

# Cumulative Long-term Potentiation in the Rat Dentate Gyrus Correlates With, But Does Not Modify, Performance in the Water Maze

Kathryn J. Jeffery and Richard G. M. Morris

Department of Pharmacology, University of Edinburgh Medical School,  
Edinburgh, U.K.

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## ABSTRACT

The electrically induced increase in hippocampal synaptic strength known as long-term potentiation (LTP) is thought to involve some of the same mechanisms as those mediating information storage during spatial learning. Physiological saturation of synaptic weights might therefore be expected to occlude spatial learning. In support of this, Castro et al. (Castro CA, Silbert LH, McNaughton BL, Barnes CA, 1989, *Nature* 342:545–548) reported that repeated induction of LTP to asymptotic levels, over a 14-day period, blocked the ability of rats to learn the position of a hidden platform in a water maze shortly afterwards. The authors have attempted to replicate this finding in two experiments. In Experiment 1, rats were given either 400 Hz stimulation as 50 trains/day over a 5-day period, to induce LTP, or were given the same number of stimulus pulses at 1 Hz. Two hours after the last stimulation session they underwent eight spatial learning trials, occurring at 2-hour intervals. In Experiment 2, the procedure of Castro et al. was followed exactly, with LTP induced by 10 trains of 400 Hz stimulation daily for 14 days. Spatial learning trials began 10 minutes after the last stimulation session and consisted of 12 trials with alternating 30-second and 2-minute intervals. In neither experiment was a significant impairment of spatial learning observed. However, there was a strong positive correlation, in both experiments, between the final level of LTP and subsequent performance in the water maze.

**Key words:** hippocampus, synaptic plasticity, spatial learning, LTP saturation

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Long-term potentiation (LTP) is an experimentally induced increase in synaptic strength following high-frequency electrical tetanization of intrinsic pathways of the hippocampal formation. It has attracted attention because it may represent artificial induction of synaptic weight changes of the type that have been postulated to occur naturally during memory formation. If this is so, then disruption of the ability of synapses to alter their weights should interfere with the types of learning for which the hippocampus is necessary, such as spatial learning. One way of achieving this might be to force synaptic weights to their maximum values, by repeated tetanization, so that further learning-induced changes are blocked and memory formation thereby impaired. Just such a saturation-related learning impairment using two spatial tasks and tetanization paradigms has been reported by McNaughton et al. (1986, in the circular platform maze) and Castro et al. (1989, in the water maze). We have sought to replicate this apparently robust effect, as it provides a nonpharmacological means of interfering with plasticity in selected pathways of the hippocampus, and hence of exploring further the contribution of

synaptic weight changes to learning. In the two experiments outlined below, the water maze was chosen as the spatial task of interest because it allows for fast learning within a narrow time window, and also for comparison with other studies in this laboratory on the effects of pharmacological agents that block LTP (Davis et al., 1992).

## MATERIALS AND METHODS

### Subjects

Subjects were 50 male Lister hooded rats (250–400 g) from the laboratory breeding stock, housed in groups (Experiment 1) or individually (Experiment 2) with *ad libitum* food and water, on a 14/10 hour light-dark cycle.

### Surgery

Two groups of rats served as handled but unoperated controls (Experiment 1,  $n = 14$ ; Experiment 2,  $n = 8$ ). The remaining 28 rats were premedicated with midazolam and then anesthetized with tribromoethanol. They were implanted bilaterally, using conventional stereotaxic techniques, with 75  $\mu\text{m}$  teflon-coated stainless steel electrodes in the angular bundle of the perforant path (stimulating electrode, coordinates

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Correspondence and reprint requests to Dr. K. J. Jeffery, Department of Pharmacology, University of Edinburgh Medical School, 1 George Square, Edinburgh EH8 9JZ U.K.

7.5 mm posterior and 4.0 mm lateral to bregma) and hilus of the dentate gyrus (recording electrode, coordinates 3.8 mm posterior and 2.2 mm lateral to bregma). In Experiment 1, the stimulating electrodes were a twisted pair, with tips separated vertically by 0.5–1.0 mm. In Experiment 2, the stimulating electrodes were monopolar with 0.5–1.0 mm insulation scraped from the tip. Two jeweler's screws fixed to the skull anteriorly and two posteriorly served as ground and/or stimulus return. Electrodes and screws were attached to gold pins (Augat) with silver electrical paint, and the pins were coated in nail lacquer and attached to a plastic connector that was cemented to the skull with dental acrylic. The rats were given an intramuscular injection of buprenorphine for postoperative analgesia, and allowed at least 2 weeks to recover from surgery before electrophysiological testing began.

### Electrophysiology

After an acclimatization period of 20 minutes, during which time the rats stopped moving around the recording chamber and sat quietly, evoked waveforms were sampled from each hemisphere consecutively at 0.1 Hz. Stimuli were biphasic square-wave pulses of 100  $\mu$ s half-cycle duration, supplied by a constant-current stimulus isolator at an intensity adjusted to give a population spike of 1–3 mV (200–800  $\mu$ A). The signal was amplified 200 times by a Grass amplifier, filtered (1 Hz–10 kHz) and fed via an A-D converter (Wild Vision, Newcastle) to a computer for analysis and storage. Analysis of the waveform consisted of measurement of the slope of the rising phase of the excitatory postsynaptic potential (EPSP) between two fixed time points, using linear regression, and the height of the population spike from the minimum of the spike to a line drawn between the two local maxima of the field potential. Ten waveforms were sampled on each day from each hemisphere and the values averaged.

Rats were assigned either to a high-frequency (HF) group (Experiment 1,  $n = 8$ ; Experiment 2,  $n = 8$ ), to receive LTP-inducing stimulation, or to a low-frequency (LF) control group (Experiment 1,  $n = 7$ ; Experiment 2,  $n = 5$ ). Those with small or unilateral EPSPs were accepted into the LF group providing both stimulating electrodes were well placed (i.e., could evoke a population spike). The recording protocol consisted of a 5-day baseline period followed either by 5 days (Experiment 1) or 14 days (Experiment 2) of daily tetanization. Values for the EPSP were expressed as a percentage increase over the mean of the 6 baseline days (including the prestimulation value on day 6). Because the size of the population spike prior to tetanization was kept as uniform as possible, and because proportional changes in this measure are generally very large, values for the population spike were expressed as the absolute deviation, in mV, from the baseline. Both EPSP and population spike were averaged across the two hemispheres where possible. Tetanic stimulation was administered as follows.

#### Experiment 1

Stimulus pulses were increased to 250  $\mu$ s half-cycle duration and given as 10-pulse trains at 400 Hz. Five such trains were given at 1-second intervals followed by a 1-minute pause, and this procedure was repeated to a total of 50 trains.

Low-frequency controls received the same total number of pulses at 1 Hz. Stimulation was administered daily for 5 days.

#### Experiment 2

Ten-pulse trains at 400 Hz were given as above at 10-second intervals for a total of 10 trains. This paradigm was repeated on each of 14 days. In LF controls, the 10-pulse train was replaced by a single pulse.

A poststimulation baseline was recorded 30 minutes after the last train on each day. In Experiment 2, poststimulation recordings were omitted on the 14th day of tetanization to allow for behavioral training shortly afterwards.

### Behavioral training

Behavioral training was carried out in a 2 m diameter water maze as previously described (Morris, 1984). Prior to tetanization all rats were pretrained in the water maze with curtains drawn around the pool to obscure room cues. The position of the slightly submerged platform was indicated by the experimenter, and over six trials the rats became accustomed to swimming in the pool and learned to climb onto the platform.

Spatial training began after the final tetanization session. The curtains were drawn back to reveal the room, and the rats were trained to swim from various starting positions to the platform, which was hidden in a fixed location with respect to the now visible room cues. In Experiment 1, a total of 8 trials were given at 2-hour intervals, beginning 2 hours after the last HF session. In Experiment 2, training began 10 minutes after the last LTP session and 12 trials were given with alternating 30-second and 2-minute intervals, as in Castro et al. (1989). A transfer test was given 2 hours or 2 minutes after the final trial for Experiments 1 and 2, respectively. In this trial, the platform was removed from the pool and the rats were allowed to search for 60 seconds. The percentage of time spent searching in the appropriate quadrant of the pool was used as an index of spatial learning.

### Histology

Seven rats from the HF group in Experiment 1 were used for histological analysis. They were deeply anesthetized with sodium pentobarbital and perfused intracardially with 10% formalin, and the brains removed and stored in formalin for later sectioning. Sections (40  $\mu$ m thick) were mounted and stained with cresyl violet to allow visualization of the stimulating electrode placements.

## RESULTS

### Experiment 1

#### Electrophysiology

Eight rats received tetanization, 7 rats received LF stimulation, and 14 unoperated control rats received an equivalent amount of handling. A typical evoked potential is shown in Figure 1A before and after HF stimulation. Table 1 shows the mean values for EPSP and spike in the HF and LF groups, over the baseline period and the last 3 days of the LTP induction phase.

The effects of the tetanic vs. LF stimulation on the normalized values for EPSP and population spike are shown in Fig-

**Table 1.** Mean Raw Values ( $\pm$  SEM) for EPSP, Population Spike, Stimulus Intensity, and Training Quadrant Times for Rats in Both LF and HF Groups in Experiments 1 and 2

	Experiment 1		Experiment 2	
	LF (n = 7)	HF (n = 8)	LF (n = 5)	HF (n = 8)
EPSP (before) (mV/ms)	1.92 (0.34)	2.88 (0.55)	2.37 (0.44)	2.67 (0.14)
Spike (before) (mV)	2.85 (0.27)	2.64 (0.35)	2.05 (0.13)	1.99 (0.28)
EPSP (after) (mV/ms)	1.65 (0.31)	3.80 (0.81)	2.39 (0.23)	3.97 (0.25)
Spike (after) (mV)	1.85 (0.27)	6.02 (1.21)	2.05 (0.46)	6.69 (0.85)
Stimulus intensity ( $\mu$ A)	539 (85)	588 (59)	505 (77)	336 (46)
Quadrant time (%)	41.29 (6.10)	41.18 (4.95)	48.18 (6.07)	39.44 (2.75)

EPSP, excitatory postsynaptic potential; LF, low-frequency; HF, high-frequency.

ure 1B,C. Analysis of variance of the evoked potentials in the HF and LF groups during the baseline and LTP induction phases revealed a significant interaction between groups and phases reflecting the increase in the HF group, following tetanization, in both the EPSP [ $F(1, 13) = 12.54, P < .01$ ] and the population spike [ $F(1, 13) = 12.41, P < .01$ ]. The values before and after 1 Hz stimulation were analyzed further for the LF group using a one-tailed paired *t*-test. This revealed a significant decrease in the population spike ( $P = .002, t = 4.50$ ) but not in the EPSP ( $P > .1$ ). There was no change across the last 3 days of tetanization in the LTP group for EPSP [ $F(2, 14) = 1.20, NS$ ] or population spike [ $F(2, 14) < 1, NS$ ], suggesting that both measures had reached a stable level, with increases of 30% and 3–4 mV, respectively.

### Behavior

Figure 2A shows the acquisition curve for rats in each group over the eight water maze trials. All groups showed a significant decrease in escape latency [ $F(7, 182) = 14.01, P < .001$ ] with no difference between the groups [ $F(2, 26) = 1.03, NS$ ]. Figure 2B shows the performance of rats searching for the absent platform during the transfer test.

All three groups spent significantly more time in the quadrant of the pool where the platform had been located [ $F(3, 78) = 18.66, P < .001$ ]. There was no effect of LTP induction on time spent in the training quadrant [ $F(2, 26) < 1, NS$ ; Table 1].

### Behavior–LTP correlation

The behavioral and electrophysiological results from the HF group were analyzed further. Pearson product-moment correlation revealed a highly significant correlation between the final level of EPSP LTP (as measured 30 minutes after the fifth tetanization session) and the percentage of time spent in the training quadrant (Pearson's  $r = .95, P < .001$ ; Fig. 5A). Rats from the LTP and LF groups combined were assigned on the basis of time spent in the training quadrant into good ( $n = 7$ ) and poor ( $n = 8$ ) learners (greater or less than the mean, respectively). Analyses of variance showed no significant relationship of learning with stimulus intensity, baseline EPSP, or baseline population spike values [ $F(1, 14) = 0.34, 1.58, \text{ and } 0.27, \text{ respectively, } NS$ ].

## Experiment 2

### Electrophysiology

Eight rats received tetanization, five rats received LF stimulation, and eight unoperated control rats received an equivalent amount of handling. Mean raw values for the EPSP and

population spike are shown in Table 1, and normalized values over the 14 days of LTP induction are shown in Figure 3A and B. The cumulative level of LTP in the HF group was higher than in Experiment 1, reaching a final increase of 50% for the EPSP and 5–6 mV for the spike (Fig. 3A,B), despite the rats having received a smaller total number of trains (140 vs. 250). Analysis of variance of the evoked potentials during the baseline and LTP induction phases revealed a significant interaction for both the EPSP [ $F(1, 11) = 23.31, P < .001$ ] and the population spike [ $F(1, 11) = 48.43, P < .001$ ]. There was no change across the last 3 days of tetanization in the LTP group for EPSP [ $F(2, 14) = 1.02, NS$ ] or population spike [ $F(2, 14) = 1.00, NS$ ], again suggesting that both measures had reached a stable level.

### Behavior

Acquisition curves for the water maze training (Fig. 4A) revealed a significant decrease, over the 12 trials, in latency to find the platform [ $F(11, 198) = 6.26, P < .001$ ], and all groups spent significantly more time in the training quadrant during the transfer test [ $F(3, 54) = 47.89, P < .001$ ]. There was no significant difference between groups either for acquisition [ $F(2, 18) = 2.21, NS$ ], or during the transfer test [ $F(2, 18) = 1.76, P > .10$ ]. The slight decrease in time spent in the training quadrant shown by the HF group as compared with the LF and unoperated control groups (Fig. 4B) was analyzed further by considering only the first 30 seconds of the transfer test, but still failed to reach significance [ $F(2, 18) = 1.79, P > .10$ ].

### Behavior–LTP correlation

Pearson product-moment correlation again revealed a significant correlation between the final level of LTP of the EPSP and the percentage of time spent in the training quadrant (Pearson's  $r = 0.80, P < .05$ ; Fig. 5B).

LTP and LF rats were assigned on the basis of time spent in the training quadrant into good ( $n = 5$ ) and poor ( $n = 8$ ) learners (greater or less than the mean, respectively). One-way analyses of variance again showed no significant relationship of learning with stimulus intensity, baseline EPSP, or baseline population spike values [ $F(1, 12) = 0.37, 0.32 \text{ and } 0.33 \text{ respectively, } NS$ ].

### Histology

All seven brains examined (including those from both good and poor learners) showed stimulating electrode placements within the angular bundle bilaterally.

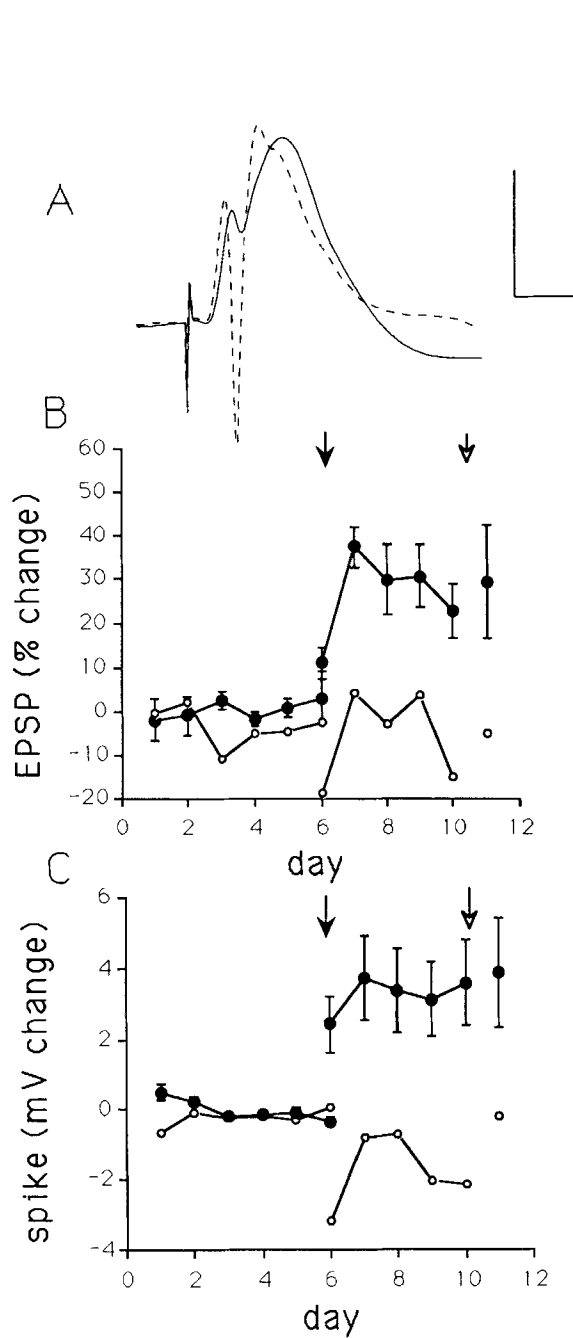


Fig. 1. Experiment 1. (A) Representative evoked waveform in the dentate gyrus before (solid line) and after (dotted line) 5 consecutive days of tetanic stimulation of the perforant path. Calibration bar; 5 mV, 5 ms (B) Normalized values for EPSP and (C) for population spike before, during, and 24 hours after 5 consecutive days of high-frequency (50 10-pulse trains at 400 Hz,  $n = 8$ ) or low-frequency (500 pulses at 1 Hz,  $n = 7$ ) stimulation. Posttetanization values were recorded 30 minutes after the last train on each day. Each point represents the group mean ( $\pm$  SEM) of 10 evoked waveforms. The LTP induction phase commenced on day 6 (solid arrow) and behavioral training began 2-4 hours after the fifth tetanization session (open arrow). The small decrease in EPSP and population spike recorded 30 minutes following 1 Hz stimulation had recovered completely by 24 hours after the final stimulation session (day 11 time point).

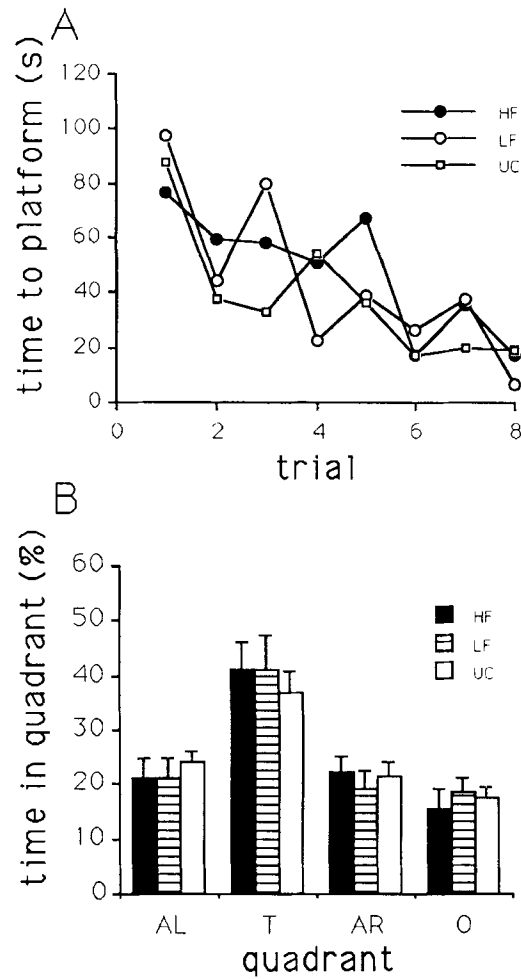


Fig. 2. (A) Acquisition curves during water maze training for high-frequency (HF;  $n = 8$ ), low-frequency (LF;  $n = 7$ ), and unoperated control (UC;  $n = 14$ ) groups in Experiment 1. Data points represent the group mean latencies to find the hidden platform in each of the eight trials. Intertrial interval was 2 hours. (B) Transfer test 2 hours following the eighth trial. Values shown represent the group mean percentage time ( $\pm$  SEM) spent in each of the four quadrants of the pool while searching for the absent platform. AL, adjacent left; T, training; AR, adjacent right; O, opposite.

### DISCUSSION

Two results emerged from this study. First, induction of LTP in the dentate gyrus to apparently asymptotic levels had no effect on the ability of rats to learn a water maze task. Second, the cumulative value of the EPSP LTP correlated highly and *positively* with performance on the task. These two findings will be considered in turn.

#### LTP "saturation"

In the experiments reported here, no significant learning impairment was seen following induction of LTP to levels as high as those reported by Castro et al. (50% increase in EPSP in Experiment 2). There was a slight, nonsignificant trend towards a spatial learning impairment seen in the HF group in Experiment 2, as reflected both in the acquisition curve

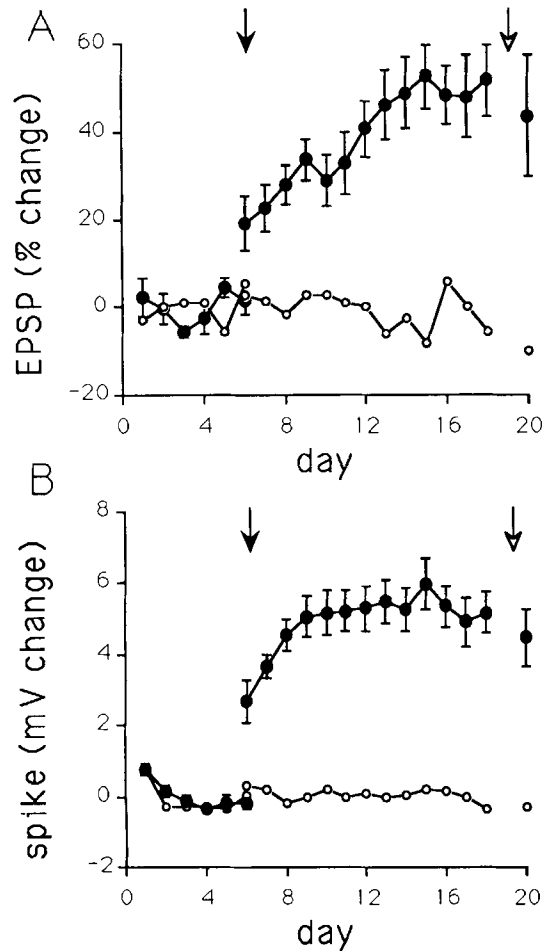


Fig. 3. Experiment 2. Normalized values for the EPSP and population spike (A and B) before, during, and 24 hours after 14 consecutive days of high-frequency (10 10-pulse trains at 400 Hz,  $n = 8$ ) or low-frequency (10 pulses at 0.1 Hz,  $n = 5$ ) stimulation. Each data point represents the group mean ( $\pm$  SEM) of 10 evoked waveforms. The LTP induction phase commenced on day 6 (solid arrow) and behavioral training took place 10 minutes after the last session on day 19 (open arrow, physiological recordings omitted).

(Fig. 4A) and the transfer test (Fig. 4B). This may be because the induction of LTP had moved synapses sufficiently far toward saturation to produce the beginnings of a spatial learning deficit. It is also possible, however, that the effect was due to chance assignment of naturally poor learners to the HF group. In support of this interpretation, there is in this group (unlike Experiment 1) an uneven distribution of LTP levels with a bias towards the lower end of the range (Fig. 5B). If synaptic plasticity is related to spatial learning (see below), it is possible that rats scoring low on both of these measures were overrepresented in this group by chance alone.

The absence, in our experiments, of a spatial learning impairment after apparently maximal induction of LTP raises the question of whether there are methodological or theoretical reasons for this pattern of results. We shall consider three possible explanations for the discrepancy from the previous reports (McNaughton et al., 1986; Castro et al., 1989).

The first explanation concerns the extent to which the en-

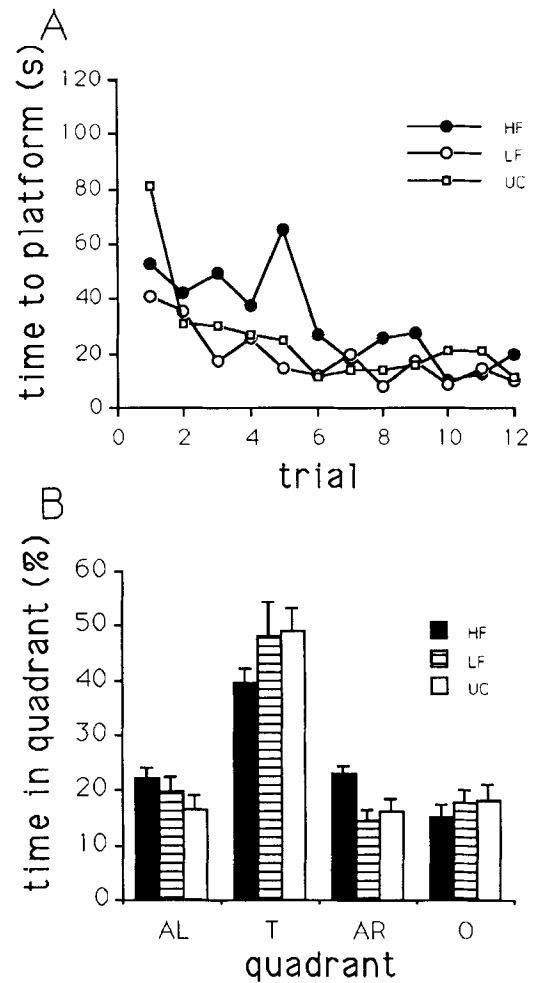


Fig. 4. (A) Latency data for the 12 water maze trials for the high-frequency (HF;  $n = 8$ ), low-frequency (LF;  $n = 5$ ), and unoperated control (UC;  $n = 8$ ) groups in Experiment 2. Inter-trial interval alternated between 30 seconds and 2 minutes and trials began 10 minutes after the last LTP induction session. (B) Transfer test 2 minutes after the last trial (group mean % quadrant time  $\pm$  SEM). The HF group showed a slight trend towards spending less time in the training quadrant but this difference was not significant.

tire length of the hippocampus is activated by the stimulating positions that we, and others, have used. Histological examination of the stimulating electrode tracks in a subset of rats showed the electrodes to be located in the angular bundle of the perforant path, which projects to a large part of the rostrocaudal extent of the dentate gyrus (Amaral and Witter, 1989). However, the two components of the perforant path do not project equivalently to all parts of the hippocampus, the medial path contribution becoming progressively less in more ventral regions (Gall et al., 1984). Thus, an electrode located principally in the medial perforant path might evoke a strong response in the dorsal hippocampus (DHC), from where our recordings were made, but a weaker response (and hence less LTP) in the ventral hippocampus (VHC). In support of this, data from immediate-early gene induction suggest that the LTP-associated increase in *zif-268* following tetanization at comparable stimulating locations is more intense in the

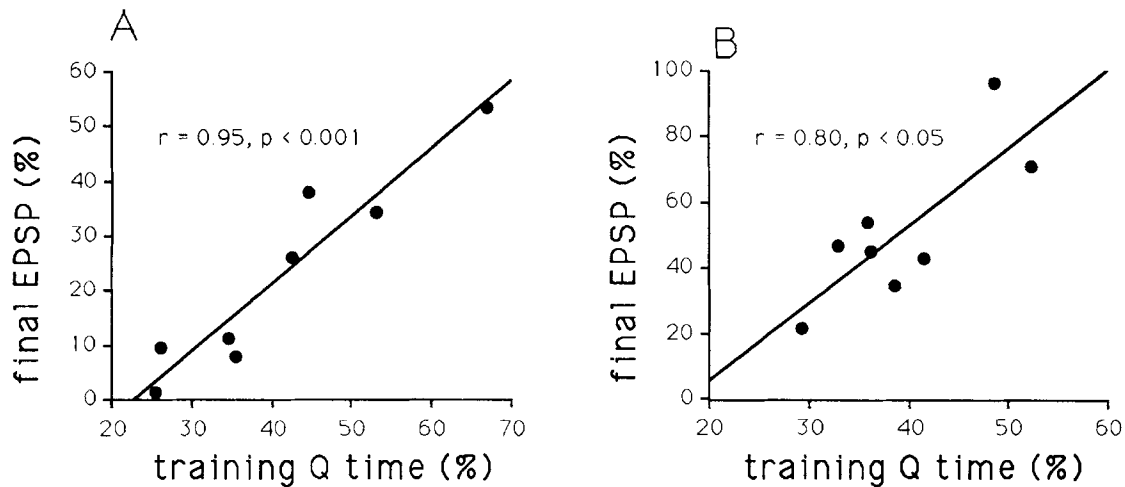


Fig. 5. Linear correlation between the cumulative level of EPSP LTP (y axis) and percentage time spent in the training quadrant during the transfer test (x axis), for Experiments 1 (A) and 2 (B).

DHC than the VHC (Cole et al., 1989). To investigate whether robust LTP is being induced throughout the hippocampus, it would be necessary to record evoked potentials from several sites along its length. Preliminary experiments in our laboratory suggest that it is not easy to record such potentials from ventral regions using our stimulation sites. An explanation, therefore, for our failure to observe an LTP-related learning impairment may be that we have failed to evoke a sufficiently strong ventral response to enable saturation of LTP in this region, thus allowing LTP in the VHC to compensate for the loss of dorsal plasticity. Arguing against this is the observation that while good spatial learning may be supported by the DHC in the absence of the VHC, the converse does not appear to be true, at least following aspiration lesions (Moser et al., 1990). This suggests that the contribution of the VHC to spatial learning may be small under normal circumstances. Whether the VHC is able to take over the spatial learning role of the DHC following fiber-sparing lesions to the latter, or following blockade of LTP, remains to be demonstrated.

The second point is how close is "maximal LTP" to the true saturation level attainable by synapses under natural learning conditions? Our findings are that repeated tetanization over many days results in a variable increase in the EPSP slope that stabilizes, on average, at only 50% above baseline. Data from hippocampal slices (e.g., Barrionuevo and Brown, 1983) show that under some conditions (such as in the presence of picrotoxin), nearly 200% increases in the EPSP are possible—considerably higher than the maximum found by ourselves or by Castro et al. (1989). The findings of different "maximal" LTP under different conditions raises the question of where lies the true limit to synaptic weights, and under what circumstances, if any, this limit may be reached *in vivo*.

Third, how closely must the synapses approach their maximum weights in order for a learning deficit to ensue? The contribution of synaptic weight changes to learning depends both upon the magnitude of their change following a given input, and their lability (i.e., how readily they change in response to the input). Learning impairments could, in theory, be induced either by limiting the magnitude of weight change

possible (e.g., by forcing synapses up towards a ceiling level and thus restricting their dynamic range), or by decreasing their readiness to change (e.g., by blocking the NMDA receptor). There may be a functional difference in effects on learning between these two factors. Studies of LTP blockade with the NMDA-receptor antagonist AP5 suggest that synaptic plasticity need only be reduced by as little as 50% before a learning impairment becomes detectable (Davis et al., 1992). However, it is not known whether forcing synaptic weights even to near-maximal levels produces a similar reduction in their lability. It may be the case that, provided synapses are able to distribute their weights with relative ease, they can function within a narrow range. In addition, negative synaptic changes (long-term depression) may supervene at higher LTP levels so as to enable sufficient information storage for the task to be learned.

Although there is considerable uncertainty surrounding conditions under which LTP induction could alter the rate of learning, previous demonstrations that posttetanization learning changes have been obtained with a variety of protocols (Berger, 1984; McNaughton et al., 1986; Castro et al., 1989) suggest that physiological manipulation of synaptic plasticity is a feasible tool with which to investigate the processes underlying learning and memory. It is therefore surprising that the experiments reported here have failed to find any such effect. Further work needs to be done to identify the factors responsible for these discrepant results.

#### Correlation of LTP with behavior

The second main result of the present study is that the cumulative level of LTP following repeated tetanization correlated highly and positively with the ability of the rats to learn a spatial task. Rats receiving LTP-inducing stimulation performed at the same level as control rats overall, suggesting that LTP was not modifying learning but rather covaried with it. This finding provides additional circumstantial evidence to support the hypothesis that the learning of the water maze task is mediated, at least in part, by synaptic weight changes in the dentate gyrus. Individual variation in the overall plastic-

ity of these synapses may help determine the effectiveness of learning. Laroche et al. (1989) have provided experimental evidence that changes in synaptic efficacy correlate directly with learning in a task in which those synapses are unequivocally involved: that is, when perforant path tetanization itself serves as the stimulus. In these experiments, learning of a tetanus-footshock association was shown to correlate directly with the magnitude of LTP resulting from the tetanus, and treatments that reduced LTP produced a proportional decrease in the strength of learning.

Several other possible explanations for the LTP-behavior correlation need to be considered. One is that it is not LTP *per se* that covaries with learning, but rather an associated parameter, such as prior dentate gyrus excitability. In our experiments input-output (IO) curves were not measured, and the stimulus intensity was set so as to evoke a population spike of a predetermined size. This same intensity, at a longer duration, was used to induce LTP. Thus, if there had been a correlation between the EPSP-spike relationship *prior* to tetanization and a given rat's subsequent performance in the water maze, this correlation could result in a secondary association of learning with LTP, either as an artifact of the position on the IO curve at which the responses were measured or as a real difference in the amount of LTP induced. Analysis of EPSP, population spike, and stimulus intensity for good and poor learners, however, suggested that these parameters did not differ between the two groups. Nevertheless, comparison of the posttetanization shift of the whole IO curve with water maze performance is warranted. These experiments are currently underway. Preliminary results suggest that our method of measuring LTP correlates well with shift of the entire IO curve and that both measures correlate with learning.

Another possible explanation for the LTP-learning correlation is that plasticity in the hippocampus may correlate with plasticity in other parts of the cortex, and that the spatial learning is really taking place elsewhere. Alternatively, both phenomena may correlate with nonspecific brain states such as arousal. A more interesting possibility is that those rats that showed only low levels of LTP induction were those whose synapses were already approaching maximal levels, either because they had already undergone considerable naturally occurring potentiation, or because they had intrinsically lower ceiling levels of synaptic strength. Thus, the poor spatial learning performance may represent a true saturation-related effect. In support of this, comparison of LTP acquisition curves in Experiment 2 for good learners (greater than mean performance on the transfer test,  $n = 3$ ) vs. poor learners (less than the mean,  $n = 5$ ) shows that LTP in the poor learners appears already to have reached a plateau, whereas that in the good learners has yet to do so (Fig. 6). However, if this plateau represents true LTP saturation and is the explanation for the poor performance of these animals, the HF group as a whole would be expected to show an overall impairment in learning as compared with controls. This was not the case, although there was a nonsignificant decrease in time spent in the training quadrant in the HF group in Experiment 2. This matter could be resolved conclusively by means of experiments in which spatial training takes place *before* LTP induction. If the impaired performance is truly a result of greater

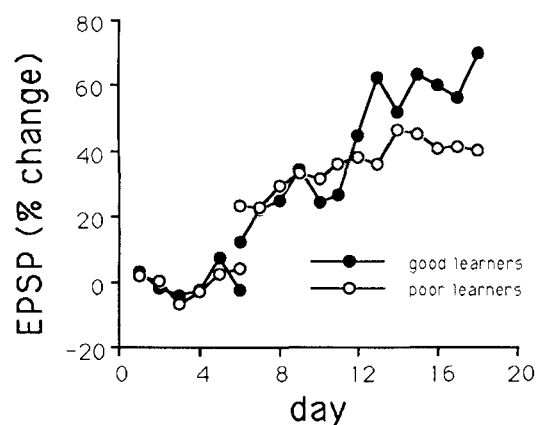


Fig. 6. Comparison of the acquisition of EPSP LTP in Experiment 2 for good learners ( $n = 3$ ) and poor learners ( $n = 5$ ). LTP induction began on day 6. By the final day of tetanization, LTP appears to have stabilized at a low level in the poor learners but is still increasing in the good learners.

LTP saturation in those animals who are closer to their synaptic ceilings to begin with, then the correlation should disappear if training takes place first, and all animals should perform equally well in the water maze. On the other hand, if both the impaired performance and the lower LTP levels result from some characteristic property of the synapses, intrinsic to the animals, then the correlation should be preserved.

Barnes (1979) found that 3 days of repeated daily tetanization induced higher levels of LTP in young rats (good spatial learners) than in old ones (poorer learners). This difference was due to the gradual slowing of LTP decay in the young rats, resulting in accumulation of total LTP levels. When the tetanization was continued for 12 days, however, the durability of LTP in the old rats also gradually increased, though more slowly, resulting in the same asymptotic levels of LTP as in the young rats by day 12 (Barnes and McNaughton, 1980). In the experiments reported here, by contrast, final levels of LTP varied between good and poor learners even after a prolonged period of daily tetanization. It may be that the lower LTP levels seen in the poor spatial learners in our experiments are an artifact of slower LTP acquisition such as that observed by Barnes and McNaughton. However, comparison of the acquisition of EPSP LTP for the three good and five poor learners (Fig. 6) suggests that in Experiment 2, poor learners appear to have reached asymptote *sooner* than the good learners, and at a lower overall level of LTP. Thus, the apparent difference in final LTP does not seem to be an artifact of varying acquisition time constants. The reason for the discrepancy of our data with those of Barnes and McNaughton is uncertain. One possibility is that the LTP protocol that they used succeeded in forcing synaptic weights up to their ceiling, whereas ours failed to achieve true saturation. Thus, the variability in cumulative LTP levels seen in the present experiments may have been masked in their study by a saturation effect. In this context it is worth noting that the same authors have produced the only studies reporting a spatial learning impairment following LTP induction (McNaughton et al., 1986; Castro et al., 1989). If this is the case, then the association of LTP with learning seen in our experiments

should begin to disappear as synaptic weights approach their upper limits.

A second explanation is that learning may depend on an interaction between both the magnitude and the durability of LTP, and that only the latter varied between the young and old rats in the Barnes and McNaughton (1980) study. The behavioral protocol in their experiments involved training over several days, whereas the experiments reported here required the rats to remember information only for minutes to hours. Possibly LTP induction contributes to acquisition of a spatial task (learning), whereas its maintenance contributes to retention (memory). It may be possible to dissociate the relative contributions of these two parameters by means of pharmacological manipulation of LTP induction (e.g., with NMDA-receptor blockade) and maintenance (e.g., with sodium pentobarbital, Jeffery et al., 1990).

The only way to determine unequivocally whether a causal relationship exists between the magnitude of dentate LTP and spatial learning is selectively to intervene in plasticity in that pathway, and to look for a corresponding change in learning. One method of achieving this is to modulate plasticity using physiological methods, and this was the motivation behind the present attempt to replicate the Castro et al. finding of impaired learning in the water maze following LTP induction. Our finding of the *absence* of a learning impairment following such widespread induction of LTP in this population of synapses, which appear nevertheless to be involved in this type of learning, suggests that further work needs to be done in identifying the true saturation level attainable, and in devising a means of inducing LTP of this magnitude throughout the dentate gyrus. In addition, the observation of an apparently linear relationship between LTP and performance in the water maze suggests the possibility of interventional experiments, attempting to quantify the contribution of synaptic plasticity to spatial learning.

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#### References

- Amaral DG, Witter MP (1989) The three-dimensional organization of the hippocampal formation: a review of anatomical data. *Neuroscience* 31:571–591.
- Barnes CA (1979) Memory deficits associated with senescence: a neurophysiological and behavioral study in the rat. *J Comp Physiol Psychol* 93:74–104.
- Barnes CA, McNaughton BL (1980) Spatial memory and hippocampal synaptic plasticity in senescent and middle-aged rats. In: *The psychobiology of aging: problems and perspectives* (Stein D, ed), pp 253–272. New York: Elsevier/North Holland.
- Barrionuevo G, Brown TH (1983) Associative long-term potentiation in hippocampal slices. *Proc Natl Acad Sci USA* 80:7347–7351.
- Berger TW (1984) Long-term potentiation of hippocampal synaptic transmission affects rate of behavioural learning. *Science* 224:627–630.
- Castro CA, Silbert LH, McNaughton BL, Barnes CA (1989) Recovery of spatial learning deficits after decay of electrically induced synaptic enhancement in the hippocampus. *Nature* 342:545–548.
- Cole AJ, Saffen DW, Barbaran JM, Worley PF (1989) Rapid increase of an immediate early gene messenger RNA in hippocampal neurons by synaptic NMDA receptor activation. *Nature* 340:474–476.
- Davis S, Butcher SP, Morris RGM (1992) The NMDA receptor antagonist D-2-amino-5-phosphonopentanoate (D-AP5) impairs spatial learning and LTP *in vivo* at concentrations comparable to those that block LTP *in vitro*. *J Neurosci* 12:21–34.
- Gall C, Lynch GS, Morris RGM, Wilson D (1984) Septo-temporal distribution of medial and lateral perforant path innervation of rat hippocampus and capacity to support long-term potentiation (LTP). *J Physiol* 358:45P.
- Jeffery KJ, Abraham WC, Dragunow M, Mason SE (1990) Induction of Fos-like immunoreactivity and the maintenance of long-term potentiation in the dentate gyrus of unanaesthetized rats. *Mol Brain Res* 8:267–274.
- Laroche S, Doyere V, Bloch V (1989) Linear relation between the magnitude of long-term potentiation in the dentate gyrus and associative learning in the rat. A demonstration using commissural inhibition and local infusion of an *N*-methyl-D-aspartate receptor antagonist. *Neuroscience* 28:375–386.
- McNaughton BL, Barnes CA, Rao G, Baldwin J, Rasmussen M (1986) Long-term enhancement of hippocampal synaptic transmission and the acquisition of spatial information. *J Neurosci* 6:563–571.
- Morris RGM (1984) Developments of a water-maze procedure for studying spatial learning in the rat. *J Neurosci Methods* 11:47–60.
- Moser M-B, Moser EI, Andersen P (1990) Place learning is impaired by small lesions in the dorsal but not in the ventral hippocampus of the rat. *Eur J Neurosci Supp* 3:2248.