Fine-tuning synaptic plasticity by modulation of $Ca_V2.1$ channels with Ca^{2+} sensor proteins

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Modulation of P/Q-type Ca^{2+} currents through presynaptic voltage-gated calcium channels ($Ca_V2.1$) by binding of Ca^{2+}/calmodulin contributes to short-term synaptic plasticity. Ca^{2+}-binding protein-1 (CaBP1) and Visinin-like protein-2 (VILIP-2) are neurospecific calmodulin-like Ca^{2+} sensor proteins that differentially modulate $Ca_V2.1$ channels, but how they contribute to short-term synaptic plasticity is unknown. Here, we show that activity-dependent modulation of presynaptic $Ca_V2.1$ channels by CaBP1 and VILIP-2 has opposing effects on short-term synaptic plasticity in superior cervical ganglion neurons. Expression of CaBP1, which blocks Ca^{2+}-dependent facilitation of P/Q-type Ca^{2+} current, markedly reduced facilitation of synaptic transmission. VILIP-2, which blocks Ca^{2+}-dependent inactivation of P/Q-type Ca^{2+} current, reduced synaptic depression and increased facilitation under conditions of high release probability. These results demonstrate that activity-dependent regulation of presynaptic $Ca_V2.1$ channels by differentially expressed Ca^{2+} sensor proteins can fine-tune synaptic responses to trains of action potentials and thereby contribute to the diversity of short-term synaptic plasticity.

Neurons fire repetitively in different frequencies and patterns, and activity-dependent alterations in synaptic strength result in diverse forms of short-term synaptic plasticity that are crucial for information processing in the nervous system (1–3). Short-term synaptic plasticity on the time scale of milliseconds to seconds leads to facilitation or depression of synaptic transmission through changes in neurotransmitter release. This form of plasticity is thought to result from residual Ca^{2+} that builds up in synapses during repetitive action potentials and binds to a Ca^{2+} sensor distinct from the one that evokes neurotransmitter release (1, 2, 4, 5). However, it remains unclear how changes in residual Ca^{2+} cause short-term synaptic plasticity and how neurotransmitter release is regulated to generate distinct patterns of short-term plasticity.

In central neurons, voltage-gated calcium ($Ca_V2.1$) channels are localized in high density in presynaptic active zones where their P/Q-type Ca^{2+} current triggers neurotransmitter release (6–11). Because synaptic transmission is proportional to the third or fourth power of Ca^{2+} entry through presynaptic $Ca_V2.1$ channels, small changes in Ca^{2+} current have profound effects on synaptic transmission (2, 12). Studies at the calyx of Held synapse have provided important insights into the contribution of presynaptic Ca^{2+} current to short-term synaptic plasticity (13–17). $Ca_V2.1$ channels are required for synaptic facilitation, and Ca^{2+}-dependent facilitation and inactivation of the P/Q-type Ca^{2+} currents are correlated temporally with synaptic facilitation and rapid synaptic depression (13–17).

Molecular interactions between Ca^{2+}/calmodulin (CaM) and $Ca_V2.1$ channels induce sequential Ca^{2+}-dependent facilitation and inactivation of P/Q-type Ca^{2+} currents in nonneuronal cells (18–21). Facilitation and inactivation of P/Q-type currents are dependent on Ca^{2+}/CaM binding to the IQ-like motif (IM) and CaM-binding domain (CBD) of the $Ca_V2.1$ channel, respectively (20, 21). This bidirectional regulation serves to enhance channel activity in response to short bursts of depolarizations and then to decrease activity in response to long bursts. In synapses of superior cervical ganglion (SCG) neurons expressing exogenous $Ca_V2.1$ channels, synaptic facilitation is induced by repetitive action potentials, and mutation of the IM and CBD motifs prevents synaptic facilitation and inhibits the rapid phase of synaptic depression (22). Thus, in this model synapse, regulation of presynaptic $Ca_V2.1$ channels by binding of Ca^{2+}/CaM can contribute substantially to the induction of short-term synaptic plasticity by residual Ca^{2+}.

CaM is expressed ubiquitously, but short-term plasticity has great diversity among synapses, and the potential sources of this diversity are unknown. How could activity-dependent regulation of presynaptic $Ca_V2.1$ channels contribute to the diversity of short-term synaptic plasticity? CaM is the founding member of a large family of Ca^{2+} sensor (CaS) proteins that are differentially expressed in central neurons (23–25). Two CaS proteins, Ca^{2+}-binding protein-1 (CaBP1) and Visinin-like protein-2 (VILIP-2), modulate facilitation and inactivation of $Ca_V2.1$ channels in opposite directions through interaction with the bipartite regulatory site in the C-terminal domain (26, 27), and they have varied expression in different types of central neurons (23, 25, 28). CaBP1 strongly enhances inactivation and prevents facilitation of $Ca_V2.1$ channel currents, whereas VILIP-2 slows inactivation and enhances facilitation of $Ca_V2.1$ currents during trains of stimuli (26, 27). Molecular analyses show that the N-terminal myristoylation site and the properties of individual EF-hand motifs in CaBP1 and VILIP-2 determine their differential regulation of $Ca_V2.1$ channels (27, 29–31). However, the role of CaBP1 and VILIP-2 in the diversity of short-term synaptic plasticity is unknown, and the high density of Ca^{2+} channels and unique Ca^{2+} dynamics at the presynaptic active zone make extrapolation of results from studies in nonneuronal cells uncertain. We addressed this important question directly by expressing CaBP1 and VILIP-2 in presynaptic SCG neurons and analyzing their effects on synaptic plasticity. Our results show that CaM-related CaS proteins can serve as sensitive bidirectional switches that fine-tune the input–output relationships of synapses depending on their profile of activity and thereby maintain the balance of facilitation versus depression by the regulation of presynaptic $Ca_V2.1$ channels.

Results

CaBP1 Reduces Paired-Pulse Facilitation. CaBP1 enhances the inactivation of $Ca_V2.1$ channels, whereas VILIP-2 reduces their inactivation and enhances facilitation (26, 27). To determine whether presynaptic expression of these CaS proteins affects short-term synaptic plasticity in SCG neurons, we designed our experiments to measure synaptic transmission driven by $Ca_V2.1$ channels expressed only on the presynaptic side of the synapse, and we blocked the endogenous N-type Ca^{2+} current with ω-conotoxin GVIA. Under these conditions, synaptic transmission is mediated specifically by transfected $Ca_V2.1$ channels (22, 32). We expressed $Ca_V2.1$ channels and CaBP1 or VILIP-2

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by injecting cDNA into an identified SCG neuron, and we recorded excitatory postsynaptic potentials (EPSPs) from a neighboring synaptically connected but untransfected neuron, thereby isolating presynaptic effects. We first tested synaptic transmission under conditions of low release probability (1 mM extracellular Ca^{2+}) in which facilitation is observed in control synapses. In response to paired stimuli, we observed paired-pulse facilitation (PPF) at interstimulus intervals (ISI) beginning at 50 ms, consistent with previous findings (Fig. 1 *A* and *B*) (22). In contrast, CaBP1-expressing synapses showed paired-pulse depression (PPD) rather than PPF at the same ISI (Fig. 1 *B* and *C*). At the peak of PPF for control synapses (ISI = 80 ms), the maximum paired-pulse ratio (PPR) reached in cells cotransfected with CaBP1 was reduced significantly [WT PPR_{80ms}, 1.36 ± 0.12 (n = 23); CaBP1 PPR_{80ms}, 0.76 ± 0.12 (n = 17), $P < 0.01$] (Fig. 1*C*). Thus, a switch from facilitation to depression was evident in CaBP1-expressing synapses at intermediate ISI. In contrast, synapses expressing VILIP-2 showed PPF that was indistinguishable from PPF in controls at all ISI (Fig. 1 *B* and *C*). To confirm that the effect of CaBP1 is caused by interaction with the channel and not by a change in initial neurotransmitter release probability, we compared the amplitude of the first EPSP in control synapses, CaBP1-expressing synapses, and VILIP-2–expressing synapses. Expression of CaBP1 and VILIP-2 did not change the amplitude of the synaptic response (Fig. 1*D*). Thus, CaBP1 did not affect baseline neurotransmitter release in transfected neurons. Together, our data imply that CaBP1 blocks facilitation of the Ca^{2+} current, resulting in reduced PPF. In contrast, coexpression of VILIP-2 with $Ca_V2.1$ channels under the conditions of our paired-pulse experiments had no effect, just as we previously observed no effect for PPF of Ca^{2+} currents by VILIP-2 (27).

CaBP1 Binding to $Ca_V2.1$ Channels Is Essential for Modulation of PPF. Immunocytochemistry studies show that CaBP1 colocalizes with $Ca_V2.1$ channels and syntaxin in the CA1 region of the hippocampus and in the molecular layer of the cerebellum, although postsynaptic staining also has been observed (26). Because CaBP1 is transfected only in the presynaptic neuron of SCG synaptic pairs, its effect is limited to the presynaptic sites involved in synaptic plasticity. However, it is possible that CaBP1 may act on other presynaptic machinery involved in neurotransmitter release rather than on $Ca_V2.1$ channels. Does CaBP1 bind directly to $Ca_V2.1$ channels to block synaptic facilitation? The interaction between CaBP1 and $Ca_V2.1$ channels requires the same intracellular domain of $Ca_V2.1$ that binds CaM. To address whether CaBP1 acts directly on $Ca_V2.1$ channels, we transfected SCG neurons with mutant $Ca_V2.1$ channels in which the IM motif was mutated to AA and the CBD was deleted ($Ca_V2.1_{IM\text{-}AA/\Delta CBD}$). Coexpression of CaBP1 or VILIP-2 had no effect on these mutant $Ca_V2.1_{IM\text{-}AA/\Delta CBD}$ channels in paired-pulse experiments (Fig. 1 *E*–*G*). $Ca_V2.1_{IM\text{-}AA/\Delta CBD}$ channels showed much less PPF at intermediate ISI than did $Ca_V2.1$ channels (Fig. 1 *B* and *F*). Coexpression of CaBP1 or VILIP-2 did not alter basal neurotransmission or PPF observed for any ISI (Fig. 1 *G* and *H*). These results confirm that the effect of CaBP1 in reducing PPF results from its binding to the CaS-binding site on $Ca_V2.1$ channels.

CaBP1 Reduces Synaptic Facilitation During Trains of Activity. Activity-dependent increases in Ca^{2+} entry cause facilitation followed by inactivation of $Ca_V2.1$-channel currents (13–19). This dual regulation is caused by sequential binding of the two lobes of Ca^{2+}/CaM to the IQ-like domain and CBD of $Ca_V2.1$ channels (18, 20, 21). To investigate the effects of CaBP1 and VILIP-2 on $Ca_V2.1$ channels during trains of activity, we stimulated synapses at varying frequencies and recorded EPSPs during each

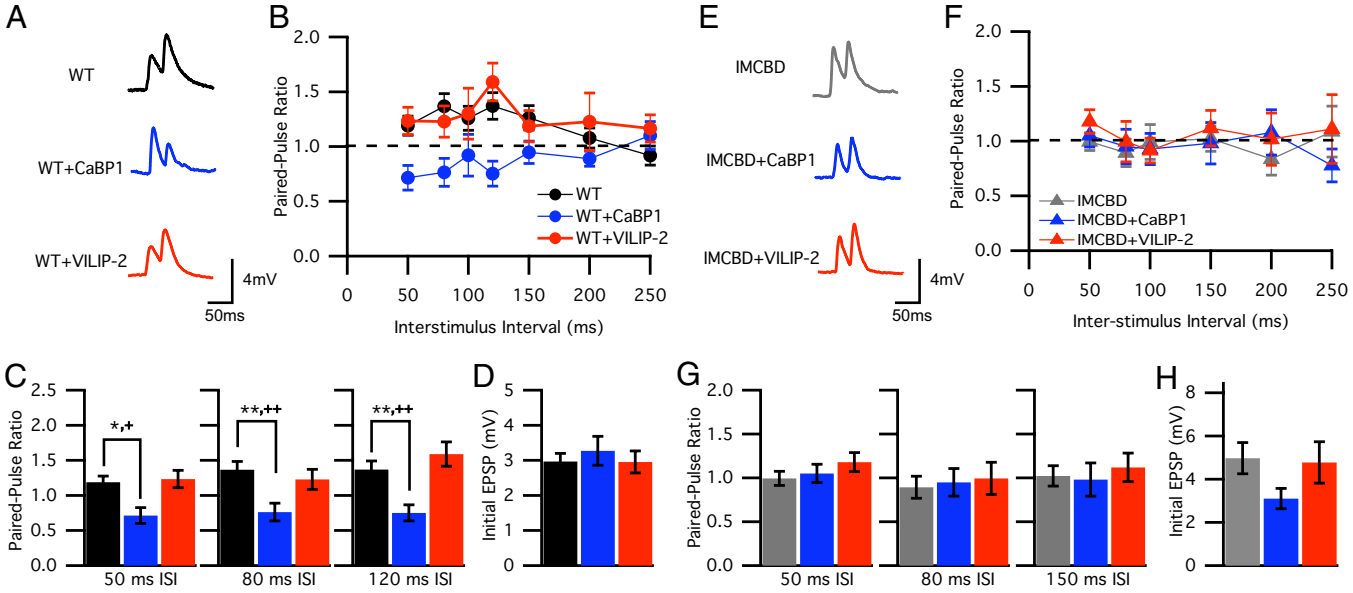

Fig. 1. CaBP1 blocks PPF. (*A*) Representative averages of 10 EPSPs evoked by paired action potentials with 50-ms ISI in the presynaptic neurons expressing $Ca_V2.1$ alone (WT) and in neurons cotransfected with CaS proteins CaBP1 (WT+CaBP1) or VILIP-2 (WT+VILIP-2) in the presence of ω-conotoxin GVIA (3 μM). (*B*) Average PPR plotted against ISI for WT (●), WT+CaBP1 (●), and WT+VILIP2 (●) neurons in 1 mM extracellular Ca^{2+}. (*C*) PPR for 50-, 80-, and 120-ms ISI for WT (black), WT+CaBP1 (blue), and WT+VILIP-2 (red) neurons. *$P < 0.05$, **$P < 0.01$, ANOVA with Bonferroni posttest. +, $P < 0.05$; ++, $P < 0.01$; ANOVA with Tukey's posttest for differences compared with WT group (absolute P values: ISI_{50ms}, $P = 0.006$; ISI_{80ms}, $P = 0.008$; and ISI_{120ms}, $P = 0.0002$). Data are shown as mean ± SEM from 10–20 synaptic pairs. (*D*) Averaged initial EPSP amplitude (mV) for WT (black), WT+CaBP1 (blue), and WT+VILIP-2 (red) neurons recorded for a 50-ms interval. (*E*) Representative averages of 10 EPSPs evoked in neurons expressing $Ca_V2.1_{IMAA\text{-}\Delta CBD}$ alone (IMCBD) or with CaS proteins CaBP1 (IMCBD +CaBP1) or VILIP-2 (IMCBD+VILIP-2) evoked by paired-action potentials with 50-ms ISI in the presynaptic neurons. (*F*) PPR plotted against ISI for IMCBD (▲), IMCBD+CaBP1 (▲), and IMCBD+VILIP2 (▲) neurons. (*G*) PPR for 50-, 80-, and 120-ms ISI for IMCBD (gray), IMCBD+CaBP1 (blue), and IMCBD+VILIP-2 (red) neurons. (*H*) Averaged initial EPSP amplitude (mV) for WT (black), WT+CaBP1 (blue), and WT+VILIP-2 (red) neurons recorded for a 50-ms interval. Data shown in *E*–*H* are mean ± SEM from 10–15 synaptic pairs.

stimulation in the presence of 1 mM extracellular Ca^{2+} to give a low probability of neurotransmitter release (Fig. 2*A*). In control synapses expressing WT $Ca_V2.1$ channels alone, we observed synaptic facilitation that then decayed at all stimulus frequencies (Fig. 2*B*). To include all the data points in our statistical analysis, the mean synaptic responses were fit to a biexponential function, the time constant for depression was derived from this fit, and the value for maximum facilitation was estimated by extrapolation to the time of the second stimulus in the train. Errors in the fits and parameter estimates were expressed as 95% confidence limits (Fig. 2*B*, dotted lines). This profile of synaptic facilitation and depression resembles the regulation of $Ca_V2.1$ channels by endogenous CaM (Fig. 2*B*) (17, 19, 22). In contrast, synapses expressing CaBP1 showed less facilitation and increased depression at each stimulus frequency (Fig. 2*B*). Peak synaptic facilitation was reduced on average from 2.22 ± 0.25 to 1.53 ± 0.15 at 30 Hz ($P < 0.05$), and synaptic depression following the peak response also was enhanced, as indicated by the more rapid decrement in EPSP amplitude (Fig. 2*B*).

Although VILIP-2 has no effect on facilitation by a single pulse, it remained possible that VILIP-2 has an important effect on synaptic transmission during trains of stimuli. Previous studies in transfected tsA-201 cells have shown that VILIP-2 reduces inactivation of Ca^{2+} currents during trains of repetitive depolarizations and thereby enhances and prolongs Ca^{2+} current facilitation (27). In contrast to our expectations from these previous results in nonneuronal cells, synapses expressing VILIP-2 showed synaptic facilitation followed by synaptic depression that was similar to that in control synapses (Fig. 2*C*). At 30 Hz, no difference in the rate of facilitation or depression was observed (Fig. 2*C*), in contrast to the marked effects of CaBP1 expression at this stimulus frequency (Fig. 2*B*). These results illustrate that effects of VILIP-2 on short-term synaptic plasticity are not extrapolated accurately from the effects observed on Ca^{2+} currents in transfected nonneuronal cells under these conditions, perhaps because of the differences in Ca^{2+} dynamics in presynaptic active zones versus nonneuronal cells. We conclude that CaBP1-expressing synapses have reduced synaptic facilitation during trains of activity but that VILIP-2 has no effect under conditions of low release probability.

CaBP1 Binding to $Ca_V2.1$ Channels Is Required for Its Effects on Synaptic Plasticity in Trains of Stimuli. To test whether direct binding of CaBP1 to $Ca_V2.1$ channels is required for the modulation of synaptic facilitation during trains of stimuli, we expressed CaBP1 with the mutant $Ca_V2.1_{IM\text{-}AA/\triangle CBD}$. SCG neurons expressing $Ca_V2.1_{IM\text{-}AA/\triangle CBD}$ show peak synaptic facilitation that is reduced from 2.22 ± 0.21 to 1.48 ± 0.12 at 30 Hz (compare Figs. 3*A* and 2*B*, 30 Hz, $P < 0.05$), and synaptic facilitation is reduced or completely blocked throughout the trains of stimuli at 10–30 Hz (Fig. 3*A* and Fig. S1). These results are consistent with the previous conclusion that normal synaptic

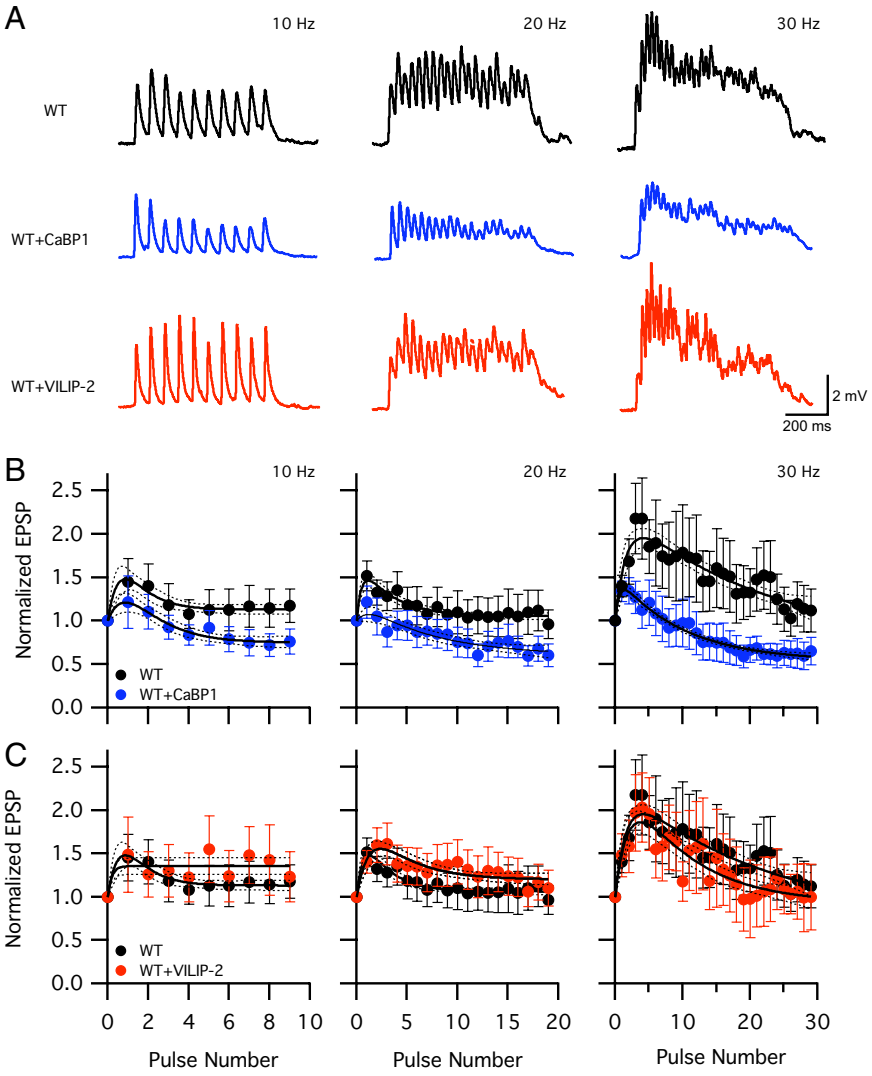

Fig. 2. CaBP1 blocks synaptic facilitation in trains of stimuli. (*A*) Representative EPSPs in 1 mM extracellular Ca^{2+} evoked by repetitive action potentials at 10, 20, and 30 Hz for 1s in the presynaptic neurons expressing $Ca_V2.1$ alone (WT, black) or cotransfected with CaBP1 (WT+CaBP1, blue) or VILIP-2 (WT+VILIP-2, red). Data from 10 sweeps repeated every 30 s at each frequency were averaged. (*B* and *C*) Mean normalized EPSP amplitude from 10–16 synaptic pairs at 10-, 20-, and 30-Hz frequency. EPSP amplitudes were normalized to the first EPSP of each train and plotted against action potential number for WT (●), WT+CaBP1 (●), and WT+VILIP-2 (●) neurons. Points represent the mean ± SEM. Solid lines are best fits of a biexponential equation as described in *SI Materials and Methods*. Dotted lines indicate 95% confidence intervals.

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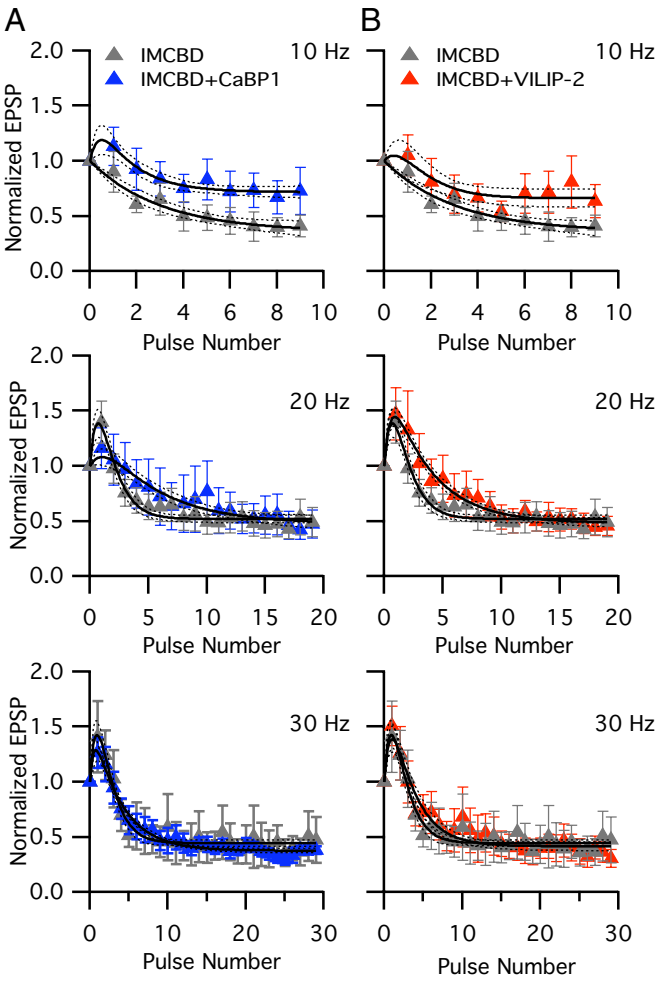

Fig. 3. Mutation of the CaS-binding site reduces modulation of short-term synaptic plasticity by CaBP1 and VILIP-2. (*A*) Normalized EPSP amplitudes for $Ca_V2.1_{IM\text{-}AA/\Delta CBD}$ (IMCBD) alone (▲) and with coexpression of CaBP1 (IMCBD+CaBP1) (▲) in 1 mM extracellular Ca^2 at a stimulation frequency of 10, 20, and 30 Hz. (*B*) Normalized EPSP amplitudes for $Ca_V2.1_{IMAA\text{-}\Delta CBD}$ alone (▲, IMCBD) and with coexpression of VILIP-2 (IMCBD+VILIP-2) (▲) at a stimulation frequency of 10, 20, or 30 Hz. Mean EPSP amplitudes were normalized to the first EPSP of the train. Data shown are mean ± SEM from 8–14 synaptic pairs.

facilitation in this model synapse requires facilitation of $Ca_V2.1$ channels via CaS proteins interacting with the IM and CBD domains (22). Coexpression of CaBP1 does not further reduce facilitation of synapses expressing the mutant $Ca_V2.1_{IM\text{-}AA/\Delta CBD}$ channel (Fig. 3*A* and Fig. S1). These data indicate that mutation of CaS-binding sites eliminates regulation by CaBP1 and therefore suggest that direct binding of CaBP1 to $Ca_V2.1$ channels is sufficient to cause a switch from synaptic facilitation to depression.

VILIP-2 was unable to increase facilitation of synaptic transmission in paired pulses or trains of stimuli under conditions of low release probability (Figs. 1 and 2). As a further control for the specificity of VILIP-2 effects, we tested VILIP-2 on SCG neurons expressing the $Ca_V2.1_{IM\text{-}AA/\Delta CBD}$ mutant channel. As expected from the results of Figs. 1 and 2, coexpression of VILIP-2 did not significantly increase facilitation during trains of stimuli at 10, 20, or 30 Hz (Fig. 3*B* and Fig. S1). These results are consistent with the conclusion that the $Ca_V2.1_{IM\text{-}AA/\Delta CBD}$ mutation completely prevents CaS protein regulation.

VILIP-2 Enhances Synaptic Facilitation at High Basal Release Probability. Facilitation during trains of stimuli in SCG neuron synapses is robust, approaching 2.22-fold at 30 Hz in 1 mM extracellular Ca^{2+} (Fig. 2). This substantial level of facilitation may represent the maximum possible at these synapses. We hypothesized that this high level of basal synaptic facilitation may occlude effects of VILIP-2. Therefore, we raised external Ca^{2+} from 1 mM to 2 mM to enhance release probability and increase synaptic depression. Under these conditions, control synapses show rapid synaptic depression at all three stimulus frequencies (Fig. 4*A* and Fig. S2), but synapses expressing VILIP-2 show significantly more facilitation and significantly less depression at 20 Hz and 30 Hz than controls ($P < 0.05$) (Figs. 4*A* and Fig. S2). At 30 Hz, VILIP-2 restores synaptic facilitation during the first five pulses followed by pulse-wise synaptic depression (1.37 ± 0.08, $P < 0.01$) (Fig. 4*A* and Fig. S2). Peak synaptic facilitation observed with VILIP-2 in 2 mM Ca^{2+} (Fig. 4*A*) was 27% less than in the corresponding control without VILIP-2 in 1 mM extracellular Ca^{2+} ($P < 0.05$) (Fig. 2*B*). The increase in facilitation during stimulus trains in 2 mM Ca^{2+}

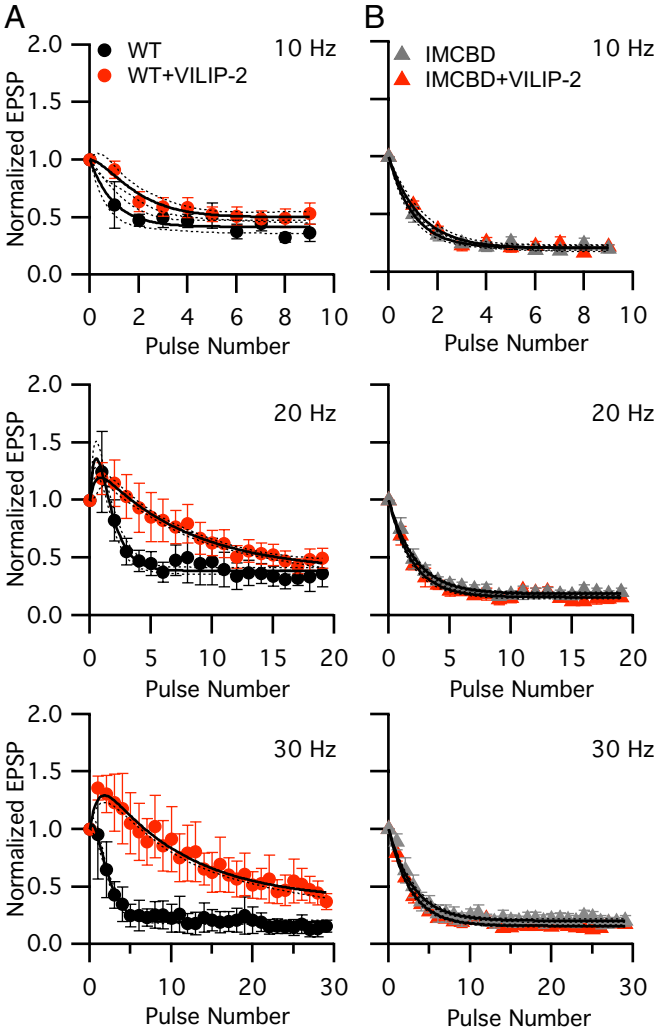

Fig. 4. VILIP-2 enhances synaptic facilitation via the CaS protein-binding site on $Ca_V2.1$ channels. (*A*) Normalized EPSPs recorded after the expression of $Ca_V2.1$ channels alone (WT) (●) and with VILIP-2 (●) in 2 mM extracellular Ca^{2+} during trains of action potentials at 10, 20, and 30 Hz. (*B*) Normalized EPSPs recorded after the expression of the $Ca_V2.1$ channel double mutant $Ca_V2.1_{IMAA\text{-}\Delta CBD}$ (IMCBD) alone (▲) and with VILIP-2 (IMCBD+VILIP-2) (▲) in 2 mM extracellular Ca^{2+} during trains of action potentials at 10, 20, and 30 Hz. The averaged first EPSP amplitude recorded in 2 mM extracellular Ca^{2+}: WT, 6.99 ± 1.9 mV (n = 10); WT+VILIP-2, 5.60 ± 0.98 mV (n = 14); IMCBD, 7.69 ± 1.01 mV (n = 8); and IMCBD+VILIP-2, 6.77 ± 1.19 (n = 12). Data shown are mean ± SEM from 5–14 synaptic pairs.

highlights the ability of VILIP-2 to facilitate neurotransmitter release specifically at high frequencies of stimulation when basal neurotransmitter release is high. This specificity of the effect of VILIP-2 for the facilitation of $Ca_V2.1$ channel activity under conditions of high release probability is unexpected from previous work in nonneuronal cells and indicates that the Ca^{2+}-signaling environment at the active zone markedly affects the ability of CaS proteins to modulate short-term synaptic plasticity. The synaptic facilitation during trains of stimuli shows that VILIP-2 binding to $Ca_V2.1$ channels slows their cumulative Ca^{2+}-dependent inactivation and thereby enhances their Ca^{2+}-dependent facilitation (27).

CaS-Binding Sites of $Ca_V2.1$ Channels Are Essential for VILIP-2 Modulation. To confirm that the effect of VILIP-2 in 2 mM Ca^{2+} is caused by the direct interaction with the CaM-binding site on the channel, we coexpressed VILIP-2 with $Ca_V2.1_{IM\text{-}AA/\triangle CBD}$ mutant channels. Under conditions of high release probability in 2 mM Ca^{2+}, $Ca_V2.1_{IM\text{-}AA/\triangle CBD}$ channels undergo rapid synaptic depression, as seen at other synapses when external Ca^{2+} is elevated (Fig. 4*B*) (22). Coexpression of VILIP-2 has no effect on depression or facilitation of $Ca_V2.1_{IM\text{-}AA/\triangle CBD}$ channels (Fig. 4*B* and Fig. S2). This result is consistent with the conclusion that, when external Ca^{2+} and basal release probability are high, VILIP-2 prevents rapid Ca^{2+}-dependent channel inactivation and enhances Ca^{2+}-dependent facilitation by interaction with the CaS protein regulatory site on $Ca_V2.1$ channels. Evidently, when bound in place of CaM, VILIP-2 can switch SCG synapses from synaptic depression to facilitation. Thus, CaBP1 and VILIP-2 provide bidirectional control of facilitation and depression by CaS proteins.

Discussion

Short-term synaptic plasticity converts the information encoded in the frequency and pattern of action potential firing in the presynaptic terminal into an analog signal for transmission to the postsynaptic neuron (3). Our results show that this information processing can be controlled in a bidirectional manner at the level of the presynaptic Ca^{2+} channel by CaS proteins, which are poised to modulate these channels and alter neurotransmitter release. Direct regulation of presynaptic $Ca_V2.1$ channels by the CaS proteins CaBP1 and VILIP-2 may serve as a bidirectional switch to control the input–output relationships of synapses in response to trains of action potentials. This conversion of synapses from depressing to facilitating and vice-versa would be expected to have profound consequences for the encoding properties of neural circuits, thereby fine-tuning the synaptic plasticity of different types of synapses (3).

Molecular Analysis of Synaptic Plasticity in SCG Neurons. Analysis of the functional effects of presynaptic Ca^{2+} channel regulation in synaptic transmission is challenging because of the need to alter regulation only in the presynaptic cell. Our experiments took advantage of the unique characteristics of SCG neurons as an expression system for studies of presynaptic effects on synaptic transmission. These neurons lack CaS proteins, which are expressed specifically in the central nervous system and retina (23, 25), and they lack $Ca_V2.1$ channels, which are expressed primarily in central neurons (6, 9, 10). Thus, SCG neurons provide a null genetic background for studies of these key components of central synapses. The large cell bodies of cultured SCG neurons allow microinjection of cDNA encoding $Ca_V2.1$ channels into individual cell nuclei without damage, giving expression only in presynaptic cells, and the level of functional expression is in the same range as the endogenous $Ca_V2.2$ channels, which can be blocked specifically and completely by ω-conotoxin GVIA (32). Facilitation followed by depression is observed in these synapses (22). Both facilitation and the rapid phase of depression are dependent on the regulation of $Ca_V2.1$ channels by CaM interaction with the IM and CBD motifs in the C-terminal domain (22). These favorable characteristics have allowed us to test directly the role of CaS proteins in the modulation of synaptic plasticity that is dependent on $Ca_V2.1$ channels and by using mutant channel constructs to demonstrate the requirement for CaS protein binding to the IM and CBD motifs.

CaS Proteins Override CaM-Dependent Synaptic Plasticity in a Ca^{2+}-Dependent Manner. Like CaM, the CaS proteins are expressed in high levels in central neurons (23). Our results show that specific expression of CaBP1 and VILIP-2 in presynaptic neurons can compete effectively with endogenous CaM, without changing peak $Ca_V2.1$ currents (Fig. S3). CaBP1 expression blocks CaM-dependent facilitation and enhances rapid synaptic depression when studied in the presence of 1 mM extracellular Ca^{2+}, where synaptic facilitation is prominent. In contrast, CaBP1 did not have detectable effects on synaptic plasticity at 2 mM Ca^{2+}, where synaptic depression is rapid ($n = 6$; $P > 0.05$). Thus, CaBP1 is able to compete with CaM for binding to their common regulatory site and can induce changes in short-term synaptic plasticity under physiological conditions, depending on the level of Ca^{2+} entry and the resulting level of release probability.

In contrast to CaBP1, VILIP-2 has no effect on synaptic plasticity in SCG neurons at 1 mM extracellular Ca^{2+}, but it reduces synaptic depression and enhances synaptic facilitation at 2 mM Ca^{2+}, where synaptic depression is rapid. These results show that VILIP-2 also can override the synaptic plasticity induced by CaM under appropriate conditions of Ca^{2+} entry. It is noteworthy that the effects of CaBP1 and VILIP-2 on synaptic plasticity respond in opposite ways to changes in the level of release probability—CaBP1 reduces facilitation and increases depression more effectively at low release probability in the presence of low extracellular Ca^{2+}, whereas VILIP-2 reduces depression and enhances facilitation more effectively at high release probability in the presence of higher extracellular Ca^{2+}. This difference in the effects of CaBP1 and VILIP-2 under different physiological conditions highlights the importance of the functional state of the synapse in dictating the response of short-term synaptic plasticity to these CaS proteins. Taken together, our results show that the CaS proteins CaBP1 and VILIP-2 can serve as a bidirectional switch controlling synaptic facilitation and depression in a push–pull manner through opposing regulation of $Ca_V2.1$ channels.

CaS Proteins and Short-Term Synaptic Plasticity. It is becoming increasingly apparent that CaS proteins play a significant role in coupling changes in intracellular Ca^{2+} to modulation of different types of ion channels and other regulatory proteins. Ca^{2+} and CaM have long been implicated in mechanisms of synaptic plasticity (33–35). In addition, the CaS proteins frequenin, neuronal Ca^{2+} sensor-1 (NCS-1), and KChIP modulate a broad range of voltage-gated and ligand-gated channels (36–40). NCS-1, which is closely related to VILIP-2, enhances P/Q-type Ca^{2+} currents in the calyx of Held and can facilitate synaptic transmission at the calyx of Held and in hippocampal synapses (39, 40), but modulation of $Ca_V2.1$ channels by direct binding of NCS-1 has not been reported. Our results indicate that CaBP1 and VILIP-2 can regulate short-term synaptic plasticity in SCG neurons directly in opposing ways by binding to the same regulatory site as CaM and differentially regulating $Ca_V2.1$ channels. The fact that these effects of CaS proteins on synaptic transmission are largely eliminated when mutant $Ca_V2.1_{IM\text{-}AA/\Delta CBD}$ channels mediate Ca^{2+} entry demonstrates the regulation of $Ca_V2.1$ channels by direct binding of CaS proteins as opposed to other possible presynaptic targets. Thus, regulated expression of these CaS proteins in different classes of synapses could work as a binary switch to change short-term synaptic plasticity from depressing to facilitating or vice versa through direct modulation of $Ca_V2.1$ channels.

Distinct Expression of CaS Proteins in the Central Nervous System. CaS proteins are expressed in a cell-specific manner in the central nervous system (23–25, 28). VILIP-2 is expressed mainly

in the caudate-putamen, neocortex, and hippocampus (28, 38). Expression of VILIP-2 is notably absent in the cerebellum and pons (28, 41). CaBP1 is expressed primarily in cerebral cortex, retina, and hippocampus, whereas CaM is expressed ubiquitously (25). Additionally, CaBP1 and $Ca_V2.1$ channels coexist in the CA1 region of the hippocampus and in presynaptic nerve terminals in the molecular layer of the cerebellum (26). Neuronal CaS proteins have Ca^{2+}-binding affinities considerably higher than those of CaM (42). Therefore, the diversity of this family of CaS proteins and their Ca^{2+}-binding properties raises the possibility that they may override the ubiquitous effects of CaM and contribute broadly to the diversity of Ca^{2+} channel regulation and synaptic plasticity.

CaS Proteins as Effectors of the Diversity of Synaptic Transmission. Different synapses show diverse patterns of facilitation and depression, and the underlying mechanism for this diversity is unknown (2, 3). CaS proteins contribute substantially to the diversity of neuronal Ca^{2+} signaling in the postsynaptic cell (23). Our results suggest a key role for CaS proteins in synaptic plasticity by showing that CaBP1 and VILIP-2 can exert opposing influences on facilitation and depression of synaptic transmission through the regulation of presynaptic $Ca_V2.1$ channels. This perspective opens the way for a broader analysis of the potential roles of other CaS proteins in control of short-term synaptic plasticity by regulation of presynaptic Ca^{2+} channels. Given the widespread distribution of $Ca_V2.1$ channels in the central nervous system, cell-specific modulation of presynaptic $Ca_V2.1$ channels by CaBP1, VILIP-2, and other CaS proteins may act as a molecular switch linking CaS-dependent facilitation and inactivation of $Ca_V2.1$ channels to the encoding of information in the frequency domain at synapses.

Materials and Methods

SCG neurons were cultured as described to allow synapse formation (43). cDNAs encoding $\alpha_1 2.1$ subunit, $\alpha_1 2.1_{IMAA/\Delta CBD}$, CaBP1, VILIP-2, and eGFP were microinjected into nuclei, and synaptic transmission was measured as described previously (22). More detailed descriptions of the experimental conditions can be found in *SI Materials and Methods*.

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