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RESEARCH ARTICLE

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# Remapping of place cell firing patterns after maze rotations

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**Abstract** When place cells are recorded from rats running on an elevated T-maze inside a curtained enclosure that contains distinct, experimenter selected stimuli, rotations of the maze plus stimuli cause equal rotations of firing fields. Here, we examined the effects of conflicting rotations of a T-maze relative to a laboratory frame that contained a large number of fixed stimuli in the environment and asked whether positional firing patterns stayed in register with the maze or the room cues or were modified in some more complex way. After maze rotations of 90°, 180° or 270°, firing fields were stable in the laboratory frame and thus shifted to a different maze arm. In contrast, rotations of 45° or –45° resulted in dramatic changes of positional firing patterns regardless of their initial position on the maze. Crucially, even cells whose fields were initially on the central platform underwent major firing pattern alterations although the view of the environment from the platform was unchanged by such rotations. Finally, we found that altering the visual appearance by removing without rotation one or two maze arms did not alter firing fields on the remaining part of the maze. Thus, the "remappings" caused by 45° rotations could result from the disturbed relationship between all arms and the room cues or from the changes in the possible paths the animal can take in the environment. Taken together, our results provide an example of combinatorial coding by the hippocampus, in which the place cell representation of the environment was seen to be modified as a unit and not piecewise according to locally available stimuli.

**Keywords** Dorsal hippocampus · Unit recordings · Place cells · Spatial navigation · Rat

## Introduction

Place cells are hippocampal pyramidal neurons whose activity is strongly tied to the location of a rat (O'Keefe and Dostrovsky 1971) or a mouse (McHugh et al. 1996; Rotenberg et al. 1996) in its environment. In a fixed environment, each place cell discharges intensely only when the rat's head is inside a cell-specific region called the "firing field" (Muller and Kubie 1987). As the rat moves around in the environment, the discharge of the place cell ensemble co-varies such that there is a unique pattern of activity at each location. This across-cell discharge profile may therefore provide a "pointer" to the animal's location on a cognitive map of the environment (O'Keefe and Nadel 1978). Previous work has shown that the location of firing fields can be controlled by both allothetic (e.g., visual cues; Muller and Kubie 1987; O'Keefe and Speakman 1987) and idiothetic (motion-related) information (e.g., Bures et al. 1997; Gothard et al. 1996; Jeffery and O'Keefe 1999; Knierim et al. 1998; McNaughton et al. 1996; O'Keefe 1976; Poucet et al. 2000; Wiener et al. 1995).

Although there is little dispute about the reality of the place cell phenomenon, the notion that place cells constitute a map-like representation of space is by no means fully accepted. For example, it has been proposed that each place cell is activated by a constellation of stimulus features that happen to be available only in the vicinity of the cell's firing field, so that the place cell population comprises a set of independent or nearly independent

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feature detectors. Thus, Shapiro et al. (1997) distinguished cells whose fields were controlled by either the local or distal stimuli from cells whose fields were tuned to the laboratory frame (see also Tanila et al. 1997). Another instance of cells found to react relatively independently from each other was found when rats were switched from a random pellet chasing task to a directed search task in the same apparatus (Markus et al. 1995). Under these circumstances, one-third of the cells immediately changed their firing location while the remaining cells had the same fields in both tasks. In the same study, however, it was found that when both the task and the apparatus were changed within the same room, almost all place cells changed their firing fields (Markus et al. 1995).

On the other hand, several studies have found that some stimulus manipulations can cause all place cells in a given animal to undergo "remapping" such that the firing pattern of each cell is dramatically altered between two very similar environments. Remappings can be induced by gross changes in the environment such as in Markus et al.'s study (1995), but also by smaller modifications (Bostock et al. 1991; Kentros et al. 1998; O'Keefe and Burgess 1996). The existence of remappings presents serious problems for the general notion that the activity of multiple place cells is independently controlled by arbitrary combinations of available stimuli (Muller et al. 1999).

Since remappings can be viewed either as the activation of distinct maps of the environment or as the use of different reference frames by the hippocampus (Redish and Touretzky 1999), it is therefore important to establish the conditions in which they occur. The issue is also important in light of the continuing conflict between the mapping and sensory-tuning interpretations of the place cell phenomenon. In the present study, we have further investigated the nature of the hippocampal representation by manipulating the orientation and components of a T-maze inside a recording room. In a first experiment, we rotated the maze by 90°, 180° or 270° relative to the laboratory and asked how firing fields are affected by changing the relationship of the maze to the distal cues. In a second experiment, we removed one or two of the arms of the maze so as to alter more strongly the relationships between the accessible space and the distal cues. These two experiments revealed that firing fields were unaffected by such manipulations. In the final experiment, we rotated the maze by 45° or –45° relative to the laboratory. Such rotations make it impossible for the animal to be in the same place in the environment when it is on any arm. In contrast, the position of the circular central platform is unchanged as is the view of distal cues from the central platform. Thus, the ±45° rotations allow us to determine if fields in a privileged part of the environment are constant or modified when the firing of cells in all other parts of the environment must be altered.

## Materials and methods

All procedures complied with guidelines for animal experimentation from international ("Principles of laboratory animal care", NIH publication no. 86–23, revised 1985) and national institutions (Council directive no. 87848 of the Direction of the Service Vétérinaire de la Santé et de la Protection Animales; permission to B.P. no. 13–76).

### Subjects

Nine Long-Evans male rats (CERJ, Le Genest-St-Isle, France) weighing 300–350 g were used. They were housed one per cage in a room that was lit for 12 h from 8 a.m. to 8 p.m. and dark otherwise; the temperature was regulated to 20±2°C. The rats had ad lib access to water during all phases of the experiment. Upon arrival in the laboratory, the rats were handled daily for 2 weeks prior to pre-surgery training. Each rat was then food deprived to 85% of ad lib body weight and trained to retrieve 20-mg food pellets from the ends of the three arms of a T-maze.

### Apparatus

The apparatus was a T-shaped plywood maze elevated 50 cm above the floor. It consisted of a circular central platform (30 cm in diameter) and three arms (40 cm long, 10 cm wide) bounded by 1-cm-high stainless steel lips. A remote-controlled food dispenser was attached to the end of each arm. To prevent the rat from interfering with the food dispensers, a transparent Perspex barrier (20 cm wide, 30 cm high) was set vertically between the end of each arm end and the corresponding dispenser. A small hole at the base of each barrier allowed 20-mg food pellets to be ejected onto the arm. Except for the barriers, all parts of the maze were painted flat black. The maze center was at the middle of a 2.8×2.8-m room in which a variety of visual cues were available (Fig. 1). The "north" wall was covered with gray wallpaper, the black "west" wall had a 1×1-m white sheet at its center, the "south" wall contained the entrance door (which was nearer the east wall) and the "east" wall contained windows with closed Venetian blinds. Illumination was provided by four 25-W light bulbs set in a square above the central platform of the maze. A radio above the central platform generated background noise (>70 dB) to mask uncontrolled directional sounds during all phases of the study. Between each session, the maze was cleaned. In addition, the arms were interchanged to randomize possible local cues.

### Food retrieval training

After weight was reduced to 85%, rats were taught to obtain food on the maze. A rat was placed on the central platform and allowed to freely explore the entire surface of the maze. During this time, the food dispensers were randomly activated to deliver a total of about 3 pellets/min. Since the arm on which the next food pellet would be delivered was unpredictable, the rat learned to run almost constantly between the ends of all arms. Three 16-min training sessions were done daily for at least 8 days prior to electrode implantation. All training sessions were made with the maze in the standard position (see "Experimental protocols"). After training was complete, the rat visited all parts of the maze many times per recording session so that firing activity could be sampled everywhere in the apparatus.

### Surgery

A preanesthetic injection of 0.3 ml atropine (0.25 mg/kg) 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 muscles were retracted and holes for four anchoring screws were drilled in the skull; the screws were inserted over right and left frontal cor-

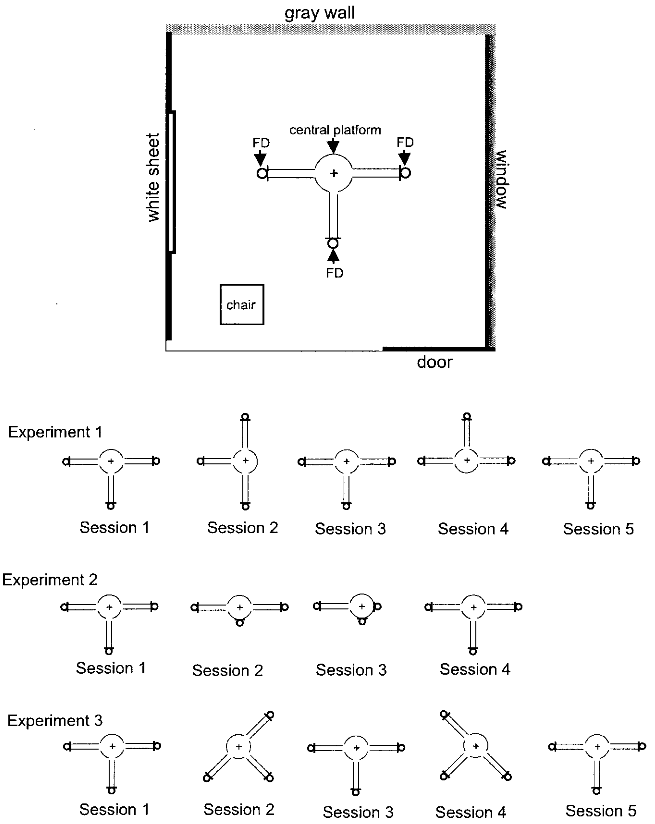

**Fig. 1** Schematic representation of the apparatus and protocol. *Top* The T-maze position was maintained constant during training and standard recording sessions. A remote-controlled food dispenser (*FD*) and a transparent barrier were attached to the end of each arm. Food pellets were ejected onto the arm through a small hole in the barrier. The axis around which the maze was rotated is indicated by a *plus sign*. *Bottom* The sequence of recording sessions is depicted for each experiment. The exact sequence of manipulations in experiments 1 and 2 was determined so that the rat could gain access to the place where the field was seen initially. For example, the sequences shown here are appropriate only for a field in the left arm during session 1. All manipulations kept the central platform stable relative to the room

tex and left cerebellar cortex. To improve the stability of the electrodes, an additional screw with the head ground to a T-shape was lowered upside down into a hole in the left parietal bone and turned 90° before being locked in place with a nut.

A movable array of ten 25-µm electrode wires (Kubie 1984) was implanted in the right dorsal hippocampus at stereotaxic coordinates 3.8 mm posterior and 3.0 mm lateral to bregma and 1.5 mm below the dura (Paxinos and Watson 1986). Sterile petroleum jelly was applied to the exposed brain surface and the guide tubing of the electrode array. The exposed skull was covered with dental cement. The anchoring screws and nut were embedded in dental cement and the bottoms of the three drive screw assemblies were bonded to the skull.

At the completion of the experiment, rats 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 µA 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 µm in thickness were taken. Every fifth section was stained with cresyl violet for verification of electrode placements.

## Recording methods

Screening and recording were done using a cable attached at one end to the rotating portion of a commutator that allowed rats to move freely on the maze. The other end of the cable was connected to a headstage whose top contained a field effect source follower (FET) for each wire and a light-emitting diode (LED) for tracking the rat's head position. The bottom of the headstage was a connector that mated to the top of the electrode connector cemented on the rat's skull.

The fixed side of the commutator was connected to a distribution panel that fed signals sequentially to amplifiers (×1000 gain, bandpass filtering between 300 Hz and 10 KHz) and then to two pairs of time-and-amplitude window discriminators (Model DIS-1, Bak Electronics). The discriminators were arranged in series, which allowed us to isolate two distinct waveforms either from the same wire or from two different wires. The discriminator settings were left unchanged between successive sessions in order to ensure that the same unit was recorded across sessions. In addition, waveforms were checked for constancy before each recording session. Accepted spikes were converted to digital pulses that were counted for 20-ms intervals. At the end of each such interval (the end of a TV frame) the spike count for one or more cells was sent to a computer.

In parallel with spike discrimination, the rat's head position was determined by tracking the LED on the headstage. In use, the LED was on the midline, 1 cm above the head and somewhat forward of the rat's eyes. Tracking was done with a TV-based digital spot follower whose input was the red channel of the RGB signal from a ceiling-mounted CCD color camera. The optical axis of the camera was aimed at the middle of the central platform of the maze. The LED was detected at 50 Hz in a grid of 32×32 square regions (pixels) with each side 35 mm, and the *X* and *Y* coordinates were stored along with the spike data.

## Experimental protocols

Beginning 1 week after surgery, the activity from each microwire was screened daily while the rat performed the pellet-retrieving task. The electrodes were lowered over a period of several weeks while searching for unit waveforms of sufficient amplitude to be isolated. If no cell could be isolated, the electrode bundle was advanced 25–50 µm. In contrast, when a unit with clear location-specific firing was isolated, several recording sessions were done to see how modifications of the environment affected its location-specific activity. Between sessions, rats were returned to a small waiting cage (40×30 cm) near the maze. The cage was covered with a black curtain so that the rat could not see the experimental manipulations. The interval between two successive sessions was 5 min on average, during which the apparatus was cleaned and the appropriate changes were made to the arm configuration.

Three experiments were conducted. For each, the applicable protocol was repeated as many times as possible for each rat but only once for each cell or cell pair. Thus, each rat was exposed several times to different configurations of the maze in a certain order. Experiments 1 and 3 were done to determine the effects of rotating the maze away from its standard position in the laboratory; all rotations were made around the middle of the central platform of the maze. Experiment 2 was done to determine the effects of removing one or more parts of the maze. In all three experiments, the first session was a "standard session" such that the maze was placed in the laboratory with the cross part of the T nearest the south wall of the recording room (i.e., the situation experienced by the rat throughout training).

In experiment 1, the protocol consisted of five 16-min sessions in which standard sessions alternated with sessions such that the maze was rotated 90°, 180° or 270° from its standard position. The choice of the two rotation sessions was made according to where the cell's firing field was during the initial standard session. If the firing field of a cell was on an arm, the two rotations were selected so that an arm was always in the position of the field in the first standard session. If the field was on the central platform of the maze, the two rotations were randomly selected from the three possible rotations. These selection rules were slightly modified if two cells were available for simultaneous recording. If the cells had fields on different arms, the rotations were selected using the larger amplitude unit. If one field was on an arm and the other in the center, the rotations were selected using the arm field. Finally, random selection was done if both fields were on the central platform. The purpose of maze rotations was to measure effects induced by the conflict between the maze position and the distal environment, thereby allowing us to assess the contribution of the internal structure of the maze. The standard sessions allowed us to test if firing fields were stable in fixed conditions.

In experiment 2, the protocol consisted of four 16-min sessions. The T-maze was in its standard position in all sessions, but one arm was removed in session 2 and two arms were removed in session 3. Session 4 was a standard session with all maze arms. Selection rules for removals were similar to the rules used in experiment 1. Thus, if the field of the larger amplitude cell was on an arm, we removed, by random choice, either of the other two arms in session 2 and both of the non-field arms in session 3. After the arms were removed in sessions 2 and 3, their associated food pellet dispensers and Perspex barriers were reattached to the central platform and operated randomly to keep the reinforcement conditions as constant as possible.

In experiment 3, the five 16-min sessions consisted of a standard session, a +45° rotation, a standard session, a –45° rotation and a final standard session. Again, maze rotations were used to assess the contribution of the internal structure of the maze while standard sessions allowed us to test if firing fields were stable in fixed conditions.

In all experiments, the rat was simply required to retrieve food pellets randomly delivered at each arm end (i.e., the traditional pellet chasing task). Although no formal analysis of pellet chasing behavior was conducted, visual observations indicated that the rats were not disturbed in any obvious way by the changes made to the maze during the experiments. Even when arms were removed, rats just continued to run back and forth between food dispensers to get the pellets.

### Data presentation and analysis

To obtain a positional firing rate distribution, the total time the headlight was detected in each pixel (dwell time) and the total number of spikes fired in each pixel were accumulated for the session duration (usually 16 min). The rate in each pixel was the number of spikes divided by the dwell time. Color-coded rate maps were used to visualize positional firing distributions. In such maps, yellow pixels represented locations where the firing rate was exactly 0 Hz for the whole session. The higher firing rates were encoded in the order orange, red, green, blue and purple. Pixels that were never visited during a session were encoded white. 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 contiguous pixels with firing rate above the grand mean (position-independent) rate. Visual inspection of rate maps was used to classify firing fields into two categories. “Arm fields” had more than 80% of their active pixels in an arm during the first recording session. “Center fields” had more than 80% of their active pixels in the central platform. Cells whose field pixels were more evenly distributed were not analyzed. Cells with two fields in the maze were classified according to the location of their largest field.

Examination of the positional firing pattern of a cell in successive sessions was done to further classify cells into one of three categories orthogonal to the arm field/center field distinction:

1. Cells whose fields were stable relative in the laboratory frame regardless of how the maze was manipulated.
2. Cells whose fields were stable relative in the maze frame regardless of how the maze was manipulated.
3. Cells whose firing patterns remap. The field of such cells changed so that it was not in a constant position relative to either the laboratory frame or the maze frame, or so that cell became silent and no longer had a field anywhere on the maze.

Judgments of field positions based on visual inspection of rate maps were confirmed by numerical measurements of field rotation between session pairs. Pixel-by-pixel cross-correlations calculated as the positional firing patterns for the first and second sessions in the pair were rotated in 22.5° steps in opposite directions (resulting in 45° rotations of the firing patterns relative to each other).

## Results

### Experiment 1

In this experiment we examined the effects of 90° rotations of the maze so as to determine the contributions of the structure of the maze and of distal stimuli in the laboratory to location-specific firing. For example, if a field is tuned to a maze arm, maze rotation should be accompanied by equal field rotation. In contrast, a field tuned to the laboratory frame should be seen at the same place in the room regardless of which arm is rotated to that place. Note that rotations provide little information about whether fields on the central platform are predominantly controlled by the maze structure or the distal cues.

Twenty-four complex-spike cells (14 from CA1 and 10 from CA3) selected for robust location-specific firing were recorded from 4 rats. In 9 instances (3 rats contributing 18 cells), two cells were recorded simultaneously while in the remaining 6 cases, only one cell was recorded at a time. Thus, 15 sequences (range: 1–7/rat) of 5 recording sessions were analyzed.

When the maze was rotated by 90°, 180° or 270°, the fields of all cells were found in the same place in the laboratory, regardless of whether they were on an arm ($n$=13) or on the central platform ($n$=11). Thus, arm fields were not perturbed by the changed appearance of the maze-laboratory stimulus constellation nor by the change in the physical arm in the constant laboratory location (Fig. 2). Field stability in the laboratory frame was confirmed by calculating angular correlations which showed that it was not necessary to rotate one map against the other to produce high pixel-by-pixel correlations between pairs of standard sessions or pairs made up of one standard and one rotated session (Table 1). Moreover, rotations had no effect on either field size or field firing rate (Table 2). Last, the stability of firing fields was unaltered by the rats’ repeated experiences with maze rotations. Thus, firing fields were as stable on the first rotation as they were on the last rotation in the three rats that were subjected to several maze rotations over time.

In summary, place cell firing fields were stable following maze rotations of multiples of 90°. Although this outcome was obtained from a relatively small sample of cells, it was consistent across both hippocampal regions (i.e., CA1 and CA3) and recording sessions. Thus, it is very unlikely that the observed field stability is a sample from a population of unstable cells.

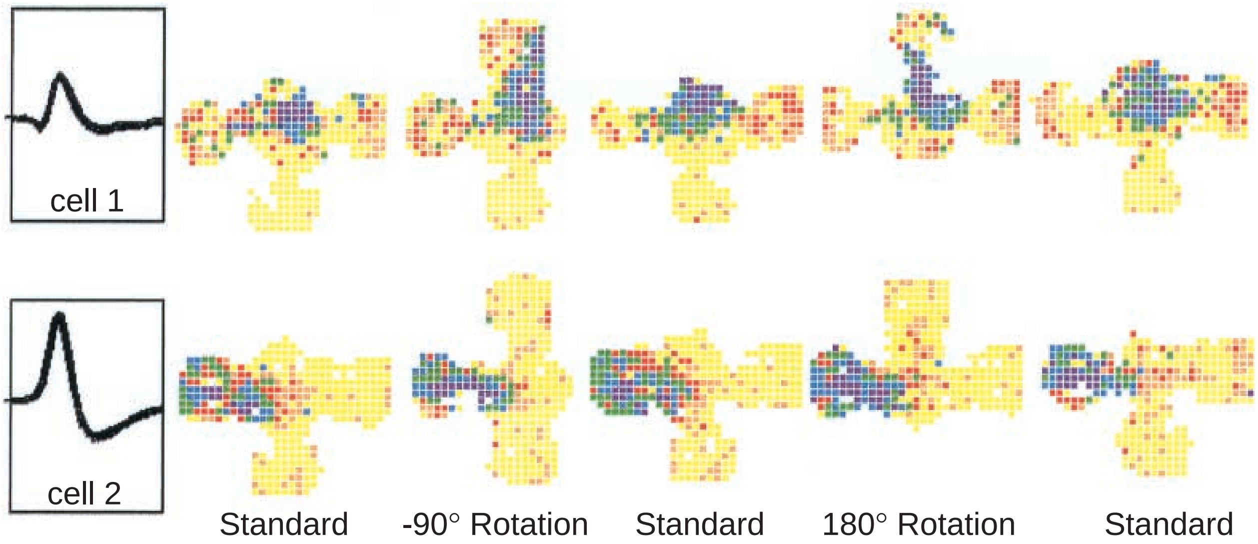

**Fig. 2** Firing rate maps of place cells in experiment 1. Cell 1 had its field in the central platform while cell 2 had its field in a maze arm. Both fields stayed stable relative to the laboratory after maze rotations, although an extension of the field of cell 1 in the arm was observed during rotation sessions. Waveforms for each cell are shown in *insets* (height of inset from baseline is 500 µV; width is 0.8 ms). Median firing rates for colors – cell 1: yellow, 0.0; orange, 0.8; red, 1.6; green, 3.1; blue, 7.1; purple, 18.9 AP/s – cell 2: 0.0; 0.5; 2.5; 6.1; 9.9; 16.9 AP/s. In all rate maps, the width of the visited area appears greater than the width of the actual arms and central platform due to the rats leaning over the apparatus

## Experiment 2

The first experiment revealed that firing fields are unaffected by 90° rotations of the maze, perhaps because only one arm is out of register with the laboratory. In the second experiment, we asked if firing fields would be perturbed by the more drastic manipulation of removing one or two maze arms with the maze left in its standard position. Arm removal affects the view of the environment from the firing field location and modifies the motions that are possible to the animal.

**Table 1** Mean angular correlation coefficients (± SE) in experiment 1. The large correlations between the rate maps of standard and rotation sessions indicate that the fields stayed stable relative to the laboratory

| | Center fields (*n*=11) | Arm fields (*n*=13) |
|---|---|---|
| Standard vs standard | 0.58±0.03 | 0.59±0.03 |
| Standard vs rotation | 0.49±0.05 | 0.59±0.04 |

Twenty-four place cells (16 from CA1 and 8 from CA3) recorded from the same four rats as in experiment 1 were again selected for robust location-specific activity in an initial standard session. Since two cells were simultaneously recorded in several instances, the number of recording sessions used for analysis was 15 in both one-arm and two-arm removal conditions (range: 1–7).

In 23/24 cases, the fields of these cells were unaffected by removing one or two of the maze arms, regardless of whether the field was on an arm or on the central platform (Fig. 3). The remaining cell ceased to fire after two arms were removed. However, the other five cells recorded from the same animal during sessions that occurred before and after the critical session were unaffected.

The lack of effect of arm removals was confirmed by pixel-by-pixel correlations between removal sessions and standard sessions; the average correlation was no different than for pairs of standard sessions (standard vs standard: $r$=0.36±0.03; standard vs one-arm removal: $r$=0.40±0.03; standard vs two-arm removal: $r$=0.32±0.04; $F_{2,23}$=3.02, NS). No changes in field firing rate were caused by arm removal although a small decrease in field size was observed, probably due to the reduced size of the apparatus (Table 3).

We conclude that arm removal left firing fields intact despite changes in the appearance of the environment and in possible trajectories. The consistency of this finding makes it unlikely that our sample of stable cells is drawn from an unstable population.

**Table 2** Mean field size and mean field firing rate (± SE) across successive sessions (experiment 1). No significant change was found in either measure of firing ($F_{(4,23)}$=0.05, NS, and $F_{(4,23)}$=2.22, NS, respectively)

| | Session 1 (standard) | Session 2 (rotation) | Session 3 (standard) | Session 4 (rotation) | Session 5 (standard) |
|---|---|---|---|---|---|
| Field size (pixels) | 54.3±6.5 | 54.2±6.7 | 55.4±6.3 | 55.5±6.8 | 55.6±7.2 |
| Field firing rate (Hz) | 5.9±0.6 | 7.3±0.6 | 6.7±0.6 | 7.3±0.7 | 6.9±0.8 |

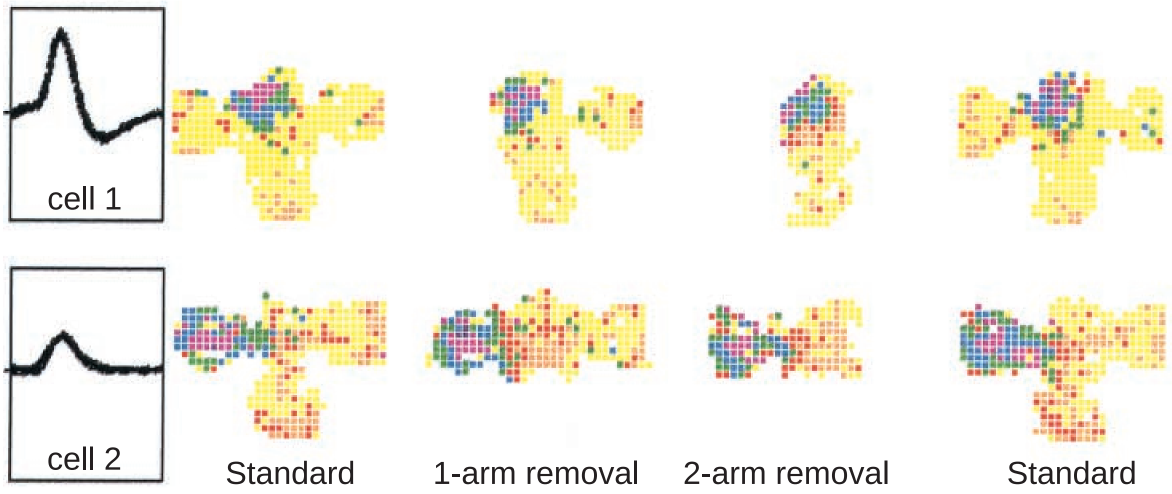

**Fig. 3** Firing rate maps of place cells in experiment 2. Cell 1 had its field in the central platform. Removal of either one arm or two arms did not alter the field characteristics. Cell 2 had its field in an arm. Removal of the other two arms also failed to alter the field characteristics. Waveforms for each cell are shown in *insets* (height of inset from baseline is 500 μV; width is 0.8 ms). Median firing rates for colors – cell 1: yellow, 0.0; orange, 0.4; red, 1.2; green, 3.6; blue, 6.6; purple, 14.9 AP/s – cell 2: 0.0; 0.5; 2.5; 6.1; 9.9; 16.9 AP/s

**Table 3** Mean field size and mean field firing rate (± SE) across successive sessions. No significant change was found in field firing rate ($F_{(3,22)}$=1.42, NS). A significant effect of session was found for field size ($F_{(3,22)}$=5.45, $P$<0.01). Field size was significantly diminished in session 3 when two arms were removed

| | Session 1<br>(standard) | Session 2<br>(one arm removed) | Session 3<br>(two arms removed) | Session 4<br>(standard) |
|---|---|---|---|---|
| Field size (pixels) | 54.6±7.3 | 50.7±5.9 | 38.7±4.3* | 55.6±6.3 |
| Field firing rate (Hz) | 6.4±0.8 | 7.1±0.6 | 7.9±0.8 | 7.4±0.7 |

*$P$<0.01 compared to session 1

### Experiment 3

This experiment examined the effects of +45° or –45° rotations of the maze. These rotations put the arms into new positions in the environment without affecting the position of the central platform. If the main sensory control over place cell firing were exerted by the layout of the maze whose asymmetric structure disambiguates all possible positions, we would expect firing fields to be unchanged in the reference frame of the maze (i.e., to rotate with the maze). In contrast, if distal stimuli are prepotent, we would expect major positional firing pattern changes for cells whose fields are on the arms. Intermediate cases are also possible; for example, if maze and distal cues exert similar amounts of control, cells might continue to fire in the standard-session fields but at lower rates.

For this experiment, 35 place cells were recorded from 5 rats. Twenty cells were from CA1 and 15 from CA3. Since no obvious difference was observed among cells in these two regions, they were all treated the same. The fields were nearly evenly divided between arms ($n$=18) and central platform ($n$=17).

Examples of spatial firing patterns for two arm cells are shown in Fig. 4, where it is clear that a 45° rotation can cause such a field to be suppressed (cell 1) or to undergo a drastic change in position with respect to both the maze and laboratory frames (cell 2). By inspection, the fields of 16 of the 18 arm cells were dramatically altered after both 45° rotations. The impression of strong arm field modifications by 45° rotations was corroborated by the high mean pixel-by-pixel correlation of 0.48 between pairs of standard session firing patterns and the low mean correlation of 0.09 between a 45° rotation session and a standard session (Table 4). The finding that 45° rotations strongly alter positional firing patterns on the arms is compatible with the idea that stimulus control over place cell discharge is exerted mainly by distal stimuli.

The conclusion that distal stimuli are prepotent can be directly tested by looking at the effects of 45° rotations on center fields where the distal stimuli are unchanged. The 17 cells with a center field were obtained from 4 rats in a total of 15 recording sequences (range: 3–5). In two instances (from two rats), two cells were simultaneously recorded. Typical firing patterns for center fields are shown in Fig. 5, where it is seen that a 45° rotation can also cause a center field to be suppressed (cell 1) or to move to a new position relative to both the maze and laboratory frames (cell 2). Inspection of firing

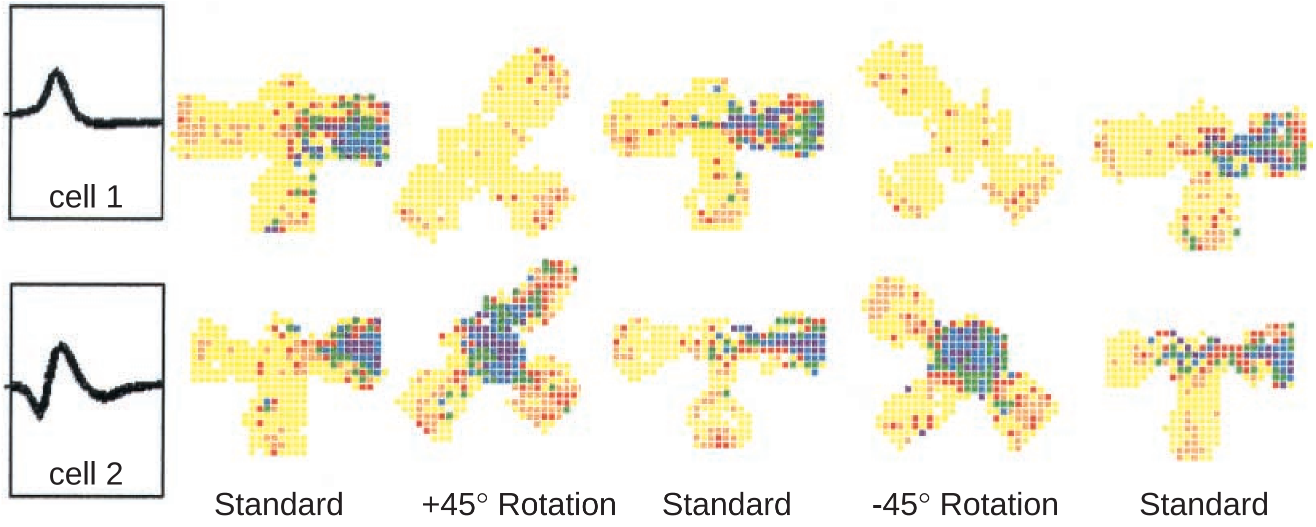

**Fig. 4** Firing rate maps of place cells with arm fields in experiment 3. Both cells had their fields in a maze arm. During rotation sessions, firing of cell 1 was considerably reduced whereas cell 2 developed a new field. The new patterns of activity were consistent across rotation sessions. Waveforms for each cell are shown in *insets* (height of inset from baseline is 500 μV; width is 0.8 ms). Median firing rates for colors – cell 1: yellow, 0.0; orange, 0.6; red, 2.0; green, 4.6; blue, 8.8; purple, 16.7 AP/s – cell 2: 0.0; 0.9; 2.0; 3.9; 12.0; 22.0 AP/s

**Table 4** Mean angular correlation coefficients (± SE) in experiment 3. The measure of interest for comparing two standard sessions is the correlation for a 0° rotation. The measures of interest for comparing a standard session to a rotation session are (1) the correlation when the two maps are rotated (i.e., superimposed) for either center or arm fields, but only (2) the correlation for a 0° rotation for center fields. These two measures show that the fields did not follow the 45° rotation of the maze and did not stay stable in the laboratory frame. The rate maps after 45° rotations were not simple rotational transforms of rate maps before rotations; rather completely new maps were obtained

| | Center fields ($n$=17) | Arm fields ($n$=18) |
|---|---|---|
| Standard vs standard | 0.48±0.04 | 0.48±0.05 |
| Standard vs 45° rotation | (1) 0.08±0.03<br>(2) 0.05±0.03 | (1) 0.09±0.04<br>NA |

rate maps indicates that the great majority (15 of 17) of center fields underwent major firing pattern changes. Once again, this impression is reinforced by pixel-by-pixel correlations; the mean was 0.48 for pairs of standard sessions and 0.05 for a 45° rotation session and a standard session (Table 4). Thus, the 45° rotations affected center fields as strongly as arm fields, despite the much greater changes of the maze + laboratory appearance from the arms than from the central platform. Regardless of field location, the changes induced by 45° rotations were reversible; all fields returned to the configuration seen in the first standard session during standard session 3 and, when still recordable, during standard session 5.

Since the rats were repeatedly exposed to 45°-maze rotations, it is possible that they came to “expect” the manipulation. No effect of temporal order on field changes was found, however. First, the two instances of center fields that were unaffected by maze rotation were bracketed by recording sessions in which strong changes were observed. Second, one of these two stable cells was recorded simultaneously with a cell whose firing pattern changed, suggesting a partial remapping in this case.

It is also interesting to compare positional firing patterns after the +45° to the patterns seen after the –45° rotation. This comparison was possible for 22/35 cells; 3 others were lost by the second rotation session and the remaining 10 had arm fields that happened to be accessible in only one of the two rotation sessions. For this subsample of 22 cells, we found that the mean correlation between +45° and –45° sessions was 0.39±0.05 when one maze image was superimposed on the other, a correlation that largely exceeds the value expected if the two maze images were uncorrelated. However, a $t$-test revealed that this mean correlation for +45° and –45° rotations was significantly less than the mean correlation found for 90° rotations in experiment 1 ($r=0.54\pm0.04$; $t_{44}=2.30$, $P<0.05$). The somewhat greater stability after the 90° than 45° rotations may be due to the repeated exposures of the animal to the standard position during pre-surgery training, cell screening and actual recording sessions. Figure 5 (cell 3) provides an illustration of the reduced stability of fields in the two 45° rotation sessions.

In conclusion, place cells switch between two distinct activity states, one for the standard maze position or its 90° rotation variants, and another for the +45° maze position and its 90° rotated variant (–45° position). These two states apply to most fields, even if they are on the central platform of the maze where the view of the environment is relatively constant. The consistency of these observations which were made in 13 out of 14 sequences in which a center field was seen makes it very unlikely that unstable center fields were drawn from a population of stable center fields.

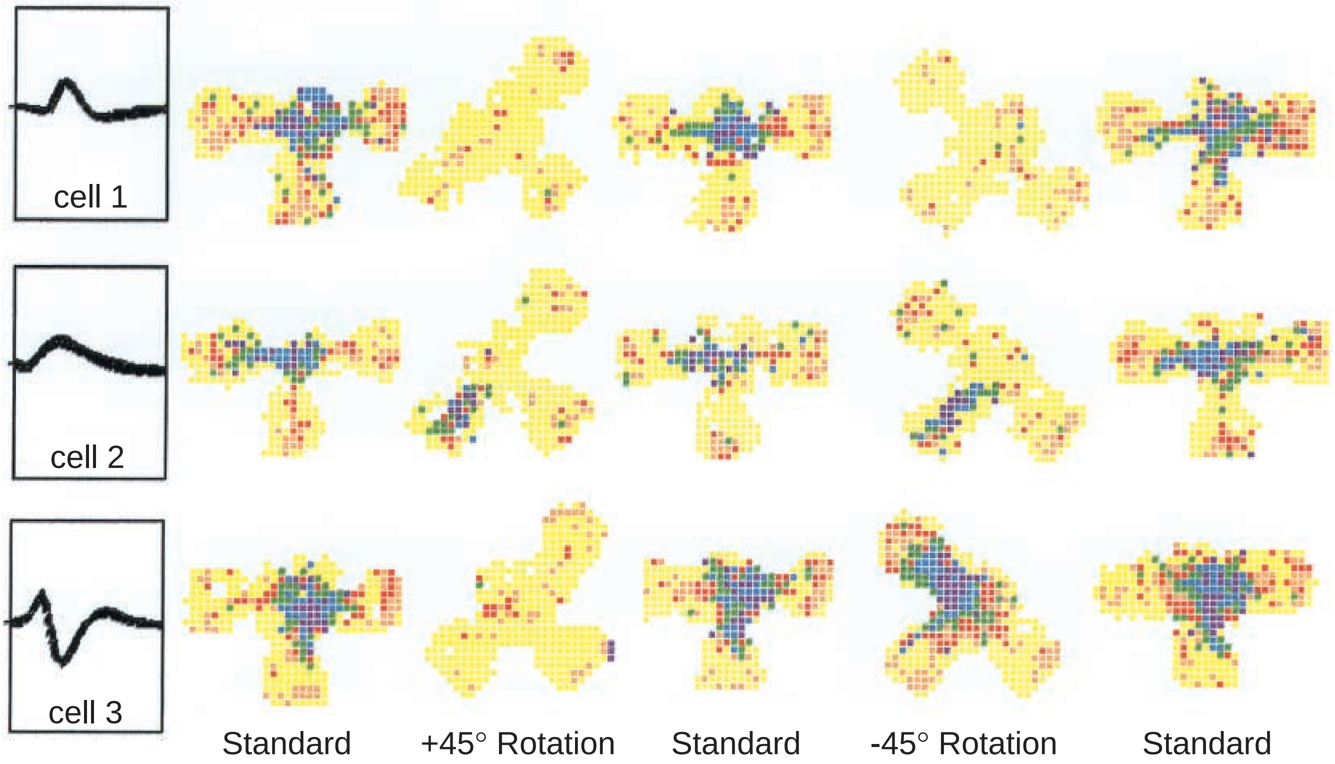

**Fig. 5** Firing rate maps of place cells with center fields in experiment 3. During rotation sessions, firing of cell 1 was considerably reduced; cell 2 developed a new field. For these two cells, the new patterns of activity were consistent across rotation sessions. In contrast, cell 3 extinguished its activity during the first (+45°) rotation session but developed a new field in the second (–45°) rotation session. Waveforms for each cell are shown in *insets* (height of inset from baseline is 500 µV; width is 0.8 ms). Median firing rates for colors – cell 1: yellow, 0.0; orange, 0.5; red, 1.6; green, 3.1; blue, 5.8; purple, 16.7 AP/s – cell 2: 0.0; 0.4; 1.0; 3.3; 7.8; 10.5 AP/s – cell 3: 0.0; 0.7; 2.2; 5.4; 10.0; 17.3 AP/s

## Discussion

Two main findings are reported in this paper. First, when the orientation of a T-maze in a laboratory room is altered by multiples of 90°, virtually all place cells continue to fire in the original position in the laboratory frame (experiment 1). For fields on the central platform, this is not a surprising outcome. For fields on an arm, it seems as if the only requirement for maintained firing was for the 90° rotation to bring one of the other two arms into the position at which the original arm lay during the standard session. Moreover, if the maze was rotated so as to not bring the arm into the position of the field, the only consequence was for the cell to become silent, as if the stimulus conditions necessary for discharge were never satisfied. Thus, even though the chirality of the T-maze provides enough information to distinguish all accessible locations from each other, place cells do not use this information. The lack of influence of the maze structure is reinforced by the constancy of firing field locations in the laboratory frame despite removal of one or two arms with the maze in its standard position (experiment 2). Two speculations about the minimal role of the maze structure are in order: (1) we imagine that adding a fourth arm to turn the T-maze into a + maze would have yielded similar results to experiment 1 so long as three of the arms lined up with the standard position of the arms of the maze; and (2) the relative importance of the T-maze structure in previous experiments (Shapiro et al. 1997; Tanila et al. 1997) might have been enhanced by putting differently textured surfaces onto each arm.

Our second main finding, anticipated by a single example in O'Keefe and Conway (1978), concerns the powerful effect of rotating the maze away from its standard position by +45° or –45°. Given the predominant control by distal stimuli, it is perhaps not unexpected that the firing patterns of fields on the arms are modified beyond recognition when the rat can get to previously inaccessible parts of the laboratory. In contrast, the idea that proper configurations of distal stimuli trigger activity in individual, independent place cells fails entirely to explain the remapping of cells with fields on the central platform where the available stimuli are largely unchanged.

These results suggest that there are two distinct activity states for place cells, one engaged by putting a rat on the maze in the standard position and the 90° variants and the other engaged by putting a rat on the maze in one of the 45° rotated positions. Thus, the place cell representation of the environment is modified as a unit and not piecewise according to whether locally available stimuli are very different or quite constant. The dramatic effects of 45° rotations might be due to setting up a conflict both between the maze position and room cues and between external sensory information and self-motion information. Thus, it should be recognized that 45° rotations disturb the relationship between all arms and the laboratory frame but also change the possible paths the animal can take in the environment. Both types of conflict may equally explain the effects observed after 45° rotations. In this view, the lack of effect in experiments 1 and 2 may simply mean that the changes brought to the maze did not generate a conflict great enough to activate a different hippocampal map. Alternatively, the effects of 45° rotations might be due to setting up a conflict between the directional and positional portions of the navigational system (e.g., Jeffery et al. 1997). With regard to this latter interpretation, it would be of great interest to see how preferred directions for head direction cells are affected by 90° and 45° rotations.

Why should 45° rotations produce such powerful effects on both arm cells and central platform cells despite great differences in the extent to which locally available stimuli are modified? We speculate that the answer lies in the mutually excitatory connections among CA3 place cells which may allow the hippocampus to form stable, cooperative ensembles to represent entire environments; these ensembles may be seen as attractors (Touretzky and Redish 1996; Samsonovitch and McNaughton 1997). In this view, the lack of correspondence for arm fields between the sensory stimuli available when the ensemble formed and the sensory stimuli available after 45° rotations provides an insufficient basis for recognition of the environment so that the ensemble for the standard position and its 90° variants is not activated. Moreover, because of the cooperative, mutually reinforcing nature of the proposed attractor, no part of the original representational ensemble is activated, even though the proper sensory stimuli are available for center fields. Instead, when the rat is put onto the maze after a 45° rotation, a new ensemble is formed and subsequently stabilized (Kentros et al. 1998).

It is important to note that whatever process is involved in activating an existing representational ensemble (or map) or otherwise forming a new map takes place rapidly. Thus, even though rats were always put onto the central platform of the maze at the start of all sessions, we saw no indication of initial firing of central platform cells followed by a switch to either silence or to a new field location. Although we have not done a formal analysis, this rapid establishment of the steady-state firing pattern may have occurred even on the first exposure to the 45° rotated maze, but surely was seen once the 45° orientation was familiar. This suggests that the rat can distinguish between the standard and 45° orientations as it is carried towards the maze or almost immediately upon touchdown. A similar effect was seen by Sharp et al. (1990), who showed that changing by 180° the entry point of a rat into a cylinder with two visually identical cue cards set 180° apart is the critical determinant of whether the field will be in register with one card or the other. In that study, self-motion (i.e., path integration) information may have set the session-specific relationship between firing fields and stimuli. In the present case, the fact that the animal was carried into the environment in the same way and always put onto the central platform plus the very fast activation of the relevant map suggests that visual stimuli may be sufficient to select a session-specific map. Note, however, that a role for idiothetic cues interacting with visual information cannot be formally discarded since, as discussed above, the changes induced by 45° maze rotations precluded the rat from using previously available moving directions in the laboratory.

In conclusion, the present study provides another example of combinatorial coding by the hippocampus by demonstrating that the firing patterns of place cells are sensitive to the orientation of a T-maze relative to the laboratory frame. Additionally, the hippocampal map of the environment was found to be modified as a unit and not piecewise. These findings open up several possibilities for further experiments. For example, would similar effects be observed if the sensory conflicts are generated by rotating controlled cues while keeping the maze stable relative to the curtained environment? We speculate that the outcome will depend on the degree of control exerted by the manipulated cues. Another issue is whether turning a + maze into a eight-arm radial maze will yield similar remappings even though a fraction of the maze arms point in familiar directions.

In addition, the present findings suggest that there may be a functional interaction between the directional and locational components of spatial information processing. Such an interaction is presumably supported by the strong coupling that seems to exist between hippocampal place cells and head direction cells (Knierim et al. 1995; Taube 1998). Obviously, it will be important to record head direction cells under similar circumstances to confirm this hypothesis.

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