Dendritic Calcium Nonlinearities Switch the Direction of Synaptic Plasticity in Fast-Spiking Interneurons

Olivier Camiré and Lisa Topolnik

Department Biochemistry, Microbiology and Bio-informatics, Université Laval; Axis of Cellular and Molecular Neuroscience, IUSMQ, Québec, Canada

Postsynaptic calcium (Ca^{2+}) nonlinearities allow neuronal coincidence detection and site-specific plasticity. Whether such events exist in dendrites of interneurons and play a role in regulation of synaptic efficacy remains unknown. Here, we used a combination of whole-cell patch-clamp recordings and two-photon Ca^{2+} imaging to reveal Ca^{2+} nonlinearities associated with synaptic integration in dendrites of mouse hippocampal CA1 fast-spiking interneurons. Local stimulation of distal dendritic branches within stratum oriens/alveus elicited fast Ca^{2+} transients, which showed a steep sigmoidal relationship to stimulus intensity. Supralinear Ca^{2+} events required Ca^{2+} entry through AMPA receptors with a subsequent Ca^{2+} release from internal stores. To investigate the functional significance of supralinear Ca^{2+} signals, we examined activity-dependent fluctuations in transmission efficacy triggered by Ca^{2+} signals of different amplitudes at excitatory synapses of interneurons. Subthreshold theta-burst stimulation (TBS) produced small amplitude postsynaptic Ca^{2+} transients and triggered long-term potentiation. In contrast, the suprathreshold TBS, which was associated with the generation of supralinear Ca^{2+} events, triggered long-term depression. Blocking group I/II metabotropic glutamate receptors (mGluRs) during suprathreshold TBS resulted in a slight reduction of supralinear Ca^{2+} events and induction of short-term depression. In contrast, blocking internal stores and supralinear Ca^{2+} signals during suprathreshold TBS switched the direction of plasticity from depression back to potentiation. These data reveal a novel type of supralinear Ca^{2+} events at synapses lacking the GluA2 AMPA subtype of glutamate receptors and demonstrate a general mechanism by which Ca^{2+} -permeable AMPA receptors, together with internal stores and mGluRs, control the direction of plasticity at interneuron excitatory synapses.

Key words: calcium; dendrite; interneuron; intracellular release; plasticity; synapse

Introduction

Hebb's learning rule requires a tight temporal association of input and output at activated synapses. The backpropagating action potential (AP) informs the dendrites about the occurrence of neuronal output and provides the critical level of membrane depolarization necessary for the activation of voltage-dependent Ca^{2+} mechanisms and generation of supralinear Ca^{2+} signals (Magee and Johnston, 1997). However, in most inhibitory interneurons, AP backpropagation is largely attenuated with distance from the soma because of the nonuniform distribution of voltage-gated ion conductances (Goldberg et al., 2003a; Hu et al., 2010; Evstratova et al., 2011). As a result, backpropagating APs can generate significant Ca^{2+} elevations and, accordingly, control the induction of Hebbian forms of plasticity only at synapses located proximally. Local upregulation of AP-evoked Ca^{2+} tran-

sients by changes in synaptic activity can rescue the induction of Hebbian plasticity at interneuron excitatory synapses (Topolnik et al., 2009). Alternatively, the induction of associative plasticity at distal synapses may rely on local regenerative activity. Studies on pyramidal neurons revealed dendritic Ca^{2+} spikes initiated via activation of NMDA receptors (NMDARs) and voltage-gated Ca^{2+} channels (VGCCs) as a mechanism for coincidence detection at distal synapses (Regehr and Tank, 1990; Markram and Sakmann, 1994; Schiller et al., 1997; Golding et al., 2002; Losonczy and Magee, 2006; Remy and Spruston, 2007; Tsay et al., 2007). In addition, pyramidal neurons exhibit dendritic Ca^{2+} nonlinearities in the form of Ca^{2+} waves resulting from the activation of metabotropic glutamate receptors (mGluRs) and a subsequent Ca^{2+} release from internal stores (Wang et al., 2000; Nakamura et al., 2002; Watanabe et al., 2006). In interneurons, the mechanisms of dendritic regenerative activity remain largely unknown, although dendritic Ca^{2+} spikes have been reported in neocortical low threshold spiking cells and hippocampal CA1 stratum radiatum interneurons (Goldberg et al., 2004; Katona et al., 2011). In particular, it remains unclear how different patterns of synaptic activity can take part in the initiation of dendritic regenerative signals and how these events can regulate the efficacy of transmission at distal synapses.

Here, we used a combination of whole-cell patch-clamp recordings and two-photon Ca^{2+} imaging in the distal dendritic branches of mouse hippocampal CA1 fast-spiking (FS) cells to

Received May 27, 2013; revised Jan. 9, 2014; accepted Feb. 4, 2014.

Author contributions: L.T. designed research; O.C. and L.T. performed research; O.C. and L.T. analyzed data; O.C. and L.T. wrote the paper.

This work was supported by the Canadian Institute of Health Research, the Natural Sciences and Engineering Research Council of Canada (NSERC), and the Savoy Foundation. O.C. was supported by the NSERC PhD fellowship. L.T. is the recipient of the NSERC Faculty Award for Women. We thank Dimitry Kullmann and Marco Capogna for stimulating discussions and comments on the previous version of this manuscript, and Dimitry Topolnik for excellent technical assistance.

Correspondence should be addressed to Lisa Topolnik, Axis of Cellular and Molecular Neuroscience, 2601 Ch. de la Canardière, CRULRG, Québec, PQ, G1J 2G3, Canada. E-mail: Lisa.Topolnik@cruirg.ulaval.ca.

DOI:10.1523/JNEUROSCI.2253-13.2014

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explore the mechanisms and functional relevance of Ca^{2+} nonlinearities evoked by the activation of glutamatergic synapses. In marked contrast to previously described mechanisms, synaptically evoked Ca^{2+} nonlinearities in FS interneurons involved primarily the activation of GluA2-lacking AMPA receptors with a subsequent Ca^{2+} release, were highly localized within individual dendritic branches and switched the direction of plasticity from long-term potentiation (LTP) to short-term depression (STD) or long-term depression (LTD), with the duration of depression depending on the additional recruitment of the group I/II mGluRs.

Materials and Methods

Slice preparation and patch-clamp recordings. Hippocampal slices (300 μm) were prepared from CD1 mice of either sex (P13–P21) as described previously (Evstratova et al., 2011) in accordance with the animal welfare guidelines of the Université Laval. During experiments, slices were perfused with standard artificial CSF (ACSF) containing the following (in mM): 124 NaCl, 2.5 KCl, 1.25 NaH_2PO_4 , 26 NaHCO_3 , 2 MgSO_4 , 2 CaCl_2 , and 10 glucose saturated with 95% O_2 and 5% CO_2 , pH 7.4, at 30–33°C. In all experiments, GABAergic transmission was blocked by the GABAA and GABAB receptor antagonists gabazine (1 μM) and CGP55845 (2 μM), respectively. In some experiments, DL-AP5 (100 μM), 1-naphthyl acetyl spermine (NASPM; 3–60 μM), philanthotoxin 433 (PhTx, 1 μM), NBQX (10 μM), *d*-tubocurarine (*d*TC; 20 μM), nifedipine (10 μM), NNC 55-0396 (10 μM), Ni^{2+} (50 μM), CPA (30 μM), ryanodine (30 μM), SNX-482 (30 nM), or E4CPG (500 μM) were included in the ACSF and caffeine (1 mM) was puff applied (50 ms). As NASPM, PhTx, and nifedipine are use-dependent blockers (Koike et al., 1997; Tóth and McBain, 1998; Shen et al., 2000), the following procedure was applied when using these pharmacological agents: after an initial period of recording of control responses, the slice was incubated in a solution containing the blocker for 10 min. After this period, synaptic stimulation was resumed, and the current amplitude as well as the peak Ca^{2+} transient were blocked in a use-dependent manner during the next 20 min.

Whole-cell patch-clamp recordings were obtained from interneurons located in the CA1 stratum oriens adjacent to the stratum pyramidale, which were identified using a 40 $\times$ water-immersion objective and infrared differential interference contrast (IR-DIC) microscopy. Recording pipettes (3–6 M Ω) were made from borosilicate glass capillaries (IB100F-4; World Precision Instruments). For whole-cell current-clamp recordings, the pipette solution contained the following (in mM): 130 KMeSO₃, 2 MgCl_2 , 10 diNa-phosphocreatine, 10 HEPES, 2–4 ATP-Tris, 0.2 GTP-Tris, 0.2–0.3% biocytin, and Ca^{2+} -insensitive (Alexa Fluor 594, 20 μM ; Invitrogen) and Ca^{2+} -sensitive [Fluo-5F, 300 μM or Oregon Green BAPTA 5N (OGB-5N), 500 μM ; Invitrogen] dyes, pH 7.2–7.3, 275–290 mOsm/L. In some experiments, spermine (100 μM) or QX-314 (2–5 mM) was included in the intracellular solution.

Two-photon Ca^{2+} imaging. Dendritic Ca^{2+} imaging was performed using a TCS SP5 two-photon laser-scanning microscope (Leica Microsystems) based on a Ti-sapphire laser (Chameleon Ultra II; Coherent; >3 W, 140 fs pulses, 80 Hz repetition rate) tuned to 800 nm. A long-range water-immersion objective (40 $\times$; NA 0.8) was used to collect photons in the epifluorescence mode with external photomultiplier tubes. Neurons were filled with indicators via the patch electrode for 20–30 min before imaging. Red fluorescence from Alexa Fluor 594 was used to locate dendrites of interest. To measure Ca^{2+} signals, green and red fluorescence was collected during 500 Hz line scans along or across dendritic segments of 2–15 μm . Fluorescence changes were quantified as increases in green fluorescence from the baseline normalized to the red fluorescence: $\Delta G/R = (G - G_0)/R$ or as changes in green fluorescence from the baseline $\Delta F/F = (F - F_0)/F_0$.

Synaptic stimulation and plasticity induction. To study the rules of synaptic integration in dendrites of interneurons, we took advantage of physiological synaptic stimulation using a local bipolar microelectrode. Although two-photon glutamate uncaging allows for the temporally precise activation of a defined number of synaptic inputs in pyramidal cells, it can produce undesirable effects (activation of perisynaptic and extra-

synaptic receptors) in aspiny dendrites of interneurons. Local activation of distal synaptic inputs (80–200 μm from the soma) in the stratum oriens/alveus was achieved using a bipolar stimulating electrode made from the borosilicate theta-glass capillaries (BT-150–10; Sutter Instruments) and filled with ACSF containing 5–10 μM Alexa Fluor 594. The electrode was positioned 7–20 μm from the dendritic branch of interest under visual control (Figs. 1A, 7C) and connected to a constant current isolation unit (A360LA; World Precision Instruments). Dendritic Ca^{2+} transients and corresponding somatic responses were evoked by brief bursts of synaptic stimulation (three stimuli at 100 Hz, 0.1 ms each) of various intensities applied once every 30 s. The threshold stimulation was defined as the minimal intensity of stimulation necessary to evoke post-synaptic responses. The stimulus strength was then increased gradually with a step of 10–15% up to 200% of the threshold and both somatic and dendritic responses were recorded. In 31 of 40 cells examined, a 10–100% increase in stimulus intensity from the threshold resulted in a supralinear dendritic Ca^{2+} event (Fig. 2), which was associated with a generation of 2–4 APs in the soma.

In synaptic plasticity experiments, the pipette solution had the same composition as in Ca^{2+} imaging experiments, except for adding spermine (100 μM) and omitting the Ca^{2+} indicator. The plasticity induction protocol was applied within 8 min of whole-cell recording to avoid extensive dendritic washout. Similar to Ca^{2+} imaging experiments, the stimulating electrode was filled with ACSF containing 5 μM Alexa Fluor 594 and positioned 7–20 μm from the distal dendritic branch under visual guidance. Voltage-clamp recordings of baseline EPSCs were performed for 3 min at a subthreshold (for somatic AP initiation) level of stimulation. In one series of experiments (Fig. 8B), three theta-burst trains of stimuli (TBS: 3 pulses/100 Hz repeated eight times at 4 Hz for 2 s) of the same baseline intensity were applied at 30 s intervals to the cell recorded in current-clamp mode at -70 mV. This induction paradigm was defined as subthreshold TBS. Thereafter, voltage-clamp recordings of EPSCs were resumed for a further 20–30 min. To mimic the conditions necessary for the generation of dendritic Ca^{2+} nonlinearities, in the second series of experiments (Fig. 8C–E), the stimulation intensity during TBS was raised to 200% (to ensure the induction of supralinear Ca^{2+} events). Accordingly, such induction paradigm was defined as suprathreshold TBS. Thereafter, the stimulation intensity was reduced to the baseline level and voltage-clamp recordings of EPSCs were resumed.

To obtain the *I*–*V* relationship of EPSCs, cells were recorded in a voltage-clamp configuration using a Cs⁺-based internal solution containing spermine (100 μM) and QX-314 (2–5 mM) and EPSCs were evoked by single stimuli at different levels of membrane potential (from -80 to $+60$ mV with a step of 20 mV, liquid junction potential not corrected).

Data acquisition and analysis. Data acquisition (filtered at 2–3 kHz, digitized at 10 kHz) was performed using Clampex 10.2 and analyzed using Clampfit 10.2 (Molecular Devices), Leica LAS, Igor Pro (WaveMetrics), and Excel (Microsoft). Series resistance was 10–25 M Ω . Pipette capacitance and series resistance (or bridge balance in current-clamp experiments) were compensated. Series resistance was verified every 5 min and readjusted if necessary. Cells with resting membrane potentials more positive than -50 mV, fluctuations in holding current, or unstable series resistance were discarded. In current-clamp experiments, interneurons were held at ~ -70 mV by injecting current (-20 to -50 pA), if necessary.

To measure $\Delta G/R$ peak amplitude, we averaged the values around the peak over a 10 ms time window. In summation experiments, both $\Delta G/R$ peak amplitude and the stimulus intensity were normalized to their threshold values (the minimal intensity necessary to evoke the minimal Ca^{2+} response). In the experiments illustrated in Figure 5, the Ca^{2+} nonlinearity threshold was determined as the minimal stimulus intensity necessary to evoke a supralinear Ca^{2+} event. In these experiments, subthreshold $\Delta G/R$ amplitude was determined as the $\Delta G/R$ peak amplitude associated with a subthreshold (for AP generation) somatic response. Spontaneous EPSCs (sEPSCs) were analyzed using the search events algorithm of Clampfit 10.2. Only those events that were not contaminated by ambiguous deflections at their rising or decaying phases, and decayed back to the baseline, were selected for the analysis of kinetics. All events

were counted for frequency analysis. Typically, 200 events per cell were chosen. sEPSC amplitudes were measured at the peak of the waveform. The rise time was defined as the duration of 20–80% of the sEPSC peak amplitude. The decay phase of the sEPSCs was fitted with a single exponential. Summary data are shown as means $\pm$ SEM. Significance between groups was assessed using the paired *t* test, the Mann–Whitney test, or one-way ANOVA at $p < 0.05$.

Morphological and neurochemical identification of interneurons. For anatomical reconstruction, neurons were filled with biocytin (Sigma) during whole-cell recordings. Slices with recorded cells were fixed overnight with 4% paraformaldehyde (PFA) at 4°C. To reveal biocytin, slices were permeabilized with 1.5–3% Triton X-100 and incubated overnight at 4°C with streptavidin-conjugated Alexa Fluor 546 or 488 (1:200; Jackson ImmunoResearch). Sections were mounted in Dako fluorescence medium and confocal images of biocytin-filled interneurons were obtained using a Leica TCS SP5 imaging system coupled with 543 nm He-Ne and 488 nm Argon lasers. Interneuron Z stacks were acquired using a 1 μm step. Anatomical reconstruction was performed using the Neurolucida 8.26.2 software (MBF Bioscience). The morphological identification of recorded interneurons was achieved by the analysis of their axonal arborization.

For neurochemical identification, slices with recorded neurons were fixed with 4% PFA and re-sectioned (thickness, 50 μm) using a Leica VT1000 vibratome (Leica Microsystems). Sections were permeabilized with 0.3% Triton X-100 in TBS and incubated in blocking solution containing 4% bovine serum albumin and 10% normal serum for 1 h. After this step, sections were incubated with streptavidin-conjugated Alexa Fluor 546 (1:200; Jackson ImmunoResearch) to reveal biocytin and a mouse monoclonal anti-parvalbumin antibody (P3088; Sigma) at 4°C for 24 h. The following day, slices were incubated with an anti-mouse secondary antibody (DyLight-647; Jackson ImmunoResearch) for 2–4 h, rinsed, and mounted on microscope slides. Confocal images of labeled processes were obtained using a Leica TCS SP5 imaging system and a 63 $\times$ (NA 1.4) oil-immersion objective (Leica Microsystems).

Results

Postsynaptic Ca^{2+} mechanisms in distal dendrites of hippocampal CA1 FS interneurons

We performed whole-cell patch-clamp recordings from hippocampal CA1 FS interneurons tentatively identified based on their morphological properties in the IR-DIC image: large-diameter fusiform cell body located within the stratum oriens adjacent to the stratum pyramidale. Only cells that exhibited nonaccommodating FS firing pattern [with an average frequency of 207.7 ± 7.8 Hz (at 30–33°C) in response to a 1 s 1 nA current pulse; $n = 84$; Figure 1B] were included in this study. All recorded interneurons were filled with biocytin for *post hoc* anatomical and neurochemical identification (Fig. 1C). Among 84 FS cells recorded, 61 neurons were labeled successfully and reconstructed. Based on anatomical criteria, 25 recorded neurons were identified as basket cells (BCs; Fig. 1C, top left) and 30 neurons were identified as bistratified cells (BISs; Fig. 1C, top right). A subset of recorded cells was processed for parvalbumin (PV) immunoreactivity. As our typical Ca^{2+} imaging experiment lasted for at least 1 h, we were only able to detect PV expression in neuronal processes (distal dendrites and axonal branches) due to a rapid washout of cytoplasmic Ca^{2+} buffers from perisomatic compartments during whole-cell recordings (Müller et al., 2005). Nevertheless, seven of seven examined neurons revealed PV immunoreactivity in axonal branches (Fig. 1C, bottom). Consistent with previous findings (Sik et al., 1995; Buhl et al., 1996; Ali et al., 1998; Glickfeld and Scanziani, 2006), FS interneurons recorded here had a resting membrane potential of -63.6 ± 0.9 mV, input resistance of 99.3 ± 6.1 M Ω and a membrane time constant of 9.9 ± 0.5 ms ($n = 55$). Furthermore, as reported previously (Buhl et al., 1996), BCs exhibited a significantly lower

input resistance and faster time constant when compared with BISs [BC: $R_{in} = 84.4 \pm 7.1$ M Ω , $\tau = 8.4 \pm 0.4$ ms, $n = 25$; BISs: $R_{in} = 114.2 \pm 5.1$ M Ω , $\tau = 11.5 \pm 0.5$ ms, $n = 31$; $p < 0.05$; Mann–Whitney test].

Because in many types of interneurons dendritic Ca^{2+} transients evoked by backpropagating APs (bAP-CaTs) decline with distance from the soma, we first examined the spatial profile of bAP-CaTs in CA1 FS interneurons. Cells were loaded with a medium-affinity Ca^{2+} indicator (Fluo-5F) and a Ca^{2+} -insensitive morphological dye (Alexa Fluor 594) for 20–30 min (Fig. 1A). After the loading period, small trains of APs were initiated by somatic current injection (0.8–1 nA, 2 ms; 10 APs at 100 Hz) and corresponding bAP-CaTs were examined in dendritic shafts at different distances from the cell body (Fig. 1A, white lines). Consistent with previous findings in cortical FS interneurons (Goldberg et al., 2003a) and dentate gyrus BCs (Aponte et al., 2008), bAP-CaTs in CA1 FS interneurons were highly restricted within proximal dendritic sites (Fig. 1D).

To determine the sources of postsynaptic Ca^{2+} elevations that may operate in distal dendritic branches (80–200 μm from the soma), a theta-glass bipolar stimulating electrode was positioned at 7–20 μm from the branch of interest within the stratum oriens/alveus (Figs. 1A, 7C, 8A). Therefore, excitatory responses analyzed in this study were evoked primarily by the activation of local CA1 pyramidal cell (PC) input (Takács et al., 2012). First, to examine the presence of NMDARs at these synapses, EPSCs were evoked by single shocks (15–50 μA , 0.1 ms; Fig. 1E) in low Mg^{2+} (0.5 mM) ACSF. Surprisingly, in 6 of 11 interneurons, EPSCs evoked in such conditions showed a strong inward rectification [Rectification Index (*RI*) = 0.11 ± 0.04 ; Figure 1E, black traces and symbols], consistent with the major role of GluA2-lacking Ca^{2+} -permeable AMPA receptors (CP-AMPA) and with the absence of NMDAR component at these synapses. The remaining cells exhibited a mixed contribution from CP-AMPA and NMDARs, as their EPSCs showed two components: one with a linear *I*–*V* relationship, which was sensitive to the NMDAR antagonist DL-AP5 (Fig. 1E, red traces and symbols), and the second, which exhibited a strong inward rectification (*RI* = 0.17 ± 0.02), with insensitivity to DL-AP5. Furthermore, in all cells examined, EPSCs were sensitive to NASPM (Fig. 1F), a synthetic analog of the Joro spider toxin, which is a selective inhibitor of CP-AMPA (Koike et al., 1997). Importantly, CP-AMPA-dominating synapses with variable contribution from NMDARs (range: 0–45%) were observed in both BCs and BISs. Moreover, when NMDARs were present, we found no significant difference in the ratio of the CP-AMPA to NMDAR EPSC components between the two cell types (BCs: 1.7 ± 0.04 , $n = 4$; BISs: 1.8 ± 0.06 , $n = 4$, $p > 0.05$, Mann–Whitney test). Thus, for the rest of the study, the data obtained from BCs and BISs were pooled together.

To examine the relative contribution of NMDARs versus CP-AMPA at these synapses under more physiological conditions, we studied their role in synaptically evoked Ca^{2+} elevations in normal ACSF (Mg^{2+} , 2 mM). Interneurons were filled with a low-affinity Ca^{2+} indicator (OGB-5N) and a morphological dye Alexa Fluor 594, and postsynaptic Ca^{2+} transients were evoked by a brief burst of synaptic stimulation (three pulses at 100 Hz) at -70 mV (Fig. 1G). Under these conditions, local subthreshold (for somatic AP initiation) synaptic stimulation produced fast Ca^{2+} transients (decay time constant: 63.3 ± 11.2 ms, $n = 6$; Figs. 1G, 2A, 5A) that were highly localized within dendritic microdomains (6.0 ± 0.9 μm , $n = 6$; Figs. 1G, 7B), consistent with previous findings in aspiny dendrites of cortical FS interneurons

Figure 1. Postsynaptic Ca^{2+} mechanisms in distal dendrites of hippocampal CA1 FS interneurons. **A**, Two-photon image (maximal projection) of an interneuron filled with Alexa Fluor 594 and OGB-5N. White arrows indicate the axon. White lines point to locations where bAP-CaTs were measured. White square indicates the dendritic area where postsynaptic Ca^{2+} signals were detected. Scale bar, 100 μm . **B**, Firing pattern of a typical FS interneuron. **C**, Neurolucida reconstructions of a BC and of a BIS (axon indicated in red; top) and confocal images of an axonal terminal labeled with biocytin (left) and immunoreactive for PV (middle), with an overlay of both images (right). **D**, Representative examples of bAP-CaTs (**D1**) evoked by a train of 10 APs at different distances from the soma. The summary plot (**D2**) shows the decrease in amplitude of bAP-CaTs relative to the distance from the soma. Data from individual cells are shown in black; average data are shown in red. The dotted line indicates the threshold level below which the signal was indistinguishable from noise. **E**, Summary plot of the I - V relationship of EPSCs evoked at distal synapses. EPSCs were recorded at different levels of membrane potential (-80 to 60 mV) in 0.5 mM Mg^{2+} ACSF and in the presence of NMDAR antagonist DL-AP5 (100 μM). Insets show representative examples of the two types of synaptic responses identified with (red traces) or without (black traces) the NMDAR component. Summary plots include data obtained in BCs and BISs. **F**, Summary dose-response plot showing EPSC sensitivity to NASPM. Inset, Examples of EPSCs recorded in the control condition (normal ACSF, left), in the presence of NASPM (25 μM ; middle), and following its washout (right). Red trace represents the average. **G**, Representative examples of postsynaptic Ca^{2+} signals evoked by subthreshold synaptic stimulation in the control condition (left) and in the presence of DL-AP5 (middle) and NASPM (right). The summary data underneath show changes in the peak amplitude of postsynaptic Ca^{2+} signals in the presence of DL-AP-5 (black symbols) or NASPM (red symbols). O/A, Oriens/alveus; PYR, stratum pyramidale; RAD, stratum radiatum.

(Goldberg et al., 2003b). Furthermore, the application of the NMDAR antagonist DL-AP5 slightly reduced the postsynaptic Ca^{2+} transients at some synapses and had no effect at others (Fig. 1G), which is in line with a variable contribution of NMDARs at these synapses. When put together, these results revealed a small but significant decrease of postsynaptic Ca^{2+} transients in the presence of DL-AP5 (to $77.1 \pm 7.1\%$ of control; $n = 6$; Fig. 1G), indicating that NMDARs make a minor contribution to postsynaptic Ca^{2+} influx at local CA1 synapses onto FS interneurons under physiological conditions. In contrast, postsynaptic Ca^{2+} transients exhibited a greater sensitivity to the CP-AMPA inhibitor NASPM (decrease to $21.6 \pm 9.9\%$ of control; $n = 7$; Fig. 1G), which was also successful in blocking the residual DL-AP5-resistant component of subthreshold postsynaptic Ca^{2+} transients ($n = 6$; Fig. 1G). Together, our data indicate that it is CP-AMPA, not NMDARs, that provide the major contribu-

tion to postsynaptic Ca^{2+} signals at CA1 local collateral synapses on FS interneurons.

Dendritic Ca^{2+} nonlinearities in FS interneurons

Previous studies in CA1 pyramidal cells showed that burst stimulation of distal synapses elicits local dendritic Ca^{2+} spikes (Golding et al., 2002; Tsay et al., 2007). To determine whether any type of local regenerative activity can be initiated synaptically in dendrites of FS cells, we examined the summation of postsynaptic Ca^{2+} transients during synaptic stimulation of increasing intensity using a low-affinity Ca^{2+} dye (OGB-5N; Fig. 2). Similar to findings in pyramidal cells (Golding et al., 2002; Tsay et al., 2007), distal Ca^{2+} signals in interneurons exhibited a steep sigmoidal relationship to stimulus intensity (Fig. 2A,B), consistent with the generation of nonlinear regenerative responses. At low stimulus intensities, subthreshold for somatic AP generation, postsynaptic

Figure 2. Supralinear Ca^{2+} signals in distal dendrites of FS interneurons. **A**, Line scan images (top) with corresponding postsynaptic Ca^{2+} transients and voltage responses evoked by synaptic stimulation of varying intensity. Black arrowheads on top indicate the onset of synaptic stimulation. Insets, Expanded view of the somatic voltage responses (with APs truncated). **B**, Representative example (**B1**) and summary plot (**B2**) of Ca^{2+} transient peak amplitude versus stimulus intensity. Red line shows the linear fit between the first three values. Dotted line indicates the Ca^{2+} nonlinearity threshold. **C**, Representative somatic responses before (black) and during the induction of supralinear Ca^{2+} events (red). **D**, Representative examples of the somatic response to gradually increasing stimulation (red trace corresponds to supralinear Ca^{2+} event). **E**, **F**, Summary plots showing the normalized EPSP slope (**E**) and the number of APs (**F**) versus the stimulus intensity (dotted lines indicate the Ca^{2+} nonlinearity threshold). Red lines show the linear fits. Note that both EPSP slope and AP number show a gradual increase in response to increasing stimulus intensity.

Ca^{2+} transients had a small amplitude ($\Delta\text{G/R}$ peak amplitude: 0.06 ± 0.004 ; Fig. 2*A*, *B1*). However, increasing the stimulus intensity by 10–100% was associated with a generation of supralinear Ca^{2+} signals that were almost five times higher than subthreshold postsynaptic Ca^{2+} transients ($\Delta\text{G/R}$ peak amplitude: 0.26 ± 0.02 , $n = 28$; Fig. 2*A*, *B2*). Generation of supralinear Ca^{2+} signals was always associated with somatic spiking (2–4 APs; Fig. 2*A*, *C*, *D*). However, both the slope of somatic voltage responses (Fig. 2*E*) and the number of somatic APs (Fig. 2*F*) increased linearly, indicative of the gradual recruitment of an increasing number of synapses. Dendritic Ca^{2+} nonlinearities were induced in both BCs and BISs as well as in some other unidentified CA1 FS interneurons, which were not included in this study. Importantly, BCs and BISs exhibited a similar degree of summation of postsynaptic Ca^{2+} signals, pointing to a common mechanism for their initiation in different subtypes of FS interneurons.

Mechanisms of Ca^{2+} nonlinearities

To study the mechanisms of Ca^{2+} nonlinearities, we next examined their sensitivity to the NMDAR and AMPAR blockers. Con-

sistent with a minor role of NMDARs at these synapses (Fig. 1), blocking NMDARs had only a small effect on the amplitude of Ca^{2+} nonlinearities (decrease to $77.3 \pm 7.2\%$ of control, $n = 19$, $p < 0.05$; t test; Fig. 3*A*, *B*). The presence of the NMDAR antagonist slightly decreased the degree of summation of Ca^{2+} transients but the summation remained supralinear ($p < 0.05$; $n = 16$, ANOVA; Fig. 3*C*, *D*), indicating that NMDARs contribute to supralinear Ca^{2+} signals but are not essential for their generation. In contrast, blocking CP-AMPA receptors resulted in a strong inhibition of these events (NASPM: decrease to $23.1 \pm 9.6\%$ of control, $n = 10$, $p < 0.001$, t test; PhTx: decrease to $19.5 \pm 5.0\%$ of control, $n = 4$, $p < 0.001$, t test; Fig. 3*A*, *B*). The effect of PhTx was not associated with blocking nicotinic acetylcholine receptors (nAChRs) as the nAChR antagonist *d*TC had no effect on supralinear Ca^{2+} transients ($p = 0.32$, t test, $n = 3$; Fig. 3*B*). Furthermore, summation of Ca^{2+} transients became linear in the presence of the CP-AMPA antagonist NASPM ($n = 7$; Fig. 3*C*, *D*). The residual NASPM-resistant component of supralinear Ca^{2+} signals was blocked by the broad-spectrum AMPA/kainate receptor antagonist NBQX (Fig. 3*A2*, *B*), indicative of a partial blockade of CP-AMPA receptors by NASPM (Le Roux et al., 2013) or of

Figure 3. Supralinear Ca^{2+} signals are mediated by Ca^{2+} -permeable AMPA receptors, with a minor contribution from NMDARs. **A**, Representative examples of supralinear Ca^{2+} signals and corresponding somatic voltage responses (with APs truncated) evoked in control (Ctl; left) and in the presence of glutamatergic receptor blockers DL-AP5 (**A1**, middle), NASPM (**A1**, right; **A2**, middle), and NBQX (**A2**, right). **B**, Summary data showing changes in the amplitude of supralinear Ca^{2+} signals in the presence of pharmacological blockers, as a percentage of control. **C**, Representative examples of supralinear Ca^{2+} signals evoked in three different cells by ramping up the stimulation in control (left), in the presence of DL-AP5 (middle), and in the presence of NASPM (right). **D**, Summary plot showing the effect of DL-AP5 and NASPM on the summation of postsynaptic Ca^{2+} signals.

an additional recruitment of kainate receptors (Cossart et al., 1998). In any case, this residual component's amplitude was small and was insufficient for the induction of supralinear Ca^{2+} signals (Fig. 3C,D). These results demonstrate that CP-AMPA receptors, not NMDARs, play a major role in synaptically evoked supralinear Ca^{2+} signals in distal dendrites of FS interneurons.

As CP-AMPA receptors could contribute to supralinear Ca^{2+} transients by providing postsynaptic depolarization necessary for the activation of VGCCs, we next examined the role of VGCCs in the generation of Ca^{2+} nonlinearities. Blocking L- or T-type VGCCs had no effect on the summation of postsynaptic Ca^{2+} transients. The L-type VGCC antagonist nifedipine (10 μM) reduced the amplitude of supralinear Ca^{2+} events ($78.1 \pm 5.1\%$ of control, $p = 0.053$, $n = 5$, t test) but did not affect the summation of postsynaptic Ca^{2+} transients (Fig. 4A,B,F). No effects on the amplitude or summation of postsynaptic Ca^{2+} transients were found with T-type VGCC inhibitor NNC 55–0396 (10 μM ; Fig. 4C,D,F) or nickel (Ni^{2+} ; 50 μM ; data not shown), consistent with a postnatal downregulation of the T-type VGCC Cav3.1 in FS interneurons (Iftinca and Zamponi, 2009; Okaty et al., 2009). The use of the R-type VGCC antagonist SNX-482 (30 nM) resulted in a strong inhibition of somatic responses ($n = 3$; data not shown), preventing us from studying the specific role of these channels in Ca^{2+} nonlinearities. Furthermore, postsynaptic Ca^{2+} transients summated nonlinearly in the presence of the sodium channel blocker QX-314 ($n = 3$; Fig. 4E). These results indicate that CP-AMPA receptors do not act via activation of sodium and L- or T-type calcium channels but, instead, may provide a direct Ca^{2+} influx involved in the generation of Ca^{2+} nonlinearities.

To test this hypothesis, we next examined the calcium dependency of supralinear Ca^{2+} events (Fig. 5). We found that the threshold for initiation of supralinear Ca^{2+} signals was lower in cells that exhibited a higher subthreshold postsynap-

tic Ca^{2+} influx (Pearson correlation: $r = -0.73$, $p = 0.0113$; Fig. 5A–C, red traces and symbols), pointing to the Ca^{2+} sensitivity of supralinear Ca^{2+} signals. The latter was not a result of an additional contribution of NMDARs at some synapses, as higher NMDAR content had no effect on the amplitude of subthreshold Ca^{2+} influx (Pearson correlation: $r = 0.29$, $p = 0.44$) or on the threshold for initiation of supralinear Ca^{2+} signals (Pearson correlation: $r = -0.38$, $p = 0.32$; Fig. 5D,E). These data point to a specific role of CP-AMPA-mediated Ca^{2+} influx in generation of Ca^{2+} nonlinearities. As supralinear Ca^{2+} signals were associated with a substantial somatic depolarization (Fig. 2), which could hamper CP-AMPA-mediated Ca^{2+} influx in the presence of intracellular polyamine, we wondered whether excluding spermine from the intracellular solution had any effect on the generation of Ca^{2+} nonlinearities. Both the amplitude of supralinear Ca^{2+} events and the threshold for their initiation were not affected by omitting spermine from the intracellular solution ($\Delta G/R$ peak amplitude with spermine: 0.27 ± 0.01 , $n = 19$; $\Delta G/R$ peak amplitude without spermine: 0.26 ± 0.03 , $n = 22$; threshold with spermine: $45.6 \pm 6.7 \mu\text{A}$; threshold without spermine: $45.0 \pm 10.9 \mu\text{A}$, $p > 0.05$, Mann–Whitney test). These findings indicate that intracellular polyamines have no effect on dendritic Ca^{2+} nonlinearities, likely due to the insufficient membrane depolarization achieved in distal dendritic branches during initiation of supralinear Ca^{2+} events. In contrast, increasing the CP-AMPA Ca^{2+} influx by membrane hyperpolarization during synaptic stimulation lowered the threshold for initiation of Ca^{2+} nonlinearities (stimulation threshold at -70 mV : $69.0 \pm 4.1 \mu\text{A}$; stimulation threshold at -90 mV : $43.3 \pm 2.4 \mu\text{A}$, $n = 3$). Collectively, these results indicate that synaptically generated supralinear Ca^{2+} events rely on Ca^{2+} influx via CP-AMPA receptors.

Figure 4. Ca^{2+} nonlinearities in distal dendrites do not involve voltage-gated Ca^{2+} and Na^{+} channels. **A**, Representative examples of supralinear Ca^{2+} events (top) and corresponding voltage responses (bottom; with APs truncated) elicited with a 20% raise in stimulation intensity, in control (Ctl; **A1**) and in the presence of L-type VGCC antagonist nifedipine (Nifed; **A2**). **B**, Summary plot showing the effect of nifedipine on the initiation of Ca^{2+} nonlinearities (dotted line indicates the linear fit between the first two values). **C**, Representative examples of nonlinear Ca^{2+} events (top) and corresponding voltage responses (bottom; with APs truncated) elicited with a 20% raise in stimulation intensity, in control (**C1**) and in the presence of T-type VGCC antagonist NNC 55–0396 (NNC; **C2**). **D**, Summary plot showing the effect of NNC 55–0396 on the initiation of Ca^{2+} nonlinearities (dotted line indicates the linear fit between the first two values). **E**, Nonlinear Ca^{2+} events (top) and corresponding voltage responses (bottom; with APs truncated) elicited with a 14% raise in stimulation intensity, in the presence of Na^{+} channel blocker QX-314. Summary plot is shown underneath. **F**, Summary data showing the effect of Ca^{2+} channel blockers (nifedipine, NNC 55–0396, and Ni^{2+}) on the amplitude of supralinear Ca^{2+} events, as a percentage of control. Note that, in all cases, a 14–20% increase in the stimulation intensity was associated with the generation of supralinear Ca^{2+} responses, indicating that VGCCs and sodium channels are not involved in the generation of dendritic Ca^{2+} nonlinearities.

Previous evidence indicates that Ca^{2+} -induced Ca^{2+} release (CICR) from intracellular stores can amplify the postsynaptic Ca^{2+} signal (Wang et al., 2000; Nakamura et al., 2002; Watanabe et al., 2006). To study whether this mechanism can operate in distal dendrites of FS interneurons, we examined the sensitivity of Ca^{2+} nonlinearities to pharmacological manipulations of Ca^{2+} release (Fig. 6). Our data showed that the summation of postsynaptic Ca^{2+} transients became linear in the presence of CPA, a specific blocker of smooth endoplasmic reticulum Ca^{2+} /ATPases (SERCA; Fig. 6A,E), or ryanodine (Fig. 6B,E). Moreover, priming the intracellular stores by local puff application of caffeine (1 mM, 50 ms), the ryanodine receptor agonist, decreased the threshold for the initiation of supralin-

ear Ca^{2+} transients ($p < 0.05$, $n = 4$, t test; Fig. 6C) without affecting their amplitude ($p > 0.05$, $n = 4$, t test; Fig. 6E). These data point to a major role of CICR in the generation of Ca^{2+} nonlinearities. As both NMDARs and L-type VGCCs made a significant contribution to supralinear Ca^{2+} signals (Figs. 3B, 4F) and together could provide Ca^{2+} influx necessary to induce Ca^{2+} release, we examined next whether blocking these two Ca^{2+} sources simultaneously may have a significant impact on the generation of Ca^{2+} nonlinearities. Our data showed that, compared with control recordings (Fig. 2B), the degree of summation of Ca^{2+} transients was reduced but the summation remained supralinear ($n = 4$, $p < 0.05$, ANOVA; Fig. 6D), suggesting that CICR initiated by the spe-

Figure 5. Calcium dependence of Ca^{2+} nonlinearities. **A**, Representative examples of postsynaptic Ca^{2+} transients (black arrows: Ca^{2+} nonlinearities) and corresponding voltage responses evoked by synaptic stimulation of different intensities [from subthreshold stimulation (for somatic AP generation) to 45% increase]. **B**, Summary plot of Ca^{2+} transient peak amplitudes versus stimulus intensities (dotted lines: Ca^{2+} nonlinearity threshold). Note that a cell with subthreshold Ca^{2+} transients of higher amplitude (shown in red) had a lower threshold for the initiation of supralinear Ca^{2+} events. **C**, Summary data for a population of cells showing a significant negative correlation between the stimulus intensity required to evoke a supralinear Ca^{2+} event and the amplitude of subthreshold Ca^{2+} transients. **D**, **E**, Summary plots showing the absence of correlation between the contribution of the NMDAR to postsynaptic Ca^{2+} response and the amplitude of subthreshold Ca^{2+} transients (**D**) or the threshold to induce a supralinear Ca^{2+} event (**E**). NMDAR contribution was determined as the DL-AP-5-sensitive component. The threshold to induce a supralinear Ca^{2+} event was determined as the minimal intensity of stimulation (μA) necessary to evoke the supralinear Ca^{2+} event.

Figure 6. Ca^{2+} nonlinearities are produced by Ca^{2+} -induced Ca^{2+} release. **A–C**, Representative examples of supralinear Ca^{2+} responses with corresponding voltage responses (with APs truncated; top) and summary plots (bottom) illustrating the summation of postsynaptic Ca^{2+} transients under different conditions (**A**, CPA; **B**, Ryanodine, Ryan; **C**, caffeine, Caf). Vertical dotted lines on the summary plot at **C** indicate the threshold for initiation of supralinear Ca^{2+} signals. **D**, Summary plot illustrating the summation of postsynaptic Ca^{2+} transients in the presence of the NMDAR blocker DL-AP5 and the L-type VGCC inhibitor nifedipine. **E**, Summary data showing the effects of CPA, ryanodine, and caffeine on the peak amplitude of supralinear Ca^{2+} signals, as a percentage of control (Ctl). Note that Ca^{2+} responses summated linearly in the presence of Ca^{2+} release blockers CPA and ryanodine.

cific activation of CP-AMPA is sufficient for the generation of Ca^{2+} nonlinearities. We did not observe the participation of group I/II mGluRs in supralinear Ca^{2+} signals induced by single bursts of synaptic stimulation because these events were

completely blocked by a combination of DL-AP5, NASPM, and NBQX ($n = 3$; Fig. 3A2,B) and were not sensitive to the group I/II mGluR antagonist E4CPG ($n = 5$, $p > 0.05$, t test; Fig. 3B). Thus, intracellular Ca^{2+} release initiated by Ca^{2+} influx

Figure 7. Spatial profile of Ca^{2+} nonlinearities. **A**, Simultaneous line scan imaging of three neighboring dendritic segments during different intensities of stimulation in control (Ctl; 70–110 μA) and in the presence of CPA (110 μA ; left) and corresponding Ca^{2+} responses detected in these segments under different conditions (right). The arrowhead to the left of the line scan images indicates the location of the stimulation pipette. The arrowheads to the right point to dendritic nonlinearities. **B**, Spatial profile graphs illustrating the spatial extent of subthreshold (Sub; top) and supra-linear (Supra; middle) Ca^{2+} responses, as well as supra-linear responses in the presence of CPA. Data are summarized alongside, with the SD from the peak serving as an approximation of spatial extent. **C**, Two-photon image of distal branches showing the location of the line scan measurements used to assess the spatial extent of Ca^{2+} nonlinearities (left), along with their corresponding responses (right, top), and summary plot of the Ca^{2+} nonlinearity peak amplitude versus distance from the stimulation site (right, bottom). Red curve indicates a Gaussian fit. **D**, **E**, Summary plots showing the relationship between the amplitude of supra-linear Ca^{2+} signals and distance from the soma (**D**) or dendrite diameter (**E**).

through CP-AMPA is a primary mechanism of supra-linear Ca^{2+} signals generated in FS interneurons.

Spatial profile of dendritic Ca^{2+} nonlinearities

The spatial extent of Ca^{2+} release events can vary from spatially restricted Ca^{2+} “sparks” to regenerative Ca^{2+} waves (Berridge, 1998; Callamaras et al., 1998; Watanabe et al., 2006; Manita and Ross, 2009; Miyazaki and Ross, 2013). To study the spatial profile of Ca^{2+} nonlinearities in dendritic branches of FS interneurons, we first performed fast line scan imaging along dendritic segments of 10–15 μm -length. Upon initiation, supra-linear Ca^{2+} signals were restricted within small dendritic microdomains ($6.0 \pm 0.9 \mu\text{m}$, $n = 6$; Fig. 7A) and remained localized within individual dendritic branches when a supra-linear Ca^{2+} event was generated ($11.2 \pm 2.3 \mu\text{m}$, $n = 6$; Fig. 7A,B). The SERCA pump inhibitor CPA removed the supra-linear component of Ca^{2+} events but did not affect significantly the spatial extent of Ca^{2+} events ($7.8 \pm 0.8 \mu\text{m}$, $n = 6$, $p > 0.05$, t test; Fig. 7A,B), consistent with a major role of Ca^{2+} release in local Ca^{2+} nonlinearities. To further examine the possible propagation of supra-linear Ca^{2+} events, we performed line scan imaging across dendritic

segments at different locations from the stimulation site (Fig. 7C, left). The focal plane was adjusted according to the z-position of the dendrite. The summation of postsynaptic Ca^{2+} transients was examined in each location by raising the stimulus intensity gradually up to 200% from the initial stimulus strength necessary to evoke the postsynaptic Ca^{2+} signal. Under these recording conditions, supra-linear Ca^{2+} signals were also seen within individual dendritic branches ($13.5 \pm 2.1 \mu\text{m}$; Fig. 7C, right). In addition, we found that supra-linear Ca^{2+} events increased their amplitude with distance from the soma (Pearson correlation: $r = 0.62$, $p = 0.0003$; Fig. 7D) and were significantly higher in secondary and tertiary dendritic branches because of the smaller dendritic diameter (Pearson correlation: $r = -0.53$, $p = 0.0024$; Fig. 7E). This shows that in FS interneurons, supra-linear Ca^{2+} events occur primarily within distal secondary and tertiary dendritic branches and remain localized.

Functional role of dendritic Ca^{2+} nonlinearities

At excitatory synapses of pyramidal neurons, supra-linear Ca^{2+} events have been consistently associated with induction of LTP (Magee and Johnston, 1997; Koester and Sakmann, 1998; Gold-

Figure 8. Ca^{2+} nonlinearities control the direction of plasticity. **A**, Two-photon image showing the location of the stimulation electrode used to induce synaptic plasticity. **B–E**, Representative examples of somatic responses and dendritic Ca^{2+} fluctuations evoked by different types of TBS under different conditions (**B1–E1**) along with summary time plots of normalized EPSC amplitude (**B2–E2**, left) and with representative examples of EPSCs recorded before and after TBS (**B2–E2**, right). The black arrowhead above plots indicates the onset of TBS. In different experiments, TBS was applied at a subthreshold (for somatic AP generation) stimulation level (**B1**), a suprathreshold stimulation level producing Ca^{2+} nonlinearity (**C1**), a suprathreshold stimulation level in the presence of CPA (**D1**), or a suprathreshold stimulation level in the presence of the group I mGluR antagonist E4CPG (**E1**). **F**, Summary graph showing the changes in Ca^{2+} fluctuations (area under the curve) during TBS for the four conditions. Ctl, control. O/A, Oriens/alveus.

ing et al., 2002; Dan and Poo, 2004). To test whether Ca^{2+} nonlinearities can regulate synaptic efficacy at excitatory synapses of FS interneurons, we examined how their generation at theta frequency, a natural pattern of hippocampal activity, affects the efficacy of transmission at distal excitatory synapses (Fig. 8). Interneurons were voltage clamped at -70 mV and EPSCs were evoked by local bipolar stimulation of distal inputs in the stratum oriens/alveus (100 – 250 μm from the soma; Fig. 8A). After a baseline recording, TBS (three trains at 4 Hz for 2 s with 30 s intervals) was applied to interneurons held in current-clamp mode, after which the voltage-clamp recordings of EPSCs were resumed. Consistent with previous findings (Lamsa et al., 2007; Oren et al., 2009; Nissen et al., 2010; Griguoli et al., 2013), low-intensity (subthreshold for somatic AP generation) TBS at rest ($V_m = -70$ mV) applied within 8 min of whole-cell recording produced LTP (EPSC amplitude, $264.3 \pm 26.3\%$ of control; Fig. 8B2; $n = 7$, $p < 0.05$, t test). Postsynaptic Ca^{2+} transients evoked by the same stimulation paradigm in a separate series of experiments had a small amplitude ($\Delta\text{G/R}$ peak amplitude: 0.07 ± 0.01 ; Fig. 8B1,F), consistent with the absence of supralinear Ca^{2+} events under basal conditions. Surprisingly, increasing the stimulation inten-

sity during TBS (100% from the baseline level) to trigger dendritic Ca^{2+} nonlinearities (Fig. 8C1,F) induced LTD at these synapses (EPSC amplitude: $70.2 \pm 13.2\%$ of control; Fig. 8C2; $n = 7$, $p < 0.05$, t test). Ca^{2+} signals evoked by such suprathreshold TBS in a separate series of experiments had a large amplitude ($\Delta\text{G/R}$ peak amplitude: 0.28 ± 0.02 , $n = 5$), consistent with the induction of supralinear Ca^{2+} events. Blocking internal Ca^{2+} release by CPA during suprathreshold TBS resulted in a significant decrease of supralinear Ca^{2+} events and in a switch in plasticity direction from LTD back to LTP (EPSC amplitude: $141.4 \pm 4.6\%$ of control; Fig. 8D,F; $n = 5$, $p < 0.05$, t test). To test whether the CPA application affects presynaptic Ca^{2+} release (Simkus and Stricker, 2002; Martín and Buño, 2003), which could contribute to plastic changes, we analyzed the properties of sEPSCs and evoked EPSCs (eEPSC) as well as the area under curve of TBS-evoked somatic responses in control and in the presence of CPA. Our data showed that the amplitude, frequency, and kinetics of sEPSCs were not affected by CPA application (amplitude control: 18.8 ± 0.4 pA; amplitude CPA: 17.7 ± 0.3 pA; frequency control: 5.1 ± 0.8 Hz; frequency CPA: 6.8 ± 1.7 Hz; rise control: 0.36 ± 0.01 ms; rise CPA: 0.35 ± 0.03 ms; decay control: $2.33 \pm$

0.09 ms; decay CPA: 2.25 ± 0.06 ms; $n = 4$, $p < 0.05$, Mann–Whitney test). Moreover, CPA had no effect on the paired-pulse ratio (PPR) of eEPSCs (PPR control: 1.85 ± 0.12 , $n = 7$; PPR CPA: 1.86 ± 0.19 , $n = 5$; $p < 0.05$, Mann–Whitney test). Furthermore, somatic voltage responses evoked by suprathreshold TBS were also not affected by CPA application (area under curve control: 1070 ± 236 mV $\times$ ms; area under curve CPA: 1315 ± 171 mV $\times$ ms, $n = 5$, $p < 0.05$, Mann–Whitney test). Together, these data indicate that, similar to other types of excitatory synapses (Carter et al., 2002), presynaptic Ca^{2+} stores do not contribute to transmitter release at CA1 synapses to FS interneurons.

As suprathreshold TBS could activate postsynaptic group I mGluRs, which also contribute to internal Ca^{2+} release, we examined the sensitivity of supralinear Ca^{2+} signals to the group I/II mGluR antagonist E4CPG (Fig. 8E1,F). Whereas E4CPG had no effect on the peak amplitude of supralinear Ca^{2+} events evoked by single bursts (Figs. 3B, 8E1; first burst in the train), it decreased significantly the area under the curve of the TBS-induced supralinear Ca^{2+} elevation (Fig. 8F; $n = 5$, $p < 0.05$, t test), indicating that mGluR-mediated postsynaptic Ca^{2+} release contributes to this signal. Accordingly, blocking group I/II mGluRs during suprathreshold TBS resulted in STD (EPSC amplitude $62.4 \pm 13\%$ of control; $n = 4$, $p < 0.05$, t test) with a complete recovery of EPSC amplitude within 15 min after induction (Fig. 8E2). Hence, supralinear Ca^{2+} events resulting from the Ca^{2+} -induced Ca^{2+} release following the activation of CP-AMPA receptors switch the direction of synaptic plasticity from LTP to STD. Furthermore, additional recruitment of mGluRs, which takes place during repetitive theta-like activity, converts STD to a longer lasting depression.

Discussion

Here, we investigated the mechanisms and functional significance of supralinear Ca^{2+} signals generated by synchronous activation of excitatory synapses in distal dendritic branches of hippocampal CA1 FS interneurons. Our major findings are that supralinear Ca^{2+} signals (1) require Ca^{2+} influx through CP-AMPA receptors with a subsequent Ca^{2+} release; (2) remain localized within individual dendritic branches; and (3) switch the direction of synaptic plasticity from LTP to STD or LTD, depending on the additional recruitment of group I/II mGluRs. We conclude that, by linking together different signaling cascades, intracellular Ca^{2+} release in dendrites of FS interneurons provides a reliable and dynamically regulated source of Ca^{2+} that may signal coincident activation of synaptic inputs and, thus, represents an important mechanism in the regulation of synaptic strength.

Mechanisms of Ca^{2+} nonlinearities in FS interneurons

Our results provide new insights into mechanisms of regenerative activity in distal dendrites of interneurons. Consistent with previous findings in neocortical FS cells and hippocampal CA1 PV-positive interneurons (Goldberg et al., 2003b; Le Roux et al., 2013), we found that the GluA2-lacking CP-AMPA receptors provide a primary source of postsynaptic Ca^{2+} influx. CP-AMPA receptors were solely responsible for postsynaptic Ca^{2+} influx in 60% of synapses examined and mediated up to 80% of postsynaptic Ca^{2+} influx at rest. Accordingly, CP-AMPA receptors played a major role in the induction of Ca^{2+} release resulting in the generation of dendritic supralinear Ca^{2+} events. NMDARs and L-type VGCCs made a minor contribution to Ca^{2+} nonlinearities but were not sufficient for their generation. Therefore, unlike the mechanisms described pre-

viously (Schiller et al., 1997; Wang et al., 2000; Wei et al., 2001; Golding et al., 2002; Losonczy and Magee, 2006; Watanabe et al., 2006; Tsay et al., 2007), supralinear Ca^{2+} signals in FS interneurons do not rely on the activation of voltage-dependent Ca^{2+} sources but require instead Ca^{2+} mechanisms activated mainly at rest. This mechanism may predominate in interneurons because of the highly variable distributions of voltage-gated Ca^{2+} sources (e.g., NMDARs; Lei and McBain, 2002; Goldberg et al., 2003b; Nyíri et al., 2003; Le Roux et al., 2013). We did not find significant differences in the NMDAR contribution to synaptic responses between BCs and BISs, pointing to the lack of cell type-specific differences in NMDAR distribution within a population of FS interneurons. However, other excitatory projections that terminate within CA1 O/A could be recruited in our experiments (Somogyi and Klausberger, 2005; Takács et al., 2012), pointing to the synapse-specific variations in NMDAR distribution in FS interneurons. This question will require further examination using selective optogenetic targeting of specific excitatory projections. Alternatively, limited functional availability of voltage-dependent Ca^{2+} mechanisms in distal dendritic branches resulting from the failure of AP back-propagation and insufficient membrane depolarization can explain the minor role of these mechanisms in supralinear Ca^{2+} signals (Goldberg et al., 2003a; Aponte et al., 2008; Hu et al., 2010). Under these conditions, AMPAR-store coupling allows the coincidence detection of activated synapses and the site-specific regulation of synaptic strength depending on a number of synapses activated synchronously. Given the size of unitary excitatory responses at CA1 PC to FS interneuron synapses (~ 2 mV; Lacaille et al., 1987; Ali et al., 1998), the size of EPSPs evoked during supralinear Ca^{2+} signals (~ 10 – 15 mV) and the heavy innervation of FS cells (Gulyás et al., 1999), we estimate that at least ~ 5 – 7 synapses are required to generate a nonlinear Ca^{2+} event in dendritic compartment of ~ 5 μm (Fig. 7A,B). It should be pointed out that this measure is an approximation since the amplitude measurements during supralinear events were contaminated to some extent by sodium spikes riding on top of EPSPs. Further increase in the stimulation intensity was associated with recruitment of additional synapses and/or local spread of Ca^{2+} signals (Fig. 7C). However, no conclusion can be made regarding the actual number of synapses that may experience supralinear Ca^{2+} elevations under physiological conditions as the spatial extent of Ca^{2+} signals was likely overestimated due to the high mobility of synthetic Ca^{2+} indicators (Goldberg et al., 2003c).

Our data showed that ryanodine receptors (RyRs) mediate release of Ca^{2+} from internal stores in FS interneurons. RyR1 and, to a lesser degree, RyR2 are expressed in the CA1 hippocampal region (Hertle and Yeckel, 2007) and trigger fast and spatially localized Ca^{2+} “sparks” in dendrites of PCs (Manita and Ross, 2009; Miyazaki et al., 2012; Miyazaki and Ross, 2013), which appear similar to Ca^{2+} release events detected here. It should be pointed out that spontaneous Ca^{2+} release events have not been observed in our study, and their existence and degree of similarity with Ca^{2+} sparks described in cardiac myocytes (Cheng and Lederer, 2008) and hippocampal PCs (Manita and Ross, 2009; Miyazaki and Ross, 2013) need to be examined using a higher affinity Ca^{2+} indicator. Furthermore, we found that group I mGluR-mediated Ca^{2+} release makes an additional contribution to supralinear Ca^{2+} signals during repetitive suprathreshold TBS but not during single burst. The mGluR-mediated Ca^{2+} release likely involved the activation of mGluR5 (van Hooft et al., 2000) and

RyRs (Topolnik et al., 2006, 2009), as PV-positive interneurons in this area do not express IP3 receptor 1 (Hertle and Yeckel, 2007) but exhibit RyR-mediated Ca^{2+} release following the activation of G_q -PLC signaling cascade (Lee et al., 2011). The absence of IP3 receptors, together with fast Ca^{2+} extrusion in PV-positive interneurons (Goldberg et al., 2003c), may explain why traveling Ca^{2+} waves were not observed in our study even following the group I mGluR activation during repetitive synaptic activity (Watanabe et al., 2006; Miyazaki and Ross, 2013).

Functional role of dendritic Ca^{2+} nonlinearities

Postsynaptic Ca^{2+} influx via CP-AMPA receptors has been associated with the induction of distinct forms of synaptic plasticity at excitatory synapses of hippocampal interneurons (Laezza et al., 1999; Lei and McBain, 2002; Lamsa et al., 2005, 2007; Oren et al., 2009; Sambandan et al., 2010). Activation of CP-AMPA receptors together with group I mGluRs or $\alpha 7$ nAChRs at a relatively hyperpolarized level of the postsynaptic membrane potential can induce the so-called “anti-Hebbian” LTP (Lamsa et al., 2007; Oren et al., 2009; Nissen et al., 2010; Le Duigou and Kullmann, 2011; Szabo et al., 2012; Griguoli et al., 2013; Le Roux et al., 2013). In addition, short- and long-term synaptic depression was observed at GluA2 AMPAR-lacking synapses following the activation of group I mGluRs (Le Duigou et al., 2011) or tetanic stimulation of excitatory inputs (Laezza et al., 1999; Lei and McBain, 2002). Together, these findings indicate that GluA2 AMPAR-lacking synapses are capable of regulating the efficacy of transmission in a bidirectional manner. It should be pointed out, however, that, as different hippocampal regions, cell and synapse types, as well as induction paradigms have been studied by different groups, the mechanisms underlying multiple forms of plasticity at GluA2 AMPAR-lacking interneuron synapses are still not clear. As postsynaptic Ca^{2+} signal is a cornerstone in plasticity induction, we focused on examining how activity-dependent Ca^{2+} fluctuations may control the induction of plasticity at CP-AMPA-dominated synapses of interneurons. Our data highlights the coexistence of multiple forms of plasticity in the same synapse by providing direct evidence for the activity-dependent recruitment of additional Ca^{2+} mechanisms downstream of the CP-AMPA Ca^{2+} influx. We demonstrate that, whereas small amplitude Ca^{2+} transients associated with subthreshold theta-like activity induce anti-Hebbian LTP, supralinear Ca^{2+} signals generated during suprathreshold theta-burst activity by the intracellular Ca^{2+} release induce LTD. The LTD induction required additional recruitment of group I/II mGluRs, as blocking these receptors converted LTD to STD. Thus, supralinear Ca^{2+} events generated by Ca^{2+} release following CP-AMPA activation were responsible for STD. The expression site of different forms of synaptic plasticity observed here and the underlying signaling mechanisms remain to be determined. Previous findings showed that anti-Hebbian LTP is expressed presynaptically and, likely, involves the activation of a yet unknown retrograde messenger (Lamsa et al., 2007). Presynaptic expression of the group I mGluR-dependent LTD was also reported. Importantly, postsynaptic group I mGluR activation and endocannabinoid release mediated LTD in CA1 FS interneurons (Péterfi et al., 2012). When compared with PCs, a higher stimulation frequency was required for this LTD induction due to the lower level of diacylglycerol lipase- α in interneurons. As application of the mGluR antagonist decreased significantly TBS-evoked post-

synaptic Ca^{2+} signals in our experiments, we assume that postsynaptic group I mGluRs were involved in suprathreshold LTD. Thus, it will be plausible to examine the role of these receptors and of endocannabinoids in the STD and LTD observed here.

In conclusion, our data shed light on the Ca^{2+} mechanisms of plasticity induction at GluA2 AMPAR-lacking synapses and revealed a novel metaplastic mechanism by which synaptic weight can be transiently adjusted depending on the number of synapses activated synchronously. Moreover, as excitatory synapses in many types of neurons throughout the CNS may incorporate GluA2-lacking AMPA receptors earlier during development or following synaptic inactivity (Kumar et al., 2002; Thiagarajan et al., 2005), this mechanism may be important for the detection of coincident synaptic inputs in the absence of voltage-dependent Ca^{2+} sources and may provide additional ways for associative learning.

Notes

Supplemental material for this article is available at www.neuronimaging.ca. This material has not been peer reviewed.

References

- Ali AB, Deuchars J, Pawelzik H, Thomson AM (1998) CA1 pyramidal to basket and bistratified cell EPSPs: dual intracellular recordings in rat hippocampal slices. *J Physiol* 507:201–217. [CrossRef Medline](#)
- Aponte Y, Bischofberger J, Jonas P (2008) Efficient Ca^{2+} buffering in fast-spiking basket cells of rat hippocampus. *J Physiol* 586:2061–2075. [CrossRef Medline](#)
- Berridge MJ (1998) Neuronal calcium signaling. *Neuron* 21:13–26. [CrossRef Medline](#)
- Buhl EH, Szilágyi T, Halasy K, Somogyi P (1996) Physiological properties of anatomically identified basket and bistratified cells in the CA1 area of the rat hippocampus in vitro. *Hippocampus* 6:294–305. [CrossRef Medline](#)
- Callamaras N, Marchant JS, Sun XP, Parker I (1998) Activation and coordination of InsP_3 -mediated elementary Ca^{2+} events during global Ca^{2+} signals in *Xenopus* oocytes. *J Physiol* 509:81–91. [CrossRef Medline](#)
- Carter AG, Vogt KE, Foster KA, Regehr WG (2002) Assessing the role of calcium-induced calcium release in short-term presynaptic plasticity at excitatory central synapses. *J Neurosci* 22:21–28. [Medline](#)
- Cheng H, Lederer WJ (2008) Calcium sparks. *Physiol Rev* 88:1491–1545. [CrossRef Medline](#)
- Cossart R, Esclapez M, Hirsch JC, Bernard C, Ben-Ari Y (1998) GluR5 kainate receptor activation in interneurons increases tonic inhibition of pyramidal cells. *Nat Neurosci* 1:470–478. [CrossRef Medline](#)
- Dan Y, Poo MM (2004) Spike timing-dependent plasticity of neural circuits. *Neuron* 44:23–30. [CrossRef Medline](#)
- Evstratova A, Chamberland S, Topolnik L (2011) Cell type-specific and activity-dependent dynamics of action potential-evoked Ca^{2+} signals in dendrites of hippocampal inhibitory interneurons. *J Physiol* 589:1957–1977. [CrossRef Medline](#)
- Glickfeld LL, Scanziani M (2006) Distinct timing in the activity of cannabinoid-sensitive and cannabinoid-insensitive basket cells. *Nat Neurosci* 9:807–815. [CrossRef Medline](#)
- Goldberg JH, Tamas G, Yuste R (2003a) Ca^{2+} imaging of mouse neocortical interneurone dendrites: Ia-type K $^{+}$ channels control action potential backpropagation. *J Physiol* 551:49–65. [CrossRef Medline](#)
- Goldberg JH, Yuste R, Tamas G (2003b) Ca^{2+} imaging of mouse neocortical interneurone dendrites: contribution of Ca^{2+} -permeable AMPA and NMDA receptors to subthreshold Ca^{2+} dynamics. *J Physiol* 551:67–78. [CrossRef Medline](#)
- Goldberg JH, Tamas G, Aronov D, Yuste R (2003c) Calcium microdomains in aspiny dendrites. *Neuron* 40:807–821. [CrossRef Medline](#)
- Goldberg JH, Lacefield CO, Yuste R (2004) Global dendritic calcium spikes in mouse layer 5 low threshold spiking interneurons: implications for control of pyramidal cell bursting. *J Physiol* 558:465–478. [CrossRef Medline](#)
- Golding NL, Staff NP, Spruston N (2002) Dendritic spikes as a mechanism for cooperative long-term potentiation. *Nature* 418:326–331. [CrossRef Medline](#)

- Griguoli M, Cellot G, Cherubini E (2013) In hippocampal oriens interneurons anti-Hebbian long-term potentiation requires cholinergic signaling via $\alpha 7$ nicotinic acetylcholine receptors. *J Neurosci* 33:1044–1049. [CrossRef Medline](#)
- Gulyás AI, Megias M, Emri Z, Freund TF (1999) Total number and ratio of excitatory and inhibitory synapses converging onto single interneurons of different types in the CA1 area of the rat hippocampus. *J Neurosci* 19:10082–10097. [Medline](#)
- Hertle DN, Yeckel MF (2007) Distribution of inositol-1,4,5-trisphosphate receptor isoforms and ryanodine receptor isoforms during maturation of the rat hippocampus. *Neuroscience* 150:625–638. [CrossRef Medline](#)
- Hu H, Martina M, Jonas P (2010) Dendritic mechanisms underlying rapid synaptic activation of fast-spiking hippocampal interneurons. *Science* 327:52–58. [CrossRef Medline](#)
- Iftinca MC, Zamponi GW (2009) Regulation of neuronal T-type calcium channels. *Trends Pharmacol Sci* 30:32–40. [CrossRef Medline](#)
- Katona G, Kaszás A, Turi GF, Hájós N, Tamás G, Vizi ES, Rózsa B (2011) Roller Coaster Scanning reveals spontaneous triggering of dendritic spikes in CA1 interneurons. *Proc Natl Acad Sci U S A* 108:2148–2153. [CrossRef Medline](#)
- Koester HJ, Sakmann B (1998) Calcium dynamics in single spines during coincident pre- and postsynaptic activity depend on relative timing of back-propagating action potentials and subthreshold excitatory postsynaptic potentials. *Proc Natl Acad Sci U S A* 95:9596–9601. [CrossRef Medline](#)
- Koike M, Iino M, Ozawa S (1997) Blocking effect of 1-naphthyl acetyl spermine on $\text{Ca}(2+)$ -permeable AMPA receptors in cultured rat hippocampal neurons. *Neurosci Res* 29:27–36. [CrossRef Medline](#)
- Kumar SS, Bacci A, Kharazia V, Huguenard JR (2002) A developmental switch of AMPA receptor subunits in neocortical pyramidal neurons. *J Neurosci* 22:3005–3015. [Medline](#)
- Lacaille JC, Mueller AL, Kunkel DD, Schwartzkroin PA (1987) Local circuit interactions between oriens/alveus interneurons and CA1 pyramidal cells in hippocampal slices: electrophysiology and morphology. *J Neurosci* 7:1979–1993. [Medline](#)
- Laezza F, Doherty JJ, Dingledine R (1999) Long-term depression in hippocampal interneurons: joint requirement for pre- and postsynaptic events. *Science* 285:1411–1414. [CrossRef Medline](#)
- Lamsa K, Heeroma JH, Kullmann DM (2005) Hebbian LTP in feed-forward inhibitory interneurons and the temporal fidelity of input discrimination. *Nat Neurosci* 8:916–924. [CrossRef Medline](#)
- Lamsa KP, Heeroma JH, Somogyi P, Rusakov DA, Kullmann DM (2007) Anti-Hebbian long-term potentiation in the hippocampal feedback inhibitory circuit. *Science* 315:1262–1266. [CrossRef Medline](#)
- Le Duijgou C, Kullmann DM (2011) Group I mGluR agonist-evoked long-term potentiation in hippocampal oriens interneurons. *J Neurosci* 31:5777–5781. [CrossRef Medline](#)
- Le Duijgou C, Holden T, Kullmann DM (2011) Short- and long-term depression at glutamatergic synapses on hippocampal interneurons by group I mGluR activation. *Neuropharmacology* 60:748–756. [CrossRef Medline](#)
- Lee SY, Földy C, Szabadics J, Soltesz I (2011) Cell-type-specific CCK2 receptor signaling underlies the cholecystokinin-mediated selective excitation of hippocampal parvalbumin-positive fast-spiking basket cells. *J Neurosci* 31:10993–11002. [CrossRef Medline](#)
- Lei S, McBain CJ (2002) Distinct NMDA receptors provide differential modes of transmission at mossy fiber-interneuron synapses. *Neuron* 33:921–933. [CrossRef Medline](#)
- Le Roux N, Cabezas C, Böhm UL, Poncer JC (2013) Input-specific learning rules at excitatory synapses onto hippocampal parvalbumin-expressing interneurons. *J Physiol* 591:1809–1822. [CrossRef Medline](#)
- Losonczy A, Magee JC (2006) Integrative properties of radial oblique dendrites in hippocampal CA1 pyramidal neurons. *Neuron* 50:291–307. [CrossRef Medline](#)
- Magee JC, Johnston D (1997) A synaptically controlled, associative signal for Hebbian plasticity in hippocampal neurons. *Science* 275:209–213. [CrossRef Medline](#)
- Manita S, Ross WN (2009) Synaptic activation and membrane potential changes modulate the frequency of spontaneous elementary Ca^{2+} release events in the dendrites of pyramidal neurons. *J Neurosci* 29:7833–7845. [CrossRef Medline](#)
- Markram H, Sakmann B (1994) Calcium transients in dendrites of neocortical neurons evoked by single subthreshold excitatory postsynaptic potentials via low-voltage-activated calcium channels. *Proc Natl Acad Sci U S A* 91:5207–5211. [CrossRef Medline](#)
- Martín ED, Buño W (2003) Caffeine-mediated presynaptic long-term potentiation in hippocampal CA1 pyramidal neurons. *J Neurophysiol* 89:3029–3038. [CrossRef Medline](#)
- Miyazaki K, Ross WN (2013) Ca^{2+} Sparks and puffs are generated and interact in rat hippocampal CA1 pyramidal neuron dendrites. *J Neurosci* 33:17777–17788. [CrossRef Medline](#)
- Miyazaki K, Manita S, Ross WN (2012) Developmental profile of localized spontaneous $\text{Ca}(2+)$ release events in the dendrites of rat hippocampal pyramidal neurons. *Cell Calcium* 52:422–432. [CrossRef Medline](#)
- Müller A, Kukley M, Stausberg P, Beck H, Müller W, Dietrich D (2005) Endogenous Ca^{2+} buffer concentration and Ca^{2+} microdomains in hippocampal neurons. *J Neurosci* 25:558–565. [CrossRef](#)
- Nakamura T, Lasser-Ross N, Nakamura K, Ross WN (2002) Spatial segregation and interaction of calcium signalling mechanisms in rat hippocampal CA1 pyramidal neurons. *J Physiol* 543:465–480. [CrossRef Medline](#)
- Nissen W, Szabo A, Somogyi J, Somogyi P, Lamsa KP (2010) Cell type-specific long-term plasticity at glutamatergic synapses onto hippocampal interneurons expressing either parvalbumin or CB1 cannabinoid receptor. *J Neurosci* 30:1337–1347. [CrossRef Medline](#)
- Nyíri G, Stephenson FA, Freund TF, Somogyi P (2003) Large variability in synaptic N-methyl-D-aspartate receptor density on interneurons and a comparison with pyramidal-cell spines in the rat hippocampus. *Neuroscience* 119:347–363. [CrossRef Medline](#)
- Okaty BW, Miller MN, Sugino K, Hempel CM, Nelson SB (2009) Transcriptional and electrophysiological maturation of neocortical fast-spiking GABAergic interneurons. *J Neurosci* 29:7040–7052. [CrossRef Medline](#)
- Oren I, Nissen W, Kullmann DM, Somogyi P, Lamsa KP (2009) Role of ionotropic glutamate receptors in long-term potentiation in rat hippocampal CA1 oriens-lacunosum moleculare interneurons. *J Neurosci* 29:939–950. [CrossRef Medline](#)
- Péterfi Z, Urbán GM, Papp OI, Németh B, Monyer H, Szabó G, Erdélyi F, Mackie K, Freund TF, Hájós N, Katona I (2012) Endocannabinoid-mediated long-term depression of afferent excitatory synapses in hippocampal pyramidal cells and GABAergic interneurons. *J Neurosci* 32:14448–14463. [CrossRef Medline](#)
- Regehr WG, Tank DW (1990) Postsynaptic NMDA receptor-mediated calcium accumulation in hippocampal CA1 pyramidal cell dendrites. *Nature* 345:807–810. [CrossRef Medline](#)
- Remy S, Spruston N (2007) Dendritic spikes induce single-burst long-term potentiation. *Proc Natl Acad Sci U S A* 104:17192–17197. [CrossRef Medline](#)
- Sambandan S, Sauer JF, Vida I, Bartos M (2010) Associative plasticity at excitatory synapses facilitates recruitment of fast-spiking interneurons in the dentate gyrus. *J Neurosci* 30:11826–11837. [CrossRef Medline](#)
- Schiller J, Schiller Y, Stuart G, Sakmann B (1997) Calcium action potentials restricted to distal apical dendrites of rat neocortical pyramidal neurons. *J Physiol* 505:605–616. [CrossRef Medline](#)
- Shen JB, Jiang B, Pappano AJ (2000) Comparison of L-type calcium channel blockade by nifedipine and/or cadmium in guinea pig ventricular myocytes. *J Pharmacol Exp Ther* 294:562–570. [Medline](#)
- Sik A, Penttonen M, Ylinen A, Buzsáki G (1995) Hippocampal CA1 interneurons: an *in vivo* intracellular labeling study. *J Neurosci* 15:6651–6665. [Medline](#)
- Simkus CR, Stricker C (2002) The contribution of intracellular calcium stores to mEPSCs recorded in layer II neurones of rat barrel cortex. *J Physiol* 545:521–535. [CrossRef Medline](#)
- Somogyi P, Klausberger T (2005) Defined types of cortical interneurone structure space and spike timing in the hippocampus. *J Physiol* 562:9–26. [CrossRef Medline](#)
- Szabo A, Somogyi J, Cauli B, Lambolez B, Somogyi P, Lamsa KP (2012) Calcium-permeable AMPA receptors provide a common mechanism for LTP in glutamatergic synapses of distinct hippocampal interneuron types. *J Neurosci* 32:6511–6516. [CrossRef](#)
- Takács VT, Klausberger T, Somogyi P, Freund TF, Gulyás AI (2012) Extrinsic and local glutamatergic inputs of the rat hippocampal CA1 area differentially innervate pyramidal cells and interneurons. *Hippocampus* 22:1379–1391. [CrossRef Medline](#)

- Thiagarajan TC, Lindskog M, Tsien RW (2005) Adaptation to synaptic inactivity in hippocampal neurons. *Neuron* 47:725–737. [CrossRef Medline](#)
- Topolnik L, Azzi M, Morin F, Kougioumoutzakis A, Lacaille JC (2006) mGluR1/5 subtype-specific calcium signalling and induction of long-term potentiation in rat hippocampal oriens/alveus interneurons. *J Physiol* 575:115–131. [CrossRef Medline](#)
- Topolnik L, Chamberland S, Pelletier JG, Ran I, Lacaille JC (2009) Activity-dependent compartmentalized regulation of dendritic Ca^{2+} signaling in hippocampal interneurons. *J Neurosci* 29:4658–4663. [CrossRef Medline](#)
- Tóth K, McBain CJ (1998) Afferent-specific innervation of two distinct AMPA receptor subtypes on single hippocampal interneurons. *Nat Neurosci* 1:572–578.
- Tsay D, Dudman JT, Siegelbaum SA (2007) HCN1 channels constrain synaptically evoked Ca^{2+} spikes in distal dendrites of CA1 pyramidal neurons. *Neuron* 56:1076–1089. [CrossRef Medline](#)
- van Hooft JA, Giuffrida R, Blatow M, Monyer H (2000) Differential expression of group I metabotropic glutamate receptors in functionally distinct hippocampal interneurons. *J Neurosci* 20:3544–3551. [Medline](#)
- Wang SS, Denk W, Häusser M (2000) Coincidence detection in single dendritic spines mediated by calcium release. *Nat Neurosci* 3:1266–1273. [CrossRef Medline](#)
- Watanabe S, Hong M, Lasser-Ross N, Ross WN (2006) Modulation of calcium wave propagation in the dendrites and to the soma of rat hippocampal pyramidal neurons. *J Physiol* 575:455–468. [CrossRef Medline](#)
- Wei DS, Mei YA, Bagal A, Kao JP, Thompson SM, Tang CM (2001) Compartmentalized and binary behavior of terminal dendrites in hippocampal pyramidal neurons. *Science* 293:2272–2275. [CrossRef Medline](#)