Hippocampal CA1-region-restricted Knockout of NMDAR1 Gene Disrupts Synaptic Plasticity, Place Fields, and Spatial Learning

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It has long been hypothesized that memory storage in the mammalian brain involves modifications of the synaptic connections between neurons. In 1949, Hebb introduced an influential theory consisting of principles that neurons must exhibit for implementing associative memory. The most important principle, known as Hebb's rule, is that when the presynaptic and the postsynaptic neurons are active simultaneously, their connections become strengthened. Subsequently, a synaptic phenomenon termed long-term potentiation (LTP) was discovered in the hippocampus that fits Hebb's rule. Hippocampal LTP, and the more recently discovered long-term depression (LTD), have been subjected to excruciating molecular and cellular studies as the promising candidate mechanisms for memory storage. A large body of research has shown that various artificial protocols in vitro and in vivo can induce LTP or LTD.

The hippocampus is also well situated for its role in memory from the anatomical point of view. It is a high-level multimodal association cortex that has reciprocal connections to many other cortical regions (Amaral and Witter 1989). Numerous neurophysiological 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 order discrimination tasks (O'Keefe and Nadel 1978; Morris et al. 1982; Eichenbaum et al. 1988; Jarrard 1993).

Does the hippocampal synaptic plasticity, LTP and LTD, indeed underlie natural learning and memory? Two types of experiments have been carried out to address this central issue. Morris and his coworkers reported that an NMDA receptor antagonist delivered to the hippocampus caused spatial learning deficits similar to hippocampal lesions and prevented LTP induction (Morris et al. 1986; Morris 1989; Davis et al. 1992). Although qualified later (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,b; Stevens et al. 1994; Chen and Tonegawa 1997). For instance, mice with a deletion in the gene encoding the α subunit of Ca^{++}/calmodulin-dependent protein kinase II (αCaMKII) displayed impaired LTP and LTD in the CA1 region of the hippocampus and a deficit in spatial learning (Silva et al. 1992a,b). Although the results of these pharmacological and genetic experiments are consistent with the notion that hippocampal synaptic plasticity underlies spatial memory, other interpretations could not be excluded because of the lack of regional (and in the case of the mutant studies, temporal) specificity of molecular blockade. It is possible that the observed behavioral defects arose from the functions of NMDA receptors or αCaMKII in processes other than the induction of hippocampal synaptic plasticity. Furthermore, even if the linkage between hippocampal synaptic plasticity and spatial memory is established, one would like to know more about how the former enables the latter.

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 will 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 locations of these place receptive fields change 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. The question then is, Does hippocampal LTP (or LTD) subserve spatial memory by enabling the formation of spatial fields?

To address these questions critically, it is highly desirable to establish a method that permits postdevelopmental elimination of a crucial gene exclusively in a subset of hippocampal cells. Using the *Cre/loxP* recombination system derived from the phage P1, we

recently produced mice in which the gene encoding NMDA receptor subunit 1, an essential component of all known forms of NMDA receptors, is knocked out exclusively in the pyramidal cells of the hippocampal CA1 region. We show that these mice lack NMDA-receptor-mediated synaptic currents and LTP specifically in the CA1 synapses and exhibit impaired spatial memory. Furthermore, 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. These results demonstrate that NMDA-receptor-mediated synaptic plasticity is necessary for the proper representation of space in the CA1 region of the hippocampus and suggest that such representation is essential for spatial memory.

METHODS

All of the methods used in this paper are described in Tsien et al. (1996a,b) and McHugh et al. (1996).

RESULTS

Generation of CA1-restricted NMDAR1 Mutant Mice

Figure 1A illustrates the general strategy for cell-type-restricted, gene knockout technology. Using the transcriptional promoter derived from the αCaMKII gene to drive *Cre* gene expression, we recently produced a transgenic *Cre* mouse line, T29-1, with which one can accomplish *Cre/loxP*-mediated gene knockout efficiently and exclusively in the CA1 pyramidal cells of the hippocampus. This is demonstrated in Figure 1B using a *lacZ* reporter mouse in which the expression of the transgenic *lacZ* gene depends on *Cre-loxP* recombination.

To apply this cell-type-restricted gene knockout technology to the NMDAR1 gene, we constructed a targeting vector in which two *loxP* sequences were inserted into the NMDAR1 gene as shown in Figure 2A. Using this vector, mice homozygous for the *loxP*-NMDAR1-*loxP* sequences (henceforth named "floxed NR1" or "fNR1" mice) were generated through standard embryonic stem (ES) cell gene-targeting procedures (Bradley 1987; Capecchi 1989).

We crossed fNR1 mice with heterozygous *Cre* transgenic mice, T29-1, which have the capacity to mediate *Cre/loxP* recombination exclusively in the CA1 pyramidal cells (Tsien at al. 1996a). After crossing, we obtained mice carrying the *Cre* transgene and the homozygous fNR1 gene (*Cre/+, fNR1/fNR1*), that is CA1-KO mice, as well as various types of siblings (see Methods). From the latter, wild-type (wt) mice (+/+, +/+), T29-1 mice (*Cre/+*, +/+), and homozygous fNR1 mice (+/+, *fNR1/fNR1*) were used as experimental controls. The proportions of mice of the various genotypes indicated that (1) *Cre* and *fNR1* segregate as independent Mendelian loci and (2) the CA1-KO mice and the homozygous fNR1 mice are not lethal at the embryonic or perinatal stage. Indeed, these two types of mice grow and mate normally, and their overall behavior is indistinguishable from that of wt and T29-1 mice. These characteristics of the CA1-KO and the homozygous fNR1 mice are in contrast to the perinatal lethality of NMDAR1 KO mice produced by conventional gene knockout techniques (Forrest et al. 1994; Li et al. 1994). The absence of a gross behavioral phenotype suggests both that the *loxP* insertions do not interfere with normal expression of the fNR1 gene and that the NMDAR1 deletion in CA1-KO mice must be regionally restricted, as expected. Histochemical examination showed that the brains from the CA1-KO mice did not exhibit any obvious abnormalities at the macroscopic level (data not shown).

We confirmed the CA1-restricted deletion of the NMDAR1 gene in the CA1-KO mice by in situ hybridization with a probe whose sequence should be deleted at the DNA level by the *Cre/loxP* recombination. The CA1 region of the mutant mice appeared to lack NMDAR1 mRNA (Fig. 2B), whereas the level of this RNA in other brain regions (such as CA3, dentate gyrus, and neocortex) was indistinguishable from that of control mice (Fig. 2B).

Synaptic Transmission in CA1 and Dentate Gyrus

Synaptic responses of CA1 pyramidal neurons were evoked by stimulation of Schaffer collateral/commissural (Scc) afferents in acute slices. Whole-cell recordings revealed that the CA1-KO cells lacked the slow component of the excitatory postsynaptic current (EPSC) that is mediated by NMDARs (Hestrin et al. 1990a,b). Conversely, the early component of the EPSC that is mediated by AMPA receptors was intact in the mutants. Blockade of AMPA receptors with CNQX (20 μM) resulted in a complete inhibition of the EPSC in the mutant cells, whereas the control cells displayed a robust isolated NMDAR-dependent EPSC (data not shown).

We used field recordings of excitatory postsynaptic potentials (EPSPs) to further examine the synaptic properties of mutant slices. The time courses of the EPSPs measured in CA1 were similar between mutant slices (Fig. 3A, left) and control slices (not shown) when they were bathed with standard saline solution. However, addition of a solution that isolates the NMDARs resulted in the complete blockade of the EPSPs in mutant slices (n = 12, Fig. 3A, left). Conversely, the control slices showed a distinct isolated NMDA EPSP in CA1 (not shown).

A key control experiment was to examine whether functional NMDARs are present in other brain structures in the CA1-KO mice. We studied the synaptic responses of granule cells in the dentate gyrus upon

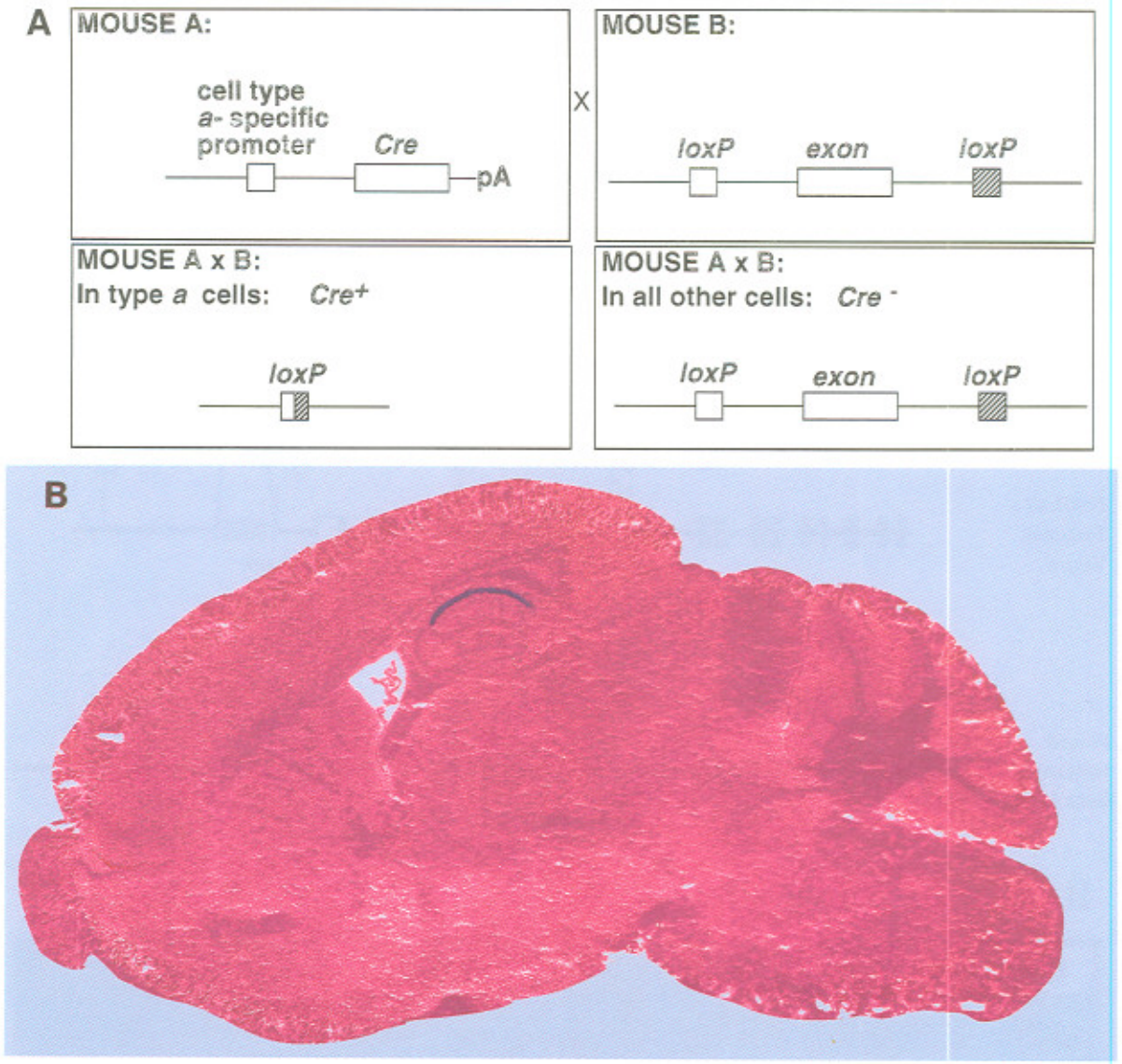
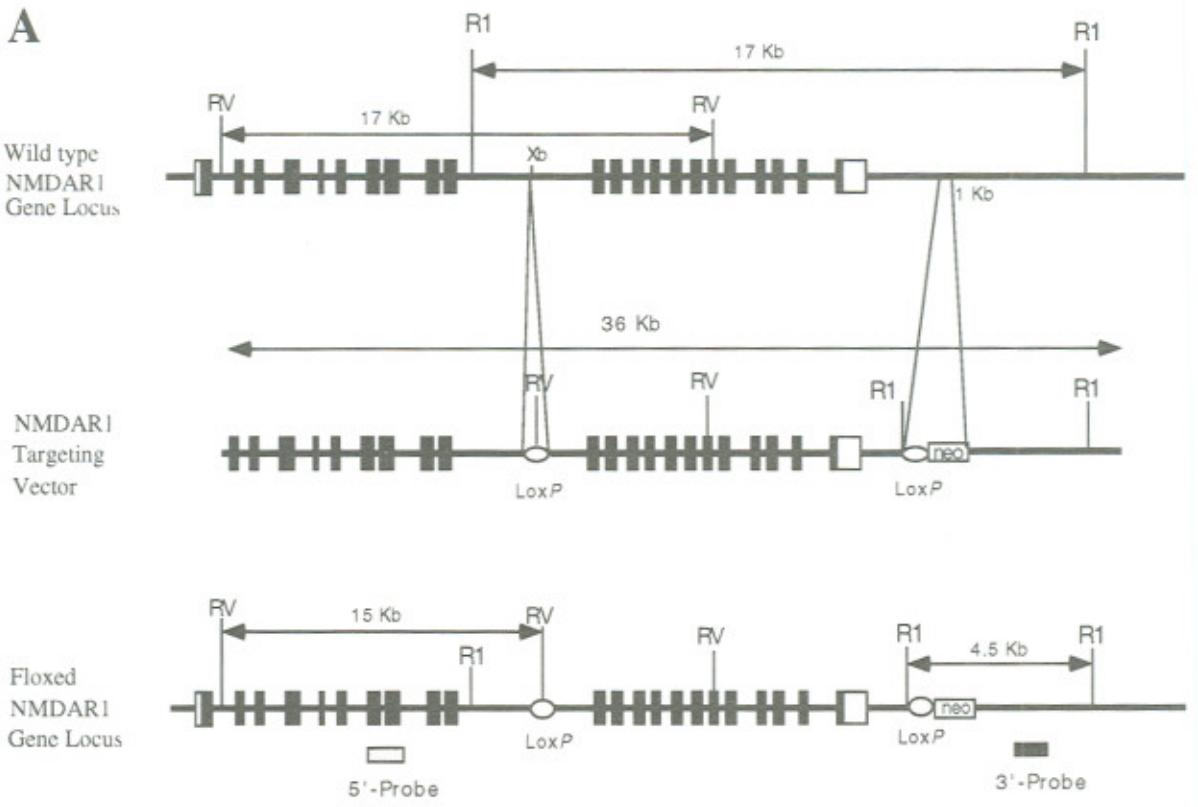


Figure 1. Strategy for cell-type-restricted gene knockout and *Cre/loxP* recombination pattern in the brain for a T29-1/*lacZ* mouse. (*A*) Strategy. *Cre* is a site-specific recombinase that mediates deletional recombination on a pair of tandemly repeated *loxP* DNA sequences. Mouse A carries the *Cre* transgene under the control of a tissue-type or cell-type-specific transcriptional promoter. Mouse B carries a pair of *loxP* sequences targeted to the gene of interest (dubbed "floxed gene") such that the mouse is homozygous with the floxed gene and the insertion itself does not interfere with the expression of the gene. This mouse can be made by the standard ES cell homologous recombination techniques. In progeny that are homozygous for the floxed gene and that carry the *Cre* transgene, the floxed gene will be deleted via *Cre/loxP* recombination but only in the tissue or cell type in which the *Cre* gene-associated promoter is active. (*B*) *Cre/loxP* pattern in T29-1/*lacZ* mouse. T 29-1 is a mouse line with a *Cre* transgene under the control of the αCaMKII promoter. This mouse was crossed to a *lacZ* reporter mouse in which β-galactosidase expression depended on *Cre/loxP* recombination. The sagittal brain section of the T29-1/*lacZ* mouse was stained with X-gal and then counterstained with eosin. Blue X-gal stain is observed only in the CA1 pyramidal cell layer of the hippocampus.

stimulation of the lateral perforant path (Hanse and Gustafsson 1992). We found that the time courses of field EPSPs in mutant slices (Fig. 3A, right) looked normal when compared with control slices (not shown). Importantly, after addition of a solution that isolates NMDARs, the mutant slices showed clear NMDA EPSPs (n =12, Fig. 3A, right) as well as the control slices (not shown). In conclusion, our electrophysiological analysis reveals that the CA1-KO mice lack functional postsynaptic NMDARs in the CA1 pyramidal cells. In contrast, postsynaptic AMPA receptors seem to operate normally in CA1. Finally, the CA1-KO mice show normal NMDAR function in the dentate gyrus.



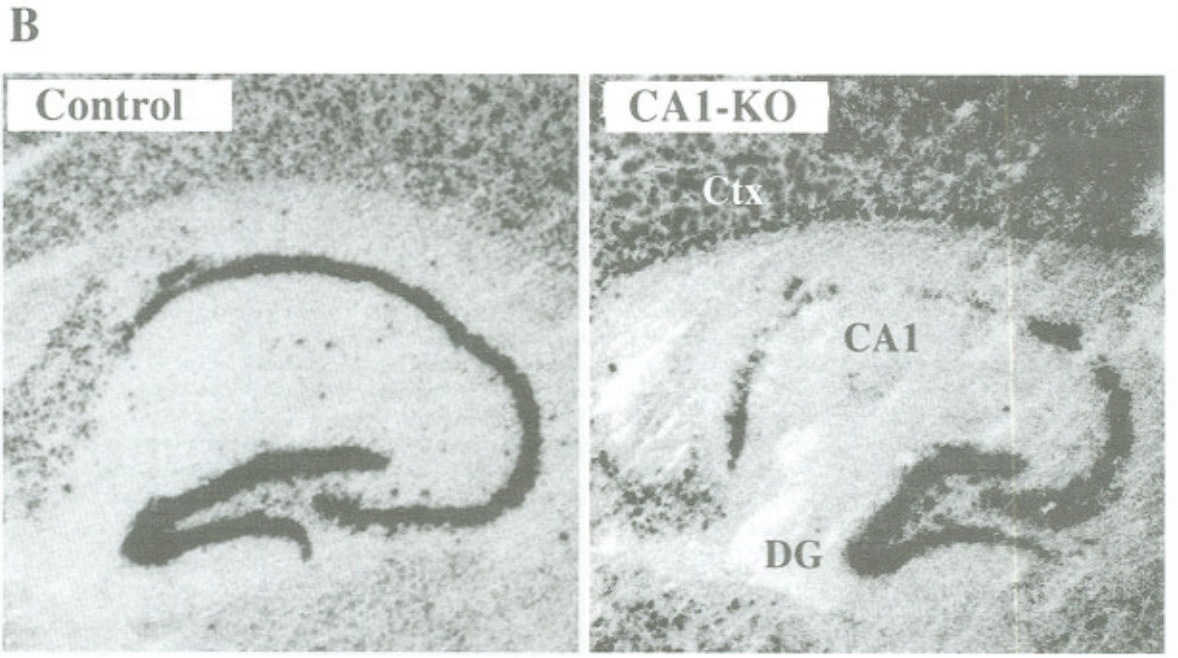


Figure 2. Strategy and demonstration of CA1-restricted deletion of the NMDAR1 gene. (*A*) The NMDAR1 locus and targeting construct. The top drawing represents the wild-type genomic region that contains the intron between exons 10 and 11 of the NMDAR1 gene. The middle drawing represents the NMDAR1 targeting vector that contains two *loxP* sequences. The lower drawing represents the floxed NMDAR1 gene. (*B*) In situ hybridization of NMDAR1 mRNA from wild-type and CA1-KO brains.

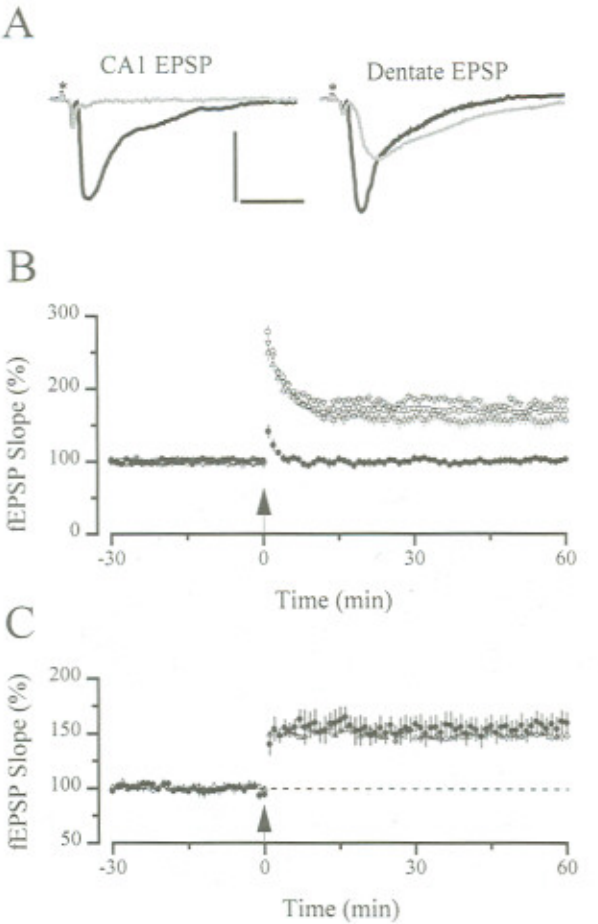


Figure 3. Field EPSPs and LTP in CA1 and dentate gyrus. (*A*) Representative traces (average of 10 responses) of field EPSPs measured in the stratum radiatum of the CA1 region (*left*) and in the stratum moleculare of the dentate gyrus (*right*) in CA1-KO slices. The black traces correspond to EPSPs measured in standard saline, and the gray traces correspond to EPSPs measured in CNQX (20 μM), 2-OH-saclofen (200 μM), glycine (1 μM) and nominally Mg^{++} free. The isolated NMDA EPSP is absent in CA1 (*left*) and clearly distinguishable in dentate (*right*). (*B*) The graph represents the mean (±S.E.M.) field EPSPs in the four groups tested for LTP induction. The CA1-KO (*filled circles*, $n = 21$) did not show LTP, whereas the control groups presented clear LTP (T29-1, *open circles*, $n = 12$; fNR1, *up triangles*, $n = 4$; wt, *inverted triangles*, $n = 5$). (*C*) The graph represents the mean (±S.E.M.) field EPSPs in the CA1-KO (*filled circles*, $n = 10$) and fNR1 (*open triangles*, $n = 6$) dentate gyrus. Significant LTP was elicited in both groups after the tetanus.

Synaptic Plasticity in CA1 and Dentate Gyrus

Application of tetanic stimulation (100 Hz for 1 sec) failed to induce LTP in the CA1-KO slices. Moreover, short-term potentiation (STP) was not detectable in the mutant slices. On average, the synaptic responses were unchanged in CA1-KO slices 45 minutes after the tetanus (100.4 ± 4.9%, $n = 21$, Fig. 3B), whereas LTP was readily obtained in control slices (T29-1, 168.5 ± 4.9 %, $n = 12$; fNR1, 156.9 ± 8.3%, $n = 4$; wt 177.7 ± 6.2%, $n = 5$; Fig. 3B).

Furthermore, we attempted to induce NMDAR-dependent LTD in CA1-KO slices by giving a low frequency train of 1 Hz for 10 minutes (Dudek and Bear 1992). This stimulation was unable to produce LTD (94.8 ± 12.6%, 45 min after train, $n = 4$; not shown). In contrast, we were able to elicit NMDAR-independent LTP in mutant slices by giving a very high frequency tetanus (four trains, each train was 250 Hz for 0.2 sec; 5 sec between trains). This paradigm induced a slowly rising synaptic increase (35.2 ± 8.4%, 60 min after tetanus, $n = 4$, $p < 0.01$, t-test; not shown), demonstrating the preservation of an NMDAR-independent LTP mechanism in the CA1 region (Grover and Teyler 1990).

Finally, we examined whether LTP could be induced in the mutant dentate gyrus. Lateral perforant path (LPP) and medial perforant path inputs onto granule cells were stimulated at 0.1 Hz (Hanse and Gustafsson 1992). Tetanic stimulation (40 shocks at 100 Hz) was able to induce LTP in either of the two inputs. On average, the LPP pathway showed a statistically significant increase in synaptic efficacy 45 minutes after the tetanus (154.6 ± 10.1%, $n = 10$, $p < 0.01$, t-test, Fig. 3C). In a few experiments, we confirmed that this LTP is NMDAR-dependent because addition of AP5 (50 μM) completely blocked its induction (99.1 ± 3.1%, $n = 3$; data not shown).

Spatial Memory

To test for spatial memory, we trained mice in a water maze to find a fixed, hidden platform by using distal cues (Morris et al. 1982; Huerta et al. 1996). We found that the CA1-KO mice were deficient in learning this task when compared to control siblings. The control mice showed a significant decrease in their latencies from block 1 to block 12. The CA1-KO mice also exhibited a significant decrease in their latencies as the training proceeded, but they never reached the level of learning displayed by the control mice.

Even though the mutant mice showed an improvement in their escape latencies, they could have been relying on nonspatial strategies to find the hidden platform. Thus, we used a "transfer" test (TT) to determine whether the mice had formed a spatial memory of the task. During this test, the platform is absent and the mice swim for 60 seconds in the pool. If the animals use a spatial strategy (i.e., they map the position of the hidden platform by using the relations among the distal visual cues around the pool), it is expected that they should spend a significantly greater amount of time than chance actively searching for the platform in the location where they were trained to find it. In contrast, if the mice use nonspatial strategies, they should spend approximately an equal amount of time in each quadrant of the pool. This could occur, for instance, if the mice learned that the platform is at a

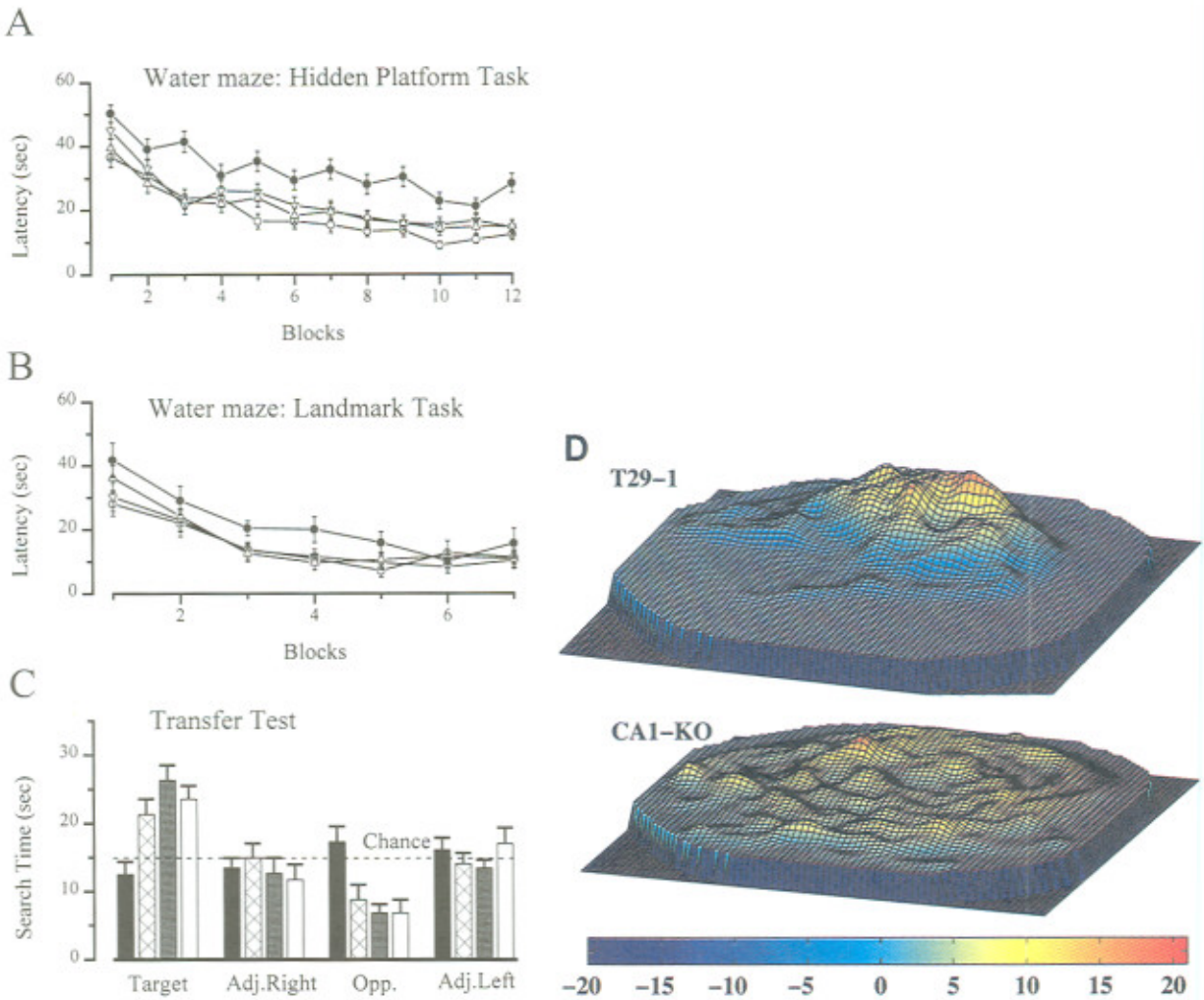


Figure 4. Morris water maze analysis. (*A*) CA1-KO mice are slower in learning the hidden platform version of the Morris water maze. The graph represents the escape latencies of mice trained to find a hidden platform in a water maze by using the distal cues surrounding it. The CA1-KO mice (*filled circles*, n =11) display a longer latency in every block (4 trials per day) than the control mice (T29-1, *open circles*, n =12; fNR1, *up triangles*, n = 13; wt, *inverted triangles*, n = 12). In addition, CA1-KO mice do not reach the optimal performance attained by the control mice, even though the mutants show some improvement. (*B*) CA1-KO mice appear normal in the performance of the landmark task in the water maze. The graph represents the escape latencies of mice trained to find a hidden platform in a water maze by using the proximal cue (a black rectangle attached to the pool wall). The CA1-KO mice (*filled circles*, n = 6) display a somewhat longer latency during the initial block (3 trials per day) but improved their performance to the same level as the control mice (T29-1, *open circles*, n = 6; fNR1, *up triangles*, n = 6; wt, *inverted triangles*, n = 6). (*C*) Transfer test. Average time (± S.E.M.) in each quadrant for the four groups (CA1-KO, *dark*; T29-1, *hatch*; fNR1, *gray*; wt, *white*). The CA1-KO mice spent equal amounts of time in every quadrant, whereas the control groups spent significantly more time than chance in the target quadrant. (*D*) Three-dimensional graphs representing the total occupancy by six T29-1 mice and six CA1-KO mice during the transfer test. The control mice focused their search in the trained location (where the platform used to be during training), whereas the mutant mice visited the whole maze area equivalently.

certain distance from the edge of the pool, a memory independent of hippocampal function. The CA1-KO mice did not show any place preference for the target quadrant. In contrast, the control groups showed a marked preference for the target quadrant (T29-1, 22.6 ± 2.7 sec; fNR1, 27.6 ± 1.5 sec; wt, 20.2 ± 2.4 sec; TT3: T29-1, 21.4 ± 2.2 sec; fNR1, 23.6 ± 1.9 sec; wt, 26.5 ± 2.2 sec; $p < 0.0001$ for all the values, paired t-test, Fig. 4C). Analysis of the navigational strategies of mice demonstrated that the control mice focused their search in the trained area and thus produced a high total occupancy peak in the position in which the platform was previously located (Fig. 4D, upper panel), whereas the mutants navigated all over the pool's area (Fig. 4D, lower panel). Finally, to ensure that our apparatus was well suited for measuring spatial memory, we tested homozygous αCaMKII knockout mice. These mice are known to be deficient in spatial memory (Silva et al.

1992b) and indeed showed, in our apparatus, the same complete deficit in spatial memory as the CA1-KO mice (data not shown).

To assess whether the impaired behavior of the mutants in the hidden platform version of the water maze was due to some sensorimotor or motivational deficit, we trained the mice in a simpler task in the water maze (Kolb et al. 1994). In this task, the animals are required to find a slightly submerged platform whose location is marked by a large proximal cue, known as the "landmark." We found that the mutant mice learned the task at a somewhat slower rate, but reached the same level of optimal performance as the control mice (Fig. 4B).

In conclusion, our behavioral analysis reveals that the CA1-KO mice display a selective and significant deficit in spatial memory, because they are unable to develop a navigational strategy in the Morris water maze. In contrast, they could perform nonspatial learning tasks that rely on simple association strategies.

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

We employed multi-electrode recording techniques to characterize hippocampal activity in CA1-KO mice 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 (Fig. 5). 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. We evaluated basic spike waveform and firing characteristics of these cells 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. This was a weak effect, but it is consistent with the lack of an excitatory NMDA current. 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.

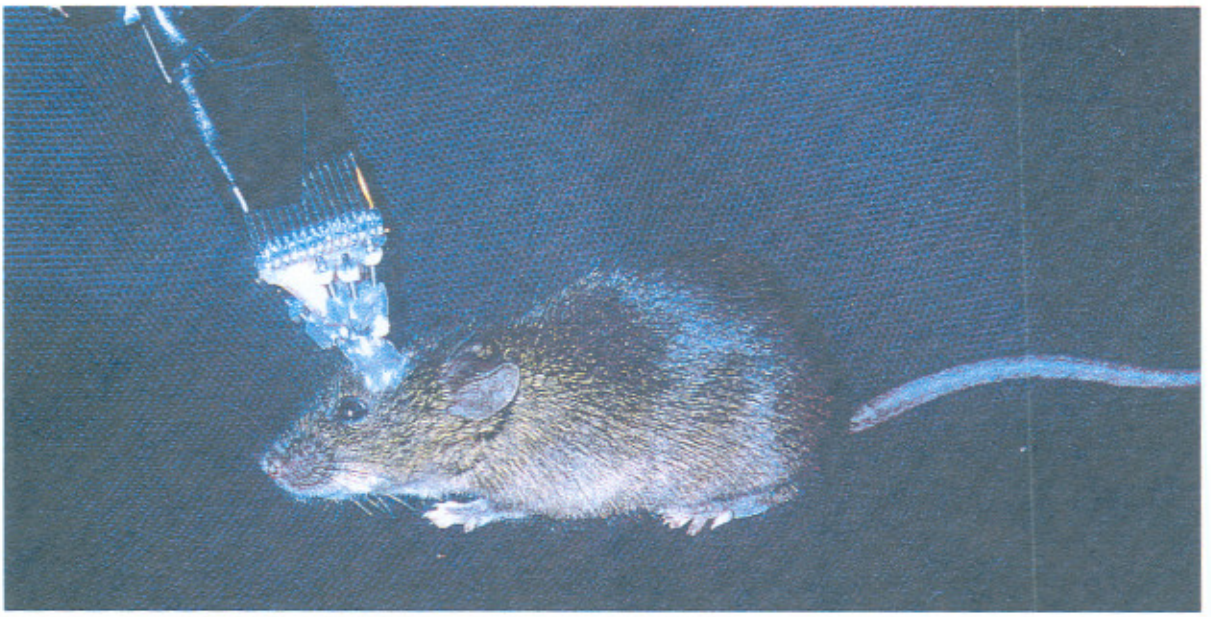


Figure 5. NMDAR1 CA1-KO mouse with implanted microdrive. A microdrive array housing six independently adjustable four-channel tetrodes was affixed to the mouse's skull directly above the hippocampus (2.0 mm L 1.8 mm P bregma coordinates), allowing large numbers of individual cells to be recorded during behavior (see Methods). All mice subjected to multiple electrode recording were littermates with CBA-C57BL/6J-129/SV background and consisted of three T29-1 mice, five CA1-KO mice, one floxed-NMDAR1 mouse, and one wild-type mouse of between 4 and 6 months of age and between 24 and 34 grams in weight.

Place Fields Are Larger in CA1-KO 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 6A 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, although 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 x 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 a few times. We therefore restricted our analysis to data from the linear and L-shaped tracks where sampling was high and uniform. Figure 6B 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 and knockout fields averaging 140 pixels. 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 for both high and low rate cells. The median firing rate for pyramidal cells that fired significantly while animals were 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 6C shows that for both high and low rates, the place fields of knockout pyramidal cells are larger than those of controls.

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 (variance-normalized covariance) of firing rates averaged over 200-msec windows between pairs of cells with overlapping fields. Average covariance coefficient was computed as $< [(\Sigma_i (R_i - R_{avg})(r_i - r_{avg})] / [\sqrt{(\Sigma_i (R_i - R_{avg})^2}\ \sqrt{(\Sigma_i (r_i - r_{avg})^2}\ ]>$, where $< >$ is an average over cell pairs, i is an index that runs over all included 200-msec bins in a session, and R and r refer to different cells in a pair. Figure 7A shows that pairs of knockout cells exhibit completely uncorrelated firing whereas 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-msec 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, i.e., 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 its fields and spike trains alone. Figure 7B 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, we may have overestimated the performance of the knockout animal ensembles. Even for ensembles with uncorrelated firing, the reconstruction improves with larger ensembles. Figure 7C shows an example of trajectory reconstruction. Ensembles of cells from the knockouts are more likely to make large errors in 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.

Model: Place Cells in CA1-specific NMDAR1 Knockout Mice

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 this: Assuming 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 (K.I. Blum and M.A. Wilson, in prep.) 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 Fig. 8). We used numbers

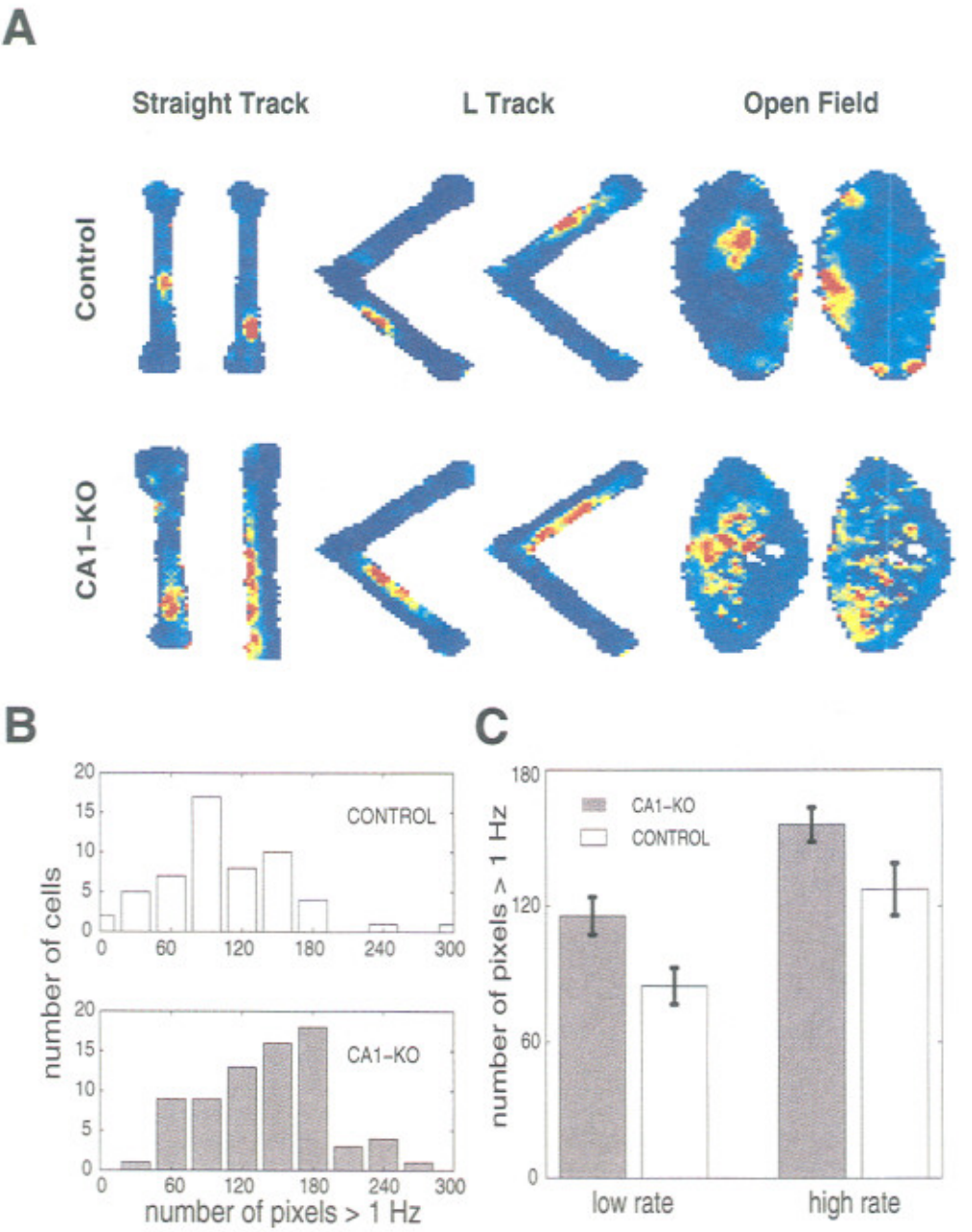


Figure 6. Place fields of NMDAR1 CA1-KO mice are significantly larger in all behavioral environments. (*A*) Rate maps of place-specific activity of two pyramidal cells from control animals and two pyramidal cells from knockout animals in each behavioral environment. The peak rate of each panel has been 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 two-dimensional open field 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 on the basis of average spike width and complex spike index score (see Results). The mean field size in NMDAR1 CA1-KO animals was 140.3 pixels (~560 cm^2) and in control animals the mean size was 106.0 pixels (~420 cm^2). (*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 NMDAR1 CA1-KO animals have significantly larger fields.

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

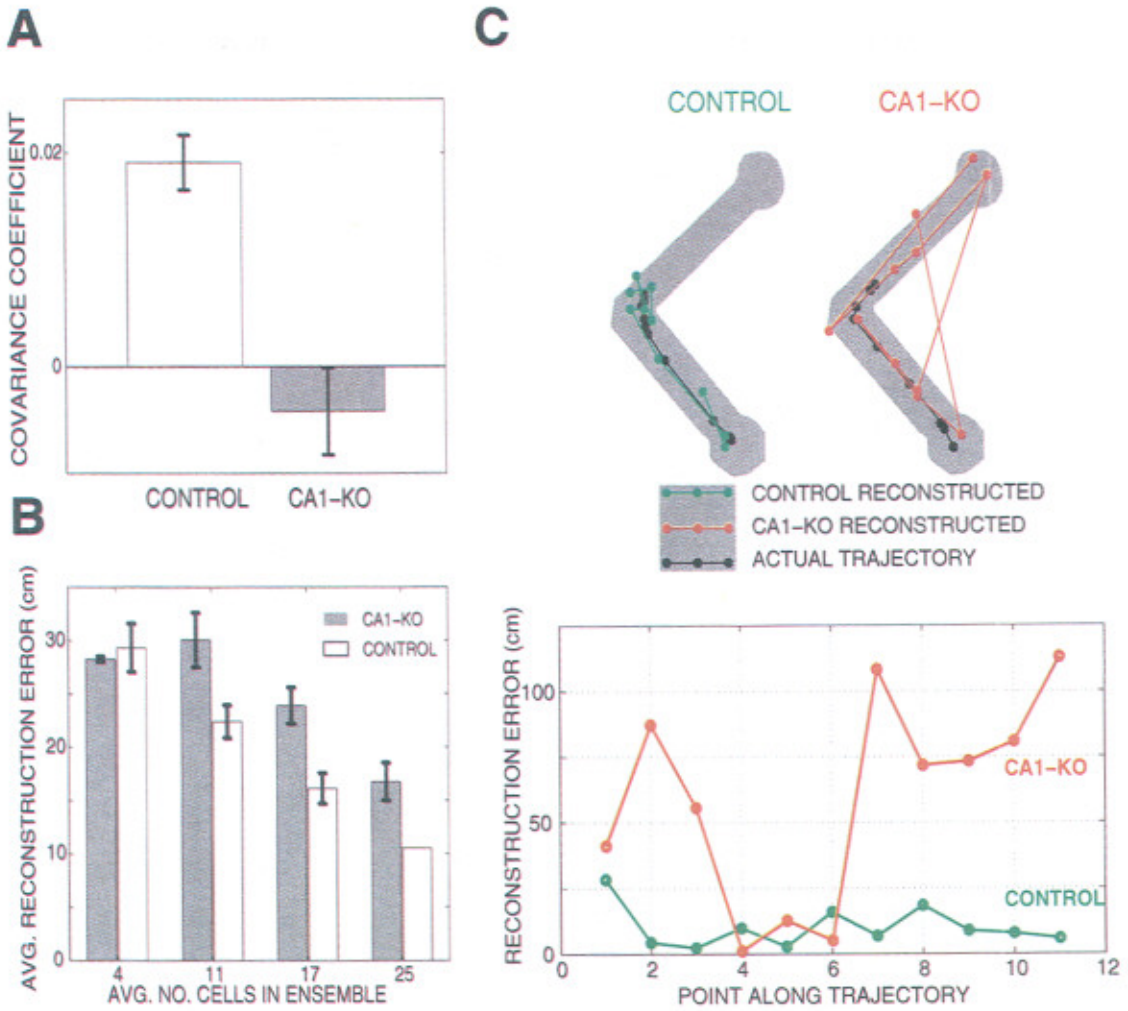


Figure 7. Ensemble coding properties of CA1 pyramidal cells in NMDAR1 CA1-KO and control mice. (*A*) The average covariance coefficient of firing rates between overlapping pairs of control and pairs of knockout pyramidal cells. Pairs of cells in knockouts fired randomly with respect to each other when their place fields overlapped. The average firing rate of each neuron was calculated for successive 200-msec bins over one 15- to 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 S.E.M. 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. 16 knockout and 13 control data sets were grouped by number of active place cells in that session. Reconstruction errors were averaged over the entire 15- to 20-min RUN sessions. Error bars represent S.E.M.; the number of degrees of freedom was taken to be the number of data sets, 4 on average. The same trend was observed in straight and L-shaped tracks, so the data were pooled. The position was reconstructed every 2 sec by comparing a list of the average firing rate of each cell for a 2-sec bin with a list of the average rates 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-sec bin. (*C*) Examples of trajectory reconstruction. The upper panel illustrates trajectories reconstructed for control and CA1-KO animals for a 20-sec 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 mutant cells. The average reconstruction errors over 15–20 min were 17 cm and 23.5 cm, respectively.

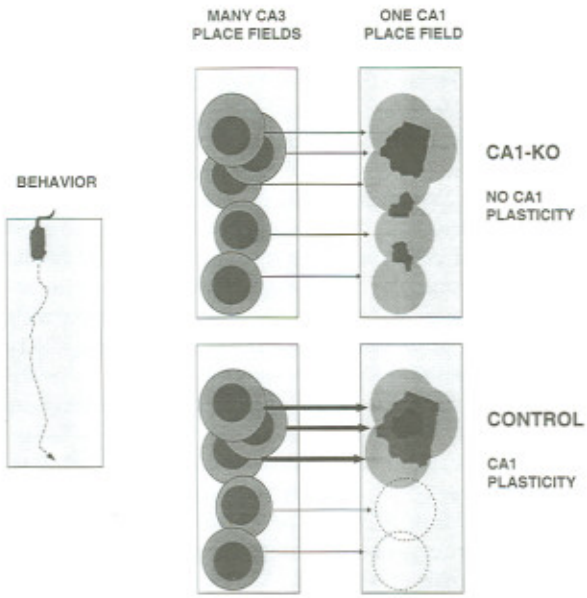


Figure 8. Schematic view of a model detailing possible role of NMDA-receptor-mediated synaptic plasticity in the refinement of place fields in CA1 pyramidal neurons. Each large rectangular panel is a copy of the environment. As the animals move around, various place cells in CA3 are activated, and these 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.

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. 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 model without plasticity. The model suggests that plasticity may also increase signal to noise in CA1 and raise the covariance coefficient for cell pairs.

DISCUSSION

CA1 LTP Underlies the Formation of Spatial Memory

We have generated a knockout mouse strain lacking the NMDAR1 subunit in the CA1 region of the hippocampus. These mice seem to grow normally and do not present obvious behavioral abnormalities. We have shown that the mutant mice lack NMDAR-mediated EPSCs and LTP in the CA1 region and are impaired in the hidden platform version of the Morris water maze (a measure of spatial memory), but not in nonspatial learning tasks. Previously, it was suggested that NMDA-receptor-dependent LTP underlies the acquisition of new memories in the hippocampus (for review, see Morris et al. 1991; Bliss and Collingridge 1993). Our results provide a strong support for this view and make it more specific: The NMDA-receptor-dependent LTP in the CA1 region is crucially involved in the formation of certain types of memory. We cannot exclude the possibility that the NMDA-receptor-dependent synaptic plasticity crucial for memory formation is LTD, given that the CA1-KO mice seem to lack NMDAR-dependent LTD. However, we think that this is unlikely because spatial learning is apparently intact in knockout mice deficient in protein kinase A (PKA) that lack CA1 LTD (Brandon et al. 1995).

Gene Knockout Studies Point to a Pivotal Role of CA1 Synaptic Plasticity

Previous studies examined the correlation between spatial memory and the site of hippocampal LTP (i.e., CA1, CA3, and dentate gyrus) by using a variety of conventional knockout mice (for review, see Chen and Tonegawa 1997). These studies found a correlated impairment in Scc-CA1 LTP and spatial memory (but see Conquet et al. 1994). It is important to point out that the only exceptional case, namely that of the mGluR1 mutant mice, is complicated by the fact that two groups have generated and analyzed these mutants independently and obtained opposing results (Aiba et al. 1994; Conquet et al. 1994; Chen and Tonegawa 1997). In contrast, it has been shown that impairments of mossy fiber-CA3 LTP (Huang et al. 1995) and perforant path-dentate LTP (Nosten-Bertrand et al. 1996) are not correlated with spatial memory deficit.

Our new evidence, although still correlational, is much stronger than the earlier reports because we have singled out the CA1 synapses as a site of plasticity impairment. What structural and functional features could explain this pivotal role of CA1 synapses as sites of plasticity underlying spatial memory? It is well known that the CA1 region is a crucial component of the "hippocampal formation" system that is involved in the acquisition of certain types of memory. In rodents, this system consists of several structures connected within a loop that encompasses the entorhinal cortex (EC), with inputs from higher sensory cortices;

dentate gyrus (DG), with inputs from EC; CA3, with "external" inputs from EC and DG and "internal" inputs from CA3; CA1, with inputs from CA3 and EC; and subiculum (SUB), with inputs from CA1 and outputs to EC (Lorente de Nó 1934; Amaral and Witter 1989). In view of the evidence currently available, it would seem that the minimal system required as a locus for memory acquisition would be the EC-CA3-CA1-SUB-EC loop. This is because (1) the plasticity in the EC-DG and DG-CA3 synapses seems to be dispensable (Huang et al. 1995; Nosten-Bertrand et al. 1996); (2) the direct EC-CA1 connection appears to be too weak to sustain the EC-CA1-SUB-EC loop as the locus for memory (Empson and Heinemann 1995); and (3) the evidence presented in this paper points to the special role of the CA3-CA1 synapses.

Interestingly, the plasticity of the EC-CA3 synapses has not been well studied, but our proposed scenario predicts that they are important in implementing the memory system. Most probably, both the EC-CA3 pathway and the EC-DG-CA3 pathway provide parallel inputs to the CA3 network during learning, and the CA3-CA3 synapses work as autoassociative memory devices, as it has long been proposed (Marr 1971). Thus, it is desirable to generate a CA3-region-specific knockout of the NMDA receptor to allow a direct examination of the contribution of these synapses to learning.

Loss of Place Field Specificity as a Mechanism for Behavioral Impairment

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 reflect, however, that the CA1 pyramidal cells of CA1-KO mice do retain a fairly high degree of spatially restricted activity. Although fields in CA1-KO mice are present and stable, there are significant alterations of the quality and size of individual fields. These alterations, although appearing rather small when compared to the magnitude of the behavioral deficit reported for these mice (Tsien et al. 1996b), 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 Fig. 5A) of cell pairs with overlapping place fields is approximately zero 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. 1996b).

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.

CONCLUSION

Creation of cell-type-restricted gene knockout techniques made it possible to examine the relationship between the function of NMDA receptors, synaptic plasticity, place fields in the hippocampal CA1 region, and spatial memory. 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. On the basis of 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. This approach promises to establish the specific relationship between targeted cellular physiological mechanisms, regional neuronal function, and behavior.

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