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Impaired Hippocampal Representation of Space in CA1-Specific NMDAR1 Knockout Mice

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Summary

To investigate the role of synaptic plasticity in the place-specific firing of the hippocampus, we have applied multiple electrode recording techniques to freely behaving mice with a CA1 pyramidal cell–specific knockout of the *NMDAR1* gene. We have discovered that although the CA1 pyramidal cells of these mice retain place-related activity, there is a significant decrease in the spatial specificity of individual place fields. We have also found a striking deficit in the coordinated firing of pairs of neurons tuned to similar spatial locations. Pairs have uncorrelated firing even if their fields overlap. These results demonstrate that NMDA receptor–mediated synaptic plasticity is necessary for the proper representation of space in the CA1 region of the hippocampus.

Introduction

The hippocampus has long been identified as a brain structure that is critical for forming and storing certain types of memory. It has hitherto been technically difficult to connect molecular, cellular, and network descriptions of hippocampal memory processes. Mice have recently been generated whose NMDA receptors are deleted only in pyramidal cells of the CA1 subregion of the hippocampus. This has allowed us to examine, in vivo, the electrophysiology of natural learning processes in an unusually well-controlled system.

The hippocampus is anatomically well-situated for its role in memory. It is a high-level multimodal association cortex that has reciprocal connections to many other cortical regions (Amaral and Witter, 1989). Numerous neuropsychological studies have revealed that humans with hippocampal lesions are severely impaired in their ability to acquire new long-term memories of people, places, and events (Scoville and Milner, 1957; Zola-Morgan et al., 1986). Rodents with hippocampal lesions are impaired in a variety of spatial and odor discrimination tasks (O'Keefe and Nadel, 1978; Morris et al., 1982; Eichenbaum et al., 1988; Jarrard, 1993). These results suggest that the hippocampus plays an evolutionarily conserved role in learning and memory.

In vivo hippocampal electrical recordings reveal that as a rodent moves freely throughout its environment, pyramidal cells in the hippocampus fire when the animal is within highly restricted regions (O'Keefe and Dostrovsky, 1971). Each cell has its own region of elevated firing, termed a place field, and large numbers of hippocampal place cells tile each environment with overlapping place fields. Information about the location of the animal is of high enough quality that the position can be well-estimated by simultaneously examining the firing patterns of many hippocampal neurons (Wilson and McNaughton, 1993). The relative location of these place-receptive fields changes in different environments, and thus, place fields clearly must be learned anew in each environment with spatial information represented in the firing of ensembles rather than single cells. Recording place fields thus allows the electrophysiological examination of a natural form of learning in a freely behaving animal. Furthermore, place fields are a robust and well-defined phenomenon. These combined factors make hippocampal pyramidal cells an ideal system in which to examine the relationships among synaptic plasticity, in vivo electrophysiology, and behavior.

At a cellular and molecular level, a major focus of studies of plasticity has been on the phenomena of long-term potentiation (LTP) and depression (LTD) (Bliss and Lomo, 1973). These terms are umbrellas for multiple mechanisms to change the strength of synapses (reviewed by Bliss and Collingridge, 1993; Bear and Malenka, 1994). A large body of research shows that various artificial protocols in vivo and in vitro can increase or decrease synapse strengths. This is in agreement with much theoretical work suggesting that synapse strengths are a good place to store information (Hertz et al., 1991); but are the mechanisms underlying the various forms of LTP and LTD actually involved in natural learning? Morris et al. (Morris et al., 1986; Morris, 1989; Davis et al., 1992) reported that an NMDA antagonist delivered to the entire brain caused spatial learning deficits similar to hippocampal lesions and prevented LTP induction. Later qualified (Bannerman et al., 1995; Saucier and Cain, 1995), this work raised the possibility that NMDA-dependent plasticity would affect the natural learning of spatial information. This correlation between impairments in hippocampal synaptic plasticity and spatial learning has also been observed in several mutant mice generated by conventional gene knockout techniques (Grant et al., 1992; Silva et al, 1992a, 1992b; Stevens et al., 1994; for review, see Chen and Tonegawa, 1997). While the results obtained with pharmacologically or genetically manipulated mice are consistent with the notion that hippocampal synaptic plasticity underlies spatial learning, the techniques were limited by the lack of region specificity of their effects. We recently obtained much stronger evidence for the connection between hippocampal plasticity and spatial learning by analyzing new mutant mice in which the *NMDAR1* gene is knocked out exclusively in the pyramidal cells of the CA1 subregion of the hippocampus (NMDAR1 CA1-KO or CA1-KO mice). For a report of these results, see the accompanying paper by Tsien et al. (1996 [this issue of *Cell*]).

To further study the role of synaptic plasticity in hippocampal-dependent spatial learning, we have recorded

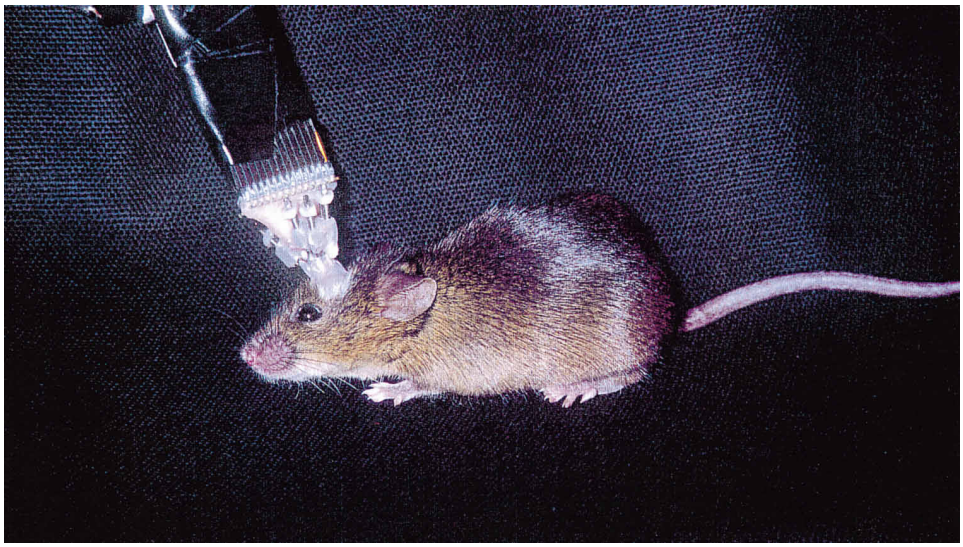

Figure 1. NMDAR1 CA1-KO Mouse with Implanted Microdrive

A microdrive array housing six independently adjustable four-channel tetrodes was affixed to the skull of the mouse, directly above the hippocampus (2.0 mm L, 1.8 mm P bregma coordinates), allowing large numbers of individual cells to be recorded during behavior (See Experimental Procedures).

from the hippocampus of CA1-KO mice using the multielectrode recording technique (Wilson and McNaughton, 1993). This technique permits us to simultaneously record the activity of large numbers of individual neurons in a freely behaving mouse and has been used to monitor place fields in the hippocampus as the animal explores an environment. The findings reported in this paper suggest that NMDA receptor–dependent synaptic plasticity plays an important role in the refinement of the place specificity of individual CA1 pyramidal cells and in the coordination of ensemble representations in this region. Since CA1-KO mice are severely impaired in spatial learning (see accompanying paper, Tsien et al., 1996), our results also suggest that refined place fields and coordinated firing may be essential for spatial learning.

Results

We employed multielectrode recording techniques to characterize hippocampal activity in mice with a CA1 restricted deletion of the *N*-methyl-D-aspartate receptor subunit 1 gene and in their littermate controls. Mice were fitted with microdrive arrays containing six independently adjustable tetrodes capable of simultaneously recording the activity of large numbers of neurons (Figure 1). We measured a variety of basic characteristics of both excitatory and inhibitory hippocampal CA1 neurons in NMDAR1 CA1-KO and control mice. While most electrophysiological properties of neurons in knockout and control animals were indistinguishable, two major differences in neuronal responses during spatial exploration were found. The first finding was that while place-related firing of CA1 pyramidal cells is substantially preserved in the CA1-KO mice, spatial specificity of the place fields is clearly poorer than in littermate controls. Second, the representation of space based on the coordinated activity of ensembles of cells in knockouts is significantly impaired.

Most Electrophysiological Properties of Control and Knockout Neurons In Vivo Are Indistinguishable

We recorded activity from 112 complex spiking cells and 13 interneurons in five control animals during 12 recording sessions, and 198 complex spiking cells and 16 interneurons in five CA1 knockout animals during 16 recording sessions (see Experimental Procedures). We evaluated basic spike waveform and firing characteristics of these cells (see Tables 1 and 2) and found that pyramidal cells and interneurons in CA1-KO animals are largely indistinguishable from those of the control animals. Overall firing rates of pyramidal cells were approximately 2 Hz while running and 1 Hz while resting or sleeping. Overall firing rates of inhibitory interneurons were 20–35 Hz while running and 10–15 Hz while resting or sleeping. Most neurons in control and knockout animals showed rhythmic modulation of firing rate in the theta frequency range (8–10 Hz) while the animals were running. Thus, basic neuronal firing characteristics appear normal in knockout animals during behavior. We observed no large shifts in the balance of excitation and inhibition in the CA1-KO animals, although we detected a small reduction in the firing rate of pyramidal cells during sleep (see Table 1). This was a weak effect, but is consistent with the lack of an excitatory NMDA current.

To further confirm that there were no gross changes in hippocampal function in the CA1-KO mice, we conducted EEG recordings during both behavior and sleep. During quiet wakefulness and sleep, dendritic sharp-wave field potentials, which result from synchronous discharge of CA3 pyramidal cells, were observed in CA1 (Ylinen et al., 1995). The frequency of these events was

Table 1. Electrophysiological Properties of CA1 Region Hippocampal Neurons in Control and NMDAR1 CA1-KO Animals

	RUN		SLEEP	
	Control (n = 56, D = 12, N = 5)	CA1 NMDAR1 KO (n = 74, D = 16, N = 5)	Control (n = 112, D = 12, N = 5)	CA1 NMDAR1 KO (n = 198, D = 16, N = 5)
Rate (Hz)	2.114 ± 0.177 $p < 0.49$	2.312 ± 0.214 $p < 0.49$	1.294 ± 0.126 $p < 0.03$	0.958 ± 0.065 $p < 0.03$
Complex Spike Index (csi)	14.48 ± 1.26 $p < 0.36$	12.84 ± 1.20 $p < 0.36$	21.67 ± 1.13 $p < 0.78$	21.22 ± 1.02 $p < 0.78$
Width (μs)	544.3 ± 5.0 $p < 10^{-4}$	508.5 ± 6.1 $p < 10^{-4}$	535.4 ± 4.1 $p < 10^{-4}$	511.7 ± 3.4 $p < 10^{-4}$
Field Size (pixels)	**106.0 ± 7.40** $p < 0.0004$	**140.3 ± 6.07** $p < 0.0004$	N/A	N/A

The table details the properties of CA1 pyramidal cells in control and mutant animals during both active behavior (RUN) and quiet rest (SLEEP). There were no statistically significant differences detected in overall rates or complex spike index scores between CA1-KO and control neurons. Spike width shows a small but significant difference. The field size measurement showed a 29% increase in the mutant animals. This increase passes Student's test for statistical significance ($p < 0.0001$).

approximately 1–2 Hz. These sharp waves were accompanied by high-frequency (165 ± 25 Hz) EEG oscillations in the pyramidal cell layer of CA1, as has been reported in the rat (Buzsaki, et al., 1992). Three of five mutant animals showed especially large-amplitude sharp wave–related EEG signals during these periods, but this phenomenon was not correlated with differences in any physiological variable, observed behavior, or measure of performance in navigational tasks.

We measured peak-to-trough widths of all spike waveforms and found a small but significant difference between pyramidal cells in CA1-KO and control animals. Spike widths of pyramidal cells from knockout animals were, on average, 7% smaller while running and 4% smaller while sleeping than those from control counterparts (see Table 1).

It is well known that pyramidal cells can fire bursts of action potentials in vivo, and bursting protocols are often used to induce LTP in vitro. It has been speculated that these bursts are necessary to cause plastic changes in synapses (Buzsaki, 1986; Chrobak and Buzsaki, 1996). To check that bursting activity was normal, we examined the complex bursting of cells in all animals. Pyramidal cells in both control and knockout animals showed simple spiking and complex bursting during behavior, sleep, and quiet wakefulness. Within an extracellularly recorded burst of action potentials from a pyramidal cell, interspike intervals are short and amplitudes of successive spikes decrease. We defined a complex spike index (CSI) as the percentage of first lag interspike intervals that fall between 2 ms and 15 ms and whose second spike is smaller than the first. The average CSI of pyramidal cells was approximately 14 in both control and CA1-KO animals while running and 21 while resting or sleeping (see Table 1). The average CSI of interneurons was less than 1 in all animals, awake or asleep. In addition, we believe it is important to note that although NMDA-mediated synaptic plasticity is absent in the CA1-KO mice, two endogenous hippocampal firing patterns that have been implicated in synaptic modification, the theta modulation of hippocampal activity and the complex spiking of pyramidal cells, are intact (Larson et al., 1986, Huerta and Lisman, 1993). The retention of these firing patterns is a further indication that there has been no gross alteration of hippocampal physiology.

Place Fields Exist in CA1-Knockout as Well as Control Animals and Are Stable in Both

Despite having no NMDA-dependent plasticity, CA1 pyramidal cells in knockout animals showed a surprising degree of place-related firing. In CA1-KO animals, 74 of 198 complex spiking cells recorded (37%) showed significant spatial activity during behavioral testing. In control animals, 56 of 112 complex spiking cells (49%) showed significant spatial activity. Both are consistent with previous reports of place field activity in the rat (Wilson and McNaughton, 1993). Figure 2 shows representative fields from both control and knockout animals on an L-shaped track. In two animals, a small number of cells in area CA3 were also recorded to determine whether the perturbations of spatial firing observed in

Table 2. Electrophysiological Properties of CA1 Region Hippocampal Interneurons in Control and NMDAR1 CA1-KO Animals

	RUN		SLEEP	
	Control (n = 14, D = 12, N = 5)	CA1 NMDAR1 KO (n = 16, D = 16, N = 5)	Control (n = 13, D = 12, N = 5)	CA1 NMDAR1 KO (n = 16, D = 16, N = 5)
Rate (Hz)	22.13 ± 3.60 $p < 0.08$	32.81 ± 4.42 $p < 0.08$	5.20 ± 1.38 $p < 0.18$	9.17 ± 2.24 $p < 0.18$
Width (μs)	215.5 ± 8.5 $p < 0.88$	214.0 ± 6.0 $p < 0.88$	208.2 ± 8.1 $p < 0.99$	208.3 ± 5.5 $p < 0.99$
Field Size (pixels)	249.1 ± 20.50 $p < 0.31$	224.8 ± 12.96 $p < 0.31$	N/A	N/A

The table details the properties of inhibitory interneurons in control and mutant animals. Rate, field size, and width were not significantly changed in the CA1-KO animals.

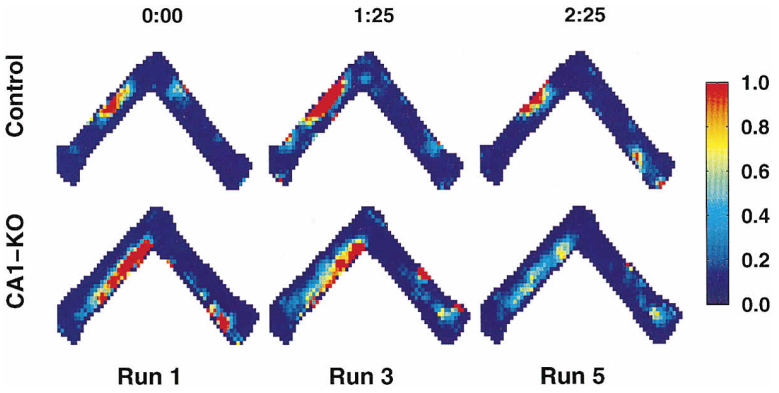

Figure 2. CA1 Pyramidal Cells of NMDAR1 CA1-KO Mice Demonstrate Stable Place-Specific Firing Patterns

Examples of a place-specific firing pattern of an individual CA1 pyramidal neuron from a control animal and NMDAR1 CA1-KO animal. Recordings from each animal lasted three hours and contained five behavioral "RUN" sessions on an L-shaped linear track. Each panel of the figure shows the firing rate of the identified cell as a function of the position of the animal on the track for runs 1, 3, and 5 respectively. The legend at the top indicates real time between exposures (hour:minutes), and the key on the right shows the firing-rate scale in spikes/s. The stability of place fields in NMDAR1 CA1-KO mice was qualitatively indistinguishable from that observed in control mice.

CA1 were the result of CA3 disruption. Preliminary evaluation did not reveal any significant disturbance of CA3 activity in the knockout animals. While we cannot rule out place field formation in CA1 via a form of plasticity not dependent on the NMDA receptors, we argue in the Results and Discussion sections that some place-related firing can be generated without any synaptic plasticity.

The format of all of our experiments consists of multiple behavioral "RUN" sessions bracketed by "SLEEP"' periods, which allows recording stability to be assessed (see Experimental Procedures). This protocol allows us to determine the stability of the spatial pattern of firing of pyramidal cells over repeated exposures to the same environment. Figure 2 shows examples of place fields recorded from CA1-KO and control animals over a period of 2.5 hr. Only pyramidal cells whose clusters were stable were used in all analyses, and our results indicate that these cells exhibit stable place fields for at least one hour. Firing rates sometimes varied, but the spatial pattern remained constant. The stability of place fields in CA1-KO animals indicates that these mice are not remapping the environment upon every exposure and suggests that they recognize the environment from previous experience.

Place Fields Show Directional Selectivity When Control and Knockout Animals Are in Linear Environments

As rats traverse a linear track, individual place fields only fire when the animal is running in one of the two directions (McNaughton et al., 1983). We therefore examined the data for an impairment in the directional selectivity of CA1-KO place cells. The presence of two light-emitting diodes on the heads of the animals allowed the head direction to be monitored at 30 Hz. Figure 3A shows examples of the directional dependence of place field firing when the animal is running on a straight track. Both control and CA1-KO animals had directionally selective place fields.

We quantified this effect to investigate the matter further. The average firing rate as a function of absolute head orientation was calculated for each cell with a resolution of 11.25°. We defined a directionality index (DI), which is a measure of the relative difference in firing rate as the animal runs in opposing directions that ranges from 0 (nondirectional) to 1 (strongly directional) (see Figure 3 legend). Figure 3B shows the DI for control and knockout animals. Both show a high degree of directional selectivity, but the knockouts are significantly impaired. We suggest that the diffuse spatial firing seen in the knockouts (see below) produces some firing even when the animal moves in the "wrong" direction.

Place Fields Are Larger in CA1-Knockout Animals

In our efforts to identify possible impairments of hippocampal function that could contribute to the behavioral deficit identified in CA1-KO mice, we first examined the spatial specificity of pyramidal cell activity as these animals explored an environment. We found that although pyramidal cells from CA1-KO animals fired in a place-related manner, the specificity was markedly poorer than the firing pattern of cells from control animals. Figure 4A shows representative examples of place fields recorded from control and knockout animals in three environments. Place fields recorded from control animals usually had a single salient peak. Place fields recorded from knockout animals, while sometimes individually indistinguishable from control fields, were more likely to be broad and diffuse, with multiple peaks.

We measured place field size by counting the number of pixels (pixels cover a 2 cm $\times$ 2 cm area) where average firing rates exceeded 1 spike per second. The open field environment most closely approximates the environment of a Morris water maze, but has the drawback that the animal visits each pixel few times. We therefore restricted our analysis to data from the linear and L-shaped tracks where sampling was high and uniform. Figure 4B shows the distribution of field sizes measured for both control and knockout animals. A significant difference in the means was found with control fields averaging 106 pixels, while knockout fields averaged 140 pixels (see Table 1). The distributions also appear roughly Gaussian with similar variances; this ensures that Student's t-test will be meaningful. We find that the probability that the means are the same is less than 0.0004.

To check the possibility that this effect may arise as a consequence of the slight increase in firing rates in the knockout animals, we calculated the place field size

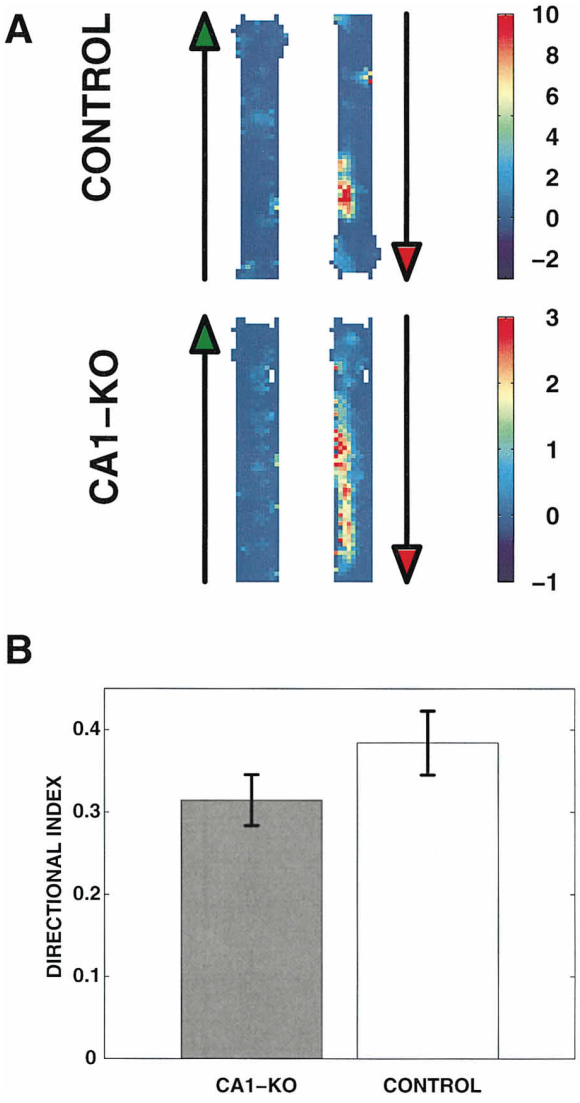

Figure 3. Directionality of Place Fields in Control and NMDAR1 CA1-KO Mice

(A) Examples of directionally specific CA1 pyramidal cell place activity during behavior on a one-dimensional linear track. The keys on the right show the scales of the firing rate for each cell. The left panel is a plot of the firing rate of the cell as a function of the location of the animal on the track as the animal traversed the track in the upward direction (green arrow). The rate maps indicate that the cells stay virtually silent. The right panels reveal that as the animal runs down the track (red arrow), the cells fire in a spatially restricted pattern. This property of directional specificity has been demonstrated in both rats and wild-type mice and is retained in the NMDAR1 CA1-KO animals.

(B) Control animals score significantly higher than mutant animals on a measure of directionality (DI). Both the control and CA1-KO animals show a high degree of directional selectivity, but the knockouts are significantly impaired. We suggest that the diffuse spatial firing seen in the knockouts produces some firing even when the animal moves in the "wrong" direction. DI = max $(R - r)/(R + r)$ over 32 directional bins, where (R) and (r) are average rates in opposite directions.

for both high- and low-rate cells. The median firing rate for pyramidal cells that fired significantly while running in an environment was approximately 1.6 Hz in both knockout and control animals. We divided these cells into two categories: those that fired, on average, between 1 Hz and 1.6 Hz and those that fired, on average, between 1.6 Hz and 6 Hz. Figure 4C shows that for both high and low rates, the place fields of knockout pyramidal cells are larger than those of controls (see Table 1).

We measured place field size in a variety of additional ways and saw an increase in size for CA1-KO animals in each. The number of pixels with a firing rate greater than half the average firing rate showed a 31% increase in knockouts over controls with a significance of $p < 0.0005$. The percentage of pixels with a firing rate greater than 1 Hz showed a 21% increase with a significance of $p < 0.01$. The number of pixels with a firing rate greater than ten percent of the maximum firing rate showed a 22% increase with a significance of $p < 0.09$. The spatial spread of suprathreshold pixels from the place field center was also significantly increased (16% increase, $p < 0.007$). From these results, we conclude that the increase in spatial extent of pyramidal cell firing in CA1-KO mice is a significant and robust effect.

Neuronal Ensemble Effects

It is likely that the coordinated firing of large numbers of place cells is necessary to accurately communicate the location of the animal to downstream brain regions. We took advantage of our multiple-electrode array to measure the covariance coefficient of firing rates averaged over 200 ms windows between pairs of cells with overlapping fields. The average covariance coefficient is computed as:

$$\langle[\Sigma_i(R_i - R_{avg})(r_i - r_{avg})]/[\sqrt{\Sigma_i(R_i - R_{avg})^2}\sqrt{\Sigma_i(r_i - r_{avg})^2}]\rangle$$

where < > is an average over cell pairs, (i) is an index that runs over all included 200 ms bins in a session, and (R) and (r) refer to different cells in a pair. Figure 5A shows that pairs of knockout cells exhibit completely uncorrelated firing, while control pairs exhibit significant correlations. As an animal moves through a region covered by several place cells, the firing of those cells is nevertheless uncorrelated over 200 ms windows. We have observed this effect for time bins ranging from milliseconds to one second. This dramatic effect in CA1-KO mice means that downstream regions cannot use these correlations to learn about place.

This point is elaborated by examining the estimate of the location of the animal conveyed simultaneously by multiple cells—the ensemble representation of location. We were able to record from as many as 29 neurons simultaneously and were thus able to estimate the position of the animal from knowledge of their fields and spike trains alone (see Figure 5B legend). Figure 5B shows that for small numbers of cells, the ensemble of neurons in knockout animals carries as much information about location as the ensemble in controls. For large numbers of cells, however, where place fields begin to overlap, an ensemble of CA1-KO neurons represents the location of the animal more poorly than an ensemble of control neurons. Because we always chose the best matching location, however poor the match (see Figure

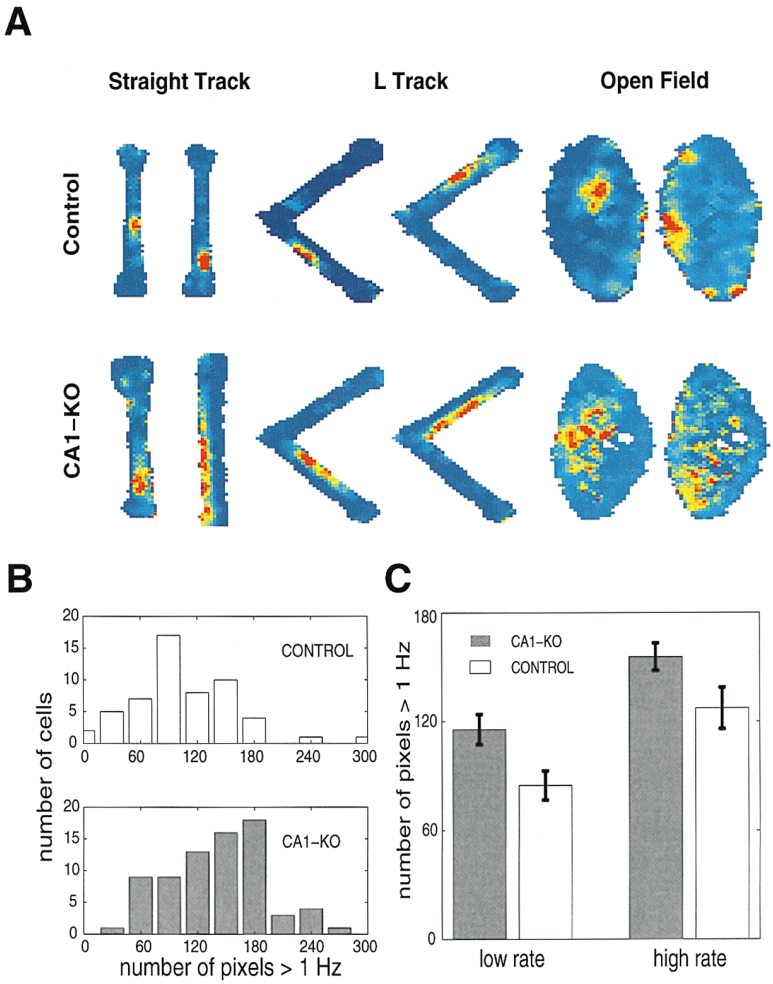

Figure 4. Place Fields of NMDAR1 CA1-KO Mice Are Significantly Larger in All Behavioral Environments

(A) Rate maps of place-specific activity of two pyramidal cells from control animals and two pyramidal cells from knockout animals in each behavioral environment. The peak rate of each panel has been adjusted to reveal areas of highest activity. The field sizes of the pyramidal cells of the NMDAR1 CA1-KO animals were significantly larger in both the linear track (one-dimensional) environments and the open field (two-dimensional) environment.

(B) Histogram demonstrating the distribution of CA1 pyramidal cell field sizes in control ($n = 55$ cells) and mutant animals ($n = 74$ cells). Pixels in which the average rate of firing exceeded 1 Hz were included when calculating field size. CA1 complex spike cells were identified based on average spike width and complex spike index score (see Results). The mean field size in NMDAR1 CA1-KO animals was 140.3 pixels ($\sim$560 cm^2), while in control animals the mean size was 106.0 pixels ($\sim$420 cm^2) (See Table 1).

(C) Increase in place field size in NMDAR1 CA1-KO animals is not caused by a general increase in rate. The histogram shows place field size for low rate (< 1.6 Hz) and high rate (> 1.6 Hz) cells from both mutant and control animals. In both cases the NMDAR CA1-KO animals have significantly larger fields.

5B legend), we may have overestimated the performance of the knockout animal ensembles. Even for ensembles with uncorrelated firing, the reconstruction will improve with larger ensembles. Figure 5C shows an example of trajectory reconstruction. Ensembles of cells from the knockouts are more likely to make large errors estimating the location of the animal. Cells that ought to fire together because they are tuned to similar locations do not robustly do so in the knockouts.

Results of a Model of CA1 Place Field Formation

How can there be place fields in CA1 without NMDA receptors? We cannot rule out the possibility that other forms of plasticity are sufficient to generate place fields with poor specificity. It is also possible that a residue of fixed topologically organized input to CA1 exists. We consider both of these to be unlikely. The puzzle is that if we assume that CA3, the major input to CA1 (Amaral and Witter, 1989), has normal place fields, the random unchanging connections from CA3 to CA1 that should exist in the CA1 knockouts might be expected to destroy all place-specific responses in CA1.

To address this problem, we constructed a model (Blum and Wilson, unpublished data) of the random hard-wired CA3 network connections to a single postsynaptic CA1 cell, similar in architecture to what exists in the CA1-KO animals (see Figure 6A). We used numbers of neurons and connections that match literature values for these brain regions (Amaral and Witter, 1989; Paxinos, 1995). We assumed that place fields are normal in CA3. Random connections generate an input to CA1 that fluctuates in a place-related manner but with an amplitude of only 5%–10%. We therefore added another ingredient to the model: homeostatic firing rates (Turrigiano et al., 1994; Miller, 1996). A firing threshold that adapts to average or peak postsynaptic activity will tend to stay in the middle range of a fluctuating response. Place-specific variations in CA1 response will thus be amplified. Figure 6B shows our preliminary results. Without plasticity but with an adapting firing rate, we obtain location-dependent firing in CA1 cells with poor spatial specificity. Furthermore, the model suggests that without plasticity, CA1 firing may be more variable and pairs may exhibit less correlated firing.

What is gained with plasticity? We assumed that the adapting threshold for firing is also an adapting threshold for plasticity (Bienenstock et al., 1982; Kirkwood et al., 1996). We also employed a conventional "Hebbian" learning rule: if the postsynaptic response was large, all input synapses were strengthened proportionally to their contribution to the response. With these simple ingredients, the model produced a more specific place field transmitted from CA3 to CA1 compared to the

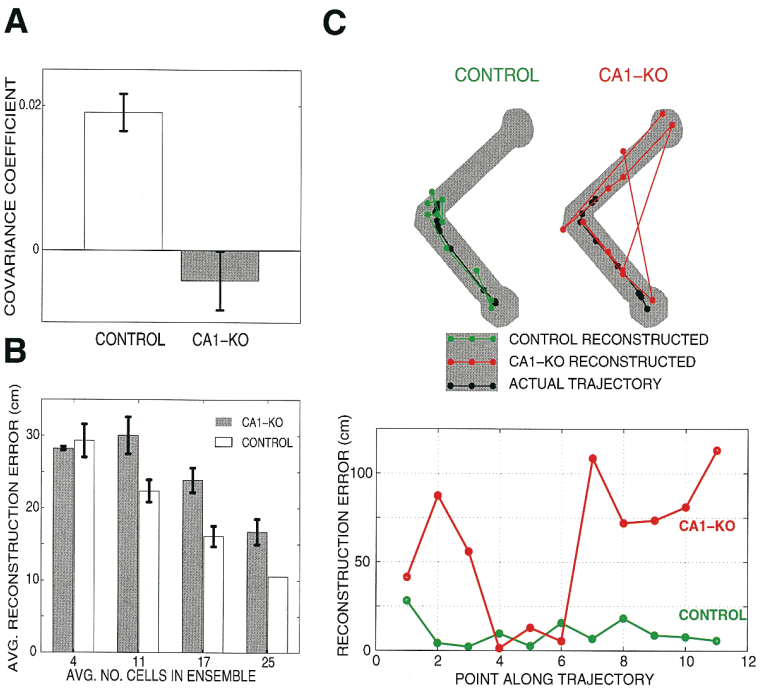

Figure 5. Ensemble Coding Properties of CA1 Pyramidal Cells In NMDAR1 CA1-KO and Control Mice

(A) The average covariance coefficient of firing rates between overlapping pairs of control and pairs of knockout pyramidal cells. Pairs of cells in knockouts fired randomly with respect to each other when their place fields overlapped. The average firing rate of each neuron was calculated for successive 200 ms bins over one 15–20 min RUN session. The firing rate covariance coefficient of all pairs of cells on different tetrodes with place fields that overlapped by 10 or more pixels (161 control pairs, 555 CA1-KO pairs) was calculated only when the animal was visiting common pixels. Common pixels were those where both cells fired at least one spike. Control pairs overlapped by 21.8 ± 2.2 pixels; CA1-KO pairs overlapped by 22.5 ± 3.1 pixels. Error bars represent standard errors of means for 8 control and 11 CA1-KO data sets.

(B) Average error in path reconstruction. Trajectory reconstruction error is larger in CA1-KO mice. With few simultaneously recorded cells there was no significant difference between knockouts and controls. With large numbers of cells, the chance of overlapping fields increased, and the lack of covariance in the knockouts appeared as an increased reconstruction error compared to controls. Knockout (16) and control (13) data sets were grouped by number of active place cells in that session. Reconstruction errors were averaged over the entire 15–20 min RUN sessions. Error bars represent standard errors of means; the number of degrees of freedom was taken to be the number of data sets, four on average. The same trend was observed in straight and L-shaped tracks, so the data were pooled. The position was reconstructed every 2 s by comparing a list of the average firing rate of each cell for a 2 s bin with a list of the average rates at each pixel over the entire session and finding the position that gave the closest match. Firing rates of each cell were normalized to their peak values, and match was determined by the angle between these rate vectors. Error at each point was calculated by computing the distance between the estimated location and the average position during the 2 s bin.

(C) Examples of trajectory reconstruction. The upper panel illustrates trajectories reconstructed for control and CA1-KO animals for a 20 s stretch of behavior. The ensemble firing of place cells of knockouts does not coincide with the actual location of the animal. Points indicate locations at which position estimates and measurements were made. Lines connect successive points in time. Each arm of the L-track was 75 cm long. The lower panel shows the differences between the reconstructed and actual locations for the same data. The knockouts had a highly variable reconstruction error with occasional large values. The data sets used in this figure had 19 control and 26 pyramidal cells. The average reconstruction errors over 15–20 min were 17 cm and 23.5 cm, respectively.

model without plasticity (see Figure 6B). The model suggests that plasticity may also increase signal to noise in CA1 and raise the covariance coefficient for cell pairs.

We stress that this is a mechanical explanation of how plasticity affects the faithful transmission of place field information out of CA3. It is not an attempt to explain the function of CA1 or the hippocampus under normal conditions. Nor does it determine whether the observed navigational deficit (Tsien et al., 1996) in the knockout animals is due to their poorer place fields. We believe it is more likely that the behavioral deficit is also due in part to the inability of CA1 neurons to effectively teach downstream brain regions and to the inability of CA1 neurons to learn associations between CA3 and other inputs, in particular, entorhinal inputs.

Discussion

Evaluations of the performance of knockout animals in the Morris water maze indicated clear deficits in the spatial hidden platform search, with apparently normal performance in the nonspatial, visually cued platform search (Tsien et al., 1996). One objective of this study was to identify the underlying neurophysiological characteristics that might account for the observed behavioral disruption. We have considered several plausible electrophysiological mechanisms behind the observed behavioral impairment.

Place Fields without NMDA-Mediated Synaptic Plasticity

We tested the hypothesis that place fields would be uniform or even absent. We found that the fields are present but degraded. The existence of place fields in region CA1 despite the lack of NMDA-mediated plasticity clearly indicates the sufficiency of established connectivity and the convergence of place-related inputs to provide some degree of spatial specificity in CA1 pyramidal cells. Given the high degree of convergence and divergence of inputs onto these cells (Amaral and Witter, 1989), it is somewhat surprising that even modest selectivity can emerge. Modeling has revealed that the use of an adapting threshold in conjunction with random convergence of place-related input is sufficient to produce cells with the type of broad spatial selectivity we have observed (Blum and Wilson, unpublished data).

Stability of Place Fields Suggests That CA1 Plasticity Is Not Involved in Recognition

A lack of stability in place representations across multiple exposures to the same environment would interfere

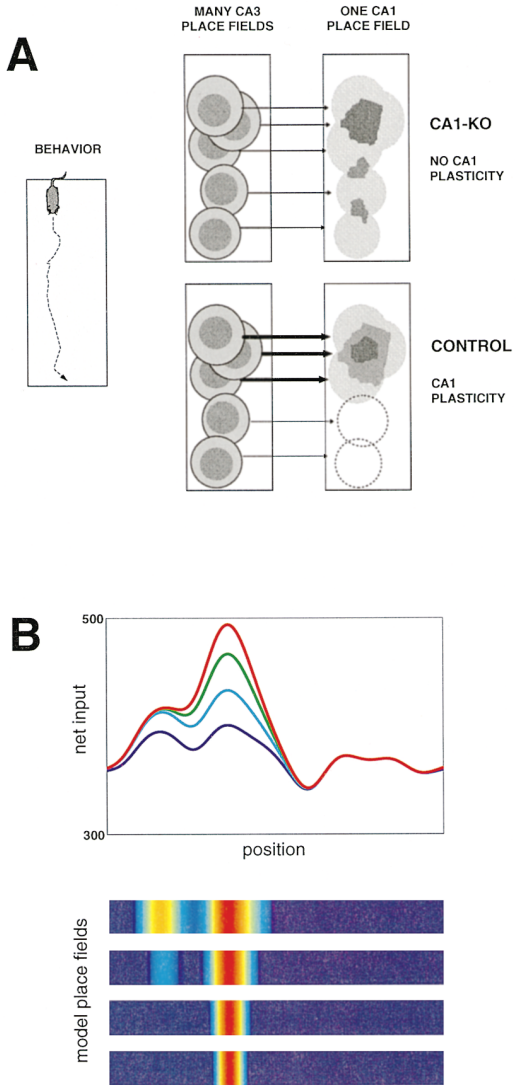

Figure 6. Model Detailing Possible Role of NMDA Receptor–Mediated Synaptic Plasticity in the Refinement of Place Fields in CA1 Pyramidal Neurons

(A) A schematic view of the model. Each large rectangular panel is a copy of the environment. As the animals move around, various place cells in CA3 are activated, and these in turn drive CA1 cells to fire. In knockout animals, CA1 cells have place-related activity because some regions of space happen by chance to be densely covered by place fields and because a sliding threshold for firing amplifies these variations. In control animals, those synapses are strengthened that connect simultaneously highly active CA3 and CA1 cells.

(B) Results of the model. The strength of the net input to a single CA1 cell is plotted in the upper panel. The lowest curve shows the small spatial variations in the input without plasticity. The higher curves show the development of the input with "Hebbian" synapses and a plasticity threshold that rises as the net input rises. Below is a color representation of the firing activity of the CA1 cell. As the synapses are strengthened (top to bottom) the place field is refined.

with the establishment of robust associations between spatial location and behavioral actions—a critical component of navigational tasks. Repeated exposures, rather than reinforcing a single stable representation, would be forced to operate on constantly changing representations. Our finding of stable place fields following repeated exposures to the same environment in both controls and CA1-KO animals suggests that CA1 plasticity is not required for hippocampal recognition of previously encountered environments. This is consistent with models of hippocampal function that have emphasized the role of CA3 and its extensive set of intrinsic collaterals as providing stability of representations through the processes of pattern completion and generalization.

Loss of Place Field–Specificity as a Mechanism for Behavioral Impairment

A second physiological mechanism capable of interfering with spatial learning is the impairment of spatial information represented by individual cells of the hippocampus. If the spatially restricted activity of a place cell was so poor that no field could be reasonably discerned, then it could be concluded that all spatial learning should be lost. Our data reflects, however, that the CA1 pyramidal cells of CA1-KO mice do retain a fairly high degree of spatially restricted activity. While fields in CA1-KO mice are present and stable, there are significant alterations of the quality and size of individual fields. These alterations, while appearing rather small when compared to the magnitude of the behavioral deficit reported for these mice (Tsien et al., 1996), may have significant effects on several levels. The fields observed in CA1-KO mice may convey locally unstable information due to their lack of a true salient peak or center. This ambiguity, when combined with the increased field size, may cause a significant decrease in the quality of information each cell can convey to the rest of the brain. If appropriate behavior in navigational tasks requires that specific spatial representations be recalled, and if plasticity within CA1 allows unambiguous association of refined place representations with appropriate behavioral response, then the reduced spatial specificity seen in many CA1 pyramidal cells of the knockout animals may account for the behavioral impairment found in the spatial water maze task.

Reduction in Covariance of Firing between Cells Disrupts Ensemble Coding

The observation that 30%–50% of cells within the hippocampus become active within a given environment strongly indicates that rodents use ensemble representations of location rather than mapping places to individual cells. For an ensemble code to provide accurate spatial information, there must be robust covariance of the firing of cells that have overlapping spatial fields. We found that the covariance coefficient (variance normalized covariance; see Figure 5A legend) of cell pairs with overlapping place fields is approximately 0 in CA1-KO animals and is significantly lower than in controls. This would radically impair the ability of the animal to use a hippocampal ensemble code as a robust indicator of spatial location. "Hebbian" learning rules operating in downstream brain regions will fail to learn anything about place from CA1 in the knockouts. We propose

that this can explain the navigational deficit observed in these animals (Tsien et al., 1996).

We have further evidence that the combination of more broadly tuned firing with decreased covariance yields a neuronal representation that may not be sufficiently robust to serve as the basis for mnemonic associations needed to perform navigational tasks such as the water maze. Impaired trajectory reconstruction in knockout animals reveals the inability of groups of neurons to coordinate their firing on a time scale relevant to tasks like the Morris water maze. Impaired trajectory reconstruction has also been seen in rats exploring a novel environment (Wilson and McNaughton, 1993). We speculate that knockout animals may be unable to establish coherent representations of an environment following novel exposure.

Loss of Spatial Specificity May Be Related to a Deficit in Heteroassociative Memory Function

CA1 is the site of convergent inputs from several sources, which include CA3, entorhinal cortex, and subcortical modulatory areas. NMDA mediated plasticity may be required for heteroassociative memory function between these inputs. Direct projections from entorhinal cortex that terminate in CA1 are particularly likely to provide inputs to CA1 that must be mixed with CA3 inputs. Therefore, the observation of reduced specificity in CA1 place firing may hint at a broader failure of CA1 neurons to learn associations between entorhinal inputs and place. Such a failure would be expected to have a significant impact on performance; hippocampal representations would not be correctly associated with cortical representations needed to guide behavior.

Conclusion

This work describes the impairment suffered by CA1 place cells without NMDA receptors and suggests a link between behavior and synaptic plasticity in this region. The absence of plasticity decreases the spatial specificity of place fields, severely damages the correlated firing of CA1 cells, and may indicate a more general loss of the ability to associate behaviorally relevant cortical and hippocampal inputs. We are convinced that for the first time controlled changes in synaptic plasticity can be linked to electrophysiological changes that can explain a behavioral impairment. Based on our experiments, we propose that the uncorrelated activity of CA1 place cells during exploration causes downstream navigational learning impairments. We also suggest that cortical inputs must be associated in CA1 with CA3 inputs. In subsequent work we will examine hippocampal activity during the acquisition of more complex spatial memory tasks in which neuronal activity may be more directly linked to behavioral performance. Overall, this approach promises to establish the specific relationship between targeted cellular physiological mechanisms, regional neuronal function, and behavior.

Experimental Procedures

Strains and Animal Care

NMDAR1 CA1-KO mice were constructed and bred as described in the accompanying paper. In situ hybridization and brain slice electrophysiological analyses of these mice confirmed that the knockout of NMDA receptors was restricted to region CA1 of the hippocampus (see accompanying paper). A total of ten mice were used for this study. All animals were littermates derived from a hybrid CBA-C57BL/6J-129/Sv background and had been previously employed in the Morris water maze study reported in the proceeding paper (Tsien et al., 1996). The mice consisted of three Cre transgenic mice of line T29-1 (*Cre*/+, +/+), five NMDAR1 CA1-KO mice (*Cre*/+, *fNR1/fNR1*), one floxed-NMDAR1 mouse (+/+, *fNR1/fNR1*), and one wild-type (+/+, +/+) mouse. No significant differences in basic electrophysiological or place field measurements were observed between the wild-type, floxed, and Cre animals; however, the floxed animal did demonstrate decreased activity and motivation during behavior. This affect may be due to strain variation (see accompanying paper [Tsien et al., 1996] for discussion). At the time of drive implantation, all mice were between four and six months of age and their weights varied between 24 and 34 g. Following surgery, all animals were individually housed. They were provided with food and water ad libitum and remained on a 12 hr light/dark cycle.

Construction and Surgical Implantation of Microdrive Array

Eleven animals were chronically implanted with miniature microdrive arrays. Each drive is hand-constructed from dental acrylic (Hygenic Co.) and stainless steel cannuli of several sizes (19 ga, 23 ga, and 30 ga; Small Parts Inc.). A complete six-drive array weighs approximately 3.5 g and stands 3 cm in height. The array houses six independently adjustable fine wires. Each microelectrode was composed of four individually insulated nichrome wires (13 mm diameter) that were twisted together and bonded to form a single tetrode of approximately 35 μm in total diameter. The tips of the tetrode were trimmed to a blunt end and electroplated with gold to an impedance of 200–300 KΩ. A manually adjustable threaded rod (0090; Small Parts Inc.) was used to advance and retract each tetrode independently. For each array, six 30 ga (125 μm inner diameter) cannuli were arranged in a bundle to provide an interelectrode spacing of 250–300 μm. Individual microdrives were placed onto the 30 ga cannuli and bonded to form a single implantable device. All surgical procedures were performed following NIH guidelines in accordance with Institutional Animal Care and Use approved protocols. Animals were anesthetized with 15 ml/kg of 2.5% tribromoethanol solution periodically supplemented with inhaled methoxyflurane. The skull was exposed and five miniature jeweler's screws were inserted into the bone for structural support. A hole 1.5 mm in diameter was made over the right hemisphere (2.0 mm L, 1.8 mm P bregma coordinates) and the array was positioned at the brain surface. Space between the edge of the array and the bone was filled with bone wax, and the surface of the skull was covered with dental acrylic. The wound was closed around the base of the array and the animal was allowed to recover. Following recovery, electrodes were advanced into superficial neocortex.

Hippocampal Recording Protocol

Initial advancement of electrodes for recording purposes began 24 to 48 hr following surgery. Mice were placed on a platform in the recording area and fitted with a preamplifier chip (Multi-Channel Concepts) connected to flexible fine-wire cables that carry the neuronal activity to a bank of multichannel amplifiers (Neuralynx) and then to a series of five 486DX4-100 PCs running data collection software written by Matthew Wilson and Loren Frank (MIT). The amplifying chip and cabling are supported from above via a counterweight pulley system that enhances the freedom of movement of the animal. Individual tetrodes were independently advanced over the course of 2–5 days into the hippocampus while the animal was quietly resting. Neural activity was monitored as the tetrodes were lowered, and characteristic hippocampal activity patterns such as sharp-wave ripples and theta modulation were used as landmarks of the position of the tetrodes. The tips of the electrodes were placed into the cell body layer of region CA1 where the activity of up to 15 individual pyramidal cells and inhibitory interneurons could be detected on each tetrode. Actual placement of electrodes was subsequently verified by postmortem histological examination of 80 μm cresyl violet–stained brain sections. Once the tetrodes were stable

in the cell layer (typically 4–7 days after surgery), recording sessions were begun. Data used for identification of cells was taken during a 20–30 min baseline resting period prior to each behavioral session. This was compared with data taken during a comparable 20–30 min baseline period following each behavioral session in order to assess recording stability. During sleep and quiet wakefulness, cells within the hippocampus exhibit spontaneous activity that allows them to be identified independently of any behavioral bias they may subsequently exhibit. This procedure allows the pool of potentially active cells to be identified prior to testing. Each behavioral testing session was bracketed by these baseline periods in which the animal sat quietly on a small platform approximately 30 cm from the testing arena. For behavioral evaluation, the animal was transferred by hand from the resting platform to the testing apparatus. All experiments were conducted in a rectangular curtained area (1 m $\times$ 2 m) with subdued overhead lighting and large, high-contrast cues placed on the walls. The location of the animal was tracked using a pair of infrared diodes placed on the head of the animal centered on the array. The diodes were positioned approximately 2 cm from the head of the animal and were separated by 2 cm. By alternately illuminating each diode at a rate of 30 Hz each, both position and head direction could be measured. This signal was detected using an overhead video camera attached to tracking hardware (Dragon Tracker) that provided approximately 0.5 cm positional resolution. Behavioral testing consisted of spatial exploration of either open field or linear track environments for periods of 10 to 30 min. The open field consisted of a low-walled (4 cm) arena 60 cm in diameter. The linear track was 1.2 meters in length and 3 cm in width. An "L" version of the linear track was also used in which a novel arm was added to the end of the original track at a 90° angle. Each day of recording typically consisted of two behavioral sessions bracketed by sleep periods.

Isolation and Identification of Individual Unit Activity

Classification of spikes was performed using computer software developed by M. Wilson for manual clustering of spike waveforms based on the amplitude and waveform of individual spikes as measured on each of the four recording surfaces of the tetrode. Each action potential was simultaneously measured on the four wires of one tetrode. The amplitudes and waveforms detected from one cell are relatively unvarying, while those from different cells are often distinct. Each spike was thus assigned to a particular cluster of spike waveforms that defined an individual cell. Spikes whose assignments were ambiguous were excluded from further analysis. If more than 0.5% of spike intervals within a cluster fell within a 1 ms refractory period, the entire cluster was excluded from further analysis. Cells were further categorized as excitatory pyramidal cells or inhibitory interneurons according to known characteristics of these cell types (Ranck, 1973). Pyramidal cells were identified as those cells that had both a broad waveform (peak-to-trough width $>$320 μs) and a significant fraction of spikes involved in complex spiking bursts. Interneurons were identified as those cells that had both a narrow waveform (95 μs $<$ width $<$ 255 μs) and the absence of complex spiking bursts.

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