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# An Examination of the Relations Between Hippocampal Long-term Potentiation, Kindling, Afterdischarge, and Place Learning in the Water Maze

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## ABSTRACT

Two approaches were used to study the relations between the acquisition of place learning in the Morris water maze and long-term potentiation (LTP), kindling, and afterdischarge (AD). In the first, the possibility of behavioral LTP in the dentate gyrus field potential evoked by stimulation of the perforant path was evaluated in rats that showed robust place learning in the water maze. There was no effect of place learning on the field potential, and field potential measures did not correlate with place learning acquisition measures. In the second approach, the effect of bilateral saturation of LTP on subsequent place learning in the water maze task, begun within 5 minutes of the last LTP session, was evaluated. The effect of kindled seizures evoked bilaterally from the perforant path, or of a single unilateral AD, on acquisition of the water maze task (begun within 10 minutes) were also evaluated. Bilateral LTP saturation did not affect place learning, and the bilateral LTP group learned as readily as controls. In contrast, the kindled and AD groups were severely impaired in their performance of the place learning task. A second day of training in the water maze without any further electrical stimulation indicated that these groups had acquired considerable information on the first day of maze training and were not distinguishable from controls on the second day of training. This indicated that the deficit in these groups on the first day of training was temporary and likely resulted from a temporary perturbation of normal brain function due to the seizures. The results indicate that bilateral saturation of LTP in the dentate gyrus does not affect place learning in the water maze. They also indicate that recent hippocampal seizures, but not kindling, disrupt place learning in this task.

**Key words:** afterdischarge, kindling, LTP, place learning, Morris water maze, seizures

Current interest in electrophysiological models of synaptic plasticity has led us to reexamine the relation between long-term potentiation (LTP) phenomena and associative learning in intact animals. Two approaches that have been used to explore this relation are the behavioral LTP and the prior LTP paradigms.

In the behavioral LTP paradigm, a baseline measure of evoked field potential magnitude is obtained from a brain circuit thought to be involved in the behavioral task under study. The animal is then trained in the behavioral task, and the evoked potential is remeasured afterwards. Any LTP-like augmentation of the field potential that results from the learning could be taken as evidence of a functional link between the two phenomena. Behavioral LTP has been reported to occur as a result of learning a variety of discrimination and conditioning tasks (Ruthrich et al., 1982; Weisz et al., 1982; Roman et al., 1987; Skelton et al., 1987). Exposure to novel or complex environments also has been reported to cause behavioral LTP, although the effects have not always been consistent across experiments (Sharp et al., 1985a; 1985b; 1986; Green and Greenough, 1986; Keith et al., 1988; Hoesing et al., 1991). However, we failed to find evidence of behavioral LTP after the learning of the eight-arm radial maze or one-way active avoidance tasks (Hargreaves et al., 1990).

The second approach, the prior LTP paradigm, involves first saturating the inducible LTP in a neural circuit and then training the animal in a learning task. A comparison is then made between the rate of learning by animals that have received prior LTP saturation and the rate of controls. If learning is disrupted as a result of prior LTP, this could indicate that LTP saturated the available plasticity, thus disrupting the establishment of new memory. There are reports of disruption

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of learning by prior LTP saturation of the perforant path input to the dentate gyrus (PP-DG) in the Barnes circular platform task (McNaughton et al., 1986) and the Morris water maze task (Castro et al., 1989). However, LTP saturation did not disrupt acquisition of new spatial information in animals that had first been trained in the procedural requirements of the maze tasks (McNaughton et al., 1986; Sutherland et al., 1991), indicating that prior knowledge of task requirements might eliminate the disruptive effects of LTP saturation on acquisition of new spatial information. Other studies using this approach have shown that prior LTP can facilitate some forms of learning in which electrical stimulation of the PP serves as a discriminative stimulus (Berger, 1984; Skelton et al., 1985), although it is not clear that LTP was saturated in these studies.

In view of the mixed results of these experiments, we decided to study both paradigms in rats undergoing place learning in the water maze (Morris, 1984). This spatial task offers the advantage that the training normally proceeds very quickly and can be completed in 1 day. Successful acquisition of this task depends on the integrity of the hippocampus, especially the dentate gyrus (Morris et al., 1982; Sutherland et al., 1983), which was the site studied.

The behavioral LTP experiment was intended to detect the occurrence of LTP-like changes in the PP-DG evoked field potential after place learning. At the end of the experiment, the ability of the recording arrangements to detect electrophysiologically induced LTP was evaluated using the same electrodes and conventional stimulation and recording techniques.

In addition to examining the effect of prior bilateral LTP of the PP-DG circuit in rats learning the water task for the first time, we examined the effects of epileptiform activity on two groups of rats. One group experienced a single epileptiform afterdischarge (AD) in response to electrical stimulation of the PP, while the other group was first kindled (Racine, 1978) by stimulation of the PP in each hemisphere and then trained in the maze.

## MATERIALS AND METHODS

### Surgical procedures

Male hooded rats weighing 300–400 g received implantation of a bipolar stimulating electrode aimed for the PP (AP: −8.1 mm, ML: 4.4 mm) and a recording electrode in the hilus of the DG (AP: −3.8 mm, ML: 2.4 mm). Measurements were from bregma, with bregma and lambda in the same horizontal plane. Electrodes were made from Teflon-coated stainless steel wire 127 μm in diameter. Recordings from the monopolar hilus electrode were referenced to a surgical screw in the skull. Final placement was optimized by recording evoked potentials before cementing the electrodes in place. Rats were selected for this study if they displayed an evoked field potential, consisting of a positive excitatory postsynaptic potential (EPSP) upon which was superimposed a negative population spike (PS), at an intensity not greater than 100 μA.

### Electrophysiological and behavioral procedures

#### *Behavioral LTP experiment*

Rats (n = 10) used in this experiment carried implants in one hemisphere. After recovery from the surgery, an input/output (I/O) curve was determined using five or six pulse intensities. From this full I/O curve, three test pulse intensities were selected (low, medium, and high) to be used in all subsequent I/O curves (see Fig. 1). This was done to reduce the total number of pulses delivered, and therefore the possibility of potentiation effects due to delivery of pulses at low frequency (Skelton et al., 1983; unpublished observations), and to allow evaluation of field potential properties near the bottom and top, as well as the middle of the I/O curve. Test pulses were biphasic, with each phase 0.1 ms in duration. Stimulation was delivered bipolarly, with current applied to one pole of the stimulating electrode and returned through the other pole. Ten responses to pulses of each intensity were averaged and analyzed by a microcomputer and software developed in our laboratory. In line with previous studies from this laboratory, evoked potential recordings were taken while the rats were behaviorally "clamped"; that is, all pulses were delivered only when the rats were awake and immobile, with their head held up against gravity (Hargreaves et al., 1990). The measures of interest were: (1) the maximum slope of the rising phase of the population EPSP and (2) the amplitude of the dentate granule cell PS measured as the length of a line joining the negative peak and a line drawn tangent to spike onset and offset (see Fig. 1). Abbreviated I/O curves were determined daily until the responses were stable, at which point a final baseline I/O curve was taken.

On the day after the final baseline, rats began acquisition of the location of a hidden platform in a circular water maze (Morris, 1981) located in a room that contained various distal cues. The maze was 1 m in diameter, painted black, and the hidden platform was obscured with a layer of small woodchips floating on the surface, which were renewed after every trial. Place learning acquisition in this maze is very similar to acquisition observed in a larger (2 m diameter) maze (unpublished observations). Animals first received a baseline probe trial that consisted of 60 seconds in the maze without a hidden platform.

Training in the water maze task was begun 1 hour later. Rats received four blocks of four trials each during which they learned to escape onto a platform hidden approximately 1 cm beneath the surface from one of four starting positions 90 degrees apart. Individual trials were terminated if the rat did not find the platform within 60 seconds, and the rat was then placed on the platform for 15 seconds. The blocks of trials were 1 hour apart. All trials were videotaped for later analysis.

Thirty minutes after the last training trial a post acquisition abbreviated I/O curve was obtained. Fifteen to 30 minutes later a postacquisition probe trial with the platform removed was conducted to evaluate whether the rats had learned the location of the platform. A circle centered on the platform location and consisting of 25% of the total area of the water maze was placed on the video monitor. Time spent swimming with the head in this circle in each 60 second probe trial was recorded and used as an indicator of whether the rat had learned the location of the platform.

In order to obtain a 24-hour retention probe trial that was not contaminated by the last unreinforced probe trial, beginning 5 minutes after the postacquisition probe trial we allowed the rats a fifth block of four reacquisition trials with the hidden platform in the same place as for the previous four blocks of reinforced trials. Twenty-four hours later a final probe trial

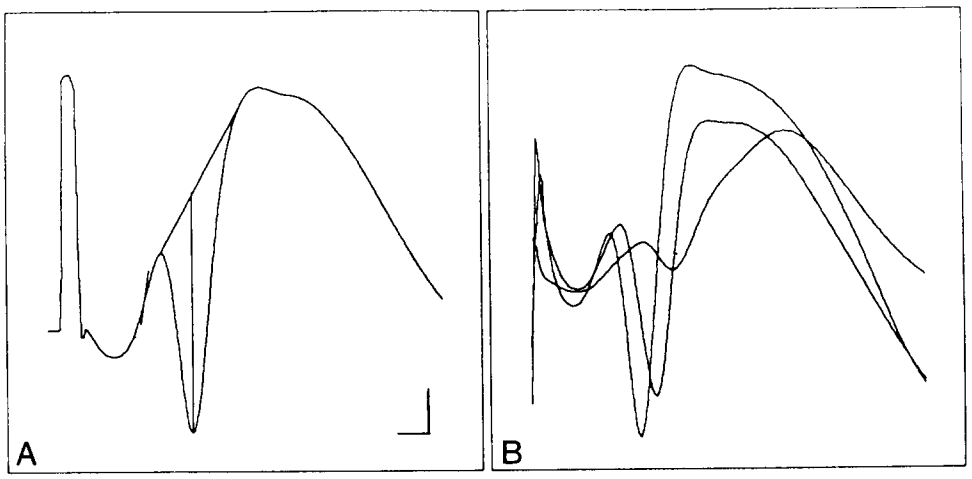


Fig. 1. (A) Typical field potential evoked in the dentate gyrus by stimulation of the perforant path, showing measures of the EPSP maximum slope and amplitude of the population spike. Calibration, 1 ms and 2 mV. (B) Field potentials evoked from a rat in Experiment 1 by single test pulses of low, medium, and high intensity.

was run again with the platform removed. This was followed by a final abbreviated I/O curve.

In order to confirm that the stimulation/recording arrangements would support electrophysiologically induced LTP in these rats, high-frequency trains were administered 2 weeks later. These consisted of 20 trains spaced approximately 20 seconds apart, delivered only when the rat was immobile. Each train consisted of eight pulses at 400 Hz, and each pulse was 0.1 ms in duration. Input/output curves were obtained immediately prior to, and 1 and 24 hours after administration of the high-frequency trains, in a manner that paralleled the earlier learning phase of the experiment.

### *Prior LTP experiment*

Procedures were similar to those in the behavioral LTP experiment except that most of the rats received bilateral PP-DG electrode implants (see below). Only animals with acceptable evoked responses (as defined above) from both implants were used. Once accepted into the study on the basis of the initial evoked responses, all rats were carried through the entire experiment, unless indicated otherwise.

Rats in the LTP Group (n = 14) received bilateral PP-DG electrode implants. After determination of I/O curves as described above, abbreviated I/O curves were determined from each hemisphere for 5 baseline days. During the next 14 days the following procedure was applied each day to each hemisphere: determination of an abbreviated I/O curve, followed by 10 trains of high-frequency pulses (each train consisting of 10 biphasic pulses, each phase 0.1 ms in duration, delivered at 400 Hz), followed immediately by determination of another abbreviated I/O curve. The test pulses and high-frequency trains were applied every 10 seconds without regard for the behavior of the rat at the time of train delivery. Two rats in the LTP group failed to complete the experiment because the field potential in one hemisphere deteriorated before the end of the procedure; these 2 rats were eliminated, leaving 12 rats in that group. High-frequency pulses were delivered to the PP either mono- or bipolarly for different rats in the LTP Group, but as there was no difference in the amount of LTP that resulted, the data from these rats were analyzed as a group. Most rats received high-frequency pulses at an intensity that was between 80% and 100% of that required to evoke maximal PS amplitude. However, to ensure complete saturation of LTP, a number of rats received high-frequency pulses at an intensity that was triple this value. Routine recording of electrographic activity on a polygraph during the induction of LTP indicated that at no time was afterdischarge (AD) evoked in any rat as a result of the electrical stimulation. Abbreviated I/O curves were similarly determined in both hemispheres of control group rats (n = 3), but no high-frequency trains were applied. A further control group (n = 5) received bilateral implantation of electrodes but were not electrically stimulated.

Additional groups of rats with unilateral (n = 5) or bilateral (n = 5) PP-DG implants experienced either a single epileptiform AD (AD group) or underwent bilateral kindling (kindled group), respectively. Electrical stimulation (1 second of 60 Hz biphasic square pulses, each pulse 1.0 ms in duration) was applied in an ascending series of intensities beginning at 200 μA until a single AD was elicited from the AD group rats. These rats were then trained in the water maze (see below). The kindled group rats were stimulated similarly until an AD was elicited from each PP electrode. The stimulation intensity that was effective for evoking AD at each electrode was then applied once daily to that electrode until fully developed stage 5 seizures (Racine, 1972) occurred. When two brain sites are stimulated alternately each day in this manner, typically only one site generates stage 5 convulsions; the other site generates weaker convulsions (Burchfiel and Applegate, 1989). Therefore, the kindled group was kindled until two or three stage 5 convulsions had been evoked from one hemisphere; at this point the contralateral site generated stage 1–4 convulsions when stimulated.

Shortly after completion of these treatments rats began acquisition of the water maze task. Procedures were the same as for the behavioral LTP experiment, with the following exceptions. Training consisted of six blocks of two trials; blocks were separated by a 2-minute interval. The first trial was pre-

ceded by, and the last trial was followed by, single 60-second trials with the platform removed. These procedures followed those of Castro et al. (1989). In addition to the initial day of water maze training, the probe and training trials were repeated a second time, on the following day. On day 2 of training the rats received no additional electrical stimulation of any kind, and the platform position was the same as on day 1 of maze training. Day 2 of maze training was undertaken to measure retention from the training session on day 1, and to evaluate the persistence of any performance deficits that occurred on that day.

In a pilot experiment, rats (n = 8) that had received bilateral LTP saturation began water maze training approximately 18 hours after the completion of LTP saturation. All of the experimental rats learned to find the hidden platform as quickly as controls, and the performance of the groups did not differ (unpublished observations). In order to maximize the possibility of observing a disruptive effect of LTP saturation on water maze performance, we began water maze training in the LTP and control groups within 5 minutes of the completion of the LTP treatment. The AD and kindled groups began water maze training after the postictal suppression of the hippocampal electroencephalogram had subsided and after behavior had normalized (i.e., within 10 minutes after the last AD).

## RESULTS

### Behavioral LTP experiment

#### *Water maze acquisition*

MANOVA within-subject repeated measures analyses were conducted on the latencies required to find the platform over the 16 trials. This showed that the rats mastered the task, as indicated by a significant reduction in the latency to find the platform over trials [$F(3,27) = 21.08$; $P < .0005$; see Fig. 2]. The mastery of the task was further confirmed by the probe trial data, which indicated that the rats spent significantly more time swimming in the location of the removed platform after the fourth block of training trials and after the fifth block of reacquisition trials compared to the baseline probe trial [$F(2,18) = 87.56$; $P < .0005$; see Fig. 2].

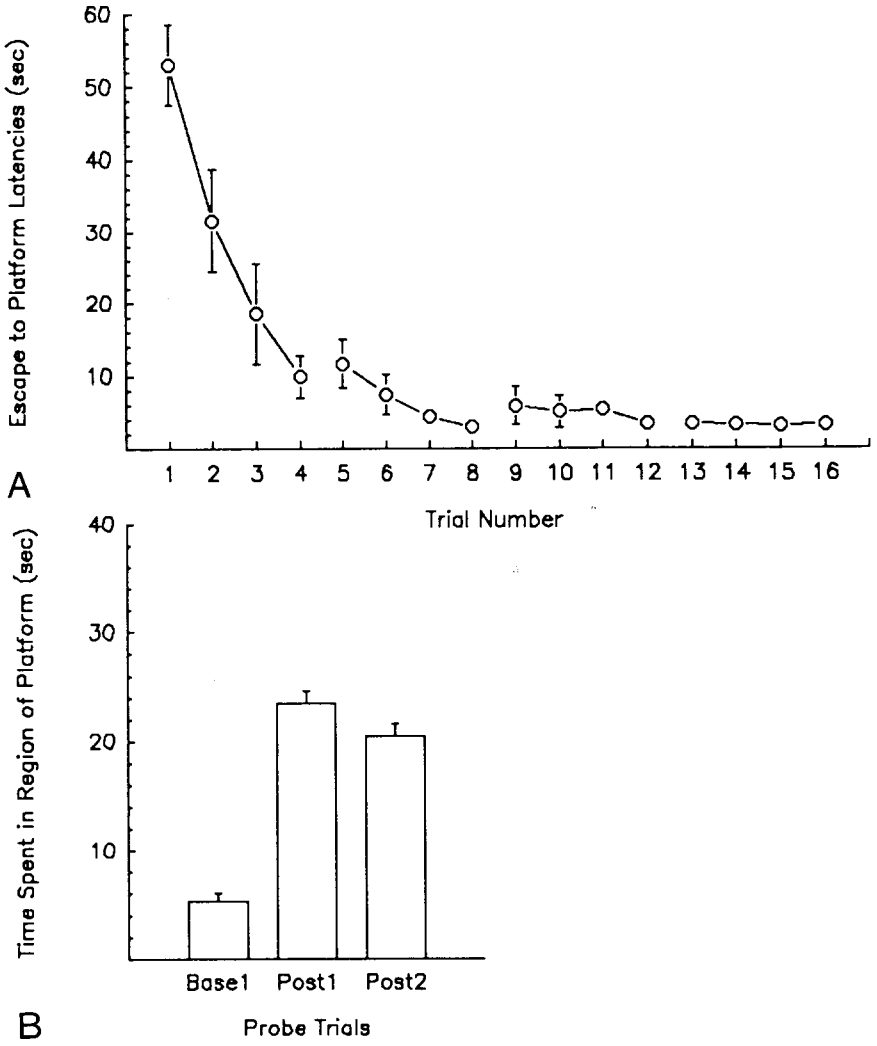


Fig. 2. (A) Mean latencies required to escape onto the hidden platform in each of 16 trials in the water maze. (B) Probe trial measures of time spent swimming in the location of the removed platform before training (Base 1), 45–60 minutes after the fourth block of training trials (Post 1), and 24 hours later (Post 2).

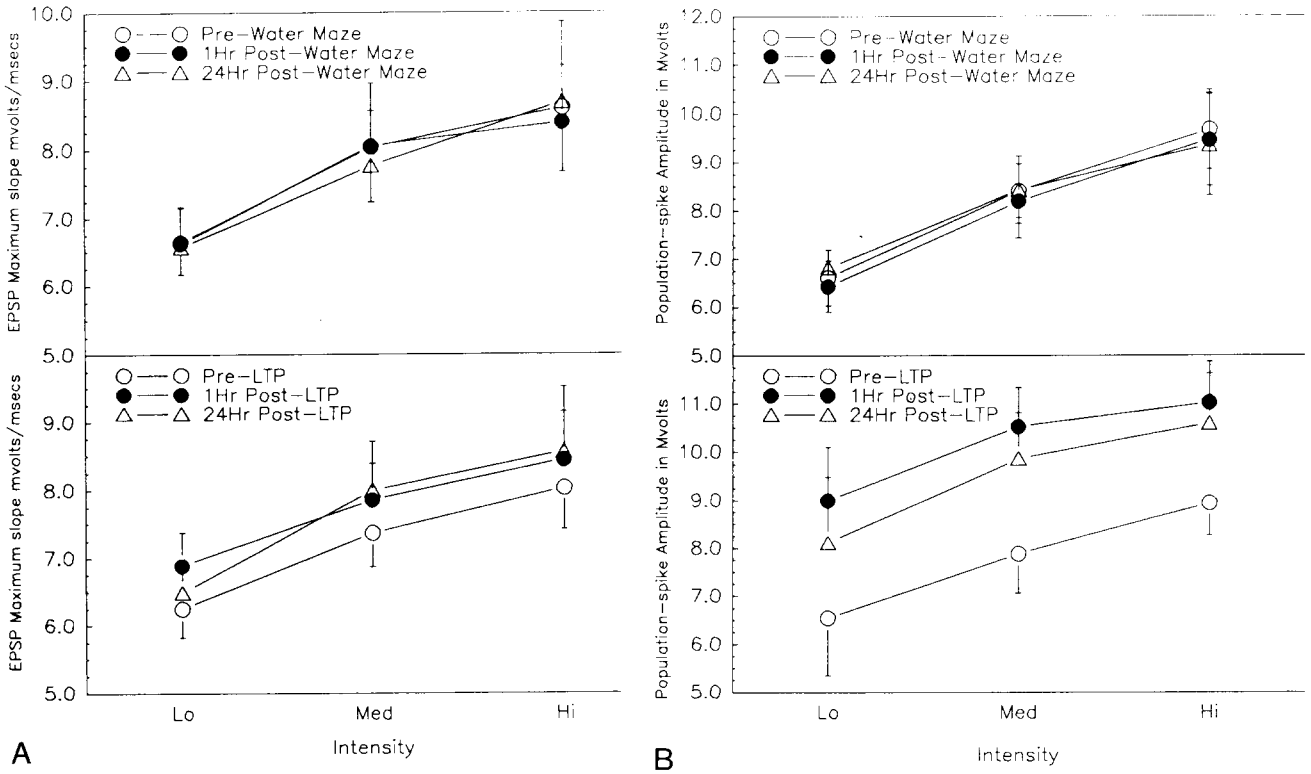


Fig. 3. Abbreviated I/O curves of EPSP (A) and population spike (B) measures obtained before and at 1 and 24 hours after water maze training (top) and electrophysiological LTP (bottom).

### *Evoked field potential responses*

Abbreviated I/O curves were obtained at baseline (premaze training), 1 hour posttraining, and 24 hours posttraining (see Fig. 3). MANOVA within-subject repeated measures analyses were conducted on both EPSP and PS raw data together. There was no effect of time (pre- and posttraining) on the measures [$F(4,36) = .77$; $P > .55$]. Similar analyses on the EPSP and PS data transformed to difference scores and to proportional change scores (relative to baseline) similarly failed to yield results that approached significance (data not shown).

### *Electrophysiological LTP*

MANOVA within-subject repeated measures analyses were conducted on both EPSP and PS raw data together (see Fig. 3). There was a significant main effect of time [$F(4,36) = 5.535$; $P < .001$], indicating that the measures taken before, 1 hour, and 24 hours after application of the high-frequency trains differed significantly. Univariate repeated measures F tests for the EPSP and PS data indicated highly significant changes in each of these individual measures [$F(2,18) = 16.82$; $P < .0005$ and $F(2, 28) = 24.38$; $P < .0005$ respectively]. Similar analyses on the EPSP and PS data transformed to difference scores and to proportional change scores (relative to baseline) also yielded highly significant main effects and significant univariate results for the EPSP and PS measure taken alone (data not shown).

### *Correlations between LTP and behavioral measures*

In a further evaluation of the relation between water maze performance, evoked responses taken before and after water maze acquisition, and electrophysiological LTP, product-moment correlations were calculated between water maze performance measures and the evoked field potential measures. Performance measures included summed escape latencies from the first two blocks of four trials, the last two blocks of trials, all blocks of trials, and changes in probe trial search time from the pretraining probe trial to both posttraining probe trials. Both percent change (relative to pretraining baseline) and difference scores (absolute value of change from pretraining baseline to posttraining measures) were calculated for both EPSP and PS measures in both the behavioral LTP and electrophysiological LTP situations. A total of 240 correlation coefficients were calculated. Three of these, relating maze acquisition and subsequent electrophysiological LTP measures, were statistically significant; two were negative and one was positive. No pattern of correlations relating maze acquisition and evoked field potential or electrophysiological LTP measures emerged from these correlational matrices.

## Prior LTP experiment

### *Electrophysiological and LTP results*

Table 1 presents some of the electrophysiological and LTP results of the LTP group from the first LTP session. The mean PS amplitude evoked by the high-intensity test pulses was more than 12 mV, which indicates that the responses were robust. Table 1 shows that the increase in PS amplitude, both in terms of amplitude in mV and in percent increase, was greatest for responses evoked by low-intensity test pulses and smallest for responses evoked by high-intensity test pulses.

Data collected after the first and last LTP sessions were

**Table 1.** Electrophysiological Results of LTP Group From First LTP Session

| Stimulation Intensity | Test Pulse Intensity (μA) | Population Spike Amplitude on Last Baseline Day (mV) | Population Spike Amplitude Increase Due to LTP (mV) | Population Spike Amplitude Increase Over Baseline Due to LTP (%) |
|---|---|---|---|---|
| Low | 125.0 + 15.2 | 4.2 + 0.67 | 3.2 + 0.59 | 130 + 39.0 |
| Medium | 335.5 + 48.6 | 8.9 + 0.94 | 2.7 + 0.38 | 40 + 9.5 |
| High | 862.5 + 48.6 | 12.3 + 0.97 | 1.2 + 0.46 | 13 + 6.0 |

Values represent the overall mean and SEM of both left and right hemisphere data.

analyzed separately by within-subjects factorial repeated-measures MANOVAs. Within each analysis EPSP slope and PS amplitude data were analyzed simultaneously. However, because data for the lowest intensity test pulses were unavailable for 2 rats for the last LTP session, data for only 10 rats were used in this analysis. The effect of the high-frequency pulses was significant for both the first [$F(2,10) = 35.7$; $P < .0005$] and last LTP sessions [$F(2,8) = 8.4$; $P < .01$] compared to the last baseline measure, indicating that LTP occurred as a result of the first LTP session and that LTP was present after the last LTP session. The main effect of hemisphere was not significant for either analysis [$F(2,10) = 0.56$; $P = .59$ and $F(2, 8) = 0.57$; $P = 0.59$], indicating that there was no significant difference between the amount of LTP obtained in the left and right hemispheres. A significant interaction of intensity X time for the data from the first LTP session [$F(4,44) = 4.8$; $P < .003$] confirms the impression from Table 1 that the amount of LTP obtained was significantly related to test pulse intensity.

### *AD and kindling*

A single AD occurred in all rats in the AD group (mean duration = 29.8 seconds, SEM = 8.1; mean convulsion stage = 0.2, range = stage 0–1). Afterdischarges and kindled seizures occurred in all rats in the kindled group. One hemisphere kindled fully to stage 5 in all but one rat in this group (mean convulsion stage = 4.6, SEM = 0.4). Stimulation of the contralateral hemisphere evoked a submaximal convulsion (mean convulsion stage = 1.4, SEM = 0.7).

### *Water maze acquisition*

The water maze acquisition results appear in Figure 4. MANOVA repeated-measures analyses were conducted on the latencies required to find the platform over six blocks of two trials each. Because the performance of the stimulated and unstimulated control groups did not differ, their data were collapsed for the analyses. There were overall significant effects of blocks on both the first [$F(5,130) = 29.20$; $P < .0005$] and second days of training [$F(5,130) = 17.61$; $P < .0005$], and overall significant differences among groups on the first day [$F(3,26) = 12.58$; $P < .0005$] but not on the second day of training [$F(3,26) = 1.08$; $P > .05$]. Specific $t$ value contrasts were then performed on the overall summed escape latencies using separate variance estimates to compensate for the heterogeneity of variance in different groups. These analyses revealed that both the kindled group and the AD group, but not the LTP group, differed significantly from the control group on the first day of training ($P < .02$, $P < .05$, and $P > .05$, respectively). Similarly $t$ value contrasts between groups were done for the probe trial data before and after training on the first day and for probe trial retention data 24 hours later, just before training on the second day. The omnibus F tests in these analyses are equivalent to group X time interactions across the probe trials. Results for the probe trial data indicated a significant overall effect for before vs. after training on the first day [$F(3,26) = 7.77$; $P < .0007$] and for before training on the first day vs. before training on the second day (24-hour retention) [$F(3,26) = 4.55$; $P < .01$]. The $t$ value contrasts showed that for both of these comparisons, both the kindled and AD groups were impaired with respect to the control group (all $P < .05$), but that the LTP group did not differ from the control group for either comparison (both $P > .05$). A final comparison at the end of training on the second day showed that all groups were equivalent in the amount that they retained (all $P > .05$).

These results show that the LTP and control groups acquired the task equally quickly, but that the kindled and AD groups were significantly impaired. By trial 6, and on all subsequent trials, the LTP and control groups were able to find the platform in a mean of less than 10 seconds (Fig. 4). Retention measures provided by the probe trial data were consistent with these findings in that they showed impairment in the kindled and AD groups relative to the control and LTP groups, which did not differ between themselves. By the end of the second day all groups had mastered the task to an equal degree.

### *Relations between LTP and water maze acquisition*

The amount of LTP induced in the LTP Group did not appear to predict any aspect of their water maze performance. In order to examine this further, we attempted to relate measures of LTP in both hemispheres of each rat to that rat's acquisition performance in the water maze. The behavioral measures used were: summed latency to find the platform on training day 1; final probe trial score minus initial probe trial score on training day 1; initial probe trial score on training day 2 minus initial probe trial score on training day 1. The LTP measures were: change (compared to baseline) in EPSP slope or PS amplitude on LTP day 1, LTP day 14, or LTP days 1 and 14. Left and right hemisphere data for the low- and medium-intensity test pulse intensities were averaged, and both percent change and difference scores (see Hargreaves et al., 1990) were calculated. Thirty-six product-moment correlation coefficients were calculated between all pairs of behavioral and potentiation measures. The coefficients ranged between −0.59 and +0.35. None of the coefficients was statistically significant, and there was no pattern in the correlation matrix that suggested a functional relation between the

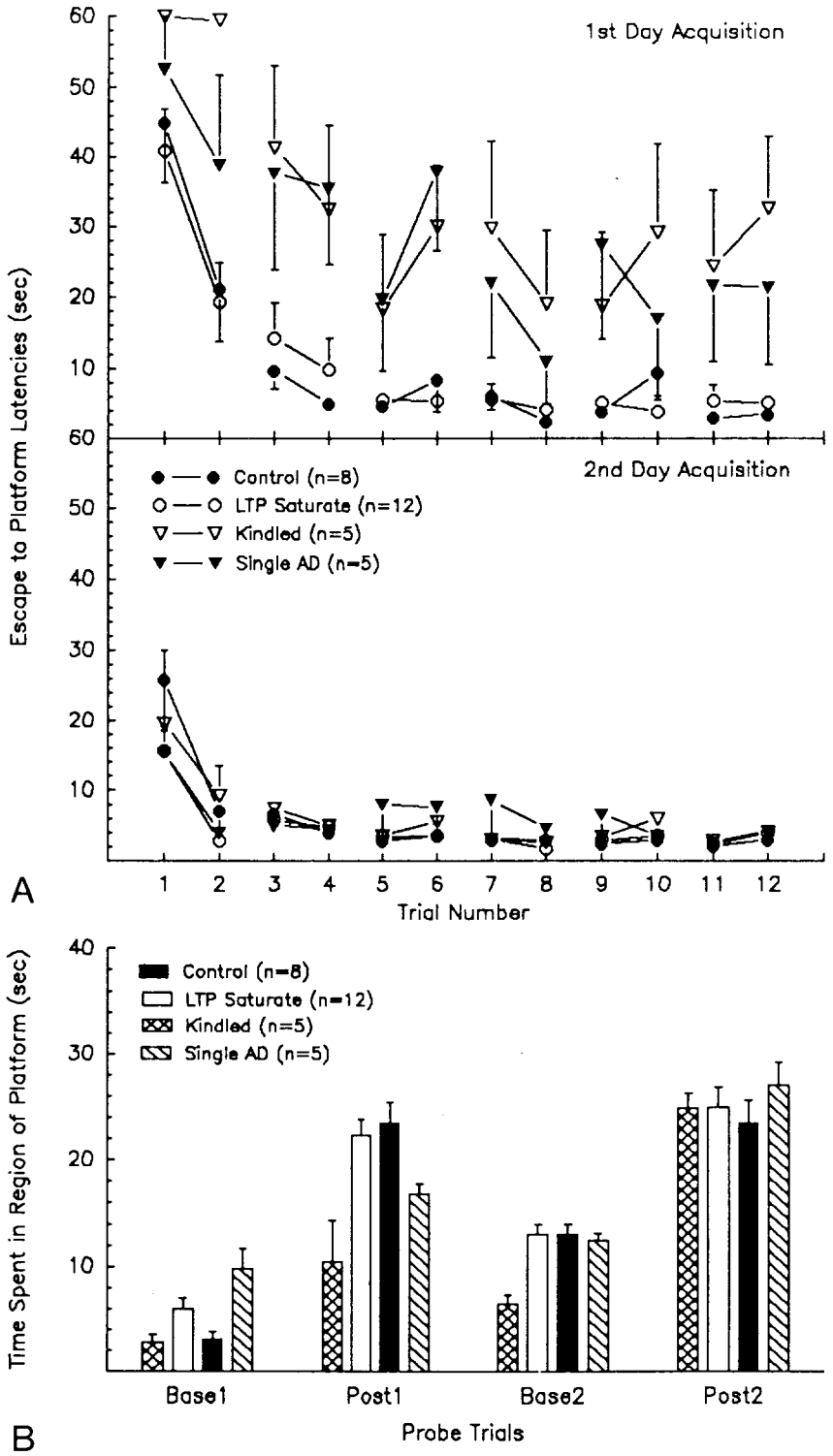


Fig. 4. (A) Mean latencies required to escape onto the hidden platform in each of 12 trials on the first and second days of training in the water maze task. (B) Probe trial measures of time spent swimming in the location of the removed platform before training (Base 1), after the first day of training (Post 1), before training on the second day (Base 2), and after training on the second day (Post 2).

two classes of measure. Further correlations with composite measures of LTP that corrected for differences in the amount of LTP induced in the two hemispheres and for short-term potentiation effects similarly revealed no relation between the measures (data not shown). Finally, individual rats that exhibited increases during the saturation period of >20% in the slope of the population EPSP and >70% increases in the amplitude of the PS in both hemispheres performed as well as controls in the water maze task, and as well as rats that exhibited smaller increases in the field potentials.

### *Relations between AD, kindling, and water maze acquisition*

Analyses of the relation between water maze acquisition performance and seizure strength were performed by relating water maze acquisition, expressed as the sum of escape latencies on the first day of training, to a composite of the strength of the *last* seizure evoked in the rats in the kindled and AD groups before training in the water maze on that day. For the kindled group, the composite of seizure strength was obtained by multiplying the duration of the last AD by the behavioral convulsion stage. For the AD group, the composite of seizure strength was obtained by multiplying the AD duration by 1 for rats that exhibited no convulsive behavior, and multiplying by 2 for rats that exhibited stage 1 convulsions (the maximal convulsion stage observed in this group). The correlation coefficients were +0.91 for the kindled group and +0.73 for the AD group; there was no relation between these measures and retention. These results indicate that the more severe the seizure on the first day of water maze training, the greater the summed latencies to find the platform on that day.

## DISCUSSION

There was no change in the field potential measures in the behavioral LTP experiment despite evidence of robust place learning. Subsequent induction of normal LTP in the same rats using the same electrodes showed that the experimental arrangements were appropriate for the detection of potentiation effects. Of the 240 correlation coefficients examined, only 3 were significant, all relating to electrophysiological LTP induced after behavioral testing was completed. Two of the significant correlations were negative, and therefore suggestive of an inverse relation between learning and subsequent capacity for electrophysiological LTP. The third coefficient, while positive, was isolated. The fact that the three significant coefficients were not part of a consistent pattern relating water maze performance to either behavioral or electrophysiological LTP suggests that the significance obtained for these three correlation coefficients was most likely spurious. The number of significant coefficients closely matches the number that would be expected by chance alone from a group of 240 coefficients (i.e., 2.4). The nature of the three significant coefficients failed to support a functional link between behavioral learning and LTP.

Thus we fail to find evidence of behavioral LTP in a neural circuit known to be important for place learning in the water maze, and we fail to find a correlation between behavioral learning and subsequent LTP. This is consistent with earlier failures to find evidence of behavioral LTP after training in spatial tasks (McNaughton et al., 1986, control data; Castro et al., 1989, control data; Hargreaves et al., 1990; Robinson, 1992, control data). The present study extends the failure to observe behavioral LTP after place learning in the water maze to rats whose stimulation/recording arrangements were subsequently confirmed to be capable of inducing normal electrophysiological LTP. This suggests that the previous failure to detect behavioral LTP after water maze training (Castro et al., 1989, control data) was not the result of problems with the stimulation/recording arrangements.

The prior LTP experiment failed to yield evidence of a disruptive effect of prior bilateral LTP saturation on place learning, despite the use of procedures similar to those of an earlier study that reported this finding (Castro et al., 1989). Further, there was no relation between the strength of LTP saturation and behavioral performance in the maze. These basic results are similar to those of Korol et al. (1992), Sutherland et al. (1991, 1992), and Robinson (1992), who also failed to find an effect of prior LTP saturation on place learning in the water maze and eight-arm radial maze.

In contrast, kindled seizures had a pronounced disruptive effect on performance of the task, as was shown by elevated escape latencies and a decrease in time spent swimming in the vicinity of the platform on day 1 of training. This is consistent with the results of earlier studies showing that seizures result in deficits in the performance of spatial tasks (Lopes da Silva et al., 1986; Leung et al., 1990) and that hippocampal lesions have a disruptive effect on the water maze task (Morris et al., 1982; Sutherland et al., 1983). Thus, the behavioral measures used were able to detect performance deficits of the kind normally measured in the water maze task.

The disruption of water maze performance was temporary, however, and on day 2 of training the performance of the kindled group was indistinguishable from that of the LTP and control groups, suggesting that information about platform location had been acquired on day 1. The disruptive effect of kindled seizures could not have been due to decay of kindling, since we and others have shown that kindling is essentially permanent (e.g., Wada et al., 1974; Dennison et al., 1990). A more likely explanation is that a temporary perturbation of normal brain function caused by the seizure that was evoked just before training temporarily disrupted performance of the task. This conclusion is strengthened by our finding that the water maze data of the AD group were indistinguishable from those of the kindled group. It is further strengthened by the positive correlations that were found between measures of seizure strength and latencies to find the hidden platform, which indicated that the strength of the evoked seizures was related to the deficit in performance of the task. Our basic findings agree with those of other studies using the water maze and eight-arm radial maze tasks (McNamara et al., 1992a, 1992b; Robinson et al., 1992).

The lack of an effect of LTP saturation on spatial learning reported here and by others (Sutherland et al., 1991, 1992; Korol et al., 1992; Robinson, 1992) contrasts with earlier reports of such effects in the circular platform and water maze tasks (McNaughton et al., 1986; Castro et al., 1989). The reasons for this discrepancy are not clear. A number of variables may affect whether a disruptive effect of LTP saturation is observed in studies of this kind.

The interval between the end of the last LTP session and

the commencement of spatial learning might be an important variable. Presumably a long interval would allow for the partial decay of LTP to levels below saturation, which might allow sufficient plasticity within the PP-DG circuit for the encoding of spatial information. Castro et al. (1989) did not indicate the interval between the last LTP session and the beginning of behavioral training. We failed to observe a disruptive effect of prior LTP saturation in both a pilot study with rats that began water maze training 18 hours after the completion of LTP saturation (unpublished observations), and in the present study in which a 5-minute interval was used. McNaughton (Korol et al., 1992, and personal communication, June 1992) used intervals of either 24 hours or 5 minutes, and in both cases there was no disruptive effect of prior LTP saturation on water maze acquisition. Similarly, Sutherland et al. (1991) used a 24-hour interval between LTP-inducing stimulation and acquisition of the location of a hidden platform that was changed daily and found no effect (personal communication, September 1992). McNaughton et al. (1986) had previously reported that bilateral LTP applied to the PP-DG within 5 minutes of retraining disrupted acquisition of new spatial information in the circular platform task in rats that had previously acquired the task. The trains induced a 37% increase in EPSP amplitude and a 25% increase in PS area, which may not have achieved saturation. Nevertheless, the trains disrupted acquisition of the new spatial information for at least 5 days. McNaughton et al. (1986) also reported that prior LTP applied over 12 days disrupted acquisition, begun 24 hours later, of the circular platform task, and that the disruptive effect lasted for 16 days. Taken as a whole, these data indicate that the interval between the last LTP session and behavioral training on the spatial task does not predict whether prior LTP will disrupt spatial learning. This discussion leaves aside the further issue whether short-term potentiation effects might play a role in any disruption of spatial learning in studies in which brief intervals between LTP and behavioral training were used.

Given that a single AD disrupted performance as much as fully kindled seizures did, and approximately as much as LTP saturation did in the report of Castro et al. (1989), it is conceivable that AD, perhaps inadvertently evoked by the high-frequency trains, might have caused the disruption in spatial learning that was found in some studies. Routine monitoring of the electroencephalogram for the occurrence of AD was carried out in some but not all of the studies cited above. However, our observations indicate that it is nearly impossible to evoke AD with trains of 400 Hz pulses that are less than 400–500 ms long (unpublished observations), a train duration that far exceeds that used for induction of LTP in these studies. For the occurrence of undetected AD to actually underlie the disruptive effect of LTP saturation on maze acquisition, the AD would have to occur close to the time of training. Given the great difficulty in evoking AD with brief high-frequency trains of the kind used to induce LTP, it seems unlikely that undetected AD is the cause of the disruptive effects of LTP on maze acquisition.

The extent of LTP saturation throughout the DG might be an important variable in these studies. If the induceable LTP was not saturated in all parts of the DG in both hemispheres, there might be sufficient available plasticity for the rat to acquire the task. Less than complete saturation in some portion of the DG conceivably could explain our failure to observe a deficit in spatial learning. Unfortunately, as desirable as such information would be, confirmation of the completeness of LTP saturation in all parts of the DG is an extreme challenge, and to our knowledge no one has attempted it.

The further issue of the amount of bilateral LTP achieved also might be important. Should smaller amounts of asymptotic LTP saturation yield smaller deficits in spatial learning? Are there criterion levels of asymptotic LTP saturation that must be achieved before a spatial learning deficit is to be expected? Do modest levels of asymptotic LTP reflect a failure to achieve saturation of LTP? In cases where LTP levels are subasymptotic, perhaps due to decay of LTP over 1 or a few days, should spatial learning deficits disappear? Must robust potentiation of both the EPSP and PS occur before a deficit is to be expected? In cases in which potentiation of the PS is robust, but potentiation of the EPSP is weak or absent, should a facilitation of learning be expected due to an increase in the probability of DG granule cells firing in response to a given PP input? Answers to these questions would be required for a complete understanding of the usefulness of the prior LTP approach in the study of relations between LTP and normal learning, but firm answers are so far lacking.

Comparison of the amount of LTP reported in different experiments is complicated by the fact that the amount reported can differ depending on how the measure is determined (Hargreaves and Cain, 1991, and unpublished observations) and, as we have shown here, the test pulse intensity that is used to measure it. Measures taken near the bottom of the I/O curve yield greater values of LTP than measures taken near the middle or top of the curve in the same animal. This can result in relatively small amounts of LTP being obtained when the test pulse intensity is near the top of the curve (e.g., Hargreaves et al., 1990). Any conclusions regarding the questions posed in the previous paragraph will necessarily need to take this into account.

Given that the amount of prior LTP induced in different animals can differ markedly, an important question is whether this amount predicts subsequent performance on spatial tasks. Castro et al. (1989) achieved very substantial amounts of bilateral LTP of both the EPSP (a 40–50% increase over baseline) and PS (a 150–200% increase over baseline) and measured a significant deficit in spatial learning. However, McNaughton et al. (1986, Experiment 3A) also measured a lasting (16 day) deficit in spatial learning as a result of more modest levels of LTP (EPSP: 11% over baseline; PS: 35% over baseline). Based on LTP decay data (McNaughton et al., 1986), the levels of LTP present toward the end of the 16-day behavioral training period would be substantially less than those measured just after the last LTP session. In contrast, Robinson (1992) failed to find a spatial learning deficit despite levels of bilateral LTP that were higher than those reported by McNaughton et al. (1986) in their Experiment 3A. We obtained a high level of LTP in both hemispheres of some rats, but these rats performed as well as control rats, and there was no relation between the amount of LTP induced and subsequent performance in the water maze. Taken together, the available data suggest that there is no relation between the amount of LTP induced and subsequent deficits in spatial tasks.

In the Castro et al. (1989) study, animals were used that had previously participated in an enriched environment experiment (B. McNaughton, personal communication, June 1992). It has been shown that such animals exhibit larger PP-DG field potentials (Green and Greenough, 1986; Sharp et al., 1987) and acquire the water maze task quicker than individually housed controls (Park et al., 1991). We have confirmed the latter result, and have also found that enriched rats exhibit significantly greater LTP of both the EPSP and PS than individually housed littermates (Hargreaves et al., 1992). Taken together, these data show that enriched rats are different in their ability to acquire the water maze and to support PP-DG LTP. It is possible that the interaction between LTP saturation and water maze learning is different in enriched rats, and that LTP saturation has a more debilitating effect on maze acquisition in enriched rats than it does in individually housed rats. This question is currently under study by McNaughton and colleagues (Korol et al., 1992).

Our results do not rule out the possibility that potentiation phenomena, defined as increases in the efficacy of specific synapses, might underlie certain forms of learning. Long-term potentiation as a phenomenon is defined and measured by recording and analyzing the field potential that is evoked by the synchronous activation of many neural fibers by an electrical pulse. Surely this method is not adequate for fully evaluating all possible changes in neural function that might be induced in particular individual synapses by various inputs, both natural and experimental. It is conceivable that changes in neural function of an LTP-like nature are important for learning, but are not adequately measured by the methods currently used to measure LTP (Hargreaves et al., 1990). What the bulk of available data show is that the PP-DG field potential does not change as a result of the acquisition of spatial learning tasks, and that LTP saturation, as conventionally induced and measured in the PP-DG circuit, is not associated with deficits in spatial learning tasks.

Because of the inherent uncertainties and limitations of the LTP phenomenon as currently studied, it might be more fruitful to examine the functional relations between LTP and learning through the use of the blockade/facilitation strategy described by Morris and Baker (1984). However, when pharmacological treatments are used for this purpose, their effects on the unlearned behavior that is necessary to demonstrate acquisition of the task under study deserve the greatest care and attention (Morris, 1989; Hargreaves and Cain, 1992).

We conclude that neither the behavioral LTP nor the prior LTP paradigms have provided firm and unequivocal support for a functional link between LTP phenomena and behavioral learning phenomena.

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