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Endocannabinoid-Mediated Metaplasticity in the Hippocampus

Vivien Chevaleyre and Pablo E. Castillo*
Department of Neuroscience
Albert Einstein College of Medicine
Bronx, New York 10461

Summary

Repetitive activation of glutamatergic fibers that normally induces long-term potentiation (LTP) at excitatory synapses in the hippocampus also triggers long-term depression at inhibitory synapses (I-LTD) via retrograde endocannabinoid signaling. Little is known, however, about the physiological significance of I-LTD. Here, we show that synaptic-driven release of endocannabinoids is a highly localized and efficient process that strongly depresses cannabinoid-sensitive inhibitory inputs within the dendritic compartment of CA1 pyramidal cells. By removing synaptic inhibition in a restricted area of the dendritic tree, endocannabinoids selectively "primed" nearby excitatory synapses, thereby facilitating subsequent induction of LTP. This induction of local metaplasticity is a novel mechanism by which endocannabinoids can contribute to the storage of information in the brain.

Introduction

Dendritic processing of excitatory and inhibitory synaptic inputs critically influences neuronal excitability, synaptic plasticity, and ultimately, cognitive processes such as learning and memory (Magee et al., 1998; Shepherd, 2003; Yuste and Tank, 1996). By regulating the level of depolarization and by shunting depolarizing currents, dendritic inhibition controls the functional gain and modifiability of nearby excitatory synapses (Freund and Buzsaki, 1996; Miles et al., 1996). Therefore, it has been postulated that inducing enduring changes in GABAergic synaptic strength (Gaiarsa et al., 2002) also changes subsequent synaptic modifiability, thereby forming the basis of some forms of metaplasticity (Abraham and Bear, 1996; Abraham and Tate, 1997). However, evidence for this type of inhibition-dependent metaplasticity has been lacking. We have recently reported that in hippocampal CA1 pyramidal neurons, repetitive activation of presynaptic glutamatergic fibers induces long-term depression of inhibitory synapses via retrograde endocannabinoid signaling, a phenomenon that we call I-LTD (Chevaleyre and Castillo, 2003). Although the mechanism of induction of this form of plasticity seems to be clear, its physiological relevance is less well understood. We sought to determine whether I-LTD could mediate metaplasticity in the hippocampus.

Endocannabinoids inhibit GABA release via type 1 cannabinoid receptors (CB1Rs) that are found presynaptically at the axon terminals of a subset of inhibitory interneurons (for a recent review, see Freund et al., 2003). To activate these receptors, endocannabinoids must diffuse from the postsynaptic cell where these messengers are produced (Di Marzo et al., 1998; Elphick and Egertova, 2001; Howlett et al., 2002; Piomelli, 2003; Freund et al., 2003; Kreitzer and Regehr, 2002; Petrocellis et al., 2004; Piomelli, 2003; Wilson and Nicoll, 2002; for a review on endocannabinoid-mediated retrograde signaling, see Alger, 2002). Both the heterosynaptic nature of I-LTD and its dependence on retrograde signaling by diffusible messengers raise questions about the synapse specificity of this form of plasticity. The degree of localization of the endocannabinoid-mediated effect is a function of the diffusion of endocannabinoids and/or the biochemical signals involved in their production. While several reports have investigated the spread of endocannabinoid signaling between neighboring cells (Chevaleyre and Castillo, 2003; Gerdeman et al., 2002; Kreitzer et al., 2002; Robbe et al., 2002; Wilson et al., 2001), little is known about the lateral diffusion of endocannabinoids, or their precursors, along the dendrite. Theoretically, I-LTD could spread as a result of (1) diffusion of 2-arachidonoyl glycerol (2-AG), the endocannabinoid likely mediating this form of plasticity (Chevaleyre and Castillo, 2003); (2) spread of postsynaptic signals downstream from metabotropic glutamate receptor (mGluR) activation; or (3) glutamate spillover (Kullmann and Asztely, 1998). Whether I-LTD is a local or diffuse phenomenon has important implications for the dendritic integration of synaptic inputs and the potential control of plasticity at excitatory synapses. Hence, it is critical to know whether local activation of inputs impinging on a restricted area of the dendrite can trigger I-LTD, and if so, whether this depression spreads to neighboring synapses.

In this study, using focal stimulation in rat hippocampal slices, we measured the spread of I-LTD along the dendritic compartment of CA1 pyramidal cells and found that the endocannabinoid signaling that mediates this form of plasticity is remarkably restricted to a very small dendritic area. By selectively monitoring the subset of GABAergic synapses that contain CB1Rs, we show that these synapses are efficiently depressed by patterns of synaptic activity that may occur normally in vivo. We demonstrate that I-LTD can be induced without triggering LTP at neighboring excitatory Schaffer collateral inputs. Furthermore, we show that by inducing I-LTD, subsequent induction of LTP at neighboring excitatory synapses is locally and persistently facilitated. Thus, by removing synaptic inhibition in a restricted dendritic area, endocannabinoids can mediate metaplasticity.

Results

I-LTD Is a Highly Localized and Efficient Phenomenon

In investigating the role of I-LTD, we first determined the degree of localization of this form of plasticity within the dendritic tree of CA1 pyramidal cells. By stimulating a small number of synaptic inputs—excitatory and inhib-

*Correspondence: pcastill@aecom.yu.edu

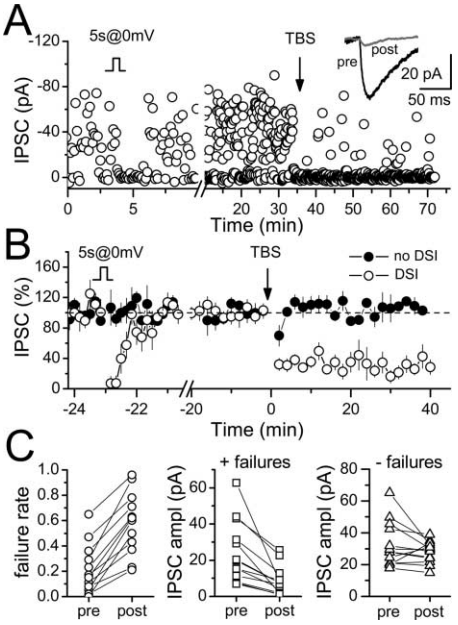

Figure 1. I-LTD Can Be Induced by Focal Stimulation

(A) Single experiment in which IPSCs were evoked by focal stimulation (Vh = −60 mV). The input sensitivity to endocannabinoids was initially verified by the presence of DSI (triggered with a 5 s depolarizing step from −60 to 0 mV). Subsequent TBS application induced a persistent increase in the failure rate.

(B) Time course summary graphs of 12 experiments performed as in (A) (white circles), and 5 experiments that show no DSI (black circles).

(C) The failure rate (left) and the mean IPSC amplitude, with (center) or without (right) failures, are plotted before (pre) and 30 min after (post) TBS for each individual experiment that showed DSI.

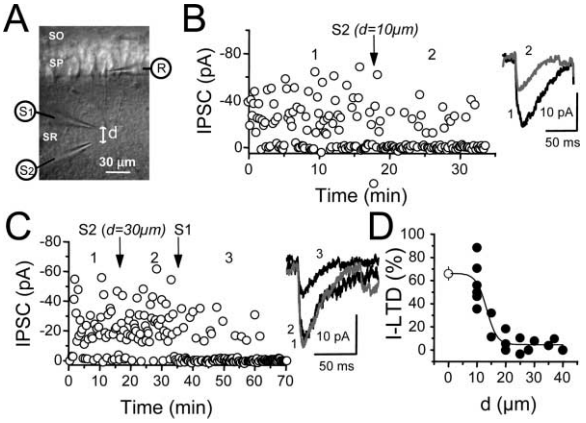

Figure 2. Endocannabinoid Signaling that Mediates I-LTD Is Highly Localized along the Dendritic Tree

(A) Picture showing the arrangement of the recording and stimulating pipettes used to address the degree of localization of I-LTD. Two stimulating pipettes (S1 and S2) were positioned along the apical dendrite of the recorded cell (~20 μm from the apical dendrite axis); IPSCs were evoked through S1 while S2 was only used to deliver tetanic stimulation (TBS) and positioned at a variable distance (d) from S1.

(B) Single experiment in which TBS was applied through S2 pipette at 10 μm from S1. Averaged sample traces taken at the time indicated by numbers are shown on the right side of the plot.

(C) In this example, TBS applied 30 μm from S1 was unable to trigger I-LTD. However, when TBS was delivered through S1, it did induce I-LTD.

(D) Summary graph of I-LTD magnitude versus distance (d). The magnitude of I-LTD induced with the same stimulating pipette, and under identical recording conditions, is also plotted for comparison (open circle, 12 cells).

itory—close to the dendritic tree, we sought to determine whether signals mediating I-LTD in a subset of synapses confined to a small area could diffuse along the dendrite and reach more distant synapses. Because activation of only a few excitatory fibers may induce little endocannabinoid release, we first verified that weak, local stimulation can still induce I-LTD. Focal stimulation (see Experimental Procedures) was achieved by low stimulus strength delivered through a patch-type pipette located within 20 μm of the apical dendrite of the CA1 pyramidal cell. A likely consequence of reducing stimulus strength is that some sets of recruited GABAergic fibers may not express I-LTD, as they do not contain CB1Rs. For this reason, in all experiments in which inhibitory postsynaptic currents (IPSCs) were evoked by focal stimulation, we first verified the presence of functional CB1Rs by testing for DSI (depolarization-induced suppression of inhibition), a transient depression of inhibition (Llano et al., 1991; Pitler and Alger, 1992) that is also mediated by CB1Rs (Diana et al., 2002; Ohno-Shosaku et al., 2001; Wilson and Nicoll, 2001). We found robust DSI in about 20% of stimulated inhibitory inputs under these experimental conditions (i.e., using focal stimulation and placing the stimulating pipette randomly in *stratum radiatum*). As illustrated in a representative experiment in Figure 1A, a 5 s depolarization to 0 mV transiently suppressed inhibitory synaptic transmission; after IPSC recovery, theta-burst stimulation (TBS) induced a robust and persistent depression of IPSCs. On average, in those experiments that showed DSI (93.1% ± 9.5% inhibition, n = 12 cells; Figure 1B), the average failure rate after I-LTD induction was increased (169.7% change in the mean failure rate, $p < 0.001$; or 322.3% ± 107.7%, mean change in failure rate, $p < 0.00001$; Figure 1C, left) and the synaptic efficacy—mean IPSC amplitude including failures—was decreased (to 34.3% ± 5.8% of baseline, $p = 0.0006$; Figure 1C, center), whereas no significant change in synaptic potency—mean IPSC amplitude excluding failures—was observed (92.7% ± 8.0% of baseline, $p = 0.24$; Figure 1C, right). The enhancement of IPSC failure rate with no change in synaptic potency after induction of I-LTD is consistent with a presynaptic mechanism of depression (Katz, 1969; Korn and Faber, 1991), thereby supporting the notion that I-LTD is due to a persistent reduction of GABA release (Chevaleyre and Castillo, 2003). As expected for a CB1R-mediated phenomenon, and consistent with our previous report (Chevaleyre and Castillo, 2003), where DSI could not be induced, I-LTD was also absent (103.4% ± 3.7% of baseline following TBS, n = 5, Figure 1B). These results are remarkable for the large magnitude of the long-term depression of CB1R-sensitive GABAergic inputs that is produced by synaptically driven release of endocannabinoids, even after weak, local stimulation.

To test the spread of I-LTD to more distant synapses, weak stimulation was delivered via two pipettes posi-

tioned along the apical dendrite of the recorded cell (Figure 2). IPSCs were evoked with one stimulating pipette while the other pipette was used to deliver TBS at different distances along the dendrite (Figure 2A). We found that if the tetanizing pipette (S2) was 10 μm away from the pipette (S1) that was used to evoke IPSCs (Figures 2B and 2D), the magnitude of I-LTD (to 42.2% ± 7.7% of baseline, mean amplitude including failures, n = 6) was indistinguishable from control (i.e., using the same pipette for evoking test IPSCs and delivering TBS, p = 0.46; open circle shown in Figure 2D). In contrast, TBS induced no I-LTD when delivered ≥20 μm away (Figures 2C and 2D). In all these cases, we verified that I-LTD could still be induced by delivering TBS through the same pipette (S1) used to evoke IPSCs (to 40.2% ± 6.4% of baseline, n = 10). Thus, using focal stimulation, I-LTD was only induced in those inhibitory inputs close to the stimulation site (~10 μm). Our findings indicate that I-LTD is a highly localized process within the dendritic tree that persistently and efficiently decreases GABA release from CB1R-containing synapses.

Selective Induction of I-LTD

It is well established that synaptic inhibition plays a key role in the induction of LTP at excitatory synapses (Wigstrom and Gustafsson, 1983). Given the robust and enduring depression of inhibitory transmission that occurs as a result of I-LTD induction, this form of synaptic plasticity could facilitate induction of LTP in response to subsequent activity of excitatory inputs. A simple way to explore this possibility is by testing whether LTP is facilitated by previous induction of I-LTD ("priming"). Ideally, this test requires that both forms of plasticity can be triggered independently. Because the stimulation protocols used thus far to induce I-LTD—i.e., theta burst stimulation (TBS) or high-frequency stimulation (HFS)—also induce LTP at Schaffer collateral to CA1 pyramidal cell synapses (Sch-CA1) (Chevaleyre and Castillo, 2003), we first sought another protocol capable of inducing I-LTD selectively. Using bulk stimulation as in our previous report (Chevaleyre and Castillo, 2003), we tested different frequencies of stimulation (200 stimuli total in all cases, see Experimental Procedures), as summarized in a frequency-response graph (Figure 3A). Interestingly, I-LTD could be triggered not only by stimulation frequencies that triggered LTP at Sch-CA1 synapses, but also by lower frequencies that did not induce LTP. At 10 Hz, repetitive stimulation induced a robust long-term depression of IPSCs (Figure 3A; 67.0% ± 3.8% of baseline, p = 0.0004, n = 5) equal in magnitude to the depression induced by HFS or TBS (Figure 3A). In addition, the 10 Hz-induced depression of IPSCs was associated with paired-pulse ratio (PPR) enhancement (152% ± 11.9%, p = 0.0024, data not shown), and it was also blocked by the CB1R antagonist AM251 (Figure 3B; 101.9% ± 1.9% of baseline, p = 0.67, n = 6). Thus, this 10 Hz-induced depression, similar to that produced by HFS and TBS, is likely due to a reduction of GABA release via endocannabinoid retrograde signaling (Chevaleyre and Castillo, 2003). We verified that the 10 Hz protocol induced I-LTD in current clamp mode, that is, under more physiological experimental conditions that mimic the normal behavior of CA1 pyramidal cells (Figure 3C, 71.8% ± 1.1% of baseline, p < 0.001, n = 4). No changes in membrane potential were observed before and after I-LTD induction (−55.6 ± 2.2 mV and −55.9 ± 2.0 mV, respectively, n = 4, data not shown). In addition, focal stimulation—as performed in Figures 1A and 1B—with a 10 Hz protocol also induced robust I-LTD (35.7% ± 8.0% of baseline, p = 0.0005, n = 5, data not shown). Finally, we confirmed that the 10 Hz stimulation protocol does not induce long-term synaptic plasticity at excitatory synapses (Figure 3D), whether under control conditions (97.1% ± 3.8% of baseline, n = 10, p = 0.38) or even when LTP induction was facilitated by pharmacological blockade of $GABA_A$ receptors (Wigstrom and Gustafsson, 1983) with 100 μM picrotoxin (96.3% ± 3.2% of baseline, n = 10, p > 0.2). In conclusion, we identified a stimulation protocol that selectively triggers I-LTD without inducing long-term plasticity at excitatory synapses.

Facilitation of LTP Induction by a Priming Stimulation that Triggers I-LTD

We next investigated whether priming synapses with the 10 Hz stimulation protocol would facilitate subsequent induction of LTP by repetitive synaptic activity.

LTP was induced by TBS in two groups of hippocampal slices. In one group, TBS was delivered 30–40 min after priming (Figure 4A, upper panel), whereas the other group received no priming stimulation and served as the control (Figure 4A, lower panel). In these and the next series of experiments, to try to follow the stimulation procedures of Figures 1 and 2, low-magnitude fEPSPs (0.2–0.4 mV) were evoked by activating Schaffer collaterals with stimulating patch-type pipettes at low stimulus strength. We found that the magnitude of LTP after priming was significantly larger than in control experiments (Figure 4B; 125.2% ± 2.5% after priming, n = 11, compared to 108.4% ± 1.7% in control, n = 8, p = 0.0004). The relatively small magnitude of LTP probably results from applying TBS under conditions of intact synaptic inhibition (see below and Figure 6A). Thus, a 10 Hz repetitive stimulation protocol that by itself does not trigger synaptic plasticity at excitatory synapses can still facilitate subsequent induction of LTP.

If the 10 Hz-induced facilitation of LTP is due to disinhibition that occurs as a result of I-LTD induction, a minimum requirement is that our stimulation in *s. radiatum* should have activated CB1R-sensitive inhibitory inputs. In our previous experiments (Figures 1 and 2), we reported that the probability of recruiting these inputs using focal stimulation is around 20%. However, different experimental conditions between these two sets of experiments may have affected this value. For example, ionotropic glutamate receptor antagonists used in the experiments of Figures 1 and 2 could not be used to investigate the effects of priming on LTP induction. As a result, additional di- or polysynaptic recruitment of inhibitory fibers may have occurred, which could recruit CB1R-sensitive inputs more efficiently than low-strength, monosynaptic stimulation. In addition, in order to evoke 0.2–0.4 mV fEPSPs, the stimulus strength must be slightly higher (5.0–15.0 V, 0.1 ms) than in the IPSC experiments where we used focal stimulation (2.0–10.0 V, 0.1 ms). It is therefore conceivable that the increase in

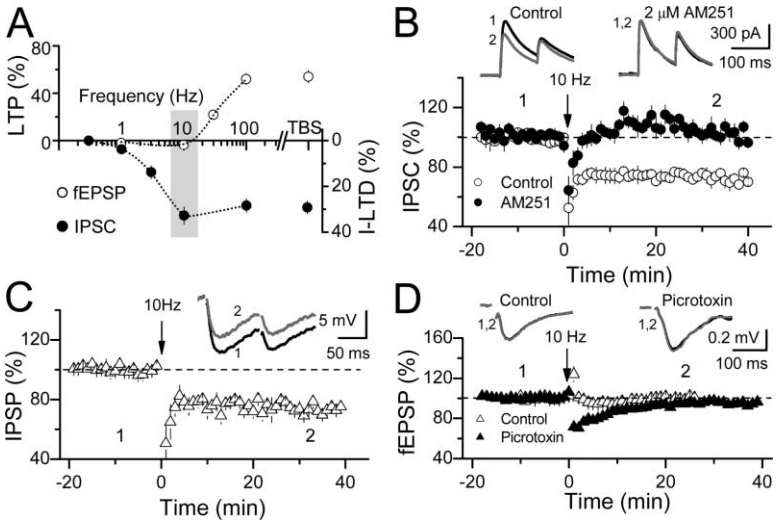

Figure 3. I-LTD Can Be Induced without Triggering Long-Term Plasticity at Excitatory Synapses

(A) Frequency-response plot for excitatory (white circles) and inhibitory synapses (black circles). In all cases, the induction protocol included 200 stimuli delivered at different frequencies (5–10 experiments were performed for each frequency value). All fEPSP experiments were performed in the presence of 100 μM picrotoxin.

(B) Two trains of 100 pulses at 10 Hz-induced I-LTD in control condition (white circles, n = 5) but not in the continuous presence of 2 μM AM251 (black circles, n = 6).

(C) I-LTD is also observed in current clamp mode.

(D) The 10 Hz induction protocol did not induce long-term changes of the amplitude of excitatory synaptic responses (fEPSP) in control conditions (white triangles, 10 slices) or after blockade of inhibitory synaptic transmission with 100 μM picrotoxin (black triangles, 10 slices).

(B–D) Averaged sample traces taken before and after repetitive stimulation as indicated by numbers are shown above each panel.

stimulus strength may have modified the relative contribution of CB1R-sensitive fibers. To determine the probability of recruiting such fibers under the same experimental conditions used to investigate the effect of priming on the induction of LTP, we tested for DSI while monitoring IPSCs and fEPSPs in the same slice and by stimulating at different locations in *s. radiatum* (Figure 5A). Consistent with previous anatomical reports (Egertova and Elphick, 2000; Hajos et al., 2000; Katona et al., 1999) that showed a dense mesh of CB1R-postitive fibers in *s. radiatum,* DSI was detected in most cases (~65%) under these experimental conditions. Furthermore, the time course, amplitude, and occurrence of DSI were similar in three different locations along the apical dendrite (Figure 5B). As expected for the recruitment of CB1R-negative fibers, DSI magnitude (37.8% ± 4.9%, n = 8 cells, tested in three different locations per cell) was smaller than that observed after selecting inputs by focal stimulation (see Figure 1). To summarize, weak, focal, presumably monosynaptic stimulation would sometimes be expected to recruit single fibers or small groups of fibers that are cannabinoid insensitive. However, in our field potential experiments, higher stimula-

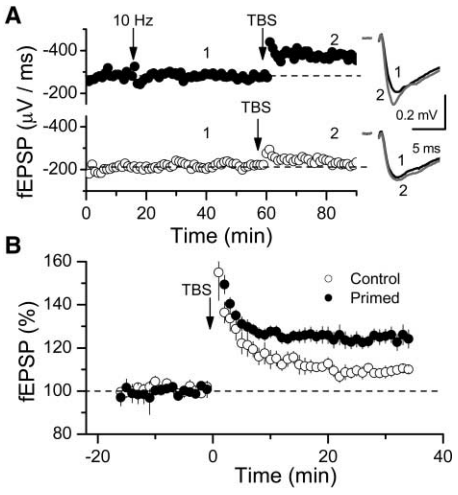

Figure 4. Priming with a 10 Hz Stimulating Protocol Facilitates Subsequent Induction of LTP at Schaffer-CA1 Pyramidal Cell Synapses

(A) Representative experiments (field potential recordings) in which TBS was applied either 40 min after priming with a 10 Hz stimulation protocol (black circles) or to naive slices (white circles).

(B) Summary graph of experiments performed as in (A). LTP induction by TBS was facilitated in slices previously primed with a 10 Hz stimulation protocol (black circles, n = 11) compared to naive slices (white circles, n = 6).

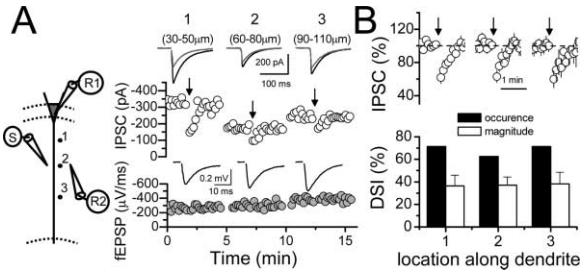

Figure 5. High Probability of Recruiting CB1R-Containing Fibers by Extracellular Stimulation in *s. radiatum*

(A) Representative experiment in which IPSCs and fEPSPs were monitored in the same slice by two recording pipettes (R1 and R2, respectively); these synaptic responses were evoked by a single stimulating pipette (S). Both S and R2 were moved along the dendritic axis of the recorded cell (1, 30–50 μm; 2, 60–80 μm; 3, 90–110 μm from the cell body). The stimulus intensity was adjusted in order to obtain 0.2–0.4 mV fEPSP, and the inhibitory responses were tested for DSI (vertical arrows). Averaged IPSCs (before and immediately after depolarization of the pyramidal cell) and fEPSPs are shown above each plot.

(B) Summary graph of the time course (top), occurrence, and magnitude (bottom) of DSI observed in the three different locations along the dendrite (8 cells total, 6 with all 3 locations, 2 with only 2).

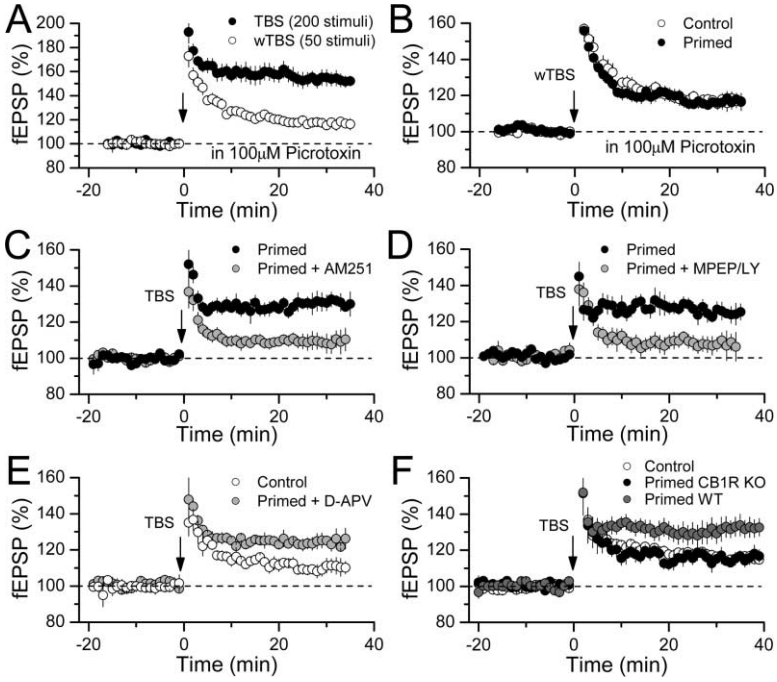

Figure 6. LTP Facilitation Triggered by Priming Exhibits Several Properties Identical to I-LTD

(A) In the presence of 100 μM picrotoxin, TBS (200 stimuli total) induced robust LTP (TBS, black circles), whereas weak TBS (50 stimuli total) induced submaximal LTP (wTBS, white circles).

(B) Priming stimulation in picrotoxin did not facilitate the induction of LTP by a weak TBS (n = 12 slices for each group).

(C and D) Priming stimulation delivered in the presence of AM251 (2 μM, n = 10 slices) or a cocktail of MPEP/LY367385 (4 μM/100 μM, n = 7 slices) failed to facilitate the induction of LTP.

(E) Transient blockade of NMDARs with 50 μM D-APV during priming did not prevent LTP facilitation (n = 5) when compared to interleaved nonprimed slices (n = 6).

(F) 10 Hz priming stimulation facilitated subsequent induction of LTP in wild-type mice (wt, gray circles) but not in CB1R knockout mice (CB1R KO, black circles). Because the magnitude of LTP (without priming) was identical in wt and CB1R KO mice (see main text), "Control" LTP (white circles) are data pooled from experiments performed in both groups of animals.

tion strength and polysynaptic activation, by increasing the number of recruited fibers, makes it likely that a significant proportion of inhibitory inputs will always be cannabinoid sensitive. This situation made it possible for an endocannabinoid-mediated disinhibition to reliably facilitate subsequent LTP induction.

If the 10 Hz-induced facilitation is due to I-LTD, this effect should be blocked in presence of the $GABA_A$ receptor antagonist picrotoxin. However, in picrotoxin, TBS (200 stimuli) triggered robust and presumably saturating LTP (Figure 6A; 154.7% ± 4.2%, n = 7). Therefore, to make it possible to detect the effects of priming, we first identified a short TBS protocol (50 stimuli) that induced submaximal LTP (Figure 6A). Using this induction protocol, we found that in the presence of picrotoxin, 10 Hz priming stimulation failed to facilitate LTP (Figure 6B; LTP control: 116.6% ± 2.5%, n = 12; LTP after priming: 115.4% ± 2.0%, n = 12; p = 0.7). This observation indicates that the LTP facilitation induced by 10 Hz priming requires intact GABAergic inhibition.

If the facilitation of LTP is due to I-LTD, it should be eliminated by manipulations known to block I-LTD induction, such as pharmacological blockade of CB1Rs or group I mGluRs (Chevaleyre and Castillo, 2003). Priming performed in the presence of the CB1R antagonist AM251 abolished LTP facilitation (Figure 6C) when compared to control interleaved slices (108.3% ± 2.7%, n = 10 in AM251; 130.3% ± 3.6%, n = 5 in control slices; p = 0.0004), indicating that CB1R activation is necessary for LTP facilitation by priming. Similarly, pharmacological blockade of the group I mGluRs by a combination of MPEP (4 μM) and LY 367385 (100 μM), to block mGluR5 and mGluR1, respectively, also abolished the priming-induced effect (Figure 6D; 108.8% ± 3.3% in presence of MPEP and LY 367385, n = 7; 131.9% ± 5.6%, n = 6 in control interleaved slices, p = 0.003). In addition, as expected for an NMDAR-independent phenomenon such as I-LTD, priming in the presence of D-APV still facilitated the LTP induced after D-APV washout (Figure 6E; 123.4% ± 1.9% in D-APV, n = 5; 110.8% ± 2.9% in nonprimed interleaved slices, n = 6, p = 0.005). Using CB1R knockout mice, we confirmed that both I-LTD and priming-induced facilitation of LTP require CB1R activation. As suggested by our pharmacological results (Chevaleyre and Castillo, 2003), I-LTD was absent in CB1R knockout mice (96.3% ± 2.7% of baseline, 6 slices, 4 animals, p = 0.3, data not shown), in contrast to the robust I-LTD we recently described in wild-type mice (Mato et al., 2004). The magnitude of nonprimed LTP was the same in wild-type and CB1R knockout mice (wild-type mice: 116.2% ± 4.1%, 8 slices, 4 animals; CB1R knockout mice: 115.5% ± 2.8%, 8 slices, 4 animals, p = 0.88), consistent with previous reports showing that blockade of CB1R does not affect LTP induction (Carlson et al., 2002; Chevaleyre and Castillo, 2003; but see Bohme et al., 2000). In addition, priming with a 10 Hz protocol clearly facilitated subsequent induction of LTP in wild-type mice (Figure 6F; 130.4 ± 4.8%, 8 slices, 4 animals, p = 0.04), but not in CB1R knockout mice (113.6% ± 2.6%, 8 slices, 4 animals, p = 0.63) as expected for a CB1R-dependent phenomenon. To summarize, LTP facilitation by priming requires intact inhibition, and similar to I-LTD is NMDAR independent but mGluR1/5 and CB1R dependent. These findings strongly suggest that this facilitation is due to the disinhibition resulting from I-LTD induction.

Finally, if I-LTD underlies the facilitation induced by priming, this effect should share I-LTD's spatial and temporal features, that is, it should be localized to the postsynaptic region exhibiting I-LTD and show a similar time course. To investigate the time course of facilitation, LTP was induced at different delays after 10 Hz

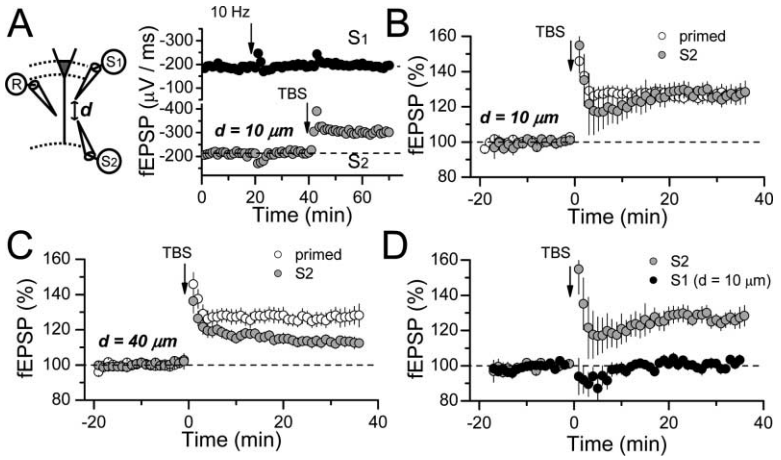

Figure 7. Local LTP Facilitation Induced by Priming

(A) Representative experiment (right) in which two independent sets of fibers were activated with two stimulating pipettes (S1 and S2). These pipettes were positioned in *s. radiatum* 10 μm apart as indicated in the scheme (left). A 10 Hz stimulation protocol was delivered through one stimulating pipette (S1, black circles), and the effect of this stimulation on LTP induction was evaluated in the other pathway (S2, gray circles).

(B) A summary graph of six experiments performed as in (A) (S2, gray circles) is superimposed to control LTP facilitation that was obtained in 16 interleaved slices (primed, white circles).

(C) Summary graph comparing the magnitude of LTP when priming was applied while both pipettes were 40 μm apart (S2, gray circles) versus control LTP facilitation (primed, white circles) as in (B).

(D) Summary graph demonstrating the independence of excitatory inputs and the input specificity of LTP. TBS was applied to S2 (gray circles) but not S1 (black circles) when both stimulating pipettes were 10 μm apart as in (A).

priming (data not shown). We found that the priming effect of 10 Hz stimulation is established as early as 5–10 min postpriming (129% ± 4.5% after priming, n = 7, compared to 113.4% ± 2.6% in control, n = 5, p = 0.023) and lasts for at least 60–90 min (127.2% ± 1.8% after priming, n = 8, compared to 113.2% ± 1.7% in control, n = 8, p = 0.0006), a time course similar to that of I-LTD. To test whether this facilitation is similar to I-LTD spatial profile also, we used a two-independent pathway approach and explored whether priming one pathway would affect the induction of LTP in the other pathway. Thus, two independent sets of inputs were activated, using two stimulating pipettes placed in *s. radiatum* (Figure 7A). The distance between these two pipettes was either 10 or 40 μm while extracellular synaptic responses were monitored with a third recording pipette, which was placed between the other two. We found that priming one pathway facilitated the induction of LTP (20–40 min later) in the other pathway only when the stimulating pipettes were 10 μm apart (Figure 7B; 127.2% ± 3.8%, n = 6; compared to facilitation obtained in control interleaved slices: 125.5% ± 3.7%, n = 10, p = 0.77). However, if the stimulating pipettes were 40 μm away, priming stimulation did not facilitate LTP in the other pathway (Figure 7C; 111.1% ± 2.1%, n = 16, p = 0.002 when compared to a control, primed pathway in the same slice). These results suggest that similarly to I-LTD, the priming effect is highly localized and does not affect synaptic inputs distant from the stimulation site. Furthermore, when the stimulating electrodes were 10 μm apart, LTP induction was still input specific (Figures 7A and 7D); that is, TBS of one pathway triggered LTP only in that specific pathway (126.7% ± 3.8%) but not in the other (99.5% ± 2.1%, n = 6, p = 0.0001). This observation clearly suggests that under our experimental conditions, LTP is even more restricted than I-LTD.

LTP Is More than a Persistent Change in Glutamatergic Synaptic Strength

Our experiments show that TBS of Schaffer collaterals triggers both LTP at Sch-CA1 synapses and I-LTD. If LTP is indeed a more localized process than I-LTD, there would be an area surrounding the stimulation site where excitatory inputs do not express LTP but are still primed for subsequent LTP due to the induction of I-LTD. To test this hypothesis, two stimulating electrodes were placed 10 μm apart along the apical dendrite. TBS was delivered to one pathway, and 30–40 min later, TBS was delivered to the other pathway (Figure 8A). As shown in Figure 8B, the magnitude of LTP induced by the second TBS was larger (TBS2: 126.8% ± 2.6%; TBS1: 111.3% ± 3.7%, n = 5, p = 0.009), as if the first TBS in one pathway had facilitated LTP induction in the other pathway. To verify that this facilitation was due to I-LTD that was triggered by the first TBS, we repeated these experiments under conditions known to block I-LTD (Figure 8C). We found that when the first TBS was delivered in presence of the CB1R antagonist AM251, it had no effect on the magnitude of the LTP induced subsequently in the second pathway (TBS1: 114.6% ± 2.5%; TBS2: 110.2% ± 3.9%; n = 5, p = 0.36). This result strongly suggests that the facilitation triggered by priming with TBS is mediated by endocannabinoids. In conclusion, our findings can be summarized as follows (Figure 8D): repetitive activation of excitatory inputs can trigger LTP in a restricted area (<10 μm) of the dendrite, but at the same time, it induces I-LTD at inhibitory inputs in a more extended area (≥10 μm), thereby facilitating subsequent LTP induction at surrounding Sch-CA1 synapses.

All previous experiments were performed at 25°C and in relatively high extracellular Ca^{2+} (2.5 mM). Because these parameters may affect the threshold for the induction of synaptic plasticity, as well as the diffusion and uptake of glutamate (Asztely et al., 1997; Bergles and Jahr, 1998; Wadiche and Kavanaugh, 1998) and 2-AG (Hajos et al., 2004), we verified that most relevant findings of our study could also be observed at a more physiological 35°C and 1.8 mM extracellular Ca^{2+} (Figure 9). We found that 10 Hz repetitive stimulation induced robust I-LTD (Figure 9A, 77.4% ± 2.6% of baseline, n = 5) and that the threshold for I-LTD and LTP induction was unchanged under these recording conditions (Figure 9B). Furthermore, using a two-pathway paradigm

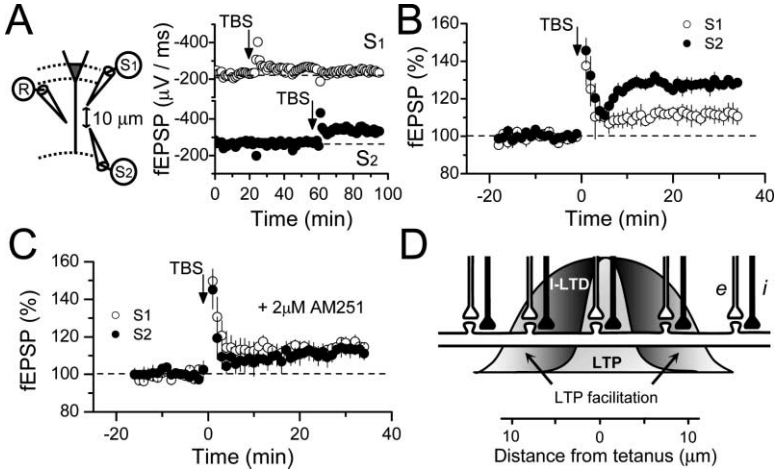

Figure 8. LTP Is Associated with a Long-Lasting and CB1R-Dependent Facilitatory Effect on Surrounding Synapses

(A) Experimental paradigm in which two stimulating electrodes were placed 10 μm apart in the middle third of *s. radiatum* along the apical dendrite of CA1 pyramidal cells and synaptic responses were recorded extracellularly. Stimulus strength was set to evoke identical synaptic responses in both pathways. The same TBS was first applied to one pathway (S1) and then to the other pathway (S2) 35 min later.

(B) Superimposed summary graphs of five experiments performed as described in (A). The first tetanus was delivered either to the proximal or distal stimulating pipette (n = 2 and n = 3 slices, respectively).

(C) Summary graphs showing that LTP facilitation was abolished when TBS to S1 was delivered in presence of 2 μM AM251 (n = 5 slices).

(D) Model that summarizes the local facilitatory effects of I-LTD on LTP induction at Sch-CA1 synapses. The cartoon illustrates excitatory (*e*, white) and inhibitory (*i*, black) inputs impinging on the apical dendrite of a CA1 pyramidal cell. Local activation of excitatory inputs triggers LTP in a highly restricted area (<10 μm from the stimulating site) and at the same time, it triggers I-LTD in a slightly larger area, thereby facilitating LTP induction of neighboring excitatory inputs.

and placing stimulating pipettes 40 μm apart in *s. radiatum*, the 10 Hz stimulation protocol facilitated the induction of LTP only in the primed pathway, suggesting that I-LTD is also a localized phenomenon under these more physiological conditions (Figure 9C, primed pathway: 134.3% ± 4.4%; nonprimed pathway: 115.4% ± 3.4%, n = 8; p = 0.004). Finally, using an experimental paradigm similar to that shown in Figures 8A and 8B (two stimulating pipettes 10 μm apart), we found that the first TBS induces LTP in the tetanized pathway only (not shown), while at the same time, it facilitates the subsequent induction of LTP by TBS of the other pathway (Figure 9D; TBS1: 115.9% ± 4.6%, TBS2: 135.2% ± 2.5%, n = 7, p = 0.003). This observation strongly suggests that even under more physiological experimental conditions, I-LTD is less localized than LTP. Taken together, these results not only indicate that the main properties of I-LTD and the associated facilitation of LTP induction remain unchanged under more physiological conditions, but they also support the idea that these endocannabinoid-mediated phenomena may occur in vivo.

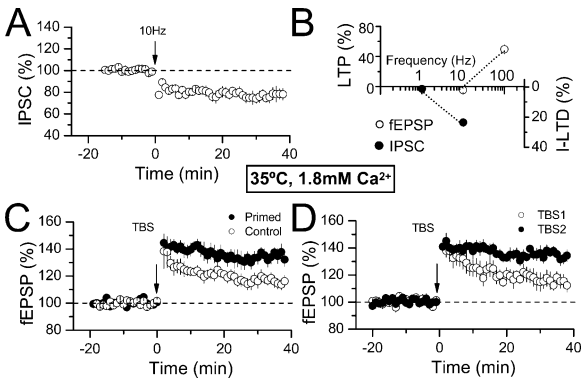

Figure 9. I-LTD and the 10 Hz-Induced Facilitation of LTP Induction Occur under Physiological Experimental Conditions

All experiments in this figure were performed in presence of 1.8 mM extracellular Ca^{2+} (instead of 2.5 mM) and at 35°C (instead of 25°C).

(A) A 10 Hz tetanus (bulk stimulation as in Figure 3B) triggered normal I-LTD.

(B) The threshold for the induction of I-LTD and LTP was explored as described in Figure 3A (6–8 experiments/frequency value).

(C) Summary graph of a set of experiments in which two independent pathways were activated by two stimulating pipettes that were placed 40 μm apart in *s. radiatum*. The magnitude of LTP was significantly larger in the pathway that received 10 Hz stimulation, indicating that the priming effect is input specific.

(D) Summary graph of experiments performed as in Figures 8A and 8B. A first tetanus (TBS1) facilitated subsequent induction of LTP by a second tetanus (TBS2) that was delivered 20–40 min later.

Discussion

It is becoming clear that endocannabinoids play an essential role in the induction of long-term plasticity in different brain structures (Chevaleyre and Castillo, 2003; Gerdeman et al., 2002; Marsicano et al., 2002; Robbe et al., 2002; Sjostrom et al., 2003; for a review, see Gerdeman and Lovinger, 2003). In the hippocampus, endocannabinoids mediate I-LTD, an activity-dependent form of long-term depression at inhibitory synapses (Chevaleyre and Castillo, 2003). Three major novel observations arise from our present study. First, physiologically relevant stimulation that triggers I-LTD can virtually remove the contribution of CB1R-sensitive inhibitory synapses within a restricted area of the dendritic tree of CA1 pyramidal cells. Second, I-LTD can be triggered not only by the same stimulation protocols that trigger LTP, but also by stimulation protocols that are normally subthreshold for the induction of long-term plasticity at excitatory synapses. Third, by suppressing inhibition in a restricted dendritic area, endocannabinoids can locally and persistently facilitate the induction of LTP at excitatory synapses. Thus, the disinhibition caused by synaptically driven release of endocannabi-

noids not only enhances excitability (Chevaleyre and Castillo, 2003) but also mediates metaplasticity (Abraham and Bear, 1996). Our findings stress the relevance of endocannabinoids in synaptic plasticity and reveal the variety of mechanisms by which these messengers contribute to the storage of information in the brain.

Local and Efficient Regulation of Hippocampal GABAergic Synaptic Transmission by Endocannabinoids

We have recently reported a ~25% depression of inhibitory synaptic transmission following I-LTD induction (Chevaleyre and Castillo, 2003). By more selectively monitoring cannabinoid-sensitive fibers (Figures 1 and 2), we now show that a weak and physiological stimulation pattern such as TBS induces a massive depression (~65%) of IPSCs. The different magnitude of I-LTD is likely due to the particular stimulation protocol used in each study. In our initial report, use of bulk stimulation of inhibitory inputs, including a large proportion of endocannabinoid-insensitive inputs, means that the magnitude of the depression we reported did not give a full sense of the massive depression that takes place at cannabinoid-sensitive inputs. The large magnitude of I-LTD underscores the high efficacy of endocannabinoids in regulating inhibitory synaptic transmission and clearly suggests that I-LTD profoundly affects the dendritic processing of synaptic inputs.

Our data suggest that the spread of signals required for I-LTD induction is restricted to synapses impinging on a small portion of the CA1 pyramidal cell dendritic tree (Figure 2). In this regard, our findings are consistent with recent observations in the cerebellum showing spatial restriction of transient endocannabinoid-mediated inhibition (Brown et al., 2003). Our results also demonstrate that local synaptic activity can trigger sufficient release of 2-AG, the putative endocannabinoid that mediates I-LTD (Chevaleyre and Castillo, 2003), to strongly suppress inhibitory inputs impinging close to the endocannabinoid release site (within ~10 μm).

I-LTD can be triggered by stimulation protocols, such as HFS and TBS, that commonly induce LTP at excitatory synapses (Chevaleyre and Castillo, 2003). In addition, we report here that a 10 Hz stimulation protocol can selectively induce I-LTD (Figure 3) without triggering long-term plasticity at excitatory synapses. Interestingly, this frequency was previously reported to be the point of crossover from LTD to LTP at excitatory synapses (Dudek and Bear, 1992). It is conceivable that under conditions of intact synaptic inhibition, repetitive synaptic activity that is subthreshold for LTP/LTD induction at excitatory synapses can still induce long-term depression at inhibitory synapses. The fact that I-LTD can occur independently of long-term plasticity at excitatory synapses and can be easily triggered by weak stimulation (Figures 1 and 2) clearly emphasizes the relevance of activity-dependent plasticity at inhibitory synapses under physiological conditions. It remains to be seen whether LTP can occur independently of I-LTD.

Endocannabinoids as Mediators of Metaplasticity

It is commonly believed that the history of activity of a given neuron influences its future responses to synaptic inputs (Bienenstock et al., 1982). Previous studies have suggested that prior synaptic activity can elicit a persistent modification (≥1 hr) in the ability of synapses to undergo changes in strength in response to subsequent episodes of synaptic activity, a phenomenon known as metaplasticity (Abraham and Bear, 1996). The mechanisms underlying metaplasticity are certainly diverse. These may include modification of postsynaptic membrane potential and/or calcium dynamics, kinase/phosphatase function, as well as gene expression (Abraham and Tate, 1997). Regardless of the mechanism, changes in the induction threshold for LTP/LTD are commonly specific to those synapses that were previously active. We show here an endocannabinoid-mediated form of metaplasticity in the hippocampus that can be triggered by brief repetitive activation of glutamatergic fibers. Although this metaplasticity is a highly localized phenomenon, it may target adjacent naive synapses that are not necessarily activated during its induction. Conditioning stimulation of Schaffer collateral fibers has previously been shown to modify subsequent induction of LTP and LTD (Wang and Wagner, 1999), and mild priming stimulation was shown to facilitate LTP induction of lateral perforant path afferents to the dentate gyrus in vivo (Christie et al., 1995). It has also been reported that prior activation of mGluRs by an agonist facilitates LTP induction (Bortolotto et al., 1994; Cohen and Abraham, 1996; Miura et al., 2002), although this effect seems to be independent of synaptic inhibition (Cohen and Abraham, 1996). However, endocannabinoid-mediated metaplasticity is unique in that it is the long-lasting reduction of synaptic inhibition, triggered by transient mGluR activation (Chevaleyre and Castillo, 2003; Liu et al., 1993) that facilitates subsequent induction of LTP. Thus, our results not only uncover a novel form of activity-dependent metaplasticity in the CA1 area of the hippocampus that is mediated by the dendritic release of endocannabinoids, but they also underscore the relevance of local GABAergic inhibition for the induction of long-term synaptic plasticity.

Previous studies in the hippocampus have reported that cannabinoid agonists impede the induction of LTP (Collins et al., 1995; Misner and Sullivan, 1999; Stella et al., 1997; Terranova et al., 1995) and LTD at excitatory synapses (Misner and Sullivan, 1999). The mechanism underlying these effects is not entirely clear, although it could result from decreased glutamate release (Misner and Sullivan, 1999). While these studies demonstrate an effect of exogenous application of cannabinoids on hippocampal LTP/LTD, it is less clear how endocannabinoids regulate the induction of long-term synaptic plasticity in vivo. Given the well-documented reduction of GABA release induced by CB1R activation in the hippocampus (Freund et al., 2003; Hajos et al., 2000; Hoffman and Lupica, 2000; Katona et al., 1999; Schlicker and Kathmann, 2001) and the profound facilitation of LTP induction by reduced synaptic inhibition (Wigstrom and Gustafsson, 1983), it is expected that endocannabinoids may facilitate the induction of LTP. The temporal and spatial properties of this facilitation will depend on the time course of endocannabinoid-mediated effects and the targeted synapses. Accordingly, it has been recently shown that the endocannabinoid release that occurs during DSI may transiently (less than 30 s) facilitate the

induction of LTP (Carlson et al., 2002). As expected for the nonselective release of endocannabinoids in the somatic and dendritic compartments (Chevaleyre and Castillo, 2003; Martin et al., 2001), the DSI-related facilitation of LTP induction most likely affects synaptic inputs more widely. Our findings show that patterns of synaptic activity that are commonly observed in the hippocampus (Larson et al., 1986; Rose and Dunwiddie, 1986) also facilitate the induction of LTP via endocannabinoids. In contrast to the global and transient facilitation triggered by depolarization-induced release of endocannabinoids (i.e., DSI), synaptic activity-dependent facilitation is local and long-lasting. Indeed, inhibitory synaptic inputs impinging on the dendritic tree might be instrumental in controlling synaptic plasticity at neighboring excitatory synapses (Miles et al., 1996). The close anatomical proximity between glutamatergic and CB1R-containing GABAergic synapses on the dendritic tree (Hajos et al., 2000) is entirely consistent with the interaction between excitatory and inhibitory inputs that is mediated by local endocannabinoid signaling. The fact that the activation of a subset of glutamatergic fibers triggers local release of endocannabinoids clearly suggests that this process is well suited to regulate neighboring synapses within a narrow band of the dendrite.

One emerging aspect from our study is the local interaction between long-term plasticity at excitatory and inhibitory synapses. It has been commonly assumed that LTP is an input-specific phenomenon. However, using a pairing protocol to induce LTP and a perfusion system to locally restore synaptic transmission in slices, it was reported that this specificity is lost when inputs are closer than 70 μm (Engert and Bonhoeffer, 1997). Using focal stimulation with a pattern that mimics neuronal activity during hippocampal theta rhythm (TBS), we show here that LTP fades 10 μm away from the stimulation site (Figures 7A and 7D). Our findings place tight constraints on models of the potential spread of LTP.

Conclusions

In this study, we describe a novel endocannabinoid-mediated mechanism by which previous activity can modify subsequent induction of synaptic plasticity. We provide evidence that the induction of LTP under physiological conditions of intact synaptic inhibition also primes the surrounding area for subsequent LTP induction (Figure 7D). This facilitation is long lasting and is most likely mediated by the local release of endocannabinoids and subsequent induction of I-LTD at nearby synapses. Thus, the activity patterns that lead to LTP induction trigger not only a change in the strength of excitatory synapses but also a persistent facilitation in the ability of surrounding synapses to be potentiated.

Experimental Procedures

Transverse hippocampal slices were prepared from 3- to 4-week-old Sprague Dawley rats or C57BL/6J mice as described elsewhere (Chevaleyre and Castillo, 2003). Animals were killed by decapitation in accordance with institutional regulations. Slices (400 μm thickness) were cut on a vibratome (Dosaka, Kyoto, Japan) in ice-cold extracellular solution containing (in mM): 238 sucrose, 2.5 KCl, 10 glucose, 25 $NaHCO_3$, 1.25 NaH_2PO_4, 2.5 $CaCl_2$, and 1.3 $MgCl_2$. The cutting medium was gradually switched to the recording solution (ACSF) that contained (in mM) 124 NaCl, 2.5 KCl, 10 glucose, 25 $NaHCO_3$, 1.25 NaH_2PO_4, 2.5 $CaCl_2$, and 1.3 $MgCl_2$. The slices were kept at room temperature for at least 1.5 hr before being transferred to the recording chamber. Cutting and recording solutions were both saturated with 95% O_2 and 5% CO_2 (pH 7.4). Unless otherwise stated, experiments were performed at 25.0°C ± 0.1°C.

IPSCs were recorded in CA1 pyramidal neurons voltage clamped at −60 mV with a pipette (3–5 MΩ) containing (in mM): 125 CsCl, 8 NaCl, 1 $CaCl_2$, 10 EGTA, 10 HEPES, 10 glucose, 5 ATP, 0.4 GTP (pH 7.2; 280–290 mOsm) or voltage clamped at +10 mV with the same solution but containing 123 cesium gluconate instead of CsCl. Series resistance (typically 8–15 MΩ) was monitored throughout each experiment with a −4 mV, 80 ms pulse, and cells with more than 10% change in series resistance were excluded from analysis. For current-clamp recordings, IPSPs were recorded with a patch pipette containing in (mM): 135 $KMeSO_3$, 5 KCl, 1 $CaCl_2$, 5 EGTA-Na, 10 HEPES, 10 glucose. IPSCs/IPSPs were monitored in the continuous presence of NMDA and AMPA/Kainate receptor antagonists (50 μM D-APV and 10 μM NBQX, respectively). Synaptic responses were evoked by monopolar stimulation with a patch-type pipette filled with ACSF and placed in the middle third of *s. radiatum*. I-LTD was induced after 20 min of stable baseline by a 10 Hz stimulation (two trains of 100 pulses at 10 Hz, 20 s apart) or by theta-burst stimulation (TBS) consisting of a series of 10 bursts of 5 stimuli (100 Hz within the burst, 200 ms interburst interval), which was repeated 4 times (5 s apart). DSI was evoked by a 5 s voltage step from −60 to 0 mV. IPSCs were monitored every 6 s for DSI and every 20 s for I-LTD. For focal stimulation experiments, a patch-type pipette was placed at the same depth as the recording pipette within 20 μm of the apical dendrite of the CA1 pyramidal cell (Vh = −60 mV). The stimulus strength (2.0–10.0 V, 0.1 ms) was reduced to elicit small amplitude (~30 pA) IPSCs with >10% failures. When exploring the spread of I-LTD (Figure 2), the independence of the two inhibitory inputs was insured by the presence of DSI in only one input. The stimulating pipette that activated the DSI-sensitive input was used as the test pipette, whereas the other was used to deliver the I-LTD inducing tetanus. A patch-type pipette filled with 1 M NaCl was used to record field excitatory synaptic potentials (fEPSPs) from *s. radiatum*. fEPSPs were evoked with a patch pipette and the simulation strength adjusted in order to obtain fEPSPs of 0.3–0.4 mV. The stimulating and recording pipettes were positioned at the same depth in the slice and close to each other (within 50 μm). In the two excitatory pathway experiments, the interaction between these pathways was systematically evaluated at the beginning of all the experiments by paired-pulse stimulation.

Whole-cell and extracellular recordings were performed with a MultiClamp 700A (Axon Instruments, Union City, CA), and output signals were filtered at 3 kHz. Data were digitized (5 kHz) and analyzed online using a macro written in IgorPro (Wavemetrics, Lake Oswego, OR). Results are reported as mean ± SEM. The magnitudes of I-LTD and LTP were estimated by comparing averaged responses 30–35 min after induction with baseline-averaged responses before induction protocol. Statistical comparisons were performed using Student's t test. The generation of CB1R knockout mice is described elsewhere (Zimmer et al., 1999). All drugs were bath-applied following dilution into the external solution from concentrated stock solutions. D-APV, NBQX, MPEP, LY367385, and AM251 were obtained form Tocris-Cookson. All other chemicals and drugs were from Sigma-Aldrich.

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