles are located at a distance from $Ca_V2.1$ channels such that the Ca^{2+} entering during the action potential does not reach them (Wadel et al., 2007).

In transfected SCG neurons (Mochida et al., 2008), deletion of the CaM-binding domain (CBD) in the intracellular C terminus of full-length $Ca_V2.1$ channels, a mutation known to reduce Ca^{2+}-dependent inactivation in heterologous expression systems (Lee et al., 1999, 2003), blocked paired-pulse depression (Figure 6) and reduced synaptic depression during trains up to 40 Hz (Mochida et al., 2008), suggesting that binding of CaS proteins to the CBD induces inactivation of presynaptic $Ca_V2.1$ channels, resulting in rapid synaptic depression. During trains at 30 Hz and 40 Hz, a slower phase of synaptic depression was observed that may have been caused by vesicle depletion. Together, the data from the calyx of Held and transfected SCG neurons suggest that Ca^{2+}-dependent inactivation of presynaptic Ca^{2+} channels, mediated by Ca^{2+}-dependent binding of CaS proteins to the C-terminal of Ca_V2 channels, is a conserved mechanism generating rapid synaptic depression evoked by stimuli of physiological rate and duration ($\leq$40 Hz for 1 s) at multiple synapses.

Results of studies with cultured hippocampal neurons also support an important role for modulation of Ca_V2 channels in synaptic plasticity. Expression of $Ca_V\beta$ subunits has a strong influence on synaptic facilitation in hippocampal synapses through their effects on Ca^{2+} channel function (Xie et al., 2007). Overexpression of $Ca_V\beta4a$ favors facilitation whereas overexpression of $Ca_V\beta2a$ favors depression (Xie et al., 2007). Similarly, modulation

of the rate of inactivation of $Ca_V2.2$ channels by overexpression of the 14-3-3 protein also modulates the rate of synaptic depression in cultured hippocampal neurons, adding further support for a role of inactivation of presynaptic Ca^{2+} channels in rapid synaptic depression (Li et al., 2006).

Regulation of Synaptic Transmission and Synaptic Plasticity by Ca^{2+} Regulatory Proteins

The short-term facilitation and depression of synaptic transmission observed in transfected SCG synapses resembles regulation of $Ca_V2.1$ channels by CaM (Mochida et al., 2008). Because regulation of $Ca_V2.1$ channels by CaBP1 and VILIP-2 is strikingly different from CaM (Lautermilch et al., 2005; Lee et al., 2002), it would be predicted that differential expression of different nCaS proteins in different synapses would result in different ratios of synaptic facilitation and depression. Consistent with this idea, injection of the nCaS protein NCS-1, a close relative of VILIP-2, into presynaptic nerve terminals at the calyx of Held synapse promotes facilitation of P/Q-type Ca^{2+} currents, and activity-dependent facilitation of P/Q-type Ca^{2+} currents at this synapse can be prevented by injection of NCS-1-inhibitor peptides (Tsujimoto et al., 2002). Similarly, NCS-1 can enhance facilitation of synaptic transmission in cultured hippocampal neurons (Sippy et al., 2003). Although these experiments did not identify the site of NCS-1 action, these effects of NCS-1 on P/Q-type Ca^{2+} currents and synaptic facilitation suggest that local expression of nCaS proteins can markedly alter Ca^{2+} channel activity, synaptic function, and synaptic plasticity, thereby fine-tuning the encoding properties of different classes of synapses. It will be of great interest to determine whether NCS-1 is acting at the same site as CaM, CaBP1, and VILIP-2 in regulating $Ca_V2.1$ channels and to define the range of regulatory properties of different nCaS proteins on the activity of Ca_V2 channels, synaptic transmission, and synaptic plasticity.

CaMKII has an important presynaptic effect on synaptic plasticity (Chapman et al., 1995; Lu and Hawkins, 2006), in addition to its well-known postsynaptic effects. The unexpected noncatalytic effect of CaMKII binding to enhance the activity of $Ca_V2.1$ channels described above (Jiang et al., 2008) raises the possibility of a role for this noncatalytic mechanism of regulation of $Ca_V2.1$ channels in presynaptic plasticity. Studies of mutant mice with knockout and knockin mutations in CaMKII provide evidence for a possible synaptic counterpart of this novel form of channel regulation. Deletion of CaMKII increases augmentation and decreases synaptic fatigue (Chapman et al., 1995; Hojjati et al., 2007), whereas loss-of-function CaMKII mutations do not (Hojjati et al., 2007), indicating that loss of CaMKII protein, but not loss of CaMKII activity, increases augmentation and reduces depression. As augmentation is measured as a ratio of stimulated EPSP amplitude over basal EPSP amplitude, it is possible that loss of CaMKII protein decreases the strength of basal synaptic transmission by a noncatalytic mechanism and thereby increases the subsequent ratio of augmentation by trains of stimuli. A noncatalytic decrease in basal synaptic strength caused by CaMKII (Hojjati et al., 2007) could result from loss of its noncatalytic enhancement of the activity of $Ca_V2.1$ channels (Jiang et al., 2008) in the knockout mice. The role of CaMKII in presynaptic plasticity is an important area for future research.

Targeting of Presynaptic Ca^{2+} Channels to the Active Zone

In the postsynaptic membrane, dynamic internalization and reinsertion of glutamate receptors mediated by SNARE proteins is a crucial mechanism of regulation of synaptic strength in long-term potentiation and depression (Luscher et al., 2000). Comparable dynamic regulation of the presynaptic membrane by regulated internalization and reinsertion of Ca^{2+} channels has not yet been described. However, interactions with SNARE proteins and several other novel interacting proteins are required for efficient targeting and specific localization of presynaptic Ca^{2+} channels in active zones in the presynaptic plasma membrane.

Deletion of the *synprint* site on the $Ca_v2.1$ channel results in a substantial reduction in neurotransmitter release in transfected SCG neurons, correlated with reduced localization of the mutant channels to presynaptic terminals (Mochida et al., 2003a, 2003b). Transfer of the *synprint* sequence from $Ca_v2.1$ to $Ca_v1.2$ channels, which conduct L-type Ca^{2+} currents and are ineffective in supporting synaptic transmission, was sufficient to allow these channels to initiate synaptic transmission. Similarly, mouse pheochromocytoma cells (MPC 9/3L), which lack voltage-gated Ca^{2+} channels, require the *synprint* site of $Ca_V2.2$ channels for efficient reconstitution of secretion (Harkins et al., 2004). In addition, in transfected neurons in cell culture, localization of Ca_V2 channels to nerve terminals was substantially reduced when the *synprint* site was partially or completely deleted (Szabo et al., 2006). Finally, the SNARE protein regulator RIM increases the number of docked readily releasable vesicles in pheochromotocytoma cells through interactions with $Ca_V\beta$ subunits (Kiyonaka et al., 2007). These studies support the concept that the SNARE protein binding to Ca_V2 channels is necessary for efficient presynaptic localization and functional coupling of $Ca_v2.1$ and $Ca_v2.2$ channels to vesicle exocytosis.

While these studies show that interaction of the *synprint* site of presynaptic Ca^{2+} channels with SNARE proteins enhances the efficiency of neurotransmitter release in vertebrate neurons, three lines of evidence indicate that this interaction is unlikely to be the primary mechanism for targeting presynaptic Ca^{2+} channels to nerve terminals. First, inhibition of *synprint* interaction or deletion of the *synprint* site on both $Ca_v2.1$ and $Ca_v2.2$ channels reduces the efficiency of exocytosis but does not completely abolish synaptic transmission or hormone secretion (Harkins et al., 2004; Mochida et al., 1996, 2003b; Rettig et al., 1997). Second, although invertebrate Ca_v2 channels effectively initiate synaptic transmission, they lack a *synprint* site (Spafford et al., 2003). Third, SNARE proteins are not selectively localized at nerve terminals themselves, making it unlikely that SNARE proteins provide the primary targeting information. Evidently, interactions with other proteins are also involved in targeting and trafficking of presynaptic Ca^{2+} channels.

Consistent with this idea, the active zone scaffolding proteins Mint and CASK play critical roles in the presynaptic targeting of $Ca_V2.2$ channels in cultured mammalian neurons via direct interactions with PDZ and SH3 targeting motifs in the C-terminal tail (Maximov and Bezprozvanny, 2002). Moreover, for snail Ca^{2+} channels that lack a *synprint* site, Mint and CASK are required for targeting of presynaptic Ca^{2+} channels to the synapse (Spafford et al., 2003; Spafford and Zamponi, 2003). In addition, the

Drosophila protein *Bruchpilot*, a large coiled-coil domain structural protein, is required for formation of functional neuromuscular synapses and clustering of Ca^{2+} channels in the presynaptic membrane (Kittel et al., 2006). The presynaptic plasma membrane glycoprotein neurexin is similarly required for development of functional synapses containing presynaptic Ca^{2+} channels in mice (Missler et al., 2003). From these multiple studies, it seems that interactions with Mint, CASK, *Bruchpilot* and neurexins, as well as interactions with SNAREs and the SNARE regulator RIM, are all involved in efficient and accurate targeting and trafficking of presynaptic Ca^{2+} channels. It will be interesting to see whether these proteins also participate in dynamic regulation of Ca^{2+} channels in active zones, in analogy to the dynamic regulation of glutamate receptors in the postsynaptic membrane (Luscher et al., 2000; Shepherd and Huganir, 2007).

Because presynaptic Ca^{2+} channels must form specific interactions with multiple scaffolding and targeting proteins for efficient and accurate localization in active zones, it is possible that they must occupy a fixed number of sites in or near active zones in order to be effective in release of neurotransmitters. Evidence in favor of the idea of "slots" for presynaptic Ca^{2+} channels comes from experiments in which wild-type and mutant $Ca_V2.1$ channels were found to "compete" functionally for initiation of synaptic transmission in cultured neurons (Cao et al., 2004; Cao and Tsien, 2005). Expression of inactive mutant channels reduced the efficiency of synaptic transmission, as if they had displaced wild-type channels from a fixed number of saturable sites (Cao and Tsien, 2005). This apparent competitive interaction between wild-type and mutant channels may reflect saturable competitive interactions of transfected Ca^{2+} channels with any of their essential binding partners that are available in limited amounts in neurons, including the auxiliary $\alpha_2\delta$, β, and γ subunits; any of the individual trafficking, targeting, and scaffolding proteins discussed above; or preformed slots in the presynaptic plasma membrane containing these interacting proteins.

Our working hypothesis is that the SNARE proteins and targeting/scaffolding proteins play complementary roles at the active zone. The targeting and scaffolding proteins (Mint, CASK, *Bruchpilot* neurexin, etc.) are essential for targeting Ca_V2 channels to the presynaptic terminal and for formation of the structure of the active zone, which brings together Ca_V2 channels and other fixed components of the exocytosis machinery. No doubt the protein composition of the active zone is dynamic, like the postsynaptic density, but there is no clear evidence for that at present. We suppose that dynamic changes in active zone composition and structure would take place on the relatively slow timescale of seconds and longer. We hypothesize that the interactions of Ca_V2 channels with SNARE proteins have complementary roles. For Ca_v2 channels containing a *synprint* site, binding of SNARE proteins to newly synthesized Ca_V2 channels is necessary in a permissive sense for efficient targeting of Ca_V2 channels to presynaptic terminals, but it does not provide the primary targeting information. More importantly, dynamic interactions between Ca_V2 channels and SNARE proteins at the active zone bring docked synaptic vesicles close to the source of entering Ca^{2+} and regulate the activity of Ca_V2 channels such that those channels with nearby docked vesicles are more likely to open. These proposed complementary roles for targeting/scaffolding proteins versus SNARE proteins are also consistent with their modes of binding—high affinity and therefore relatively slowly reversible binding for targeting/scaffolding proteins versus lower affinity and therefore more rapidly reversible and dynamic binding for SNARE proteins.

Presynaptic Ca^{2+} Channels and Inherited Neurological Disease

As expected from the essential role of presynaptic Ca_V2 channels in synaptic function and plasticity and the exquisitely detailed regulation of their activity, mutations in these channels cause human diseases. Missense mutations in $Ca_V2.1$ channels cause familial hemiplegic migraine (FHM), a rare inherited form of migraine with aura and hemiparesis (Ophoff et al., 1996; Pietrobon and Striessnig, 2003). Studies of these mutations by expression in nonneuronal cells and neurons provided evidence for both loss of function owing to reduced levels of expression and gain of function owing to negatively shifted voltage dependence of activation (Hans et al., 1999; Kraus et al., 1998, 2000), suggesting a complex mechanism of action in causing migraine. FHM mutant $Ca_V2.1$ channels with reduced functional expression also reduced synaptic transmission in transfected neurons, apparently by competing with endogenous wild-type $Ca_V2.1$ channels for essential interacting proteins or slots in the presynaptic plasma membrane (Cao et al., 2004; Cao and Tsien, 2005). This apparent diversity of mutational effects was resolved in part by single-channel recording studies in transfected neurons, which revealed that all FHM mutations cause an increase in the single-channel Ca^{2+} current at the foot of the activation curve, even though their reduced level of expression decreases the peak of the whole-cell Ca^{2+} current at positive membrane potentials (Tottene et al., 2002). As activation at more negative potentials has the greatest effect on the rapid Ca^{2+} entry that initiates synchronous neurotransmitter release, this effect of FHM mutations would provide a gain of function in synaptic transmission. Moreover, it is likely that the trafficking and scaffolding mechanisms described above assure that a correct number of Ca^{2+} channels are inserted at each mature active zone, even if fewer Ca^{2+} channels are present in the cell soma, so the gain of single-channel function is likely to be the dominant effect at mature active zones. Incorporation of FHM mutations into the mouse genome has confirmed that they indeed have a gain-of-function effect in synaptic transmission and in increasing the cortical spreading depression that is a key pathological element in migraine with aura (Pietrobon, 2007; van den Maagdenberg et al., 2004). Thus, it seems most likely from the current results that the gain-of-function negative shift of the voltage dependence of activation of presynaptic $Ca_V2.1$ channels caused by FHM mutations in turn causes enhanced synaptic transmission, cortical spreading depression, and hemiplegic migraine with aura (Pietrobon, 2007).

Mutations in $Ca_V2.1$ channels also cause episodic ataxia (EA) type 2 and spinocerebellar ataxia (SCA) type 6 (Ophoff et al., 1996; Zhuchenko et al., 1997), which result from cytotoxicity to cerebellar neurons. Loss-of-function truncations and missense mutations that have dominant-negative effects are the cause of EA-2 (Guida et al., 2001; Jeng et al., 2006; Mezghrani et al.,

2008; Raike et al., 2007). Polyglutamine expansions in the C-terminal domain of $Ca_V2.1$ channels are the likely pathogenic mechanism for SCA-6. These polyglutamine expansions increased Ca^{2+} channel activity when expressed in cultured cells (Piedras-Renteria et al., 2001). However, insertion of a SCA-6 mutation into the mouse genome caused ataxia and neurodegeneration, but did not cause any change in $Ca_V2.1$ channel activity (Watase et al., 2008). The expanded $Ca_V2.1$ polyglutamine tract did accumulate with age, and the accumulation of mutant protein is the likely cause of cytotoxicity in this disease rather than gain-of-function alterations in the expression or function of Ca^{2+} channels.

The Presynaptic Ca^{2+} Channel as a Regulatory Node in Dynamic, Activity-Dependent Control of Synaptic Transmission

Regulation of synaptic transmission is central to the function of the nervous system in learning, memory, and physiological control. In molecular systems biology, regulatory networks are represented as a series of nodes with connecting regulatory pathways that lead to specific physiological endpoints (Bromberg et al., 2008; Eungdamrong and Iyengar, 2004). Nodes in such regulatory networks serve as decision points to integrate many incoming signals and initiate specific physiological events. On the postsynaptic side of excitatory synapses, receptors for glutamate are the major transmembrane signaling proteins that receive neurotransmitter and transduce its binding into electrical excitation of the postsynaptic cell. They form large signaling complexes—including SNARE proteins, scaffolding proteins, Ca^{2+}-binding proteins, and CaMKII—which send downstream signals into the postsynaptic cell and mediate feedback regulation of synaptic transmission. Dynamic regulation of function and localization of glutamate receptors mediate many complex forms of postsynaptic plasticity, including long-term potentiation of synaptic strength, long-term depression of synaptic strength, and coupling of synaptic activity to regulation of gene expression (Luscher et al., 2000; Shepherd and Huganir, 2007). Thus, postsynaptic glutamate receptors serve as a regulatory node for dynamic control of synaptic transmission on the postsynaptic side of the membrane (Weng et al., 1999).

Because of the power law relationship between Ca^{2+} entry and neurotransmitter release, regulation of the Ca^{2+} channel is a natural control point for synaptic transmission on the presynaptic side of the synapse. In analogy to glutamate receptors, presynaptic Ca^{2+} channels are the major transmembrane transducers of the electrical signal of the action potential to a chemical signal—Ca^{2+} entry and neurotransmitter release—and they form a large signaling complex containing SNARE proteins, Ca^{2+}-binding proteins, CaMKII, and scaffolding proteins. However, far less attention has been given to the possibility that the presynaptic Ca^{2+} channel may be a major site of synaptic regulation and presynaptic plasticity. Based on the work reviewed here, we propose that the presynaptic Ca^{2+} channel signaling complex serves as a regulatory node to mediate multiple layers of control of synaptic transmission, presynaptic plasticity, and feedback regulation of Ca^{2+} entry (Figure 7A), analogous to the well-established role of the glutamate receptor signaling complex at the postsynaptic side of the membrane.

Presynaptic Ca_V2 channels have three crucial functions. First, the presynaptic Ca^{2+} channel provides the rapid, spatially focused Ca^{2+} entry that initiates rapid and synchronous synaptic transmission. Second, through specific protein-protein interactions with SNARE proteins and scaffolding proteins, the presynaptic Ca^{2+} channels bring docked synaptic vesicles close to the source of Ca^{2+} entry, allowing them to respond efficiently to the microdomain of high Ca^{2+} concentration. Third, through specific protein-protein interactions with Ca^{2+}-binding proteins and CaMKII, the presynaptic Ca^{2+} channels respond to residual Ca^{2+} in nerve terminals and mediate multiple forms of short-term synaptic plasticity. Each of these Ca^{2+} channel functions serves as a substrate for regulation of neurotransmitter release in a multilayered, activity-dependent network that controls synaptic function in response to neurotransmitters, synaptic vesicle dynamics, and cytosolic Ca^{2+} (Figure 7B).

In the broadest layer of this activity-dependent regulatory system, neurotransmitters in the extracellular milieu bind to G protein-coupled receptors in the presynaptic nerve terminal, activate heterotrimeric Gi/Go proteins, catalyze release of their $G\beta\gamma$ subunits, and thereby inhibit activation of presynaptic Ca^{2+} channels and reduce the Ca^{2+} entry that initiates synaptic transmission. This broad layer of regulation allows neurotransmitters released homosynaptically from the same synaptic terminal or heterosynaptically from other synaptic terminals to regulate presynaptic function and synaptic transmission on the timescale of milliseconds to minutes. This form of regulation integrates inputs from many neurons in the control of presynaptic function.

In a second layer of activity-dependent regulation, SNARE proteins and SNARE protein regulators like synaptotagmin and RIM tune the function of presynaptic Ca^{2+} channels in response to locally docked synaptic vesicles. Interaction with free plasma membrane SNARE proteins (syntaxin or SNAP-25) inhibits channel activity, whereas formation of a complete SNARE complex with synaptotagmin and RIM bound to it relieves this inhibition and enhances Ca^{2+} channel activity. This form of regulatory interaction serves to draw docked synaptic vesicles into the microdomain of high Ca^{2+} required for their efficient exocytosis, reduce the activity of Ca^{2+} channels distant from docked vesicles, and enhance the activity of Ca^{2+} channels interacting with nearby docked synaptic vesicles through binding of SNARE proteins.

The third layer of this activity-dependent regulatory network depends on residual Ca^{2+} near the active zone and mediates classical short-term synaptic plasticity in response to trains of action potentials. This form of regulation allows information contained in the frequency and pattern of action potential generation to be transmitted to the postsynaptic cell as a change in the amplitude of the postsynaptic response. Surprisingly, recent research has provided strong evidence that synaptic enhancement by facilitation, augmentation, and possibly PTP are all mediated, at least in part, by Ca^{2+}-dependent facilitation of presynaptic $Ca_V2.1$ channels. Moreover, rapid synaptic depression during brief trains of impulses is also mediated, at least in part, by Ca^{2+}-dependent inactivation of presynaptic Ca^{2+} channels. Both of these regulatory processes depend on binding of CaM and related nCaS proteins to a bipartite regulatory site in the C-terminal domain of $Ca_V2.1$ channels. This protein interaction site

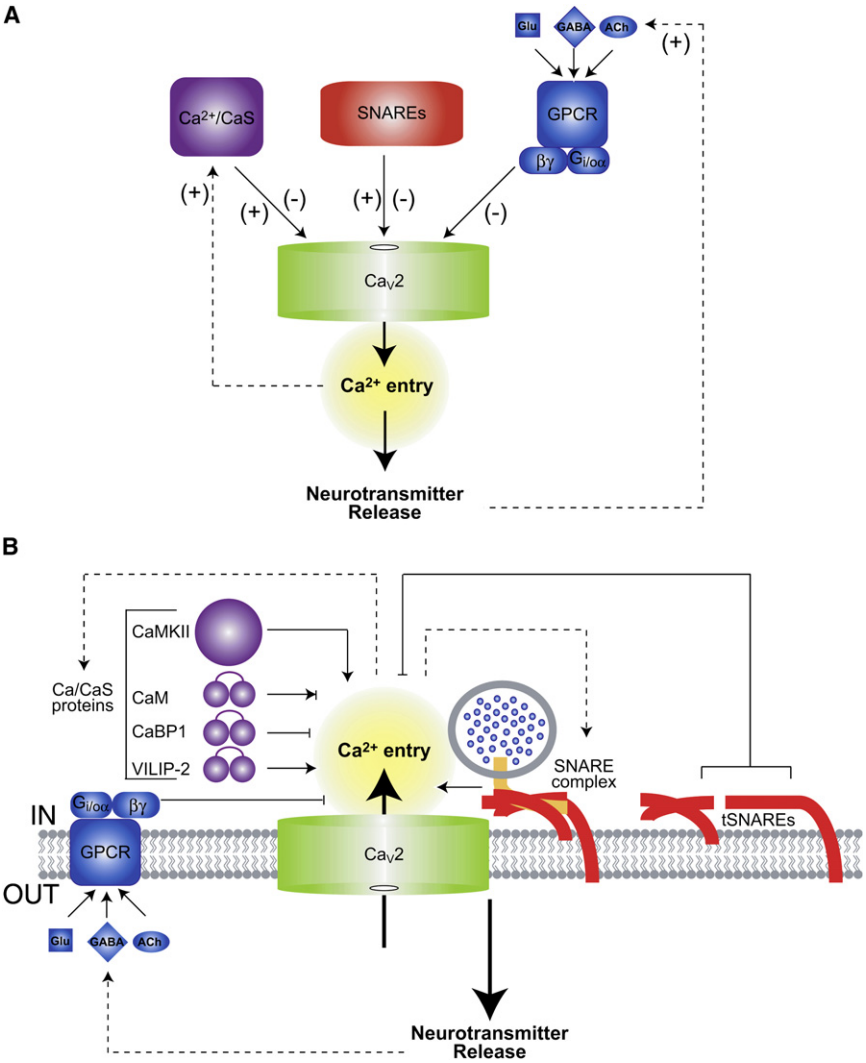

Figure 7. A Regulatory Network that Controls Neurotransmitter Release

(A) The pathway diagram of the Ca^{2+} channel regulatory network. The two major outputs generated by this regulatory network are Ca^{2+} entry into the presynaptic terminal and neurotransmitter release. The three signaling pathways regulating these outputs are (1) modulation of G protein bg subunits of the Gi/o family coupled to receptors activated by neurotransmitters like glutamate (Glu), GABA, and acetylcholine (ACh) (blue); (2) regulation by SNARE proteins either binding as individual tSNAREs (syntaxin or SNAP-25, red) or as a complete SNARE complex with its associated docked vesicle (red and orange); and (3) modulation by Ca^{2+}, CaM, and nCaS proteins as well as CaMKII (purple). Arrows indicate forward flow of regulatory information. Positive and negative regulatory effects are denoted by (+) and (−). Dashed lines indicate feedback loops.

(B) Diagram showing the details of the regulatory network and the location of its components relative to the plasma membrane. Activating interactions are indicated by arrows; interactions leading decreased channel activity are indicated by bars.

therefore serves as a key transducer of information encoded in the frequency and pattern of presynaptic action potentials into changes in the EPSPs generated in the postsynaptic cell.

Each tier of this multilayered regulatory network has been shown to be operative in multiple types of synapses in cell culture and in native synapses isolated from the calyx of Held. Therefore, it is likely that many native synapses are controlled in this way in situ. However, the integration of these three different layers of regulation of presynaptic function has not yet been extensively analyzed, and the impact of differential expression of multiple subtypes of G proteins, SNARE proteins, and CaS proteins within this regulatory network has not been extensively explored. In addition, the impact of this regulatory network on neural function and animal behavior in vivo has not yet been addressed by incorporating mutations into the genomes of mice or other model organisms. We look forward to the results of these new generations of experiments that will further define the multifaceted roles of presynaptic Ca^{2+} channels in regulation of synaptic function.

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