

Evidence for a Relationship Between Place-Cell Spatial Firing and Spatial Memory Performance

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ABSTRACT: The rat hippocampus contains place cells whose firing is location-specific. Although many properties of place cells have been uncovered, little is known about their actual contribution to the animal's spatial performance. In this study, we addressed this issue by recording place cells while rats solved a continuous spatial alternation task in which they had to alternate between the two arms of a Y-maze to get a food reward in the third (goal) arm. By manipulating the information available to the animals, we induced the cells to establish their fields in locations that were out of register relative to their standard position, thus making them inconsistent with the learned spatial task. When this happened, the rats' performance in the alternation task was markedly decreased. In addition, the nature of the behavioral errors during inconsistent field placements also changed dramatically in a way that was highly indicative of the rats' spatial disorientation. These results suggest that there is a functional relationship between the spatial firing patterns of place cells and the spatial behavior of the rat, thus strengthening the idea that these cells are part of a navigational system. *Hippocampus* 2001;11:377–390. © 2001 Wiley-Liss, Inc.

KEY WORDS: hippocampus; unit recordings; place cells; spatial learning; spatial memory; alternation behavior

INTRODUCTION

The ability of rats to flexibly navigate in space suggests that they can form representations of their environment (Gallistel, 1990; Poucet, 1993). An influential theory about how such representations are implemented in the brain is the cognitive map theory, based on the existence of hippocampal place cells (O'Keefe and Nadel, 1978). A place cell is functionally characterized by location-specific firing and discharges only when the rat's head is in a continuous, simply shaped part of the environment called the "firing field" (O'Keefe and Dostrovsky, 1971; O'Keefe, 1979). Since fields are distributed over the surface of the apparatus in an overlapping fashion (Muller, 1996), the place-cell population can be viewed as a neural system that provides a representation of the current environment. A central question is whether this representation indeed is used for spatial memory and navigation.

In support of the cognitive map theory, lesion work shows that destruction of the hippocampus disrupts the performance of rodents in spatial tasks (Jarrard, 1978, 1983; Morris et al., 1982, 1990; Save et al., 1992; Save and Poucet, 2000). This evidence, however, does not directly implicate the place cells. Thus, while the hippocampus is essential for successful navigation behavior (Nadel, 1991), there is as yet little understanding of exactly how the hippocampal place cells contribute to this function. So far, the only bridge from place cells to the navigational behavior of the whole animal comes from a single study (O'Keefe and Speakman, 1987). In that experiment, rats used environmental cues to go to one arm of a four-arm radial maze designated as a goal arm from any of the three other arms. On infrequent trials, no cues were presented. Nevertheless, animals chose one arm as a goal. Under these ambiguous circumstances, place cell firing fields remained in register with the rat's random choice of goal, suggesting that place cell activity and spatial behavior are coupled. However, the discrete nature of the trials in O'Keefe and Speakman (1987) precluded numerical characterization of spatial firing patterns. Also, only a small number of such trials were done. Lastly, the question arises as to the across-task generality of this result.

The present study was aimed at further documenting the existence of a functional relationship between the activity of hippocampal place cells and the animal's performance of a spatial alternation task, known to depend on the integrity of the hippocampus (e.g., Stevens and Cowey, 1973). Briefly, we reasoned that if the hippocampal place-cell system provides position information useful for performing the task, then information that is not in register with the animal's spatial task should be accompanied by incorrect spatial behavior. Also, if the positional signals are in register with the animal's spatial task, one would expect correct spatial performance. Finding such outcomes would certainly add strong support to the hippocampal mapping function, while finding contrary evidence would put the theory in great difficulty.

In the present experiment, rats were trained to perform a continuous spatial alternation task in a Y-shaped maze in which the only available cue was a prominent white

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cue card. Once the rats performed well in the presence of the cue (“standard condition”), place cells were recorded in several sessions after the cue was manipulated by rotating and removing it. These manipulations were designed with the expectation that, in some sessions, the angular positions of firing fields would be out of register with the positions seen in the standard condition. Our purpose was to determine if the rats’ performance was worse when fields were out of register relative to their standard position. Based on this reasoning, our results suggest that there is an intimate relationship between purposive spatial behavior and the activity patterns of hippocampal place cells.

MATERIALS AND METHODS

Subjects

Twelve Long-Evans male rats (Janvier, St.-Berthevin, France) weighing between 300–350 g were used. They were housed one per cage in a room on a natural light-dark cycle (temperature, $20 \pm 2^\circ\text{C}$). They had water ad lib during all phases of the experiment. Upon receipt, the rats were handled daily for 2 weeks prior to presurgery training. To permit assessment of both the rats’ spatial performance and positional firing rates everywhere in the apparatus, the rats were food-deprived to 80–85% of ad lib body weight and trained in a continuous spatial alternation task in a Y-maze apparatus (see below) for 3 weeks prior to electrode implantation. Briefly, the task required the rat to visit two arms of the Y-maze in alternation with the third arm, where they could get a 20-mg food pellet. After training was complete, the rat visited all parts of the Y-maze many times per recording session, so that firing activity could be sampled everywhere in the apparatus.

Apparatus

The apparatus was a Y-shaped plywood maze elevated 50 cm above the floor. It consisted of a circular central platform (30 cm in diameter) and three arms (36 cm long, 10 cm wide) bound by 1-cm raised stainless steel edges. The angle between each pair of arms was 120° . A small remote-controlled food dispenser was attached to the end of each arm. To prevent the rat from accessing the food dispensers, a transparent Plexiglas vertical barrier (20 cm wide, 30 cm high) was inserted between each arm end and the corresponding food container. A small hole at the base of the barrier allowed 20-mg Noyes food pellets to be ejected onto the arm end. Except for the barriers, all parts of the maze were painted flat black. The maze was visually isolated from the rest of the laboratory by a concentrically placed cylindrical curtain 250 cm in diameter and height. Within the curtained area, illumination was provided by three 25-W light bulbs above the maze, so that each light bulb was exactly between two arms. The floor of the laboratory was covered entirely with bed sheets, so that the cables connected to the food dispensers were not visible. A radio tuned to an FM station was fixed above the central platform of the maze, producing back-

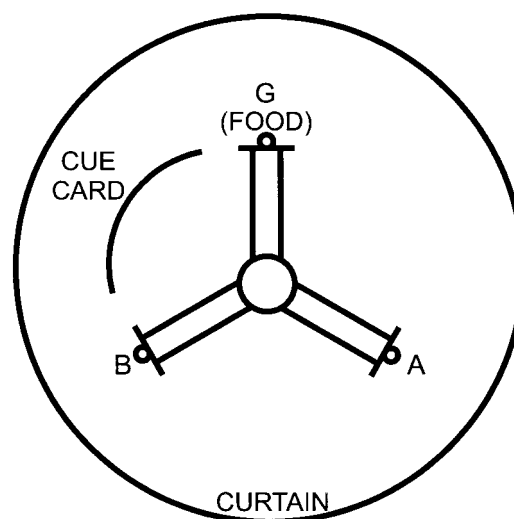


FIGURE 1. Schematic representation of experimental apparatus. Cue card is shown as curved line. Circles at end of each arm indicate food dispensers. Food rewards were delivered only at the end of arm G, if the rat had alternated visits to the other two arms A and B.

ground noise >70 dB to mask noncontrolled directional sounds during all phases of the study.

A single visual landmark was used to mimic the appearance of the white cue card used in earlier cylinder experiments (Muller et al., 1987; Cressant et al., 1997; Save et al., 2000). This was a piece of white cardboard (70 cm wide, 50 cm high), bent to form the arc of a circle 132 cm in diameter. The card stood on a pole that was located 36 cm from the outer edge of the center platform and between two arms, so that its center was exactly 60° away from the end of each arm. The lower edge of the card was at the same height as the maze. When present, the card was always at the same position relative to the arm where food was available (Fig. 1).

The experimenter was in an adjacent room that contained the unit recording setup, the computer, a TV monitor to watch the rat moving in the maze in the experimental room, and a panel that controlled the food dispensers.

Behavioral Procedures

Behavioral training was done before electrode implantation. Each rat was first pretrained to retrieve 20-mg food pellets from the end of all arms. The rat was placed on the center platform and allowed to freely explore all arms. Each food dispenser was randomly activated (overall mean delivery rate, 3 pellets per min). Since the arm where a food pellet was available was unpredictable, the rat learned to run almost constantly to the end of all arms. Three 15-min pretraining sessions were done daily for 3 days.

Training in the continuous spatial alternation task began on day 4. As during pretraining, the rat was first placed on the center platform and allowed to freely move on the apparatus. Now, however, only one arm (arbitrarily labeled “G” for goal) was associated with delivery of food rewards. The goal arm was consistently at the same location relative to the card. Similarly, the goal arm was consistently located at the same place in the laboratory during training.

To get a food pellet on the goal arm, the rat had to visit the other two arms, A and B, in alternation: after a visit to arm G, the rat had to visit the arm not immediately visited previously. Thus, a typical sequence of correct choices was A-G-B-G-A-G-B (underline indicates reward). If the rat visited the correct alternation arm, it could get a single food pellet in arm G. Delivery of the reward required the rat to return to the goal arm, i.e., the food dispenser was switched on only after the rat had fully entered arm G. The rat was not rewarded upon its return to arm G if it repeatedly visited the same alternation arm (e.g., A-G-A-G). After an incorrect choice of alternation arm, it was allowed to self-correct by choosing the other alternation arm on its next choice before going to the goal arm (e.g., the rat would be rewarded at the end of the sequence G-A-G-A-B-G, but not after G-A-G-A-B-A-G). Although food was never delivered in the two alternation arms, it was nevertheless present in the food dispensers which were occasionally turned on. However, an obstruction block in the container prevented the pellet from being released on the arm. To further equate the arms, the maze (i.e., arms and food dispensers) was disassembled daily and its components were shuffled. Last, the maze was cleaned between each rat. These procedures were adopted to exclude possible effects of differential odors and sounds associated with each arm.

An arm entry was registered if the rat placed its four paws beyond a line drawn on the TV screen 5 cm from the center platform (i.e., its head was less than ≈ 15 cm from the arm end). Three 15-min presurgery training sessions were done daily for 3 weeks. At the end of this period, the average number of visits to arms was about 100 per session, and performance scores were 85–95% correct.

Surgery

Surgery and postsurgical care were conducted according to institutional guidelines. Surgery was done after training in the continuous spatial alternation task was complete. An injection of 0.3 ml of atropine was given to prevent respiratory distress. Next, rats were anesthetized with pentobarbital (45 mg/kg) and placed in a Kopf stereotaxic apparatus. After a midline incision of the scalp, the skin and the muscles were retracted and holes were drilled in the skull at appropriate locations. A movable array of 10 25- μ m electrode wires (Kubie, 1984) was stereotaxically implanted in the dorsal hippocampus at the following stereotaxic coordinates: 3.8 mm posterior and 3.0 mm lateral to bregma, and 1.5 mm below the dura (Paxinos and Watson, 1986). Miniature screws were placed over the right and left frontal cortex and the left cerebellar hemisphere. To improve stability, an additional screw with the head ground to a T-shape was lowered upside down into another hole in the left parietal bone and turned 90° before being locked in place with a nut. For protection from the dental cement, sterile petroleum jelly was applied to the exposed brain surface and the guide tubing of the electrode array. Dental cement was applied over the jelly and around the guide tubing. The exposed skull was covered with dental resin cement. The screws and nut were then embedded in dental cement, and the bottom of the three drive screws assembly was cemented to the skull.

At completion of the experiment, animals were injected with a lethal dose of pentobarbital and perfused intracardially with 0.9% saline followed by 10% formalin. Just prior to death, positive current (15 μ A for 30 s) was passed through one of the microwires to deposit iron that could be visualized following reaction with potassium ferrocyanide (Prussian blue). The brains were removed and stored for 1 day in 3% ferrocyanide. Later, coronal sections 40 μ m in thickness were taken. Every fifth section was stained with cresyl violet for verification of electrode placements.

Recording Methods

Beginning 1 week after surgery, the activity from each microwire was screened daily while the rat was retrained. The electrodes were lowered over a period of several weeks while searching for unit waveforms of sufficient amplitude to be isolated. Since several days were necessary before reaching the first recordable cells in the hippocampus, enough time passed for the performance at recording time to be similar to that observed preoperatively. Once a unit was isolated, it was recorded during several successive sessions (see protocol below). Such multiple sessions are possible because the same cell can be reliably recorded for days or even weeks (Muller et al., 1987). This makes it possible to compare the firing of an individual cell after the environment has been changed many times.

Screening and recording were done with a cable attached at one end to a commutator that allowed the rat to turn freely. The other end of the cable was connected to a light-emitting diode (LED) for tracking the rat's head position, a head stage with a field effect transistor amplifier (FET) for each wire, and a connector that mated with the electrode connector cemented to the rat's skull. The FETs were used to amplify signals before they were led to the commutator via the cable. The fixed side of the commutator was connected to a distribution panel. From the panel, the desired signals were amplified 10,000-fold with low-noise differential amplifiers, band-pass filtered from 0.3–10 kHz, and sent to a 250-kHz analog-to-digital (A/D) board in an 80486 computer. The data acquisition system (DataWave, Longmont, CO) recorded a 1-ms sample of the data that exceeded an experimenter-defined threshold (sampling frequency, 32 kHz). Before the initial recording session, spike discharges of single units were separated by using on-line clustering software (DataWave Discovery) so as to facilitate further off-line separation. Briefly, scatterplots of waveform parameters (e.g., spike height, spike amplitude, and spike width) were generated from the signals emanating from cells simultaneously recorded on the same channel.

The head position of the rat was tracked by locating the LED that was secured to the animal head stage. The LED was positioned on the midline ≈ 1 cm above the head and 1 cm behind the head stage. This placement was designed to minimize electrical noise induced by contacts of the rat's head with the transparent barrier while the animal was getting food pellets. The LED was tracked with a TV-based digital spot follower that received the RGB signals from a CCD color camera fixed to the ceiling of the experimental room above the center of the Y-maze. The LED was detected in a grid of 32 \times 32 square regions (pixels) 35 mm on a side. The X and Y coordinates were stored at 50 Hz (TV frame rate).

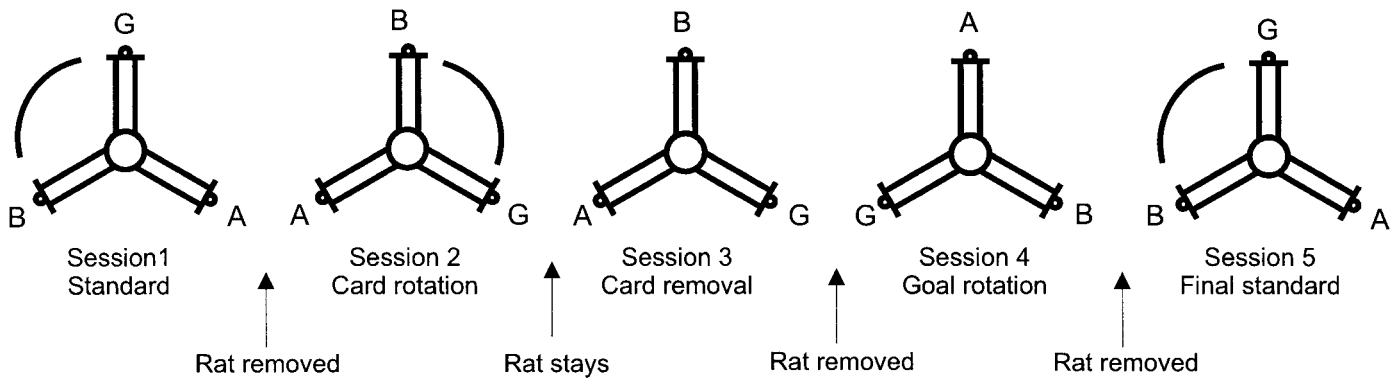


FIGURE 2. Schematic representation of protocol. Five sessions were run in succession. Between sessions 1 and 2, card and goal arm were rotated 120° CW. Between sessions 2 and 3, card was removed. Between sessions 3 and 4, goal arm was rotated 120° CW relative to its

position during session 3 (card was not returned). Session 5 was a final standard session similar to session 1. Except between sessions 2 and 3, the rat was returned to its home cage between sessions. G indicates goal arm, and A and B the two alternating arms.

Testing Protocol

The electrodes in each rat were checked three times a day while the rat performed the task. This ensured that asymptotic performance was maintained even if no recording was made for several days. If no recordable cell could be isolated, the electrode bundle was advanced 25–50 μm . Cells selected for recording were well-discriminated complex-spike cells that showed clear location-specific firing. Once a waveform of sufficient amplitude ($>100 \mu\text{V}$ with a background noise level $<30 \mu\text{V}$) was well-isolated, five 12-min recording sessions were run consecutively. Before each session, the waveform and firing pattern were briefly inspected to check for constancy.

The sequence of five recording sessions is illustrated in Figure 2. During all sessions, the rat performed the usual alternation task. The first *standard* session was made with the Y-maze and card in their standard positions relative to the laboratory.

Following the standard session, the rat was disconnected and returned to its home cage. The maze was cleaned, and both the card and experimenter-defined position of the goal arm were rotated 120° clockwise around the center platform. The rat was brought back to the apparatus and connected to the cable, and a second session was made (*card rotation*).

When the card rotation session was finished, the experimenter entered the experimental room, cleaned the maze with a wet towel, and removed the card while the rat was in the maze. Care was taken not to disturb the animal during this phase. The experimenter then began the third (*card removal*) session. Although the card was removed, the rat could still perform the task using two types of information: 1) internal memory of the goal location (since the goal arm was not changed between sessions 2 and 3) combined with the use of motion-related idiothetic cues, and 2) external feedback provided by the availability of food in only one arm.

Following session 3, the rat was returned to its home cage. The maze was cleaned. The rat was then brought back to the apparatus and connected to the cable, and a fourth (*goal rotation*) session was made, with the experimenter-defined goal arm position rotated by 120° clockwise relative to session 3. Since the card was *not* replaced,

the only cue available to help the rat was the location where rewards were delivered (i.e., the goal arm).

After session 4, the rat was disconnected and returned to its home cage. The maze was cleaned and the card was put back. The card and goal arm positions were as in the first session. This *final standard* session was made to test if the original firing fields were still present at the same locations in the maze as during the initial standard session, regardless of whatever occurred during sessions 2–4.

The rationale for this protocol was to progressively restrict the amount of information available to the rat. Our expectation was that, by reducing information across sessions, we could induce the positional signals of place cells to be out of register with the spatial task that the animal had to perform. Thus, while information was complete in sessions 1 and 5, session 2 eliminated the possibility of the rat using background (uncontrolled) cues emanating from the laboratory. During session 3, the rat could not use the controlled visual cue but instead could use information from its recent experience in combination with the goal-related spatial information. Session 4 restricted useful information to the positional feedback provided by food delivery at a consistent location in the maze.

Data Presentation and Analyses

Unit analyses

The first step in off-line analyses was to refine the cluster boundaries for waveforms that had been determined on-line. The most useful parameters were spike height and amplitude, spike width, and A/D values at selected points of the waveforms. Once single units were well-separated, positional firing rate distributions were calculated. The total time the light was detected in each pixel (dwell time), and the total number of spikes in each pixel was accumulated for the session duration (12 min). The rate in each pixel was the number of spikes divided by the dwell time. For each session, a firing rate map was constructed, using the method described by Muller et al. (1987) to visualize the positional firing rate distribution. In such maps, pixels in which no spikes occurred

during the whole session are displayed as white. The highest firing rate is coded as black, and intermediate rates are shown as light gray, medium gray, and dark gray from low to high. The values used as boundaries between gray categories were determined for the map of the first session recorded for a given cell. To permit comparisons among positional firing distributions across several sessions for a cell, the rate categories used for subsequent sessions were the same as for the first session.

A firing field was defined as a set of at least four pixels contiguous by an edge, and with firing rate above the grand mean rate. Visual assessment of field positions was complemented by numerical estimations of field rotation between session pairs. Pixel-by-pixel cross-correlations were calculated as the positional firing patterns, for the first and second sessions in the pair were rotated in 60° steps in opposite directions (resulting in 120° rotations of the firing patterns relative to each other, i.e., the angular separation between arms). Thus, correlations were calculated at rotations of 0°, 120° clockwise (CW), and 120° counterclockwise (CCW). The rotation associated with the highest correlation (R_{Max}) was taken as the rotation of the field between the two sessions.

Behavioral analyses

For each recording session, the number and sequence of arms visited were recorded. The sequence of arms was further analyzed to score the type and number of errors. Two types of errors were considered: alternation errors and orientation errors. Alternation errors were the most common errors during asymptotic performance. They presumably reflect incorrect storage of the last visited arm in short-term (working) memory. Such errors were scored when the rat repeated a visit to the same alternation arm. For example, in the sequence G-A-G-A-G-B-G, the second (incorrect) visit to A was scored as an alternation error (visits are underlined to indicate a reward and in bold to indicate an error). Since the rat was allowed to self-correct after alternation errors, the first incorrect choice was scored as an alternation error, but the second choice was scored as correct provided the rat returned to the goal arm immediately afterwards. For instance, the rat would be scored one alternation error but would still get the reward after the sequence G-A-G-A-B-G.

All other types of errors (perseverations and failures to return to the goal) presumably reflect a deeper disturbance of the rat's behavior than alternation errors. They were all classified as "orientation errors." Such errors were scored when the rat visited the same alternation arm more than twice (e.g., G-A-G-A-G-A-G). In this sequence, the second (incorrect) visit to A was scored as an alternation error, while the third was scored as a perseveration (hence, an orientation error). Orientation errors were also scored when the rat failed to follow the basic rule of returning to the goal arm after a visit to an alternation arm. For instance, the sequence G-B-G-A-B-G includes a correct visit to A but also an incorrect visit to B, a departure from the learned rule. Although it was generally easy to judge when the behavior was back in register with the alternation rule, the rats sometimes produced very complex sequences of errors, making it difficult to know when to reward it. In those instances, we considered, as a principle, that the alternation was reset

on visits to G. For example, in the sequence A-B-A-B-A-B-G-A-G, the first visit to G was considered the start of a new alternation sequence, and the rat was rewarded upon returning to G after a visit to either A or B.

Errors were counted as incorrectly chosen arms (i.e., choices of arms that were either alternation or orientation errors). Because the rat was allowed to self-correct and because of the complex nature of the task, random behavior was not expected at 50%. Monte Carlo simulations (1,000 repetitions of 100 random choices) revealed that a rat behaving fully randomly on the task would be scored 55% correct, given our method for counting errors. For each session, the percentage of errors (number of incorrect choices \times 100/total number of choices) was calculated. The distribution of errors was analyzed by dividing the number of alternation or orientation errors by the total number of errors.

The data (or their arcsine transform for percentages) were analyzed using repeated measures analyses of variance (VAR3 program; Rouanet and Lépine, 1970). In addition to the effects of the main factors and their interactions, this program allows for planned contrasts and detailed analyses of the significant effects. For simple paired comparisons, Student's *t*-tests were conducted. Finally, chi-square analyses were used for comparison of frequencies.

RESULTS

General Considerations

Because the study required stable place-cell recordings while the rat performed the behavioral task, several animals had to be eliminated from the data analysis. Four rats were removed from the analysis because they lost their head stage before any recording session, or because no useful signal was ever detected from their electrodes. The data are thus based on 47 complex-spike cells from the remaining eight rats. To be included in the sample set, cells had to satisfy the following criteria: 1) their spike amplitude had to be larger than 100 μ V (\approx 3 times the background noise); 2) they had to have a clearly identifiable field in the maze; and 3) the field location had not to be centered in the maze (so that it was possible to measure its angular location).

Of the 47 recorded place cells, 41 were recorded in 21 complete sequences of five sessions. Six other cells were recorded in four incomplete sequences. In these instances, recording had to be stopped either because the waveforms were lost or because they changed so much that we could not be confident it was the same unit. Changes of this type usually occurred while connecting or disconnecting the rat from the recording cable (e.g., after session 4 in two cases). In two cases, however, large changes happened after session 2 when the card was removed.

Below, we will first show the effects of cue manipulations on cell firing, before addressing the relationships between changes in cell firing patterns and changes in behavior. Our method for assessing between-session changes in cell firing was first to visually inspect the associated rate maps and then to confirm our judgment by

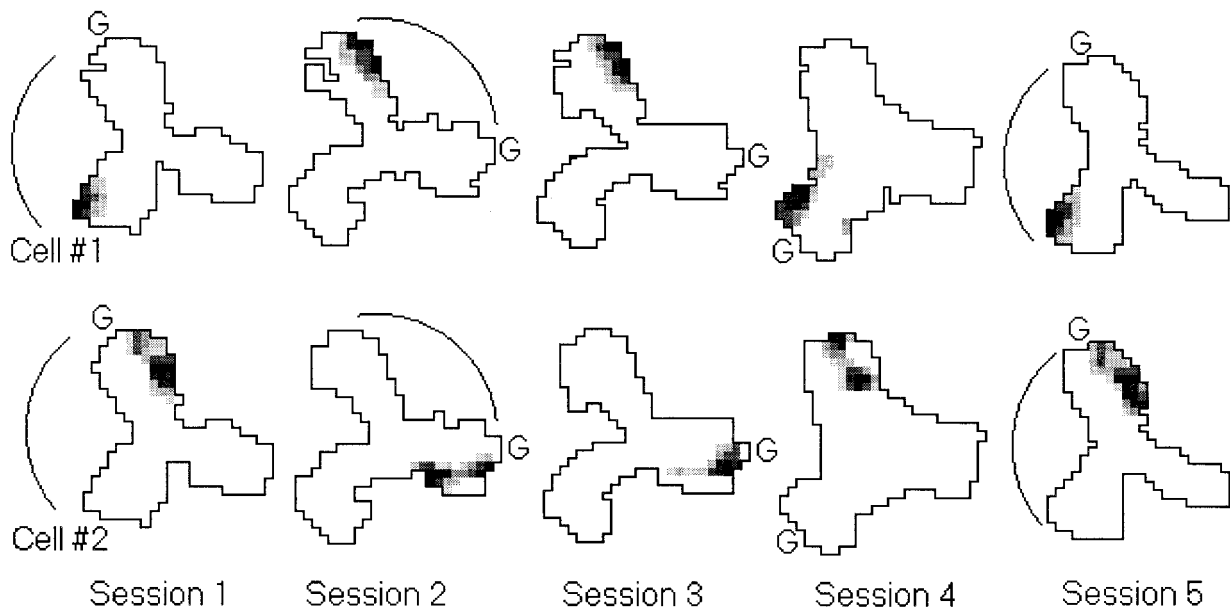


FIGURE 3. Firing rate maps of two simultaneously recorded place cells during five successive sessions of a whole sequence. Fields of both cell 1 (top row) and cell 2 (bottom row) behaved similarly across sessions. These were controlled by card rotation (session 2), and were stable when the card was removed (session 3). They incor-

rectly rotated back to the standard position after the 120° CW goal rotation (session 4), and finally were observed to stay in the standard position when the card was returned (session 5). For clarity, only the principal field of each cell (in gray) is shown. G, goal arm.

calculating angular correlations (see Materials and Methods). For such calculations, only one cell among those simultaneously recorded was used for each session pair, to avoid bias due to recording varying number of cells in different session pairs. In addition, in all instances but one, there was equivalent rotation of the fields of all simultaneously recorded cells (e.g., see Fig. 3). This observation was confirmed by the large correlations found between spatial firing patterns for the same rotation angle (Table 1). This result suggests that the entire representation acts as a unit. Therefore, both for the sake of simplicity and to avoid biased sampling, changes associated with cue manipulations will be presented below by considering only one representative cell for each session pair. The selected cell was the one that had the greatest spatial coherence (whose field was the smoothest) during the first standard session. The results of angular correlation analyses are summarized in Table 2.

Effects of Cue Manipulations on Firing Fields

Card rotation

Card rotation was made on 25 occasions. In 17/25 session pairs (68%), the fields rotated with the card (Fig. 4, cell 1), and the maximum correlation (R_{Max}) between the rate maps of sessions 1 and 2 was found at the expected angle of 120° CW (Table 2). Since the number of fields controlled by the card was above chance (33%) ($\chi^2 = 13.53$; $df = 1$; $P < 0.001$), the card exerted significant cue control over field locations.

In the eight remaining session pairs, six resulted in the fields staying on the same arm as during the standard session (Fig. 4, cell

2), and the R_{Max} was found at 0°. In one other session pair, a rotation of the fields in the direction opposite to the card rotation was observed (R_{Max} at 120° CCW). The remaining session pair

TABLE 1.

*Angular Correlation Coefficients (\pm SEM) for Simultaneously Recorded Cells Before and After Card Rotation**

Field rotations	0°	120° CCW	120° CW
Representative cells (n = 12)	0.54 \pm 0.06	-0.02 \pm 0.05	-0.03 \pm 0.04
Simultaneously recorded cells (n = 17)	0.42 \pm 0.05	-0.05 \pm 0.03	-0.02 \pm 0.02

*In 12 sequences of sessions, several cells were recorded simultaneously. From these simultaneous recordings, only one representative cell per sequence was considered for further analysis, since other simultaneously recorded cells behaved the same way as the representative cells (except for one remapping instance not included in this analysis; see text). This table shows the relative effect of card rotation on the representative cell and the other simultaneously recorded cells. The between-session rotation angle observed for the representative field after the rotation of the card was used as the reference angle (shown as 0° in the first column), against which the correlation coefficients of other fields were plotted. That the correlation coefficients for the simultaneously recorded fields were also the greatest at 0° (i.e., at the same rotation angle as the representative field) indicates that they had rotated the same amount as the representative field.

TABLE 2.

Mean Angular Correlation Coefficients (\pm SEM) Between Firing Patterns*

		0°	120° CCW	120° CW
Cue card rotation (sessions S1/S2)	Controlled fields (n = 17)	-0.06 \pm 0.05	0.04 \pm 0.06	0.43 \pm 0.06
	Uncontrolled fields (n = 8)	0.23 \pm 0.09	-0.04 \pm 0.02	-0.06 \pm 0.05
Cue card removal (sessions S2/S3)	Stable fields (n = 15)	0.59 \pm 0.06	0.01 \pm 0.05	0.03 \pm 0.06
	Unstable field (n = 1)	-0.05	0.23	-0.03
Goal arm rotation (sessions S3/S4)	Controlled fields (n = 2)	0.00 \pm 0.13	0.10 \pm 0.06	0.50 \pm 0.09
	Uncontrolled fields (n = 13)	0.18 \pm 0.10	0.29 \pm 0.08	0.01 \pm 0.05

*Angular correlations were calculated by pairing the spatial firing for different amounts of rotation. After cue card rotation, the maximum correlation for controlled fields was found at the expected rotation of 120° CW (i.e., card rotation angle). The maximum correlation for uncontrolled fields was found at 0°, because a majority of these fields stayed stable after card rotation. After card removal, the maximum correlation for stable fields was seen at 0°. The correlation for the unstable field was maximum at 120° CCW (the field returned to the arm location of session 1). After goal arm rotation, the maximum correlation for controlled fields was found at the expected rotation of 120° CW (i.e., goal arm rotation angle). The correlation for uncontrolled fields was maximum at both 0° and 120° CCW, because half the fields were stable while the other half relocated to the maze arm of session 1.

resulted in a complete remapping of the field locations such that the fields of six simultaneously recorded cells were markedly different in sessions 1 and 2, with no clear spatial relationship between

the two sets (therefore leading us to discard the entire sequence from further analyses). The mean correlation value for the fields representative of these eight session pairs was low for the 120° CW rotation (Table 2), confirming that they did not rotate with the card. The greatest average value was found for a rotation of 0°, simply because fields were stable in 6/8 cases.

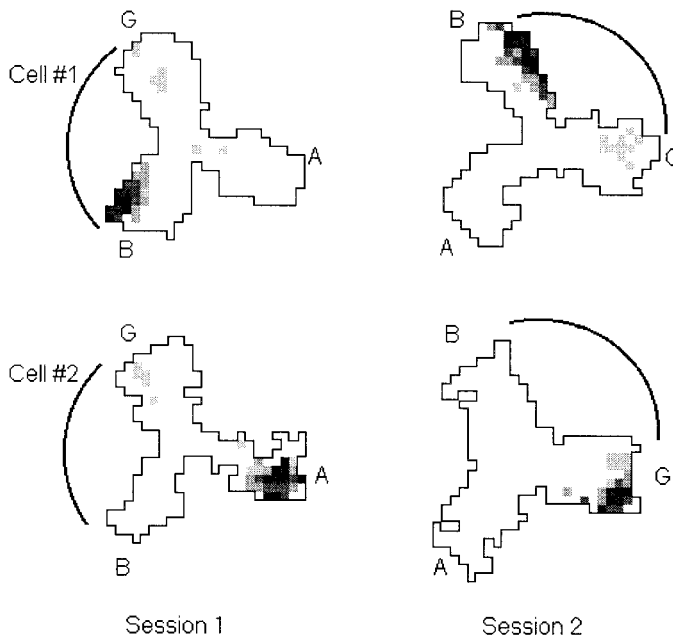


FIGURE 4. Firing rate maps of two place cells during sessions 1 and 2. Cell 1 (top row) had its field controlled by card rotation. Cell 2 (bottom row) had a field that did not rotate after card rotation. G, goal arm; A and B, the two alternating arms. Pixels in which no firing occurred during the whole session are white. Highest firing rate is coded as black, and intermediate rates are shown as light gray, medium gray, and dark gray from low to high. Peak firing rate (black pixels): cell 1, 28.1 AP/s; cell 2, 19.6 AP/s.

Card removal

Although card removal was made on 23 occasions, only the 17 fields that were controlled by the card during session 2 were considered for further analysis, so as to avoid the problem of determining which session should be used as the reference. One cell stopped firing following card removal and was discarded. In 15 of the remaining 16 session pairs (93.75%), fields were stable after card removal (Fig. 5, cell 1), and the R_{Max} between the rate maps of sessions 2 and 3 was found at the expected angle of 0° (Table 2). In only one session pair (Fig. 5, cell 2) did the field location change after card removal (R_{Max} at 120° CCW).

A chi-square test showed that the number of stable fields after card removal was well above chance ($\chi^2 = 26.3$; $df = 1$; $P < 0.001$), thus suggesting that the combination of motion-related idiothetic information and feedback provided by the goal location was sufficient to maintain a stable firing field. Interestingly, this was also true of cells that were uncontrolled by the card during session 2. Although these cells were not taken into account in the above analyses, all had fields that were unaffected by the removal of the card during session 3.

Goal rotation

During session 4, the rat was introduced into the maze in the absence of the card after the goal arm had been rotated. Represent-

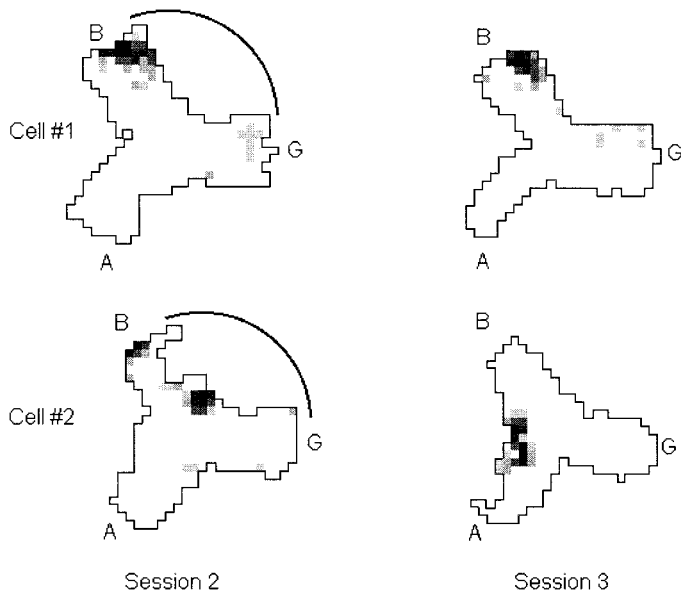


FIGURE 5. Firing rate maps of two place cells during sessions 2 and 3. Cell 1 (top row) had a stable (consistent) field when the card was removed, while cell 2 (bottom row) had an inconsistent field that returned to its standard position after card removal. G, goal arm; A and B, the two alternating arms. Pixels in which no firing occurred during the whole session are white. Highest firing rate is coded as black, and intermediate rates are shown as light gray, medium gray, and dark gray from low to high. Peak firing rate (black pixels): cell 1, 14.1 AP/s; cell 2, 13.6 AP/s.

tative rate maps are shown in Figure 6. The fields were observed at the location expected on the basis of the goal arm location in only 2/15 session pairs (R_{Max} at 120° CW; Fig. 6, cell 1). In the remaining 13 session pairs (86.67%), the fields were seen on the same arm as during session 1 (seven session pairs; R_{Max} at 120° CCW; Fig. 6, cell 2), or on the same arm as during session 3 (six session pairs; R_{Max} at 0°; Fig. 6, cell 3). As a result, the mean correlation values of these fields have two maxima (see Table 2).

A chi-square test showed that the distribution of fields across the three possible arm locations was not different from a random distribution ($\chi^2 = 2.8$; $df = 2$; NS). This suggests that the feedback information provided by the goal arm (where the rat received food rewards) was not sufficient to spatially anchor the location of firing fields, as confirmed by the comparison of controlled vs. noncontrolled fields ($\chi^2 = 2.7$; $df = 1$; NS).

Final standard

This session was a replication of the initial standard session. Its purpose was to see if the fields were similar in location and spatial extent in spite of the disturbances generated by the manipulations of sessions 2–4. Visual inspection of the firing rate maps revealed that the firing patterns were very similar in the two standard sessions for 19 of the 21 session sequences that could be completed (see General Considerations, above). Angular correlations confirmed these impressions. The R_{Max} value for each of the 19 representative fields was at 0°. The mean correlation values for 0°, 120° CW, and 120° CCW were 0.53 ± 0.06 , -0.02 ± 0.04 , and

-0.02 ± 0.04 , respectively. These results show that most alterations of firing fields across sessions 2–4 were reversible.

Finally, we looked for the possible existence of regularities in field relocations across testing. Since several complete sequences of recording sessions were made in four rats, it was of interest to see if a given cell's response to an environmental manipulation could be predicted from the response of cells recorded during previous sequences. In fact, no consistent pattern was seen across repeated testing of an individual rat. For example, a field was found to be uncontrolled by the card rotation for rat 9 during recording sequences 3 and 4. However, the same rat showed cells whose fields followed the card during recording sequences 1 and 2 as well during recording sequence 5. Looking at the other manipulations (i.e., card removal and goal rotation) failed to reveal any regular pattern in the cells' reaction to environmental manipulations across repeated testing.

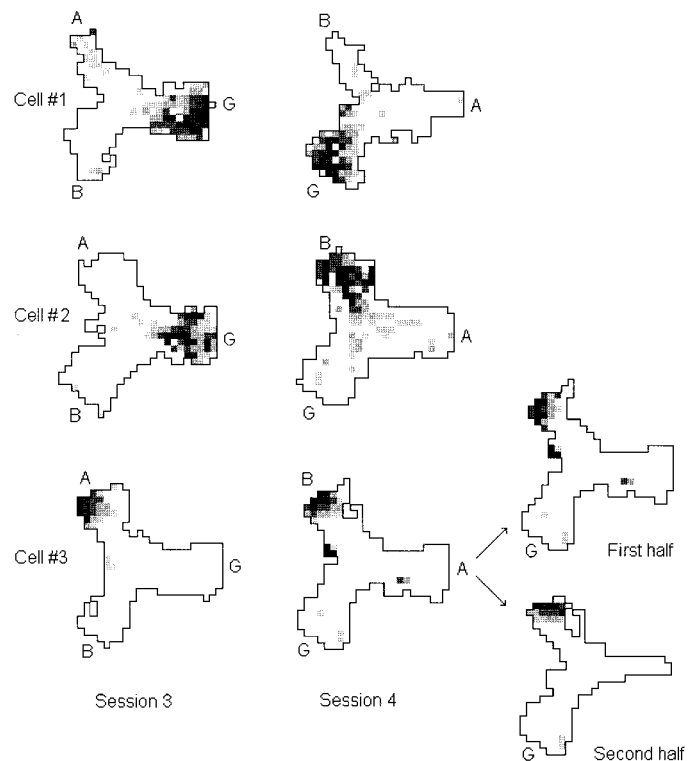


FIGURE 6. Firing rate maps of three place cells during sessions 3 and 4. Cell 1 (top row) had a consistent field that rotated appropriately after goal rotation. Cell 2 (middle row) had an inconsistent field that rotated in the direction opposite to goal rotation. Cell 3 (bottom row) had an inconsistent field that did not move between sessions 3 and 4. For that cell, session 4 was further broken down into two halves of equal duration. Rate maps for each half-session show that the field stayed stable throughout the session, even though behavioral performance improved from 68% during the first half to 88% correct during the second half of the session. G, goal arm; A and B, the two alternating arms. Pixels in which no firing occurred during the whole session are white. Highest firing rate is coded as black, and intermediate rates are shown as light gray, medium gray, and dark gray from low to high. Peak firing rate (black pixels): cell 1, 23.4 AP/s; cell 2, 5.9 AP/s; cell 3, 8.3 AP/s.

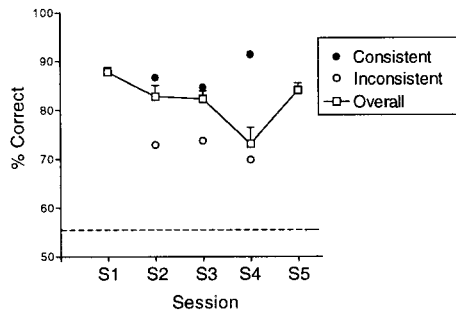


FIGURE 7. Across-session time course of behavioral performance. There was a general effect of sessions. After a gradual deterioration of performance from sessions 1 to 4 ($P < 0.001$), an abrupt increase of correct responses was observed between sessions 4 and 5 ($P < 0.01$). Circle show performance when consistent and inconsistent field placements were separated. Dashed horizontal line indicates score of a rat behaving fully randomly on the task, calculated according to Monte Carlo simulations (see details in Data Presentation and Analyses).

Behavioral Performance and Spatial Firing Patterns

Across-session time-course of behavioral performance

The percentage of correct choices during each session is shown in Figure 7. A smooth, consistent degradation of performance scores occurred from sessions 1 to 4, followed by an abrupt improvement during session 5. An analysis of variance conducted on these data yielded a significant effect of Session ($F(4, 76) = 7.15$; $P < 0.001$). More important, planned comparisons confirmed the decrease of correct choices from session 1 to session 4 ($F(1, 19) = 18.78$; $P < 0.001$) and the improvement between sessions 4 and 5 ($F(1, 19) = 8.44$; $P < 0.01$). Nonetheless, a small yet significant difference was found between the first and last sessions ($F(1, 19) = 7.41$; $P < 0.025$). The similarity of performance scores during the two standard sessions shows that the decrease between sessions 1 and 4 cannot be caused only by a time-related state of decreased motivation (caused by satiety or fatigue).

Classification of spatial firing patterns

The effects of cue manipulations on place-cell spatial firing patterns show that, under some circumstances, fields shift out of register with the goal (and thus with the overall task). This makes it possible to analyze the link between place-cell firing patterns and behavior. To do so, pairs of sessions were placed into one of two categories.

In the first, the “consistent” category, the placement of the field relative to the goal arm is the same during both the first (called “reference” hereafter) and second (called “test” hereafter) sessions in the pair. The rate maps for cell 1 in Figure 4 illustrate this situation. The cell initially fires in the arm 120° CCW to the goal arm during the reference session (standard). After the 120° CW rotation of card + goal, the field rotates 120° CW in the test

TABLE 3. Mean Angular Correlation Coefficients (\pm SEM) for Consistent and Inconsistent Session Pairs*

Session pairs	Expected position	120° CCW	120° CW
Consistent (n = 41)	0.50 ± 0.04	-0.05 ± 0.03	0.00 ± 0.04
Inconsistent (n = 27)	-0.03 ± 0.03	0.20 ± 0.06	0.15 ± 0.06

*The expected angular position for the second session in the pair was calculated by adding the rotation of the goal arm to the angular field location on the first session in the pair. Contrary to inconsistent session pairs, the maximum correlation for consistent pairs was found at the expected angular position. Note that two maximal values were found for inconsistent pairs, due to the even distribution of field locations in the 120° CCW and 120° CW arms.

session (card rotation), so that its location is still 120° CCW relative to the goal arm location.

In the second, the “inconsistent” category, the placement of the field relative to the goal arm is different in the test and reference sessions. The rate maps for cell 2 in Figure 4 illustrate this situation. The cell initially fires in the arm 120° CW to the goal arm during the reference (standard) session. After the 120° CW rotation of card + goal, the field does not rotate 120° CW, but instead stays on the same arm as in the reference session. Thus, the field location in the test session is now 0° relative to the goal arm location.

This classification was done only for sessions 1–4, since session 5 was usually preceded by an inconsistent session pair, thus precluding clear prediction of the field position during that session. Twelve additional session pairs (repeats of incomplete sequences of the previous day) were also included. Based on the rate maps and the correlation coefficients associated with session pairs (Table 3), 41 session pairs were classified as consistent and 27 pairs were classified as inconsistent. Noticeably, no systematic change in several basic firing properties was observed for cells recorded during inconsistent sessions (besides the erroneous location of their fields). For example, the overall firing rate (in spikes per second) of these cells was not significantly different during the inconsistent session relative to the preceding session (0.92 ± 0.17 vs. 1.05 ± 0.28 , respectively; $t = 0.59$, $df = 26$, NS).

Overall behavior and spatial firing patterns

Behavioral performance was examined for both consistent and inconsistent session pairs. First, the total number of errors was found to be greater during the test session than during the reference session of inconsistent pairs, while no change was seen for consistent pairs (Fig. 8A). This effect can also be seen in Figure 7, in which the performance of sessions 2–4 was plotted separately for consistent and inconsistent field placements.

An analysis of variance conducted on these data yielded significant effects of Session (reference vs. test: $F(1, 66) = 18.67$; $P <$

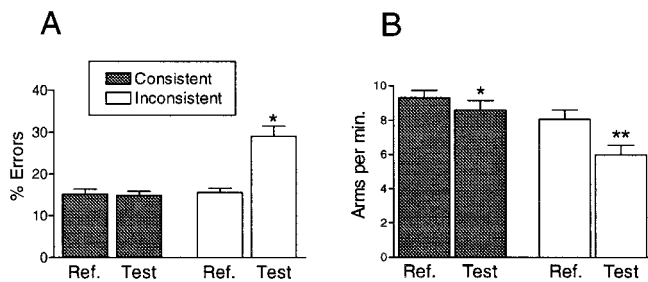


FIGURE 8. Behavioral scores and spatial firing patterns. **A:** Mean percent errors (\pm SEM) during reference and test sessions of consistent and inconsistent session pairs ($*P < 0.001$ compared to reference session). **B:** Mean number of arms visited per minute (\pm SEM) during reference and test sessions of consistent and inconsistent session pairs. A significant decrease was found for both consistent and inconsistent session pairs. Session \times Pair Type interaction was significant at $P < 0.02$, thus showing a greater reduction of visits during inconsistent than consistent session pairs. $*P < 0.05$ and $**P < 0.001$, compared to reference session.

0.001), Pair Type (consistent vs. inconsistent: $F(1, 66) = 19.13$; $P < 0.001$), and Session \times Pair Type interaction ($F(1, 66) = 30.22$; $P < 0.001$). Planned comparisons revealed a significant effect of Session for inconsistent pairs ($F(1, 26) = 33.46$; $P < 0.001$), but not for consistent pairs ($F(1, 40) = 0.02$; NS). Lastly, a statistical difference between consistent and inconsistent pairs was found for the test session ($F(1, 66) = 41.79$; $P < 0.001$), but not for the reference session ($F(1, 66) = 0.09$; NS). Overall, these results show that inconsistent field placements during the test session were accompanied by reduced performance scores. Such changes were independent of the exact session in the sequence when an inconsistent field placement was observed (Fig. 7). In contrast, consistent field placements did not result in any strong change in performance.

Not only did the rats make more errors after inconsistent field placements, but they also reduced their number of choices to a much greater extent than after consistent field placements (Fig. 8B). An analysis of variance conducted on the number of arms visited during each session revealed significant effects of Session

(reference vs. test: $F(1, 66) = 21.96$; $P < 0.001$), Pair Type (consistent vs. inconsistent: $F(1, 66) = 9.5$; $P < 0.01$), and Session \times Pair Type interaction ($F(1, 66) = 6.22$; $P < 0.02$). Planned comparisons revealed a significant effect of Session for both inconsistent ($F(1, 26) = 20.82$; $P < 0.001$) and consistent ($F(1, 40) = 4.73$; $P < 0.05$) pairs. A statistical difference between consistent and inconsistent pairs was found for the test session ($F(1, 66) = 14.13$; $P < 0.001$), but not for the reference session ($F(1, 66) = 3.45$; NS).

In summary, inconsistent field placements during the test session were predictive of a rat's reduced ability to solve the task. Although they were impaired, rats still performed the task above chance. Their mean score during inconsistent test sessions was $70.9 \pm 2.4\%$ of correct responses, i.e., well above 55%, the score of a randomly behaving animal ($t = 8.8$, $df = 26$, $P < 0.001$; see Materials and Methods). This incomplete deterioration could be explained by the feedback resulting from obtaining rewards, which allowed the animal to adapt its behavior and to relearn the task within a session (see below).

Error type analysis and spatial firing patterns

At asymptotic performance, rats mostly made alternation errors ($\approx 75\%$ of total number of errors). Orientation errors (failures to return to the goal arm and perseverations) were much less common. This trend was maintained for consistent field placements during the test session (Fig. 9). Thus, for consistent fields there were more alternation errors than orientation errors during both reference and test sessions ($t = 5.87$ and $t = 7.08$, respectively; $df = 40$; $P < 0.001$), and there was no variation in this distribution between the reference and test sessions (both $t < 1$).

In contrast, a complete reversal in the distribution of errors was seen for inconsistent field placements (Fig. 9). While there was also a majority of alternation errors during the reference session for inconsistent fields ($t = 6.46$; $df = 26$; $P < 0.001$), there were more orientation errors than alternation errors during the test session ($t = 2.31$; $df = 26$; $P < 0.02$). The increase in orientation errors and correlative decrease in alternation errors were statistically confirmed ($t = 6.47$; $df = 26$; $P < 0.001$). This suggests that rats did

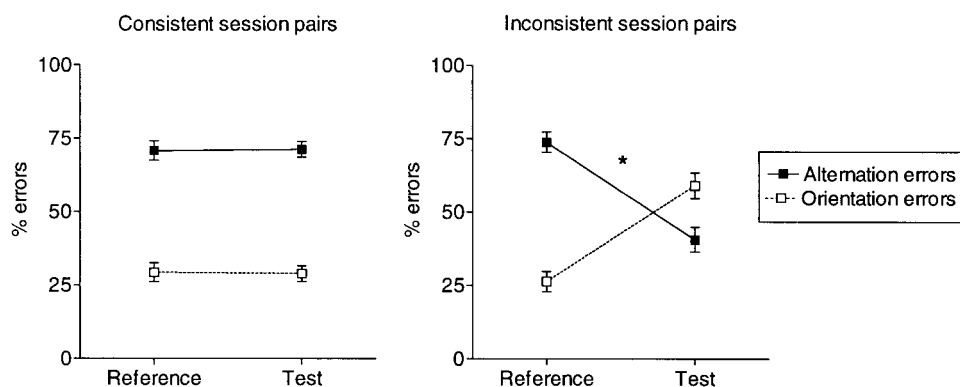


FIGURE 9. Error types for consistent and inconsistent session pairs. Rats usually made more alternation errors than orientation errors at asymptotic level. This trend was reversed for inconsistent field placements. $*P < 0.001$, reference vs. test sessions.

not just fail to remember their last alternation choice, but were confused about what to do to solve the problem.

This interpretation was further substantiated by the observation that field location predicted behavior. In other words, the locus of most errors during the test session of inconsistent pairs was in agreement with the arm where the field was seen. Thus, a comparison of errors made in the two nongoal arms revealed a significant bias towards the arm indicated by the cell (66.5% vs. 33.5%; $\chi^2 = 10.85$; $df = 1$; $P < 0.001$). In cell 2 in Figure 6, rats made more errors towards arm A than towards arm B, a bias predicted by the misplacement of the field by 120° CCW. Similarly, in the example shown in cell 3 in Figure 6, rats made more errors towards arm B than towards arm A, a bias consistent with the stability of the field in the two sessions. Actually, some remnants of the alternation task were found when behavior was measured with the arm predicted by the field location being considered as the goal. This was tested by assuming that each of the two alternation arms could act as the goal arm. The comparison of scores obtained in each case revealed that rats were slightly, but significantly, more correct for the arm predicted by the field location than for the other arm ($58.3 \pm 2.6\%$ vs. $48.7 \pm 1.4\%$ of correct responses, respectively; $t = 4.47$; $df = 26$; $P < 0.001$).

Within-session relearning during test sessions (inconsistent pairs)

Although rats were disturbed during the test session of inconsistent session pairs, they slightly improved their performance within the session. A comparison of error scores during the first and second halves of test sessions revealed a significant improvement (from 69.87% to 76.11% of errors; $t = 4.83$; $df = 26$; $P < 0.001$). Such a change was not observed for the reference session of inconsistent pairs (respectively, 85.25% and 84.08%; $t = 1.29$; $df = 26$; NS). However, full recovery of performance was usually not achieved before the following session. Noticeably, this within-session improvement was *not* accompanied by any meaningful change in cell firing patterns. This conclusion was reached when cell activity was analyzed separately during the first and second halves of inconsistent test sessions. We compared the cell firing rate and spatial coherence (an index of the local smoothness of place fields; see Muller and Kubie, 1989) during each session half and found no significant difference (firing rate in spikes per second: 1.1 ± 0.2 vs. 0.9 ± 0.2 , $t = 1.79$, $df = 26$, NS; spatial coherence: 0.57 ± 0.03 vs. 0.59 ± 0.04 , $t = 0.46$, $df = 26$, NS). In addition, the pixel-by-pixel correlation between rate distributions associated with each session half was relatively high (mean correlation, 0.49 ± 0.04), thereby indicating that the firing pattern was very similar across the two session halves. Together, these results show that, once the field was seen somewhere in the maze, it was stable until the end of the session, independent of any behavioral change (e.g., cell 3 in Fig. 6). Similarly, although we have not made a formal analysis of this aspect, we did not observe that behavioral errors occurring during inconsistent field placements were accompanied by extrafield firing. In other words, there was no adaptation of cell firing to the rat's current behavior on a short time-scale.

DISCUSSION

The purpose of this study was to determine if there is a relation between rats' behavior in a spatial memory task and the spatial firing patterns of place cells. To do so, we performed cue manipulations that revealed interesting aspects of place-cell firing. Thus, we first examine the effects of the different cue manipulations on firing fields before turning to the behavioral evidence.

Effects of Cue Manipulations

Cue control by the card

Compared to cylinder experiments, the present study shows that the cue card has less effective (though still significant) control over firing fields. Whereas 68% of fields rotated with the card in our Y-maze experiment, as many as 90% do so in cylinder experiments (Muller and Kubie, 1987; Cressant et al., 1997). Why the control exerted by the card is different in the two situations is unclear. The possibility that the rat could use local cues in our study can be discarded, since arms were exchanged and cleaned between sessions. A more plausible possibility is that the behavioral task interfered in some way with the ability of the rat to attend to the visual cue (Markus et al., 1995). Thus, while the pellet-chasing task used in cylinder experiments to keep the animal moving does not require explicit self-localization (although the rat presumably does so spontaneously), the present alternation task required the animal to consistently monitor its location. Because the rat had to pay attention to its location, it may have collected more information about its spatial environment than in the cylinder. That the fields not rotating with the card were usually stable in the laboratory frame suggests that they were tuned to uncontrolled background cues. One factor that may have enhanced the influence of such background cues relates to the environment itself. For example, the wall of the cylinder greatly restricts the collection of distant information, therefore enhancing the salience of the cue card. In the Y-maze, on the contrary, the curtain ring constituted the boundary of the visual environment, and the cue card was simply embedded within this area. Since previous studies suggest that distant landmarks provide better directional anchors than do closer cues (Cressant et al., 1997, 1999), it is possible that surface irregularities on the curtains provided enough information to control field positions. In any event, we notice that cues that appear to be very subtle for an experimenter's eyes can overcome the use of the seemingly more salient landmark. That such cues arise from remote parts of the environment bolster our previous emphasis on the important contribution of distal cues in the angular anchoring of place-cell firing patterns (Poucet and Cressant, 1998).

Effects of card removal

In the vast majority of sessions, removing the card while the animal was in the maze had no effect on firing fields. Fields were seen on the same arm as before cue removal, and were stable throughout the session. Since olfactory cues were avoided by clean-

ing the maze between sessions, this stability was unexpected, because we recently found that firing fields do not stay stable for more than a few minutes when both visual and olfactory cues are neutralized in a cylinder environment (Save et al., 2000). With no external cues, the use of idiothetic motion-related cues is the only viable strategy for an animal to update its position (Mittlestaedt and Mittlestaedt, 1982). However, the integration of motion-related cues is prone to cumulative error when recalibration via an external reference is precluded (McNaughton et al., 1991). Thus, fields should be subject to positional drifts that reflect this computational error. The present observation of field stability therefore deserves some discussion. First, one could imagine that local cues on the arms provided enough information to maintain stable firing fields. This is an unlikely possibility, however; the arm arrangement was varied several times during a session, thus precluding the learning of a constant configuration.

Although, in the absence of local cues, the major source of information available to the rat came from its own motion, several other pieces of information might have helped the animal. First, food reward was delivered at a constant location, thus potentially providing a reliable feedback signal. Second, the 3-fold symmetry of the maze may have helped positional recalibration by reducing the number of reference directions for polarizing the hippocampal representation (Cressant et al., 1999). Lastly, the maze restricted the range of possible trajectories. In contrast, the cylinder environment does not provide any polarizing cue by itself, and allows for a wide range of complex trajectories, thus putting more burden on path integration than the simple paths that a rat can travel in the maze. In support of these hypotheses, Markus et al. (1994) demonstrated that place cells recorded from rats running on a radial arm maze had stable spatial firing patterns when the light was switched off and olfactory cues were made irrelevant, an outcome also observed in the present study.

To sum up, a variety of factors may have allowed the rat to make efficient use of motion-related idiothetic cues to update its position in the maze. Thus, a combination of the simple maze structure and a well-defined goal location may have been sufficient to maintain firing field stability in the absence of the visual cue.

Effects of goal rotation

When the goal was relocated to a new position and the rat was to solve the task in the absence of the visual cue, place cells established their fields on an arbitrary arm, and their spatial distribution did not differ from random. Once established, fields were stable throughout the session, even though they were consistently out of register with the behavioral task. This observation complements the observation of Speakman and O'Keefe (1990) that shifting the goal relative to extramaze cues in a spatial learning task did not induce a corresponding shift in firing field locations. Rather, fields remained in the same position relative to the extramaze cues, whatever the location of the goal. One possible interpretation of these previous data, however, was that the salient extramaze cues overshadowed the possible control of firing fields by the goal location. Clearly the present results allow us to rule out this possibility since, even in the absence of any external cue, firing fields were not

reliably controlled by the goal location (see Zinyuk et al., 2000 for similar results). Thus, the mere presence of a reward at a consistent location does not seem to be sufficient to anchor place cell firing.

Evidence for a Relationship Between Spatial Performance and Place-Cell Firing

The cue manipulations described sought to produce observable changes of the firing fields that could be related to the rats' performance in the spatial memory task. We reasoned that if place-cell firing supports spatial behavior in some manner, then spatial performance should deteriorate when fields occupy locations out of register relative to their standard position. If this outcome is not observed, then the hypothesis that the spatial firing patterns of place cells are useful for navigation would be considerably weakened. For example, take a cell that fires on the goal arm during the initial session and that, during a subsequent session, fires on the arm west of the goal arm. A failure of this change in spatial firing to alter the rat's performance would indicate that the spatial information provided by that cell does not contribute significantly to behavior. Conversely, the occurrence of a systematic deterioration of performance under those circumstances would provide strong evidence for a relationship between the information carried by the cell and the information required for solving the task. Our results bolster the latter view, since the signals generated by hippocampal place cells were highly coherent with spatial behavior.

Evidence supporting this assertion was first provided by the changes in rats' overall behavior when the fields occupied inconsistent (i.e., erroneous) positions. The number of visited arms was decreased by about 25%, and the proportion of correct choices was decreased by about 25%. In contrast, no such changes were found when field positions were consistent (correct). The combined effect of the two behavioral changes observed for inconsistent field placements resulted in the rats being rewarded, on average, half as often as during sessions with consistent field placements. Explanations of these effects in terms of decreased hunger or decreased motivation to solve the problem could be ruled out, since they were observed with equal likelihood in all sessions, whatever their rank order in the sequence. Also, performance markedly improved during the last (standard) session, when fields usually returned to their initial location. This improvement is not compatible with the interpretation that the decreased performance in previous sessions was simply due to satiety.

The nature of the errors made during consistent and inconsistent field placements was also different. Because the rats were well-trained to solve the alternation task, they usually made simple alternation errors and rarely made more complex orientation errors. Whereas this trend was maintained for consistent field placements, it was markedly changed for inconsistent field placements. The animals either made repeated choices of the same alternation arm, or failed to return to the goal arm, thus no longer relying on the basic alternation rule to solve the task. Moreover, the erroneous placement of the firing field predicted erroneous visits to an arm. Overall, this behavioral pattern seems to reflect a more complete disorganization than just making occasional alternation (working memory) errors.

Together, these results suggest that inconsistent field placements were accompanied by disorganized spatial behavior. Noticeably, errors in field placements concerned their angular, but not radial positions. In other words, there was no evidence that cue manipulations resulted in activating a different representation. In addition, simultaneous recordings suggested that all cells behaved the same way on a specific session, i.e., if the field of one cell was seen to rotate at some angle, the fields of other simultaneously recorded cells were seen to rotate at the same angle. Thus, a reasonable assumption is that the observed behavioral effects were caused by the mismatch between the orientation provided by the hippocampal representation and the learned task, rather than by the activation of a distinct representation. Interestingly, inconsistent angular placements for fields did not prevent the rat from relearning the task, since some improvement was seen within sessions with an inconsistent field placement. Such relearning was not accompanied by a change in the position of the field in the course of the session; nor were errors accompanied by extrafield firing. In other words, once the fields were seen at a given position, they stayed there for the entire session. The instantaneous behavior did not appear directly related to the cells' current firing state. Thus, rats reorganized their behavior on the basis of the hippocampal representation, even though this representation was out of register with the standard condition. These observations support the notion that the hippocampal spatial representation implemented by place cells allows for fast and flexible new spatial learning (O'Keefe and Nadel, 1978), an assumption strongly supported by the inability of rats with hippocampal disruption to easily learn new locations in the Morris navigation task (e.g., Steele and Morris, 1999).

FINAL COMMENTS

Our results provide direct evidence for a relationship between the firing patterns of place cells and the observable spatial behavior of the animal. Although the alternation task used here is, at first glance, more complicated than the spatial learning task used by O'Keefe and Speakman (1987), basic results were the same, thus showing across-task generality of the involvement of place cells in behavior. O'Keefe and Speakman (1987) demonstrated that the instantaneous discharge of place cells during single-trial performance of a spatial task in a four-arm radial maze was predicted if erroneous performance was assumed to be correct. We extend this observation by demonstrating that once established, firing fields remained stable, even though they were incorrectly located relative to the goal. In other words, the rat did not use the potential feedback signal provided by the presence of rewards on a specific arm to update its representation. That, under these circumstances, the activity of place cells was still a good predictor of the animal's performance emphasizes the importance of position signals for the generation of spatial behavior.

We therefore believe that these data support the view that place cells are part of a system for representing space, and that this representation is useful for solving spatial tasks (O'Keefe and

Nadel, 1978; see also Muller et al., 1999). This system would also involve postsubicular and anterior thalamic head direction cells, whose activity was recently demonstrated to correlate with spatial behavior on a radial maze (Dudchenko and Taube, 1997). Since the task was spatial in nature, however, the present data certainly do not speak to alternative views of hippocampal function. For example, Eichenbaum et al. (1999) proposed that the hippocampus is concerned with the encoding of all kinds of events in episodic memory, with space only one particular kind of information to be memorized. Probing this general memory function would require showing that place cells can also encode nonspatial aspects of the events to be stored (Wiener et al., 1989). In any event, the functional relationship seen in the present study between the spatial activity of place cells and the rat's spatial performance in the alternation task is strong evidence that these cells do play a direct role in spatial behavior.

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