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Homosynaptic and Heterosynaptic Inhibition of Synaptic Tagging and Capture of Long-Term Potentiation by Previous Synaptic Activity

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Long-term potentiation (LTP) is an enhancement of synaptic strength that may contribute to information storage in the mammalian brain. LTP expression can be regulated by previous synaptic activity, a process known as "metaplasticity." Cell-wide occurrence of metaplasticity may regulate synaptic strength. However, few reports have demonstrated metaplasticity at synapses that are silent during activity at converging synaptic inputs. We describe a novel form of cell-wide metaplasticity in hippocampal area CA1. Low-frequency stimulation (LFS) decreased the stability of long-lasting LTP ["late" LTP (L-LTP)] induced later at the same inputs (homosynaptic inhibition) and at other inputs converging on the same postsynaptic cells (heterosynaptic inhibition). Significantly, heterosynaptic inhibition of L-LTP also occurred across basal and apical dendrites ("heterodendritic" inhibition). Because transient early LTP (E-LTP) was not affected by previous LFS, we examined the effects of LFS on the consolidation of E-LTP to L-LTP. The duration of E-LTP induced at one set of inputs can be extended by capturing L-LTP-associated gene products generated by previous activity at other inputs to the same postsynaptic neurons. LFS applied homosynaptically or heterosynaptically before L-LTP induction did not impair synaptic capture by subsequent E-LTP stimulation, suggesting that LFS does not impair L-LTP-associated transcription. In contrast, LFS applied just before E-LTP (homosynaptically or heterosynaptically) prevented synaptic tagging, and capture of L-LTP expression. Thus, LFS inhibits synaptic tagging to impair expression of subsequent L-LTP. Such anterograde inhibition represents a novel way in which synaptic activity can regulate the expression of future long-lasting synaptic plasticity in a cell-wide manner.

Key words: **metaplasticity; heterosynaptic plasticity; synaptic capture; synaptic tagging; LTP; long-term potentiation; hippocampus**

Introduction

Long-term potentiation (LTP) is a form of activity-dependent synaptic enhancement that can be regulated by the previous history of synaptic activation (Bliss and Lømo, 1973; Bliss and Collingridge, 1993; Abraham and Bear, 1996). For example, the duration of transient LTP induced at one set of synapses can be extended by capturing late LTP (L-LTP)-associated gene products from previous activity at other synapses on the same postsynaptic neurons (i.e., heterosynaptic facilitation) (Frey and Morris, 1997). Whereas L-LTP-stabilizing gene products may be distributed throughout the cell, it is proposed that they can be captured and used only at synapses that have been "tagged" by previous activity (Sossin, 1996; Frey and Morris, 1997; Barco et al., 2002; Martin and Kosik, 2002) [see also *Aplysia* work on long-term facilitation (Martin et al., 1997; Casadio et al., 1999)]. Because stimuli that are insufficient for inducing L-LTP on their own can nonetheless generate a synaptic tag, triggering L-LTP-associated transcription can result in a cell-wide decrease in the threshold for inducing long-term plasticity (Frey and Morris, 1997,1998b).

Unregulated facilitation of synaptic strength can saturate synaptic strength across neural networks of connections, thereby impeding the storage of new information (Abraham and Robins, 2005). Thus, mechanisms should exist to regulate synaptic tagging and limit L-LTP expression. Indeed, various patterns of synaptic activity can impair expression of subsequent L-LTP (Christie and Abraham, 1992; Huang et al., 1992; Fujii et al., 1996; Woo and Nguyen, 2002). However, it is unknown whether previous synaptic activity can inhibit subsequent L-LTP by suppressing synaptic tagging. In addition, inhibition of LTP by previous activity has typically been limited to the previously activated synapses (i.e., homosynaptic inhibition) (Christie and Abraham, 1992; Huang et al., 1992; Fujii et al., 1996; Woo and Nguyen, 2002). In contrast, for effective homeostatic regulation of synaptic weights, metaplastic changes should be expressed cell-wide and should occur for all synapses terminating on the affected neurons (Bienenstock et al., 1982; Abraham et al., 2001).

We report a novel form of heterosynaptic depression and cell-

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wide inhibition of subsequent L-LTP. Low-frequency stimulation (LFS) at 5 Hz for 3 min produces transient depression of field EPSPs (fEPSPs) in area CA1 of mouse hippocampal slices. Although this brief LFS did not persistently alter synaptic transmission, it decreased the stability of L-LTP induced later at the same synapses (Woo and Nguyen, 2002). We show here that these metaplastic effects of previous LFS also occur at synapses that did not contribute to postsynaptic activation (i.e., heterosynaptic inhibition). Heterosynaptic inhibition of L-LTP also occurred across basal and apical dendrites, showing that LFS regulates L-LTP in a cell-wide manner. Furthermore, our data indicate that LFS may selectively affect L-LTP, and not transient forms of LTP, by impairing synaptic tagging that would otherwise permit capture of somatic gene products required for stabilizing L-LTP. Our study reveals that the synapse specificity of L-LTP, and spatiotemporal integration of synaptic events over time, can be critically influenced at a cell-wide level by previous activity.

Materials and Methods

Hippocampal slice preparation. All experiments were conducted on female C57BL/6 mice (aged 10–14 weeks) (Charles River, Montreal, Quebec, Canada) housed at the University of Alberta Animal Care Facility. Care and experimental procedures were in accordance with guidelines approved by the Canadian Council on Animal Care. Animals were cervically dislocated and decapitated, and their brains were removed and immersed in ice-cold (4°C) artificial CSF (ACSF) bubbled with a "carbogen" mixture of 95% O_2 and 5% CO_2. The ionic composition of our ACSF was the same as in previous studies (Woo and Nguyen, 2003), consisting of the following (in mM): 124 NaCl, 4.4 KCl, 1.3 $MgSO_4 \cdot 7H_2O$, 1.0 $NaH_2PO_4 \cdot H_2O$, 26.2 $NaHCO_3$, 2.5 $CaCl_2$, and 10 D-glucose. The hippocampi were dissected free and transverse slices (400 μm thickness) were cut on a manual tissue chopper (Stoelting, Wood Dale, IL). Slices were transferred onto a nylon mesh in an interface chamber where they were perfused with carbogenized ACSF (1 ml/min). The temperature of the interface chamber was maintained at 28°C. Slices were allowed to recover for at least 60 min before experiments commenced.

Electrophysiology. Extracellular fEPSPs were recorded in the stratum radiatum (SR) of area CA1 with a glass microelectrode (A-M Systems, Carlsborg, WA) filled with ACSF (electrical resistances, 2–4 MΩ). Extracellular stimulation of the Schaeffer collateral pathway was accomplished with two nickel–chromium (A-M Systems) bipolar stimulating electrodes (diameter, 130 μm) placed on either side of a single recording electrode in the stratum radiatum (see Fig. 1*A*). Where indicated, one stimulating electrode was placed in the stratum oriens (SO), with a second stimulating electrode and a single recording electrode in the stratum radiatum (see Fig. 7*A*).

Evoked fEPSPs were amplified, digitized (DigiData 1200B Interface; Molecular Devices, Foster City, CA), and analyzed using Axon Clampex 7.0 (Molecular Devices). The test stimulus intensity was adjusted to produce "baseline" fEPSP sizes that were 40% of maximal evoked fEPSP amplitude (Grass S48 Stimulator). Test stimuli were delivered once per minute (0.08 ms stimulus duration) to the Schaeffer collaterals with a 200 ms separation between stimulation through the two electrodes (stimulating electrodes S1 and S2). To ensure that fEPSPs evoked through each stimulating electrode resulted from activation of two independent synaptic pathways, we positioned the electrodes so that no paired-pulse facilitation (PPF) was evident after sequential activation of S1 and S2. Interpathway PPF was assessed at various time intervals (40, 50, 75, 100, 150, and 200 ms) during baseline acquisition and at the end of experiments. Sample data from one experiment (i.e., one slice) are shown in Figure 1*B*.

LTP was elicited by delivering "weak" stimulation (one tetanic train; 1 s duration at 100 Hz) to induce early LTP (E-LTP) (Huang and Kandel, 1994) or "strong" stimulation (four tetanic trains; 1 s duration at 100 Hz; intertrain interval, 3 s) to induce L-LTP (Woo et al., 2003). LFS consisted of 5 Hz stimulation for 3 min (Woo and Nguyen, 2002). We used this same LFS to induce depotentiation (reversal of LTP) (Barrionuevo et al., 1980; Stäubli and Lynch, 1990; Woo and Nguyen, 2002).

Drugs. Actinomycin D (Act D) (BioShop Canada, Burlington, Ontario, Canada), a transcription inhibitor, was added to ACSF to a final concentration of 25 μM from 25 mM stock prepared in dimethylsulfoxide (DMSO) (Sigma, St. Louis, MO). At 25 μM, Act D has been shown to block transcription by >70% in hippocampal slices (Nguyen et al., 1994). All drugs were bath-applied. Experiments with actinomycin D were performed in dimmed light conditions. Final concentration of applied DMSO was 0.01%. At this concentration, baseline fEPSP slopes were not significantly affected (data not shown) (Woo and Nguyen, 2002, 2003).

Statistical analysis. In general, our data analysis followed procedures described by Woo and Nguyen (2003). The initial fEPSP slope was measured and expressed as a percentage of the averaged pretreatment baseline. The latter was obtained by averaging 20 min of fEPSPs measured during baseline acquisition. Data are plotted as mean ± SEM. Student's *t* test was used to compare mean fEPSP slopes within paired data sets, with a significance level of $p < 0.05$ (denoted on graphs with an asterisk). Data sets with more than two comparison groups were analyzed with ANOVA. Tukey–Kramer multiple comparisons test was completed if ANOVA analysis indicated a significant difference between groups ($p < 0.05$; denoted on graphs with an asterisk). Kolmogorov–Smirnov and Bartlett's tests were done to determine normality and SDs, respectively, of all test groups. Where indicated, the Kruskal–Wallis test (nonparametric ANOVA) was applied accordingly. In all electrophysiological data, *n* indicates number of slices.

Results

Transient homosynaptic and heterosynaptic depression evoked by low-frequency stimulation

Previous experiments demonstrated homosynaptic inhibition of subsequently induced L-LTP by 5 Hz stimulation for 3 min (Woo and Nguyen, 2002). To investigate heterosynaptic effects of 5 Hz stimulation, we made recordings using two stimulating electrodes (S1 and S2) positioned on either side of the recording electrode, all placed in the stratum radiatum of area CA1 in mouse hippocampal slices (Fig. 1*A*). Figure 1*B* shows sample traces and data from a representative experiment in which successive stimulation through S1 and S2 revealed no significant difference in the initial fEPSP slope in S2 at all time intervals tested (40–200 ms). A lack of interpathway paired-pulse facilitation thus confirmed the independence of the two inputs.

LFS at 5 Hz for 3 min produced a transient synaptic depression of fEPSPs in area CA1, both in the pathway receiving the conditioning stimulation [i.e., homosynaptic (Hom) inhibition; S1; 64 ± 4%; $n = 5$; $F_{(2,12)} = 29.381$; $p < 0.0001$] (Fig. 1*C*, time point b, open squares) and at other synapses converging on the same postsynaptic cells [i.e., heterosynaptic (Het) inhibition; S2; 69 ± 7%; $n = 5$; $F_{(2,12)} = 6.893$; $p = 0.0102$] (Fig. 1*C*, time point b, open circles). *Post hoc* tests revealed a significant depression of initial fEPSP slope, compared with baseline values, immediately after LFS [S1, Hom, $p < 0.001$ (Fig. 1*D*, open squares); S2, Het, $p < 0.05$ (time point b, open circles)]. Recovery to baseline values occurred within 10 min of LFS onset (S1, Hom, 106 ± 5%, $n = 5$, $p > 0.05$; S2, Het, 93 ± 3%, $n = 5$, $p > 0.05$) (Fig. 1*C*,*D*, time point c).

Previous LFS selectively impairs homosynaptic and heterosynaptic L-LTP but not E-LTP

Because LFS elicits both homosynaptic and heterosynaptic depression, we asked whether the inhibitory effects of previous LFS also occurred at nonstimulated inputs. A single tetanus of 100 Hz (1 s duration) induces transient E-LTP that decays within 1–2 h and requires covalent modification of existing proteins (Rey-

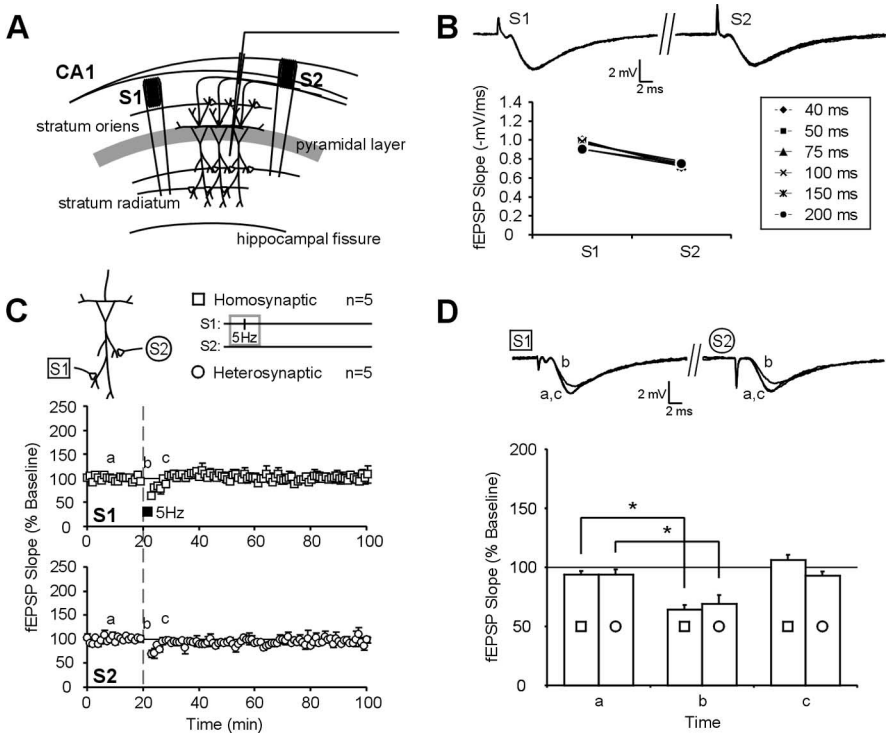

Figure 1. LFS produces transient homosynaptic and heterosynaptic depression. ***A***, Schematic of area CA1 in a mouse hippocampal slice, showing positions of two stimulating electrodes (S1 and S2) and a single recording electrode placed in the stratum radiatum. ***B***, Sample fEPSP responses and analysis of interpathway PPF from a representative experiment. A lack of PPF demonstrates the independence of S1 and S2 inputs. ***C***, LFS at 5 Hz for 3 min produced a transient depression in both homosynaptic (open squares) and heterosynaptic (open circles) pathways. ***D***, Sample fEPSP responses and comparison of mean fEPSP slopes from homosynaptic (open squares) and heterosynaptic (open circles) pathways during baseline (a), immediately after 5 Hz stimulation (b), and 10 min after onset of 5 Hz stimulation (c). *Statistical significance, $p < 0.05$. Error bars indicate SE.

mann et al., 1985; Huang and Kandel, 1994). To examine the effects of LFS on subsequently induced E-LTP, we applied 5 Hz stimulation to one pathway. Ten minutes after the beginning of LFS, a time point in which fEPSPs had recovered to baseline values (S1; 30 min; controls, 96 ± 4%, $n = 7$; Hom, 92 ± 2%, $n = 6$; Het, 95 ± 5%, $n = 6$; $F_{(2,16)} = 0.3350$; $p = 0.7203$) (Fig. 2*A*,*C*, time point b), one train of tetanus was given to either the pathway that had received LFS (i.e., homosynaptic) (open squares) or to a separate pathway (i.e., heterosynaptic) (open circles). Previous LFS did not affect the amount of potentiation observed 60 min after E-LTP induction, compared with controls that did not receive previous LFS (S1; 90 min; controls, 119 ± 5%, $n = 7$; Hom, 119 ± 6%, $n = 6$; Het, 116 ± 13%, $n = 6$; Kruskal-Wallis ANOVA, 0.2090; $p = 0.9008$) (Fig. 2*A*,*C*, time point c).

Multiple trains of 100 Hz stimulation induce L-LTP that requires transcription and *de novo* protein synthesis (Frey et al., 1988; Huang and Kandel, 1994; Nguyen et al., 1994). Again, we applied LFS to one set of inputs and then waited 10 min after LFS onset for fEPSP values to return to baseline values (S1; 30 min; controls, 99 ± 1%, $n = 12$; Hom, 94 ± 2%, $n = 7$; Het, 99 ± 3%, $n = 7$; $F_{(2,23)} = 1.659$; $p = 0.2123$) (Fig. 2*B*,*D*, time point b). Next, we stimulated either the pathway that had received LFS (i.e., homosynaptic) (open squares) or a separate pathway (i.e., heterosynaptic) (open circles) with four tetanic trains. Previous LFS significantly impaired L-LTP as measured 120 min after LTP induction (S1; 150 min; controls, 149 ± 7%, $n = 12$; Hom, 108 ± 7%, $n = 7$; Het, 97 ± 9%, $n = 7$; $F_{(2,23)} = 13.582$; $p < 0.001$) (Fig. 2*B*, time point c). *Post hoc* tests revealed a significant impairment of homosynaptic ($p < 0.01$) (open squares) and heterosynaptic ($p < 0.001$) (open circles) L-LTP compared with control slices that received L-LTP stimulus without previous LFS (Fig. 2*D*, time point c). Because previous LFS selectively impairs L-LTP, we hypothesized that LFS may be modulating processes required for the cellular consolidation of E-LTP to L-LTP.

Synaptic capture of L-LTP and immunity to depotentiation

According to the "synaptic tag" hypothesis, input-specific L-LTP expression requires (1) a signal to trigger transcription and cell-wide delivery of plasticity-related proteins (PRPs), and (2) a synaptic tag to mark activated synapses, thereby allowing capture of LTP-stabilizing PRPs (Sossin, 1996; Frey and Morris, 1997, 1998b; Barco et al., 2002; Martin and Kosik, 2002; Kelleher et al., 2004). To probe the mechanism by which previous LFS inhibits subsequent L-LTP, we modeled our protocol on previous experiments that examined synaptic tagging and L-LTP expression (Frey and Morris, 1997). E-LTP produced by a single 100 Hz train (weak stimulation) decays to baseline values within 2 h of induction (S2; 101 ± 6%; $n = 8$) (Fig. 3*A*, time point c, filled diamonds) and can be reversed to baseline values, or depotentiated (Dpt), by low-frequency stimulation applied 5 min after tetanus (S2; 60 min; Dpt, 97 ± 6%; $n = 5$) (Fig. 3*A*, time point b, open diamonds). Mean fEPSP slopes from Dpt slices did not differ significantly from time-matched mean values obtained in nondepotentiated slices (S2; 60 min; controls, 115 ± 9%; $n = 8$; $p > 0.05$) (Fig. 3*A*, time point b, filled diamonds). Pairing one tetanic train (weak stimulation) in S2 with four tetanic trains (strong stimulation) at an independent set of inputs (S1) results in nondecremental LTP of the weak S2 pathway, although it received only E-LTP-inducing stimuli (S2; 120 min; 158 ± 9%; $n = 8$) (Fig. 3*B*, time point c, filled circles). In addition to its extended duration, LTP in S2 resembles L-LTP in its immunity to depotentiation (Woo and Nguyen, 2003). Mean fEPSP slopes recovered to potentiated levels within 55 min of depotentiating LFS (S2; 60 min; 139 ± 9%; $n = 7$) (Fig. 3*B*, time point b, open circles), and they did not differ significantly from nondepotentiated controls (S2; 60 min; 144 ± 10%; $n = 9$; $p > 0.05$) (Fig. 3*B*,*C*, time point b, filled circles).

These data show that pairing strong (four-train) with weak (one-train) LTP stimulation results in L-LTP expression at both sets of synaptic inputs. Successful synaptic capture by one-train stimulation at S2 is evident as a persistent potentiation that is immune to depotentiation. More importantly, for our later experiments, expression of this stable L-LTP, and its immunity to depotentiation, can thus be used to gauge successful synaptic tagging and capture of L-LTP.

A critical period of transcription is needed for synaptic capture of L-LTP

Late-phase LTP induction triggers transcription and translation of plasticity-related proteins, which can be captured at many syn-

apses (Frey and Morris, 1997; Barco et al., 2002; Kelleher et al., 2004). Because previous LFS impairs L-LTP in a cell-wide manner, we hypothesized that it may be inhibiting transcription. We began by using a transcriptional inhibitor, Act D (25 μM), to examine the effects of blocking transcription on synaptic capture of L-LTP. This concentration has previously been shown to block transcription by >70% in hippocampal slices (Nguyen et al., 1994). Strong tetanization (S1 pathway) in the presence of Act D produced LTP that decayed to baseline values within 2 h (S1; 120 min; 104 ± 7%; $n = 6$). Weak tetanic stimulation of an independent pathway 30 min after strong tetanization of S1 also produced decremental LTP (S2; 120 min; 100 ± 6%; $n = 6$) (Fig. 4*A*, time point c, filled diamonds). In contrast, application of Act D during the weak LTP tetanus in S2, but after strong tetani in S1, did not affect L-LTP expression in either pathway (S1, 120 min, 153 ± 10%, $n = 6$; S2, 142 ± 4%, $n = 6$) (Fig. 4*B*, time point c, filled circles). These results define a critical time period for triggering transcription by strong tetanic stimulation, from immediately after L-LTP induction to 20 min after tetanus. Our data mirror previous findings indicating a specific time window for transcription in L-LTP expression (Nguyen et al., 1994).

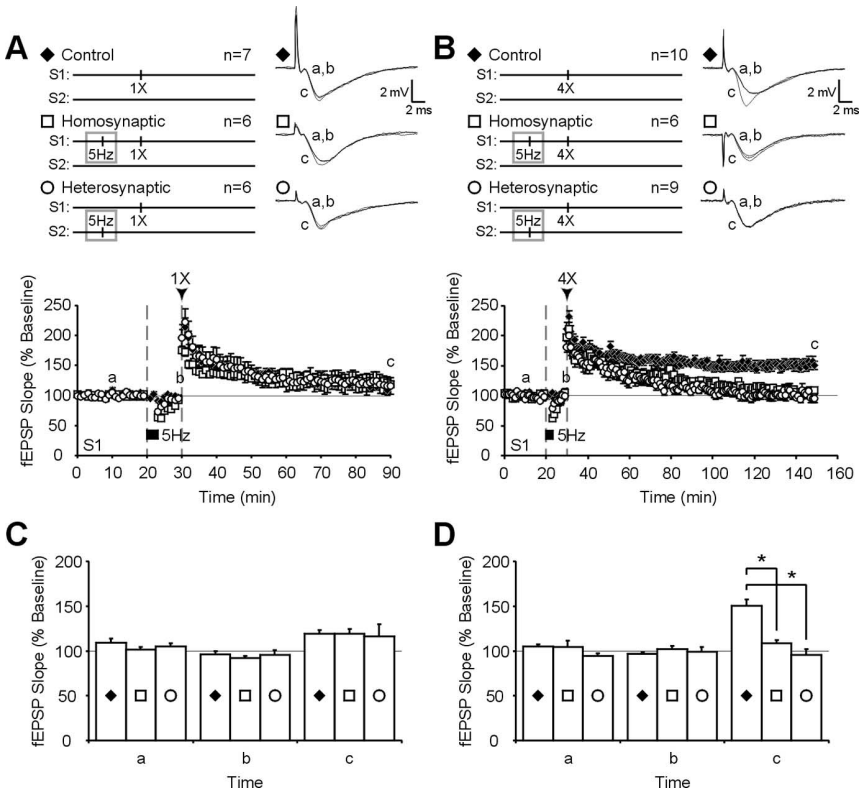

Figure 2. Previous LFS impairs subsequent homosynaptically and heterosynaptically induced L-LTP. ***A***, LFS did not affect subsequent E-LTP elicited by a single 100 Hz tetanus in the homosynaptic (open squares) or heterosynaptic (open circles) pathway (control, filled diamonds). ***B***, LFS impaired subsequent L-LTP induced with four 100 Hz trains applied to the homosynaptic (open squares) or heterosynaptic (open circles) set of inputs (control, filled diamonds). ***C***, Summary of LFS effects (homosynaptic, open squares; heterosynaptic, open circles) on one-train E-LTP (control, filled diamonds). Columns represent mean fEPSP slopes during baseline (a), 10 min after onset of LFS (b), and 60 min after LTP induction (c). ***D***, Summary of LFS effects (homosynaptic, open squares; heterosynaptic, open circles) on four-train L-LTP (control, filled diamonds). Mean fEPSP slopes are shown from baseline (a), 10 min after onset of LFS (b), and 120 min after L-LTP induction (c). *Statistical significance, $p < 0.05$. Error bars indicate SE.

Our results support the synaptic tag model in which L-LTP that is captured by weak tetanic stimulation does not depend on activating transcription. The duration of transient E-LTP induced at one set of synapses is extended by capturing L-LTP-associated gene products produced by previous synaptic activity at other synapses on the same postsynaptic neurons. The experiments of Figure 4*B* show that transcription is not required in the synaptic tagging process, and they also show that Act D application does not result in a general run-down of LTP under these conditions.

Previous LFS does not affect somatic gene expression associated with L-LTP expression

Pairing strong and weak tetanization allows for the dissociation of metaplastic effects on somatic gene expression versus effects on local synaptic tagging. If LFS inhibits L-LTP by impairing transcription, then synaptic capture by subsequent weak LTP stimuli should be unsuccessful because there will be no L-LTP gene products for activated synapses to capture. In other words, application of LFS before strong tetanization should exert effects that mirror those seen by applying Act D during strong tetanization. Like Act D application during strong tetanization, LFS applied homosynaptically (i.e., to S1) (filled diamonds) or heterosynaptically (i.e., to S2) (filled squares) before strong tetanus in S1, effectively blocked L-LTP expression in S1 (S1; 120 min; Hom, 103 ± 6%, $n = 6$; Het, 118 ± 7%, $n = 6$) (Fig. 4*C*,*D*). However, unlike the results observed with Act D application, weak tetanus to S2 given 30 min later (i.e., 40 min after start of LFS) elicited stable and persistent potentiation (S2; 120 min; Hom, 146 ± 13%, $n = 6$; Het, 142 ± 5%, $n = 6$) (Fig. 4*C*,*D*, time point c). In addition, LTP of S2 inputs acquired immunity to depotentiation (Fig. 4*C*,*D*,*F*, time point b). ANOVA revealed significant differences between levels of potentiation elicited by weak LTP stimulation (S2) with delayed Act D or previous LFS application, compared with Act D application during initial strong tetanus ($F_{(3,20)} = 6.586$; $p = 0.0028$). *Post hoc* tests indicate that significantly more potentiation was seen with delayed Act D application ($p < 0.05$) (filled circles) and both homosynaptic ($p < 0.01$) (filled diamonds) and heterosynaptic ($p < 0.05$) (filled squares) LFS, than with Act D application during strong tetanus (Fig. 4*E*, filled triangles). Student's *t* tests confirmed that weak tetanus applied to S2 elicited LTP that acquired immunity to depotentiation despite homosynaptic (S2; 60 min; controls, 143 ± 8%, $n = 6$; Dpt, 139 ± 4%, $n = 6$; $p > 0.05$) (Fig. 4*C*,*F*, time point b, diamonds) or heterosynaptic (S2; 60 min; controls, 139 ± 7%, $n = 6$; Dpt, 138 ± 6%, $n = 6$; $p > 0.05$) (Fig. 4*D*,*F*, time point b, squares) inhibition of L-LTP in S1 by previous LFS.

Contrary to our original hypothesis, metaplastic effects of LFS are not mediated by inhibition of L-LTP-associated gene expression. Thus, we hypothesized that LFS may be interfering with synaptic tagging.

Previous LFS impairs synaptic capture of L-LTP expression and acquired immunity to depotentiation

Does LFS affect synaptic tagging? We first addressed this hypothesis by pairing strong (S1) with weak (S2) stimulation. If previous

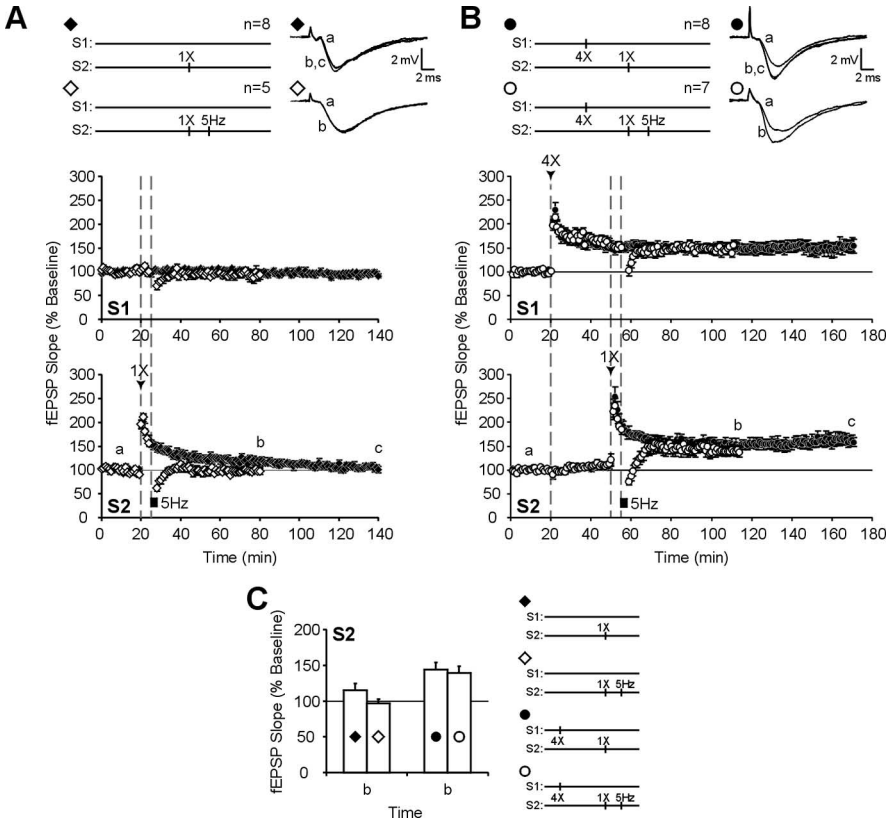

Figure 3. Successful synaptic capture of L-LTP by weak LTP stimuli can be assayed by prolonged potentiation and a newly acquired immunity to depotentiation. ***A***, E-LTP induced by one-train (weak) tetanus (S2) is input specific and decays to baseline within 120 min of induction (c, filled diamonds). E-LTP is sensitive to depotentiation and can be reversed to baseline values by LFS (b, open diamonds). ***B***, When four-train (strong) tetanus is first established at one set of inputs (S1), one-train (weak) tetanus to S2 elicits potentiation that is persistent and stable at 120 min after tetanus (c, filled circles). The potentiation elicited by one-train tetanus (S2) is now resistant to depotentiation; after Dpt LFS, mean fEPSP slopes gradually recovered to potentiated levels (b, open circles). ***C***, Summary of depotentiation data. Mean fEPSP slopes 55 min after depotentiation (open symbols) are compared with nondepotentiated controls (filled symbols) of both tetanus protocol groups (one train alone, diamonds; four train plus one train, circles). Error bars indicate SE. a, Pretetanus baseline.

LFS impairs tagging of activated synapses, then LFS given just before weak tetanus in S2 (and after L-LTP stimuli in S1) should prevent S2 from capturing L-LTP expression. Indeed, we found that LFS applied homosynaptically (i.e., to S2) (Fig. 5*A*, filled squares) or heterosynaptically (i.e., to S1) (Fig. 5*B*, filled triangles) before weak tetanus impaired L-LTP expression in S2. Mean fEPSP slopes decayed close to baseline by 120 min after LTP induction in S2 when LFS was applied homosynaptically (i.e., to S2) before weak S2 stimulation (S2; 120 min; 109 ± 9%; $n = 7$) (Fig. 5*A*, time point c, filled squares). Heterosynaptic LFS (i.e., to S1) before weak S2 stimulation also impaired LTP expression in S2 (S2; 120 min; 129 ± 7%; $n = 10$) (Fig. 5*B*, time point c, filled triangles). Although heterosynaptic inhibition of LTP in S2 was not as dramatic as homosynaptic inhibition, statistical analysis demonstrated that both homosynaptic and heterosynaptic effects of LFS on LTP expression in S2 were significant. ANOVA revealed a significant difference ($F_{(3,39)} = 10.542$; $p < 0.0001$) in mean fEPSP slopes in S2 from LFS-treated slices compared with slices that received weak tetanus alone (S2; 120 min; 101 ± 6%; $n = 8$) and slices that received strong plus weak tetanus without previous LFS (S2; 120 min; 158 ± 9%; $n = 8$). *Post hoc* tests showed that LTP elicited by weak tetanus alone ($p < 0.001$) (Fig. 5*C*, filled diamonds), or paired strong plus weak tetanus with LFS [homosynaptic, $p < 0.001$ (filled squares); heterosynaptic, $p < 0.05$ (filled triangles)] were significantly lower than LTP from paired strong plus weak tetanus without previous LFS (filled circles). There was no significant difference ($p > 0.05$) between levels of potentiation elicited by weak tetanus alone (filled diamonds) and in either LFS-treated groups [homosynaptic (Fig. 5*C*, filled squares); heterosynaptic (filled triangles)]. These data support the hypothesis that homosynaptic and heterosynaptic application of LFS impairs synaptic tagging, and capture of L-LTP expression.

However, depotentiation experiments highlighted an important difference between homosynaptic and heterosynaptic application of LFS within the strong plus weak stimulation protocol. Homosynaptic application of LFS before weak tetanus impaired LTP expression in S2 (Fig. 5*A*, filled squares). LTP in S2 could be depotentiated to baseline levels (S2; 60 min; 104 ± 6%; $n = 6$) (Fig. 5*A*, time point b, open squares) and were comparable with non-Dpt controls (S2; 60 min; 117 ± 6%; $n = 6$; $p > 0.05$) (Fig. 5*A*,*D*, time point b, filled squares). These results are consistent with homosynaptic inhibition of synaptic tagging, and capture of L-LTP expression. Heterosynaptic application of LFS before weak tetanus also significantly impaired LTP expression in S2 (Fig. 5*B*, filled triangles). However, mean fEPSP slopes in S2 recovered after depotentiation (S2; 60 min; 133 ± 11%; $n = 8$) (Fig. 5*B*, time point b, open triangles) and stabilized at levels that were comparable with non-Dpt controls (S2; 60 min; 130 ± 6%; $n = 10$; $p > 0.05$) (Fig. 5*B*,*D*, time point b, filled triangles). The LTP and depotentiation results suggest that, although synaptic tagging and capture are significantly impaired by heterosynaptic application of LFS in this protocol, the amount of inhibition was not as substantial as with homosynaptic application of LFS. This may have resulted from differences in the state of inputs to which LFS was applied. With heterosynaptic application of LFS (i.e., to S1) within the strong (S1) plus weak (S2) stimulation protocol, LFS was applied to already potentiated inputs, because L-LTP induction in S1 has already occurred (i.e., LFS equals depotentiation) (Fig. 5*B*). In contrast, with homosynaptic application of LFS (i.e., to S2) within the strong (S1) plus weak (S2) stimulation protocol (Fig. 5*A*), LFS was applied to "naive" inputs. Indeed, many studies have shown that LFS can recruit different signaling cascades depending on the initial state of the stimulated synapses (i.e., naive vs potentiated) (Katsuki et al., 1997; Lee et al., 2000; Jouvenceau et al., 2003).

Therefore, we tested a second model of synaptic capture that would allow for heterosynaptic application of LFS to naive inputs. Frey and Morris (1998a) first showed that synaptic capture could also occur when the order of strong plus weak LTP stimuli is reversed. When weak tetanus (S1) is applied first, synaptic capture in S1 can occur provided that L-LTP is induced by strong tetanus (S2) within the lifetime of the synaptic tag in S1 (Frey and Morris, 1998a; Sajikumar and Frey, 2004a). We applied one-train

(weak) tetanus to S1 followed by four-train (strong) tetanus to S2. LTP in S1 was stable and persistent 120 min after induction (S1; 120 min; 144 ± 7%; $n = 6$) (Fig. 5*E*, time point b, filled inverted triangles). Heterosynaptic application of LFS (i.e., to S2) before weak tetanus within the weak (S1) plus strong (S2) stimulation protocol significantly impaired LTP expression in S1 (S1; 120 min; 104 ± 11%; $n = 6$; $p < 0.01$) (Fig. 5*E*, time point b, open pentagons). These results show that LFS can heterosynaptically impair synaptic tagging.

Our results demonstrate that previous LFS homosynaptically and heterosynaptically impairs synaptic tagging, and capture of L-LTP expression. Our data also reveal a limited time window and temporal direction in which LFS can inhibit synaptic capture. LFS applied 10 min (Fig. 5*A*,*B*,*E*), but not 40 min (Fig. 4*C*,*D*), before weak tetanus significantly impaired synaptic capture of L-LTP expression. Previous work showed that anterograde homosynaptic inhibition of L-LTP by previous LFS is effective when LFS is applied 20 min, but not 40 min, before L-LTP induction (Woo and Nguyen, 2002). Significantly, LFS did not affect L-LTP expression when applied 20 min after strong tetanus (S1; 120 min; Hom, relative strong tetanus, 136 ± 15%, $n = 11$; Het, relative strong tetanus, 148 ± 19%, $n = 7$) (Fig. 5*A*,*B*). This suggests that there is a limited time window during which the newly set synaptic tag is sensitive to disruption. Indeed, retrograde "resetting" of synaptic tags set by weak E-LTP-inducing stimulation is ineffective when LFS is applied beyond 5 min after tetanus (Sajikumar and Frey, 2004a).

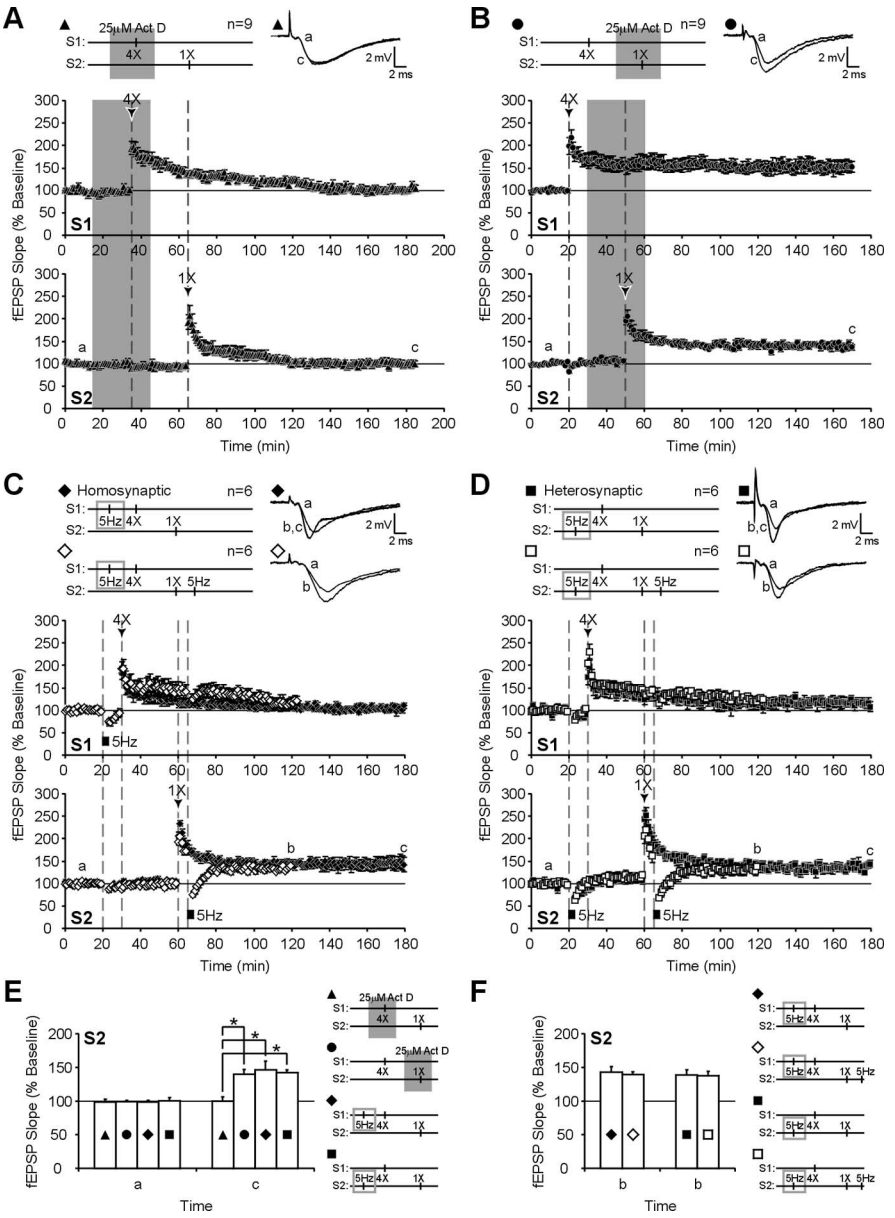

Figure 4. Previous LFS does not affect transcription associated with L-LTP expression. Four-train (strong) tetanus was applied to S1 followed by one-train (weak) tetanus to S2. ***A***, Application of Act D (25 μM) during four-train L-LTP induction prevented L-LTP expression in both S1 and S2 pathways (filled triangles). ***B***, Act D applied during one-train tetanus (after four-train tetanus), did not affect L-LTP expression in either pathway (filled circles). ***C***, ***D***, LFS applied before four-train tetanus impaired L-LTP expression in S1 but did not affect L-LTP expression by one-train tetanus in S2. LFS was applied to the homosynaptic (diamonds) and heterosynaptic (squares) pathway, relative to four-train tetanus. One-train tetanus (S2) elicited stable potentiation (filled symbols) that was immune to depotentiation (open symbols). ***E***, Summary of four-train plus one-train LTP data from the S2 pathway of all four treatment groups. Mean fEPSP slopes are taken during baseline (a) and 120 min after one-train tetanus (c). ***F***, Summary of depotentiation data from LFS-treated groups, 60 min after one-train tetanus (b). Mean fEPSP slopes in S2 after depotentiation (open symbols) are compared with non-Dpt controls (filled symbols), within treatment groups (homosynaptic, diamonds; heterosynaptic, squares). *Statistical significance, $p < 0.05$. Error bars indicate SE.

Previous LFS does not affect dendritic protein synthesis that confers local immunity to depotentiation

As demonstrated previously (Fig. 3*B*) and in previously published accounts (Barco et al., 2002; Woo and Nguyen, 2003), cell-wide distribution of L-LTP-transcriptional products can provide immunity to depotentiation. However, strong depolarization, such as that associated with L-LTP tetani, can also trigger dendritic protein synthesis independent of transcription, to confer immediate, synaptically localized immunity to depotentiation (Ouyang et al., 1999; Woo and Nguyen, 2003). Our experiments showed that previous LFS can impair synaptic capture of immunity to depotentiation that requires transcription. Do these effects extend to depotentiation mediated by local protein synthesis? After LFS conditioning to one set of inputs, L-LTP tetani were given to either the same (homosynaptic) (open squares) or a separate (heterosynaptic) (open circles) pathway. Five minutes after LTP induction, depotentiating LFS was given to the tetanized pathway (Fig. 6*A*). We found that previous LFS did not affect the resistance of L-LTP to depotentiation (60 min; Hom, 131 ± 14%, $n = 6$; Het, 133 ± 9%, $n = 6$) (Fig. 6*A*, time point b). Both groups recovered to mean potentiated values that were comparable with control slices that did not receive LFS before L-LTP induction (60 min; controls; 138 ± 10%; $n = 6$; $F_{(2,14)} = 0.1395$; $p = 0.8709$) (Fig. 6*A*,*B*, time point

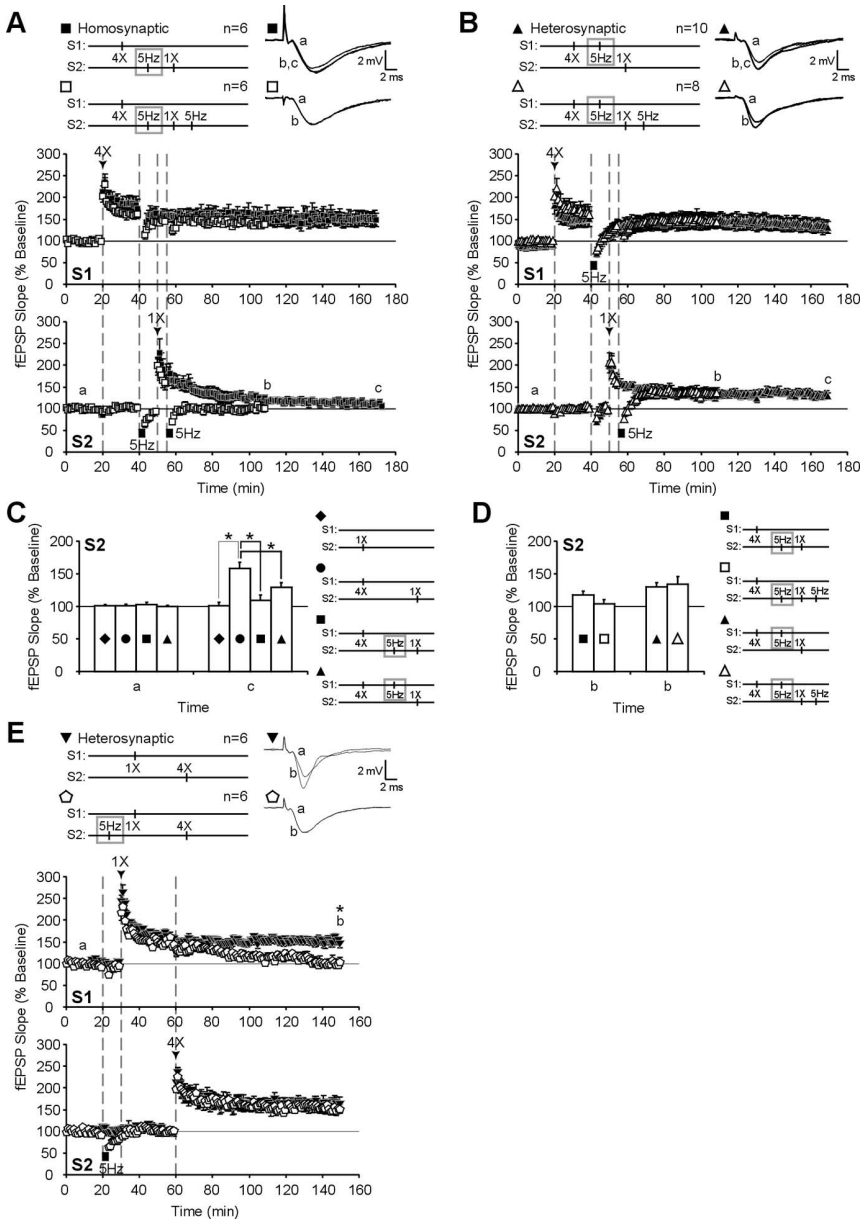

Figure 5. Previous LFS impairs synaptic capture of L-LTP expression and acquired immunity to depotentiation. ***A***, ***B***, Four-train (strong) tetanus was applied to S1 followed by one-train (weak) tetanus to S2. LFS was given before one-train tetanus in S2. ***A***, Homosynaptic application of LFS (S2, naive pathway) before one-train tetanus impaired LTP expression in S2 (filled squares). LTP in S2 depotentiated to baseline levels (open squares). ***B***, Heterosynaptic application of LFS (S1, potentiated pathway) before one-train tetanus impaired LTP expression in S2 (filled triangles), although mean fEPSP slopes remained elevated above baseline values. LTP in S2 recovered to potentiated levels after depotentiation (open triangles). ***C***, Comparison of mean fEPSP slopes in S2 during baseline (a) and 120 min after one-train tetanus (c): one-train alone (filled diamonds), one train when paired with four trains (filled circles), and four train plus one train with LFS before one train (homosynaptic, filled squares; heterosynaptic, filled triangles). LFS significantly impaired L-LTP expression in S2 when applied homosynaptically (filled squares) or heterosynaptically (filled triangles) before one-train tetanus. Mean fEPSP slopes from LFS-treated groups did not differ significantly from one-train controls. ***D***, Summary of depotentiation data from LFS-treated groups, 60 min after one-train tetanus (b). Mean fEPSP slopes in S2 after depotentiation (open symbols) are compared with non-Dpt controls (filled symbols), within treatment groups (homosynaptic, squares; heterosynaptic, triangles). ***E***, One train of tetanus was applied to S1 followed by four trains of tetani to S2. L-LTP is expressed in both pathways (filled inverted triangles). LFS applied heterosynaptically (S2, naive pathway) relative to one-train tetanus in S1, significantly impaired L-LTP expression in S1 (open pentagons). Mean fEPSP values were at baseline levels 120 min after tetanus (b). *Statistical significance, $p < 0.05$. Error bars indicate SE.

b). Because LFS impairs synaptic capture of immunity to depotentiation, these results suggest that the dendritic translation that underlies local synaptic immunity to depotentiation (Woo and Nguyen, 2003) is unaffected by previous LFS.

Heterodendritic inhibition of L-LTP by previous LFS

One theory of metaplasticity predicts that metaplastic effects should be expressed in a cell-wide manner to effectively regulate synaptic plasticity (Bienenstock et al., 1982). Previously, we showed that LFS impairs L-LTP in a heterosynaptic, non-input-specific manner (Fig. 2*B*). However, as both stimulating electrodes were placed in the stratum radiatum, metaplasticity effects of LFS may be limited to the apical dendrites of CA1 neurons. To determine whether the inhibitory effects of LFS on L-LTP are cell-wide, we examined whether LFS applied to Schaeffer collaterals in the stratum oriens could impair L-LTP that was subsequently induced in the stratum radiatum (i.e., heterodendritic inhibition). We made recordings using two stimulating electrodes (one positioned in SO and the other in SR) placed on either side of the recording electrode in the stratum radiatum (Fig. 7*A*). Low-frequency stimulation applied to SO at 5 Hz for 3 min produced a transient synaptic depression in both SO (70 ± 8%; $n = 8$) and SR (79 ± 4%; $n = 8$). Mean fEPSP slopes of both pathways recovered to baseline values within 10 min of LFS onset (SO, 99 ± 6%, $n = 8$; SR, 100 ± 3%, $n = 8$) (Fig. 7*B*, time point b), at which point four-train tetanus was applied to SR to induce LTP. Previous LFS to SO significantly impaired L-LTP expression in SR (120 min; SR; 107 ± 6%; $n = 8$) when compared with control slices that did not receive LFS before four-train tetanus (120 min; SR; 148 ± 6%; $n = 8$; $p < 0.001$). These results show that L-LTP expression can be regulated in a cell-wide manner by previous synaptic activity.

Discussion

Our findings have revealed a novel form of cell-wide metaplasticity that regulates the expression of long-lasting LTP. Late LTP, but not E-LTP, was impaired by previous LFS at 5 Hz. Homosynaptic and heterosynaptic inhibition by previous LFS occurred in a time-dependent manner and did not affect previously established L-LTP. Our results show that LFS impairs subsequent L-LTP, not by blocking transcription, but by inhibiting synaptic tagging of activated inputs that would otherwise allow for input-specific capture of L-LTP-stabilizing gene products. Previous LFS also blocked transcription-dependent, acquired immunity of L-LTP to depotentiation. In contrast, depotentiation immunity arising from local dendritic translation was unaffected by previous LFS.

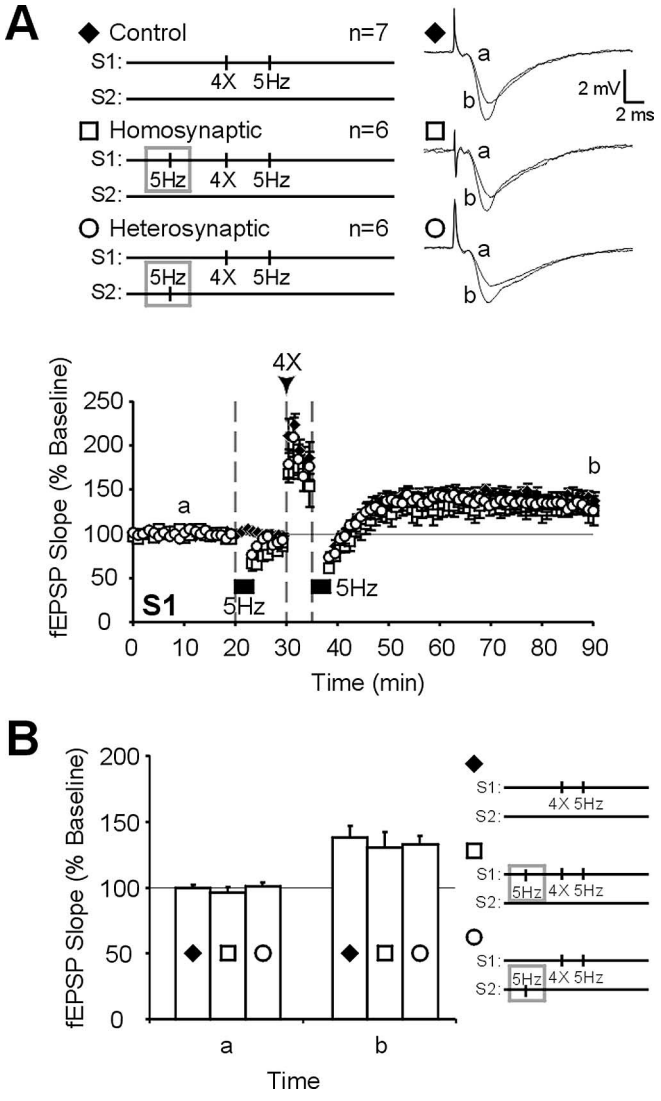

Figure 6. Immunity of four-train L-LTP to depotentiation is not affected by previous LFS. ***A***, Four-train tetanus elicits L-LTP that is immune to depotentiation (filled diamonds). LFS applied homosynaptically (open squares) or heterosynaptically (open circles) did not affect the immunity of four-train LTP to depotentiation. ***B***, Comparison of mean fEPSP slopes during baseline (a) and 55 min after depotentiating LFS (b). Mean fEPSP slopes in slices that received LFS before four-train tetanus (homosynaptic, open squares; heterosynaptic, open circles) recovered after depotentiation to levels that were comparable with slices that did not receive previous LFS (filled diamonds). Error bars indicate SE.

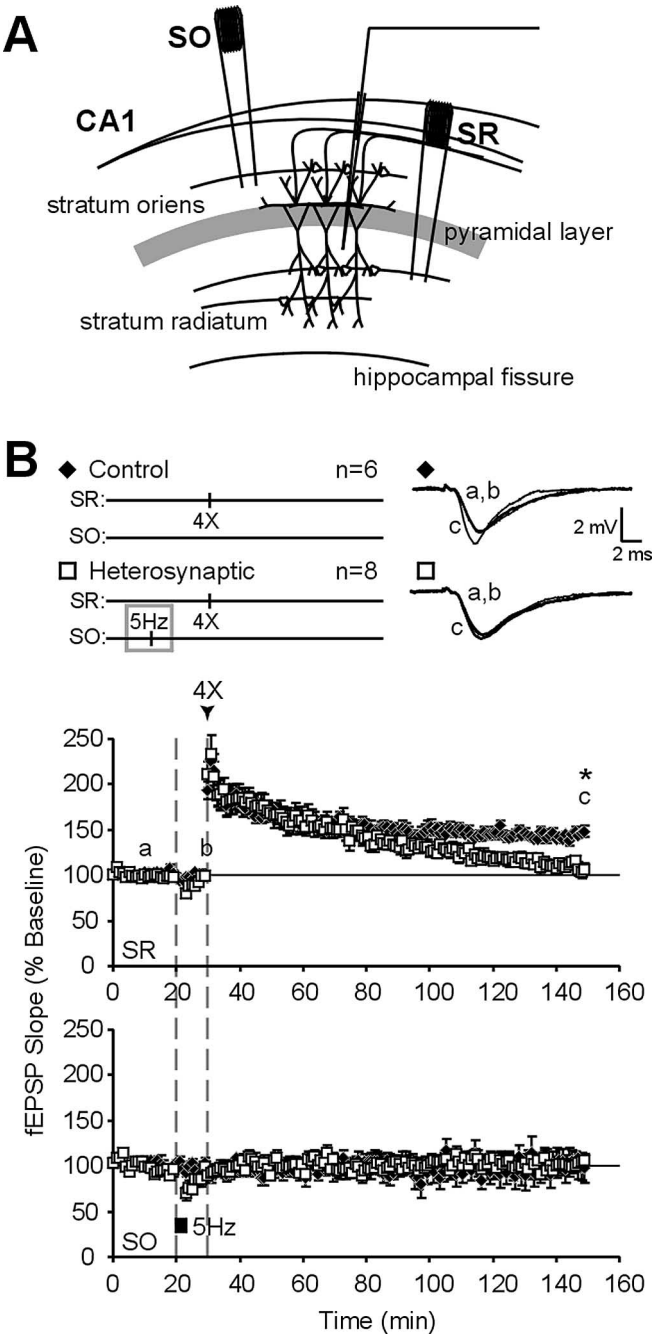

Figure 7. LFS impairs subsequently induced L-LTP in a heterodendritic manner. ***A***, Schematic of area CA1 in a mouse hippocampal slice, showing positions of two stimulating electrodes (one in SO and one in SR) and a single recording electrode placed in stratum radiatum. ***B***, LFS in SO impaired subsequent L-LTP in SR (open squares). LFS applied to SO elicited a transient synaptic depression in both pathways. Mean fEPSP slopes recovered to baseline values (a) within 10 min of LFS onset (b). Four tetanus trains applied to SR after recovery elicited a transient potentiation that decayed close to baseline values by 120 min after tetanus (c). Mean fEPSP slopes in SR were significantly lower than those in control slices that did not receive LFS before four-train tetanus (filled diamonds). *Statistical significance, $p < 0.05$. Error bars indicate SE.

LFS regulation of synaptic tagging and capture of L-LTP gene products

The synaptic tag hypothesis proposes that input-specific L-LTP expression requires (1) gene expression and cell-wide distribution of newly synthesized PRPs and (2) an activity-dependent synaptic tag that allows for input-specific capture of LTP-stabilizing proteins (supplemental Fig. 1*B*, available at www.jneurosci.org as supplemental material) (Sossin, 1996; Frey and Morris, 1998b). Once transcription and protein synthesis have been triggered by appropriate synaptic activity at a subset of inputs, a period of cell-wide, reduced threshold for L-LTP ensues. Thus, weak tetanic stimulation that normally produces E-LTP can elicit L-LTP by capturing PRPs from L-LTP induced (with strong tetanus) at separate inputs (Figs. 3*B*, 5*E*) (supplemental Fig. 1*C*, available at www.jneurosci.org as supplemental material) (Frey and Morris, 1997, 1998b). Because previous LFS impairs L-LTP in a cell-wide manner, we hypothesized that LFS may be inhibiting transcription.

We began by characterizing the role of transcription in synaptic capture using the strong (S1) plus weak (S2) tetanus protocol. Application of a transcriptional inhibitor, Act D, during strong tetanus (S1) prevented L-LTP expression at those inputs. Weak tetanus applied to an independent pathway (S2) 30 min later produced transient potentiation, presumably because there were no LTP-stabilizing gene products for those synapses to capture (Fig. 4*A*) (supplemental Fig. 1*D*, available at www.jneurosci.org as supplemental material). Like Act D, application of LFS (S1, homosynaptic; S2, heterosynaptic) before strong tetanus (S1) also impaired L-LTP in the S1 pathway, but subsequent weak tetanus to the S2 pathway remained successful in capturing L-LTP that was immune to depotentiation (Fig. 4*C*,*D*). Because the lowered threshold for L-LTP remains intact, these results suggest that LFS does not affect L-LTP-associated transcription but, instead, impairs the ability of activated synapses to generate a synaptic tag and capture LTP-stabilizing PRPs (supplemental Fig. 1*E*, available at www.jneurosci.org as supplemental material). Consistent with this idea, LFS applied homosynaptically or heterosynaptically before weak tetanus prevented synaptic capture of L-LTP expression from separate inputs that received strong tetanus (Fig. 5*A–E*) (supplemental Fig. 1*F*, available at www.jneurosci.org as supplemental material).

Synaptic capture of L-LTP encompasses many different processes, including the distribution of plasticity-related gene products, generation of the synaptic tag, and capture of PRPs at tagged synapses (for review, see Martin and Kosik, 2002) [see also *Aplysia* work on long-term facilitation (Martin et al., 1997; Casadio et al., 1999)]. Previous studies show that a synaptic tag can last as long as 1–2 h after synaptic activation (Frey and Morris, 1997, 1998a). In contrast, LFS impairs L-LTP expression when applied 10 min (Fig. 5*A*,*B*,*E*), but not 40 min, before L-LTP induction (Fig. 4*C*,*D*). If LFS acted on PRP distribution or capture processes, and not on synaptic tagging, the synaptic tag should outlive the metaplastic effects of LFS on PRP distribution or capture processes. Hence, no L-LTP deficits would be apparent because the intact synaptic tag should still be available to capture L-LTP expression once inhibition of PRP distribution/capture ceased after 40 min. Thus, our results suggest that LFS likely impairs L-LTP expression by preventing formation of a synaptic tag during a critical time window that extends up to 40 min after LTP induction.

Our data also highlight the stability of synaptic tags arising from different patterns of synaptic activation. After weak (E-LTP) stimulation, there is a 5 min window during which the newly generated synaptic tags can be reset by LFS, and inducing L-LTP subsequently at separate inputs will not enhance LTP in the reset pathway (Sajikumar and Frey, 2004a). We show that this window of instability can be eliminated if successful synaptic capture occurs. Once L-LTP has been induced at one set of inputs, subsequent weak tetanus can capture L-LTP expression and stabilize the synaptic tag such that LFS applied 5 min later (i.e., depotentiation) does not persistently reverse L-LTP expression (Figs. 3*B*, 4*C*,*D*). Thus, our results support the rapid generation of a synaptic tag, and capture of preexisting PRPs, within minutes of synaptic activation.

A transient form of LTP that is immune to depotentiation

With strong tetanic stimulation, both somatic gene expression and local protein synthesis act to stabilize L-LTP expression by conferring immunity to depotentiation (Woo and Nguyen, 2003). Strong tetanus applied after LFS elicits LTP that decays to baseline with a similar time course as E-LTP induced with weak tetanic stimulation (within 1–2 h) (Fig. 2*B*). However, whereas E-LTP reverses to baseline after depotentiating stimuli, LTP induced with LFS plus strong tetanus remains resistant to depotentiation (Figs. 3*A*, 6*A*). Immunity to depotentiation may enable a cell to distinguish between synaptic changes resulting from repeated episodes of synaptic activity versus a single isolated episode, such as that used to induce E-LTP (Zhou and Poo, 2004). Thus, the prevailing depotentiation immunity of transient L-LTP after LFS may allow those inputs to retain the experience of multiple episodes of synaptic activation, even if those episodes do not elicit long-lasting LTP. In addition, depotentiation of E-LTP resets synaptic tags and synaptic strengths to baseline values (Fig. 3*A*) such that subsequent LTP can be readily induced in those inputs (Sajikumar and Frey, 2004a). In contrast, L-LTP induced with strong tetanic stimulation does not readily depotentiate, and repeated induction of L-LTP at the same set of inputs requires several hours between episodes for recovery of functional plasticity (Fig. 6*A*) (Frey et al., 1995; Woo and Nguyen, 2003). This continued resistance to erasure may be important in determining the capacity for inducing subsequent LTP at those inputs. Therefore, additional potentiation may be limited at synapses with decaying L-LTP (i.e., LFS plus strong tetanus) until the inhibitory effects on synaptic tagging wear off and synaptic strength returns to less potentiated levels.

Homosynaptic and heterosynaptic depression

What are the mechanisms underlying heterosynaptic regulation of L-LTP? In the present study, metaplastic effects are associated with a novel form of heterosynaptic synaptic depression. Stent (1973) proposed that synaptic connections would weaken when they are inactive at the same time that a postsynaptic neuron is active (i.e., heterosynaptic depression). Interestingly, stimulation of Schaeffer collaterals at the 5 Hz (theta) frequency can induce complex spike-like bursts of action potentials in CA1 neurons (Thomas et al., 1998). Backpropagation of these action potentials into apical and basilar dendrites may activate signaling cascades independent of synaptic activation at these dendrites. Indeed, heterosynaptic depression exists at synapses "silent" during strong postsynaptic depolarization associated with LTP induction (Lynch et al., 1977; Alger et al., 1978; Kerr and Abraham, 1993; Holland and Wagner, 1998; Wang and Wagner, 1999; Abraham et al., 2001). Voltage-gated calcium channels and intracellular calcium stores have been implicated in conveying heterosynaptically generated signals to silent synapses (Wickens and Abraham, 1991; Artola and Singer, 1993; Nishiyama et al., 2000), and similar mechanisms may underlie LFS-mediated heterosynaptic metaplasticity. Various second messenger systems have been proposed to detect, and translate, the moderate changes in Ca^{2+} associated with nonassociative depression, into changes in synaptic efficacy. Protein phosphatases are preferentially activated by low-frequency stimulation (Mulkey et al., 1993, 1994; Morishita et al., 2001), and significantly, LFS has been shown to engage protein phosphatase-1 and -2A during homosynaptic anterograde inhibition of L-LTP (Woo and Nguyen, 2002).

Functional significance of metaplasticity and synaptic tagging

In contrast to the temporal summation of synaptic potentials that underlie associative LTP (in milliseconds), metaplastic effects of

LFS regulate the integration of synaptic events over much longer periods of time (in minutes). Activity-dependent changes in gene expression (e.g., with L-LTP induction) may prime synapses of a neuron to form lasting associations with many inputs by reducing the threshold for inducing long-lasting plasticity at convergent inputs (Frey and Morris, 1998b; Morris et al., 2003; Richter-Levin and Akirav, 2004; Sajikumar and Frey, 2004b). Synaptic tagging refines the expression of long-lasting plasticity by limiting changes to activated inputs. Our data show, for the first time, that L-LTP-associated transcription and the expression of prolonged potentiation can be differentially regulated by previous synaptic activity. By regulating synaptic tagging, synaptic activity at the theta (5 Hz) frequency may therefore act to regulate the input-specificity of L-LTP. Furthermore, because 5 Hz LFS regulates L-LTP expression across multiple synaptic inputs, the maximum flexibility, and capacity, for synaptic plasticity can be maintained in the neuron as a whole.

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