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Retrograde Amnesia for Spatial Memory Induced by NMDA Receptor-Mediated Long-Term Potentiation

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If information is stored as distributed patterns of synaptic weights in the hippocampal formation, retention should be vulnerable to electrically induced long-term potentiation (LTP) of hippocampal synapses after learning. This prediction was tested by training animals in a spatial water maze task and then delivering bursts of high-frequency (HF) or control stimulation to the perforant path in the angular bundle. High-frequency stimulation induced LTP in the dentate gyrus and probably also at other hippocampal termination sites. Retention in a later probe test was disrupted. When the competitive NMDA receptor antagonist 3-(2-carboxypiperazin-4-yl)propyl-1-phosphonic acid (CPP) was administered before the high-frequency stimulation, water maze retention was unimpaired. CPP administration blocked the induction of LTP. Thus, high-frequency stimulation of hippocampal afferents disrupts memory retention only when it induces a change in the spatial pattern of synaptic weights. The NMDA receptor dependency of this retrograde amnesia is consistent with the synaptic plasticity and memory hypothesis.

Key words: memory; spatial memory; synaptic plasticity; hippocampus; dentate gyrus; LTP; NMDA receptor; CPP; water maze; rat; saturation; retrograde amnesia

Long-term potentiation (LTP) refers to the major cellular model of the mechanisms underlying activity-dependent plasticity in the hippocampus (Bliss and Lømo, 1973; Malenka and Nicoll, 1999), and the "synaptic plasticity and memory" hypothesis captures the notion that this type of plasticity occurs during and is necessary for hippocampus-mediated memory functions such as spatial memory formation in the rodent (Martin et al., 2000). Although growing evidence suggests that hippocampal LTP is required for some aspects of spatial learning (Morris et al., 1986; Silva et al., 1992; Bourtchuladze et al., 1994; Tsien et al., 1996; Minichiello et al., 1999), the extent to which memory is also stored by hippocampal LTP remains unresolved. On the one hand, the primary role of the hippocampus may be to facilitate transfer of information from a short-term to a long-term store in the neocortex, without actual storage in the hippocampus as such (Wickelgren, 1979; Delacour, 1995). On the other hand, specific information may be stored within the hippocampal formation itself, in which case successful retrieval might require that the pattern of synaptic connection strengths induced by a particular learning experience be retained (Marr, 1971; McNaughton and Morris, 1987). Such storage may be temporary, with neocortical circuits taking over the storage slowly (Marr, 1971; McClelland et al., 1995; Squire and Alvarez, 1995), or it may be permanent (Nadel and Moscovitch, 1997).

If information is stored as patterns of synaptic weights inside the hippocampus, induction of LTP in some or all of the remaining unpotentiated synapses should interfere with the ability to retrieve memory already stored in the network (Fig. 1). To test this idea, McNaughton et al. (1986) trained rats to find an escape tunnel in a Barnes circular maze, then induced LTP by tetanic stimulation within the angular bundle, and finally tested memory of the escape location. They reported that only short-term memory was affected, suggesting that spatial long-term memory was stored elsewhere or by mechanisms different from those of LTP. However, it is also possible that long-term storage in the hippocampus was spared because too few fibers were activated by the tetanization. Here, we repeated the experiment with a more efficient stimulation protocol that allowed LTP to be induced in fibers throughout the angular bundle (Moser et al., 1998). We trained rats over several days to find a hidden platform at a constant location in a water maze memory task and subsequently induced LTP through a multielectrode array in and around the angular bundle until no further potentiation was obtained. We then tested whether this LTP disrupted retention in the water maze relative to appropriate control groups.

Impaired retention might be caused by effects of high-frequency (HF) stimulation other than LTP. Accordingly, some animals received tetanic stimulation in the presence of the NMDA receptor antagonist 3-(2-carboxypiperazin-4-yl)propyl-1-phosphonic acid (CPP).This should block the induction of LTP without necessarily affecting other consequences of the stimulation. The prediction is that this drug treatment, which ordinarily disrupts learning when given during training, should cancel out the LTP-inducing effect of tetanization and leave water maze retention unimpaired.

MATERIALS AND METHODS

Sixty-nine naïve male Long–Evans rats (350–500 gm at implantation and testing) were housed in groups of four to six in large transparent Plexiglas cages (54 × 44 × 35 cm height) with food and water available *ad libitum*. The animals were kept on a 12 hr light/dark schedule and tested in the dark phase.

The general procedure consisted of (1) surgical removal of one hippocampus, (2) implantation of electrodes, (3) pretraining in the water maze task, (4) electrophysiological stimulation involving LTP induction

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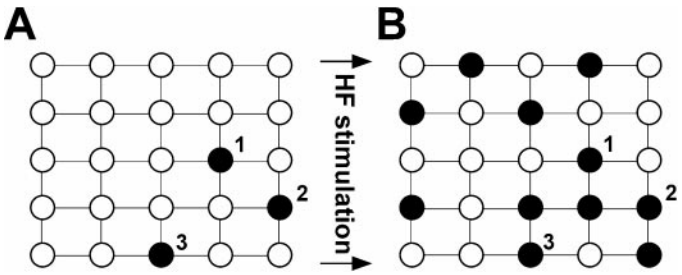

Figure 1. Hypothesized disruption of synaptic weight distribution in a hippocampal neural network by widespread LTP induction. *A*, Network after spatial learning, showing potentiation of a few synapses (*1–3*). *Lines* are neuronal processes that intersect as synapses. Potentiated synapses are *black*; unpotentiated synapses are *white*. *B*, Same network after subsequent induction of LTP in a subset of the synapses. Synapses that were potentiated during learning (*1–3*) may not be distinguishable from those that were potentiated by electrical stimulation.

in some animals, (5) retention test in the water maze, (6) retraining (Experiment 1 only), (7) monitoring residual LTP, and (8) histological assessment of lesions and electrode locations.

Surgery. To increase the proportion of afferent fibers that would receive high-frequency stimulation, we first decreased the volume of hippocampal tissue by making unilateral ibotenic acid lesions of the hippocampus and dentate gyrus, as described previously (Jarrard, 1989; Moser and Moser, 1998).

At least 10 d later, electrodes for field potential stimulation and recording were implanted according to methods described previously (Moser et al., 1998). For stimulation, three concentric bipolar electrodes (SNEX 100; Rhodes Medical, Woodland Hills, CA) were placed in the medial, middle, and lateral parts of the angular bundle of the intact hemisphere and 3.0, 4.0, and 5.0 mm, respectively, lateral to the lambda (~7.0 mm posterior to bregma) (Fig. 2). Two recording electrodes (twisted, 70 μm Teflon-insulated stainless steel wires; Goodfellow, Cambridge, UK) were implanted in the dentate hilus or granule cell layer (3.5 mm posterior and 2.4 mm lateral to the bregma). Each electrode was advanced until waveforms with a large positive field EPSP and a superimposed negative population spike could be evoked by 100 μsec pulses (100–500 μA) to the perforant path. The electrodes were fixed in place with dental acrylic, which was anchored to the skull by jeweler's screws. All rats were anesthetized with Equithesin (pentobarbital and chloral hydrate; 1.0 ml/250 gm body weight) during both surgical events.

Behavioral training. All rats were trained in an open-field water maze, a white circular polyvinylchloride tank (2 m diameter, 50 cm deep) filled to a depth of 40 cm with water at 25 ± 1°C (Morris, 1984). Latex liquid was added to make the water opaque. The maze contained four pneumatically controlled escape platforms (10 cm diameter) that could be moved by remote control between an available level (1.5 cm below the water surface) and an unavailable level (22 cm below the surface). The choice of platform was varied from rat to rat, but each individual rat was always tested with the platform in the same location (NW, NE, SW, or SE).

Pretraining started 10–14 d after electrode implantation and consisted of two daily sessions of four consecutive trials for 5 consecutive days. There was a minimum of 4 hr between sessions. The rats were released from the perimeter of the pool at N, W, S, or E in a predetermined pseudorandom order. If the rats had not entered the platform after 120 sec, the experimenter guided them onto it. The rats were left for 30 sec on the platform after each trial. At the end of pretraining (day 6), a spatial probe test was conducted, during which the platform was submerged and unavailable for the first 60 sec before being raised.

After the electrophysiological stimulation was completed, another probe test was conducted. The interval between stimulation and probe test was 2 hr in the initial study without drugs and 24 hr in the CPP study. In the first experiment, the animals were trained on a new water maze task immediately after the retention test. Partition walls were introduced, and all major visual cues were moved to new locations or replaced by other cues. The platform was in the quadrant opposite to that used during training. The animals received three blocks of two consecutive trials at an interblock interval of 30 min, with a 60 sec spatial probe test 30 min after the final training trial.

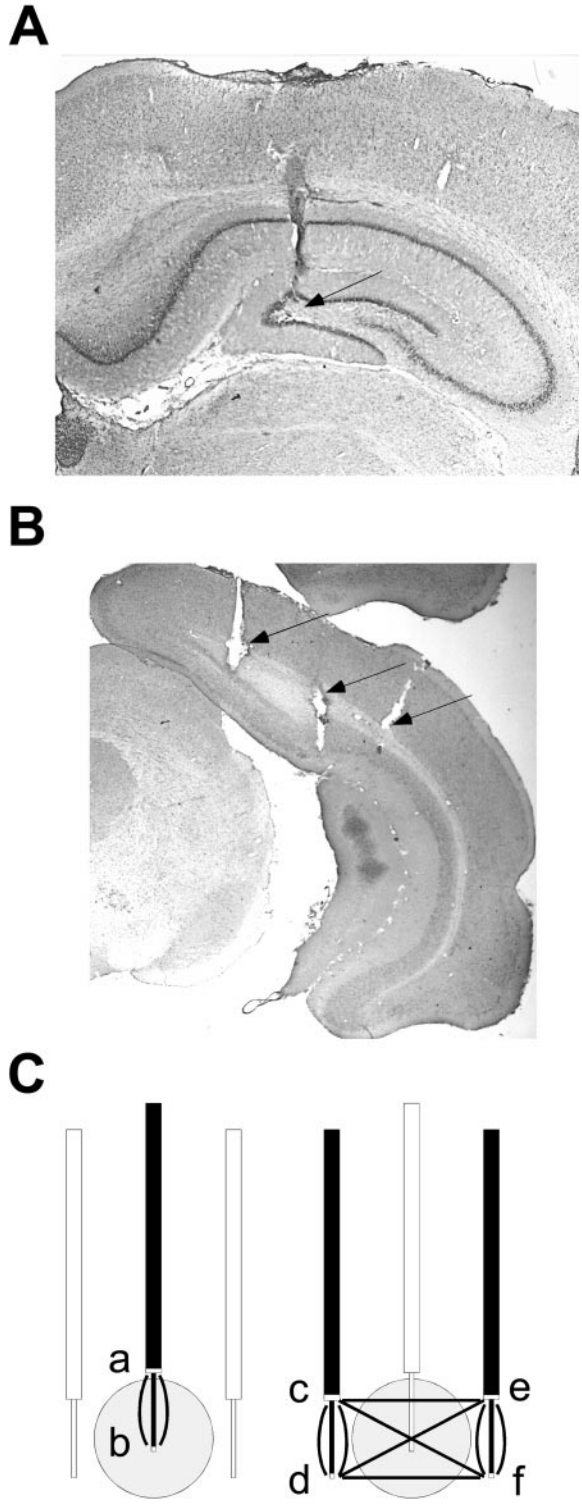

Figure 2. Position of electrodes. *A*, *B*, Traces (indicated by *arrows*) of the recording electrode in the dentate gyrus (*A*) and three bipolar stimulation electrodes (medial, central, lateral) in the angular bundle (*B*) (coronal sections, cresyl violet). *C*, Stimulation protocols. *Left*, Single test pulses were delivered through the central electrode in the angular bundle (*circle*), with anode and cathode at different depths (*ab*). *Right*, Induction of LTP by tetanic stimulation across the angular bundle, with anode and cathode varied between six stimulation sites (*c–f*). Sequence of stimulation: *ce*, *df*, *cf*, *de*, *cd*, *ef* (first and third stimulation session) and *ec*, *fd*, *da*, *fc*, *dc*, *fe* (second and fourth stimulation session). For each combination, eight trains (high-frequency group) or eight single pulses (low-frequency group) were delivered, with combinations separated by 30 sec intervals.

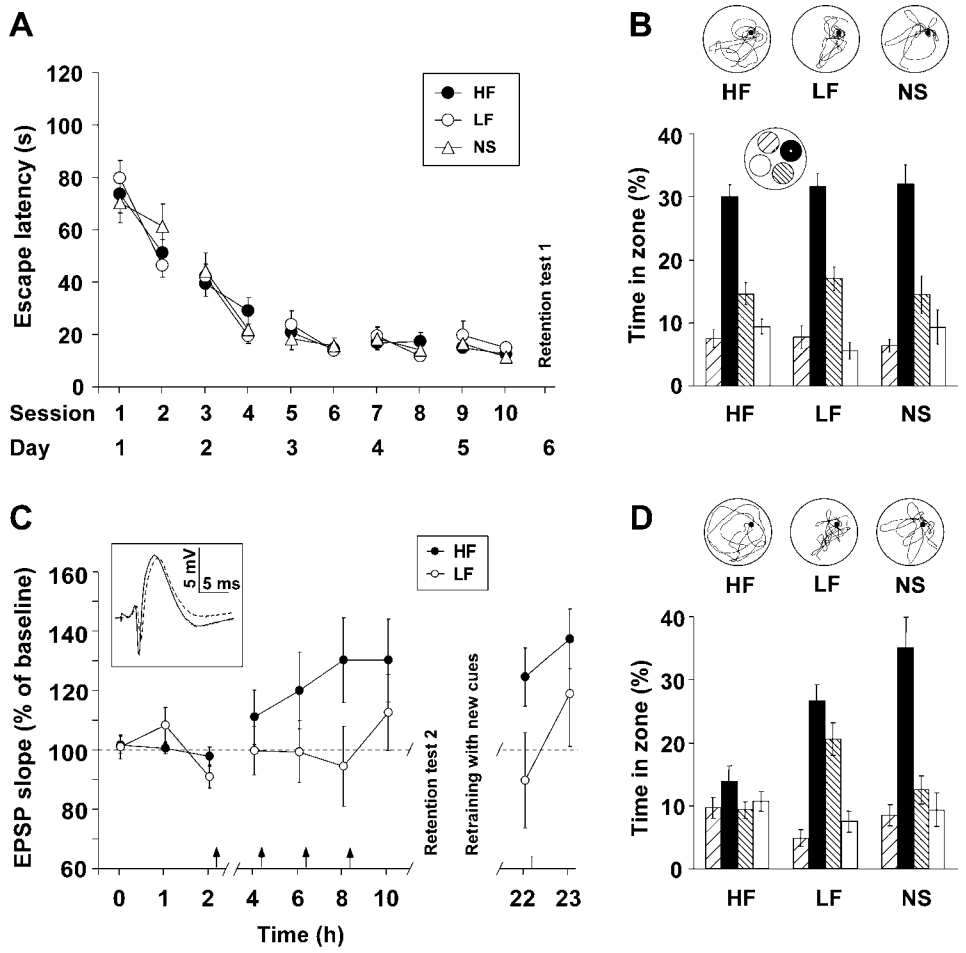

Figure 3. Effect of LTP on retention of spatial memory in a water maze (means ± SEM). *A*, Latency to enter the hidden platform during the 5 d of pretraining before electrophysiological stimulation started (2 sessions of 4 trials on each day). *B*, Retention test at the end of the pretraining. *Top*, Records of the search pattern of a representative animal from each group during 60 sec of swimming with the platform submerged to an unavailable level. *Bottom*, Time spent inside a circle (32 cm radius) around the platform position (*black*) and in corresponding, equally large zones in the three other pool quadrants during these 60 sec. All rats searched extensively in the platform zone. *C*, Induction of LTP by tetanic stimulation between multiple electrodes in the medial and lateral parts of the angular bundle as indicated in Figure 2*C*. The diagram shows the normalized values for field EPSP slope (amplitude difference at 2 fixed latencies) before and after HF and LF stimulation (means ± SEM, 6 responses per animal per session). Cross-bundle stimulation (*closed arrows*) was delivered immediately after recording at 2, 4, 6, and 8 hr. High-frequency stimulation gradually increased the EPSP slope values. The animals were tested in the water maze at 12 hr and retrained with new cues between 12 and 14 hr (Fig. 4). The evoked potentials were unchanged as a consequence of testing in the water maze (22 hr). Residual LTP was estimated as the relative change in EPSP slope from 22 to 23 hr, after tetanic stimulation through the naïve central electrode (*open arrow*). *Inset*, Representative evoked potentials before tetanic stimulation (*broken line*) and after the final stimulation session (*solid line*). *D*, Retention test 2 hr after termination of stimulation (same rats as in *B*). Symbols are the same as in *B*.

On each probe test, we calculated the percentage time spent in a circular zone around the platform position and in corresponding zones of the three other quadrants. Each zone had a radius of 32 cm and comprised 10.5% of the pool surface.

Electrophysiology: Experiment 1. Implanted rats were assigned to either a HF ($n = 13$) or a low-frequency (LF) ($n = 12$) stimulation group. The animals received seven electrophysiology sessions at 1–2 hr intervals. Before each session, the rat was placed in a black chamber ($44 \times 30 \times 37$ cm) to which it had been habituated on the preceding 5 d. Counterbalanced wires for recording and stimulation were connected to the implanted electrodes. During each session, evoked waveforms were sampled in the dentate gyrus in response to constant square-wave pulses (100 μsec, 0.2 Hz, 70–1000 μA) to the middle perforant path electrode (Fig. 2*C*, *left*). Immediately after the third through sixth recording sessions, the rats received either HF or LF stimulation. HF stimulation consisted of a sequence of eight trains of eight pulses of 400 Hz (2 sec between trains; 30 sec between sequences). To activate a maximum number of fibers (Moser et al., 1998), stimulation was given between all six possible anode–cathode combinations of the two bipolar peripheral electrodes (Fig. 2*C*, *right*). The pulse intensity was 1500 μA when anode and cathode were on different sides of the angular bundle, and 800 μA when the poles were on the same side. LF frequency rats received similar stimulation at 0.5 Hz. After testing in the water maze, synaptic responses were sampled again, and all animals received HF stimulation (two sequences of eight 400 Hz trains at 1000 μA) through the naïve middle stimulation electrode. The effect on the EPSP slope was determined 1 hr later. Nonstimulated (NS) rats ($n = 10$) received the same habituation and handling as implanted rats and spent the same amount of time in test boxes during the stimulation sessions.

Electrophysiology: Experiment 2. To determine whether the behavioral effects of LTP were NMDA-receptor-mediated, another 34 implanted animals were given HF or LF stimulation after intraperitoneal injections of either saline (SAL) or the NMDA receptor antagonist CPP. This gave four groups: HF/CPP ($n = 8$), HF/SAL ($n = 11$), LF/CPP ($n = 7$), and LF/SAL ($n = 8$). CPP has previously been reported to block induction of hippocampal LTP for at least 3 hr at a dose of 10 mg/kg (Abraham and Mason, 1988; Kentros et al., 1998). In the present study, each animal received 15 mg/kg CPP or an equivalent volume of saline intraperitoneally 20 min before recording started. However, because CPP has been estimated to decay with a half-time of 90–190 min in pigs (Kristensen et al., 1995), the interval between baseline recordings was reduced to 30 min, and the interval between stimulation sessions was reduced to 1 hr; a second injection (7.5 mg/kg CPP or saline) was given 150 min after the first (after session 4). Because the animals were not tested behaviorally until 24 hr later, slightly higher stimulation intensities were used (2000 μA when anode and cathode were on different sides of the angular bundle, and 1500 μA when they were on the same side). After the retention test on the next day (24 hr after stimulation), synaptic responses were sampled again, and HF stimulation was given through the naïve middle stimulation electrode as above. In all other respects, stimulation and recording were conducted as in Experiment 1.

Histology. The rats were killed with an overdose of Equithesin and perfused intracardially with saline and 4% formaldehyde. The brains were extracted and stored in formaldehyde, and frozen sections (30 μm) were cut coronally, mounted, stained with cresyl violet, and examined for hippocampal and extrahippocampal damage. Volume of residual hippocampal tissue on the lesioned side was determined as described previously (Moser and Moser, 1998).

RESULTS

Retention is disrupted by high-frequency stimulation

Before electrophysiological stimulation, the rats were trained to find a hidden platform at a constant location in the water maze

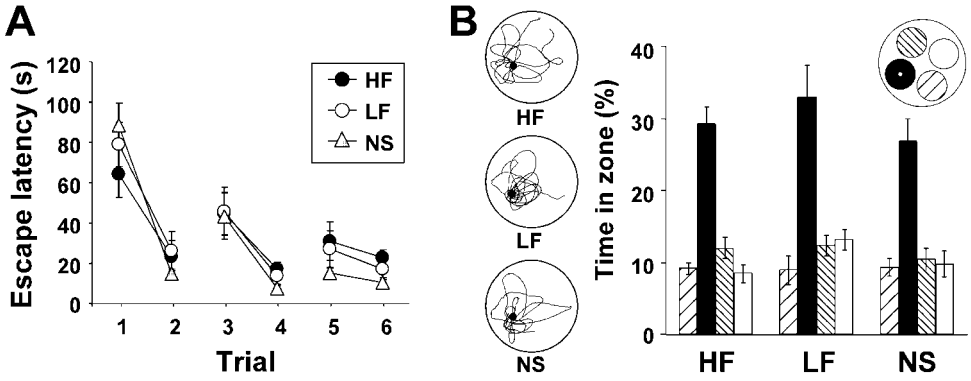

Figure 4. Effect of LTP on new spatial learning in the water maze (means ± SEM). *A*, Latency to enter the hidden platform. *B*, Representative search patterns (same rats as in Fig. 3*B*,*D*) on a probe test at the end of training in the new task (*top*) and time in the center of each quadrant on the probe test (*bottom*). All symbols as in Figure 3.

over a period of 5 d (10 blocks of trials). All rats learned to swim directly to the hidden platform well before training was completed and generally reached asymptotic escape latencies before the sixth session (Fig. 3*A*). On day 6, a spatial probe test was conducted in which the platform remained unavailable at the bottom of the pool for the first 60 sec and then rose to near the surface to become available as a refuge. All animals spent a large portion of the trial searching within a small zone centered on the platform (Fig. 3*B*).

Cross-bundle stimulation resulted in cumulative LTP in the HF group, with waveforms showing a gradual increase in the early rising portion of the extracellular field potential over the course of the recording period (Fig. 3*C*). The potentiation reached ~130% of the baseline values. The field EPSP slope measured 2 hr after the last cross-bundle stimulation session was significantly elevated compared with the pretetanization baseline ($t_{(9)} = 2.2; p < 0.05$). There was no significant potentiation of the EPSP in the LF group.

Two hours after the last stimulation session, the effect of LTP on retention was tested in the water maze (Fig. 3*D*). The platform was not available during the initial 60 sec of this test. As before, LF and NS animals searched more in the previous platform zone than in the corresponding zones of the other quadrants. Rats that had received HF stimulation failed to demonstrate any significant preference for the platform region. A repeated-measures ANOVA of time spent in the four quadrant zones showed a significant groups × zones interaction ($F_{(6,96)} = 6.7; p < 0.001$), with significant group differences in the target zone (one-way ANOVA; $F_{(2,32)} = 10.4; p < 0.001$). Subsequent pairwise comparisons [Tukey honestly significant difference (HSD)] showed that the HF group had lower target zone times than the LF group (studentized $t_{(32)} = 3.8; p = 0.02$) and the NS group (studentized $t_{(32)} = 6.4; p < 0.001$), whereas the control groups themselves did not differ (studentized $t_{(32)} = 2.6; p = 0.20$).

The same HF rats, however, were able to learn a new task in the water maze after the platform was moved to the opposite quadrant, partition walls were inserted, and all major visual cues were moved randomly to new locations (Fig. 4). Training in the new environment consisted of three blocks of two trials at an interblock interval of 30 min, with a probe test 2 hr after the retention test in the original environment. There was no difference in preference for the platform zone on the probe test (groups × zones: $F < 1$). Rats with hippocampal lesions fail to learn when trained in this protocol (Moser and Moser, 1998). Thus, the intact new learning of the HF animals suggests that their retention deficit was not caused by major structural or functional damage to the hippocampus after synchronized stimulation of a large number of afferent fibers.

The evoked potentials were unchanged as a consequence of testing in the water maze (Fig. 3*C*) (22 hr after the start of recording, 8 hr after the last water maze trial). The potentiation of the field EPSP was maintained in the HF group (125 ± 10%; mean ± SEM), whereas the EPSP of the LF group remained near the baseline value (90 ± 16%). The group difference was significant ($t_{(15)} = 2.0; p < 0.05$). We finally estimated the extent to which cumulative LTP had been induced in fibers outside the immediate vicinity of the induction electrodes. Tetanic stimulation was delivered through the middle electrode in the angular bundle (Fig. 2*C*, *left*), which so far had been used for single-pulse stimulation only. We found that some LTP could still be induced through this naïve electrode in several (but not all) HF animals (Fig. 3*C*, *23* hr), but the amount was significantly less than in the LF control group ($p < 0.005$), suggesting that the cross-bundle stimulation protocol had induced LTP in fibers throughout the angular bundle but not to a level that resulted in "saturation" of LTP. Residual LTP in the HF group ranged from −14.5 to 47.4%, but the correlation with time in the platform zone on the retention test was not significant ($r_{(12)} = -0.41$). The consistent impairment of retention in the HF group indicates that its disruption requires only that the spatial pattern of synaptic weights be altered, not that LTP be saturated (Fig. 1).

All animals had substantial hippocampal damage to the lesioned hemisphere (residual hippocampal tissue in HF group: 8.7 ± 2.7%; LF group: 8.9 ± 2.8%; NS group: 5.5 ± 1.6%; means ± SEM of total contralateral hippocampal volume).

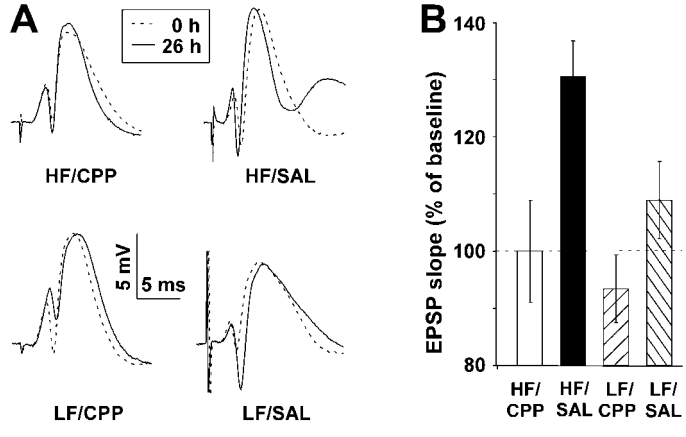

Figure 5. Blockade of dentate LTP in animals injected with the competitive NMDA receptor antagonist CPP. *A*, Representative evoked potentials before (*broken line*) and after (*solid line*) cross-bundle stimulation at low or high frequency (*LF*/*HF*) in animals treated with either saline (*SAL*) or *CPP*. *B*, Normalized values for field EPSP slope 22 hr after the last session of HF or LF stimulation and >2 hr after the retention test in the water maze (means ± SEM). The EPSP was strongly potentiated in the HF/SAL group but not in the other groups.

Disruption of retention is NMDA receptor-mediated

In the second experiment, the rats reached asymptotic swim latencies well before the pretraining was terminated (see Fig. 6*A*). They showed a clear preference for the platform zone on the probe test (see Fig. 6*B*).

As in the first study, the EPSP slope was significantly enhanced in HF-stimulated animals that did not receive the drug (131 ± 6%) (Fig. 5) but not in the other groups. The HF-induced enhancement was completely blocked by CPP (100 ± 9%), the EPSP slopes of the LF groups were not different (LF/CPP: 93 ± 6%; LF/SAL: 109 ± 7%), and the slope of the HF/CPP group did not differ from the values of the LF groups. We conducted a repeated-measures analysis of the increase in the EPSP slope. The LF groups were combined to increase the power of the analysis. The analysis revealed a significant group difference ($F_{(3,27)} = 5.4$; $p = 0.005$). Pairwise comparisons (Tukey HSD) confirmed that the HF/SAL group had a steeper EPSP slope function than the LF groups (studentized $t_{(25)} = 3.7$; $p < 0.02$) and the HF/CPP group (studentized $t_{(25)} = 4.5$; $p < 0.02$). The HF/CPP group was not different from the LF groups (studentized $t_{(25)} = 0.8$).

The retention test was conducted the day after tetanic stimulation, when the cerebral CPP concentration would have declined to near zero. Animals that had received HF stimulation in the presence of CPP performed just as well on the probe test as animals that received LF control stimulation (Fig. 6*C*). Both the HF/CPP group and the LF groups searched more around the platform (HF/CPP: 33.2 ± 4.8%; LF/CPP: 29.7 ± 3.5%; LF/SAL: 27.7 ± 3.6%) than in corresponding zones of the other quadrants. In contrast, in rats that received HF in the presence of saline, the preference for the platform zone was significantly attenuated (17.7 ± 1.7%). A repeated-measures ANOVA of time spent in the four quadrant zones showed a significant groups × zones interaction ($F_{(6,93)} = 4.1$; $p < 0.001$; LF groups combined as above), with significant group differences in the target zone ($F_{(2,31)} = 6.8$; $p < 0.005$). Subsequent pairwise comparisons (Tukey HSD) showed that the HF/SAL group had lower target zone times than the LF groups (studentized $t_{(31)} = 3.7$; $p = 0.02$). The HF/CPP group swam as much in the target zone as the LF groups (studentized $t_{(31)} = 1.5$; $p = 0.54$), and was superior to the HF/SAL group (studentized $t_{(31)} = 5.2$; $p = 0.005$).

Subsequent tetanization through the naïve middle electrode showed that there was still significant capacity for LTP in the two LF groups (LF/CPP: 43.6 ± 4.0%; LF/SAL: 25.3 ± 4.6%) as well as the HF/CPP group (26.6 ± 6.6%). In the HF/SAL group, in contrast, the mean residual potentiation of the EPSP was attenuated (16 ± 3.8%), but the range was large (−0.5–32.5%) and the group differences were not significant.

Histological analysis showed that the four groups had comparable amounts of residual hippocampal tissue (HF/CPP: 7.0 ± 1.5%; HF/SAL: 7.0 ± 2.4%; LF/CPP: 6.7 ± 1.7%; LF/SAL: 7.8 ± 1.4%; means ± SEM of contralateral volume). Two rats in the HF/SAL group had incomplete unilateral lesions of the hippocampus (13 and 15% of total unilateral hippocampal tissue, with spared tissue both dorsally and ventrally) but were not excluded. They showed better retention (29.2 and 25.5% of search time in the platform zone) than any of the other HF/SAL rats after the tetanic stimulation (15.6 ± 1.1%). Remaining hippocampal tissue correlated significantly with search time in the platform zone in the HF/SAL group ($r_{(9)} = 0.69$; $p < 0.05$). This correlation disappeared when we excluded the two rats from the correlation analysis ($r_{(7)} = -0.07$, not significant).

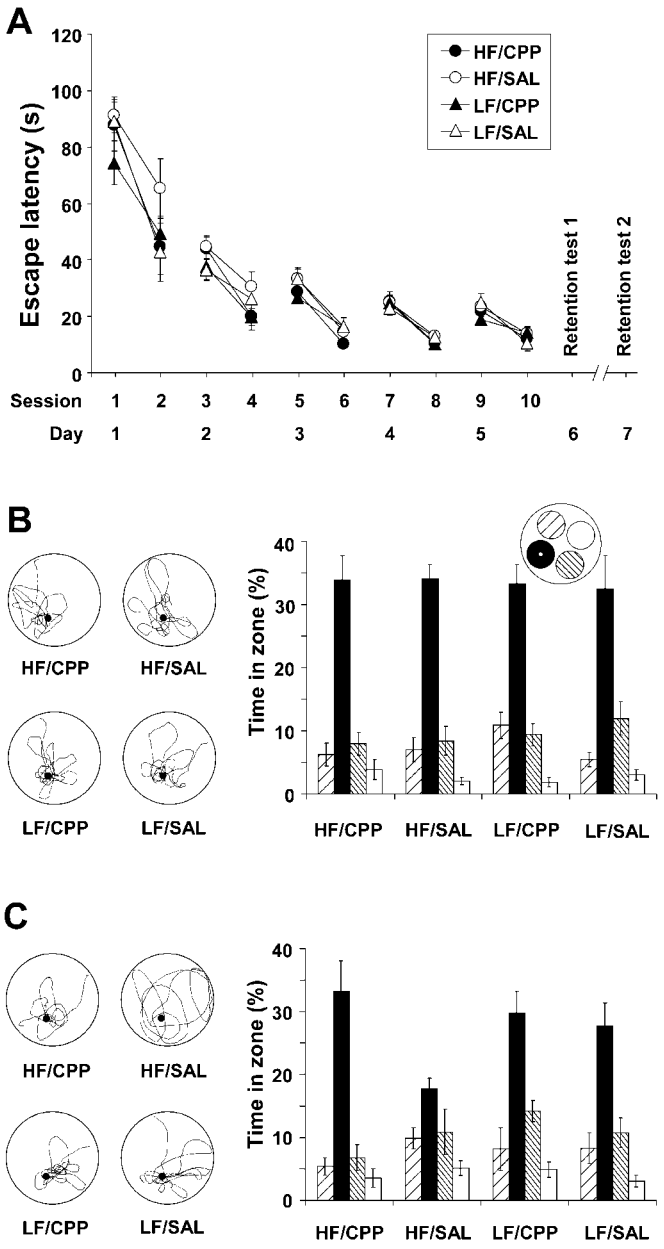

Figure 6. Effect of HF stimulation on memory retrieval in animals with LTP blocked by the competitive NMDA receptor antagonist CPP (means ± SEM). *A*, Latency to enter the hidden platform during pretraining. *B*, Retention test at the end of pretraining. *C*, Retention test 24 hr after HF stimulation. All symbols as in Figure 3.

DISCUSSION

The key finding is that retention of a recently learned spatial memory task was impaired by repeated HF stimulation of the perforant-path input to the dentate gyrus and hippocampus, one of the effects of which was to induce measurable LTP. This disruption of retention was prevented by previous application of the NMDA receptor antagonist CPP at a dose that blocked the induction of LTP. The effect on retention was retrograde and not anterograde.

Specificity of the behavioral impairment

HF stimulation of hippocampal afferent fibers caused retrograde amnesia for a task learned over several days without impairing the ability to learn a new water maze task. This suggests that the retention deficit was an interference with storage rather than encoding or retrieval and implies that critical elements of spatial memory may be stored within the hippocampus after encoding. Previous studies have reported impaired retention of spatial location after prolonged sinusoidal current in the dentate gyrus (Collier et al., 1982) or brief HF stimulation of the perforant path (McNaughton et al., 1986) when the stimulation was delivered immediately after acquisition. Information encoded during the 2 preceding weeks was unaffected (McNaughton et al., 1986). In our study, the animals were trained over a period of 5 d and approached asymptotic performance levels as early as the second or third day (Figs. 3*A*, 6*A*). Because our HF stimulation was not delivered until several days later, the hippocampal representation appears to be vulnerable to HF stimulation for a period of several days, and perhaps longer. This is a time scale more consistent with the "consolidation" period of days or weeks during which memory is disrupted by medial temporal lesions or hippocampal inactivation (Winocur, 1990; Zola-Morgan and Squire, 1990; Kim and Fanselow, 1992; Riedel et al., 1999). It is possible that the cross-bundle stimulation protocol used in the present study affected hippocampally encoded memory more efficiently than in the study of McNaughton et al. (1986) because a larger and more widespread subset of the synapses was affected (Moser et al., 1998).

Was the retention impairment caused by LTP?

Although the disruption of retention after HF stimulation could reflect induction of LTP in the hippocampal network, it is also possible that the performance was affected by other consequences of the stimulation. There were several control procedures in the experiment, and their purpose was to check on the contribution of these putative nonspecific consequences.

First, one group of animals received LF stimulation at the same intensity and between the same electrodes as in the HF group, yet retention in the LF group was not significantly impaired compared with the nonstimulated group. Admittedly, the level of retention shown by the LF group was lower, but this difference did not reach significance; variations in the design or larger group sizes might have revealed a significant behavioral impairment caused by LF stimulation alone. In any case, HF stimulation caused a significantly larger retention deficit than LF stimulation, suggesting that retention was affected by HF stimulation as such.

Second, HF trains could and almost certainly do have other physiological and biochemical consequences than the induction of LTP. For this reason, a subset of the animals received HF stimulation in the presence of CPP, which blocked NMDA receptors and prevented LTP. There was no impairment of retention in this condition, suggesting that the behavioral impairment in the non-drugged animals was caused by the alteration of synaptic weights associated with LTP induction or perhaps by nonspecific events downstream of the NMDA receptor. One major nonspecific consequence of excessive glutamate release is excitotoxic neuronal injury, which is mediated in part by NMDA receptors (Rothman and Olney, 1987). It is unlikely that the present results primarily reflect excitotoxic damage, because tetanic stimulation did not lead to reduced field potentials (Fig. 3*C*), which might be expected after damage or hypofunction in the recorded neurons.

Third, to the extent that the retention impairment does reflect the induction of NMDA receptor dependent LTP, the data confirm predictions of models proposing that spatial memory is stored as a distributed array of synaptic strengths in the hippocampal formation (Marr, 1971; McNaughton and Morris, 1987; McClelland et al., 1995). These models would predict memory performance to deteriorate by any treatment that alters the connection strengths of the network, such as LTP, provided that the treatment is administered after behavioral training. In a distributed network, poor retention would be expected even when LTP is induced incompletely (Fig. 1), which is consistent with what we have observed. Nearly all animals were impaired by the HF stimulation, regardless of whether there was residual LTP. New learning, in contrast, is disrupted only with extensive LTP induction (Moser et al., 1998), suggesting that the encoding of spatial information within the hippocampal network may be highly dynamic. That is, the encoding system may be able to selectively use intrinsic pathways at which there is the potential for synaptic enhancement, and because it uses only a subset of synapses for any given encoding task, memory is only disrupted in the anterograde domain by extensive previous LTP induction. In the retrograde domain, in contrast, once the encoding system has committed traces to a particular subset of synapses, potentiation of a small number of others will be sufficient to disrupt retention. To summarize, these data are consistent with central predictions of models of distributed memory storage, although many details have yet to be worked out for the case to be proved.

What is the function of LTP in spatial memory?

The present results showed that HF stimulation impaired memory of where the platform was located. To the extent that this implies an involvement of synaptic strengths in retention of spatial memory (Fig. 1), these results are consistent with the disruption of spatial learning after blockade of NMDA receptors and hippocampal LTP (Morris et al., 1986; Davis et al., 1992; Sakimura et al., 1995; Tsien et al., 1996).

Several observations have challenged the supposition that memory was disrupted in these experiments because NMDA receptor-dependent LTP is necessary for encoding location. First, spatial learning in the reference memory version of the water maze task remains intact in animals that receive pretraining in a different water maze before the intervention with LTP induction (Bannerman et al., 1995; Saucier and Cain, 1995; Hoh et al., 1999; Otnæss et al., 1999). Pretraining does not facilitate learning of trial-unique information during blockade of LTP (Steele and Morris, 1999). The unimpaired new learning of the HF group after massive LTP induction (Fig. 4) replicates the findings in the reference memory condition and reinforces the view that LTP may not always be necessary for spatial learning. Second, hippocampal pyramidal cells retain the ability to develop spatial firing fields in a new environment after disruption of NMDA receptor-mediated transmission (McHugh et al., 1996; Kentros et al., 1998), although the overnight stability of such firing fields is poor (Kentros et al., 1998). These spared abilities suggest that functional spatial maps, to some extent and under some circumstances, may develop without NMDA receptor-dependent hippocampal LTP. It can be speculated that in a pretrained rat, spatial information that is not trial-unique may be acquired by less efficient, LTP-independent mechanisms. Under normal conditions, however, when the NMDA receptor pathway is not blocked, both naïve and pretrained animals may store spatial and spatiotemporal relations primarily by an LTP-like mechanism

(Steele and Morris, 1999; Moser and Moser, 2000). Subsequent disruption of the pattern of synaptic strengths, such as by widespread HF stimulation, might then result in poor retention.

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