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Associative spike timing-dependent potentiation of the basal dendritic excitatory synapses in the hippocampus in vivo

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Fung TK, Law CS, Leung LS. Associative spike timing-dependent potentiation of the basal dendritic excitatory synapses in the hippocampus in vivo. *J Neurophysiol* 115: 3264–3274, 2016. First published April 6, 2016; doi:10.1152/jn.00188.2016.—Spike timing-dependent plasticity in the hippocampus has rarely been studied in vivo. Using extracellular potential and current source density analysis in urethane-anesthetized adult rats, we studied synaptic plasticity at the basal dendritic excitatory synapse in CA1 after excitation-spike (ES) pairing; E was a weak basal dendritic excitation evoked by stratum oriens stimulation, and S was a population spike evoked by stratum radiatum apical dendritic excitation. We hypothesize that positive ES pairing—generating synaptic excitation before a spike—results in long-term potentiation (LTP) while negative ES pairing results in long-term depression (LTD). Pairing (50 pairs at 5 Hz) at ES intervals of −10 to 0 ms resulted in significant input-specific LTP of the basal dendritic excitatory sink, lasting 60–120 min. Pairing at +10- to +20-ms ES intervals, or unpaired 5-Hz stimulation, did not induce significant basal dendritic or apical dendritic LTP or LTD. No basal dendritic LTD was found after stimulation of stratum oriens with 200 pairs of high-intensity pulses at 25-ms interval. Pairing-induced LTP was abolished by pretreatment with an *N*-methyl-D-aspartate receptor antagonist, 3-(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP), which also reduced spike bursting during 5-Hz pairing. Pairing at 0.5 Hz did not induce spike bursts or basal dendritic LTP. In conclusion, ES pairing at 5 Hz resulted in input-specific basal dendritic LTP at ES intervals of −10 ms to 0 ms but no LTD at ES intervals of −20 to +20 ms. Associative LTP likely occurred because of theta-rhythmic coincidence of subthreshold excitation with a backpropagated spike burst, which are conditions that can occur naturally in the hippocampus.

long-term potentiation; pairing; backpropagation; NMDA receptors; current source density; theta frequency

NEW & NOTEWORTHY

We implemented a new protocol to study spike timing-dependent plasticity at the basal dendrites of hippocampal CA1 area in vivo. Pairs of weak synaptic excitation were delivered at different time intervals from a population spike peak, at a theta frequency of 5 Hz. Pairing of spike at 0–10 ms before, but not at 10–20 ms after, excitation induced basal dendritic long-term potentiation (LTP). LTP induced only by spike-then-excitation pairing is unexpected but of biological and computational significance.

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TEMPORAL PROXIMITY between pre- and postsynaptic activities has been suggested to cause synaptic plasticity (Bliss et al. 2007; Hebb 1949; Levy and Steward 1983), which may be the basis of associative learning (Bliss et al. 2007; Brown et al. 1988). The association may depend on postsynaptic *N*-methyl-D-aspartate (NMDA) receptors that open when both glutamate (presynaptic activity) and postsynaptic depolarization are present (Mayer et al. 1984; Nowak et al. 1984). NMDA receptor opening starts a cascade of biochemical events that results in long-lasting increase or decrease in synaptic transmission, called long-term potentiation (LTP) or long-term depression (LTD), respectively (Bliss et al. 2007).

Synaptic plasticity as a function of the timing between pre- and postsynaptic activities has been studied in vitro with brain slices or slice cultures. Since coincidence of a postsynaptic spike and synaptic excitation was sufficient for LTP (Wigstrom et al. 1986), the outcome of pairing a subthreshold synaptic excitation (E) with a postsynaptic spike (S) at various ES time intervals was investigated (Dan and Poo 2006). A common pattern of spike timing-dependent plasticity (STDP) is that LTP was induced when synaptic excitation preceded a single spike (positive ES interval) and LTD was induced at negative ES intervals when synaptic excitation followed a spike (Bi and Poo 1998; Campanac and Debanne 2008; Debanne et al. 1996, 1998; Markram et al. 1997; Nishiyama et al. 2000). However, different rules of STDP may apply if the stimulus parameters or conditions were varied, and LTP could be elicited at negative ES intervals (Wittenberg and Wang 2006; Zhang et al. 2009).

Part of the variability of STDP may depend on the different techniques used in vitro, and certain interpretations may be hampered by the abnormal physiology and connections of in vitro preparations (cf. Schwartzkroin 1981; Taubenfeld et al. 2002) compared with the brain in situ. The present study is among the first to develop a physiologically relevant model for studying STDP in the hippocampus in vivo. We used two basic components of the normal hippocampal physiology in vivo—theta frequency and complex spike bursts. Theta-frequency stimulation, commonly used to induce synaptic plasticity (Capocchi et al. 1992; Larson and Lynch 1986; Magee and Johnston 1997; Thomas et al. 1998), has additional significance in vivo, because theta rhythm is a dominant oscillation in the intact hippocampus (Bland and Colom 1993; Buzsáki et al. 1983; Leung 1998). Theta-frequency stimulation at moderate intensity induces complex spike bursts from pyramidal cells

(Thomas et al. 1998), and complex spike bursts are a natural firing pattern of hippocampal pyramidal cells (O'Keefe 2007; Ranck 1973).

Specifically, in urethane-anesthetized rats, we paired a weak basal dendritic excitatory synaptic input with backpropagated spikes evoked by a strong apical dendritic excitation of hippocampal CA1 pyramidal cells (Kloosterman et al. 2001). Weak excitation paired with backpropagated dendritic spikes was an effective means to induce synaptic plasticity, likely by facilitating Ca^{2+} influx and NMDA receptor opening at the dendrites (Christie et al. 1996; Magee and Johnston 1997). We chose the basal dendritic (stratum oriens) synapse in CA1 area for this study because this synapse showed LTP in vivo after a small number of theta-frequency primed burst stimulations (~4 trains of primed bursts of 4–10 pulses; Kaibara and Leung 1993; Leung et al. 1992; Leung and Shen 1995). Spikes were evoked by strong synaptic excitation, as distinct from intracellular current injection that was commonly used in pairing in vitro. Apical dendrites of CA1 pyramidal cells receive inputs predominantly from CA3c and CA3b areas, whereas the basal dendrites receive inputs from CA3b, CA3a, and CA2 areas (Ishizuka et al. 1990; Kohara et al. 2014; Li et al. 1994; Shinohara et al. 2012). We separated basal and apical dendritic excitation by means of current source density (CSD) analysis (Kaibara and Leung 1993; Kloosterman et al. 2001). Following the common finding that LTD/LTP occurs at negative/positive ES interval, we hypothesized that positive ES pairing (excitation before spike) would result in LTP while negative ES pairing (spike before excitation) would result in LTD.

MATERIALS AND METHODS

All experimental procedures were approved by the local Animal Use Committee and conducted according to the guidelines of the Canadian Council for Animal Care. Eighty adult male Long-Evans rats of age >60 days (270–420 g) were used. A rat was anesthetized with urethane (1.5 g/kg ip) and given atropine methyl nitrate (0.02 mg/kg ip) to reduce salivation. The stimulating electrodes used were composed of Teflon-coated stainless steel wire of 125-μm diameter, insulated except at the cut ends. A stimulating electrode was lowered into stratum radiatum near the CA1/CA3 border at coordinates posterior (P) 3.2 mm, lateral (L) 2.2 mm relative to bregma, and another was lowered to stimulate stratum oriens of CA3a at P3.2, L3.2 (Fig. 1; Paxinos and Watson 1986). A silicon probe (NeuroNexus, Ann Arbor, MI) was lowered at coordinate P3.8, L1.8 to record from hippocampal CA1 region; the probe had 16 recording sites (each site covers 177-μm^2 area), separated by 50 μm, on a vertical shank. A jeweler's screw in the skull plate over the frontal cortex served as the anode, while another skull screw over the cerebellum served as a recording ground. Stimulus currents were delivered (with pulse duration of 0.2 ms) through a photo-isolated stimulus isolation unit (PSIU6, Astro-Med/Grass Instrument). Stimulation repetition rate was <0.1 Hz.

The signals were amplified by preamplifier and amplifier and acquired by custom-made software with Tucker-Davis Technologies (Alachua, FL) real-time processor system RA-16. Extracellular population excitatory postsynaptic potentials (pEPSPs) were recorded in CA1 pyramidal cells after stimulation of stratum radiatum or stratum oriens, ~2 mm away from the recording site. Low-intensity stimulation of stratum oriens and stratum radiatum evoked an excitatory sink at the CA1 basal dendrites and proximal apical dendrites, respectively (Kloosterman et al. 2001). High-intensity stimulation of stratum radiatum (typically 150–300 μA) evoked a population spike (PS) that was 50–75% of the maximum amplitude, and this PS originated at the CA1 proximal apical dendrites and propagated into the basal dendrites (Kloosterman et al. 2001).

Low-intensity test pulses ($\leq 2 \times$ pEPSP threshold intensity delivered at 0.1 Hz) were applied to stratum oriens to evoke excitation at the CA1 basal dendrites without a PS and to stratum radiatum to evoke apical dendritic excitation (Fig. 1), and average evoked potentials (AEPs; $N = 4$ sweeps) were recorded every 5 min. A baseline recording consisted of 30 min of stable test-pulse responses. After confirmation of a stable baseline, an excitation-spike pairing (ES pairing) induction protocol was applied to induce synaptic plasticity, and then basal and apical dendritic AEPs were recorded at 5-min intervals after an induction or control stimulation protocol. A frequency of 5 Hz was selected for ES pairing because of its effectiveness in evoking LTP in vitro (Capocchi et al. 1992; Thomas et al. 1998) and in vivo (Leung et al. 1992).

For the first series of experiments, a low-intensity stimulus evoking subthreshold basal dendritic excitation (E) was paired, at different ES intervals, with a high-intensity stimulation that activated a PS (S) in the CA1 apical dendrites. The ES stimulus time interval was defined as the time interval between the stimulus evoking the basal excitatory sink (E) and the high-intensity PS-evoking stimulus (S). This was close to the actual ES time interval, determined experimentally, as described below. ES pairing consisted of either 50 pairs at 5 Hz

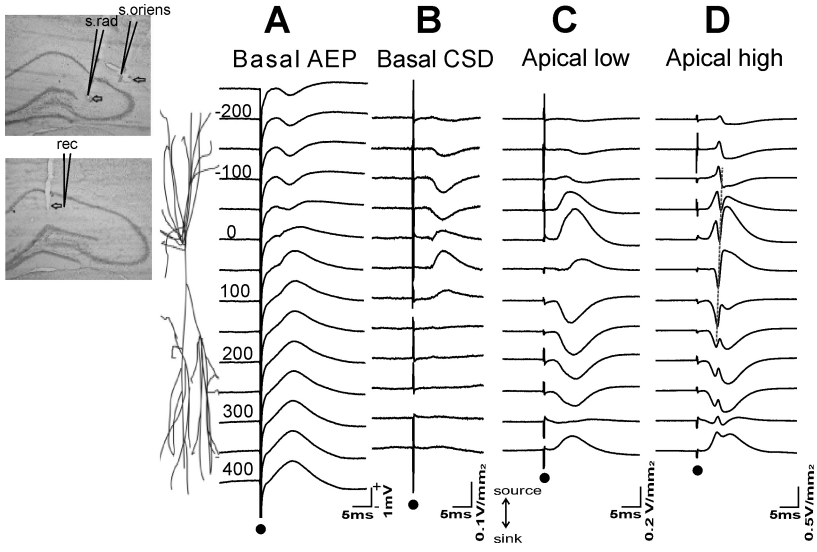

Fig. 1. Laminar profiles of average evoked potential (AEP) and current source density (CSD) transients in CA1 of a representative rat. *Inset*: schematic and histological sections illustrating location of stimulating electrodes at stratum oriens (s. oriens) and stratum radiatum (s. rad) evoking basal dendritic and apical dendritic excitation, respectively, at the CA1 recording probe (rec). *A*: AEP profile at 16-channel electrode probe with 50-μm interval between electrodes after single-pulse weak stimulation of the stratum oriens at 50 μA. Depths are indicated by a schematic CA1 pyramidal cell drawn and by the distance (in μm) away from the cell layer (+ toward the apical dendrites). *B*: CSD profile derived from AEP profile in *A*, showing sink at the basal dendrites. *C*: CSD profile after stimulation of stratum radiatum at 40 μA, which evoked subthreshold excitatory sink at the apical dendrites. *D*: same as *C* except for a high-intensity apical dendritic excitation that resulted in a population spike sink starting at proximal apical dendrites and propagating to the soma and basal dendrites; peak spike sinks are linked by a dotted line indicating propagation direction. Stimulus artifact is indicated by a solid circle. Positive potential and CSD source are upward-going.

(continuously, or 1 × 50 pairs) or 5 sweeps with 10 pairs per sweep (5 × 10 pairs), with sweeps repeating every 10 s (Fig. 2, *inset*). ES stimulus intervals were used for all groups below except for the ES = 0 ms group, in which the ES stimulus interval was deliberately set to be >0 ms (but <3 ms) so that the basal dendritic sink (E) peak would coincide in time with the S-evoked backpropagated sink at the basal dendrites. There were 12 groups, with the first 5 groups using continuous pairing (1 × 50 pulses) at ES stimulus interval of −20 ms (*group 1*), −10 ms (*group 2*), 0 ms (*group 3*), 10 ms (*group 4*), and 20 ms (*group 5*). There were two control stimulation groups: only stratum oriens stimuli given at 5 Hz (Low Basal Stimulation Only) (*group 6*) and only stratum radiatum stimuli given at 5 Hz (High Apical Stimulation Only) (*group 7*). There were three groups using discontinuous ES pairing (5 × 10 pulses) given at 5 Hz: ES stimulus interval of −10 ms (*group 8*), 10 ms (*group 9*), and 20 ms (*group 10*). *Group 11* received 60 ES pairs at 0.5 Hz, with the E pulses being single ($n = 2$), or 4 pulses at 10 ms intervals ($n = 5$); the response of the first E pulse was coincident with the first spike evoked by S. *Group 12* tested the effect of NMDA receptor antagonist 3-(2-carboxypiperazin-4-yl)propyl-1-phosphonic acid (CPP) on LTP induced by discontinuous (5 × 10) pairing at ES interval of −10 ms. CPP (10 mg/kg ip) was given at 45 min before ES pairing. Each group consisted of five to eight rats.

In a second series of experiments, a paired-pulse stimulation (PPS) protocol described by Thiels et al. (1994) was used. Two hundred pairs of pulses, with 25-ms interpulse interval, at a single-pulse stimulus intensity that evoked 50–75% of the maximal PS response, were delivered at 0.5 Hz to a stimulating electrode in either stratum oriens or stratum radiatum. Both apical and basal dendritic responses were recorded before and after PPS.

A one-dimensional CSD was calculated from the field potentials. CSD(z, t), a function of depth z and time t, was calculated by a second-order differencing formula (Freeman and Nicholson 1975; Leung 2010):

$$\mathrm{CSD}(z,t) = \sigma\left[2\Phi(z,t) - \Phi(z+2\Delta z,t) - \Phi(z-2\Delta z,t)\right]/(2\Delta z)^2 \quad (1)$$

where $\Phi(z, t)$ is the field potential at depth z and time t and Δz is the spacing (50 μm) between adjacent electrodes on the 16-channel probe. The conductivity σ was assumed to be constant, and the CSDs were reported in units of volts per square millimeter.

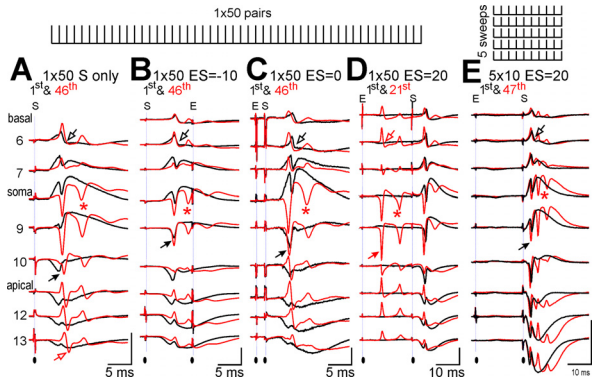

Fig. 2. Current source density (CSD) profiles during 5-Hz stimulation and excitation-spike (ES) pairing. *Inset*: illustration of continuous pairing (1 sweep × 50 pairs) and discontinuous pairing (5 sweeps of 10 pairs). *A*: CSD laminar profile in a representative rat during continuous (1 × 50) 5-Hz high-intensity stratum radiatum stimulation (S) only that evoked a population spike. Apical dendritic spike indicated by up-pointing filled arrow and subsequent basal dendritic spike indicated by down-pointing open arrow. Responses evoked by the 1st pulse are overlaid on those evoked by the 46th pulse that included a spike burst (*). *B*: same as *A* for another rat with 1 × 50 continuous pairing at ES interval of −10 ms. *C*: representative continuous pairing at ES interval of 0 ms; E and S stimuli had slightly different timing in order to evoke synchronous E and S responses in stratum oriens. *D*: representative rat showing continuous pairing at ES interval of 20 ms; the 21st pair evoked large population spikes that fired during the time of the weak basal dendritic excitation. *E*: overlaid 1st and 47th sweeps during discontinuous pairing in a representative rat, with 5 sweeps of 10 (5 × 10) pairs delivered at ES interval = 20 ms; no PS was induced during basal dendritic excitation. Stimulus artifact is indicated by a solid ellipse. Vertical calibration bar, 1 mV/mm^2. CSD source is up.

The CSD and AEP were evaluated for stability before pairing and potentiation after pairing. Four CSD measures were included: *1*) the maximal slope over a 1-ms time interval during the rising phase of the maximal excitatory sink, at stratum oriens (or radiatum) as appropriate; *2*) peak of the excitatory sink response; *3*) the sink at half the maximal peak response, typically at 5- to 7-ms latency; and *4*) the sum of sinks at the time instant of the half-maximal sink response. Two measures of the AEP were used: *5*) the maximal slope of the AEP, at the site with the maximal negative peak in stratum oriens (or radiatum); and *6*) negative peak AEP. Each response measure was normalized by the grand average of the measure during baseline before LTP induction. Stability during baseline was estimated by the standard error (SE) divided by the mean measure, and this ratio had to be <0.05 for 30 min before pairing. After 50 pairing pulses, responses were taken at 5-min intervals after pairing, from 5 to 120 min (additional recording at 1 min after pairing was recorded in the continuous pairing protocols).

Analysis in selected groups of rats indicated that all six measures of excitatory response (above) gave similar magnitudes of potentiation. The slope of the excitatory sink gave one of the more stable responses (indicated by SE-to-mean ratio) during baseline and the smallest variation (SE) across rats for the selected group (data not shown). These suggest that the maximal sink slope gave a robust measure of the local excitatory current.

Block repeated-measures analysis of variance (ANOVA) was used for statistical analysis of the normalized slope data. If a significant main or interaction effect was found, Newman-Keuls post hoc test was applied. $P < 0.05$ was considered statistically significant. For each group, change in synaptic response was assessed by comparing the normalized basal or apical excitatory sink slopes after induction with their respective baseline, by one-way ANOVA followed by post hoc Newman-Keuls test. In addition, group comparisons were made between an ES pairing group and a control group (High Apical Only and Low Basal Only).

After an experiment, the site of a stimulating electrode was lesioned by passing 0.5-mA current for 1-s duration. The rat brain was removed after intracardial perfusion with phosphate-buffered saline and 4% formalin and was later sliced into 40-μm-thick coronal sections. The stimulus and recording electrode tracks were identified in sections stained with thionin (Fig. 1).

RESULTS

Baseline basal and apical responses and spike bursts during ES pairing at 5 Hz. Single-pulse low-intensity stimulation of the stratum oriens evoked a negative pEPSP at the same layer, accompanied by a positive wave at stratum radiatum (Fig. 1*A*). CSD analysis revealed an excitatory sink at the basal dendrites and sources at the soma and proximal apical dendrites (Fig. 1*B*; Kloosterman et al. 2001). Low-intensity, single-pulse stimulation of the stratum radiatum generated a negative pEPSP at stratum radiatum (not shown), which corresponded to an apical dendritic sink accompanied by a major source at the soma and a minor source at the distal dendrites (Fig. 1*C*). High-intensity stimulation of the stratum radiatum generated a PS, which propagated from the proximal apical dendrites through the cell body and into the basal dendrites (Fig. 1*D*; Kloosterman et al. 2001). The invasion of a PS sink into the basal dendrites indicated spike backpropagation.

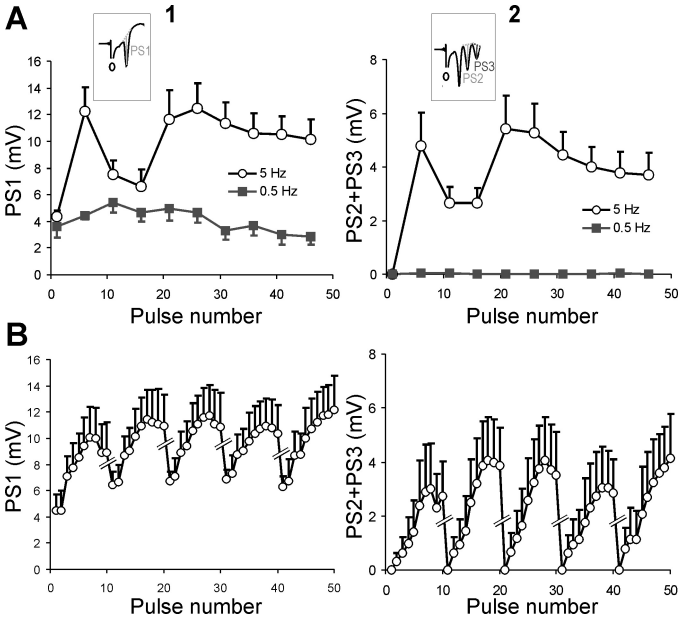

Fig. 3. Facilitation of CA1 spike bursts with continuous and discontinuous 5-Hz stimulation of stratum radiatum. *A*: mean ± SE of amplitude of 1st population spike (PS1; *1*) and sum of amplitudes of 2nd and 3rd population spikes (PS2+PS3; *2*) evoked by continuous (1 × 50) 5-Hz or continuous 0.5-Hz stimulation. Note gradual increase in PS1 amplitude and bursting, indicated by PS2+PS3, with 5-Hz but not 0.5-Hz stimulation. One in every 5 responses was averaged and plotted ($N = 9$ for 5-Hz, $N = 5$ for 0.5-Hz stimulation). *B*: mean ± SE of PS1 and (PS2+PS3) amplitudes of all 50 stimuli after a discontinuous 5-Hz stimulation (5 sweeps × 10 stimuli at 5 Hz; 10-s interval between sweeps). Note the gradual rise in PS1 and bursting (PS2+PS3) from the start of each sweep.

A 5-Hz train of 50 high-intensity stimulation pulses at stratum radiatum (High Apical Only) resulted in an increase in the amplitude of the first evoked PS during the train (Fig. 2*A*, Fig. 3*A1*). High Apical Only stimulation also increased spike bursts, which was quantified by a bursting index defined as the sum of amplitudes of the second and third PSs (PS2 + PS3) in a spike burst recorded at the cell layer (Fig. 3*A2*). An increase in PS1 amplitude and PS bursts was also observed during pairing at ES intervals of 0 ms (Fig. 2*C*), −10 ms (Fig. 2*B*), and −20 ms (not shown). Increase of PS1 or bursting index was not found during pairing at 0.5 Hz (Fig. 3*A*).

An unexpected result occurred after >10 pairs of continuous 5-Hz pairing at 10-ms ES and 20-ms ES intervals—PSs were activated by the weak basal dendritic stimulation (Fig. 2*D*). Since this violated the assumption that E preceded S, the results of these experiments were excluded. To avoid spiking during the weak excitation, a discontinuous pairing protocol (5 × 10 pairing) was used for positive ES intervals, which consisted of 10 contiguous pairs in one sweep, and five sweeps were given at 10-s intervals (Fig. 2, *inset*). Discontinuous pairing at positive ES intervals did not give rise to PSs during the weak basal excitation (Fig. 2*E*). Discontinuous pairing, shown for ES interval of −10 ms, also increased PS1 amplitude (Fig. 3*B1*) and bursting index (Fig. 3*B2*) during each sweep, with excitability returning to baseline at the start of the next sweep.

The timing of the excitatory sink and the spike bursts during pairing was examined in each rat. At the cell layer, the average peak latency of the first, second, and third PSs in a burst was 6.3 ± 0.2, 10.3 ± 0.1, and 15.3 ± 0.2 ms ($n = 9$ rats). The mean range of peak latency (maximal minus minimal latency of all occurrences) was 1.84, 3.60, and 3.36 ms ($n = 9$ rats) for the first, second, and third PSs, indicating that the peak latency of each PS was closely clustered. On average, the time of the maximal PS sink (and voltage peak) at the cell layer preceded the maximal spike sink at the basal dendrites (stratum oriens) by ~1 ms, consistent with Kloosterman et al. (2001). The interval between the peak excitatory sink (E) and the peak PS sink (S), measured at the basal dendrites, was found to be nonsignificantly smaller than the ES stimulus interval by 0.8 ± 0.6 ms ($n = 14$ rats), i.e., the actual ES interval at the basal dendrites was practically the same as the ES stimulus interval.

Table 1. *Basal dendritic response, normalized to baseline, at different times after pairing or control stimulation*

Group	*n*	Response 5 min	Response 30 min	Response 60 min	Response 120 min
−20 ms ES (1 × 50 pairs)	5	1.4 ± 0.2*†	1.10 ± 0.03†	1.09 ± 0.02†	1.05 ± 0.08
−10 ms ES (1 × 50 pairs)	5	1.33 ± 0.06*†	1.21 ± 0.03*†	1.20 ± 0.04*†	1.14 ± 0.03†
−10 ms ES (5 × 10 pairs)	5	1.20 ± 0.12	1.27 ± 0.08*†	1.28 ± 0.11*†	1.30 ± 0.06†
0 ms ES (1 × 50 pairs)	6	1.38 ± 0.13*†	1.34 ± 0.09*†	1.35 ± 0.08*†	1.24 ± 0.06*†
10 ms ES (5 × 10 pairs)	5	1.02 ± 0.07	0.99 ± 0.05	1.13 ± 0.15	1.10 ± 0.14
20 ms ES (5 × 10 pairs)	5	0.91 ± 0.06	0.97 ± 0.05	1.0 ± 0.07	0.93 ± 0.09
Low Basal Only (5 Hz)	8	0.99 ± 0.03	0.98 ± 0.02	1.03 ± 0.05	1.03 ± 0.05
High Apical Only (5 Hz)	9	1.13 ± 0.04†	1.08 ± 0.04	0.98 ± 0.03	0.94 ± 0.07
Apical 200 paired pulses	5	0.98 ± 0.02	0.98 ± 0.03	0.96 ± 0.06	0.93 ± 0.11
Basal 200 paired pulses	6	0.97 ± 0.05	1.04 ± 0.04	1.08 ± 0.05	1.10 ± 0.07

Values are mean ± SE slope (normalized to baseline) of the excitatory sink at 5, 30, 60, and 120 min after different induction protocols. ES pairing consisted of 50 pairs at 5 Hz, each with a low-intensity basal dendritic excitation (E) and a high-intensity apical dendritic spike-evoking stimulus (S), at different ES intervals. Pairing can be continuous (1 × 50 pairs) or discontinuous (5 × 10 pairs, or 5 sweeps of 10 pairs per sweep, at 1 sweep per 10 s). Control 5-Hz stimulation was either low-intensity basal dendritic (stratum oriens) stimulation alone (Low Basal Only) or high-intensity apical dendritic (stratum radiatum) stimulation alone (High Apical Only). Apical (stratum radiatum) and basal (stratum oriens) 200 paired pulses indicate 200 high-intensity pulse pairs, at 25-ms interpulse interval, delivered at 0.5 Hz. *$P < 0.05$, Newman-Keuls post hoc test, after significant group difference with Low Basal Only group (2-way repeated-measures ANOVA); †$P < 0.05$, different from unity, *t*-test.

Potentiation at the basal and apical dendritic synapses after pairing. Control stimulations at 5 Hz did not induce significant long-term changes in the basal or apical dendritic responses. High-intensity apical dendritic stimulation (High Apical Only, $n = 9$) stimulation resulted in short-term potentiation (STP) at 1 and 5 min and no clear basal dendritic LTP at 30–120 min (see Fig. 5*A*; Table 1). Low-intensity basal dendritic stimulation only (Low Basal Only, $n = 7$) induced no change in the normalized basal dendritic sink slope at any time (see Fig. 5*A*; Table 1).

After continuous (1×50) ES pairing at 5 Hz, basal dendritic potentiation was found with ES intervals of 0 ms, −10 ms or −20 ms. As illustrated by continuous pairing with coincident E and S (ES = 0 ms) in a representative rat, an increase of the slope of the basal dendritic sink (CSD, Fig. 4*B*) was observed for 120 min (Fig. 4, *A* and *B*). The CSD spatial profile shows that the potentiation of excitatory sink was restricted to the basal dendrites, except for a small proximal apical dendritic sink increase at 5 min after pairing (Fig. 4*C*). Similar magnitudes of potentiation (or the lack of) were found when the slope of the sink, the slope of the AEP, or the sum of the basal dendritic sinks was used as the metric (Fig. 4, *B* and *C*). Only results for the slope of the excitatory sink are presented below. At 30–60 min after pairing, ~35% average enhancement was found for the ES = 0 group, compared with ~20% and ~10% for the ES −10 ms and ES −20 ms groups, respectively (Fig. 5*A*; Table 1). The normalized sink slopes showed long-term (30–120 min) enhancement after pairing at ES 0 and −10 ms when compared with either the High Apical Only or the Low Basal Only group (Table 1; $P < 0.05$, Newman-Keuls post hoc test, after a significant main or interaction effect in a 2-way repeated-measures ANOVA). The ES −20 ms group resulted in a significant potentiation only at 5–10 min after pairing compared with the High Apical Only or the Low Basal Only group. Pairing at 0.5 Hz (ES = 0 ms) resulted in no significant change in the basal dendritic slope.

There was no significant potentiation after discontinuous (5×10) pairing at ES of 10 or 20 ms (Fig. 5*B*; Table 1). In contrast, discontinuous pairing at ES −10 ms resulted in significant basal dendritic potentiation for 120 min after pairing (Fig. 5*B*), similar to that after continuous pairing (Fig. 5*A*; Table 1). Basal dendritic LTP was found in experiments in which continuous (1×50) pairing at ES 10 and 20 ms resulted unexpectedly in PSs occurring during weak excitation (see, e.g., Fig. 2*D*); these results are not further analyzed.

None of the ES pairing groups showed any significant long-term change in the apical dendritic sink compared with the High Apical Only group (Fig. 6, *A* and *B*). As a group, High Apical Only stimulation induced apical dendritic STP at 5–10 min but no significant potentiation at 15–120 min. Low Basal Only stimulation induced no significant change in the apical dendritic excitatory sinks. Thus ES pairing induced input-specific LTP at the basal dendritic synapse.

Pairing LTP was blocked by NMDA receptor antagonist. To test whether pairing-induced LTP was mediated by NMDA receptors, the NMDA receptor antagonist CPP (10 mg/kg ip) was given at 45 min before ES −10 ms pairing. Single-pulse basal and apical dendritic excitatory sinks were not significantly affected by CPP injection (Fig. 7). Discontinuous 5-Hz pairing at ES −10 ms, at 45 min after CPP injection, did not induce any significant change in the normalized basal dendritic

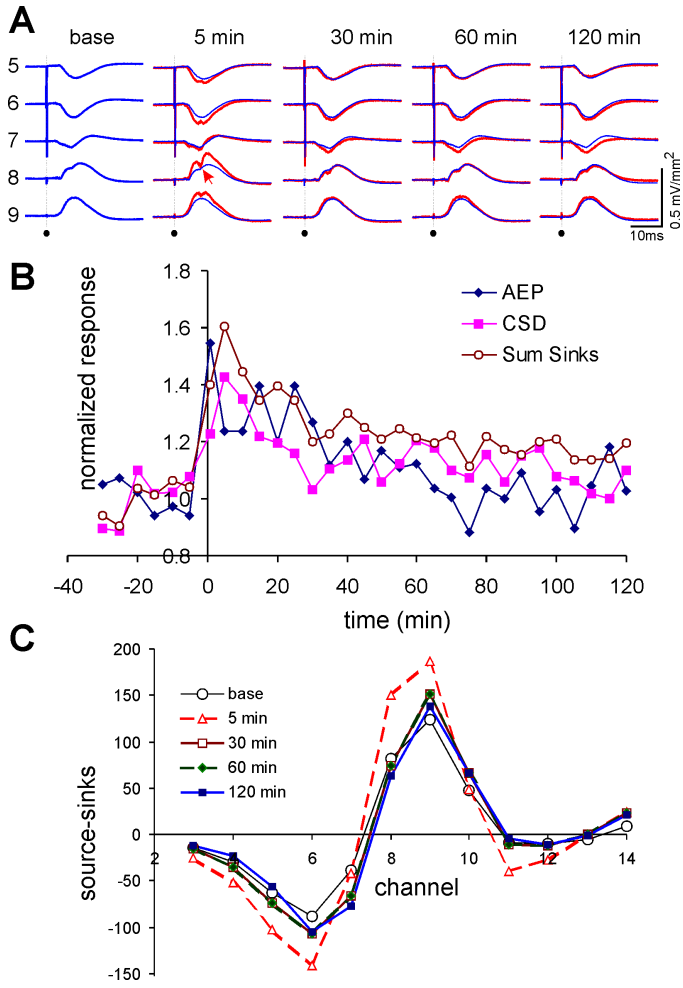

Fig. 4. Long-term potentiation (LTP) of a representative rat after excitation-spike (ES) pairing at 0 ms or weak basal dendritic excitation (E) was coincident with strong, spike-evoking stratum radiatum stimulation (S). *A*: current source density (CSD) at channels near the maximal basal dendritic sink (down) in stratum oriens recorded at *channel 6*, with adjacent channels 50 μm apart. At each time after pairing (5, 30, 60, or 120 min), red traces are the CSD responses (average of 4 sweeps) at the time indicated, overlaid with the baseline responses (light blue traces, average of 24 sweeps). Note that small population spike at *channel 8* (arrow) increased after pairing. *B*: time course of normalized response after pairing at *time 0*. Different measures gave comparable LTP, normalized to the baseline response. AEP, maximal rising slope of the negative average evoked potential at *channel 6*; CSD, maximal rising slope of the CSD sink at *channel 6*; Sum Sinks, sum of the basal dendritic sinks from *channels 3–7* shown in *C*. *C*: spatial profiles of CSD at different times after pairing. Profile was a snapshot of the spatial pattern of source-sinks at a fixed time instant that was halfway between onset and peak of sink at *channel 6* during baseline. The baseline pattern shows a basal dendritic sink (*channels 3–7*) accompanied by source near the cell layer (*channels 8–10*), with small apical dendritic sinks in stratum radiatum (*channels 11* and *12*). Basal dendritic sinks increased for up to 120 min, while apical dendritic sinks increased only for 5 min after pairing.

sink response in the group (Fig. 7*A*). The single-pulse-evoked apical dendritic sink appeared to decline after ES −10 ms pairing (Fig. 7*B*), but the decrease was not different between no-drug and CPP conditions and not statistically significant compared with the response after High Apical Only stimulation ($P > 0.05$, 2-way ANOVA).

Excitability changes during pairing were significantly affected by CPP. In the no-drug condition, PS bursts were greatly facilitated during each sweep of 10 contiguous pulses at 5 Hz

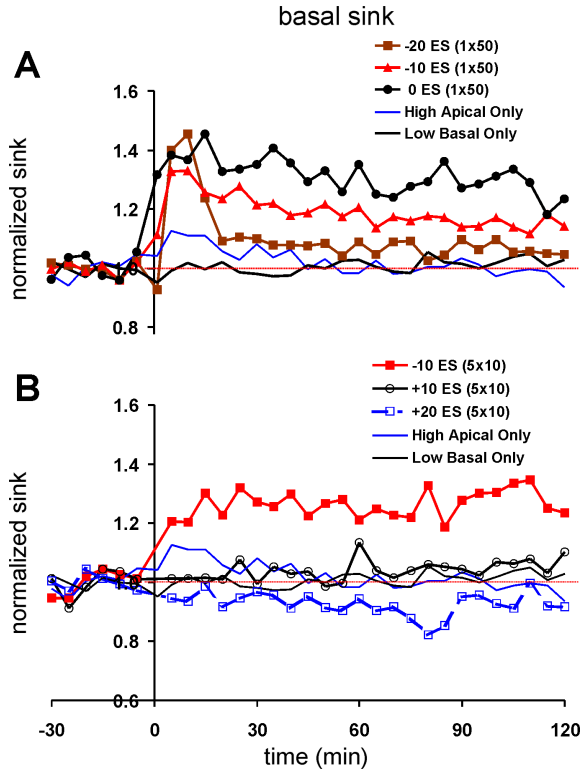

Fig. 5. Normalized CA1 basal dendritic sink following pairing of weak basal dendritic excitation (E) with strong stratum radiatum-evoked spiking (S), at different ES intervals. Basal excitatory sink slope was normalized by the average baseline amplitude; ES pairing occurred at *time 0*. *A*: 50 contiguous (1 × 50) pairs at 5 Hz induced long-term potentiation (LTP) at ES intervals of 0 and −10 ms, compared with control groups after continuous 5-Hz low-intensity basal dendritic (stratum oriens) stimulation only (Low Basal Only) or after continuous high-intensity apical dendritic (stratum radiatum) stimulation only (High Apical Only). STP of <20 min was found with ES interval of −20 ms. For clarity, SE is not shown. *B*: discontinuous 5-Hz stimulation (5 sweeps × 10 pairs) also induced significant LTP at ES interval of −10 ms but not for 10 or 20 ms. Same "Low Basal Only" and "High Apical Only" traces as in *A*.

illustrated for odd-number pulses of the first, third, and fifth sweeps (Fig. 8*A1*). The increase in bursting index (PS2 + PS3 amplitude) was smaller in the CPP condition than the no-drug condition, shown for a representative rat (Fig. 8*A2*) and for the group (Fig. 8*C*); the difference was more robust for a late (3rd to 5th) sweep than the first sweep (only 5th sweep is illustrated in Fig. 8*C*). Two-way (group × pulse number) ANOVA of the (PS2 + PS3) amplitude showed a significant interaction effect [$F(49,441) = 3.04$, $P < 0.0001$], a significant time effect, but no group effect. Unexpectedly, the amplitude of PS1 increased more during a sweep in the CPP condition than the no-drug condition, although this was statistically significant only for the first and second sweeps and not for subsequent sweeps, as confirmed by post hoc Newman-Keuls test after a significant group × time interaction effect with two-way ANOVA [$F(49,441) = 2.78$, $P < 0.0001$]. Thus CPP reduced the increase in spike bursts but not the increase in PS1 amplitude during pairing.

Basal dendritic synapses did not show LTD with paired-pulse stimulation. The lack of LTD at −10- or −20-ms ES pairing suggests that LTD did not readily occur at the basal dendritic synapse after pairing. To further characterize basal synaptic plasticity, we used a LTD induction protocol that was verified in adult rats—0.5-Hz stimulation with 200 high-intensity paired pulses at 25-ms interval (PPS).

As reported previously (Thiels et al. 1994), apical dendritic LTD was readily induced by PPS of the stratum radiatum, when paired-pulse depression of the evoked PSs was apparent during PPS (Fig. 9*A*, *inset*). Immediately after stratum radiatum PPS the slope of the apical dendritic excitatory sink decreased to 71.0 ± 3.6% ($n = 6$) of the baseline (Fig. 9*A*), and it further decreased, starting at ~50 min after PPS, to 62.4 ± 4.0% ($n = 6$) at 2 h. One-way repeated-measures ANOVA revealed a significant time effect [$F(29,145) = 20.9$, $P < 0.0001$], and post hoc Newman-Keuls tests showed significant differences for apical dendritic excitatory sink slopes at all time points (1–120 min, $P < 0.05$) compared with the baseline (Fig. 9*A*). The basal dendritic excitatory sink was not significantly affected by stratum radiatum PPS (Fig. 9*A*), indicating that the apical dendritic LTD was input specific.

Immediately (1 min) after PPS of stratum oriens, there was a decline in the slope of the basal dendritic sink to 90.8 ± 2.1% ($n = 6$) of the baseline (Fig. 9*B*; post hoc Newman-Keuls test after a significant 1-way repeated-measures ANOVA). However, the basal excitatory sink at 5–120 min after PPS was not different from the baseline (Fig. 9*B*; Table 1). The apical dendritic excitatory sink slope after stratum oriens PPS was not significantly different from baseline (Fig. 9*B*), although there was a small, nonsignificant decline of the mean apical dendritic sink from 50 to 120 min after stratum oriens PPS. A high-intensity single-pulse stimulation of stratum oriens induced PS

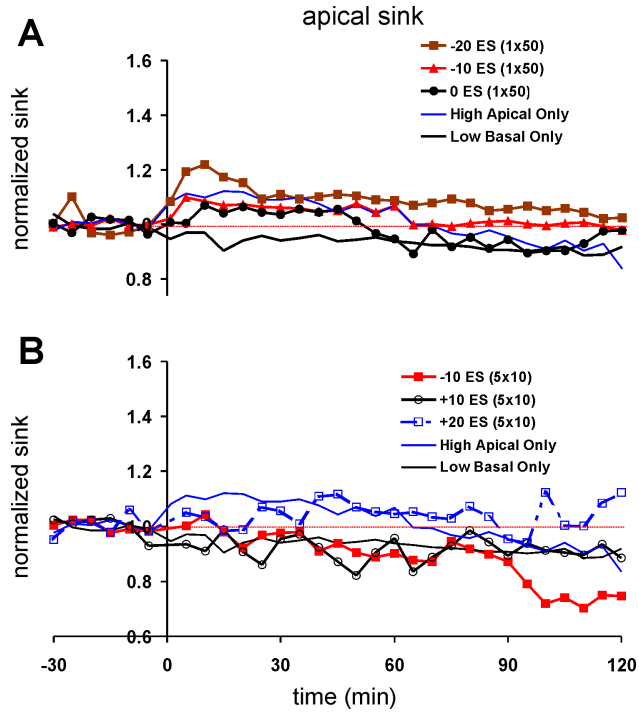

Fig. 6. Normalized CA1 apical dendritic sink following pairing of weak basal dendritic excitation (E) with strong stratum radiatum-evoked spiking (S) at different ES intervals; same format as in Fig. 5 except showing the apical dendritic sink. *A*: continuous 5-Hz ES pairing resulted in no significant potentiation of the apical excitatory sink at ES interval of 0, −10, and −20 ms, compared with High Apical Only stimulation. *B*: discontinuous 5-Hz pairing (5 × 10 pairs) also resulted in no significant potentiation of the apical excitatory sink for ES intervals of −10, 10, and 20 ms. Same "Low Basal Only" and "High Apical Only" traces as in *A*.

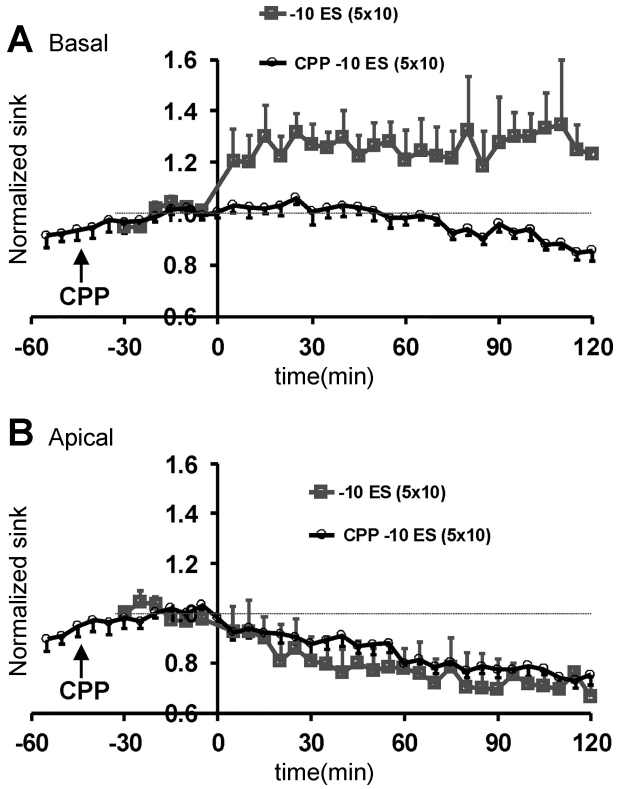

Fig. 7. *N*-methyl-D-aspartate (NMDA) receptor antagonist CPP blocked basal dendritic LTP induced by discontinuous 5-Hz pairing at ES = −10 ms. *A*: mean ± SE of the normalized basal dendritic slope after ES −10 ms (5 × 10) pairing without drug and in the presence of CPP. Same ES −10 ms pairing group as that plotted in Fig. 5*B*, except now with SE. *B*: same as *A* except for the normalized apical dendritic response following the ES −10 ms pairing, with or without CPP. There was a trend of gradual decrease of apical dendritic sink response.

amplitude (1.97 ± 0.46 mV at the CA1 pyramidal cell layer) that was not statistically different than the PS amplitude following stratum radiatum stimulation (3.22 ± 1.07 mV).

DISCUSSION

We found that 5-Hz pairing of a weak basal dendritic excitation (E) with a high-intensity spike (S) originating from the apical dendrites resulted in basal dendritic LTP at ES intervals of −10 to 0 ms and not at +10 to +20 ms (Fig. 10). ES pairing at −20 ms resulted in potentiation up to 60 min. The magnitude of LTP was not dependent on whether pairing was continuous or discontinuous, as shown for ES pairing at −10 ms. The NMDA receptor antagonist CPP blocked the LTP induced by discontinuous pairing at ES −10 ms. While LTP at ES 0 ms was consistent with our hypothesis, LTP at ES −10 ms and the lack of LTD at any ES interval were not.

LTP resulted from coincidence of spike burst and excitatory postsynaptic potentials. LTP is expected at ES = 0 because of coincidence of a backpropagated spike and weak basal dendritic excitation. We suggest that the LTP at an ES interval of −10 ms (i.e., S preceding E by 10 ms) could be explained by the duration of the spike bursts induced by the S stimulus. The S stimulus evoked a "complex spike" (Ranck 1973) rather than a single spike in hippocampal pyramidal cells, and a burst consisted of two to four action potentials at 4- to 8-ms intervals (Azouz et al. 1996; Liu and Leung 2004). Typically, a PS burst consisted of two or three spikes, with the last spike at 9–12 ms after the first spike, and in hippocampal pyramidal cells the spike burst arose from a depolarizing afterpotential (DAP) (Azouz et al. 1996; Liu and Leung 2004; Yue et al. 2005). The duration of the spike bursts and DAP extended the coincidence between spike and excitation by ~15 ms (Fig. 10). LTP induced by negative ES intervals was also found for hippocampal synapses after activation of dopaminergic receptors (Zhang et al. 2009). At the medial perforant path synapse on dentate granule cells, D1 receptor activation converted LTP at negative ES interval to LTP by decreasing K^+ current I_A (Yang and Dani 2014). Spike bursts induced by 5-Hz stimulation in the present study also likely resulted from a decrease in I_A (Watanabe et al. 2002).

In the present study, spike bursts appear to be important for pairing-induced basal dendritic LTP in sexually mature adult rats. Coincident ES pairing (ES = 0 ms) induced basal dendritic LTP when 5-Hz stratum radiatum stimulation evoked spike bursts but not when 0.5-Hz stratum radiatum stimulation evoked only a single PS. The in vivo increase in spike excitability and bursting during stimulation at 5 Hz, but not 0.5 Hz, was similar to results reported in vitro (Thomas et al. 1998). Spike bursts compared with single spikes were also critical for ES pairing-induced apical dendritic LTP in brain slices from 43-day-old rats (Meredith et al. 2003; Pike et al. 1999). However, homosynaptic apical dendritic LTP was found after 150 stimuli at 5 Hz in vitro (Thomas et al. 1998). In the present study, we found apical dendritic STP of ~5-min duration after High Apical Only stimulation at 5 Hz (50 stimuli).

The present results on basal dendritic STDP in vivo may be different from the previous literature, which mainly studied the apical dendritic synapse in CA1 in vitro. In addition, STDP in the present study resulted from pairing basal dendritic excitation with a PS arising from apical dendritic excitation, compared with current-induced spikes in single neurons in vitro. Induction of Ca^{2+} transients and NMDA spikes in the basal dendrites of neocortical pyramidal cells (Schiller et al. 2000), or NMDA-mediated DAP in the basal dendrites of CA1 pyramidal cells (Enoki et al. 2004), could be enhanced by an electrotonically compact basal dendritic tree (Antic 2003). In previous studies, a low threshold for basal dendritic LTP using theta-frequency primed bursts has been attributed partly to a layer-specific interneuronal inhibition (Arai et al. 1994; Kaibara and Leung 1993; Leung and Shen 1995). In particular, the lack of LTD at negative ES intervals may be attributed to the difficulty of inducing LTD at the basal dendritic synapse. Here we showed that high-intensity PPS in vivo did not induce basal dendritic LTD but readily induced apical dendritic LTD (Fig. 9), confirming previous results on apical dendritic LTD (Doyere et al. 1996; Thiels et al. 1994). Low-frequency (1–3 Hz) stimulation with 900 pulses could also induce apical dendritic LTD in CA1 in vivo (Heynen et al. 1996; Kemp and Manahan-Vaughan 2004).

LTP following ES pairing was NMDA receptor dependent. Pretreatment with NMDA receptor antagonist CPP was found to suppress ES pairing-induced LTP, consistent with previous studies in vitro (Debanne et al. 1998; Pike et al. 1999). Coincident ES pairing has been suggested as Hebbian and explained by gating of the NMDA receptor by both presynaptic glutamate and postsynaptic voltage (Bliss et al. 2007; Brown et al. 1988). In the present study, we could not detect any NMDA

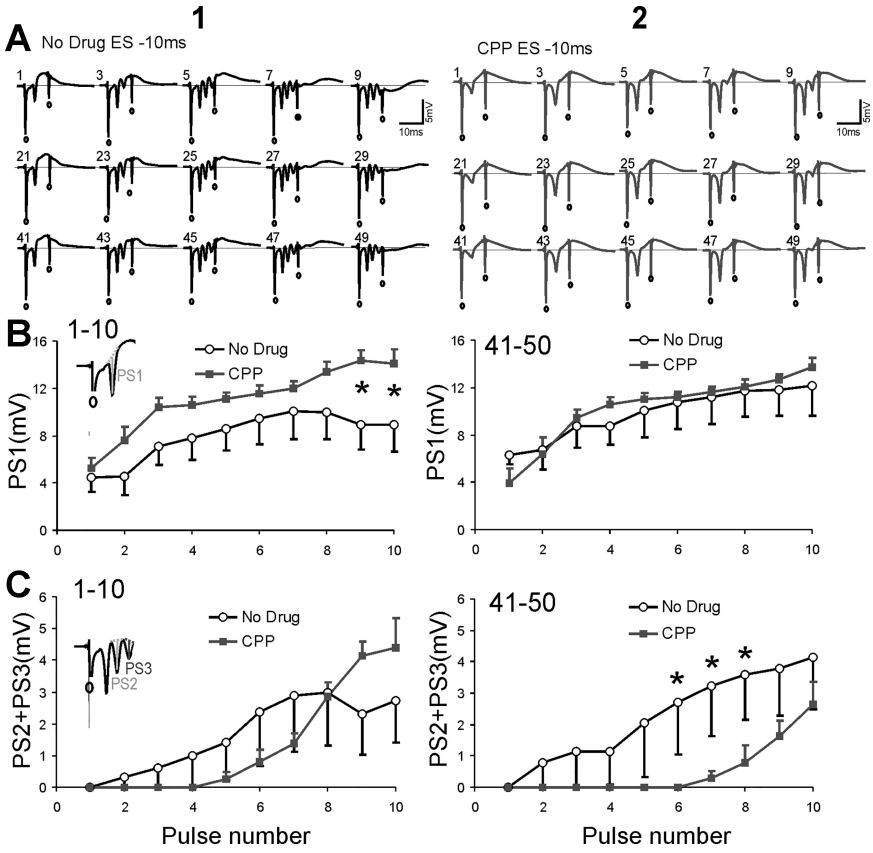

Fig. 8. Temporal facilitation of spike bursts during pairing was attenuated by NMDA receptor antagonist CPP. *A*: representative voltage response at CA1 cell layer during discontinuous pairing of 50 pulses in 5 sweeps of 10 pulses at 200-ms interpulse interval: experiment with no drug (*1*) and experiment with CPP (*2*). *Top*: responses to *sweep 1*, *pulses 1*, *3*, *5*, *7*, and *9*. *Middle*: responses to *sweep 3*, *pulses 21*, *23*, *25*, *27*, and *29*. *Bottom*: responses to *sweep 5*, *pulses 41*, *43*, *45*, *47*, and *49*. Open circle labels a shock artifact. Spike bursts increased during sweep, more for no-drug than CPP condition. Same set of no-drug data was plotted in Fig. 2. *B*: group average of the 1st population spike amplitude (PS1) evoked by each of the 10 pulses in the 1st sweep (*pulses 1–10*; *1*) and 5th sweep (*pulses 41–50*; *2*). PS1 evoked by the 9th and 10th pulses in the 1st sweep was larger in the CPP group ($N = 6$) than the no-drug group ($N = 5$). $^*P < 0.05$, post hoc Newman-Keuls test after a significant interaction of 2-way ANOVA. *C*: group average of the sum of the amplitude of the 2nd (PS2) and 3rd (PS3) population spikes in a burst for the 1st sweep (*pulses 1–10*; *1*) and 5th sweep (*pulses 41–50*; *2*). Increased bursting, indicated by (PS2+PS3) amplitude, was found in the no-drug compared with CPP group for the 5th sweep. $^*P < 0.05$, post hoc Newman-Keuls test after a significant interaction of 2-way ANOVA.

receptor-resistant basal dendritic LTP, whereas the homosynaptic apical dendritic LTP induced by 5-Hz stimulation in vitro had an NMDA receptor-resistant component (Thomas et al. 1998). Interestingly, NMDA receptor antagonist CPP not only blocked pairing-induced LTP but also reduced spike bursts during pairing, while facilitating the first PS in a spike burst (Fig. 9). The facilitation of single-PS response in the presence of CPP differed from a lack of spiking during 5-Hz stimulation in vitro (Zhou et al. 2005). However, the sensitivity of the spike bursts during 5-Hz stimulation to NMDA receptor blockade was similar in vivo and in vitro (Fink and O'Dell 2009; Thomas et al. 1998). While the site (pre- or postsynaptic) of NMDA receptor blockade is not known, it appears that both the attenuation of spike bursts and the blockade of Ca^{2+} influx through the postsynaptic NMDA receptors may be necessary to block pairing-induced LTP (Fig. 8). During NMDA receptor blockade, other mechanisms may contribute to excitability increase and bursting, which may include depression of $GABA_A$ inhibition (McCarren and Alger 1985), partly through presynaptic $GABA_B$ receptors (Davies et al. 1991; Mott and Lewis 1991), temporal summation of non-NMDA receptor excitation, and I_A decrease (Losonczy et al. 2008; Watanabe et al. 2002).

The participation of specific NMDA receptor subunits NR2A and NR2B in synaptic plasticity (Yashiro and Philpot 2008) at the CA1 basal dendrites is unclear. It has been suggested that NR2B is important for LTD and NR2A for LTP (Liu et al. 2004), but this is controversial (Yashiro and Philpot 2008), in part because NR2B-specific antagonist also blocked LTP induced by a pairing protocol (Barria and Malinow 2005; Zhang et al. 2008). We are not aware of reports of NR2A-to-NR2B ratio at the apical or basal dendrites of CA1 pyramidal cells in rats. After ventral hippocampal transection in mice, an increase in NR2A-to-NR2B ratio was found at the basal dendrites of the right CA1 area (Kawakami et al. 2003), but the consequence of this ratio increase for synaptic plasticity is not known.

A physiologically relevant pairing protocol. Pairing-induced basal dendritic LTP in vivo apparently required several conditions. First, a frequency of 5 Hz was needed, and 0.5 Hz was inadequate. Second, pairing-induced LTP depended on coincidence of a 15-ms-duration spike burst (S) with a weak postsynaptic excitation (E). Third, NMDA receptors were activated during pairing.

There are strengths and some weaknesses in using the present in vivo preparation for studying associative synaptic plasticity. Among the strengths is that the hippocampus in vivo offers a natural and physiologically relevant preparation with intact brain connections. Study of long-lasting synaptic plasticity (here up to 2 h) at a physiological temperature is relatively easier in vivo than in vitro. Strong synaptic excitation can readily induce a natural spike burst compared with using multiple current pulses to induce multiple spikes (Pike et al. 1999; Thomas et al. 1998). However, an intact in vivo preparation also comes with some intrinsic issues. Synaptically evoked inhibition is likely stronger in vivo than in vitro (Schwartzkroin 1981), and even a weak stimulus is expected to evoke inhibitory postsynaptic potentials (Kandel et al. 1961), possibly reducing spiking at positive ES intervals. Stimulation (E or S) in vivo may also evoke multisynaptic excitation that may affect the result of ES pairing. For example, the strong S stimulus may synaptically excite the basal dendrites, directly or

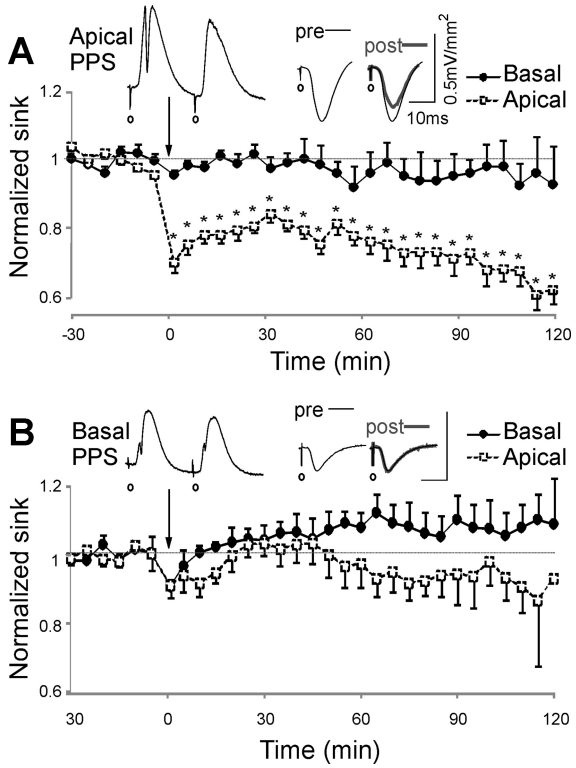

Fig. 9. Paired-pulse stimulation (PPS) of the CA1 stratum radiatum resulted in long-term depression of the apical dendritic excitatory sink, while PPS of the CA1 stratum oriens resulted in no significant change of the basal dendritic excitatory sink. *A*: group average of normalized sink (mean ± SE) at the apical and basal dendritic synapses after PPS of CA1 stratum radiatum at *time 0* (down arrow). PPS consisted of 200 pairs of high-intensity pulses at 25-ms pulse interval. *Top left* trace is the current source density (CSD) at CA1 cell layer of a representative rat in response to 1 pair of pulses during PPS; *top right* traces are apical dendritic sink response showing depression after PPS (post, thick line) compared with baseline (pre, thin line); open circle indicates shock artifact. *B*: same as *A* except that PPS was delivered to the CA1 stratum oriens. *Top* traces show small population spike sink at the CA1 cell layer (*top left* trace) during PPS (*left*) and no significant change of basal sink before (pre) and after (post) PPS in a representative rat. Graph shows time course of normalized group sink response (mean ± SE) after stratum oriens PPS. $^*P < 0.05$, Newman-Keuls post hoc comparison between normalized apical and basal dendritic responses in each panel.

indirectly through CA3, although this appears to be much weaker than backpropagated spiking. The duration of the excitatory sink, which provides a measure of polysynaptic excitation, was not typically altered after ES pairing (Fig. 4*A*), suggesting that long-latency, polysynaptic pathways did not show LTP. We used high-intensity afferent stimulation to evoke PSs following synaptic excitation, instead of current-induced spiking of single neurons in vitro. The peak latency of an evoked PS varied within a small range of ±1.8 ms (half the mean range in peak latency), and spike latencies among pyramidal cells were likely synchronized by ephaptic interaction (Yim et al. 1986). Whether pairing E with sub-spike threshold depolarization could also result in LTP (Hardie and Spruston 2009; Krasteniakov et al. 2004) remains to be studied in vivo, but 0.5-Hz pairing of submaximal PS with weak excitation in the present study did not result in LTP. In addition, urethane anesthesia in the present study was used to facilitate electrode placement, but it may reduce excitatory synaptic transmission (Hara and Harris 2002) and synaptic plasticity.

Basal and apical dendritic inputs to CA1 are known to come from different areas of CA3 (Ishizuka et al. 1990; Li et al. 1994) and CA2 (Kohara et al. 2014; Shinohara et al. 2012). We expect the E stimulus at stratum oriens to excite CA3a, CA3b, and CA2 afferents to the basal dendrites of CA1 pyramidal cells. CA2 projects selectively (but not exclusively) to deep-layer CA1 pyramidal cells (Kohara et al. 2014), and this is not likely a major factor affecting LTP in CA1 since CA2 only contributed 15–20% of stratum oriens afferents (Shinohara et al. 2012) and apparently all CA1 pyramidal cells were excited by CA2 stimulation to various degrees (Kohara et al. 2014). While electrical stimulation of afferent fibers is an experimental technique, the parameters (10 bursts at 5 Hz, repeated 5 times) used in ES pairing are within physiological limits. Spike bursts in CA1 cells, or complex spikes, may occur during normal behaviors, such as an animal running through its place field (O'Keefe 2007; Ranck 1973), accompanied by theta-frequency oscillations in the field and membrane potentials. Pairing resembles a natural situation, such as that of a rat running through a maze, with activation of hippocampal neurons within their place field. Spike bursts in CA1 may also occur, in the absence of theta rhythm, during hippocampal sharp waves, which provide a massive and synchronous excitation of the CA1 apical dendrites by CA3 neurons (Buzsáki et al. 1983).

Our results did not support a LTD/LTP relation commonly found with pairing at negative/positive ES intervals. This may be related to the CA1 basal dendritic synapse in vivo, which has not been studied before. However, our results support diverse functional relations between synaptic plasticity and ES timing (Wittenberg and Wang 2006). At the CA1 basal dendrites, LTP was induced by pairing at an ES interval of −10 to 0 ms. This suggests that pairing-induced LTP can occur with ~15-ms jitter of the ES interval, or E and S need not be precisely coincident. An example is to associate the immediate consequence of being in one location (place) with the location

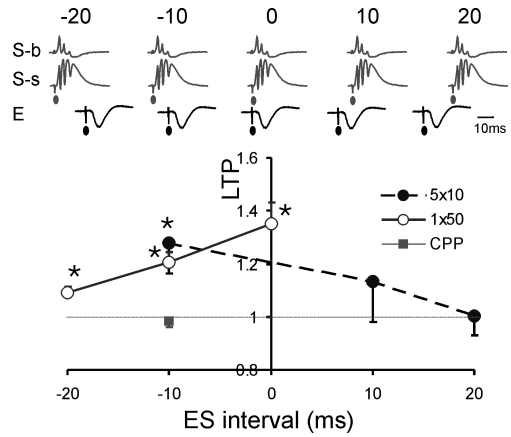

Fig. 10. Summary of long-term potentiation (LTP) at 60 min after pairing at different ES intervals. Potentiation indicated by the normalized basal dendritic excitatory sink (mean ± SE) at 60 min after pairing at different ES intervals from −20 ms to 20 ms. Open symbols, 1 × 50 continuous pairing; filled symbols, 5 × 10 (5 sweeps of 10 pulses) pairing. NMDA receptor antagonist CPP blocked LTP induced by ES-10 (5 × 10) pairing. *Top*: traces indicate temporal overlap using representative bursting current source density (CSD) temporal traces at the basal dendritic (S-b) and the somatic layer (S-s) evoked by the S stimulus (solid ellipse), below which are representative temporal traces of the excitatory sink at the basal dendrites (E) evoked by the E stimulus. $^*P < 0.05$, significantly different from unity.

itself (cf. Dragoi and Buzsáki 2006). Bursting (S) may occur in a CA1 place cell when an animal is in a particular location, while subsequent synaptic inputs on the same cell, which may signal stimuli or reward, may be associated with the place by pairing-induced LTP.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

T.K.F., C.S.L., and L.S.L. performed experiments; T.K.F., C.S.L., and L.S.L. analyzed data; T.K.F., C.S.L., and L.S.L. interpreted results of experiments; T.K.F., C.S.L., and L.S.L. prepared figures; T.K.F., C.S.L., and L.S.L. drafted manuscript; T.K.F. and L.S.L. edited and revised manuscript; T.K.F., C.S.L., and L.S.L. approved final version of manuscript; L.S.L. conception and design of research.

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