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Place Representation within Hippocampal Networks Is Modified by Long-Term Potentiation

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Summary

In the brain, information is encoded by the firing patterns of neuronal ensembles and the strength of synaptic connections between individual neurons. We report here that representation of the environment by "place" cells is altered by changing synaptic weights within hippocampal networks. Long-term potentiation (LTP) of intrinsic hippocampal pathways abolished existing place fields, created new place fields, and rearranged the temporal relationship within the affected population. The effect of LTP on neuron discharge was rate and context dependent. The LTP-induced "remapping" occurred without affecting the global firing rate of the network. The findings support the view that learned place representation can be accomplished by LTP-like synaptic plasticity within intrahippocampal networks.

Introduction

Information in the brain is believed to be encoded and stored by the strength of synaptic weights within networks of connected neurons (Fregnac et al., 1988; Bliss and Collingridge, 1993; Martin et al., 2000; Kandel and Squire, 2000). A classic example of the representation of the environment is place-related activity of hippocampal pyramidal neurons (O'Keefe and Nadel, 1978). It has been hypothesized that the physical map of the environment is represented by the synaptic connectivity within the hippocampal neuronal populations (Wilson and McNaughton, 1993; Muller et al., 1996). Indeed, the firing patterns of pyramidal cells remain stable in a familiar environment for extended periods and new map representations emerge over time in a novel environment (Thompson and Best, 1990; Wilson and McNaughton, 1993; Lever et al., 2002). A leading candidate mechanism responsible for such stability and respective change of neuronal firing patterns is use-dependent synaptic plasticity. A laboratory model of synaptic plasticity is long-term potentiation (LTP) (Bliss and Lomo, 1973; Malenka and Nicoll, 1999; Kandel and Squire, 2000). There is controversy regarding the effects of LTP on neuronal firing as well as on solving spatial memory tasks. LTP of synaptic inputs has been associated with increased population spike and increased probability of action potential discharge to test stimulation (Taube and Schwartzkroin, 1988; Chavez-Noriega et al., 1990). Furthermore, the spontaneous firing of unidentified dentate neurons was increased after perforant path LTP (Deadwyler et al., 1976). In later experiments, however, LTP did not induce an overall increase in firing rate in anesthetized animals, even when only neurons discharged by the pre-LTP test pulse stimulation were considered (Martin and Shapiro, 2000; Kimura and Pavlides, 2000).

Numerous experiments demonstrate that various manipulations that affect electrically induced LTP also have an impact on the execution of spatial memory tasks (Morris et al., 1986; Silva et al., 1992; Kentros et al., 1998; Moser et al., 1998; Nakazawa et al., 2002). However, whether and how LTP exerts an impact on the firing patterns and place-related features of neuronal assemblies is not known. We hypothesized that if spatial representation is embedded in the weights of synaptic connectivity within hippocampal networks, then alterations of these connections by artificial stimulation should lead to the disappearance of established place fields and appearance of novel place fields even in a familiar environment.

Results

Because tetanic stimulation of hippocampal pathways induces long-term enhancement of evoked activity (Bliss and Lomo, 1973), we first examined whether induction of LTP affects the global excitability of the hippocampal network as measured by the firing rates of hippocampal neurons. High-frequency stimulation of the ventral hippocampal commissure (VHC) resulted in a long-lasting change of the slope of the evoked field responses measured in CA3 and CA1 pyramidal layers (Figure 1A). The evoked responses were periodically tested before and after the LTP train in the home cage with 10–20 pulses at 0.1 Hz and averaged. These test periods were exluded from the sleep periods over which firing rates were calculated. Responses at the various recording sites were affected differentially (Figure 1B). In some rats, enhancement at some sites was accompanied by a decrease at other sites. LTP, measured at the most effective site, varied from 12% to 107% (median, 37%, measured at 60–90 min after LTP induction; $p < 0.05$; Figure 1A, bottom; $n = 7$ rats) and in most cases lasted over 6 hr ($p < 0.05$). In contrast to the potentiated evoked potentials, the spontaneous firing rate of the total neuronal population, measured during the last sleep epoch before (30 min) and the first sleep epoch after (30 min) LTP induction ($n = 229$ CA1 pyramidal cells, $n = 171$ CA3 pyramidal cells, $n = 64$ CA1-3 interneurons, $n = 7$ rats), remained unchanged for all cell types (Figure 1C; $p > 0.05$, sign test), confirming previous observations in anesthetized animals (Martin and Shapiro, 2000; Kimura and Pavlides, 2000) and in rats exposed to a novel environment (Hirase et al., 2001). The incidence of sharp wave-associated ripples measured during the same slow-wave sleep periods (Figure 1D) was also not significantly different before and after LTP ($p > 0.5$; sign test).

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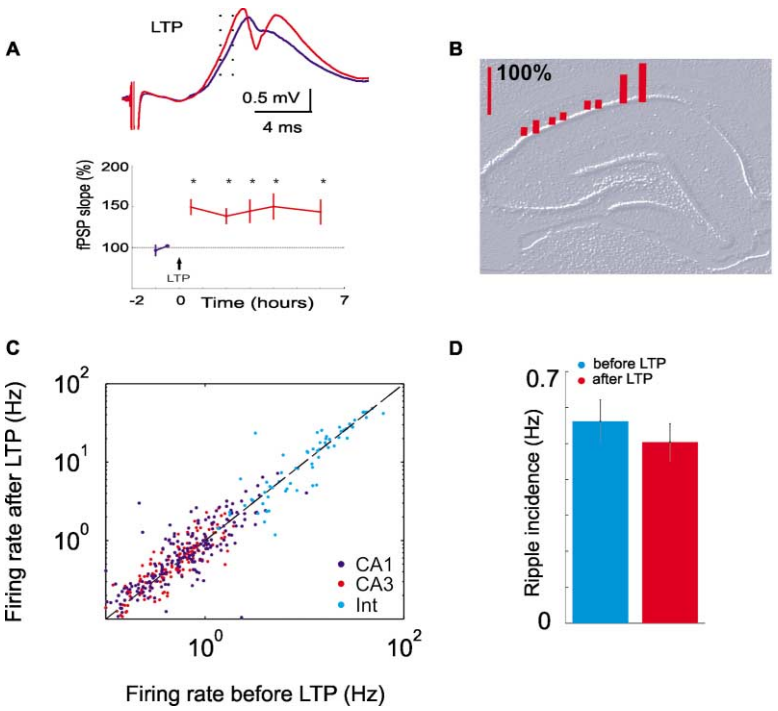

Figure 1. LTP Does Not Change the Global Spontaneous Excitability of the Hippocampal Network

(A) Example of average evoked potentials (n = 10) in CA1 pyramidal layer in response to VHC stimulation before (blue) and 90 min after (red) induction of LTP. Field postsynaptic potential (fPSP) slopes were calculated between the dotted lines. Note potentiation of EPSP slope and population spike after LTP. Below, LTP-induced slope changes at various intervals after the tetanus (n = 18 sessions in 7 rats). Averages were taken at the beginning of sleep episodes in the home cage. *$p < 0.05$, rank-sum test.

(B) Location dependence of LTP magnitude. Bar sizes reflect relative change in EPSP slopes in a single rat.

(C) Correlations between firing rate before and after LTP during sleep in the home cage. Each point corresponds to the mean firing rate of a single neuron. CA1, CA1 pyramidal cells; CA3, CA3 pyramidal cells; Int, interneurons in CA1 and CA3. Note that the grand mean firing rate of all populations is not affected by LTP.

(D) The incidence of sharp wave/ripple episodes during sleep was not affected by LTP. Ripples were detected in sleep sessions before and after (<1 hr) LTP induction.

Although LTP did not affect the global firing rate, it exerted a profound effect on the behavior-related firing patterns of place cells. The animals were tested on an elevated platform while searching for food. Place-correlated firing of parallel-recorded, multiple single neurons was determined in four test sessions. The position of the rat, local EEG, and the spiking pattern and stability of unit activity were continuously monitored throughout the experiment (Figure 2). Between the test sessions, the rats were placed back in their home cage and allowed to sleep. Evoked responses were tested at the beginning of sleep periods (10–20 pulses at 0.1 Hz; low-frequency stimulation, LFS) following the first and third test sessions. These control sessions served to assess the effects of handling, environment change (McNaughton et al., 1996), and brain stimulation on place cell activity. Between the second and third sessions, LTP was induced in the home cage (see Experimental Procedures). Since place cell activity depends on the direction of movement on linear track apparatus (McNaughton et al., 1983), clockwise and counterclockwise runs were examined separately. The effect of LTP on three representative CA1 place cells, recorded from the same tetrode, is illustrated in Figure 3. The neuron, shown in Figure 3A, had a similar place field during both directions of movement before LTP. Following the LTP-inducing trains, the neuron acquired a new place field during counterclockwise laps but remained unchanged during clockwise runs both in terms of place-related firing and "in-field" firing rate. Thus, disregarding the direction of movement, the neuron possessed two place fields after LTP, perhaps reflecting two different sets of functional inputs (Figure 3F). The second neuron was not affected by LTP and showed stable place field-related discharge in all sessions (Figure 3B). The third neuron had a well-developed place field only in the counterclockwise direction prior to LTP. After LTP, new place fields were present in both directions (Figure 3C). Importantly, 5.5 hr after LTP induction, when the slope of the evoked field response returned to pre-LTP level in this rat (Figure 3D), the neuron reinstated its direction-specific place field at the original position on the track (Figure 3E). This observation suggests that cell firing was controlled by the strongest synaptic input from a set of multiple existing synaptic pathways. Upon weakening of the potentiated set of input, control was resumed by the original set.

To allow for the simultaneous comparison of multiple neurons, the smoothed firing rates were plotted as a function of rat's position on the "linearized" track (see Experimental Procedures). In the example shown in Figure 4, the LTP-inducing train resulted in a larger potentiation of the evoked response at site A, including the emergence of a population spike, than at site B. Under tetrode A, cell #1 was "silent" before LTP. Following LTP, it acquired a robust place field when the animal ran counterclockwise (Figure 4B) but not clockwise (Figure 4A). Cell #4 lost its place field in clockwise direction after LTP (Figure 4A), but retained it while running counterclockwise (Figure 4B). Cell #3 shifted its place field for both directions of movement. Cell #2 reduced its in-field firing rate (clockwise, Figure 4A), whereas LTP had no significant effect on the remaining two place cells. The five neurons recorded with tetrode B (Figures 4C and 4D) showed only nonsignificant changes, comparable in magnitude to those observed after LFS or when a single session was split into two subsessions. These observations indicate that some neurons localized in the volume monitored by a single tetrode can be selectively affected by LTP, leaving their peers unaffected. The

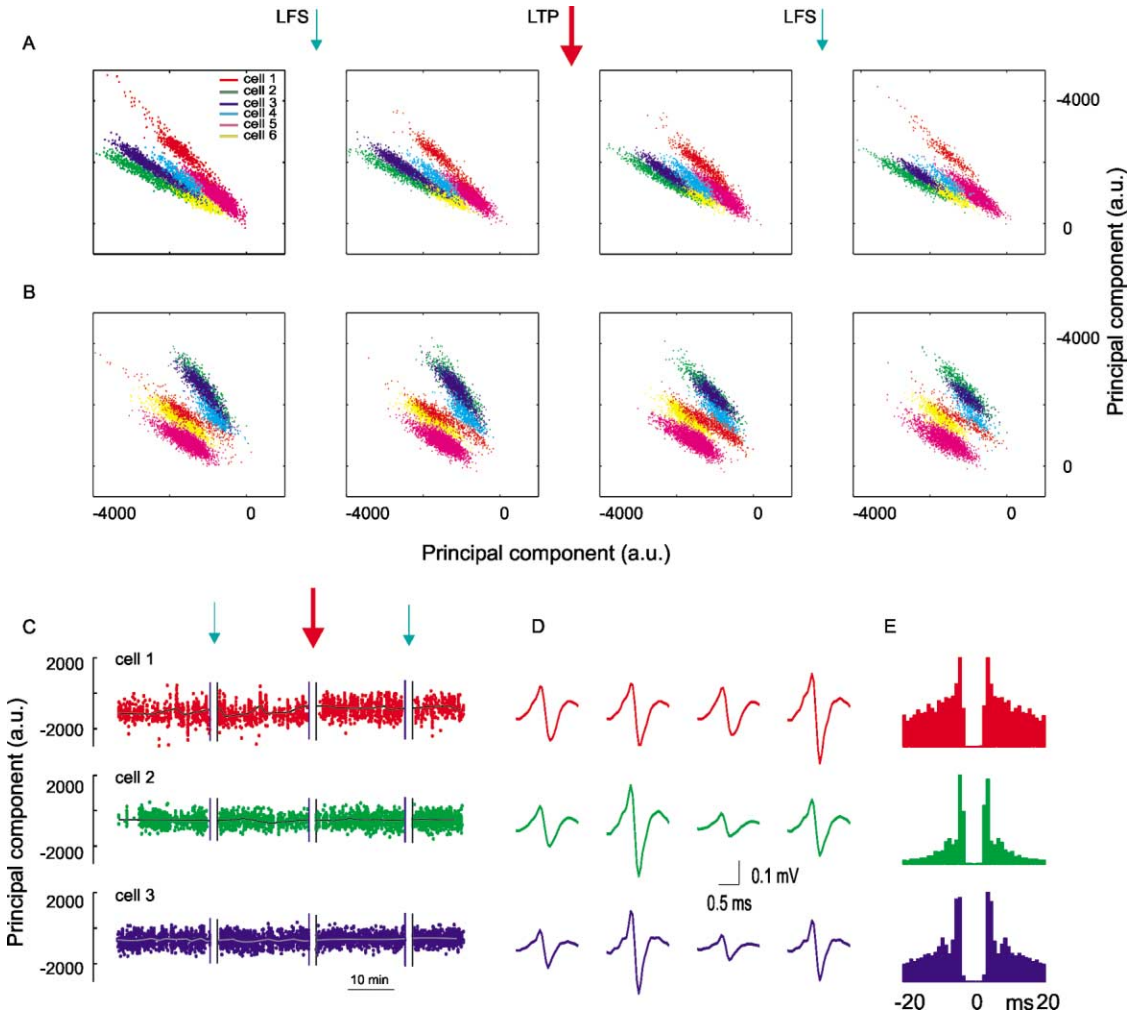

Figure 2. Stability of the Recorded Units in the Course of the Experiment

(A and B) Clusters of six CA1 pyramidal cells from a single tetrode (color-coded). Clustering was done using waveforms from all four tetrode sites. Only two projections (A and B) are shown separately for the four run sessions. *x* and *y* coordinates show the first principal components (arbitrary units; a.u.). Arrows: LFS, low-frequency stimulation; LTP, tetanic stimulation.

(C) First principal component for 3 place units (also shown in A and B and Figure 3) as a function of time. Black lines are medians of the smoothed distribution of the principal component in time. Vertical lines separate the four exploration sessions.

(D) Waveform of the three units (800 Hz–5 kHz) at each site of the tetrode.

(E) Autocorrelograms of the three units for the total duration of recording (exploration and sleep). Note peaks at 3–5 ms (complex spike bursts) and clear refractory periods.

findings also indicate that the LTP-induced effects on individual neurons cannot be accounted for by electrode drift or unit clustering errors.

To assess the effect of LTP at the population level, the difference between place field maps of CA1 and CA3 pyramidal cells before and after LTP was compared with the difference between maps computed during the first and second run sessions (LFS control). For each cell and each type of stimulation, the two place maps (one before and one after LFS or LTP) were pixel-by-pixel subtracted and the absolute values of all the pixel differences were added (see Experimental Procedures). For the place cell population, the place map differences produced by LFS were compared with those produced by LTP. LTP produced a significant change (firing rate and/or place shift) in place representation compared to the control LFS (Figure 5A; $n = 146$ CA1 and CA3 pyramidal cells; $p < 0.001$, sign test). To distinguish between shifts in place field position and in-field firing rate changes, place maps were first normalized by dividing the rate map by the sum of firing rate calculated in each pixel of the place map and the same differences were calculated and compared. Changes reflected in both nonnormalized and normalized place field maps were further interpreted as changes in place field location, whereas changes in only nonnormalized maps were considered to be rate changes within the same field. At the population level, LTP produced a significant shift in place representation compared to control LFS ($p < 0.05$, sign test). Using an arbitrary criterion (LTP-induced change at least twice as large as LFS-induced change, red dots in Figure 5A), 52 CA1 and CA3 place cells (35%

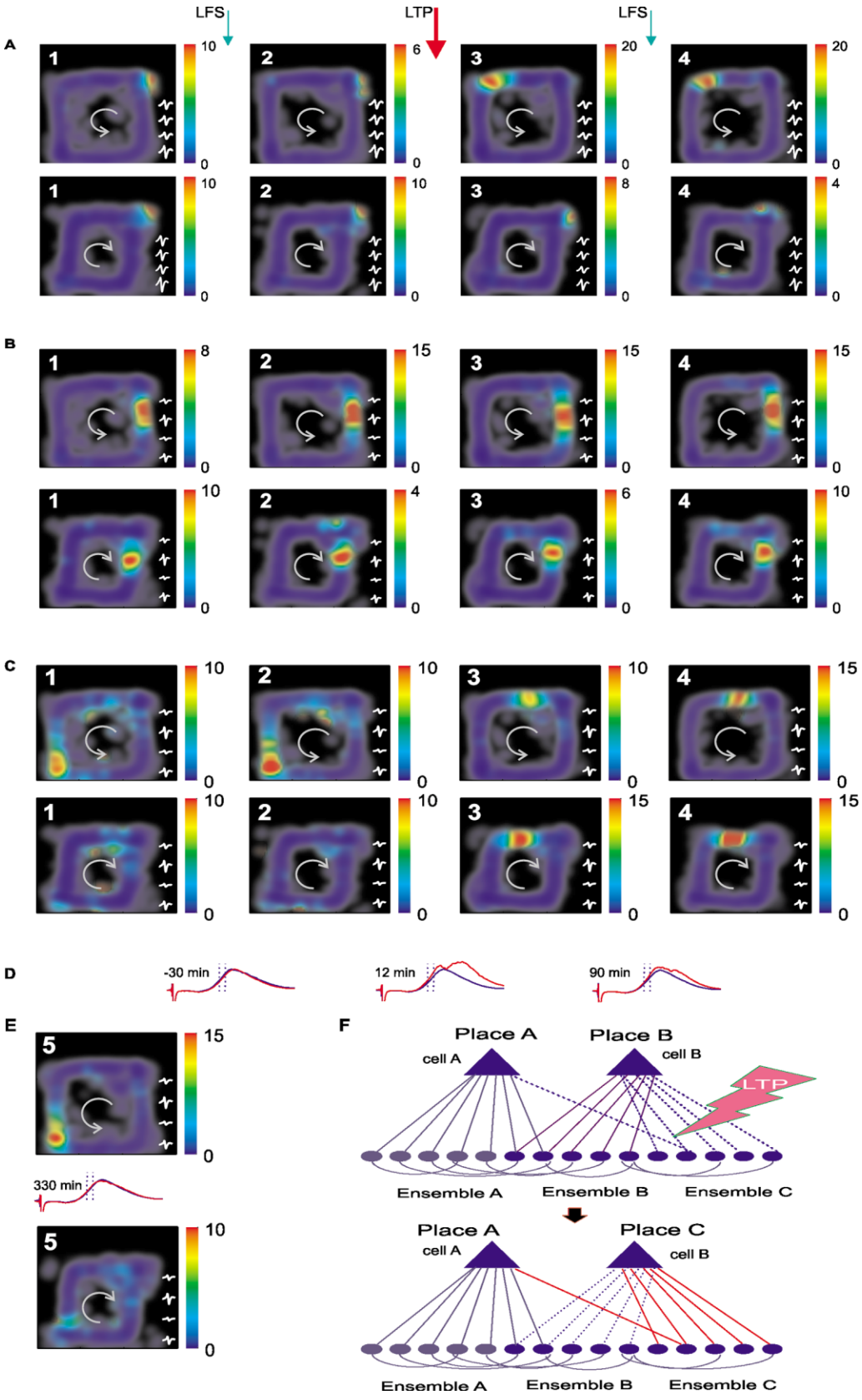

Figure 3. LTP-Induced Effects on Place Correlates of Neighboring Neurons

(A–C) Clusters of the three units are shown in Figure 2.

(A) Example place cell with unidirectional change of place field after LTP. Each map represents 10–20 min of counterclockwise or clockwise

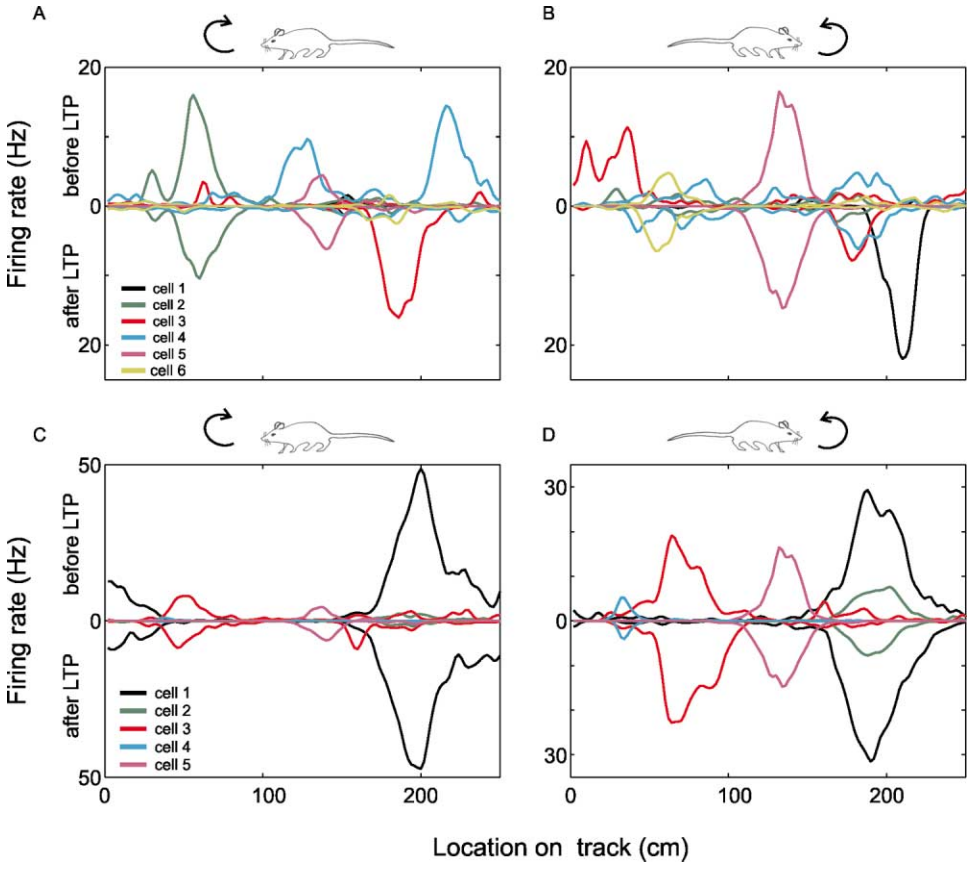

Figure 4. LTP-Induced Effects on the Place Correlates of Pyramidal Neurons

Units recorded simultaneously from two tetrodes are shown.

(A and B) The paths on the rectangular tracks were linearized. 0–250 cm represents a full turn. Smoothed firing rates before and after LTP (run sessions 2 and 3) as a function of the rat's position are shown separately for both directions; (A) is clockwise, (B) is counterclockwise. Novel place fields emerged for cell #1 and cell #3 (for counterclockwise and both directions, respectively). Cell #4 lost its double place field in clockwise direction. Cell #2 reduced its in-field firing rate, whereas no effect was observed on cells #5 and #6.

(C and D) The place fields of the five neurons simultaneously recorded by another tetrode were retained after LTP when the animal moved clockwise (C) or counterclockwise (D).

of the pyramidal cell population) were affected by LTP for at least one direction of movement. Approximately half of the affected cells shifted their place fields as well (39.4 $\pm$ 7.78 cm change after LTP versus 13.1 $\pm$ 4.97 cm after LFS; Figure 5A, inset). The majority of the affected cells showed unidirectional place field changes (changes expressed during either clockwise or counterclockwise movement), with only a minority displaying changes while moving in both directions (Figure 5A, inset). This latter finding suggests that in different contexts (e.g., direction of movement) the same cell is under the control of different sets of synaptic inputs that can be affected selectively.

To gain insight into the nature of the effect LTP exerted on the population representation of the environment, we examined the LTP effect on the distances (travelled by the rat) between initially overlapping place fields for pairs of neurons (fields with $<$30 cm distance, see be-

runs (gray arrows). Between run sessions the rat was placed back to the home cage. Arrows, LFS, low-frequency stimulation; LTP, tetanic stimulation. Color code: frequency of unit discharge (Hz). Note the difference in the colorbar scales across sessions. Blue, places visited but no spike present. Insets: average waveforms of the unit on each wire of the tetrode. Compare place fields before (1 and 2) and after (3 and 4) LTP. Note emergence of a new place field during counterclockwise runs and preservation of the pre-LTP field during clockwise runs. Note also the presence of some activity in the new place field prior to LTP (upper left corner, session 2).

(B) Example cell with no significant change in place representation after LTP. Same arrangement as in (A).

(C) Another example neuron with place field change in both directions after LTP.

(D) Average evoked potentials recorded before sessions 2, 3, and 4, superimposed on baseline recording (blue trace). Time is relative to the induction of LTP.

(E) Recovery of the original place field of cell 3 after 5.5 hr after LTP and the associated evoked field response.

(F) Schematic of hypothesized synaptic changes after LTP. Synaptic weight increases between input ensembles, representing distinct places, and target neurons are suggested to be responsible for the altered place representation. Competition of these inputs determines the place field characteristics of the pyramidal cells.

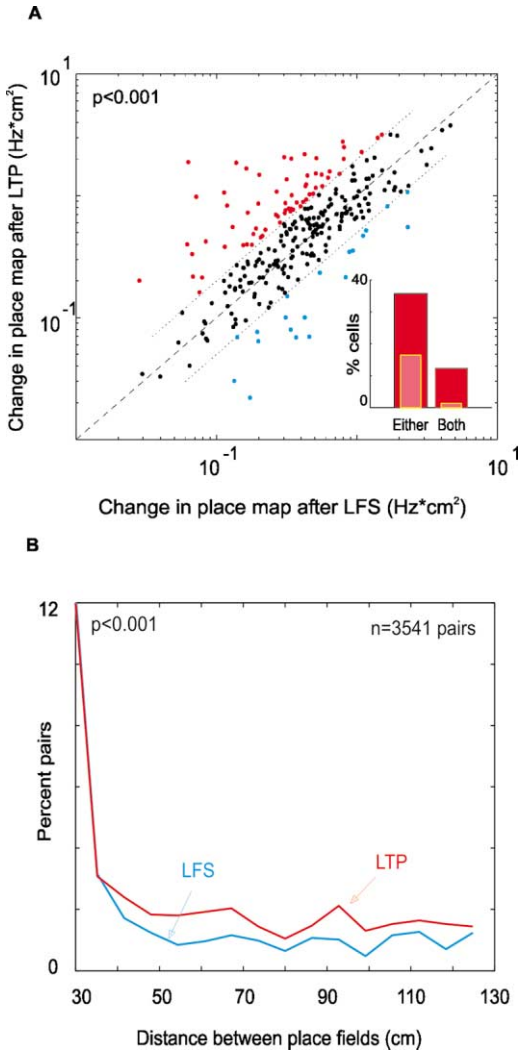

Figure 5. LTP Alters Place Field Properties

(A) Changes in place fields are significantly larger after LTP than after control LFS. Each dot corresponds to a place cell for one direction of movement. Red (blue) dots, cells for which LTP (LFS) produced a change in place map twice bigger than LFS (LTP) as marked by the corresponding dotted line. Inset: percentage of place cells that changed at least twice as much after LTP than after LFS. Red columns, in-field rate change or location change; pink columns, location change alone. Either, change in either clockwise or counterclockwise direction; Both, changes in both directions of movement. (B) LTP alters spatial relationship between neuron pairs. Histograms of distances between place field centers of pairs of neurons after stimulation. All neuron pairs included had <30 cm overlap during the respective baseline session. LTP induced shifts in a significantly larger proportion of pairs than did LFS.

low). Twenty percent of the neurons had more than one place field. For comparing distances between place fields of neuron pairs, only the field with the strongest firing rate was considered. Thirty percent of the overlapping pairs were dissociated after LTP and new pairs were formed. Spatial dissociation occurred in a significantly larger percentage of neuron pairs after LTP compared to LFS (Figure 5B; $p < 0.001$, Z-test for the equality of two proportions). This finding indicates that LTP affected not only the activity of individual cells, but also their functional connections within the network.

The change in spatial interaction by LTP occurred without a significant change in the global firing rate of the neuronal population during exploration (Figures 6A and 6B). The preservation of the grand mean rate was due to a balanced increase and decrease in firing rates of the LTP-affected neurons (Figures 6A and 6B). Moreover, the firing pattern of pyramidal cells (incidence of burst events, ratio of the number of spikes in burst versus tonic firing, number of spikes in a burst event) did not change after LTP ($p > 0.05$, paired t test). Comparison of the place field shape (mean ± SE skewness: before, 0.005 ± 0.047; after, 0.049 ± 0.049, Figure 6D) and size (mean ± SE integrated place field size: before, 156.24 ± 12.04 Hz·cm; after, 151.65 ± 12.65 Hz·cm, Figure 6E; place field width: before, 34.2 ± 1.7 cm; after, 30.5 ± 1.4 cm, Figure 6F) for all place cells active both before and after the tetanic stimulation revealed they were not significantly affected by LTP (for all comparisons, $p > 0.05$, paired t test and Kolmogorov-Smirnov test). Neither the time spent in each pixel of the track nor the power of theta during exploration (Figure 6C) was significantly different before and after LTP ($p > 0.5$, sign test). Thus, LTP induced remapping without affecting the properties of place coding.

The differential impact of LTP on single cells was assessed by comparing the magnitude of the location-specific evoked field response (Figure 1B) and the magnitude of place field changes. The site-dependent variability in the amount of potentiation within the same experimental session (Figure 1B) suggested that sites displaying larger potentiation received a stronger convergent input from the bulk of axons affected by the tetanic stimulation or were more susceptible to plastic changes. Stronger LTP is expected to exert a larger effect on place cell activity than weak LTP, as suggested by the representative example in Figure 4. At the population level, neurons recorded from sites with larger enhancement of the locally recorded evoked responses were significantly more strongly affected than those from sites with smaller changes of the field response (Figure 7A; $r = 0.41$; $p < 0.001$). Thus, in response to the same tetanus, place cells located at sites with stronger LTP displayed larger changes in their place fields than neurons recorded from less potentiated, control areas. This observation also supports the interpretation that an LTP-related process, rather than some undetected behavioral changes, was responsible for biasing place cell activity.

If the strength of synaptic inputs onto a cell is reflected by the in-field firing rate of the neuron, then strong synapses (and high firing rate cells) are expected to be more resistant to LTP than weak synapses (low firing cells). In line with this hypothesis, we found a significant negative correlation between maximal in-field discharge rate before LTP and the magnitude of place field shift (Figure 7B; $r = -0.54$, $p < 0.001$; rank correlation). To demonstrate that this relationship is not simply due to the larger discharge variability of slow firing neurons, we used the bootstrap resampling method to compare correlation coefficients obtained between sessions 2 and 3 (LTP change) and sessions 1 and 2 (LFS control variability) for the same population of place cells. The consistently larger correlation coefficients associated with LTP (Figure 7B, inset, $p < 0.001$, Kolmogorov-Smirnov test) indicate that LTP induced a differentially larger

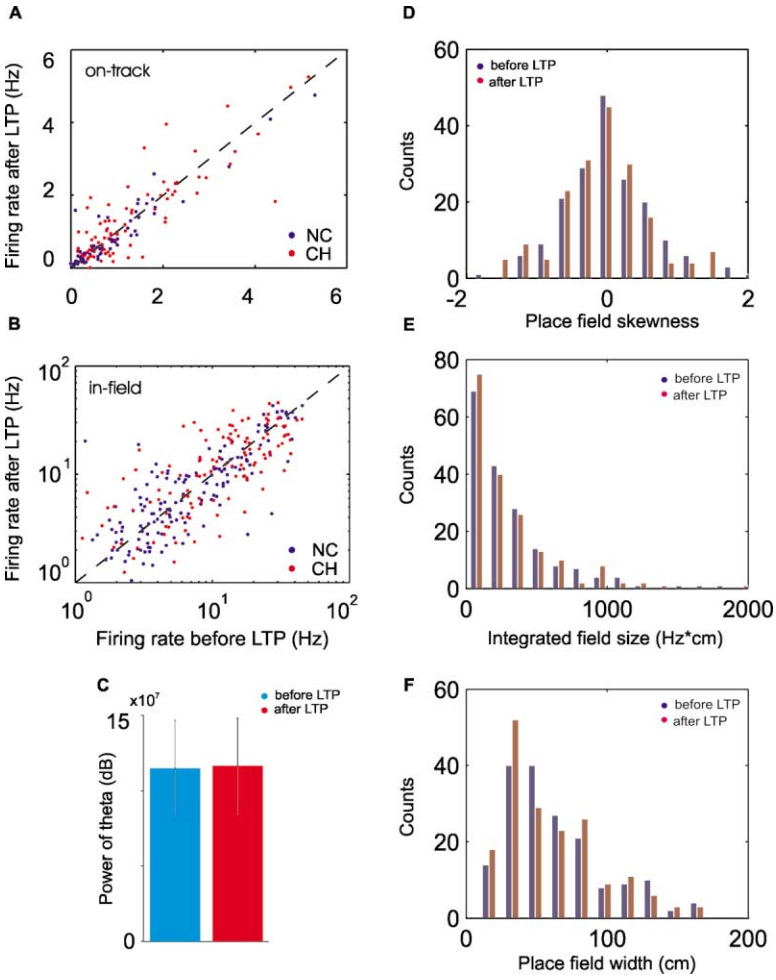

Figure 6. Preservation of the Place-Coding Properties after LTP

(A and B) Firing rate correlations before (session 2) and after (session 3) LTP during exploration for mean firing rates during the whole session on the track (A) and in-field peak firing rates (B) and for all place cells. Each dot corresponds to the mean firing rate of a single neuron. Blue dots code for neurons with no change (NC) and red dots for neurons that changed (CH) their in-field firing rate and/or location after LTP. Note that the grand mean firing rate of all populations is not affected by LTP. Note also cases when previously inactive neurons become active after LTP and vice versa.

(C) Power of theta during exploration was not changed after LTP.

(D–F) Similar distribution of place field skewness (D), integrated place field size (E), and place field width (F) before (blue bars) and after (red bars) LTP for all place cells that had a field both before and after LTP (each cell is represented for each direction of movement, i.e., twice).

change in slow firing place cells, compared to fast firing place cells, than expected on the basis of random variability. This finding indicates a competition between physiologically induced plastic changes and tetanic stimulation-induced synaptic plasticity.

To address the effect of LTP on the temporal aspects of network activity, we examined crosscorrelations between neuron pairs. Multiple place neurons, representing overlapping parts of the environment, are sequentially activated in space and time as the animal traverses the environment (Wilson and McNaughton, 1993). This spatial representation is "compressed" into individual theta cycles in the time domain by coordinated phase-precession (Skaggs et al., 1996). While firing rate and spike timing of individual cells within the theta cycle code for spatial location (O'Keefe and Nadel, 1978; Skaggs et al., 1996), the theta cycle-related temporal correlation between neuron pairs reliably predicted the distances between field centers of the neurons (Figure 8C). LTP induced a rearrangement of the hippocampal "map" but did not affect the mechanism of the theta cycle compression of place fields (Figures 8C and 8D). When the place representation of a newly generated place cell overlapped with the place field of another neuron, this newly created spatial relationship was faithfully reflected by the short-time scale (theta cycle) correlation of the neuron pair (Figures 8A and 8B). Conversely, when neurons with spatially overlapping fields were dissociated by LTP, their theta-cycle correlations were also lost. Figures 8C and 8D show the relationship between direction-corrected distance between place fields and respective temporal correlation for multiple pairs of neurons (see Experimental Procedures). The highest density of points corresponds to theta cycles (three clouds of points on the *y* axis) and reflects ~30 cm spatial coverage (*x* axis). LTP destroyed the temporal relationship of 40% of the preexisting neuronal pairs and created a similar number of new pairs (Figures 8C and 8D, insets). Nevertheless, the overall structure of place versus theta cycle-related correlation was not altered by LTP (Figure 8D).

Discussion

It has been assumed that spatial and other types of memories are encoded in the synaptic weights of hippocampal networks (O'Keefe and Nadel, 1978; Wood et al., 1999) and that modification of synapses alter memory traces and spatial representations (Kentros et al., 1998; Moser et al., 1998; Nakazawa et al., 2002). At the experimental level, Hebbian pairing of afferents and postsynaptic activity of neurons, as modeled by long-term potentiation/depression paradigms, has been suggested to be the key mechanism underlying synaptic weight

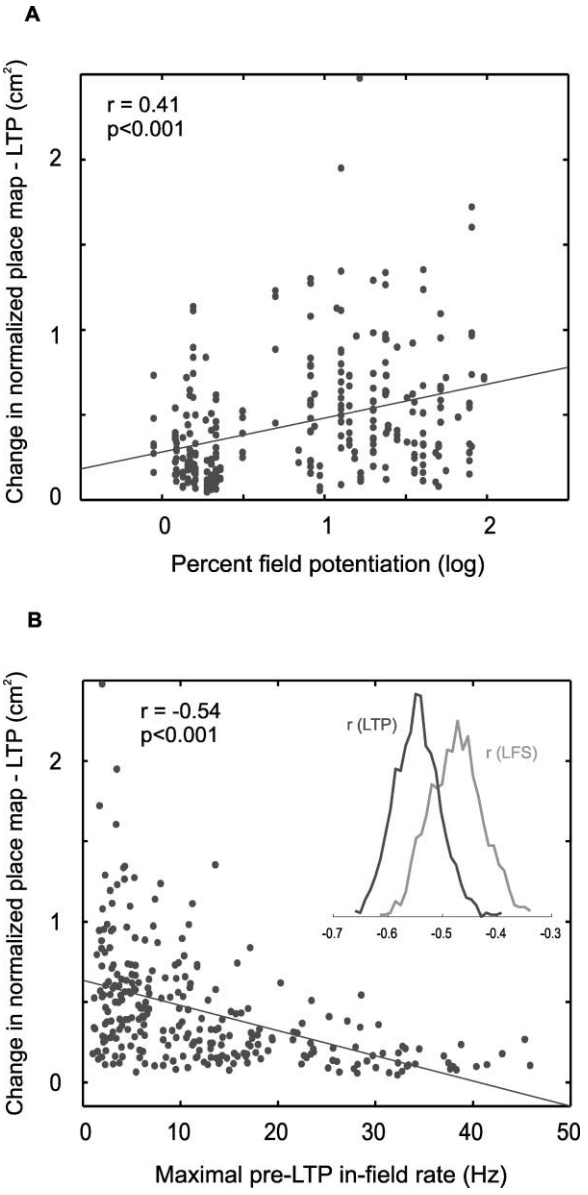

Figure 7. Synaptic Plasticity- and Firing Rate-Dependent Changes in Place Fields of Pyramidal Cells after LTP

(A) Correlation between absolute change in slope of field potentials after LTP and shift in place fields (change in normalized place maps) for neurons recorded with the same tetrode.

(B) Correlation between pre-LTP maximal in-field firing rate and magnitude of LTP-induced shifts in place fields. Inset: distributions of correlation coefficients, calculated from data points obtained after bootstrap resampling of the data in (B).

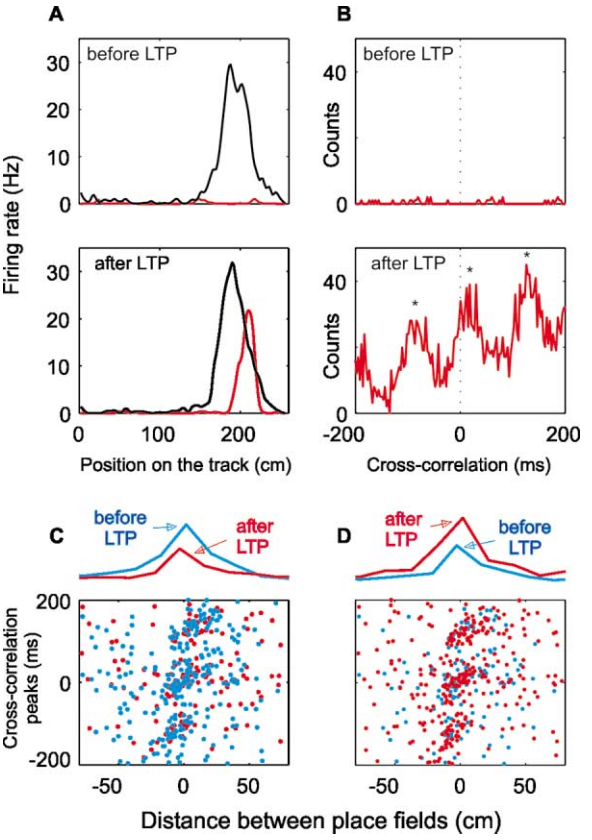

Figure 8. LTP Affects Representation of the Familiar Environment, but Does Not Alter Place Representation Rules

(A) Place fields for two simultaneously recorded units before and after LTP. Note emergence of a new place field (red), which overlaps with the unchanged place field (black).

(B) Crosscorrelation between the two neurons (reference, black neuron). Note theta frequency modulation of the crosscorrelogram after LTP (stars mark peaks).

(C) Relationship between spatial distance of place field peaks and temporal peaks of the crosscorrelograms (marked with stars for the pair in B) for all neuron pairs. Note high density of dots corresponding to three theta cycles and approximately 30 cm distance. The densities of dots are shown by the same color line histograms above, separately for before (blue) and after (red) LTP. Red dots, subgroup of pairs unchanged by LTP.

(D) Same layout as in (C) for pairs after LTP. Red, significant pairs after LTP; blue, subset of the post-LTP group that had significant correlations before LTP as well (no-change pairs).

changes (Levy and Steward, 1979; Markram et al., 1997; Magee and Johnston, 1997). Our findings provide a link between these two lines of research. Induction of long-term changes of VHC-evoked responses was associated with a "remapping" of the hippocampal representation of the environment, including creation of new place cells and abolishment of preexisting place fields, supporting the view that place features of pyramidal cells emerge within hippocampal circuits (McNaughton et al., 1996; Lever et al., 2002). LTP-induced effects were context dependent since place fields associated with one direction of movement were often selectively modified without affecting the neuron's place representation when moving in the opposite direction. These observations indicate that single neurons may be part of several representations (O'Keefe and Nadel, 1978; Markus et al., 1995; Wood et al., 1999, 2000) and inputs from these representations can be modified selectively. The same neuron can thus be part of different neuronal ensembles that may be activated by different combinations of inputs.

The changes in place field representation occurred without affecting (1) the theta cycle compression of distances between place fields, (2) the size and shape of place fields, (3) the power of theta, (4) the incidence of

sharp wave bursts, or (5) the global firing rate of the network. These findings suggest that LTP led to rearrangement of place representation in the hippocampus without altering the encoding "rules" of the network.

Because electrical stimulation might activate subcortical neurons connected with the hippocampus, one could argue that such indirect effects were responsible for the altered place representation after LTP. However, the demonstration of a reliable relationship between the magnitude of place field changes and the magnitude of change in the evoked field responses, as induced by LTP, supports the interpretation that an LTP-related process, rather than some nonspecific changes, was responsible for biasing place field activity. In addition, most units were selectively affected only during a given direction of movement, and units with large place field changes and units with nonsignificant changes could be recorded by the same tetrode. These observations also eliminate the possibility that changes due to discrete electrode drift in the brain were responsible for our findings.

The preservation of global firing rates after the LTP train is surprising since increase in the excitability of the affected pathways by LTP is expected to increase the firing rates of the target cells. Nevertheless, no changes were observed at the population level either in the sleeping animal or in the behaving animal engaged in the foraging task, even when only the subpopulation of changed cells was considered. One potential explanation of these findings is that the LTP-potentiated synapses at sites with the strongest convergence of the stimulated afferents were compensated for by a decrease in synaptic weights elsewhere (Lynch et al., 1977; Royer and Pare, 2003). Alternatively, the potentiated neurons could decrease the firing rate of the surrounding neurons by interneuron-mediated recurrent inhibition. A third possibility is that intrinsic mechanisms compensated the altered magnitude of afferent inputs, although such "rescaling" mechanisms operate on a significantly longer time scale (Turrigiano et al., 1998) than observed here. Importantly, similar preservation of global firing rates with altered individual firing patterns were observed in rats exposed to a new environment (Hirase et al., 2001), indicating that similar processes may underlie both LTP- and novel environment-related remapping. Altogether, the findings suggest that an LTP-like mechanism has the potential for abolishing and creating new functional connections in behavioral learning.

An implicit interpretation of the present findings is that arbitrary alteration of synaptic connectivity within the hippocampus should affect spatial memory. If all synaptic connections were rearranged by artificial means, such as LTP, the hippocampal representation of the same environment is expected to be entirely different. Behavioral experiments testing this hypothesis have produced conflicting results. Repeated hippocampal LTP, in an attempt to "saturate" all excitatory synapses, failed to affect spatial memory in most cases (Castro et al., 1989; Korol et al., 1993; Cain et al., 1993; McNamara et al., 1993; Jeffery and Morris, 1993). However, in those experiments the same pathways were tetanized repeatedly. It is therefore possible that only a subset of afferents and synapses were affected, leaving a large portion of the hippocampal network unaltered (Barnes et al., 1994), as was also the case in our present experiments. An explicit prediction of our observations is that if the majority of hippocampal synapses are affected, this should be associated with compromised spatial performance. Indeed, when LTP was induced in a large proportion of hippocampal afferents by several closely spaced stimulation electrodes, spatial memory in a water-maze task was impaired (Moser et al., 1998; Brun et al., 2001).

Experimental Procedures

Surgery, Recording, Stimulation, and Behavior

Seven adult male Sprague-Dawley rats (300–450 g) were implanted with eight independently movable tetrodes (3–4 mm posterior to bregma and 1.5–3.5 mm from the midline) and equipped with a single LED for position tracking (at 40 Hz with 0.8 cm spatial resolution) following NIH guidelines. Bipolar stimulating electrodes were placed in the contralateral ventral hippocampal commissure (VHC). While all seven rats were recorded during sleep, four rats were trained to run on an elevated rectangle-shaped linear track (8.5 cm wide, 70 $\times$ 60 cm sides, 30 cm above the ground) located inside a wooden box with 60 cm high walls. The rats ran clockwise or counterclockwise to retrieve bits of chocolate wafers from two fixed opposite corners. During recording sessions, the rats were placed on the track for approximately 20 min and then returned to the home cage for rest. Rest-sleep in the home cage and 20 min run sessions on the linear track were alternated several times. At the beginning of a sleep episode in the home cage, evoked responses were elicited by low-frequency stimulation (LFS) pulses (0.1 ms) delivered at 0.1 Hz (10 to 20 pulses) to VHC after the first, third, and fourth run sessions. LTP was induced by two 200 Hz trains of 10 pulses, separated by 2 s, after the second run session, and also in the home cage during sleep. Tetanic stimulation intensities were adjusted individually to evoke 25%–50% of the maximum population spike amplitude in the CA1 pyramidal layer before LTP (range, 100–200 μA). The VHC arises from CA3 pyramidal neurons. The axons travel in the ventral portion of the fimbria-fornix, and the fibers from left and right hippocampi are mixed. Thus, stimulation directly and orthodromically activates the contralateral CA3 and CA1 regions by discharging fibers of passage from the ipsilateral CA3. In addition, the stimulation backfires the contralateral CA3, whose collaterals now indirectly but also orthodromically activate CA1 (and CA3 by the recurrent collaterals). Independent of the stimulation site, there is a mixture of direct and indirect activation of the contralateral CA3 and CA1 areas (Buzsaki and Eidelberg, 1982). The effect of LTP on place cell firing was compared with that of LFS applied after the first run session. After the recording sessions, the electrodes were moved to isolate new sets of cells and a few days later a new session was recorded. After the completion of the experiments, the brains were perfused, fixed, sectioned, and stained with cresyl violet to identify all stimulation and recording electrode tracks.

Data Analysis

For the assessment of LTP, the middle 1–1.5 ms of the EPSP slope (between field onset and population spike onset) was determined before and after the tetanic trains at each recording location (Figures 1A and 1B). Single hippocampal pyramidal cells and interneurons were identified and isolated using a semiautomatic cluster cutting method (Wilson and McNaughton, 1993; Csicsvari et al., 1999; Harris et al., 2000). Stability of the neuron was assessed by spike wave shape changes: the principal component values of the spike were plotted against time for the entire recording period. In addition, the stability of two-dimensional projections of the unit clusters was examined by comparing the position of unit clusters corresponding to each session (Figure 2). Units with large progressive shifts or sudden changes were excluded from the analyses. With these criteria, 3 to 6 single units were obtained per tetrode. Action potentials associated with immobility and sharp-wave/ripple epochs on the track were excluded from the analysis. A total of 464 CA1 and CA3 units in seven rats met the stability criteria. Of these, 332 were recorded in the four rats tested in the spatial task. Of the 332 units, 146 pyramidal cells had place fields prior to the induction of LTP.

Burst events were composed by spikes with no more than 6 ms interspike interval (Harris et al., 2001). Place fields were computed by a smoothing-based method (Harris et al., 2001), where the estimated firing rate at a point $\mathbf{x}$ was computed as:

$$f(\mathbf{x}) = \frac{1}{\Delta t} \frac{\sum_t n_t w(|\mathbf{x} - \mathbf{x}_t|)}{\sum_t w(|\mathbf{x} - \mathbf{x}_t|)}.$$

Here, n_t is the number of spikes fired in a given time bin, $\mathbf{x}_t$ is the two-dimensional position of the rat in that time bin, and Δt is the time bin size. The smoothing function $w(d) = \exp(-d^2/2\lambda^2)$ is a Gaussian with a width parameter $\lambda = 5$ cm. The similarity of two place fields $f_1(\mathbf{x})$ and $f_2(\mathbf{x})$ was assessed by the L_1 distance between place maps, weighted by the geometric mean occupancy probability across the two sessions $\int\int |f_1(\mathbf{x}) - f_2(\mathbf{x})| \sqrt{p_1(\mathbf{x}) p_2(\mathbf{x})} d^2\mathbf{x}$. The weighting term ensured that the results were not biased by areas the rat visited infrequently, where rate calculation is inaccurate. In order to compare the spatial distribution of neuronal firing independent of overall changes in firing rate, normalized place fields were computed as $f'(\mathbf{x}) = f(\mathbf{x}) / \int\int f(\mathbf{x}) d^2\mathbf{x}$, and again compared with the same metric.

Place fields were defined as areas with localized increase in firing rate above 1 Hz for at least 10 contiguous pixels (20 cm). After "linearization" of the squared track into one dimension, place field borders were defined as the points where the firing rate became less than 10% of the peak firing rate for at least 10 cm. The width of the place field was defined as the distance (in cm) between the two borders of the place field. In neurons with multiple place fields, only the field corresponding to the maximum firing rate was considered for further analysis. The summed instantaneous firing rate between the borders of each place field (Hz·cm) represented the integrated place field size. The shape of the place field was evaluated by calculating its skewness (the ratio between the third moment of the place field rate distribution and the cube of the standard deviation [Mehta et al., 2000]). Crosscorrelations between neurons pairs were calculated in a 400 ms window, with 3 ms bin size. Crosscorrelograms with <0.8 count/ms were discarded. Several peaks were detected in each crosscorrelogram (marked with stars on Figure 8B). In case two or more peaks were detected in a 50 ms window, only the largest value was included in the analysis. Distances between place fields of two neurons were determined by calculating the distance travelled by the rat between the pixels with the peak firing rates of the respective place fields (Figure 8A).

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