Dendritic calcium spikes induce bi-directional synaptic plasticity in the lateral amygdala

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Abstract

Postsynaptic induction of long-term potentiation (LTP) at cortical and thalamic afferent synapses onto lateral amygdala (LA) projection neurons not only involves NMDA receptor activation, but also depends on L-type voltage-dependent calcium channels (L-VDCCs). Here we show, using whole cell recordings and two-photon Ca\(^{2+}\) imaging, that L-VDCCs contribute to the induction of dendritic Ca\(^{2+}\) spikes in LA projection neurons. Dendritic Ca\(^{2+}\) spikes can be induced in the absence of sodium spikes by supra-threshold somatic depolarization or by pairing sub-threshold depolarization with synaptic stimulation. Moreover, synaptic induction of Ca\(^{2+}\) spikes is facilitated by R-VDCCs in a pathway-specific manner. Once induced, dendritic Ca\(^{2+}\) spikes propagate into large parts of the dendritic tree. We show that pairing synaptic stimulation with single dendritic Ca\(^{2+}\) spikes can induce bi-directional plasticity, the sign of which might be determined by the anatomical location of active synaptic inputs relative to the spike initiation zone. These data suggest an important role for dendritic Ca\(^{2+}\) spikes in dendritic integration and provide a mechanism by which local synaptic activity may influence global dendritic integration in LA projection neurons.

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1. Introduction

NMDA receptor-dependent long-term potentiation (LTP) in the lateral amygdala (LA) is thought to underlie the acquisition of classical Pavlovian fear conditioning (LeDoux, 2000; Maren, 2001). Projection neurons in the LA receive converging thalamic and cortical sensory afferents that are simultaneously active during sensory experience (Carlsen and Heimer, 1988; LeDoux, 2000; Smith et al., 2000). Whereas LTP at thalamic afferents is induced and expressed postsynaptically (Weisskopf et al., 1999; Bissière et al., 2003; Humeau et al., 2005; Rumpel et al., 2005; but see: McKernan and Shinnick-Gallagher, 1997), LTP induction at cortical afferents involves both pre- and postsynaptic mechanisms (Huang and Kandel, 1998; Tsvetkov et al., 2002; Humeau et al., 2003; Humeau et al., 2005). In addition to NMDA receptors, L-type voltage-dependent Ca\(^{2+}\) channels (L-VDCCs) seem to play an important role during LTP induction at both thalamic and cortical afferents to the LA (Huang and Kandel, 1998; Weisskopf et al., 1999; Humeau et al., 2005) as well as in other nuclei of the amygdala (Fu and Shinnick-Gallagher, 2005). Consistent with these in vitro experiments, behavioral data indicate a requirement for L-VDCCs during the acquisition and extinction of conditioned fear (Bauer et al., 2002; Cain et al., 2002; Shinnick-Gallagher et al., 2003), two forms of associative learning that are thought to depend on synaptic plasticity in the amygdala. Moreover, Ca\(^{2+}\) influx through L-VDCCs has been suggested to trigger long-term memory formation by specifically activating signal transduction pathways involved in transcriptional regulation in the nucleus (Dolmetsch et al., 2001; Deisseroth et al., 2003).

In hippocampal CA1 pyramidal neurons L-VDCCs are localized across the entire somato-dendritic compartment including dendritic shafts and spines (Davare et al., 2001; Obermair et al., 2004). Similarly, the major L-VDCC isoform...
Ca,1,2 localizes to the dendrites of amygdala projection neurons (Pinard et al., 2005). We have previously shown that L-VDCCs substantially contribute to dendritic Ca2+ transients elicited by bursts of back-propagating action potentials (APs) in LA projection neurons (Humeau et al., 2005). AP back-propagation and suprathreshold synaptic input in apical and basal dendrites of hippocampal and cortical pyramidal cells can induce regenerative dendritic Ca2+ spikes (Schiller et al., 1997; Golding et al., 1999; Helmcchen et al., 1999; Häusser et al., 2000; Wei et al., 2001) involving activation of different types of VDCCs or NMDA receptors (Miura et al., 1997; Magee, 1999; Schiller et al., 2000; Kampa et al., 2006). These relatively slow events lead to massive dendritic Ca2+ influx and local depolarization that facilitates NMDA receptor activation and induction of synaptic plasticity (Golding et al., 2002; Holthoff et al., 2004; Kampa et al., 2006). On the other hand, dendritic Ca2+ spikes can propagate to the soma, giving rise to bursts of Na+ APs (Larkum et al., 1999). Thus, dendritic Ca2+ spikes are thought to play an important role in dendritic integration and synaptic plasticity by serving both as a local signal boosting plasticity as well as a long-distance signal within the dendritic tree upon coincident and spatially clustered synaptic activity.

Strong synaptic input onto amygdala projection neurons can induce dendritic Ca2+ spikes (Calton et al., 2000). The properties and functional role of dendritic Ca2+ spikes in the LA are poorly understood. Using a combined electrophysiological and two-photon Ca2+ imaging approach, we therefore investigated the mechanisms underlying the generation of dendritic Ca2+ spikes and their role during induction of synaptic plasticity at cortical and thalamic afferents, the two main sensory pathways converging on LA projection neurons (Carlsten and Heimer, 1988; Smith et al., 2000; LeDoux, 2000).

2. Methods

2.1. Slice preparation

Standard procedures were used to prepare 325-μm thick coronal slices from 3- to 4-week-old male C57BL/6J mice following a protocol approved by the Veterinary Department of the Canton of Basel-Stadt as previously described (Bissière et al., 2003). Briefly, the brain was dissected in ice-cold artificial cerebrospinal fluid (ACSF), and sliced with a vibrotome at 4 °C. Slices were maintained for 45 min at 35 °C in an interface chamber containing ACSF equilibrated with 95% O2/5% CO2 and containing (in mM): 124 NaCl, 2.7 KCl, 2 CaCl2, 1.3 MgCl2, 26 NaHCO3, 0.4 NaH2PO4, 10 glucose, 4 ascorbate, and then for at least 45 min at room temperature before being transferred to a superfusing recording chamber.

2.2. Electrophysiology

Whole-cell recordings from projection neurons located in the dorsal part of the LA were performed as previously described (Bissière et al., 2003) at 30–32 °C in a superfusing chamber. Neurons were visually identified with infrared videomicroscopy using an upright microscope equipped with a 40× objective (Olympus). Patch electrodes (3–5 MΩ) were pulled from borosilicate glass tubing and filled with a solution containing (in mM): 120 K-glucuronate, 20 KCl, 10 HEPES, 10 phosphocreatine, 4 Mg-ATP, and 0.3 Na-GTP (pH adjusted to 7.25 with KOH or CsOH, respectively, 295 mM). All experiments were performed in the presence of picrotoxin (100 μM). In current-clamp recordings membrane potential was kept manually at −70 mV. Data were recorded with a Multiclamp700B (Axon Instruments), filtered at 2 kHz and digitized at 10 kHz. In all experiments, series resistance was monitored throughout the experiment by applying a hyperpolarizing current or voltage pulse, and if it changed by more than 15%, the data were not included in the analysis. Data were acquired and analyzed with: ClampEx9.2, ClampFIt9.0 (Axon Instruments), and the LTP Program (Anderson and Collingridge, 2001). Monosynaptic excitatory postsynaptic potentials (EPSPs) exhibiting constant 10–90% rise times and latencies were elicited by stimulation of afferent fibers with a bipolar twisted platinum/10% iridium wire (25 μm diameter). LTP or LTD were quantified for statistical comparisons by normalizing and averaging EPSP slopes during the last 5 min of experiments relative to 5 min of baseline. Depicted traces show averaged EPSPs for 2 min of baseline and 2 min of LTP/LTD (22–24 min after pairing). All values are expressed as means ± S.E.M. Statistical comparisons were done with paired or unpaired Student’s t-test as appropriate (two-tailed P < 0.05 was considered significant).

2.3. Two-photon imaging

Cells were filled via a patch pipette with normal K+ based intracellular solution containing 40 μM alexa-594 (Ca2+-insensitive dye, red fluorescence) and 200 μM Fluo-5F (medium-affinity Ca2+-indicator, green fluorescence). After gaining access to the cell, dyes were allowed to equilibrate by diffusion for at least 15 min. For the optical measurements, we used a custom-made two photon laser scanning microscope based on a modified Fluoview (Olympus) confocal microscope using a 60 × 0.9NA objective (LUMPlanFL, Olympus) coupled to an ultrafast Ti:sapphire laser (Mai-Tai, Spectra-Physics) tuned to λ = 800 nm. Fluorescence was detected as epifluorescence by two internal PMTs (Olympus). SDM570 and BAS10IF and BAS65IF barrier filters were placed in the “green” and the “red” pathways, respectively, to eliminate reflected excitation light. Fluorescence intensities were acquired and analyzed with Fluoview software (FV300, Olympus). To avoid movement artifacts, a background image was acquired before and after stimulation (Oertner et al., 2002).

2.4. Drugs

Verapamil was from Tocris-Cookson, QX-314 Cl– was from Alomone Labs, SNX-482 was from Peptides International, and Fluo-5F and alexa-594 were from Molecular Probes. All other drugs were from Fluka/Sigma.

3. Results

3.1. Induction of dendritic Ca2+ spikes by sustained postsynaptic depolarization

We have previously shown that bi-directional, spike timing-dependent plasticity (STDP) can be induced at thalamic but not at cortical afferents to LA projection neurons by pairing brief bursts of EPSPs with precisely-timed bursts of postsynaptic APs back-propagating into the dendritic tree (Humeau et al., 2005). Postsynaptic induction of LTP at cortico-amygdala afferent synapses requires the postsynaptic neuron to be kept at depolarized levels in between the individual back-propagating Na+ spikes (Humeau et al., 2005). Under these conditions, LTP induction depends on NMDA receptor and L-VDCC activation and on postsynaptic Ca2+ influx (Humeau et al., 2005). We therefore compared postsynaptic responses during postsynaptic AP firing in the presence and absence of sustained depolarization. While allowing the postsynaptic neuron to depolarize in between APs resulted in the firing of three precisely timed APs, introducing sustained depolarization in between...
individual APs gave rise to broad depolarizing events reminiscent of dendritic Ca\(^{2+}\) spikes (Fig. 1A). To examine whether such events are indeed associated with a dendritic Ca\(^{2+}\) signal we used two-photon laser scanning microscopy (2PLSM) to image dendrites of LA projection neurons loaded with a Ca\(^{2+}\)-sensitive green dye (Fluo-5F) and a Ca\(^{2+}\)-insensitive dye (Alexa-594) during different pairing protocols. Comparing dendritic Ca\(^{2+}\) signals measured during postsynaptic AP firing in the presence or absence of sustained depolarization revealed that Ca\(^{2+}\) transients were doubled if the neurons were kept

**Fig. 1. Sustained somatic depolarization induces all-or-none dendritic Ca\(^{2+}\) spikes.** (A) Schematic representation of stimulation protocols (top) and associated postsynaptic waveforms (bottom). Note appearance of broad spikes in the presence of sustained inter-AP depolarization (protocol 2) (B) Dendritic Ca\(^{2+}\) transients recorded during stimulation with protocols shown in (A). Top left: Low magnification micrograph illustrating a dye-filled LA projection neuron. White box indicates the location of the imaged dendritic segment. Line indicates the position of the line scan. Bottom left: Example line scan illustrating increase of Ca\(^{2+}\) concentration in the dendritic shaft (D) and in the spine (S) over time during postsynaptic stimulation (white arrowheads). Scale bar: 0.5 s. Bottom right: Traces illustrate dendritic shaft Ca\(^{2+}\) transients during stimulation with indicated protocols. Sustained inter-AP depolarization (2) results in substantially larger Ca\(^{2+}\) transients as compared to bursts of back-propagating APs without sustained depolarization (1). (C) Example recording illustrating that increasing amounts of sustained postsynaptic depolarization lead to the induction of all-or-none waveforms associated with dendritic Ca\(^{2+}\) signals. The green trace corresponds to the threshold level of postsynaptic depolarization evoking a Ca\(^{2+}\) spike (as indicated by the broadening of the AP waveform). The red trace shows a full blown Ca\(^{2+}\) spike in the absence of superimposed Na\(^{+}\) spike. The corresponding stimulation protocols are illustrated at the bottom. (D) Dendritic Ca\(^{2+}\) transients measured in the same neuron as in (C). Increasing sustained postsynaptic depolarization beyond threshold results in a stepwise amplitude increase in the dendritic Ca\(^{2+}\) transient. (E) Quantification of traces shown in (D).
depolarized in between the APs (195 ± 29%, n = 5, P < 0.001) (Fig. 1B). To further investigate whether large dendritic Ca\textsuperscript{2+} transients reflect dendritic all-or-none events (spikes) we determined the relative thresholds at which spike waveforms and large Ca\textsuperscript{2+} transients could be triggered. Gradually increasing inter-AP depolarization resulted in the concomitant and stepwise appearance of a broad spike waveform and large dendritic Ca\textsuperscript{2+} transients (Fig. 1C–E), thus confirming the idea that sustained postsynaptic depolarization induces dendritic Ca\textsuperscript{2+} spikes in LA projection neurons.

3.2. Synaptic induction of dendritic calcium spikes

Next we examined whether induction of dendritic Ca\textsuperscript{2+} spikes could also be triggered by synaptic stimulation. We stimulated afferent fibers from the external capsule (containing cortical afferents) (Carlsen and Heimer, 1988; Huang and Kandel, 1998; Smith et al., 2000) eliciting monosynaptic low amplitude EPSPs in the presence of the intracellular Na\textsuperscript{+} channel blocker N-(2,6-dimethylphenyl-carbamoylmethy)triethylammonium chloride (QX-314; 5 mM) in the patch pipette to block postsynaptic Na\textsuperscript{+} APs. Low-intensity extracellular stimulation of the external capsule leads to the activation of about 20–30 cortical afferent synapses onto single LA projection neurons (estimation based on comparison of mEPSC amplitude with the amplitude of evoked EPSCs) (Humeau et al., 2005). Previous studies have shown cortical afferents to contact both proximal and distal parts of the dendritic tree of LA projection neurons (Farb and LeDoux, 1999; Smith et al., 2000; Humeau et al., 2005). Using the present experimental approach, however, the anatomical location of the activated synapses was not possible to determine. Stimulation of cortical afferents in conjunction with postsynaptic depolarization induced all-or-none Ca\textsuperscript{2+} spikes riding on top of the EPSPs (mean amplitude: 41 ± 3 mV; half-width: 46.7 ± 2.4 ms; peak latency from EPSP onset: 42.3 ± 1.2 ms; n = 51) (Fig. 2A). Simultaneous imaging of dendritic Ca\textsuperscript{2+} transients at about 100–150 µm from the soma—a distance at which we have previously identified active cortical inputs (Humeau et al., 2005)—revealed that synaptically triggered spike waveforms were associated with all-or-none Ca\textsuperscript{2+} transients (Fig. 2B). This indicates that Na\textsuperscript{+} AP back-propagation is not necessary, and that synaptic activity in conjunction with sub-threshold postsynaptic depolarization is sufficient for the induction of dendritic Ca\textsuperscript{2+} spikes in LA projection neurons.

3.3. Pathway-specific facilitation of dendritic calcium spikes

Given the differential thresholds for postsynaptic induction of LTP at thalamo- and cortico-amygdala afferents (Humeau et al., 2005), we compared induction of dendritic Ca\textsuperscript{2+} spikes in response to cortical and thalamic afferent stimulation. Different Ca\textsuperscript{2+} channel blockers were added to the perfusion system, resulting in a ramp-like increase in concentration in the slice during the first 10 min of the experiment (Fig. 3A). Consistent with the idea that L-VDCCs contribute to Ca\textsuperscript{2+} signaling in the dendritic shaft and that cortical and thalamic afferent
synapses are sharing the same postsynaptic dendritic segments (Humeau et al., 2005), we found that application of the L-VDCC antagonist verapamil equally reduced the probability of an EPSP to trigger a Ca$^{2+}$ spike at both inputs ($n = 6$; $P < 0.05$ for both inputs; Fig. 3A). However, in the presence of similar synaptic input, induction of Ca$^{2+}$ spikes at thalamic afferents required significantly less postsynaptic depolarization than at cortical inputs ($V_m$ to obtain a spike probability of 0.5: thalamic: $-34 \pm 5$ mV; cortical: $-23 \pm 5$ mV; $n = 6$; $P < 0.05$). Spines receiving thalamic input express increased

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**Fig. 3.** Pathway-specific facilitation of dendritic Ca$^{2+}$ spike induction mediated by R-VDCCs. (A) Top left: Example traces illustrating Ca$^{2+}$-spikes evoked by synaptic stimulation of thalamic and cortical afferents during application of the L-VDCC antagonist verapamil (in the presence of the Na$^+$ channel blocker QX-314). Panels below show the time course of PSP amplitudes during verapamil application. Dashed lines indicate EPSP and Ca$^{2+}$-spike amplitudes. Top right: Analogous experiment in the presence of the R-VDCC blocker Ni$^{2+}$. Scale bars: 10 mV and 20 ms. Bottom left: Averaged time course of Ca$^{2+}$-spike probabilities showing the equal reduction of thalamic and cortical Ca$^{2+}$-spikes in the presence of verapamil ($n = 6$). Middle: Averaged time course of Ca$^{2+}$-spike probabilities induced by cortical (open symbols; $n = 11$) or thalamic (filled symbols; $n = 10$) afferent stimulation during Ni$^{2+}$ application. Right: Averaged time course of Ca$^{2+}$-spike probabilities illustrating the predominant blockade of thalamic Ca$^{2+}$-spikes by the R-type Ca$^{2+}$ channel antagonist SNX482 ($n = 3$). (B) Top: Ca$^{2+}$-spike probabilities at thalamic and cortical afferents plotted as a function of the membrane potential ($V_m$; $n = 7$). $V_m$ was normalized at both inputs relative to the threshold potential required to elicit a Ca$^{2+}$ spike under control conditions. Bottom: Ni$^{2+}$ application induces a greater shift in the spike probability plotted as a function of $V_m$ at thalamic afferents ($n = 7$). (C) Effect of Ni$^{2+}$ and verapamil on Ca$^{2+}$-spike latency and amplitude at thalamic and cortical afferents. Ni$^{2+}$ selectively prolonged spike latency at thalamic inputs (thalamic: $n = 6$; cortical: $n = 6$). Verapamil increased spike latency and decreased spike amplitude at both inputs (thalamic: $n = 4$; cortical: $n = 4$). Scale bars: 10 mV and 25 ms.
amounts of R-type VDCCs (R-VDCCs), and R-VDCCs are necessary for induction of STDP (Humeau et al., 2005). This suggests that selective activation of R-VDCCs by thalamic synapses may facilitate induction of dendritic Ca\(^{2+}\) spikes. Consistent with this hypothesis, blockade of R-type Ca\(^{2+}\) channels with a low concentration of Ni\(^{2+}\) (10 μM) or the R-VDCC selective toxin SNX482 (100 nM) asymmetrically affected the probability to elicit a Ca\(^{2+}\) spike at thalamic vs. cortical afferents. Whereas spike probability was strongly decreased or abolished at thalamic inputs (Ni\(^{2+}\): n = 10, P < 0.01; SNX482: n = 3, P < 0.03; Fig. 3A), cortical inputs were significantly less affected (Ni\(^{2+}\): n = 11; SNX482: n = 3, P > 0.05 compared to baseline; Fig. 3A). To verify that the Ni\(^{2+}\)-induced asymmetric reduction in spike probability was not due to a saturation of spike probability at cortical inputs, we assessed the effect of Ni\(^{2+}\) on Ca\(^{2+}\) spike probabilities as a function of postsynaptic depolarization. Even at low spike probabilities, the reduction in spike probability by Ni\(^{2+}\) was significantly stronger at thalamic inputs (n = 7; Fig. 3B). This indicates that R-VDCCs asymmetrically contribute to the generation of Ca\(^{2+}\) spikes elicited by simultaneous pre- and postsynaptic stimulation. To address the question whether an asymmetric contribution of L-VDCCs may have made R-VDCC activation redundant at cortical inputs, we analyzed the effect of R- and L-VDCC antagonists on spike latency and amplitude. Whereas Ni\(^{2+}\) increased spike latency without any effect on spike amplitude specifically at thalamic inputs (thalamic: latency, 177 ± 15% of baseline, n = 6, P < 0.01; amplitude, 93 ± 5% of baseline, n = 6; P > 0.05; cortical: latency, 108 ± 5% of baseline, n = 6, P > 0.05; amplitude, 92 ± 4% of baseline, n = 6; P > 0.05; Fig. 3C), verapamil application resulted in a symmetric increase in spike latency and a reduction in spike amplitude at both inputs (thalamic: latency, 172 ± 16% of baseline, n = 4, P < 0.001; amplitude, 84 ± 2% of baseline, n = 4; P < 0.05; cortical: latency, 159 ± 11% of baseline, n = 4, P < 0.001; amplitude, 86 ± 5% of baseline, n = 4; P < 0.05; Fig. 3C). This indicates that L-VDCCs equally contribute to the generation of Ca\(^{2+}\) spikes at thalamic and cortical inputs, while R-VDCCs selectively facilitate Ca\(^{2+}\) spike induction upon thalamic afferent activity.

3.4. Ca\(^{2+}\) spike propagation in the dendritic tree

Spreading of Ca\(^{2+}\) spikes in the dendritic arbor of hippocampal pyramidal cells or cerebellar Purkinje neurons is strongly limited by dendritic voltage-dependent K\(^{+}\) conductances (Golding et al., 1999; Cai et al., 2004; Rancz and Hauser, 2006). To examine whether somatically or synaptically triggered dendritic Ca\(^{2+}\) spikes in LA projection neurons were spatially confined, or whether they propagate within the dendritic arbor, we imaged dendritic segments belonging to different dendritic branches. When triggered somatically, we found Ca\(^{2+}\) transients in all imaged dendritic segments (Fig. 4B), suggesting that Ca\(^{2+}\) spikes spread across large parts of the dendritic tree. The amplitude of somatically triggered dendritic Ca\(^{2+}\) transients recorded in different dendritic branches as well as the delay between the onset of the Ca\(^{2+}\) transients and the peak of the somatically recorded Ca\(^{2+}\) spike exhibited low variability (Fig. 4B). In comparison, dendritic Ca\(^{2+}\) transients elicited by synaptic stimulation exhibited significantly smaller amplitudes and areas (n = 33; P < 0.05) (Fig. 4C), suggesting that they may be initiated at sites different from the soma. Like with somatically triggered spikes, we did not observe any propagation failures (Fig. 4C), suggesting that synaptically triggered Ca\(^{2+}\) spikes also propagate into large parts of the dendritic tree of LA projection neurons.

To further examine the locus of synaptically induced Ca\(^{2+}\) spike initiation in LA projection neurons, we measured the latencies of dendritic Ca\(^{2+}\) transients imaged at a given dendritic location relative to the somatically recorded Ca\(^{2+}\) spike. If all Ca\(^{2+}\) spikes were initiated at the soma, the relative latencies would be expected to be independent of whether Ca\(^{2+}\) spikes are triggered by somatic depolarization or synaptic stimulation. In contrast, we found that the latencies of dendritic Ca\(^{2+}\) transients relative to the somatically recorded Ca\(^{2+}\) spikes differed depending on the trigger mode. In particular, latencies of synaptically triggered dendritic Ca\(^{2+}\) transients exhibited a much larger variance relative to spikes triggered by somatic current injection (Fig. 4C). Some dendritic Ca\(^{2+}\) transients occurred at longer latencies, consistent with the idea that synaptic stimulation can trigger Ca\(^{2+}\) spikes in distinct dendritic branches subsequently spreading, via the soma, into the other main dendrites. In other dendrites, the latency was significantly shorter, suggesting that spike initiation occurred in the same dendrite close to the imaged segment. Notably, we found that short latency dendritic Ca\(^{2+}\) transients were significantly smaller in amplitude and area than somatically induced dendritic Ca\(^{2+}\) transients (amplitude: 80 ± 5% of somatically evoked spikes; n = 16; P < 0.01; area: 69 ± 4%; n = 16; P < 0.001) (Fig. 4D). In contrast, the amplitude and area of synaptically evoked Ca\(^{2+}\) transients occurring at longer latencies did not differ from those evoked by somatic depolarization (amplitude: 104 ± 8% of somatically evoked spikes; n = 15; P > 0.05; area: 89 ± 5%; n = 15; P > 0.05) (Fig. 4D). This suggests that Ca\(^{2+}\) spikes may get boosted at the soma before propagating into the other main dendritic branches (Fig. 4E). Thus, our data suggest that somatic supra-threshold depolarization elicits Ca\(^{2+}\) spikes at or near the soma, and that these spikes invade most or all of the main dendrites of LA projection neurons. In contrast, synaptic stimulation appears to elicit local Ca\(^{2+}\) spikes at distinct locations in the dendritic tree, subsequently propagating across most of the dendritic arbor.

3.5. EPSP–Ca\(^{2+}\) spike pairing induces bi-directional synaptic plasticity

What are the implications of this scenario for the induction of synaptic plasticity? Based on our observation that different dendritic branches are invaded by synaptically triggered Ca\(^{2+}\) spikes at latencies differing up to 120 ms (Fig. 4C), this would predict that the relative timing of synaptic EPSPs and dendritic Ca\(^{2+}\) spikes, and hence the sign of synaptic plasticity, might...
depend on the particular anatomical location of active synaptic inputs relative to the spike triggering zone. Consistent with this idea we found that pairing stimulation of cortical afferents (0.2 Hz; 30 pairings) with Ca\textsuperscript{2+} spikes induced LTP in about half of the experiments (200 ± 35% of baseline; n = 6; P < 0.05) while LTD was induced in the other half (67 ± 17% of baseline; n = 5; P < 0.05) (Fig. 5A–C). Notably, we found that application of the NMDA receptor antagonist 3-(R)-2-carboxypiperazin-4-propyl-1-phosphonic acid (CPP; 20 μM) did not block bi-directional synaptic plasticity induced by EPSP–Ca\textsuperscript{2+} spike pairing. In the presence of CPP LTP was induced in two out of five experiments (302% of baseline), whereas LTD was induced in three experiments (68 ± 14% of baseline). This indicates that NMDA receptor-mediated Ca\textsuperscript{2+} influx is not necessary for induction of plasticity under these conditions. Moreover, since using the present experimental approach it is not possible to determine the localization of all stimulated synapses and the spike initiation zone within the dendritic arbor, further experiments using more controlled stimulation techniques will be necessary to address the detailed mechanisms.

4. Discussion

This study shows that synaptic activity in the presence of sustained postsynaptic depolarization elicits dendritic Ca\textsuperscript{2+} spikes in LA projection neurons. Pairing of synaptic activity with dendritic Ca\textsuperscript{2+} spikes induces bi-directional synaptic plasticity, the sign of which might depend on the location of the activated synapses relative to the spike initiation zone. While L-VDCC activation is required for the expression of dendritic Ca\textsuperscript{2+} spikes independent of which afferents are stimulated, R-VDCCs specifically facilitate Ca\textsuperscript{2+} spike induction at thalamic afferents. Finally, our data suggest that dendritic
Ca^{2+} spikes can propagate into large parts of the dendritic tree. This suggests that in the LA voltage-dependent Ca^{2+} channel-dependent dendritic spikes may be an important mechanism by which local synaptic activity may influence synaptic integration and plasticity in a cell-wide manner.

Similar to what has been described at glutamatergic synapses in the hippocampus and in the cortex (Markram et al., 1997; Debanne et al., 1998; Bi and Poo, 2001) pairing of pre- and postsynaptic APs induces bi-directional, spike timing-dependent plasticity (STDP) in the amygdala (Humeau et al., 2005). Interestingly, classical STDP (exhibiting integration time windows in the range of ±20 ms) can be induced at thalamic, but not at cortical afferent synapses. This differential sensitivity to EPSP—AP pairing can be accounted for by the preferential localization of R-VDCCs to spines contacted by thalamic afferents. R-VDCCs selectively boost spine Ca^{2+} transients and possibly spine depolarization thereby facilitating STDP induction. In contrast, L-VDCCs equally contribute to spine and dendritic shaft Ca^{2+} transients at both inputs, and contribute to LTP induction at cortico-amygdala synapses in the presence of sustained postsynaptic depolarization (Humeau et al., 2005). Dendritic Ca^{2+} spikes and somatically recorded spike waveforms are concomitantly induced in an all-or-none and stepwise manner. Consistent with our previous results, we found that R-VDCCs selectively facilitate Ca^{2+} spike induction upon stimulation of thalamic afferents. R-VDCC blockade did not affect spike amplitude, but rather led to a progressive increase in the delay, and eventually to a complete failure of spike induction. In contrast, L-VDCC blockade equally reduces the probability of Ca^{2+} spike induction and spike amplitude at both inputs. This suggests that distinct types of VDCCs contribute to spike triggering and expression. Since L-VDCC blockade did not completely abolish dendritic Ca^{2+} spikes this suggests the contribution of additional VDCC types (Magee, 1999; Häusser et al., 2000). The prominent role of L-VDCC during postsynaptic induction of LTP in LA projection neurons using a protocol involving strong and sustained postsynaptic depolarization and dendritic Ca^{2+} spikes is consistent with previous reports on the mechanisms underlying postsynaptic induction of LTP at cortical and thalamic afferents to the LA (Huang and Kandel, 1998; Weisskopf et al., 1999; Bauer et al., 2002; Humeau et al., 2005). The role of NMDA receptors during the induction of L-VDCC-dependent plasticity is less clear. Pairing brief bursts of
presynaptic stimulation with sustained postsynaptic depolarization induces L-VDCC- and NMDA receptor-dependent LTP (Humeau et al., 2005). In contrast, LTP induced with much stronger LTP induction paradigms, such repeated high frequency stimulation or voltage-clamp pairing protocols, is only partially dependent on NMDA receptor activation (Huang and Kandel, 1998; Tsvetkov et al., 2002). In the present study we found that NMDA receptor blockade does not affect bidirectional plasticity induced by pairing single EPSPs with dendritic Ca\(^{2+}\) spikes. Since we did not control for reliable synaptic Ca\(^{2+}\) spike induction in our previous study (Humeau et al., 2005), a possible interpretation could be that, in addition to NMDA receptor-mediated Ca\(^{2+}\) influx, temporal summation of NMDA receptor-mediated EPSPs during burst stimulation is required for L-VDCC activation and the initiation of dendritic Ca\(^{2+}\) spikes. In contrast, if synaptic stimulation is adjusted to reliably evoke dendritic Ca\(^{2+}\) spikes, NMDA receptor activation might not be necessary any more. Thus, induction of synaptic plasticity in the LA involves a synergistic interplay between synaptic AMPA and NMDA receptors on the one hand, and different types of VDCCs located in spines and on the dendritic shaft locally boosting depolarization and Ca\(^{2+}\) signaling (Sjöström and Nelson, 2002).

Our data suggest that Ca\(^{2+}\) spikes can be triggered in the dendrites and subsequently propagate into large parts of the dendritic tree. Moreover, the inverse correlation between Ca\(^{2+}\) transient latency and amplitude suggests that Ca\(^{2+}\) transients get boosted when they pass through the soma. The measured latencies indicate that in LA projection neurons dendritic Ca\(^{2+}\) spikes propagate at a speed of around 5 mm/s. Therefore, IP\(_{3}\)-mediated intracellular Ca\(^{2+}\) waves, which travel much slower at about 30 \(\mu\)m/s (Power and Sah, 2002), are unlikely to substantially contribute to the dendritic Ca\(^{2+}\) transients in our experiments.

Our model is not consistent with previous reports from cortical and hippocampal pyramidal cells demonstrating spatial confinement of Ca\(^{2+}\) spikes to individual dendritic segments (Golding et al., 1999; Häusser et al., 2000; Wei et al., 2001; Cai et al., 2004; Holthoff et al., 2004). Since we combined synaptic stimulation with postsynaptic depolarization to elicit Ca\(^{2+}\) spikes, it is possible that the spatial containment of dendritic spikes broke down because of inactivation of voltage-dependent K\(^+\) conductances. Nevertheless, this may indicate that dendritic Ca\(^{2+}\) spikes have the potential to propagate over large distances if CA projection neurons are sufficiently depolarized in vivo. Propagation of dendritic Ca\(^{2+}\) spikes over large distances raises the possibility that, depending on the relative location of active synapses with respect to the spike initiation zone, EPSP—Ca\(^{2+}\) spike interactions might occur at different time intervals for a population of simultaneously active synaptic inputs. Consistent with the idea that timing of pre- and postsynaptic activity can determine the sign of synaptic plasticity, we found that EPSP—Ca\(^{2+}\) spike pairing can induce both LTP and LTD (Fig. 5). Under our experimental conditions we are activating around 20—30 synapses using extracellular stimulation of cortical or thalamic afferents (estimation based on mEPSC amplitude measured in LA projection neurons) (Humeau et al., 2005). Since triggering of dendritic Ca\(^{2+}\) spikes is expected to require cooperative activation of spatially clustered synapses, this suggests that the spatial distribution of the synapses contributing to the overall somatic EPSP may determine whether LTP (if most synapses are close to the spike trigger zone) or LTD (if most synapses are on other dendrites) is observed. Although the range of Ca\(^{2+}\) spike latencies we measured (up to 120 ms) is consistent with the possibility that certain synapses might “see” dendritic Ca\(^{2+}\) spikes long enough after EPSP onset for LTD induction (Nishiyama et al., 2000), our experimental approach does not allow to determine the relative localization of all activated synapses and the spike initiation zone. More controlled stimulation techniques, as for example localized glutamate uncaging, will be required to address the detailed mechanisms. Nevertheless, this study suggests an intriguing role for dendritic Ca\(^{2+}\) spikes in differentially orchestrating dendritic integration and synaptic plasticity on local and global scales. The fact that dendritic Ca\(^{2+}\) spikes can be induced by stimulation of thalamic or cortical afferents suggests that, in addition to associative interactions at the presynaptic level (Humeau et al., 2003), dendritic Ca\(^{2+}\) spikes may contribute to associative interactions between both pathways at the postsynaptic level. Moreover, consistent with behavioral pharmacological analysis of long-term fear memory formation (Bauer et al., 2002; Shinnick-Gallagher et al., 2003), propagation of L-VDCC-mediated dendritic Ca\(^{2+}\) spikes to the soma may provide an attractive mechanism by which synaptic activity can impact gene transcription in the nucleus.

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References


