LTP Saturation and Spatial Learning Disruption: Effects of Task Variables and Saturation Levels

C. A. Barnes,^{1,2,4} M. W. Jung,⁴ B. L. McNaughton,^{1,3,4} D. L. Korol,⁴ K. Andreasson,⁵ and P. F. Worley⁵

Departments of ¹Psychology, ²Neurology, and ³Physiology, and ⁴Arizona Research Laboratories, Division of Neural Systems, Memory and Aging, University of Arizona, Tucson, Arizona 85724, and ⁵Departments of Neurology and Neuroscience, Johns Hopkins University Medical School, Baltimore, Maryland 21205

The prediction that “saturation” of LTP/LTE at hippocampal synapses should impair spatial learning was reinvestigated in the light of a more specific consideration of the theory of Hebbian associative networks, which predicts a nonlinear relationship between LTP “saturation” and memory impairment. This nonlinearity may explain the variable results of studies that have addressed the effects of LTP “saturation” on behavior.

The extent of LTP “saturation” in fascia dentata produced by the standard chronic LTP stimulation protocol was assessed both electrophysiologically and through the use of an anatomical marker (activation of the immediate-early gene *zif268*). Both methods point to the conclusion that the standard protocols used to induce LTP do not “saturate” the process at any dorsoventral level, and leave the ventral half of the hippocampus virtually unaffected. LTP-inducing, bilateral perforant path stimulation led to a significant deficit in the reversal of a well-learned spatial response on the Barnes circular platform task as reported previously, yet in the same animals produced no deficit in learning the Morris water task (for which previous results have been conflicting). The behavioral deficit was not a consequence of any after-discharge in the hippocampal EEG. In contrast, administration of maximal electroconvulsive shock led to robust *zif268* activation throughout the hippocampus, enhancement of synaptic responses, occlusion of LTP produced by discrete high-frequency stimulation, and spatial learning deficits in the water task. These data provide further support for the involvement of LTP-like synaptic enhancement in spatial learning.

[Key words: perforant path, granule cells, long-term potentiation/long-term enhancement, spatial behavior, *zif268*, maximal electroconvulsive shock]

A critical prediction of the theory that LTP/LTE (e.g., Bliss and Lomo, 1973; McNaughton et al., 1978) underlies memory is that an experimental treatment that results in maximal expression of LTP in the entire population of relevant synapses (i.e.,

“saturation”) should produce learning and memory deficits. This prediction arises from the hypothesis that neural networks store information as a specific distribution of synaptic strengths (e.g., Hebb, 1949; Marr, 1971) that would be both disrupted and prevented from further change by LTP saturation. If spatial learning, which requires an intact hippocampus (e.g., O’Keefe and Nadel, 1978; Jarrard, 1993), involves LTP at the primary perforant path inputs, it should be disrupted by saturation of their capacity for LTP. Such disruption was observed in two separate sets of experiments following asymptotic LTP induction in the perforant path (McNaughton et al., 1986; Castro et al., 1989). These studies differed primarily in the behavioral task used to assess spatial memory. One was the circular platform task (Barnes, 1979), in which rats learn to escape from a bright, open surface to a hidden dark tunnel that is fixed in relation to remote visual cues. The other was the Morris (1981) swim task, in which learning the location of a submerged platform facilitates escape from a pool of water. Both tasks are conceptually similar in that they require finding a hidden goal on the basis of distal visual stimuli. While they differ with respect to motivational and sensorimotor factors, effective learning of either task requires an intact hippocampus (e.g., Morris et al., 1982; Sutherland et al., 1982; McNaughton et al., 1989).

Recent attempts, both in this laboratory (Korol et al., 1993a) and elsewhere (Cain et al., 1993; Jeffery and Morris, 1993; Sutherland et al., 1993), have failed to replicate the impairment on the water task following bilateral LTP induction. One possible explanation is that the relationship between learning impairment and LTP saturation may be steeply sigmoidal (Fig. 1), as predicted by most models of Hebbian associative memory (e.g., Marr, 1971; Amit, 1989; Gibson and Robinson, 1992; Buckingham and Willshaw, 1993). In view of this relationship, large behavioral impairment differences could result from relatively minor differences in the magnitude and extent of LTP. (The simplest such models assume binary state synapses, in which case “saturation” level refers to the proportion of synapses that have been used. In models employing continuous but bounded synaptic weights, saturation level would be defined as the sum of all of the weights divided by the maximum possible sum.) Alternatively, however, both earlier positive results may have been due to statistical error. Thus, we have reinvestigated the effects of LTP induction on behavior in both tasks, with particular attention to assessing the physiological magnitude of the LTP induced by standard stimulation procedures. This was studied by assessing the residual LTP obtainable from a second stimulation site, following “saturation” at the standard site.

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Correspondence should be addressed to Dr. C. A. Barnes, Life Sciences North Building, Room 384, University of Arizona, Tucson, AZ 85724.

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Figure 1. The hypothetical relationship between level of synaptic saturation and memory impairment, predicted by many theoretical models, is nonlinear, as illustrated diagrammatically. Such a relationship may explain the considerable variation in behavioral results following “LTP-saturation” treatment. Moreover, the shape of the function may vary for different behavioral tasks.

We also estimated the anatomical extent of LTP “saturation” using a combination of electrophysiological recording and the activation of the immediate early gene *zif268*, which is highly correlated with LTP induction (e.g., Cole et al., 1989; Wisdon et al., 1990; Abraham et al., 1992; Worley et al., 1993; but cf. Schreiber et al., 1991). Therefore, *zif268* activation was used as an anatomical marker for the probable extent of LTP induction. *Zif268* is also activated throughout the entire hippocampus by maximal electroconvulsive shock (MECS), which has previously been shown to reduce LTP induction both in CA1 *in vitro* (Anwyl et al., 1987) and in the fascia dentata (FD) in anesthetized rats (Stewart and Reid, 1993a). The latter authors suggested that MECS may actually induce LTP, which would occlude LTP induction in subsequent tests. Electroconvulsive shock also leads to memory dysfunction in humans and rats (e.g., Madsen and McGaugh, 1961; Squire et al., 1981, 1984), which might be related to synaptic modifications taking place in the hippocampus and elsewhere. Thus, we have extended the previous studies by examining the effects of MECS on FD evoked responses, and on the spatial memory performance of the same animals.

Some of these data have been presented in abstract form (Barnes et al., 1993; Korol et al., 1993b; Stevenson et al., 1993).

Materials and Methods

Experiment 1: LTP saturation, circular platform, and water task behavior

Subjects. Three replicates of 12 rats were planned a priori. The 36 male F-344 retired breeder rats (9 months) used in this experiment were obtained in batches of 12, approximately 3 months apart. All rats were housed individually in 45 × 24 × 21 cm Plexiglas cages and were maintained on a free feeding regimen, and on a reversed 12/12 hr light/dark schedule with lights off at 10:00 A.M. Recording sessions were conducted primarily between 10:00 A.M. and noon. Behavioral testing began approximately 1 hr following recording. Rats were extensively handled and were given weekly health checks.

Surgery. Chronic implantations of bilateral recording and stimulating electrodes in the hilus of the fascia dentata and angular bundle were conducted according to NIH guidelines, essentially as described in McNaughton et al. (1986). Rats were deeply anesthetized with 40 mg/kg Nembutal (supplemented with Metofane). Small bone flaps were re-

moved over the recording (3.8 mm P, 2.0 mm L to bregma) and stimulating sites (8.1 mm P, 4.4 mm L) and burr holes were drilled for three anchor screws, a reference, two ground and two stimulus return screws. Evoked responses were optimized under electrophysiological control, and all rats had positive-going perforant path–granule cell evoked responses, characteristic of placement of the recording electrode in the hilus of the fascia dentata at the time of surgery. Postoperatively, rats were given a 10 ml saline injection subcutaneously, Children’s Tylenol for analgesic purposes, and fresh fruit.

Behavioral training. The circular platform behavioral apparatus used in this experiment was 1.2 m in diameter, with 18 equally spaced holes around the periphery (see Barnes, 1979, for more detail). One hole led to a dark escape box (43.5 × 17 × 10 cm) that was fixed in relation to the distal environmental cues. The platform surface was rotated from trial to trial to eliminate use of local olfactory, visual, or tactile cues, and was brightly illuminated from above. The bright illumination served as motivation to escape to the dark box. Performance on this task is also facilitated through the use of a large room, with the edges of the maze well away from any wall (C. A. Barnes, unpublished observations). Because the most robust effect of LTP induction in the McNaughton et al. (1986) study was on the reversal of a well-learned spatial problem on this task, a reversal protocol was also employed in the present studies.

On the first day of training (which varied between 15 and 34 d following surgery for the different batches), rats were initially placed directly into the escape box for 30 sec. Trial 1 commenced immediately afterward. Although the box itself remained in a fixed position in relation to the room, both the orientation of the platform surface and the direction in which the rat was facing in the start chamber were predetermined randomly, and changed from trial to trial. Each trial began by placing the rat into the false-bottomed start chamber, which was placed in the platform center. To begin the trial, the start chamber was raised, leaving the rat free to move on the platform surface. After a maximum of 4 min to find the location of the goal, the rat was placed next to the correct hole, and allowed to descend into the escape box. The latency was recorded from the time the start chamber was raised, to the time when the rat’s last foot left the maze surface. In addition, errors were counted (nose-head-neck deflection into a hole that was not over the escape box), and the deviation of the first error from the correct hole (number of holes away from the goal, maximum 9) was noted.

Acquisition trials on the circular platform were given once per day for 16 d before commencement of the high- and low-frequency stimulation treatment. Ten additional trials were given following the stimulation treatment, in which the goal box was shifted 135° away from the original acquisition location. Trials on which the goal was in this changed location are referred to as reversal trials.

The swim-task procedure was essentially as described in Korol et al. (1993a), and was used to test the 12 rats in batch 3 only. Rats were placed for 30 sec on the escape platform as an adaptation trial, and were then trained in sets of two trials (60 sec maximum per trial, interset interval of about 30 min) for a total of 14 trials per day for 2 d. The starting location was randomized for each trial and across rats. Path length and latency to find the fixed, submerged platform in the pool were recorded by a tracking system (HVS Systems). On trial 25, the platform was removed from the pool and each rat was allowed to swim for 60 sec (probe trial).

Electrophysiological recording. Rats were given at least 1 week for recovery from surgery before electrophysiological testing began. Evoked responses were recorded for 8, 5, and 5 d, respectively, for the three batches of rats, before circular platform testing was initiated. Low-frequency test stimuli at 0.1 Hz (30 total) were delivered daily to each hemisphere at both 250 and 500 μ A stimulus intensities as follows: stimuli 1–10 and 21–30 at 250 μ A; stimuli 11–20 at 500 μ A. In order to assess the possible confounding effects of gross EEG abnormalities and/or seizures (e.g., Leung, 1987; Cain et al., 1993) either during or after electrical stimulation, a 2.56 sec EEG sample was also collected immediately before each stimulus delivery (30 total). Two rats (batch 2) did not have acceptable perforant path–granule cell evoked field responses; thus, the final *n* was 34. Rats were removed from the colony room in pairs and were recorded from simultaneously. The time of day of testing was fixed for each pair of rats, and the interval between removal from the colony room and the first stimulus pulse was typically 1 min.

Test stimulation was given daily during the 16 acquisition trials of the circular platform (except as noted in the Results). Rats were then randomly assigned to receive either the bilateral high-frequency (ten 25 msec

bursts at 400 Hz, 100 stimuli total) or low-frequency (0.1 Hz) treatment for 3 d during the 500 μ A stimulus period (i.e., stimuli 11–20). During these 3 d, no behavioral testing was conducted. This treatment was given twice per day at 2 hr intervals. Following this 3 d period (six sessions total), high- or low-frequency stimuli were administered once per day, at least 1 hr before the beginning of the behavioral trial on the circular platform (i.e., on each of the 10 d of reversal training). For batch 3, the stimulation was continued to include the days on which the rats were tested on the spatial version of the Morris water task.

The measure of evoked response stability (for the low-frequency treatment groups) or response change (for the high-frequency treatment groups) was fractional change in the dependent variable: $(V_1/V_0) - 1$, where V_1 is the post high-frequency score and V_0 is the baseline measure. The dependent variables were the EPSP amplitude and population spike area. The EPSP was measured between a fixed temporal interval of 1 msec, on the rising phase of the EPSP, well away from the onset point. These cursor settings adequately measure changes in EPSP slope while avoiding contamination of the waveform by leftward shifts of the waveform that may occur due to brain temperature changes (e.g., Erickson et al., 1993; Moser et al., 1993) or by the onset of the population spike. The population spike was measured as the area under a tangent line joining its onset and offset. Only the average of the first 10 responses collected on any given day were included for individual daily measurements, unless otherwise specified. Statistical analyses were carried out using two-way ANOVAs, with α set at the 0.05 level for all comparisons.

Experiment 2: multiple perforant path stimulation sites

Subjects. A total of 12 young adult (6–9 months) male Fischer-344 rats were used. They were housed and maintained as in experiment 1 before the acute surgical procedures commenced.

Surgery. Animals were deeply anesthetized with Nembutal (40 mg/kg) and placed in a stereotaxic device. The skull was exposed with a midline incision and burr holes were drilled for implantation of skull screw electrodes and depth electrodes. Mineral oil was placed over the exposed brain. Supplemental doses of anesthetic (2.5 mg in 0.05 cc) were administered every 45 min. Care was taken to maintain core body temperature of the animal at 37°C throughout the experiment with a heating pad and an insulating towel. Rectal temperature measured in four experiments indicated that, with these procedures, body temperature was stable throughout the recording period (36.5–37.5°C).

Two recording and two stimulating electrodes were positioned in the dorsal hippocampus and angular bundle, respectively, in the same hemisphere. Recording and stimulating electrodes were constructed of 75 μ m diameter (conductor) Teflon-coated stainless steel wire. The stimulating electrode had 250–300 μ m of insulation removed from the tip. The final impedance of the electrodes ranged from 50 to 200 K Ω tested at 1 kHz. The more anterior recording electrode (R1; Fig. 2) was positioned on the brain surface using standard coordinates (experiment 1; McNaughton et al., 1986; Korol et al., 1993a). The more posterior recording electrode (R2) was positioned 4.5–4.7 mm posterior and 2.6–2.9 mm lateral to bregma. Under electrophysiological control, both recording electrodes were lowered into the hilus (~2.6–2.9 mm ventral to the surface of the brain). The anterior stimulating electrode (S1) was positioned at 8.1 mm posterior, 4.4 mm lateral (standard coordinates), while the second stimulating electrode (S2) was placed 8.8–9.0 mm posterior, 5.1–5.3 mm lateral to bregma (Fig. 2). Each stimulating electrode was lowered (~2.2–2.6 mm ventral to the brain surface). Both recording and stimulating electrodes were lowered to a position that resulted in the maximum response.

Electrophysiological recording. Field potentials were recorded simultaneously by electrodes R1 and R2. Responses were amplified 100 times and filtered at 1–3 Hz (high-pass) and 10 kHz (low-pass) with Grass P511 amplifiers. The responses were then digitized at 20 kHz, and stored on computer for off-line analyses. The EPSP amplitude was measured as the voltage between two fixed cursors on the initial rising phase of the response. Stimulation, consisting of unipolar rectangular pulses (constant current, 500 μ A, 320 μ sec) was delivered to S1 and S2, alternating at 0.2 Hz during baseline low-frequency test stimulation periods. These parameters are similar to those used in other experiments in which the effects of saturation of perforant path synaptic responses have been investigated (e.g., McNaughton et al., 1986; Castro et al., 1989; Robinson et al., 1992; Cain et al., 1993; Jeffery and Morris, 1993; Sutherland et al., 1993). At least 10 min of stable baseline was recorded prior to examination of stimulus input/EPSP response output (I/O) functions, and administration of the high-frequency stimulation treatment. To

Figure 2. Schematic illustration of the recording and stimulation configuration in the hippocampus used in experiment 2. S1 and R1 represent the standard stimulation and recording electrode sites, respectively, that have been used in previous “saturation” experiments. S2 and R2 are the additional sites used in the present study.

determine the I/O relationships during the baseline period for each electrode pair, nine stimulus intensities were used ranging from 15 to 600 μ A (five samples at each intensity) for S1 and S2 stimulation alone, and for combined S1 + S2 stimulation.

The high-frequency stimulation treatment consisted of ten 25 msec bursts of 400 Hz stimulation delivered at 0.1 Hz (100 pulses total). Either one (for two animals) or two (for 10 animals) episodes of the high-frequency stimulation treatment were administered to S1 or S2 stimulation sites independently. One such treatment was delivered for the combined (S1 + S2) treatment.

The overall experimental sequence was as follows: (1) 10–20 min baseline stimulation of S1 and S2 (independently); (2) I/O stimulation for S1 and S2 independently, and combined (S1 + S2); (3) 5 min low-frequency test stimulation of S1 and S2 (independently), and then high-frequency stimulation in one of two possible sequences: (a) “normal,” in which the LTP-inducing stimulation was delivered first to S1, followed by 15 min low-frequency test stimulation, and I/O stimulation on S1; similar LTP induction parameters were then delivered to S2, followed by 15 min low-frequency test stimulation, and I/O stimulation on S2; (b) “reverse,” in which the LTP-inducing stimulation was delivered first to S2, followed by S1, using the same procedure as in (a); (4) 5 min low-frequency test stimulation of S1 and S2 (independently), then high-frequency stimulation of S1 + S2 combined; (5) 15 min low-frequency test stimulation of S1 and S2 (independently); (6) I/O stimulation for S1 and S2 independently.

Both hemispheres were used in four animals in which the normal paradigm was administered for one hemisphere and the reverse paradigm for the other. Data were excluded either when greater than 10% baseline drift occurred, or greater than 5% drift occurred after the first I/O stimulation was administered. A total of 14 hemispheres from 10 rats met this criterion and were used in the final analysis. Fractional change measurements were calculated for the dependent variables of interest as described for experiment 1. One-way and multiple ANOVAs were used for statistical analyses.

Experiment 3: hippocampal distribution of zif268 mRNA following LTP-inducing stimulation

Subjects and surgery. Six male F-344 rats were housed and maintained as in experiment 1, and underwent bilateral surgical implantation of

stimulating and recording electrodes in the perforant path and hilus of FD, as described in experiment 1. At the time of surgery, evoked responses were optimized under electrophysiological control, and all rats had positive-going perforant path–granule cell evoked responses. These placements remained stable following surgical recovery. Two additional rats served as naive and MECS controls.

Electrophysiological procedure. For the *in situ* hybridization studies, the six rats were sacrificed 30 min following ten 25 msec bursts of 400 Hz stimulation (100 pulses total). EEG was monitored during this procedure (as described for experiment 1), and in one hemisphere of one rat, afterdischarges appeared following stimulation. There were no other EEG abnormalities in any of the other LTP preparations. The stimulus intensity was set at 500 μ A for all hemispheres of all rats, and the EPSP and population spike were measured as in experiment 1. The fractional change of the synaptic response was calculated by using the average of 10 responses (collected at 0.1 Hz) 30 min following high-frequency stimulation as V_i and the average of the first 10 responses collected immediately before high-frequency stimulation treatment as V_o .

In situ hybridization. *In situ* hybridization techniques were as described by Saffen et al. (1988). Rats were killed by guillotine decapitation, the brains were rapidly removed, and the hippocampi were dissected out, extended, and frozen. Serial 18 μ m sections were cut through the transverse plane of the entire hippocampus (about 1 cm) in preparation for *in situ* hybridization of zif268 mRNA. Densitometric measurements of 35 S incorporation in FD stratum granulosum were made from autoradiograms, and background levels from corresponding control (naive) sections were subtracted. The zif268 mRNA activation following LTP induction was expressed relative to the activation at the same dorsal–ventral levels following electroconvulsive shock (MECS) treatment. Standard MECS parameters were used: 100 Hz, 0.5 msec, 85 mA for 1 sec (delivered via ear-clip electrodes, ECT Unit, Ugo Basile, Italy).

Experiment 4: MECS, LTP, and spatial behavior on the water task

Subjects and surgery. Forty male F-344 rats (9 months) were housed and maintained as in experiment 1, and underwent bilateral surgical implantation of stimulating and recording electrodes in the perforant path and hilus of FD, as described in experiment 1.

Electrophysiological recording and MECS procedures. The 40 rats in this portion of the experiment were divided into four different treatment protocols that were conducted sequentially ($n = 6, 6, 12$, and 16, respectively, as described below; see also Table 1).

Protocol 1. The purpose was to compare the effects of MECS on evoked response magnitudes in FD when, for a given rat, one hemisphere received low-frequency test stimulation, and the other high-frequency stimulation. These rats received unilateral LTP treatment on one day (ten 25 msec bursts of stimuli delivered at 400 Hz, 100 pulses total), followed on the next day by LTP treatment and subsequent MECS treatment (standard parameters: 100 Hz, 0.5 msec, 86 mA, for 1 sec). Thus, LTP could be measured 24 hr following high-frequency stimulation alone, and also 24 hr following the condition in which high-frequency stimulation and MECS were given sequentially. In addition, any effects of MECS alone on the contralateral hemisphere that received only low-frequency stimulation could be assessed.

Protocol 2. The purpose was to compare directly the behavioral performance of rats that had received bilateral high-frequency stimulation with those that had received bilateral high-frequency stimulation plus standard MECS treatment. Half of these rats were given 3 d of bilateral high-frequency stimulation (ten 25 msec bursts of stimuli delivered at 400 Hz, 100 pulses total per treatment) only, followed by attachment to the MECS apparatus, without delivery of MECS. The other half were given bilateral high-frequency stimulation (parameters as above), followed on each of 3 consecutive days by the standard MECS treatment. Thus, a measure of LTP following high-frequency stimulation alone in addition to that following high-frequency stimulation plus MECS was obtained. These rats were subsequently tested on the spatial version of the Morris water task, in which behavioral testing was conducted over a single day, with a total of 14 acquisition trials. The six rats were tested at the same time, given a maximum of 60 sec to find the platform, a 30 sec exposure to the platform (after finding it, or being placed there), and were given seven blocks of two trials separated by an interblock interval of about 15 min.

Protocol 3. This protocol directly tested whether the magnitude of change in evoked synaptic responses in the hippocampus following MECS

treatment could be related to accuracy of spatial performance. The 12 rats were initially trained to acquire the spatial version of the Morris water task, in which the training was administered over 4 d (six trials per day, in blocks of two trials). All rats were then given low-frequency test stimulation to assess the size of their FD evoked responses. Eight of these rats were given MECS treatment under Metofane anesthesia for 3 consecutive days following baseline test stimulation, whereas the other four were anesthetized and not given MECS. For this experiment, two different “MECS” parameters were used, one in which overt behavioral seizures were apparent (i.e., the standard parameters of 100 Hz, 0.5 msec, 85 mA for 1 sec) and one in which behavioral seizures were not apparent (299 Hz, 0.2 msec, 42 mA, for 0.1 sec, repeated five times, once per 10 sec). Four rats were assigned to each group. Twenty-four hours after the last treatment, all rats were given 10 trials on the water task (on 1 d) with the escape platform 180° opposite its original location. After the 10th trial, a 60 sec probe trial was given with the platform removed.

Protocol 4. This experiment was conducted to assess the persistence of the behavioral and electrophysiological changes following MECS treatment. FD evoked responses were collected daily over a 1 week period to establish baseline response levels for each of the 16 rats. During that time, all rats were trained on the spatial and cued versions of the swim task. Following this, baseline low-frequency test stimulation was continued, but was immediately followed by MECS (standard parameters, 100 Hz, 0.5 msec, 85 mA for 1 sec) treatment on 5 consecutive days. After this treatment, all rats were assigned randomly to pairs for behavioral testing on a reversal problem of the water task at 1, 2, 3, 4, 5, 6, 7, or 8 d post-MECS treatment for the first reversal test. A probe trial was administered 24 hr following training (i.e., 2–9 d post-MECS treatment). For the second reversal test session, the rats were split into two groups, in which training was administered on day 31 and probe and cue training trials on day 32, or in which training was administered on day 32 and probe and cue training trials on day 33 following MECS treatment. All rats were electrophysiologically tested daily for 30 d after the MECS treatment.

Results

Experiment 1: LTP saturation and circular platform behavior

Circular platform acquisition prior to LTP induction

Six of the 36 rats showed some afterdischarge activity at the time of electrode implantation (two low-frequency groups, four high-frequency groups); however, these rats were not outliers either in the baseline electrophysiological recording parameters or in their performance on the initial acquisition of the circular platform. There were no statistically significant differences between treatment groups on the number of errors made [$F(1,32) = 1.55$, $p > 0.05$; Fig. 3A], or in the initial deviation from the correct escape hole [$F(1,32) = 0.52$, $p > 0.05$] during the initial acquisition of the circular platform task. This training was conducted prior to LTP induction in the HF group. There was, however, a significant effect of repeated trials for errors [$F(1,15) = 4.54$, $p < 0.0001$] and initial deviation [$F(1,15) = 3.33$, $p < 0.002$], indicating that the rats learned the task over days.

Circular platform and Morris water task performance following high-frequency stimulation

Analysis using a two-way ANOVA on the number of errors made during the 10 reversal trials of the circular platform task revealed a significant effect of treatment [$F(1,32) = 4.73$, $p = 0.03$], and of repeated trials [$F(1,9) = 6.83$, $p = 0.0001$]. This indicates that while both groups showed improvement over trials, the rats that received low-frequency stimulation performed significantly better (see Fig. 3B). Scheffé post hoc analysis revealed significant differences between groups on trial blocks 4 and 5 ($p < 0.0001$, and 0.05, respectively). The two-way ANOVA conducted on the deviation from the correct location of the platform indicated a significant interaction [$F(1,4) = 2.84$, $p = 0.02$], but

Table 1. Experimental summaries for the four different protocols used in experiment 4

Protocol/group	Day	Treatment/test
Protocol 1 ($n = 6$)		
Hemisphere 1	1	Unilateral LTP
	2	Unilateral LTP + MECS
	3	LF test
Hemisphere 2	1–3	LF test each day
Protocol 2 ($n = 6$)		
LTP group	1–3	Bilateral LTP
	4	Fourteen trials on spatial version of Morris water task
LTP + MECS group	1–3	Bilateral LTP + MECS
	4	Fourteen trials on spatial version of Morris water task
Protocol 3 ($n = 12$)		
Group 1: MECS, behavioral seizure	1–4	Six trials on spatial version of Morris water task per day
	5–7	Standard MECS
	8	Ten trials on 180° reversal (from days 1–4 above) on spatial version of Morris water task, plus one probe trial
Group 2: MECS, no behavioral seizure	1–4	Same as for group 1
	5–7	MECS, no behavioral seizure
	8	Same as for group 1
Group 3: ear clip	1–4	Same as for group 1
	5–7	No MECS, ear clips only
	8	Same as for group 1
Protocol 4 ($n = 15$) (all rats)		
	1–4	LF test
		Six trials on spatial version of Morris water task
	5	LF test
		Six trials on cued version of water task
		LF test + MECS
	6–10	Ten trials reversal, one spatial
	11–19	task probe trial
		Ten trials reversal, two spatial
	31–33	task probe trials

no significant effect of treatment or of repeated trials ($p > 0.05$). This outcome reflects the reduced deviation scores over trials of the controls, while the high-frequency group showed little improvement.

On the other hand, there was no effect of the same high-frequency stimulation on acquisition of the Morris water task, by the 12 rats from batch 3. The two-way ANOVA indicated no significant effect of treatment on total path length to find the hidden platform [$F(1,10) = 3.11$, $p > 0.05$; Fig. 3C], while there was a significant effect of repeated trials [$F(1,23) = 3.25$, $p = 0.001$]. Latency and probe trial data were also not significantly different between treatment groups ($p > 0.05$). A separate analysis of reversal trial errors on the circular platform for batch 3 rats indicated that the six rats that received high-frequency stimulation performed significantly worse than did the six controls

[$F(1,10) = 4.85$, $p = 0.05$]. This indicates that the same high-frequency treatment that disrupted learning of the reversal problem on the circular platform task did not affect swim task performance.

Electrophysiological measures

Evoked responses. The EPSP and population spike measures are plotted as a fractional change between the baseline and treatment days, that is, the mean of days 1–16 for each individual rat was subtracted from the corresponding mean for days 1–29, and that value was divided by the mean of days 1–16 (Fig. 4). Days 1–16 are the days that correspond to acquisition training on the circular platform. For logistical reasons, in the first batch of 12 rats, some recording sessions were missed during the pre-training period on the circular platform (recordings were taken

Figure 3. *A*, Mean and SEM number of errors during the 16 daily acquisition trials on the circular platform task (in blocks of 2 d), which was conducted during baseline low-frequency (LF) testing, prior to high-frequency (HF) treatment. There were no pretreatment behavioral differences between LF and HF groups. *B*, Mean and SEM number of errors made on the 10 daily reversal trials (in blocks of 2 d) following the LF or HF treatments. The rats given the HF treatment performed significantly worse than did the LF control rats. *C*, Mean and SEM path length (in cm) during acquisition of the spatial version of the Morris water task. No statistically significant difference was found between the LF and HF groups on this task.

on days 4, 10, 14, 15, and 16). In the other two batches, recordings were carried out every day. The rats that received low-frequency treatment only (Fig. 4*A,C*) showed excellent stability of the responses, whereas the rats that received high-frequency stimulation showed the expected increases in the amplitudes of the EPSP and population spike over the initial 3 d of twice daily high-frequency stimulation and over the subsequent days during reversal training (Fig. 4*B,D*). The stability of the responses of the low-frequency rats, tested under the same conditions, confirms that the synaptic modification observed in the high-frequency group was indeed due to the high-frequency stimulation.

EEG. Afterdischarges were not induced in any of the rats following high-frequency stimulation, as assessed by monitoring analog EEG traces on the oscilloscope during recording, by comparison of the power spectra of the EEG immediately before and after LTP-inducing stimulation, and by observing the evoked response amplitude during and after the high-frequency treatment. Tetanic stimulation-induced afterdischarge in other studies has typically been associated with a large reduction in EEG power in the hippocampus immediately following the afterdischarge episode (e.g., Leung, 1987). This was not observed in any of the animals in this study. Furthermore, evoked response amplitudes following afterdischarge are severely reduced (e.g., Worley et al., 1993), and this was not observed in any hemisphere in these rats.

To determine whether there were any subtle changes in EEG pattern between rats that received low- or high-frequency stimulation, fast Fourier transform analysis was performed on the EEG measured before each stimulus delivery (30 sample responses per recording session). The EEG power was computed for frequencies between 1 and 50 Hz, and averaged between hemispheres for a given rat. Three different approaches to examining the EEG were taken. The first question was whether there were any long-lasting changes in total power of the EEG across the 29 d of the experiment. As shown in Figure 5*A*, there was no difference between groups in total EEG power (cumulative power at all frequencies, 1–50 Hz) during the baseline period (responses 1–10) throughout the experiment [$F(1,62) = 1.19$, $p > 0.05$]. Thus, high-frequency stimulation did not lead to persistent changes in EEG over days.

The second question was whether there was an effect of the high-frequency treatment on the EEG within daily recording sessions. As can be seen in Figure 5*B*, there was a difference between groups in the ratio of total power within a session [after/before treatment, i.e., the mean of responses 21–30 divided by the mean of responses 1–10; $F(1,62) = 35.17$, $p < 0.0001$]. The mean power ratios were greater in the high-frequency group during the treatment time period (hatched bar, Fig. 5*B*).

To determine which frequencies were contributing to the increased power in the high-frequency group following treatment, and how long this alteration persisted, average power spectra (1–50 Hz) for days 17–29 were calculated before (responses 1–10) and after (responses 21–30) the high-frequency treatment. An average fractional difference score for EEG power was then calculated (Fig. 5*C,D*). These scores reveal that the increase in the high-frequency group is accounted for by changes in the higher spectral frequencies (20–50 Hz). Increases in higher frequencies of the EEG have also been observed to occur in CA1 following tetanic stimulation that leads to afterdischarge (Leung, 1987), although with the parameters used in those experiments an increase in fast activity was not observed in the hilus of FD.

Finally, to determine the durability of the EEG change in FD,

Figure 4. Perforant path–granule cell evoked responses were recorded throughout the 29 d of experiment 1. Responses to low-frequency test stimulation were obtained throughout the initial 16 d of training on the circular platform (days 1–16). Following this training, rats received either low-frequency (*A, C*) or high-frequency (*B, D*) stimulation twice per day for 3 d (days 17–19). Either low- or high-frequency treatment was then continued once per day for 10 additional days in conjunction with the 10 reversal training trials (days 20–29). The means and SEM of the fractional change of the EPSP (*A, B*) and population spike (*C, D*), relative to the first 16 low-frequency test days, indicate stability of responses over days in the control group (*A, C*), and persistent increases in the high-frequency treatment group (*B, D*). Note that standard error bars in *D* are truncated in the upward direction. *Hatched bar* indicates treatment period.

EEG power was individually calculated before each evoked response was elicited during the 5 min recording session for each rat. In Figure 5, *E* and *F* illustrate that the mean EEG power during individual recording sessions (responses 1–30), on days 17–29, did not change in the low-frequency stimulation group and changed transiently in the high-frequency stimulation group. The EEG power increased substantially during the 10 sessions of high-frequency stimulation (beginning at response 11), but declined exponentially toward control levels with a time constant of approximately 1–2 min. Thus, the stimulus-induced EEG changes would have decayed to insignificant levels by the time of behavioral training, which commenced 1 hr or more following the end of the recording session.

Electrophysiological and behavioral correlations. Because there was a relatively large number of animals for which data were available on acquisition of the circular platform task, and on induction of LTP ($n = 17$), it was of interest to determine whether there were any significant correlations between these measures. Previously, Barnes (1979) found no behavioral correlation with induction of LTP, but did find a statistically significant correlation between errors on the circular platform and LTP

Figure 5. *A*, Mean and SEM of the total EEG power (1–50 Hz) measured immediately before each of the first 10 test stimuli (responses 1–10) of each recording session over the 29 d of experiment 1. Note that there was no difference in the daily measurement of total EEG power for either the low-frequency (LF) or high-frequency (HF) treatment groups, even during the days in which the HF treatment was administered (*hatched bar*). The extra tick marks on days 17–19 indicate that recordings were made twice per day. *B*, Mean and SEM of the ratio of the EEG power sampled before each of the first 10 and last 10 test stimuli (responses 21–30/responses 1–10) throughout experiment 1. Significant differences in the EEG power ratios emerged between groups during the treatment period (*hatched bar*). The HF rats had significantly larger power ratios compared to the LF group during this time. *C* and *D*, The mean relative differences in EEG power before (days 1–16) and after (days 17–29) treatment for the LF and HF groups are shown in *C* and *D*, respectively. The frequencies contributing most significantly to the changes observed in the total power ratio for the HF group are those between 20 and 50 Hz. *E* and *F*, Time course of the changes observed in mean ($\pm$ SEM) EEG power over individual recording sessions. Total power in *E* and *F* is derived by averaging the power at each data collection point for all animals collapsed over treatment days 17–29. On average, the LF rats (*E*) did not show changes in EEG power over the entire 5 min test session. The HF rats (*F*), however, showed a sharp increase in power during the HF treatment (*hatched bar*), but this increase decayed with a time constant of 1–2 min following HF stimulation.

persistence. Although we had no measure of decay in the present experiment, we examined three different measures of LTP induction: (1) the average fractional change over the initial six LTP-inducing sessions; (2) the average fractional change during

Figure 6. *A*, Example of the results from a typical dual stimulation site experiment with responses recorded from R1 following stimulation of sites S1 and S2 (Fig. 2). The experimental sequence was as follows: 30 min baseline, stimulus input–response output functions (I/O curves) for stimulus sites 1 (S1) and 2 (S2), I/O for S1+S2, 5 min test stimulation, high-frequency stimulation (HFS) to S1 (or S2, in “reverse” paradigm), 15 min test stimulation, I/O for S1 and S2, 5 min test stimulation, HFS to S2 (or S1 in “reverse” paradigm), 15 min test stimulation, I/O for S1 and S2, 5 min test stimulation, HFS to S1+S2, 15 min test stimulation, I/O for S1 and S2. *B* and *C*, Average EPSP amplitude, across all rats, elicited by stimulation at nine different intensities at site 1 (S1), site 2 (S2), or S1+S2, recorded at R1 (*B*) and R2 (*C*). Note that regardless of recording site, S1+S2 responses are larger than responses elicited by stimulation of S1 or S2 alone, but less than the sum of the two responses. The insets are representative waveforms from the two recording locations, elicited from stimulation site 1 (calibration: 5 msec, 5 mV).

the 10 behavioral reversal trials, during which LTP was asymptotically elevated; and (3) the difference between the asymptotic value of LTP and the level of enhancement 24 hr following the first LTP stimulus session. The first measure gives an estimate of the rate of growth of LTP over the first six sessions, the second gives an estimate of the maximum enhancement attained, and the third provides an estimate of how much change occurred following the first high-frequency session in relation to the final LTP magnitude. For a measure of behavior, a cumulative error score was calculated for each of the rats from trials 6–16 during acquisition of the circular platform and was compared with the three indices of LTP for both the EPSP and population spike. Mean correlation coefficients for the EPSP and spike over the first six high-frequency stimulation trials versus cumulative errors were -0.265 and -0.282 , respectively, and were not statistically significant ($p > 0.05$). The coefficients for EPSP and spike during LTP asymptote on the 10 reversal

trials versus errors were not statistically significant either (0.134 and -0.287 , $p > 0.05$). Finally, correlation coefficients for EPSP and spike for the difference between initial magnitude of enhancement and asymptote versus errors were -0.272 and -0.156 , respectively, and again, were not statistically significant ($p > 0.05$).

Although we found no statistically significant correlation between circular platform performance and the three LTP induction parameters tested here, Jeffery and Morris (1993) have found correlations between asymptotic levels of EPSP enhancement and percentage of time spent in the training quadrant of the probe trial in the water task. The correlation coefficient between asymptotic levels of EPSP enhancement and probe trial performance of the six rats from batch 3 in the present experiment was 0.194 ($p > 0.05$); thus, no significant relationship was observed here between probe trial performance in the water task and LTP induction.

Experiment 2: multiple stimulation sites

Baseline responses

To assess the magnitude of LTP, the average EPSP amplitude during the initial 5 min period just prior to high-frequency stimulation was used as baseline and the average EPSP slope at 10–15 min afterward was used as the treatment value. Examples of response amplitudes in a typical experimental sequence resulting from each stimulating-recording electrode pair (S1R1, S1R2, S2R1, and S2R2) are shown in Figure 6*A*. The amplitudes of the responses following stimulation at 500 μ A were similar across all electrode pairings [$F(3,46) = 0.253$, $p > 0.05$]. The EPSP amplitude following simultaneous stimulation of S1 and S2 was larger than the response to either S1 or S2 alone across all stimulus intensities, but less than the sum of the responses to S1 and S2 (Fig. 6*B,C*).

LTP induced by high-frequency stimulation

The changes in EPSP amplitude following high-frequency stimulation at either stimulation site (S1 or S2) were similar between responses measured at either R1 or R2 [S1 normal paradigm: $F(1,9) = 2.47$, $p > 0.05$; S1 reversed paradigm: $F(1,4) = 0.87$, $p > 0.05$; S2 reversed paradigm: $F(1,4) = 1.66$, $p > 0.05$; S2 normal paradigm: $F(1,9) = 0.37$, $p > 0.05$]. Therefore, for simplification of presentation, the data following LTP-inducing stimulation were collapsed across R1 and R2 and expressed as a function of stimulation site only.

The EPSP amplitude was significantly increased by 16% following the high-frequency stimulation treatment to S1 in the “normal” paradigm (Fig. 7*A*). Subsequent administration of the LTP-inducing treatment to S2 alone was able to increase the synaptic response, over that induced by S1 treatment [$F(2,22) = 5.42$, $p = 0.013$; Scheffé post hoc comparison, $F = 4.32$, $p < 0.05$]; however, no additional EPSP growth was induced by the final S1+S2 high-frequency stimulation treatment (Fig. 7*A*; Scheffé $F = 0.02$, $p > 0.05$).

A similar pattern of results was also obtained in the “reverse” paradigm, in which the LTP-inducing stimulation was delivered via the S2 stimulation electrode first. That is, the EPSP amplitude was significantly enhanced by 12% after the first S2 treatment (Fig. 7*B*). High-frequency stimulation on S1 subsequently produced more enhancement, as measured via that stimulating electrode, than following the S2 treatment alone [$F(10,22) = 8.185$, $p = 0.0001$; Scheffé post hoc comparison $F = 13.448$, $p < 0.05$; Fig. 7*B*]. Again, no additional LTP was induced following the combined S1 + S2 high-frequency stimulation treatment (Scheffé $F = 0.55$, $p > 0.05$). For this paradigm, a significant decline was observed between the first high-frequency stimulation session of S2 and the combined S1+S2 session (Scheffé $F = 10.495$, $p < 0.05$). Although this could reflect significant depression of the response following repeated stimulation, more extensive testing of this stimulation site would be required to draw a strong conclusion.

Experiment 3: hippocampal distribution of *zif268* mRNA following LTP-inducing stimulation treatment

Electrophysiological data

Five of the six rats given the 100-pulse high-frequency stimulation treatment showed enhancement of the EPSP and population spike (mean fractional change at 30 min, 0.26 ± 0.06 SEM and 0.20 ± 0.09 SEM, respectively) with no afterdischarges in the EEG records. In one rat, the left hemisphere exhibited

Figure 7. Summary of changes in the amplitude of the EPSP after the first, second, and paired high-frequency stimulation sessions, for both the “normal” (*A*) and “reverse” (*B*) stimulation conditions (see text, experiment 2). No differences were obtained between recording sites R1 and R2; thus, data were combined and expressed as a function of stimulation site only. In the normal condition, the high-frequency (HF) stimulation was first delivered to S1, then to S2, and finally to both S1+S2. In the reverse condition, the high-frequency stimulation was delivered to S2 first. The main point to note is that additional modification could be induced through a second stimulating electrode beyond that produced by stimulation of an initial site.

afterdischarge and the right hemisphere did not. The right hemisphere only of that rat was included in the data presented below.

Anatomical distribution of *zif268* mRNA

Figure 8 shows representative examples of the septotemporal distribution of *zif268* mRNA activation from a rat with average LTP induction characteristics (Fig. 8*A*) and a rat that received MECS treatment (Fig. 8*B*). The average percentage *zif268* mRNA activation (11 hemispheres) following LTP induction is expressed relative to MECS control hemispheres in Figure 8*C*. This figure was constructed by aligning all hippocampal hemispheres, and averaging the values obtained from densitometric analysis along the entire 1 cm length of the structure. Although *zif268* activation in the dorsal hippocampus was approximately 60% of the MECS values, activation in the ventral hippocampus was almost zero. Thus, the average percentage activation over the entire hippocampus from these six rats was 33%, relative to the MECS values. In the one hemisphere that exhibited afterdischarge following high-frequency stimulation, the *zif268* activation showed the same distribution as MECS hemispheres.

Experiment 4: MECS, LTP, and spatial behavior on the water task

Effects of MECS on low- and high-frequency stimulus-treated hemispheres

The fractional changes of the EPSP for the following four conditions are pooled from protocols 1 and 2 described in Materials

Figure 8. *A* and *B*, Representative digitally enhanced autoradiograms illustrating the septotemporal distribution of *zif268* mRNA activation following “standard” LTP-inducing perforant path stimulation (*A*) and following MECS treatment (*B*). The section levels (in mm) at the left correspond to the x-axis of the plot in *C* (red indicates maximum activation). *C*, Mean and SEM relative percentage *zif268* mRNA activation following standard LTP stimulation parameters (compared to activation following MECS treatment) from 11 hemispheres of six rats, along the 1 cm septotemporal axis of the elongated hippocampus. These data suggest that the stimulation parameters typically used to induce LTP result in moderately strong activation restricted to dorsal hippocampal regions, and very weak activation in the ventral hippocampus.

and Methods (Table 1), and are illustrated in Figure 9*A*: (1) low-frequency stimulation alone, (2) low-frequency stimulation plus MECS, (3) high-frequency stimulation alone, (4) high-frequency stimulation plus MECS. Figure 9*A* shows that MECS treatment produced an increase in the magnitude of the EPSP above that produced by high-frequency stimulation alone, and also increased the size of the synaptic response in the hemispheres that did not receive LTP-inducing stimulation.

Effects of MECS on behavior

In the second protocol (Table 1), rats were tested in the spatial version of the swim task 24 hr after receiving either high-frequency (HF) stimulation alone, or high-frequency stimulation followed by MECS (HF + MECS) on 3 consecutive days. The HF + MECS group was significantly worse than the HF group in path length [$F(1,4) = 11.48, p < 0.03$] and latency [$F(1,4) = 10.23, p < 0.03$] to find the location of the hidden platform in the water pool (Fig. 9*B*).

In the third MECS protocol (Table 1), 12 additional rats were given pretraining on the Morris water task. Four of these rats were given the standard MECS treatment, four rats the sub-behavioral seizure treatment, and four rats the ear-clip-only (no shock) treatment for 3 consecutive days. Twenty-four hours after the last treatment, these rats were tested on a reversal

problem in the water task and were subsequently given a 60 sec probe trial in which the platform was removed. Figure 10 shows a scatter plot of the amount of time spent in the target quadrant on the probe trial versus the amount of EPSP enhancement observed in the individual rats. There was a statistically significant negative correlation between these variables ($p < 0.01$). That is, although some of the rats that showed signs of behavioral seizures following the MECS treatment did not show increased EPSP sizes, these rats also were not behaviorally impaired. Conversely, some rats that did not show behavioral seizures (subseizure parameters) did show both increased EPSPs and behavioral impairments.

In protocol 4 (Table 1), 15 of the 16 rats exhibited stable baseline responses and significant increases in the EPSP and population spike following the five daily MECS sessions (Fig. 11*A,B*). The data from the one rat that showed instability are not included here. From day 1 to 8 following MECS treatment, pairs of rats were tested on a spatial reversal problem in the water task. As can be observed in Figure 11, *A* and *B*, the evoked responses remained enhanced during this period (and for the entire 30 d of post-MECS testing). The overall performance in the water task was disrupted relative to the same rats' pre-MECS performance (Fig. 11*C*) both during the week following treatment (Fig. 11*D*; paired *t* test comparing time in target quadrant gave

Figure 9. *A*, Mean fractional change in the EPSP induced by low-frequency stimulation alone (*LF*), low-frequency stimulation plus maximal electroconvulsive shock treatment (*LF + MECS*), high-frequency stimulation alone (*HF*), and high-frequency stimulation plus MECS treatment (*HF + MECS*). Data are obtained from rats tested in protocols 1 and 2 of experiment 4 (see text). Note that synaptic enhancement was produced by MECS treatment alone, and that although additional LTP was induced in the HF group following MECS treatment, the combined treatment produced less LTP than the sum of the independent treatments. *B*, Swim path length (in cm) data for acquisition trials in the water task for rats tested in protocol 2 of experiment 4, in which either high-frequency stimulation alone (*HF*), or HF stimulation plus MECS treatment (*HF + MECS*) was given before training. The HF rats performed significantly better than did the HF + MECS rats.

$t = 2.91$, $p < 0.01$) and at the 31–33 d post-MECS test (Fig. 11E; $t = 3.99$, $p < 0.001$). On the other hand, there were no differences in mean escape latency or path length on the cued version of the swim task, in which the target was visible but was moved from trial to trial (paired t for latency, $p > 0.52$; paired t for path length, $p > 0.99$). Thus, the rats could accurately perform a nonspatial visual discrimination task, and swimming behavior was not impaired.

The time course of the MECS-induced LTP was bimodal. During the 5 d MECS treatment period, the EPSP responses exhibited an enhancement of about 20% (Fig. 11A). In the several days after the last MECS treatment, the EPSP enhancement declined somewhat; however, the initial decline was followed by a recovery back to approximately the initial asymptotic value, with very little change over the ensuing several weeks (Fig. 11A). On the other hand, the population spike did not exhibit the initial decline of MECS-induced LTP following the MECS

Figure 10. Data from experiment 4, protocol 3: water task performance versus EPSP enhancement (fractional change) following MECS or ear-clip-only treatment. The performance score is the time spent swimming in the target quadrant during the probe trial. There was a statistically significant relationship between LTP and performance. In these experiments, a subset of rats received subseizure electroconvulsive shock (*SECS*; see text).

treatment; however, following treatment, there was a gradual growth to the final asymptotic value (Fig. 11B). If the responses were decaying, the time constants would have to be on the order of 2 or more months. Examination of the EEG power at frequencies within the gamma (25–50 Hz) range (Fig. 11C) and within the theta (5–9 Hz) range (Fig. 11D) revealed an increase in the power of these EEG frequencies following the MECS treatment; however, this increase in power returned to baseline levels with a time constant of 3–5 d.

Discussion

The present results reconfirm earlier conclusions that stimulation leading to bilateral “saturation” of LTP/LTE in the dentate gyrus can cause a disruption of spatial learning performance, and shed light on why this effect may be difficult to observe under some experimental conditions. There are three principle findings: (1) the same stimulation procedures used to “saturate” LTP bilaterally at perforant path–granule cell synapses, in the absence of seizures, produce a deficit in one spatial learning task and not in another; (2) the standard stimulation procedures used to induce LTP saturation do not actually lead to full saturation at any dorsoventral hippocampal level and, in particular, leave the ventral half of the hippocampus virtually unaffected; (3) conditions (MECS) that lead to more complete saturation of LTP and that activate the entire fascia dentata produce learning impairments on a spatial task whose performance is not significantly affected by less saturating stimulation. In the case of MECS-induced LTP, the spatial performance deficit is related, at least linearly, to the amount of LTP saturation (Fig. 10); this treatment did not, however, lead to an impairment of the cued version of the task.

Rats that received bilateral, high-frequency, perforant path stimulation made significantly more errors on the circular platform task, corroborating the previous results of a similar experiment reported by McNaughton et al. (1986). It should be pointed out, however, that the effect in the present study was not as large as in the earlier experiment. On the other hand,

Figure 11. Data from experiment 4, protocol 4. Fifteen rats were recorded from daily for 43 d, and were behaviorally tested on the spatial version of the water task before, and at two times following five MECS treatments. *A* and *B*, EPSP (*A*) and population spike (*B*) amplitude normalized to the means of the pre-MECS period (open bar indicates MECS treatment days; the solid circles indicate responses 24 hr following MECS treatment). Responses were collected for 30 additional days, during which very little LTP decay occurred. (Note: data collection began on day 2.) *C* and *D*, Mean EEG power versus time (days) for frequencies in the gamma band (i.e., 25–50 Hz, *C*) and in the theta band (i.e., 5–9 Hz, *D*). The increase in power in both the theta and gamma frequencies recovered with a time constant of 3–5 d. *E–G*, Search times spent in the four quadrants of the water pool (hatched bars indicate target quadrants) are shown for the probe trials following the initial acquisition of the swim task (*E*), following the first reversal training session (days 1–8 following MECS treatment, *F*), and following the second reversal training session (days 31–33 after cessation of MECS treatment, *G*). A significantly greater amount of time was spent searching the target quadrant before MECS treatment (*E*), but there was no significant differential search during the first week, or at 30 d following this treatment (*F* and *G*, respectively). During both time periods the EPSP and population spike responses were significantly elevated above baseline (*A* and *B*, respectively), whereas the changes in EEG frequencies were only apparent during the first reversal training session (*C*, *D*). (Note: the target quadrant was different for each test; dashed line indicates chance performance levels.)

when a subset of the rats tested on the circular platform were also tested on the swim task ($n = 12$), using a procedure similar to that used in the Castro et al. (1989) study, no behavioral differences were observed between treatment groups. This result is consistent with a number of studies in which "saturation" treatment did not result in spatial learning deficits (Cain et al., 1993; Jeffery and Morris, 1993; Korol et al., 1993a; Sutherland et al., 1993). It is unclear, at present, why the two, conceptually similar, spatial learning tasks are affected differently by perforant path stimulation (and *presumably* by the resulting disruption of the perforant path synaptic weight distribution). One can only speculate either that the mnemonic resource requirements are more stringent for one task than for the other, or that the possibly stronger motivational aspects of the swim task produce more favorable conditions for storage and hence permit the animal to overcome partial LTP saturation.

Part of the answer to the more general question of why LTP saturation experiments lead to highly variable results may lie in the probable nonlinear relationship between memory disruption and saturation of the synaptic weight distribution (Fig. 1). The present combination of electrophysiological and anatomical results clearly indicate that the normal, bilateral stimulation procedures leave this distribution below the theoretical saturation point (i.e., 50%), at which memory error rate is expected to exhibit a rapid increase (e.g., Buckingham and Wilshaw, 1993). The electrophysiological observations demonstrate that saturation is not complete locally in the dorsal hippocampus (Fig. 7). Furthermore, the distribution of *zif268* mRNA activation following LTP-inducing stimulation, which can be taken as a reasonable anatomical marker of those cells affected by the stimulation event, suggests that it is unlikely that the more ventral perforant path-granule cell synapses were affected at all by the standard stimulus placements. In the present studies, *zif268* activation levels were about 33% of those that could be induced by MECS, in which the granule cells over the entire dorsoventral extent of the hippocampus are affected (Fig. 8).

Following from the fact that both LTP stimulation and MECS treatment activate *zif268* (e.g., Cole et al., 1989), and the latter does so along the entire septotemporal extent of the hippocampus, we examined the hypothesis that MECS may in fact lead to more anatomically complete LTP than the standard bilateral stimulation. A suggestion that this might be the case was previously made by Stewart and Reid (1993a) on the basis of the observation that animals given prior MECS treatment, and subsequently investigated in acute experiments, showed larger evoked responses and less LTP than controls. We examined this hypothesis directly by recording from chronically prepared rats following MECS treatment. MECS treatment did strengthen the perforant path-granule cell synaptic connection, and, as suggested by Stewart and Reid (1993a), this effect showed mutual occlusion with LTP produced by standard stimulation. Furthermore, MECS also resulted in a deficit in the spatial version of the Morris water task (cf. Stewart and Reid, 1993b). The behavioral deficit in the present study paralleled the time course of the LTP and not the transient increase in EEG power at theta and gamma frequencies that occurred in the week following the MECS treatment. Although both the behavioral deficit in the present experiment and that observed on the circular platform may result from effects other than hippocampal LTP, the water task deficit was correlated with the magnitude of LTP at the time of testing and not with whether the rats had undergone a

behavioral seizure (cf. McGaugh and Alpern, 1966) during the two types of electroconvulsive shock treatments given (Fig. 10). Furthermore, the effects of MECS appear to depend on NMDA receptor activation, as the noncompetitive NMDA receptor antagonist ketamine blocks the increase in hippocampal evoked response size following MECS treatment (Stewart and Reid, 1993c). Taken together, these results suggest that the wider distribution and possibly greater local levels of synaptic change in the hippocampus following MECS treatment may produce more reliable spatial learning deficits. The results are also consistent with the recent findings that, when rats are given unilateral hippocampal lesions, and then "saturation" treatment on the contralateral side, impairments can be observed in acquisition of the Morris water task (Mumby et al., 1993).

Interestingly, although persistence of LTP was previously correlated with performance deficits on the circular platform (Barnes, 1979), no correlation has been found with initial induction of LTP. In experiment 1 here, when a cumulative error score on acquisition of the circular platform was compared to three different measures of LTP induction in the 17 rats that were given high-frequency stimulation in that experiment, no statistically significant correlations were obtained. On the other hand, in the present studies, water task performance was negatively correlated with MECS-induced LTP. The failure to find such a correlation in the circular platform task may be due to differences in the reliability of the dependent measures in the two tasks. Differential quadrant search is a continuous measure, whereas number of errors is discrete, quite variable from trial to trial, and influenced more by the animal's initial trajectory than its confidence that it has found the correct location.

In contrast to the negative correlation with performance obtained in our MECS studies, Jeffery and Morris (1993) have obtained significant positive correlations between probe trial performance in the water task and cumulative EPSP enhancement using the standard bilateral stimulation procedure. This was not observed in our experiments, or in recent experiments by Cain et al. (1993); however, a positive correlation using the standard, incomplete saturation conditions would not be inconsistent with a negative correlation using MECS. As suggested by Jeffery and Morris (personal communication), the former method may measure essentially the intrinsic capacity for LTP, which is predicted to be positively correlated with performance, whereas LTP measured after MECS may reflect more the extent of saturation (or a combination of the two effects). Because intrinsic LTP capacity and extent of saturation would be expected to lead to opposite correlations, and are confounded with current measurement methods, variable results of correlation analyses are to be expected.

Although it is not possible to specify all the changes that may occur following repeated high-frequency stimulation treatment as administered here, it is of interest to note that no long-lasting changes were observed in the EEG. Moreover, it is clear that circular platform performance deficits were not due to afterdischarges following LTP-inducing treatment (cf. Cain et al., 1993). A transient increase in the EEG power at frequencies between 20 and 50 Hz was, however, observed immediately following high-frequency stimulation, but subsided to near baseline levels in the 100 sec following the high-frequency treatment. At present, it is not clear what this change might reflect. There has been considerable recent interest in EEG oscillations in the gamma frequency band (i.e., 20–60 Hz) as a possible mechanism of synchronizing neuronal activity in various regions of the cortex

during perceptual processing (e.g., Gray and Singer, 1989). Thus, it is possible that alterations in this frequency band may have lead to alterations in such processing mechanisms. Interestingly, the power at the theta frequencies did not change during this same time period.

In conclusion, repeated, bilateral high-frequency stimulation that induces perforant path LTP in dorsal hippocampus does lead to memory impairments in some spatial tasks. With stimulation that produces more extensive LTP (MECS), a correlation can be demonstrated between the LTP magnitude and behavioral impairment. While indirect effects of the electrical stimulation per se can never be completely excluded, one necessary condition for the hypothesis that attempts to relate LTP to learning has been reconfirmed.

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