Heterosynaptic metaplasticity in the hippocampus *in vivo*: A BCM-like modifiable threshold for LTP

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The homeostatic maintenance of the ''modification threshold'' for inducing long-term potentiation (LTP) is a fundamental feature of the Bienenstock, Cooper, and Munro (BCM) model of synaptic plasticity. In the present study, two key features of the modification threshold, its heterosynaptic expression and its regulation by postsynaptic neural activity, were tested experimentally in the dentate gyrus of awake, freely moving rats. Conditioning stimulation ranging from 10 to 1,440 brief 400-Hz trains, when applied to medial perforant path afferents, raised the threshold for LTP induction heterosynaptically in the neighboring lateral perforant path synapses. This effect recovered slowly over a 7- to 35-day period. The same conditioning paradigms, however, did not affect the reversal of long-term depression. The inhibition of LTP by medial-path conditioning stimulation was *N*-methyl-D-aspartate (NMDA) receptor-dependent, but antidromic stimulation of the granule cells could also inhibit lateral path LTP induction, independently of NMDA receptor activation. Increased calcium buffering is a potential mechanism underlying the altered LTP threshold, but the levels of two important calcium-binding proteins did not increase after conditioning stimulation, nor was *de novo* protein synthesis required for generating the threshold shift. These data confirm, in an *in vivo* model, two key postulates of the BCM model regarding the LTP threshold. They also provide further evidence for the broad sensitivity of synaptic plasticity mechanisms to the history of prior activity, i.e., metaplasticity.

Many experimental observations now support the suggestion that long-term potentiation (LTP) and long-term depression (LTD) underlie not only long-term information storage important for learning and memory but also the experience-dependent adaptations important for tuning the patterns of synaptic connectivity in the developing nervous system (1, 2). However, for these mechanisms to store information optimally, regulatory processes must exist to maintain the modifiable synapses of the network within a useful dynamic range. One proposal to account for such homeostatic regulation was made by Bienenstock, Cooper, and Munro (BCM; ref. 3) in their model of visual cortical receptive field plasticity during development. These authors suggested that the ''modification threshold'' (the level of postsynaptic response below which gives LTD and above which gives LTP) is itself dynamically regulated by the average level of postsynaptic activity. For example, if visual cortical neurons suffered a prolonged reduction in their activity because of visual deprivation, then the modification threshold would be correspondingly reduced. This adaptive response allows the preservation of a broad range of LTP and LTD responses despite treatments that restrict the firing repertoire of those neurons. A converse process, involving an elevated modification threshold, occurs if a neuron's level of activity is increased over a prolonged period. The term ''metaplasticity'' has been introduced recently to describe these and other activity-dependent changes in the ability to undergo LTP and LTD (4).

Two important characteristics of the BCM modification threshold are (*i*) the change occurs for all excitatory synapses terminating on the affected neurons, i.e., it occurs heterosynaptically, regardless of which inputs are overactive or quiescent; and (*ii*) the direction and degree of change is a function of time-averaged postsynaptic cell firing. To date, there have been no direct tests of these two key features of the BCM model *in vivo*. Although numerous studies have shown that prior activation of *N*-methyl-D-aspartate (NMDA) receptors lowers the LTD threshold and raises the LTP threshold, these effects have typically been specific to the activated synapses (reviewed in refs. 4 and 5). Recent experiments in hippocampal slices have, however, demonstrated that repeated tetanization of one input pathway can cause similar effects heterosynaptically, in an NMDA-receptor-independent manner (6, 7). Whether these effects are induced by postsynaptic cell firing, however, has not been addressed.

The present experiments, performed in the dentate gyrus of the hippocampus, were designed to test whether the LTP threshold can be modified *in vivo* according to the postulates of the BCM model. The dentate gyrus offers advantages for this type of study because it is simple to isolate two distinct synaptic inputs converging onto a common population of postsynaptic neurons. Thus, dentate granule cells were activated either by orthodromic conditioning stimulation (CS) applied to medial perforant path fibers or by antidromic CS of the granule cell mossy fiber axons, and the effect of these treatments on the ability of lateral perforant path synapses in the dentate gyrus to undergo LTP was then evaluated. The data support the BCM postulates by showing that (*i*) orthodromic CS can raise the LTP threshold heterosynaptically *in vivo*, and (*ii*) antidromic stimulation can also inhibit LTP.

Materials and Methods

Surgical Procedures. Adult male Sprague–Dawley rats (400–550 g) were prepared with chronically implanted stimulating and recording electrodes as previously described (8). The recording electrode was a monopolar stainless steel wire (75-μm tip diameter) placed in the hilus of the dentate gyrus of one hemisphere (from bregma: 3.8 mm posterior; 2.5 mm lateral) to maximize the amplitude of the positive-going perforant path evoked responses. Two stimulating electrodes (75-μm tip diameter) were placed in the ipsilateral angular bundle to separately activate the medial and lateral perforant path fibers (from lambda: 4.0 and 5.0 mm lateral, respectively). These two pathways terminate on separate but adjacent dendritic zones of the dentate granule cells and elicit characteristic field excitatory postsynaptic potentials (EPSPs) in the dentate hilus (ref. 9; Fig. 1*A Inset*). Lack of cross-facilitation with paired-pulse stimuli was used to ensure adequate separation of the two sets of fibers being stimulated, whereas a spatiotemporal

Abbreviations: BCM, Bienenstock, Cooper, and Munro; CPP, (*R, S*)-3-(2-carboxypiperazin-4-yl)propyl-1-phosphonic acid; CS, conditioning stimulation; EPSP, field excitatory postsynaptic potential; HFS, high-frequency stimulation; LTP, long-term potentiation; LTD, long-term depression; NMDA, *N*-methyl-D-aspartate; CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate.

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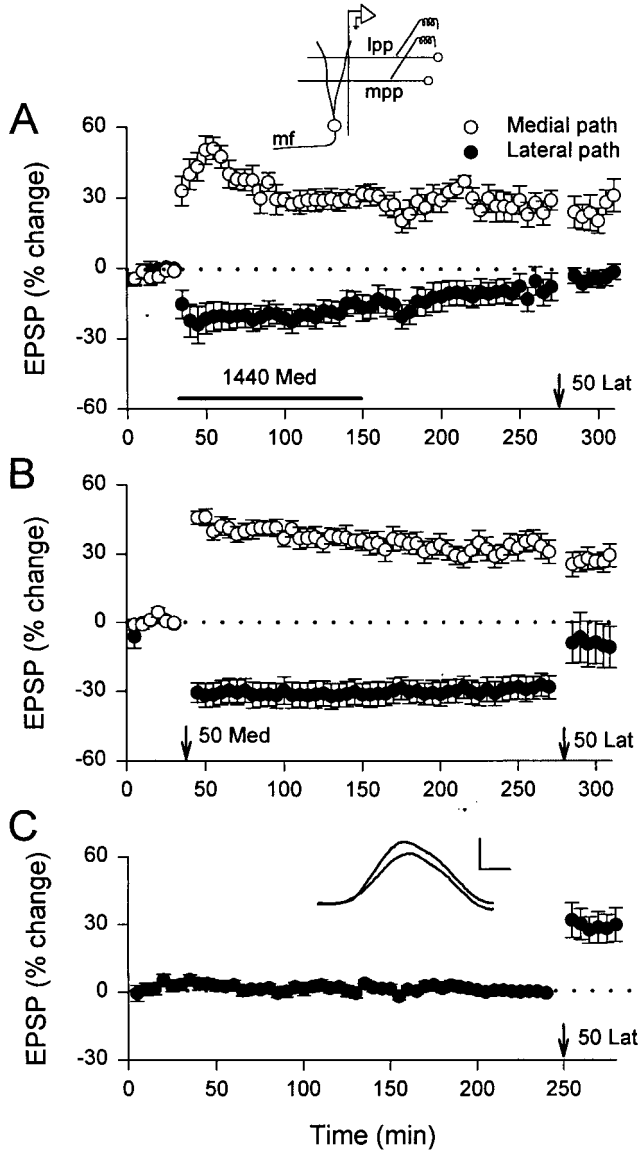

Fig. 1. CS causes heterosynaptic inhibition of LTP. CS consisting of 1,440 (n = 9; *A*) or 50 (n = 11, *B*) trains was delivered to the medial path (1,440 Med or 50 Med), and then the lateral path was probed with high-frequency stimulation (HFS) (50 Lat) for LTP induction, 4 h after commencement of the CS. HFS failed to cause LTP above the original baseline (dotted line), although it did reverse much of the LTD induced by medial path CS. This lack of LTP contrasts with LTP that is readily induced on the lateral path in naïve pathways (n = 9, *C*). Data are mean ± SEM. Waveforms represent averages of 10 sweeps, taken just before and 30 min after HFS to the lateral path, showing LTP from an animal that did not receive prior CS. (Scale bars: 2 mV, 2 ms.) (*A Inset*) The experimental preparation, including stimulating electrodes in the medial (mpp) and lateral (lpp) perforant paths and a recording electrode in the hilus near the granule cell bodies. mf, Mossy fiber axons of the granule cells.

summation test was used to verify that the two pathways converged onto a common population of granule cells (10). For some animals, the medial path stimulating electrode was replaced by a stimulating electrode implanted in the stratum lucidum of area CA3 (from bregma: 3.0 mm posterior and 2.8 mm lateral) to antidromically activate the mossy fiber axons of the dentate granule cells. All animals were allowed to recover from surgery for 2 weeks before the commencement of recordings.

Electrophysiology. Animals were placed in a recording chamber and, while they were quietly awake, recordings were made of field-potential evoked responses to 180 test pulses (150-μs duration) delivered alternately to the two stimulating electrodes at 10-s intervals. Baseline stimulation current was adjusted according to the following criteria: medial path field EPSP slopes were greater than 3.5 mV/ms, generated 2- to 3-mV population spikes, and showed paired-pulse depression; lateral path EPSPs were greater than 3 mV in amplitude, had 10–90% rise-times >2.5 ms, and showed paired-pulse facilitation; antidromically elicited population spikes were 2–4 mV in amplitude and followed brief 100-Hz stimulation with little response change. Responses were recorded 2–3 times per week until the recordings were stable over four consecutive recording sessions.

CS consisted of 10–1,440 trains of ten pulses (250-μs duration) at 400 Hz. CS was delivered in bursts of 5–6 trains at 1-s intervals, with 30–120 s between bursts, depending on the protocol. CS was delivered to either the medial path by using one of four protocols (10, 50, 360, or 1,440 trains) or the mossy fiber path (1,440 trains). The times required to complete the CS for the four protocols were as follows: 10 trains, 1 min; 50 trains, 10 min; 360 and 1,440 trains, 2 h. Responses to low-frequency test pulses were recorded on days 1, 2, 3, 4, 7, 10, 14, and weekly thereafter following CS. The test high-frequency stimulation (HFS, 50 trains, 250-μs pulses) used to probe for LTP induction was always delivered to the lateral path, unless otherwise specified. The test HFS was delivered at various times after commencement of the CS, ranging from 4 h to 35 days, to monitor the duration of any change in LTP induction. When the test HFS was given without prior CS, the baseline period was always 4 h, to match the recording period required on the day of CS. The stability of the electroencephalographic recordings and evoked responses during CS and HFS was routinely monitored to determine whether epileptiform discharges or seizures were triggered by the various stimulation protocols. No evidence of seizures was observed in any animal after orthodromic CS.

Medial and lateral path EPSP slopes and antidromic population spike heights were measured as previously described (10). The final 30 responses on each day of testing were averaged and expressed as a percent change from the mean of the last 4 days of baseline testing before CS. On the day of CS, however, all responses were expressed as a percent change from the average response (mean of 30 sweeps) recorded just before the CS on that day. Percent change data are presented as mean ± SEM.

Cycloheximide (Sigma) and (*R,S*)-3-(2-carboxypiperazin-4-yl)propyl-1-phosphonic acid (CPP; Tocris) were administered i.p. in a saline vehicle.

Immunoblot Analysis. The dorsal half of the dentate gyrus from both the unstimulated and stimulated hemispheres was dissected, and was immediately frozen in liquid nitrogen. Frozen tissue samples were homogenized in 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS)-containing extraction buffer as previously described (11), and the protein concentration was estimated by bicinchoninic acid (BCA; Pierce) assay (12). An additional fraction that contained CHAPS-insoluble proteins was obtained by boiling the pellet from the previous extraction for 10 min (in 1.25% SDS/78 mM Tris, pH 6.8), sonicating for 5 min, and centrifuging (10,000 × g for 30 min at 21°C) to clarify. Protein extracts (20 μg) were separated by SDS/PAGE on 15% polyacrylamide gels and transferred to Schleicher & Schuell Protan nitrocellulose membrane by using the Bio-Rad MiniPROTEAN II system. Western analysis was performed using primary antibodies specific to calbindin D_{28k} and the β subunit of calcineurin (Sigma), and an anti-mouse secondary antibody conjugated to horseradish peroxidase (Pierce). The immunoreactive bands were detected on preflashed Cronex film by using enhanced chemiluminescence

(ECL; Amersham Pharmacia), and quantified by using a Bio-Rad Imaging Densitometer with MOLECULAR ANALYST V 2.1 software. Analysis of each tissue extract was done at least in triplicate. For each animal, the level of calbindin or calcineurin protein in the stimulated dentate gyrus was expressed relative to that in the contralateral nonimplanted dentate gyrus. Four to six animals were used for each CS condition.

Results

Heterosynaptic Inhibition of LTP. To test whether neural activity will shift the modification threshold heterosynaptically, we delivered high-frequency CS to the medial perforant path afferents to the dentate gyrus, at an intensity above population spike threshold, and then attempted to induce LTP in the neighboring lateral perforant path synapses at various times afterward. Because it was uncertain how much stimulation would be required to produce a heterosynaptic shift in the modification threshold, we varied the total number of trains delivered (10, 50, 360, or 1,440 trains) and the time period over which they were delivered (1 min to 2 h; see *Materials and Methods*). Regardless of the protocol used, all CS paradigms produced homosynaptic LTP on the medial path and heterosynaptic LTD of the lateral path EPSP (Fig. 1 *A* and *B*). Subsequent test HFS of the lateral path, although generally effective at producing a response increase, failed to potentiate the response above the original baseline level for any of the four groups of animals (Fig. 1 *A* and *B*). Across all groups, 26 of 32 animals showed response increases that stabilized at <10% above the original baseline after lateral path HFS. The final lateral path EPSP values relative to the original (pre-CS) baseline level for the four groups were: 1,440 trains, $-2 \pm 3\%$, $n = 9$; 360 trains, $7 \pm 5\%$, $n = 6$; 50 trains, $-10 \pm 9\%$, $n = 11$; 10 trains, $-7 \pm 7\%$, $n = 6$. In contrast, test HFS of the lateral path in control animals produced $29 \pm 7\%$ LTP of the EPSP slope (Fig. 1*C*).

Our interpretation of the finding that the test HFS produced response increases, but not beyond the original baseline level, is that LTD was reversed (i.e., dedepression occurred), but LTP induction was inhibited. However, there are at least two other possible explanations. First, because the lateral path responses were depressed by the medial path CS, they may have generated less postsynaptic depolarization, and thus less LTP, during the subsequent lateral path HFS. To examine this possibility, we pooled all of the data from the four groups of conditioned animals and performed a linear regression analysis comparing the degree of LTD induced by medial path CS against the subsequent response increase induced by lateral path HFS. If animals with smaller responses were less able, in general, to produce LTP, then those animals showing the most LTD after CS should have exhibited the smallest response increase after HFS. In fact, we observed the opposite relation, i.e., there was a statistically significant negative correlation between these two parameters ($r = -0.48$, $P < 0.005$, $n = 32$), such that the greater the initial LTD, the greater the response increase after subsequent HFS. The relation between the absolute response size after CS and the subsequent response increases also showed a trend in the same direction ($r = -0.35$, $P < 0.06$). These findings argue strongly against a smaller response size being responsible for the reduction in LTP.

A second alternative explanation, based on an assumption that LTP and LTD involve different mechanisms, is that lateral path LTP did, in fact, occur, but this was masked by the initial LTD. One way to test this possibility is to examine the level of LTP exhibited by animals that did not show lateral path LTD, thus avoiding the problem. Accordingly, we selected from the conditioned groups those animals that did not show LTD (response range $\pm 5\%$ of the original baseline level just before the lateral path HFS; on average $-1 \pm 1\%$ from the original baseline, $n = 6$), and compared the level of LTP in this group against the control LTP. Strikingly, there was a complete block of lateral path LTP in the animals that had exhibited no prior LTD ($1 \pm 2\%$ change from baseline; $P < 0.01$ compared with the control group, Student's t test). Similar findings were observed 2 and 7 days after conditioning (see below). These data are therefore consistent with the view that LTP was, in fact, inhibited by the CS, and not simply masked by prior LTD.

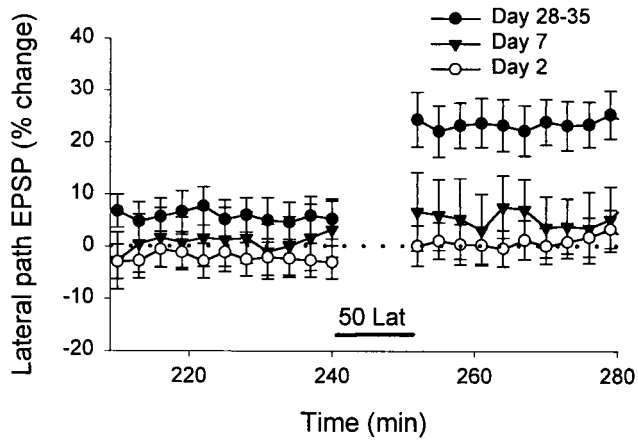

Fig. 2. Time course of LTP recovery. For 19 of 32 rats given medial path CS, lateral path HFS (50 Lat) was given on days 2, 7, and/or 28–35 after CS to track the recovery of LTP. Even though the lateral path responses were near the original baseline on day 2 ($n = 15$) and day 7 ($n = 10$), virtually no LTP was observed on either day. Significant LTP was observed when HFS was given between 28 and 35 days after CS ($n = 14$). The time values on the x axis represent the time since the start of baseline recording on the day. Whenever HFS was given, a 4-h baseline recording period was used to match the recording time before HFS on the day of CS.

Duration of LTP Inhibition. To determine how long the inhibition of LTP would last in the conditioned animals, and to establish whether LTP in the lateral path would eventually recover, 19 of 32 animals described above received additional lateral path HFS at 2, 7, and/or 28–35 days after medial path conditioning. Individual animals varied in the number of times an additional HFS was delivered. Because the recovery of LTP was similar across all four groups of animals, these data have been combined for clarity. As shown in Fig. 2, LTP was inhibited for a remarkably long time after conditioning. Virtually no LTP was observed after lateral path HFS on either day 2 or day 7 (net LTP above the original baseline on day 2, $2 \pm 3\%$, $n = 15$; day 7, $4 \pm 5\%$, $n = 10$, respectively). It is noteworthy that in both of these groups, the average pre-HFS response amplitudes were very close to the original baseline level, indicating again that the inhibition of LTP induction was not an artifact of having smaller responses. However, when lateral path HFS was given 28–35 days after medial path CS, LTP was finally observed in the majority of cases, reaching a net potentiated level of $23 \pm 4\%$ ($n = 14$). This level of LTP was not significantly different from that after lateral path HFS in control animals ($P > 0.2$).

CS Raises the Threshold for LTP Generation. Is LTP completely inhibited by the CS, or is its threshold for induction merely elevated, so that a stronger tetanic stimulation is required to observe it? To answer this question, combined HFS of the medial and lateral pathways was used to generate a stronger depolarization during HFS than lateral path stimulation alone (9). Two CS conditions were used (1,440 trains, $n = 5$; 50 trains, $n = 4$), each of which produced a large LTD of the lateral path response. Despite this strong depression, combined HFS produced robust

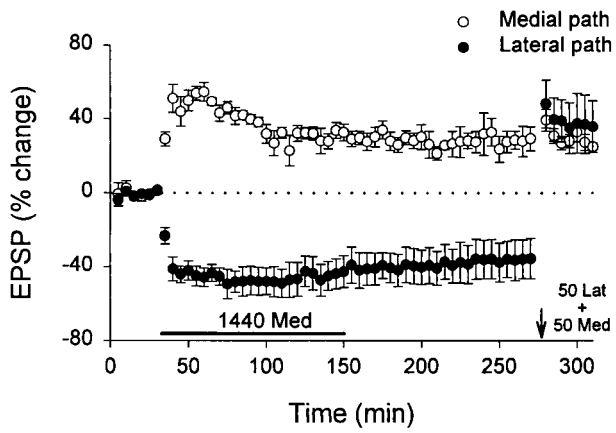

Fig. 3. CS does not completely prevent LTP. When combined HFS of the medial and lateral paths (50 Lat + 50 Med) was given after CS (1,440 Med, $n = 5$), robust LTP on the lateral path was generated. Thus, CS did not block LTP induction completely, but raised its threshold.

LTP of the lateral path responses, well above the original baseline value (38 ± 15%, and 35 ± 9% for the two groups, respectively; Fig. 3). These data indicate that CS does not cause a complete suppression of LTP; rather it elevates the threshold for LTP induction.

If LTP induction is inhibited in a cell-wide fashion after medial path CS, then the ability to induce further LTP in the medial path should also be blocked. Indeed, medial path HFS (20 trains) given 2 h after CS (1,440 trains) induced no further LTP (final level 33 ± 4%, $n = 5$) than that induced by the CS itself in the same animals (36 ± 8%), or than that induced by 20 trains in naïve animals (31 ± 11%, $n = 7$). These data are consistent with an inhibition of further LTP induction on the medial path, but could also simply reflect a saturation of LTP by the CS.

NMDA Receptors Versus Cell Firing in Adjusting the Modification Threshold. In the BCM model, the average rate of cell firing is the critical integration mechanism for computing adjustments of the modification threshold. It has been proposed, however, that activation of NMDA receptors and the associated calcium entry is the key physiological trigger for the change, rather than cell firing *per se* (13). We tested this hypothesis by giving medial path CS (1,440 trains) during NMDA receptor blockade with CPP (10 mg/kg i.p., administered 90 min before medial path stimulation). HFS was then delivered to the lateral path 2 days later, to give sufficient time for CPP to wash out. CPP effectively blocked both medial path LTP and lateral path LTD on the day of CS (Fig. 4*A*). Two days later, the lateral path response for the CPP group was near the original baseline (−5 ± 3%, $n = 5$) whereas the response for the saline group showed a small residual LTD (−14 ± 3%, $n = 8$). After HFS, a strong LTP occurred for the CPP-treated group (25 ± 1%), whereas the saline-treated group showed only dedepression back to the original baseline level (0 ± 5%). The level of potentiation was significantly different between the groups ($P < 0.05$), even when taking into account the small difference in LTD between groups before the HFS.

Although the above data indicate a contribution of NMDA receptor activation toward adjusting the modification threshold, its function may nonetheless be an indirect one by promoting cell firing through postsynaptic depolarization. According to the BCM model, if sufficient cell firing is evoked by some other means, then NMDA receptor activation should not be necessary. We tested this by antidromically driving the granule cells through stimulation (1,440 trains) of the mossy fibers running in the stratum lucidum of area CA3 (Fig. 4*B Inset diagram*). Single-

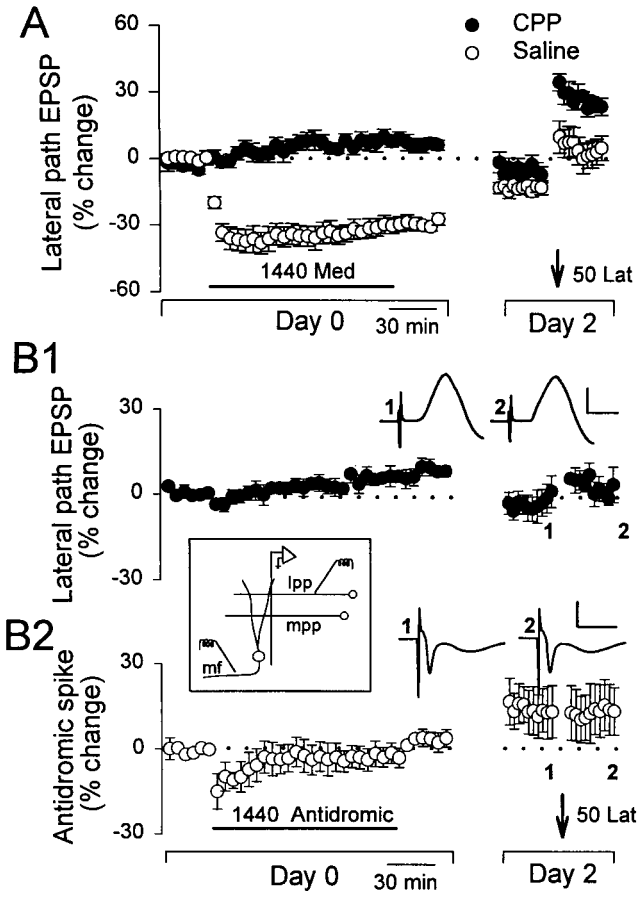

Fig. 4. (*A*) CS of the medial path was delivered after an injection of the NMDA receptor antagonist CPP ($n = 5$) or the saline vehicle ($n = 8$). CPP blocked the lateral path LTD induced by CS on the day of stimulation (left side). It also prevented the inhibition of LTP 2 days after CS (right side). LTP, but not dedepression, was blocked in the saline-treated animals. Even taking into account the slight difference in baseline levels, the CPP-treated group showed significantly greater LTP than the saline group. (*B*) Antidromic stimulation of the mossy fibers (1,440 antidromic), in the presence of CPP, produced only minor changes in either the lateral EPSP slope (*B1*) or the antidromic spike amplitude (*B2*). LTP of the lateral path responses was nonetheless inhibited 2 days after CS (right side of figure). *Inset waveforms* from a representative animal depict averages of 10 sweeps for the lateral path response (*B1*) and the antidromic spike (*B2*), obtained at the times indicated. (Bars = *B1*, 2 mV, 5 ms; *B2*, 3 mV, 5 ms.) *Inset diagram* depicts the experimental preparation using separate orthodromic stimulation of the lateral path (lpp) and antidromic stimulation of the mossy fibers (mf).

pulse stimulation of these fibers elicits a population spike in the dentate granule cell layer, without preceding synaptic activation. To reduce polysynaptic activation of the granule cells and indirect activation of NMDA receptors, the experiments were conducted in the presence of CPP, administered as described above. In these experiments, ≈50% of the animals showed epileptiform discharges during CS, and were discarded from the experiment. Seizure-free CS of the mossy fibers produced only minor changes in the lateral path EPSPs (60 min after CS: 9 ± 2%, $n = 5$; Fig. 4*B1*) and the antidromic spikes (7 ± 5%, $n = 5$; Fig. 4*B2*). Two days later, the lateral path response had remained near baseline levels (−2 ± 5%), and when a test HFS was then delivered, no LTP was generated (1 ± 5% measured 30 min after HFS; Fig. 4*B1*). This value was significantly less than that observed for the group given CS orthodromically to the medial path, also in the presence of CPP (25 ± 1%, $P < 0.002$; see

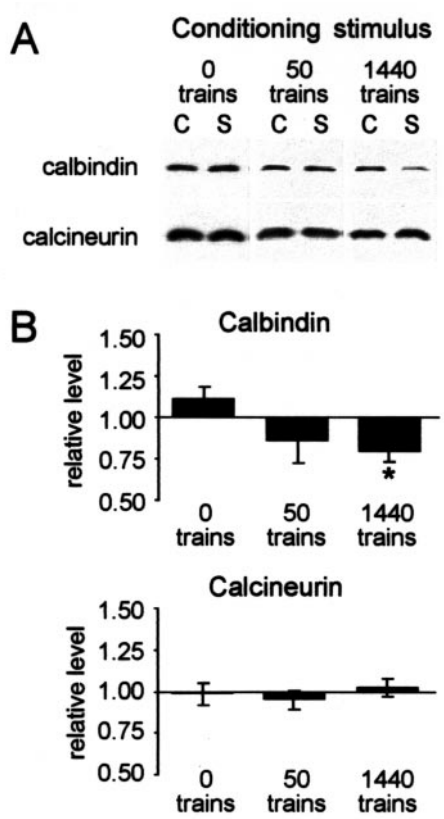

Fig. 5. Changes in calbindin D_{28k} and calcineurin protein levels in the dentate gyrus in response to CS of the medial path. (*A*) Western blot showing the change in soluble calbindin and calcineurin protein in the dentate gyrus of single animals in response to 0, 50, or 1,440 trains to the medial path. Calbindin and calcineurin levels from the same blot are pictured. C, control dentate gyrus, contralateral to the stimulated hemisphere; S, stimulated dentate gyrus. (*B*) Average change in soluble calbindin and calcineurin protein levels in the dentate gyrus in response to 0, 50, or 1,440 trains to the medial path. For each animal, the level of calbindin or calcineurin protein in the stimulated dentate gyrus is expressed relative to the control dentate gyrus. Data are means ± SEM. *, $P < 0.05$ compared with 0 trains (Student's unpaired *t* test).

above). Thus, as predicted by the model, cell firing is sufficient for computing the position of the modification threshold, even when NMDA receptors are blocked.

Calcium-Binding Proteins and Protein Synthesis. One potential mechanism mediating the shift in the modification threshold is a change in calcium buffering at the synaptic sites. It has been proposed that changes in the concentration of calcium-binding proteins such as calbindin D_{28k}, which has been shown to be regulated by activity (14, 15), might serve as a mechanism regulating free calcium levels and thus the ease of synaptic plasticity (16). Because dentate granule cells amply express calbindin D_{28k} (14), immunoblot analysis was used to test the prediction that CS of the medial path would increase levels of calbindin D_{28k}. Levels of calcineurin, a calcium- and calmodulin-dependent protein phosphatase that is located synaptically and that when activated promotes LTD and inhibits LTP (17), were also measured.

The medial path was given 0, 50, or 1,440 trains of CS, and the dentate gyrus and entorhinal cortex were dissected either immediately after the CS, or 4 h after commencement of CS, for immunoblot analysis. No increases in either soluble calbindin D_{28k} or calcineurin were observed for either brain region, at either time point after CS (Fig. 5). In fact, an unexpected, but statistically significant, reduction in calbindin D_{28k} was observed in the CHAPS-soluble fraction from the dentate gyrus when sampled 4 h after commencement of 1,440-train CS (0.79 ± 0.06, $n = 5$) compared with control tissue from other animals that received baseline stimulation but not CS (1.12 ± 0.07, $n = 4$; Fig. 5*B*). However, this effect was not reliably observed in animals receiving only 50 trains of CS (0.86 ± 0.13, $n = 6$), despite this being a paradigm equally effective at inhibiting LTP. The CHAPS-insoluble level of calbindin was not affected by CS in either group.

To test the role of protein synthesis more generally in CS-induced inhibition of LTP, additional experiments were conducted in the presence of cycloheximide (CXM, 5 mg/kg in 1 ml, i.p., injected twice, 1 h before 1,440-train CS and 1 h after CS). This dose of CXM was shown in separate animals to reduce hippocampal protein synthesis by an average of 87% compared with saline-injected controls ($n = 6$), as measured by incorporation of [^{35}S]methionine into soluble proteins. Consistent with the lack of calbindin and calcineurin up-regulation during CS, CXM did not influence the inhibition of lateral path LTP, measured 4 h after commencement of the medial path CS (final level of response after lateral path HFS, relative to the pre-CS baseline: CXM, $1 \pm 9\%$, $n = 7$; saline, $0 \pm 4\%$, $n = 8$). CXM also did not affect the degree of medial path LTP or lateral path LTD, measured over the 2 h after medial path CS, compared with saline-treated controls (data not shown).

Discussion

The BCM model is an important theoretical treatment of plasticity in the developing visual cortex that appears applicable to many other brain regions (18). A critical parameter of the model, the modification threshold, has the key feature of varying in a cell-wide (heterosynaptic) fashion, as directed by the prior history of activity in the neuron. Although there is considerable evidence that the modification threshold can be manipulated homosynaptically by prior activation of glutamate receptors (5), only a few reports from hippocampal slices have shown evidence for heterosynaptic changes (6, 7, 19). In a different approach, when young rats were reared in the dark, there was a delay of the shift in the modification threshold toward reduced LTD and enhanced LTP that normally occurs during development in the visual cortex (20). This finding is in accord with the overall predictions of the BCM model, but the experiments did not address the specificity of the neural activity required to generate the effect. Thus, despite these intriguing findings, there has remained a need to directly test *in vivo* the two critical features of the modification threshold noted above.

Here, we tested the sliding modification threshold features of the BCM model by using the perforant path to dentate gyrus synapses as a model system. We observed that the LTP threshold is indeed raised after tetanization heterosynaptically, confirming the previous findings in CA1 *in vitro* (6, 7). Unlike in CA1 slices, however, this effect was blocked by an NMDA receptor antagonist. Furthermore, we observed that antidromic activation of the granule cells also inhibited subsequent LTP, even during NMDA receptor blockade. These findings provide support for the postulate that cell firing is critical to sliding the LTP threshold, and suggest that although NMDA receptor activation may contribute to such a change, it is not an absolute requirement. A question that remains to be addressed is whether NMDA receptor activation contributes to the threshold change during orthodromic activation by depolarization-induced cell firing or by calcium influx. It will also be important to establish the effect of CS on homosynaptic LTD, because the model predicts that a facilitation of LTD induction will occur in conjunction with the inhibition of LTP.

One complicating factor encountered during the present experiments was that heterosynaptic LTD was elicited in the

quiescent lateral pathway after medial path CS. Subsequent HFS of the lateral path caused some response increase in the lateral path responses, but rarely back above the original baseline. However, lateral responses that were not depressed still showed no LTP; we conclude that although CS did not affect dedepression, it exerted a potent inhibition of LTP. Interestingly, a dissociation between dedepression and LTP has been made recently at a mechanistic level, where evidence has been put forward that LTD and LTP are separate functions of the phosphorylation status of two serine sites on glutamate receptor subunit 1, regulated by protein kinase A and calcium–calmodulin-dependent protein kinase II (CaMKII), respectively (21). According to this model, the CS used in the present experiments may act selectively to reduce the efficiency of the CaMKII-dependent processes that are involved in LTP. It is noteworthy, therefore, that the present findings are reminiscent of the reduction of LTP induced by a point mutation of CaMKII that mimicked CaMKII autophosphorylation (22), a mechanism that has been suggested to contribute to the sliding modification threshold of the BCM model and metaplasticity (22, 23). A different postulated mechanism, up-regulation of calcium-binding proteins (16), was not supported by the present experiments because calbindin and calcineurin levels did not increase after CS, nor was the inhibition of LTP affected by a protein synthesis inhibitor. These experiments, however, do not rule out a translocation of existing calcium-binding proteins to sites where they can more effectively buffer the calcium critical for LTP generation, or in other ways affect the LTP-associated signaling cascade.

A striking feature of the LTP inhibition was that it lasted between 7 and 35 days. Although the BCM model does not specify the integration period for threshold shifts, the observed time course of LTP recovery was longer than experimental estimates of this integration period made in visual cortex of young rats and kittens, which have been on the order of 2–4 days (20, 25, 26). In addition, generation of the threshold shift required fewer trains than anticipated. The experiments and models on which we based our initial hypotheses, however, have generally focused on the consequences of reduced activity on the modification threshold in developing animals, as opposed to the raised activity in adult animals investigated here. Furthermore, the modification threshold in the hippocampus may adjust at a different rate than in the previously studied visual cortex. Alternatively, the CS used in the present experiments may have generated long-lasting increases in granule cell activity (27) that continued to drive the inhibition of LTP long after termination of the CS.

The BCM theory makes several explicit assumptions about how synapses modify, and these have helped to guide experiments in a number of systems. The proposal that active synapses are modified bidirectionally as a function of the level of postsynaptic response has been explicitly tested in hippocampus, visual cortex, and elsewhere, and has received strong support. The results of the present study indicate that a second key assumption of the BCM theory is also valid, at least in the dentate gyrus: the stimulation requirements for LTP vary heterosynaptically as a function of the history of the activity of the postsynaptic neuron. The computational consequences of this form of synaptic modification have been explored extensively in models of visual cortical development. It will now be of considerable interest to understand the consequences of the BCM algorithm in computational models of hippocampus-dependent memory formation.

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