

Hippocampal CA3 NMDA Receptors Are Crucial for Memory Acquisition of One-Time Experience

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Summary

Lesion and pharmacological intervention studies have suggested that in both human patients and animals the hippocampus plays a crucial role in the rapid acquisition and storage of information from a novel one-time experience. However, how the hippocampus plays this role is poorly known. Here, we show that mice with NMDA receptor (NR) deletion restricted to CA3 pyramidal cells in adulthood are impaired in rapidly acquiring the memory of novel hidden platform locations in a delayed matching-to-place version of the Morris water maze task but are normal when tested with previously experienced platform locations. CA1 place cells in the mutant animals had place field sizes that were significantly larger in novel environments, but normal in familiar environments relative to those of control mice. These results suggest that CA3 NRs play a crucial role in rapid hippocampal encoding of novel information for fast learning of one-time experience.

Introduction

One-trial or one-experience learning is crucial for both human and nonhuman animals for the normal maintenance of day-to-day life and even for survival. In the ever changing world, events occur only once and, therefore, episodic memory—memory of an event that enables an explicit recollection (see Tulving, 1972, 2002, for reviews)—is based on this rapid form of learning. Other examples include poison avoidance (Garcia and Koelling, 1966), recognition memory (Ennaceur and Delacour, 1988), spatial learning (Morris, 1983; Panakhova et al., 1984; Whishaw, 1985; reviewed by Morris, 2001), and food caching (Clayton and Dickinson, 1998; reviewed by Griffiths et al., 1999). Studies on amnesic patients and lesioned animals have demonstrated that in both human and nonhuman animals, the hippocampus plays a crucial role in some types of one-trial learning (Zola-Morgan et al., 1986; Rempel-Clower et al., 1996; Steele and Morris, 1999; Xavier et al., 1999). In the case

of rodents, it was shown that intrahippocampal infusion of NMDA receptor (NR) antagonist, AP5, results in an impairment in delay-dependent acquisition of one-trial spatial learning (Steele and Morris, 1999; Morris and Frey, 1997). However, little is known about the hippocampal subfields and their circuits in which NRs play a crucial role in this or any other types of rapid one-trial learning.

Based on theoretical grounds, Marr and others have suggested that a recurrent network with modifiable synaptic strength could support rapid acquisition of memories of one-time experiences (Marr, 1971; McClelland et al., 1992, *Soc. Neurosci.*, abstract; McClelland et al., 1995). The CA3 subfield of the hippocampus is known to have just such a type of network, with its pyramidal cells receiving synaptic contacts from ~2% of other CA3 pyramidal cells (MacVicar and Dudek, 1980; Miles and Traub, 1986). Furthermore, NR-dependent, Hebbian-type synaptic plasticity in the form of long-term potentiation (LTP) has also been demonstrated at the recurrent collateral-CA3 synapses (Harris and Cotman, 1986; Williams and Johnston, 1988; Zalutsky and Nicoll, 1990; Berger and Yeckel, 1991). However, there has been no experimental evidence supporting the hypothesis that the recurrent CA3 network plays a crucial role in rapid one-trial learning.

Recently, we generated a conditional knockout mouse strain in which the deletion of the gene encoding the NR subunit 1 (NR1) of NRs is targeted to the CA3 pyramidal cells of adult mice (referred to as CA3-NR1 KO mice) (Nakazawa et al., 2002). These mice were indeed deficient in evoked LTP specifically at the recurrent collateral-CA3 synapses but were normal in the spatial reference memory that is acquired incrementally over several days of repeated trials. We also demonstrated that this normal acquisition of spatial reference memory in the mutants correlated well with their normal place-related activities of CA1 pyramidal cells in a simulated familiar spatial environment.

The availability of the CA3-NR1 KO mice provided an opportunity to test the hypothesis that the recurrent CA3 network with modifiable synaptic strength supports hippocampus-dependent, rapid one-trial learning. For this purpose, we subjected the mutant mice to the delayed matching-to-place (DMP) version of the Morris water maze task in which the animal's ability to acquire a novel location of the hidden platform with just one visiting trial is tested by its performance in the second trial conducted a few minutes later. We also sought physiological correlates of the behavioral phenotype by monitoring place-related activities of CA1 pyramidal cells.

Results

CA3-NR1 KO Mice Are Impaired in Acquiring Memory of Novel Platform Locations in the DMP Task

The setup of the DMP task was similar to that of the standard hidden platform version of Morris water maze

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except that the location of the platform was altered each day in a pseudo-random way (Figure 1A). We followed the trial protocol previously employed for rats with minor modifications (Steele and Morris, 1999); on each day, four trials were given with an intertrial interval of 5 min, and the time needed to reach the platform (escape latency) was recorded. The mean latencies were relatively long for the first trial of a day because the animals had no prior knowledge of the platform location. However, they can potentially shorten the mean latencies on the second trial based on the memory rapidly acquired during the first visit, and the reduced latencies in the second trial reflect the “savings” accrued from memory of the first trial (see Experimental Procedures for more details). For the data analysis and presentation, the DMP training session was subdivided into three blocks of 4 consecutive days (Figure 1B; blocks 1–3). Similarly, the data obtained during the 4 day-long testing session were combined (Figure 1C; days 13–16 for block 4). For each of the four trials (trials 1–4) conducted each day, the escape latencies were averaged over multiple animals for each genotype and over the 4 days of each training or testing block.

The mean latency curves for the DMP training session (blocks 1–3) and the testing session (block 4) are shown in Figures 1B and 1C, respectively. In Figure 1E, the latencies of trials 1 and 2 across the blocks are replotted. There were no significant differences of latencies between the two genotypes in any of the trials during the training session except for a small but significant reduction of block 3 trial 2 latencies in the mutants relative to controls. In the testing block, however, the mutants’ mean latencies of the second and subsequent trials were clearly longer than controls’ mean latencies of the corresponding trials (Figure 1C), and the mutants’ mean savings between trials 1 and 2 was significantly shorter than the controls’ (*t* test, $p < 0.04$). These results are consistent with the interpretation that CA3-NR1 KO mice are impaired in the rapid acquisition of spatial memory of novel locations of the hidden platform with just one visiting trial. However, prior to making a firm conclusion we evaluated several other possibilities. First, Figure 1D shows that the saving difference cannot be accounted for by a swim speed difference. Second, there is no significant difference in the time spent near the wall between two genotypes (Figure 1D), indicating that the mutants’ reduced saving is not due to augmented thigmotaxis. Third, mutant mice did not search the platform at or near the locations of the previous day any more than control mice (Figure 1D), indicating that the mutants’ latency prolongations are not due to increased memory interference or perseveration. This last point is also suggested by the lack of genotype-dependent latency difference in the first trials in which one expects memory interference, if any, would be most pronounced (Figure 1E). On the basis of these results, we conclude that the reduced savings observed in the mutants relative to controls during the testing session (block 4) is most probably a consequence of the mutants’ impaired capability in rapidly acquiring the spatial memory of the novel location of the platform.

Why didn’t the impairment in savings appear in the early stages of the training session (blocks 1 and 2)? Like any other task, the DMP task demands learning of

a variety of basic rules. For instance, the animals will have to learn to swim away from the walls (i.e., overcome thigmotaxis), to climb on the platform once they find it (i.e., incentive learning), and most importantly, learn that there is a single place to escape and that this place varies from one day to another. Learning these basic rules is the prerequisite for the acquisition of the robust memory of the accurate location of the novel platform. In block 1, the effect of the first trials on the mean latencies of the second trials was minimal. In block 2, it was more pronounced, but the second trial latencies were still quite long (60 and 59 s for controls and mutants, respectively), suggesting that the saving was primarily due to rule learning rather than robust acquisition of spatial memory. In blocks 3 and 4, the second trial latencies of the mutants remained at the levels attained in block 2 while those of the control mice progressively shortened, resulting in genotype-specific differences in saving (Figure 1E). Also, the fact that the first trial latencies are the same for both genotypes across all four blocks suggests normal rule learning. These data suggest that CA3 NRs are involved primarily in the rapid spatial learning rather than in DMP rule learning.

CA3-NR1 KO Mice Are Normal in Recalling the Memory of Familiar Platform Locations in the DMP Task

One of the salient features of an association to be learned rapidly with one trial or one experience is its novelty. In the water maze DMP task, the animals are required to rapidly and accurately acquire the association of the novel location of the platform with the configuration of distal cues. In order to investigate further the role of the CA3 NR and recurrent network in the novelty-associated mnemonic process, we subjected other sets of pretrained mice ($n = 15$ and 13 for mutant and control animals, respectively) to a similar testing session except that on each of the 4 days in block 4 the platform locations were those experienced 4 days earlier during training rather than novel ones (Figures 1F and 1G). Under these conditions, the first trials can serve as a reminder of the spatial memory acquired 4 days earlier, and the performance improvement (saving) in the second trials would reflect the animals’ ability to retrieve this reference memory. As shown in Figure 1F, we observed no difference in the mean latencies of not only first trials but also of the subsequent three trials between the two genotypes. Again, no significant differences were observed in the swim speed (Figure 1G), nor the time near the wall (data not shown), nor perseveration index between the two genotypes (data not shown).

A three-way ANOVA for escape latencies (KO versus control X novel versus familiar platform location X trials 2–4) based on the data from Figures 1C and 1F revealed a high significance in genotype effect [$F_{(1,220)} = 13.4$, $p < 0.0003$] as well as in the interaction between “genotype” versus “novel versus familiar platform location” [$F_{(1,220)} = 7.7$, $p < 0.006$]. Post hoc tests showed that mutant mean escape latencies of trials 2–4 were significantly longer ($p < 0.05$) than those of controls when the platform was placed at novel locations. In contrast, in trial 1 the latency differences were not significant [genotype effect, $F_{(1,220)} = 0.48$, $p = 0.49$; interaction between “geno-

type” versus “novel versus familiar platform location,” $F_{(1,220)} = 0.06$, $p = 0.81$]. No significant interaction was observed in three-way ANOVA for swim speed (KO versus control X novel versus familiar platform location X trials 2–4) based on the data from Figures 1D and 1G (interaction between “genotype” versus “novel versus familiar platform location,” $F_{(1,220)} = 0.02$, $p = 0.90$). These data demonstrate differential effects of the CA3 NR knockout on the acquisition and retrieval of reference memory and on the acquisition of single trial memory: CA3 NRs are dispensable in the acquisition of spatial memory by repeated trials and in its retrieval, but play a crucial role in rapid, one-trial acquisition of accurate memory involving novel and spatially dependent information.

Normal CA1 Place Cell Activity of Mutant Mice during Exploration in Familiar Linear Tracks

In order to investigate the cellular mechanisms that might underlie the specific behavioral impairment observed in the mutant mice, we examined the effect of novelty on spatial representations in area CA1—the output of the hippocampus proper. We chose this area because our primary goal was to determine the downstream effect of the disruption of CA3 activity that could be related to the behavioral output of hippocampus-dependent tasks. Using the tetrode recording technique (Wilson and McNaughton, 1993; Nakazawa et al., 2002), we compared the activity of CA1 pyramidal cells on familiar (track A) and novel (track B) regions of an L-shaped track (Figure 2A). After animals were familiarized with track A by running back and forth for 15 min for 1 day, data were collected from 108 complex spiking (pyramidal) cells and 21 putative interneurons from five mutant mice, and 89 complex spiking cells and eight putative interneurons from five control mice. As summarized in Table 1, under these conditions, we detected no effect of the CA3 NR1 knockout on the basic cellular properties of CA1 pyramidal cells such as spike width, complex spike index, mean firing rate, and peak firing rate, nor on the spatial tuning properties of individual place cells such as mean in-field firing rate, integrated firing rate, and place field size (see Experimental Procedures for the definition of each of these parameters). However, burst spike frequency in pyramidal cells and mean firing rate of CA1 interneurons were significantly diminished in the mutants relative to controls, presumably reflecting the reduced CA3 drive onto CA1 as a consequence of the NR1 knockout in CA3 (Nakazawa et al., 2003, in press).

Impaired Spatial Tuning of Mutant CA1 Place Cells in Novel Regions of Familiar Environment

To examine the effect of the mutation on the spatial tuning properties of CA1 place cells when animals are introduced to a novel region of the familiar environment, we let randomly chosen subsets of mutant and control mice enter into the perpendicularly attached novel track (track B) by removing the partition. We then compared the properties of CA1 place cells during the first 15 min in novel track B with those of the last 15 min in familiar track A (Figure 2A). Twenty seven and twenty nine complex spiking cells were recorded in the familiar track A,

and twenty two and twenty nine cells were recorded in the novel track B from mutant and control mice, respectively. We found that three spatial tuning measures, integrated firing rate, place field size, and number of place field peaks (see Experimental Procedures) in CA1 complex spiking cells were all significantly greater for the mutant mice in the novel track compared to the familiar track, while those of the CA1 cells of the control mice did not change significantly upon entry into the novel track (Figures 2B–2D, “Day N”). In contrast, mean in-field firing rate was not elevated in the mutants (Figure 2E), indicating that impaired spatial tuning was not simply due to over excitation but due specifically to the enlargement of the field size of the mutant CA1 place cells upon entering into the novel track.

To confirm that the observed impairment in spatial tuning was related to the novelty of exposure, the same set of mutant and control mice were subjected to another recording session on the next day in familiar track A immediately followed again by a recording session in track B. In this second pair of recording sessions, the data were collected from 24 and 26 complex spiking cells in track A and 23 and 23 complex spiking cells in track B from the mutant and control mice, respectively. In this case, there was no indication of an increase in any of the three spatial tuning measures upon reentry into track B from track A in either the control or mutant mice (Figures 2B–2D, “Day N+1”). Running velocity could affect place cell firing properties (McNaughton et al., 1983). However, average running velocity did not differ for either genotype under any of the four conditions (Figure 2F). In addition, we did not observe any overt behavioral changes in either the mutant or control mice upon any of the track shifts. Figures 2I and 2J show two examples each of the place fields of CA1 complex spiking cells from the control and mutant mice in tracks A and B, respectively. In these examples, the place fields were relatively compact in the familiar track A for both control and mutant cells (Figure 2I), while they were spatially less tuned when the mutant mice were exposed to track B for the first time (Figure 2J, “Day N”) and returned to normal on the next day (Figure 2J, “Day N+1”). We also conducted the statistical analysis per mouse and found significant interaction between “genotype” versus “condition” (four recording conditions) in integrated firing rate [$F_{(3,16)} = 7.6$, $p < 0.03$], and in place field size [$F_{(3,16)} = 3.3$, $p < 0.05$].

It is interesting to find out whether the improved spatial tuning observed in the mutant mice upon the revisit of track B on day N+1 reflects a certain consolidation process which may occur during the 24 hr-long resting period between the day N and day N+1 recording sessions. An alternative possibility is that the extent of spatial tuning depends on the amount of experience in track B throughout the day N and day N+1 recording sessions (15 min each and 30 min total). In order to address this issue, we calculated the mean integrated firing rate and the place field size every 3 min during the day N and day N+1 recording sessions. As shown in Figures 2G and 2H, there were no significant changes in either of these two spatial tuning parameters within the day N or day N+1 recording session. Thus, the levels of mutant spatial tuning were discontinuous between the last 3 min of the day N recording session and the first 3 min

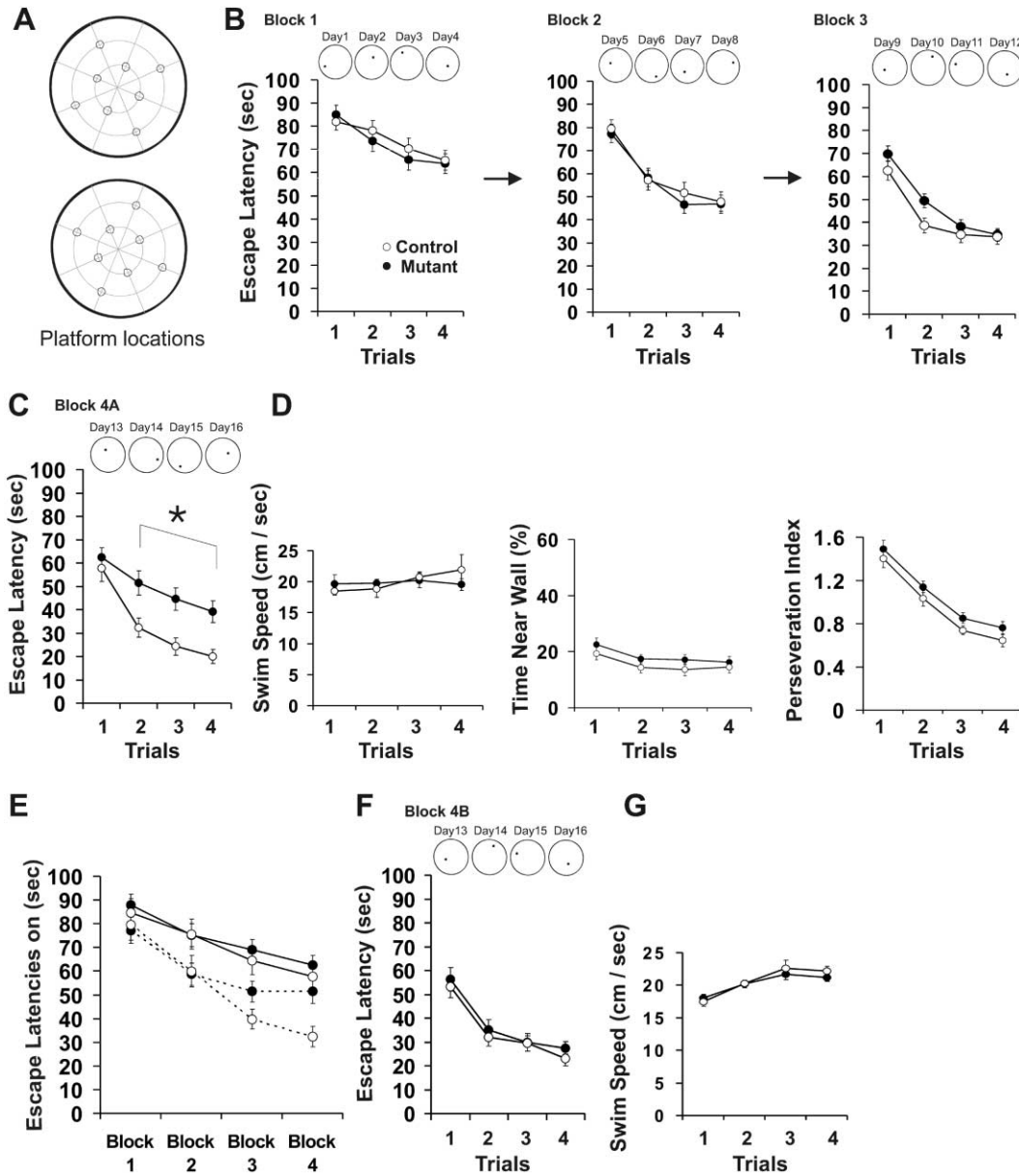


Figure 1. A Delayed Version of Matching-to-Place Task in Water Maze

(A) Sixteen platform locations employed during the 16 day-long training and testing experiments (one location per day) are displayed in two groups to avoid over crowding.

(B) Eighteen- to twenty-two-week-old male CA3-NR1 KO mice (filled circles, $N = 31$) and their fNR1 control littermates (open circles, $N = 25$) were subjected to delayed version of matching-to-place (DMP) training for 12 days, four trials per day with an intertrial interval of 5 min. The training session was subdivided into three blocks (blocks 1–3), each consisting of 4 consecutive days. The escape latencies (s) of mice of one genotype (mutant or control) were averaged for the first, second, third, and fourth trial over 4 days for each block. In this panel and in panels (C) and (F), the locations of platforms employed on each day are indicated at the top.

(C) About a half of the pretrained CA3-NR1 KO mice (filled circles, $N = 16$) and their fNR1 control littermates (open circles, $N = 12$), both randomly chosen, were subjected to 4 more days of trials, each day with a novel platform location (block 4A, days 13–16). Mutants' escape latencies were indistinguishable from those of controls in the first trial (t test, $p = 0.513$) but were significantly longer in subsequent trials (trials 2–4) compared to controls' latencies of the corresponding trials [repeated measure of ANOVA for trials 2–4, genotype effect, $F_{(1,110)} = 16.00$, $*p < 0.001$].

(D) In block 4A, there were no differences between the two genotypes in swim speed [genotype effect, $F_{(1,110)} = 0.06$, $p = 0.8$], in time (%) spent near the pool wall [genotype effect, $F_{(1,110)} = 1.9$, $p = 0.17$], and in perseveration index [genotype effect, $F_{(1,110)} = 2.8$, $p = 0.97$; see Experimental Procedures].

(E) Escape latencies of the first (solid lines) and second (dotted lines) trials during the training and testing were replotted only for those mice that received the block 4A testing (mutants, filled circles, $N = 16$; controls, open circles, $N = 12$). There was no difference in the first trial latencies between the two genotypes, suggesting no effect of the mutation on perseveration. The mutants' second trial latencies dropped in block 2 compared to block 1, but no further shortening was observed in the subsequent blocks. In contrast, controls' second trial latencies continued to drop throughout blocks, resulting in genotype-specific differences.

of the day N+1 recording session, suggesting a certain consolidation process is at work between the two sessions.

The results described in this section suggest that mutants' CA1 place cell activities are less spatially tuned compared to those of control animals, at least during the first 15 min of animals' exploration in a novel region of a familiar environment. This spatial tuning impairment is, however, restored by the time the animals explore the same region on the next day through some sort of consolidation process that occurs during the intervening resting period.

Exposure of Mutant Mice to a Novel Environment Exhibits Poor Spatial Tuning

Similar recordings were performed using a single open-field testing environment. We recorded from CA1 for 20 min as the mutant ($n = 6$) or control mice ($n = 5$) explored a novel open arena and compared the data with those obtained when the animals were returned to the same environment on the following days. The total numbers of cells recorded were 33 and 43 for day 1, 22 and 36 for day 2, and 102 and 102 for day 3 and after, for control and mutant animals, respectively. We found that the controls' place field sizes were compact on day 1 and remained compact throughout the subsequent several days, while the mutants' place field sizes were significantly greater on day 1, became compact on day 2, and remained compact on the subsequent days (Figures 3A and 3B). Similar pattern of abnormalities was found in the integrated firing rate of the mutants (Figures 3C), while neither mean in-field firing rate (Figure 3D) nor several intrinsic properties of complex spiking cells (Figure 3E and 3F) nor the animals' running velocity (Figure 3G) differed between the two genotypes.

We examined whether the mutant spatial tuning developed continuously during the day 1 and day 2 recording sessions or discontinuously between the two sessions. We found that the levels of mutant spatial tuning were discontinuous between the last 10 min of the day 1 recording session (24 cells) and the first 10 min of the day 2 recording session (16 cells) (t test, $p < 0.05$ for integrated firing rate; $p < 0.03$ for place field size). This again suggests that certain consolidation events may be at work during the intermittent resting period in the home cages.

The increases in integrated firing rate, place field size, and place field peak number observed in the mutants upon exposure to novel spatial information suggest that these animals are indeed impaired in the rapid incorporation of novel spatial information into CA1 spatial representations. The observation that the mutant's integrated firing rate, place field size, and place field shape assessed by the number of place field peaks are normal in the familiar track or arena indicate that CA3 NRs

are dispensable for reactivation of established memory representations (Brun et al., 2002). Furthermore, the observation that in mutants, these place cell parameters are normal from the very first moment upon reentry to the now familiar track or arena which was novel 1 day earlier, indicates that these mice are capable of forming memory representations of novel experiences, although they are impaired in doing so rapidly.

Discussion

In the present study, we demonstrated that CA3-NR1 KO mice were impaired in the DMP task when the escape platform was placed at a novel location and the animals were required to rapidly incorporate this novel spatially dependent information into spatial representations of the environment. In contrast, the behavior of the mutant mice was indistinguishable from the control mice when previously experienced locations of the platform were used. These results may relate to the recent finding that rats which received the NR antagonist, AP5, in CA3 displayed short-term memory deficit in a task involving a novel environment (Lee and Kesner, 2002). We also showed that spatial tuning of CA1 place cell activity in the mutant mice was impaired when they visited novel regions of an environment. This impairment was, however, overcome by the following day, showing the correlation between the time courses of behavioral deficits and impaired spatial tuning in the mutant animals.

Correlations of the Behavioral and Physiological Data

Our evaluation of the efficacy and time course of acquisition of novel information required comparison of behavioral and physiological measurements. In the DMP task, novelty lies in the new associations between the escape behavior and spatial locations within the water maze. These spatial behavioral associations were not necessarily made with novel spatial locations; the novelty arises from the specific conjunction of spatial and nonspatial information. Previous studies have shown that place cell response can reflect this type of specific conjunction (Fyhn et al., 2002; Louie, 2002). In those studies, novel spatial behavioral associations, such as stopping at a location that was previously encountered during active locomotion as a neutral site but subsequently marked by nonspatial information, resulted in establishment of distinct place cell representations, with individual place cells expressing the conjunction of both the spatial and nonspatial information present in these tasks. They demonstrated that formation of robust spatially dependent place cell responses reflects acquisition of novel spatial behavioral associations such as those established during active locomotion on the linear

(F) The other half of the pretrained mutants (filled circles, $N = 15$) and fNR1 control littermates (open circles, $N = 13$) were also subjected to 4 more days of trials, but in this case with platform locations that were experienced 4 days earlier, namely during block 3 (block 4B, day 13–16). There were no differences between the two genotypes in escape latencies throughout the trials [$F_{(1,110)} = 0.547$, $p = 0.461$ for trials 2–4; t test, $p = 0.751$ for trial 1].

(G) Swim speeds in block 4B were also indistinguishable between the two genotypes [genotype effect, $F_{(1,110)} = 0.7$, $p = 0.39$]. All the data were expressed as mean \pm SEM.

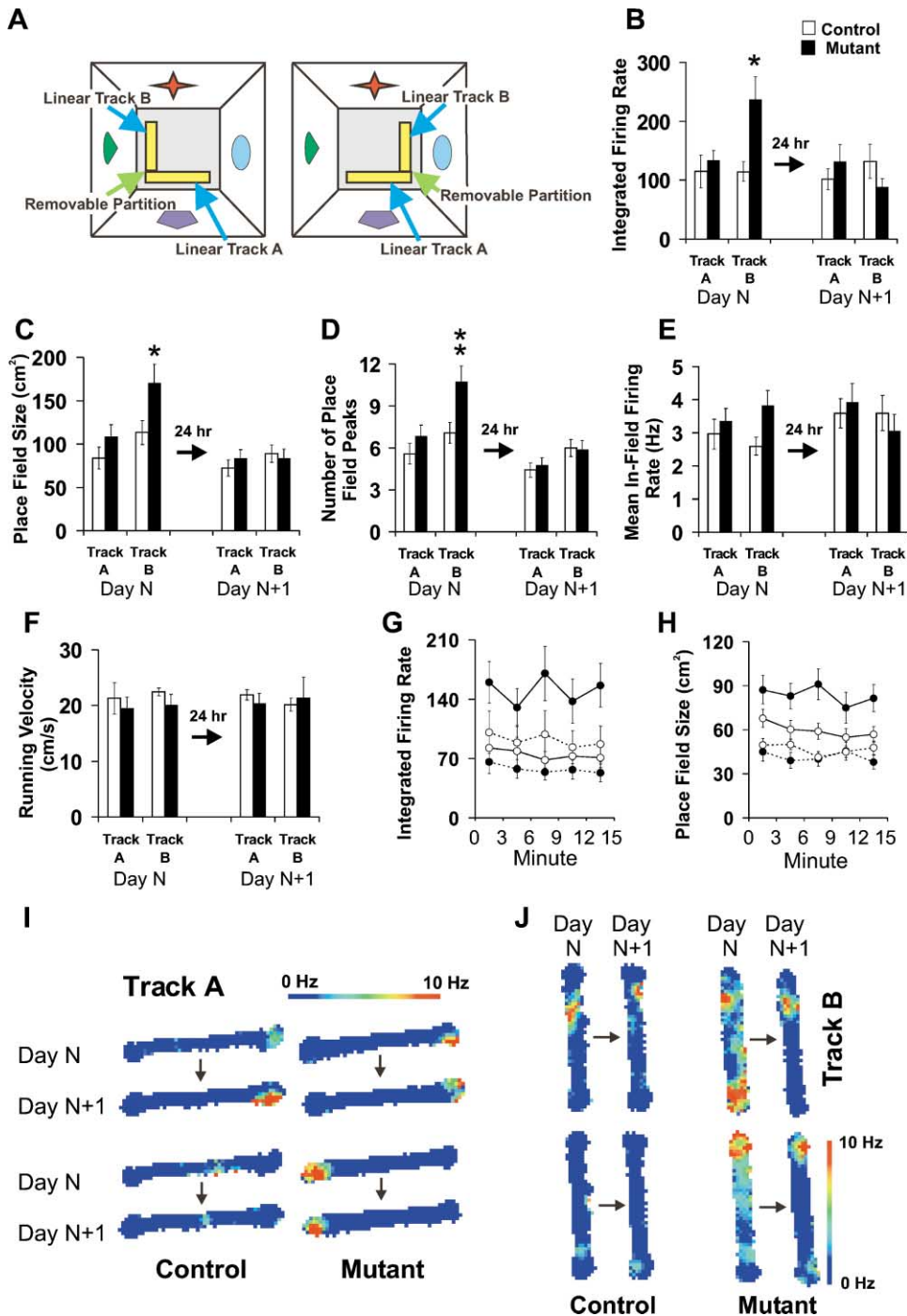


Figure 2. CA1 Place Cell Activities in Novel and Familiar Tracks

(A) Designs of the in vivo tetrode recording space (top-down view). Two linear tracks A and B were placed in an L shape in a square room. The two versions shown were alternated to counterbalance differential effects of the cue versus track configurations. Tracks A and B were used as familiar and novel areas, respectively, on day N. Immediately after the animals entered track B from track A, the partition was reinstalled to prevent them from returning to track A.

(B–E) Open bars, fNR1 controls (N = 3); solid bars, mutants (N = 3). (B) Mutants' integrated firing rates in track B on day N were significantly greater compared to all other combinations of genotype, day and track [genotype effect, $F_{(1,195)} = 3.3$, $p < 0.05$, interaction between genotype and condition, $F_{(3,195)} = 3.8$, $*p < 0.02$]. (C) Mutants' place field sizes in track B on day N were also significantly larger compared to all other combinations of genotype, day, and track [genotype effect, $F_{(1,195)} = 7.1$, $p < 0.009$; interaction between genotype and condition, $F_{(3,195)} = 2.6$, $*p < 0.05$]. (D) The number of place field peaks of the mutants in track B on day N was significantly greater compared to other combinations of day and track, while that of controls was not (Kruskal-Wallis ANOVA for number of place field peaks, $H = 16.7$, $p < 0.01$ for mutant, $H = 7.5$, $p = 0.06$ for control). Mann-Whitney U test comparison gave a highly significant difference between the genotypes in track B on day N ($U = 178.5$, $**p < 0.008$). (E) Mean in-field firing rates did not differ significantly among different combinations of conditions [genotype effect, $F_{(1,195)} = 1.1$, $p = 0.29$, interaction between genotype and condition, $F_{(3,195)} = 1.1$, $p = 0.34$].

Table 1. Properties of CA1 Pyramidal Cells and Interneurons in Familiar Linear Track

	Pyramidal Cells		Interneurons	
	Control (n = 89, D = 17, N = 5)	Mutant (n = 108, D = 18, N = 5)	Control (n = 8, D = 16, N = 5)	Mutant (n = 21, D = 15, N = 5)
Spike width (μ s)	336.1 \pm 4.4	339.7 \pm 4.6	192.0 \pm 9.8	185.5 \pm 5.5
Complex spike index	19.9 \pm 1.4	18.3 \pm 1.1	-0.7 \pm 1.3	0.7 \pm 0.3
Mean firing rate (Hz)	1.79 \pm 0.14	2.03 \pm 0.17	30.4 \pm 5.4	16.2 \pm 3.3 ^a
Peak firing rate (Hz)	9.68 \pm 0.7	9.82 \pm 0.7	ND	ND
Mean in-field firing rate (Hz)	3.35 \pm 0.26	3.28 \pm 0.23	ND	ND
Integrated firing rate [Σ (Hz/pixel)]	119.7 \pm 13.8	128.6 \pm 12.0	ND	ND
Place field size (cm ²)	85.2 \pm 6.4	98.3 \pm 5.8	ND	ND
Burst spike frequency (%)	52.1 \pm 1.3	48.2 \pm 1.4 ^a	ND	ND

All measurements are mean \pm SEM. n, number of cell; D, number of recording session; N, number of animal. ND, not determined.

^aSignificantly different from fNR1 control (Student's t-test, $p < 0.05$).

track as well as during the type of behavior that is expressed in the DMP task.

Ideally, evaluation of place cell response during the acquisition of the memory of novel spatial behavioral contingency in the DMP task would be carried out during the task itself. Unfortunately, the limited and biased nature of spatial sampling inherent in this type of navigational task, and the sensitivity of place cell activity to changes in behavior per se, complicates the interpretation of place cell response under those conditions. While place cell recording has been performed during tasks of this type (Hollup et al., 2001; Fyhn et al., 2002), the desire to examine the time course of novelty effects combined with the constraints imposed by limited sampling, and the difficulty in assessing the direct contribution of behavioral changes dictated the use of a separate protocol to robustly assess the impact of novelty on place cell response. This was achieved through the comparison of place cell activity on two separate linear tracks which allowed repeated measurement of place cell activity during exposure to novelty. Under these conditions that permit the control for sampling and behavioral variation, novelty lies in the new associations between distal cues on the second track. Therefore, in both the DMP and linear track tasks, there is the expectation that the hippocampal spatially dependent place cell representations are altered to incorporate novel associations.

While it remains possible that the mechanisms and dynamics of novel spatial behavioral associations may differ dependent upon the history of exposure to the spatial component of the association, our results suggest a strong correlation in the characteristics and time course of the behavioral and electrophysiological phe-

notypes, and point to the common involvement of CA3 NRs in both conditions.

Impaired Place Cells and Their Recovery

How does the lack of CA3 NRs lead to the decreased spatial specificity of CA1 pyramidal cells in the novel space? CA1 receives inputs both from the layer III stellate cells of entorhinal cortex (EC) via the temporoammonic pathway, and from CA3 pyramidal cells via the Schaffer collaterals. During spatial exploration, cells in the superficial layer of the EC show spatially related responses with significantly lower spatial specificity than that observed in CA3 (Barnes et al., 1990; Quirk et al., 1992; Frank et al., 2000). It has been suggested that these EC cells provide a major source of input to CA1 (Vinogradova, 1975; McNaughton et al., 1989; Brun et al., 2002), particularly during tasks that require encoding of novel information (Sybirska et al., 2000). Furthermore, the activity of the temporoammonic pathway has been shown to regulate the gating of CA1 spikes in EC-hippocampal slices (Remondes and Schuman, 2002). Our result showing that CA1 place fields are less spatially tuned in the mutant animals when new spatial representation is required is consistent with the notion that new spatial context is conveyed via temporoammonic pathway. Moreover, our finding supports a long-standing hypothesis that CA1 network acts as a comparator: detecting novelty or mismatches between actual sensory information from entorhinal cortex and expectation from memory in CA3 (Sokolov, 1963; Vinogradova, 1970; O'Keefe and Nadel, 1978; Gray, 1982; Moser and Paulsen, 2001; Fyhn et al., 2002), while the memory seems to be retrieved from area CA1 itself in the CA3-NR1 KO mice.

(F) Average running velocities during whole recording session did not differ significantly between genotypes under any of the four conditions (Kruskal-Wallis ANOVA for running velocity: $H = 3.1$, $p = 0.38$ for mutant; $H = 0.15$, $p = 0.98$ for control).

(G and H) Time courses of spatial tuning parameters averaged over 3 min bins within individual recording sessions in track B. (G), integrated firing rate; (H), place field size. Filled circles with solid lines, mutants on day N; Open circles with solid lines, controls on day N; Filled circles with dotted lines, mutants on day N+1; Open circles with dotted lines, controls on day N+1. The values for mutants on day N were consistently greater than those of other conditions [repeated measure of ANOVA, $F_{(3,93)} = 5.3$, $p < 0.003$ for integrated firing rate; $F_{(3,93)} = 7.9$, $p < 0.0001$ for place field size]. The values for mutants during the last 3 min on day N were significantly greater than those during the first 3 min on day N+1 (t test, $p < 0.004$ for integrated firing rate; $p < 0.002$ for place field size).

(I and J) Two representative place fields for each genotype recorded from the same complex spiking cells on two consecutive days in track A and track B. They were smoothed for visualization. The track A was familiar to the animals on both day N and day N+1, while the track B was novel on day N and familiar on day N+1. $N > 3$ and $N < 9$. All the data were expressed as mean \pm SEM on a per-cell basis.

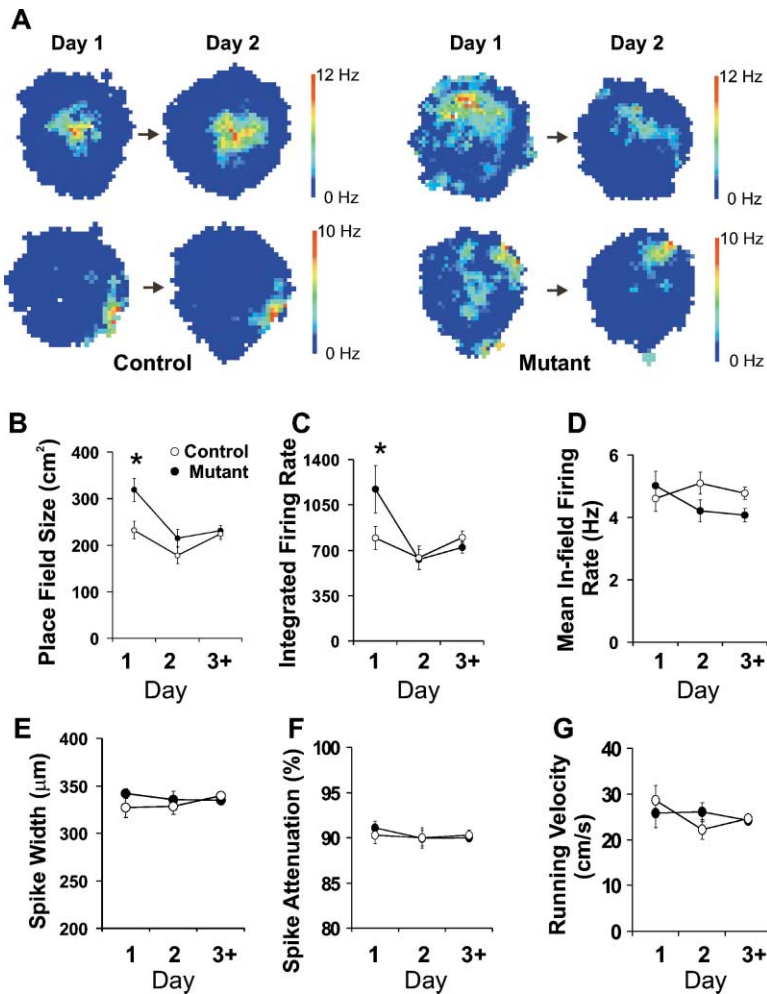


Figure 3. CA1 Place Cell Activities in Novel and Familiar Open Arena

(A) Representative place fields of the same complex spiking cells on day 1 (novel) and day 2 (familiar). They were smoothed for visualization.

(B–F) Day-to-day time course of place cell parameters. (B) The mutants' place field sizes were larger on day 1, but were indistinguishable on day 2 or subsequent days compared to the control animals [two-way ANOVA, interaction between genotype and day, $F_{(2,328)} = 3.1$, $*p < 0.045$]. (C) The mutants' integrated firing rates were greater on day 1, but were indistinguishable on day 2 or subsequent days compared to the control animals [interaction between genotype and day, $F_{(2,328)} = 3.7$, $*p < 0.026$]. (D) Mutants' in-field firing rate was indistinguishable from that of controls on day 1 and on subsequent days [interaction between genotype and day, $F_{(2,328)} = 2.0$, $p = 0.14$]. Mutants' spike width (E) and spike attenuation (F) were also indistinguishable from those of controls throughout the days of recording.

(G) The running velocity of the mutants and controls were also indistinguishable throughout the days. In (B)–(G), open circles are fNR1 controls ($N = 5$) and filled circles are mutants ($N = 6$). All the data were expressed as mean \pm SEM on a per-cell basis.

We hypothesize that during exposure to a novel context, CA1 response is initially driven by the spatially broadly tuned, direct EC input. In control animals, NR function in CA3, perhaps via recurrent connections, allows rapid formation of more spatially specific responses that can then drive correspondingly specific response in CA1 as the input through the Schaffer collaterals comes to dominate or shape the input from the EC. In CA3-NR1 KO mice, CA3 NR ablation leading to the lack of dominant CA3 input (Nakazawa et al., 2003, in press) may result in a more gradual spatial refinement of CA1 place fields implemented by other hippocampal circuit plasticity. In fact, our preliminary recording from CA3 place cells revealed a robust reduction of CA3 activity in the familiar environment, suggesting generally decreased output from CA3 (K.N., unpublished data). CA1 place field enlargement may be due to the prolonged influence of direct EC input to CA1 during this slow spatial refinement process.

Place Cells as Memory Traces

For any cellular or cell ensemble activities to qualify as memory traces, several conditions have to be met. The activities must be formed in an experience-dependent manner and be specific to the acquired information. They must outlast the period in which the animals are

exposed to the information and be reactivatable upon the presentation of recalling cues. A large number of studies have provided evidence for the qualifications of place cells as memory traces at the neuronal ensemble level (O'Keefe and Nadel, 1978; Wilson and McNaughton, 1993, 1994; Eichenbaum et al., 1999; Poucet et al., 2000; Louie and Wilson, 2001; Moser and Paulsen, 2001; Lee and Wilson, 2002). However, among these efforts, establishing the experience dependency has been most difficult. The reason is that place cells are formed so rapidly when an animal is exposed to a novel space or situation that it is difficult to catch them in the midst of making. Indeed, our data shown in Figures 2G and 2H confirmed a rapid formation of high spatial tuning place cells upon entry of control animals into the novel track, which prevented our capturing their earlier state. In contrast, the NR knockout in CA3 slowed down the process of place cell formation drastically and allowed us to catch them in an immature state (Figure 2). Although we do not know the exact time course nor the mechanism of the maturation of these place cells, our data showed that they do eventually acquire normal high spatial tuning by the time the animals reenter the space 1 day later. Assuming that these immature place cells observed in the mutant mice represent an early state of place cells in normal animals, they may provide insights into the

mechanisms that contribute to the formation of place cells. Our having captured these immature place cells reinforces evidence for the memory trace role of place cells.

Another intriguing aspect of the mutants' place cells is the rare dissociation of spatial representation and spatial encoding on day 1 (or day N). Since spatial representation is normal on day 2 (or day N+1), the animals must have encoded a sufficient amount of spatial information on day 1 that permits the subsequent formation of robust spatial representation. How this improvement of spatial representation can be achieved without a continued supply of external cues is a matter of great interest for future studies.

Conclusions

Our results have demonstrated that a specific ablation of functional elements within the hippocampal system can selectively alter the time course of acquisition of novel information and the formation of spatial memory representations. In particular, the present results provide evidence for a crucial role of CA3 NRs in the rapid formation of memory representations in CA1 as a specific pattern of place cell activities and for the rapid acquisition of one-time experience or one-trial memory. Given our previous findings demonstrating the requirement of CA3 NRs in associative memory recall, NRs expressed in the same CA3 pyramidal cells seem to play multiple roles in the hippocampus-dependent mnemonic process.

Experimental Procedures

Animals

All the experiments were carried out using male CA3-NR1 KO mice (strain C57BL/6) of 18–20 weeks of age and their floxed-NR1 (fNR1; control) littermates. By these ages, NR proteins were abolished in the mutant mice specifically in CA3 pyramidal cells (Nakazawa et al., 2002). Two to five mice were housed per cage under the conditions of a 12 hr light/dark cycle and ad libitum access to food and water. All the experiments were conducted by operators who were blind to the genotypes of the mice used. All procedures relating to animal care and treatment conformed to the Institutional and NIH guidelines.

A Delayed Matching-to-Place Task for Mice in Water Maze

The task protocol used was similar to that reported for rats (Steele and Morris, 1999). The water maze (1.6 m in diameter) and its surrounding set up was as previously described (Nakazawa et al., 2002). The experiments were conducted in a series of three phases: (1) pretraining on a cued platform task; (2) training on the delayed matching-to-place (DMP) task; and (3) DMP testing. Throughout training and testing phases, the single escape platform (10 cm in diameter) was located 1.5 cm below the surface of the water, on successive days, in 1 of 16 separate places within the pool, located symmetrically near the side wall (8 places, outer ring) or near the center (8 places, inner ring) (Figure 1A). The centers of these 16 different places were at least 26.6 cm away from the wall and separated from each other by at least 20 cm. Throughout the three behavioral phases, the locations of the platform were altered from one day to the next in a pseudo-random fashion, and the use of two locations in the same quadrant on 2 successive days was avoided. Throughout the experimental phases, the trials began at N, E, S, and W in a pseudo-random sequence with mice facing the pool wall. The experiment began with pretraining with a cued platform, which lasted for 3 days, four trials per day, with the location of the platform altered each day but marked by an attached flag (10 cm high). The flag was removed the next day (day 1) to begin DMP

training, which lasted for 12 days (days 1–12), each day with a novel location of the hidden platform. Four trials were given per day with an intertrial interval of 5 min and the four release sites (N, E, S, and W) used in a different order each day. Between trials, mice were kept in home cages under a warming light. In each trial, a mouse was allowed to search the platform for up to 120 s. In the first trial of a day, if an animal found the platform, it was allowed to stay on it for 30 s prior to transfer to the home cage. Otherwise, the search was interrupted and the animal was gently hand picked, placed on the platform, and allowed to stay there for 30 s. In the subsequent trials (trials 2–4), the same procedure was followed except that the platform-stay period was reduced to 15 s. After completion of the training phase, the mice were randomly divided into two groups for the DMP testing phase, which lasted 4 days (days 13–16). One group of mice continued to receive the DMP task, four trials per day with a new platform location each day, and the escape latency (time to reach platform), the swimming speed, time spent in an area near the wall (up to 18% radius from the wall), and averaged perseveration index were recorded. The perseveration index was defined as a ratio of averaged distance from the today's platform center to that from the previous day's platform center. The second group of mice also underwent 4 more days of the DMP task, but the platform location was the same as that experienced 4 days earlier (i.e., the platform location on day 13 was the same as that on day 9, the platform location on day 14 was the same as that on day 10, and so on). For these experiments, the mouse release sites and their order were also the same as those used 4 days earlier. The data from obvious "floaters" (less than 10% of all the mice tested) were excluded.

In Vivo Tetrode Recording

Experimental procedure for in vivo tetrode recording was previously described (Nakazawa et al., 2002). Briefly, male mice (18–24 weeks of age) were implanted with a microdrive array consisting of six independently adjustable tetrodes (stereotaxic coordinates from bregma: 2.0 mm lateral; 1.8 mm posterior). As animals randomly explored linear tracks (77 × 7 cm for each track; Figure 2A) or a low-walled open field arena (50 cm in diameter) placed near the center of a black-curtained rectangular chamber, extracellular action potentials in CA1 cell layers were recorded while the animal's position was tracked using a pair of infrared diodes above the animal's head. Following data acquisition, action potentials were assigned to individual cells based on a spike's relative amplitudes across the four recording wires of a tetrode. Putative pyramidal cells were defined as cells with relatively broad waveforms (peak to trough width > 300 μ s) and a strong tendency to produce complex spike bursts (complex spike index [CSI] > 3%; defined as the percentage above chance of spikes with first lag interspike intervals that fall between 2 and 15 ms and whose second spike is smaller than the first), whereas putative interneurons had relatively narrow waveforms (peak to trough width < 240 μ s) and few if any complex spike bursts (CSI < 3%).

Firing properties of CA1 pyramidal cells were characterized using three measures: (1) mean firing rate (Hz), (2) peak firing rate (Hz), and (3) burst spike frequency (%)—percentage of the number of spikes involved in a burst relative to the total number of spikes produced by the cell. Those pyramidal cells with mean firing rates over 0.5 Hz, indicating spatial responsiveness during running, were selected for place field analysis. Individual pixels were excluded if the animal's total occupancy time within that pixel did not exceed 0.5 s. Spatial tuning of individual cells was assessed by four measures: (1) mean in-field firing rate, defined as the average firing rate across all pixels with mean pixel firing rate exceeding 10% of the cell's peak firing rate; (2) integrated firing rate, defined as the sum of mean pixel firing rates across pixels; (3) place field size, defined as the area of pixels whose firing rate exceeding 10% of the peak firing rate; and (4) number of place field peaks (Figure 2D). Mean pixel firing rate was calculated by dividing the total number of spikes detected at a pixel location by the total occupancy time within that pixel. Place field peak number analysis was carried out by analyzing the individual firing rate maps of each place cell in the linear track. Significant peaks were counted only if their firing rates were above 20% of the cell's peak firing rate. Peaks were identified as local

maxima (locations with all surrounding pixels having lower values, Matlab image processing toolbox). As a measure of the intrinsic properties of pyramidal cells, we determined spike width (peak to trough width), and the degree of amplitude attenuation within a three spike burst. For linear track data, analysis was restricted to periods during which the animal's running speed exceeded 2.0 cm/s. Place cell examples shown were identified as the same cells with waveform profiles that remained stable across the 2 days' recording sessions (Figures 2I, 2J, and 3A). The inherently more variable behavior in the open field precluded analysis of multiple peaks.

Novelty experiment in linear tracks in Figure 2 was conducted as follows. The familiarization of track A consisted of at least two sessions of running in the track A, each session lasting for 15 min and taking place on an independent day within the maximum total period of 8 days. Immediately after the completion of the last 15 min familiarization session on day N, animals were allowed to enter the novel track B by a temporary removal of the partition and to explore it for 15 min. The activities of complex spiking cells in CA1 were recorded during the entire 30 min period before and after the track shift. The animals were then transferred to home cages and returned 1 day later (i.e., day N+1) to the track A followed by track B and the recording conducted as before.

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