On the Behavioral Significance of Head Direction Cells: Neural and Behavioral Dynamics During Spatial Memory Tasks

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Current theories assume that rats use the directional information reflected by head direction (HD) cells when performing spatial tasks. This assumption was assessed by monitoring anterior thalamic HD cell activity and relating it to the subject’s behavioral response on 2 spatial memory tasks that tested either reference memory or working memory. In both tasks, there was a significant number of trials where there was not a tight coupling between the preferred firing direction of HD cells and the direction of the behavioral response. In addition, it was possible to intentionally change the preferred direction of HD cells without affecting performance accuracy. An additional experiment showed that manipulations that affected internal, but not external, cues impaired performance on the reference memory task. These findings suggest that HD cell activity was not consistently guiding the subjects’ behavior on these 2 spatial tasks.

For most animals, the ability to navigate effectively throughout the environment is important for survival. Rodents in particular have developed a number of sophisticated navigational strategies that allow for extended journeys to and from their nests. The versatility and efficiency demonstrated by rats when solving various types of behavioral tasks led to the proposal that they construct a holistic representation of their environment (cognitive map; Gallistel, 1990; O’Keefe & Nadel, 1978; Poucet, 1993; Tolman, 1948). A representation of the directions and distances of items in the environment relative to each other, as well as to the rat’s present location, constitutes the basic elements of a cognitive map (O’Keefe & Nadel, 1978).

A fundamental question to address is how behavior that is based on cognitive map theory is instantiated at the neurobiological level. Recordings from neurons throughout the brain strongly suggest the existence of an anatomically distributed network that represents allocentric (“world-centered”) locations and directions. Specifically, neurons that preferentially discharge depending on the animal’s location (place cells) or directional heading (head direction [HD] cells) have been identified in the limbic system (for reviews, see Muller, 1996; O’Mara, 1995; Taube, 1998).

Numerous studies have examined the influence of sensory or environmental manipulations on the activity of HD and place cells (Blair & Sharp, 1996; Goodridge & Taube, 1995; Muller & Kubie, 1987; O’Keefe & Conway, 1978; Sharp, Kubie, & Muller, 1990; Taube, Muller, & Ranck, 1990b). It has been assumed that HD and place cells provide the animal with a continuous indication of its orientation in space and enable it to navigate through its environment. How HD and place cells are functionally related to an animal’s performance on spatial navigation tasks has, however, received sparse empirical attention.

In the first study of place cells that directly examined the issue of their behavioral relevance, O’Keefe and Speakman (1987) suggested that an animal’s spatial representation of its position in relation to an array of cues is preserved by the population of place cells after the cues are removed. Moreover, in a small set of control trials in which rats were not exposed to the cue array beforehand, the firing of place cells corresponded to the rat’s behavioral choice, even when the response was directed toward an incorrect location. These findings suggest that the rat used the location information provided by place cells when generating its behavioral response.

HD cells in the laterodorsal thalamic nucleus have been recorded while rats performed a spatial working memory task on the eight-arm radial maze (Mizumori & Williams, 1993). Mizumori and Williams reported that the magnitude of directional specificity displayed by these HD cells was usually inversely correlated with the number of errors committed during acquisition and under various environmental manipulations. However, in at least one clear instance, the rats performed poorly, even though the directional stability of HD cells was high, suggesting that the directional information from these cells was not always sufficient for accurate performance. Dudchenko and Taube (1997) examined the relationship between HD cell functioning and behavior in greater detail. Their findings suggested that a functional relationship between HD cell activity and the animal’s behavioral choice developed over the course of training. However, the interpretation of these findings is

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limited by the fact that the results are correlational in nature, because the animal’s behavioral choice and the preferred firing directions were both established relative to the same cue. Thus, most of the results showing that HD cells and behavioral response are correlated could also be explained by their common relationship with the cue.

The following experiments were designed to assess the extent to which the HD cell system is functionally related to the animal’s behavioral response. We approached this question by varying the orientation of HD cells and observing the behavioral consequences (Experiment 1), or by varying the learned behavioral response and observing the neuronal consequences in HD cells (Experiment 3). Experiment 2 involved environmental manipulations that assessed the types of cues the rats used to solve the task in Experiment 1.

**Experiment 1**

This experiment was based on previous findings that HD cells have different preferred firing directions when an animal is placed in a differently shaped enclosure within the same room (Taube et al., 1990b). Experiment 1 consisted of two parts. First, rats performed a spatial reference memory task while we monitored HD cell activity. Second, the orientation of the preferred directions of HD cells was intentionally shifted by exposing the rats to a novel, differently shaped environment that still permitted them to perform the well-learned memory task. For both parts of the experiment, if the rat was relying on information from the HD cell signal to generate its behavioral response, then it would be possible to accurately predict its subsequent behavioral choice by knowing the orientation of the HD cell system at the beginning of the trial.

**Method**

**Subjects**

Four female Long-Evans rats between 4 and 9 months old were used in the experiment. The rats were housed individually and maintained on a 14:10-hr light-dark cycle. For the training and data collection portions of the experiments, the rats were water restricted, receiving water ad libitum for 10–30 min/day at the end of training or testing sessions. During the cell screening and data collection phases (see below), the rats were also mildly food restricted (~15 g/day). Rats were examined regularly for signs of dehydration and/or weight loss. Occasionally, ad-lib food and water were reinstituted for 1–2 days.

**Apparatus and Training Procedures**

Rats were trained inside a gray square arena (69 cm × 69 cm, 51 cm high) that contained a white cue card along one wall. Four identical recessed opaque water cups (4.5 cm × 4.5 cm × 1.0 cm) were placed in the corners (one per corner). Removable gray photographic backdrop paper covered the arena floor. A transparent square Plexiglas corral (28 cm/ side, 28 cm high) was used to prevent the rats from searching the entire arena at the beginning of each experimental trial (described below). The arena was surrounded by a black circular curtain (2 m in diameter) that spanned from floor to ceiling. Four direct current lights were symmetrically arranged above the curtained area. A speaker hidden in the ceiling rafters and centered above the square enclosure provided constant background white noise.

The rats were handled for at least 2 days before their first exposure to the experimental apparatus. The water deprivation schedule commenced the evening before the first day of training. Rats were brought to the experimental room in their home cages, which allowed them to view portions of the room outside the circular curtains. There was no attempt to disorient the rat prior to its entry into the apparatus in either the training or test sessions.

During the initial training sessions, the rats were permitted to explore the arena for approximately 10–15 min. For each rat, one of the four corners relative to the cue card was randomly assigned to contain the water reinforcer (~0.1 ml). The remaining three corners were never baited with water. The rewarded corner assigned to each rat was maintained throughout all training and testing trials. Therefore, this experiment used a spatial reference memory procedure. After 2–4 days, the rats reliably approached a corner immediately after being placed in the center of the arena. Daily training consisted of 16 trials. The cage was located outside the arena at one of four cardinal-point positions centered along the arena wall. The rat was removed from its cage and set down in the middle of the arena facing the center of one wall (release direction). During training, the rat was allowed to search two corners when attempting to obtain the water reinforcer. After the rat made its corner choice (or choices) it was removed from the arena and returned to its home cage. With the cage placed at one of the four cardinal-point peripheral locations, four trials were conducted with the rat’s release direction varying across the four cardinal directions. Every combination of cage location and release direction was given during each block of 16 trials, in a pseudorandom order. The sequence of cage locations outside the square apparatus was also pseudorandomized across the 16 trials. An error was scored when the rat approached the water cup at an unbaited corner. Rats usually ran vigorously toward the corners and placed their heads above or inside the water cup. This behavioral response pattern allowed for the unambiguous determination of error choices. The number of errors on each trial (zero, one, or failure if the rat did not obtain the reward after two choices) was noted by the experimenter. Subjects achieved criterion performance level once they approached the baited corner on their first search for at least 13 out of 16 trials on 2 consecutive days. After a rat had achieved criterion performance levels, an array of driveable recording electrodes was surgically implanted into its anterodorsal thalamic nucleus (ADN), as described below.

**Experimental Design**

When HD cells were monitored during testing, each session began with a 4-min baseline recording session with the rat inside the square arena (see Figure 1A). The water cups were removed from the arena during the baseline session, and the rats moved freely around the arena. This baseline session was used as a reference to compare the stability of HD cell preferred firing directions across subsequent trials. After the baseline session, eight test trials were conducted on the reference memory task. During test trials, an opaque box was substituted for the rat’s cage. The first four trials were run with the box at one peripheral location and the release direction varied across the four cardinal points. The box was then moved to a different location and a second set of four trials was run, with each trial again having a different release direction. After the eight test trials, another baseline session was conducted, followed by another eight test trials that used the two box locations not used in the first eight trials. For each group of 16 standard test trials (containing every combination of box location and release direction), two baseline sessions were conducted, one before each set of eight trials. There were no indications of fewer correct responses during the reference memory trials after baseline sessions. This same pattern of alternating baseline sessions with eight trials was also used throughout the experimental manipulations described below.

The experiment consisted of three types of sessions: standard, cue rotation, and rectangle (Figure 1B). Standard and cue rotation sessions were conducted in a square enclosure, and rectangle sessions were conducted in a rectangular enclosure. Between 8 and 24 sessions were conducted on a given day. Standard sessions always preceded and followed cue rotation sessions. For each rat, standard and cue rotation sessions were recorded for at least 2 days before the rat performed any rectangle sessions.
Figure 1. Overhead view of the experimental environment. A: Example of the baseline-trial sequence used throughout the experiment. The initial baseline session served as a comparison for the subsequent eight trials. The location of the intra-arena cue card is illustrated by the vertical line to the right of the square. After eight trials, the sequence was repeated: a baseline session followed by eight more trials with the box at the other two locations. Circles indicate the location of the four water cups, one of which (filled circle) contained the water reward. Arrows indicate the four orthogonal release directions for the rat during each group of four trials. The box location is shown at the 12:00 position for the first four trials, then at the 3:00 position for the second four trials (note that the same side of the box is always facing the arena wall). B: Diagram of the apparatus setup for each of the three experimental phases. The angular label for each corner is shown in the standard session. The labels for each corner of the apparatus varied across rats, as the angular corner labels are relative to the rewarded corner, which differed across rats. The same nomenclature was used in the rotation and novel sessions, with the values shifting by 90° counterclockwise in the cue rotation session. The horizontal or vertical line in each condition indicates the position of the intra-arena cue card.

Rectangle sessions were conducted over 1–4 days and were always preceded by one or two standard sessions.

In the standard sessions, HD cells were recorded while the rat performed the reference memory task in the square arena described above. The only difference between the training and standard sessions was the introduction of the transparent Plexiglas corral (32 cm long × 22 cm wide × 28 cm high) at the start of each trial. Confining the rat inside the corral permitted an accurate measurement of an HD cell’s preferred firing direction before the rat made its behavioral response. The use of the corral did not appear to affect the preferred direction of HD cells. At the beginning of a trial, the subject was removed from the opaque box and placed directly into the corral at the center of the arena. For approximately 1 min, the rat moved freely within the confines of the corral while HD cell activity was monitored. After 1 min, the rat was lifted approximately 15 cm and the corral was quickly removed from the arena. The rat was then re-placed in the center of the arena facing one of the walls and was permitted to approach one corner. During the test trials, rats were only permitted to approach one corner. After the rat chose a corner, it was removed from the arena, placed inside the opaque holding box, and the corner it had approached was noted. A sequence of trials similar to the training sessions was performed, with the release direction and box location varied. In the rotation sessions, the cue card was affixed to an adjacent wall, ±90° from its usual position. The floor paper was also changed to remove possible lingering olfactory cues. The rat was gently spun inside the box with the lid closed (~15 rpm) in an attempt to prevent it from maintaining its orientation on the basis of internal cue sources, and then reattached to the recording cable. Previous studies have shown that under these conditions, the preferred firing direction of HD cells usually shifts in conjunction with angular rotation of the cue card (Taube et al., 1990b). The main purpose of the rotation sessions was to demonstrate that the rats were using the position of the cue card to determine which corner to approach to obtain the reward.

The purpose of the rectangle phase was to change the preferred firing direction of an HD cell and then observe the consequences on the rat’s behavioral choices (Figure 1B). The challenge was to design a manipulation that would induce a shift in the preferred directions of HD cells yet would not perturb the subject’s behavior to such a degree that it would have to acquire a new task. This goal was accomplished by introducing a novel, rectangular-shaped arena (120 cm × 60 cm) that also contained a white cue card along one wall. The change in arena shape is usually sufficient to induce a shift in preferred direction (Golob & Taube, 1997; Taube et al., 1990b), whereas the presence of a cue card and the geometric similarity to the square allow the rat to generalize its behavioral response from its experience in the square. The same corner that had been baited with water in the square, relative to the cue card’s location, was baited with water in the rectangular arena. Rats were placed into the clear rectangular Plexiglas enclosure for approximately 1 min before each trial. They were then taken out of the Plexiglas corral, placed in the center of the arena facing one wall, and allowed to approach a corner. As in the other experimental phases, the release direction and the location of the box were varied pseudorandomly. The rat’s corner choice was recorded for each trial. For 1 rat, the floor paper was replaced with a wooden floor, which was periodically cleaned with a sponge between trials.

Surgical Procedures

Before surgery, the rats were deeply anesthetized with pentobarbital (45 mg/kg) and given 0.1 cc atropine to prevent respiratory complications. Recording electrodes were constructed according to the procedure described by Kubie (1984). Briefly, 10 nichrome wires, 25 μm in diameter, were passed through a steel cannula and attached to a modified aught connector. The aught connector was in turn attached to three screws above the surface of the skull with dental acrylic, allowing the electrode array to be adjustable in the dorsoventral plane. The screws were then permanently cemented onto the rat’s skull with grip cement (Dentsply International, Milford, DE). Recording electrodes were directed toward the ADN in accordance with stereotaxic procedures from a standardized atlas (Paxinos & Watson, 1986). The coordinates relative to bregma were AP ~−1.3, ML +1.4, and DV −4.0.

HD Cell Screening and Data Acquisition

For cell screening, the rats foraged for food pellets within a 76-cm diameter cylindrical arena that contained a large white cue card occupying ~100° of arc. The arena was located inside a circular black curtain 2 m in diameter that spanned from floor to ceiling. A cable attached to the rat’s headstage was connected to an overhead commutator. This setup permitted the rats to walk uncumbered inside the arena while we monitored the activity on each of the 10 electrode wires. If an HD cell was not identified, the electrodes were advanced ventrally 30–120 μm.

Once the waveform of an HD cell was sufficiently isolated above background noise, its activity was recorded while we monitored the rat’s directional heading with a two-spot video tracking system (Eberle Electronics, Brooklyn, NY). Two LEDs, separated by ~10 cm, were used to indicate the rat’s directional heading. A red LED was positioned above the rat’s snout, and a green LED was located over the rat’s back, at the midline when its head was facing forward. The electrode signal was preamplified through a field-effect transistor in a source-follower configuration, then amplified and bandpass filtered (300 to 10,000 Hz). Cell spikes were
isolated with a series of three time-amplitude window discriminators (Bak Electronics, Germantown, MD), displayed on an oscilloscope, and saved onto a computer by using a National Instruments data acquisition board with LabView software (National Instruments, Austin, TX). Neuronal spike activity and the location of the two LEDs were sampled at 60 Hz. Video recordings of the rat's performance during the experiments were also collected from the overhead video camera.

**Data Analysis**

Graphs of firing rate versus HD were plotted by dividing the rat's HD into 60 bins of 6° each and calculating the mean firing rate of each bin. A cell's firing properties (e.g., peak firing rate, preferred direction, firing range) were determined by fitting a triangular model to the firing rate-versus-HD graphs (see Taube, Muller, & Ranck, 1990a, for details). For most trials in the experiment, electrophysiological data were continuously collected by the computer during four-trial blocks. Specific trials within a recording session were extracted from the data stream off-line by referring to the videotape. There were usually 15–30 s between trials, providing ample time to isolate the neuronal activity that occurred during a particular trial without danger of classifying the neuronal data to a different trial. This procedure removed neuronal activity that occurred during the inter-trial intervals from the analysis.

By mathematical convention, we assigned positive and negative signs for counterclockwise (CCW) and clockwise (CW) directional shifts, respectively. To quantify the difference in an HD cell's preferred direction between two sessions, or between two discrete portions within a single recording session, we used an algorithm that maximized the cross-correlation between the firing rate-versus-HD functions for the two sessions (Taube et al., 1990a). The function from one session was shifted in 6° increments relative to the function of the second session. The angular shift that yielded the maximum cross-correlation value (Pearson's r) was defined as the difference in preferred direction between sessions.

Behavioral measures were analyzed in two forms—either by descriptive categories (correct or error) or through a numerical description that assigned a degree value to each corner of the apparatus (see Figure 1B). Angular values for each corner were assigned relative to the location of the correct corner, which was denoted by 0°. As with the calculation of angular shift in preferred direction, angular values increase in the CCW direction. For simplicity, the corners in the rectangle were also assigned angular values of 0°, 90°, 180°, and 270° relative to the correct corner.

Analyses of variance (ANOVAs) were used for noncategorical data. For all statistical tests, significance was set at the .05 level.

**Histology**

The electrodes were advanced between 2.0 and 2.5 mm before the screening procedures were terminated. Once the experiments were complete, the rats were given a lethal overdose of pentobarbital. An anodal current (15 μA for 15 s) was passed through one of the electrode wires used to record an HD cell so that the recording site could be determined later by using a Prussian blue reaction. The rats were perfused with saline followed by a 10% formalin solution in saline (100 ml, 3% formaldehyde; 900 ml, 0.9% NaCl solution). The brains were then removed and soaked in 10% formalin for at least 48 hr. Potassium ferrocyanide (–2%) was added to the formalin solution for 24 hr, followed by a rinsing of the brains in formalin for 24 hr. The brains were then placed in a 20% sucrose solution for 48 hr, after which they were cut into 30-μm sections. Sections were stained with cresyl violet and examined microscopically to determine the recording site.

**Results**

HD cell activity and behavioral data were collected from 4 rats. A total of 299 trials were performed in the three experimental phases (145 standard, 48 cue rotation, and 106 rectangular arena trials). Seventeen HD cells (range: 3–6 per rat) were recorded during these sessions.

**Behavioral Results: Standard and Cue Rotation Sessions**

Training to criterion performance levels required between 204 and 250 trials. Choice responses were usually rapid, continuous movements toward one of the four corners that were initiated directly after the rats were released. The number of correct choices is expressed as a mean percentage across trials for a particular session type because the number of trials varied across rats. The mean (± SEM) percentages of correct choices on standard and cue rotation trials across all rats were 77 ± 4 and 80 ± 4, respectively. A one-way repeated measures ANOVA of percentage correct revealed no significant difference in performance across test phase, F(1, 3) = 1.65, ns. Thus, the rats appeared to be determining which corner to search on the basis of the relative position of the cue card, a finding that was a prerequisite for conducting the rectangle session phase of the experiment.

**HD cells recorded during standard trials.** Observation of the firing rate by HD plots did not reveal any systematic changes in the peak firing rate or directional firing range of HD cells across trials or as a function of the rat's corner choice. In the majority of standard session trials (77%; 112/145 trials) the preferred firing direction was similar to the value recorded during the baseline session a few minutes earlier (see Figure 2A). However, in some trials (23%; 33/145), there were large changes in the cell's preferred firing direction when compared with the baseline session (Figure 2B). The percentages of trials with directional shifts of at least 72° for each rat were 14%, 16%, 20%, and 41%. This instability was not expected, because the intertrial interval was only ~15 s, the rats were not intentionally disoriented, and previous studies have shown that the preferred direction of an HD cell is stable within an environment for several weeks of daily sessions (Golob & Taube, 1997; Taube et al., 1990b).

It is interesting that, on trials in which the preferred firing direction shifted, the magnitude of the shift was always a multiple of 90° ± 18° (i.e., 90°, 180°, 270°). Most of the shifts (88%) were either 90° or 270°. An example of HD cell responses across standard sessions in one rat is depicted in Figure 3A. On most trials (80%) the cell's preferred direction was similar to that of the previous baseline session. On two occasions, however, the cell shifted ~90° either CW or CCW for four trials before returning to the direction recorded in the baseline session. One possibility for the variability in preferred firing direction is that the holding box sometimes served as a reference point for calibrating HD cell orientation. To test this possibility, we analyzed 14 four-trial blocks from all rats that contained at least one instance of a directional shift. The box was at a constant position within the room throughout the four-trial series. Figure 3B shows the number of times a shift in preferred direction occurred as a function of trial number order in each four-trial sequence. During nine series, a directional shift was observed on the first trial after the box was moved to a different location outside of the arena. In 3 out of 4 rats, the shift always occurred on the first trial and maintained its firing direction through the fourth trial, with the remaining rat accounting for all shifts on Trials 2–4. Thus, once an HD cell
shifted its preferred direction, it usually maintained this orientation until the box was moved to a new location.

Table 1 illustrates the shift in preferred direction for the 14 four-trial blocks as a function of the angular change in box location. As an example, moving the box from the 12:00 position to the 9:00 position was categorized as a 90° angular shift; 12:00 to 6:00, a 180° shift, and so on. If the amount of preferred direction shift reflected the angular change in box location in the preferred direction, the results from most four-block trials would fall along the diagonal from the upper left to lower right corners of Table 1. As the table shows, less than half of the blocks were on the diagonal, indicating that a strong relationship was not present between the specific change in box location and the preferred direction shift.

To determine whether the instability in the preferred firing direction was related to learning the task or was instead a consequence of handling procedures during the task, we recorded 2 HD cells from 2 rats that were not trained on this reference memory task. In a series of 32 “trials” the rats were introduced into the Plexiglas corral for ~1 min, permitted to walk freely inside the entire arena for ~5 s, and then returned to the holding box. Release direction and box location were varied pseudorandomly as described above. Eleven of the 64 trials resulted in a shift in the cell’s preferred direction (four 90° shifts, four 180° shifts, and three 270° shifts). The shifts were all observed on the first trial of the four-trial blocks, usually persisted through the fourth trial, and were not related to the angular change in box location. These data suggest that the directional shifts were not a consequence of learning the reference memory task.
Table 1
Preferred Firing Direction Shift as a Function of Angular Change in Box Location

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<th>Box location (angular change)</th>
<th>90°</th>
<th>180°</th>
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<tr>
<td>90°</td>
<td>2</td>
<td>1</td>
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<td>180°</td>
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Note. Data represent the number of shifts over 14 four-trial blocks.

In summary, during the standard sessions in the square arena, HD cells usually maintained a consistent preferred direction across trials. However, in some trials, we observed large changes in preferred direction that were increments of ~90°. The observations that the directional shifts tended to occur on trials immediately after the holding box location was changed, and that the shift in preferred direction was maintained until the box was moved again, suggest that box movements can be sufficient for inducing changes in the preferred direction of HD cells. Nevertheless, the absence of a systematic relationship between changes in orientation of the box and HD cell activity suggest that the box was not used as a reference frame.

**HD cells recorded during cue rotation sessions.** Previous studies have shown that when the cue card is rotated along the inside walls of an arena, the preferred directions of HD cells usually shift by a similar amount (Taube, 1995; Taube et al., 1990b). In the present experiments, we first compared the preferred firing directions between the preceding 4-min baseline standard session and the initial baseline session in cue rotation sessions. Across five comparisons of HD cell activity in adjacent standard and rotation baseline sessions, the preferred direction shifted along with cue card rotations (either 90° or 270°) in three of five sessions. In the two sessions with inaccurate cue control, the cells shifted 180° and 270° in response to a 90° cue rotation. Although these results suggest that the cue card exerted less accurate control over the preferred firing directions than in our previous experiments, it is noteworthy that the HD cells changed their preferred directions in all five rotation sessions, indicating that the HD cell system detected the change in cue location.

When analyzed across test trials, the preferred direction was consistent with the baseline rotation session on 31 out of 48 cue rotation trials (65.6%), a value somewhat less than what was observed in the standard sessions (77%). In 13 out of the 17 directional shifts, the cell’s preferred firing direction reverted back to the orientation observed during the standard sessions, suggesting that additional cues (stable room–arena cues and/or internally generated cues) that were available during both session types competed for control of the preferred direction. Conflicts between these different cue sources caused by rotating the cue card are likely to account for the greater instability in the rotation sessions as compared with standard sessions.

Results from the rotation sessions are consistent with the instability observed across trials in standard sessions described above. The preferred firing direction inconsistency may be symptomatic of the cue card having a reduced ability to become the predominant cue source for orienting the HD cell system under these experimental conditions. Nonetheless, it is noteworthy that even with a variable preferred direction, the rats were still able to select the correct corner in the cue rotation sessions most of the time (80% correct).

**Behavioral Performance and HD Cell Activity During Rectangle Sessions**

The rectangle phase of the experiment was also designed to test the hypothesis that the rat’s behavioral response on the reference memory task is reflected by the information conveyed by HD cells. A shift in preferred direction when the rat was in the rectangular environment was a necessary component of this assessment. If the preferred direction of a recorded HD cell shifted in the rectangle, would the behavioral response (i.e., corner choice) also reflect this same shift? In 12 out of 13 rectangle sessions, the preferred direction shifted relative to the square, implying that the HD cell system was sensitive to geometric properties of the arena (Cheng & Gallistel, 1984). HD cells in all 4 rats exhibited a directional shift in at least two rectangle sessions. As was found in the square sessions, the directional shifts were also in multiples of 90° ± 18°, with 10 out of 12 shifts of ~270°.

We also examined the consistency of the preferred direction of each HD cell across trials in the rectangular arena compared with the rectangle baseline sessions. The preferred direction in the rectangle test trial was consistent with the previous rectangle baseline session in 102 out of 114 trials (90%). Three of the 4 rats did not exhibit changes in preferred direction during trials in the rectangular environment (0/90 trials). The greater stability of the HD cell’s preferred direction in the rectangle might have been facilitated by the introduction of semipolarizing geometric information from the rectangle in addition to the cue card.

The rats were first tested in the rectangular environment after receiving from 40 to 56 trials in the standard and cue rotation conditions. The rat’s corner selections indicated that they readily generalized from their training experience in the square when placed in the rectangular environment. There were 114 trials in the rectangle. In 106 of these trials (obtained from 12 sessions), there were large shifts in preferred firing direction (in multiples of ~90° ± 18°) compared with the square baseline session. The remaining eight trials were from one session in 1 rat in which the preferred direction did not shift relative to the square. These trials were not included in the analysis. Although the HD cells usually shifted their preferred direction by these large amounts, the mean percentage of correct choices in the rectangle was about the same as the value observed in the standard sessions in the square (78% vs. 77%, respectively). On the first four trials in the rectangular environment, rats approached the correct corner on 12 of 16 trials (75%), indicating that their responses were rapidly generalized from their training in the square.

The finding that HD cells adopted a different orientation in the rectangle, in conjunction with the behavioral data showing that the rats were proficient at choosing the correct corner, provides evidence that an association between the HD cell system and the rat’s behavioral response was not firmly established, if at all, during their training in the square. Thus, these results do not support the hypothesis that the rat’s behavioral choice depended on the orientation of ADN HD cells in this spatial task.
Assessment of Behavioral Choice as a Function of Preferred Direction

In the preceding analysis, we described the behavioral results that followed a shift in preferred firing direction in the rectangle. In this section, we examine the relationship between the behavioral and neuronal levels of analysis across trials in the standard sessions. HD cell activity and the rat’s corner choice on each trial were categorized according to whether the two measures were consistent with each other. The angular corner labels described above (0°, 90°, 180°, and 270°), were used to compare the rat’s corner choice on each trial with changes in the preferred direction of HD cells relative to baseline (also labeled 0°, 90°, 180°, or 270°). Behavioral and neural measures were considered to be either concordant (corner choice and preferred firing direction relative to the previous baseline session were the same) or discordant (corner choice and preferred direction relative to baseline differed). For example, if on a particular trial the rat approached the correct corner (0°), but the preferred direction shifted by ~90°, that trial would be categorized as discordant. Because the rats were trained to reliably choose the correct corner (0°), and HD cells were usually consistent across trials within a session (0° change), we would expect the majority of trials to be concordant. Error trials could also be concordant if the shift in preferred direction matched the rat’s erroneous corner choice. Three types of discordant trials were possible: (1) preferred direction shift–correct choice; (2) preferred direction consistent–choice error; or (3) preferred direction shift and choice error but not by the same angular value. The first type of discordant trial was the most interesting situation because it removed the interpretational issue of why the rat may have chosen the incorrect corner on trials for which the preferred direction remained consistent. In the first discordant condition (preferred direction shift–correct choice), we presume the rat went to the correct corner because that was the corner it was trained to approach. In the second discordant condition (preferred direction–consistent choice error), it is unclear whether the rat mistakenly approached the incorrect corner or, alternatively, whether the rat spontaneously altered its corner choice. If the rat spontaneously altered its corner choice, one might not expect a correlation between the preferred firing direction of the cell and the rat’s behavioral response. Thus, the first condition makes a stronger demonstration that HD cell information was not associated with the corner choice. In theory, if the orientation of HD cells is a major determining factor in generating the rat’s behavioral response, then nearly all the trials should be concordant.

A sequence of two trials that were classified as concordant or discordant is shown in Figure 4. The rat approached the correct corner on both trials. Figure 4A represents a concordant trial because the preferred direction did not shift and the rat chose the correct corner. In contrast, Figure 4B represents a discordant trial because the rat chose the correct corner, but the cell shifted its preferred firing direction by 270°. A representative example of changes in preferred firing direction and corner choice in 16 consecutive trials in two rats is shown in Table 2. In both rats, there are examples of concordant (e.g., Rat 1, Trials 2 and 5) and discordant (e.g., Rat 2, Trials 3 and 5) trials. For Rat 1, 5 of 16 (31%) trials were discordant (Trials 1, 3, 4, 6, and 11); the rat’s performance was correct on the first three discordant trials and incorrect on the last two discordant trials. For Rat 2, 7 of 16 (44%) trials were discordant (Trials 3, 5, 7, 8, 10, 15, and 16); of these trials, the rat’s performance was incorrect on only Trials 3 and 10.

Figure 5A illustrates the percentage of concordant and discordant trials during the standard sessions. The concordance percentage across all standard trials was 64%, indicating that the potential influence of HD cell activity on behavior was variable. The concordance percentages for individual rats ranged from 41% to 73%. Of the trials on which the rat chose the correct corner, a similar pattern of concordance–discordance percentages is apparent (78% concordant). The discordant percentage is greater for the correct trials because examining only the correct trials eliminates the possibility for discordances caused by performance errors; thus, there is only one possible type of discordance. It is important to note that Figure 5B shows that the consistency in the preferred firing direction did not vary substantially as a function of the rat’s choice. The preferred direction consistency was similar across correct trials (78%) and error trials (74%); therefore, it was just as likely that the preferred direction was consistent on error trials as
Table 2
Preferred Firing Direction Versus Behavioral Response: Examples From Two Rats

<table>
<thead>
<tr>
<th>Comparison and box location</th>
<th>Rat 1</th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td>Preferred firing direction in standard trials vs. baseline session</td>
<td>270</td>
<td>270</td>
<td>264</td>
<td>264</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>354</td>
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<td>354</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Corner choice vs. correct corner</td>
<td>0</td>
<td>270</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>90</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>270</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Box location</td>
<td>W</td>
<td>W</td>
<td>W</td>
<td>W</td>
<td>N</td>
<td>N</td>
<td>N</td>
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<td>E</td>
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<td>E</td>
<td>E</td>
<td>S</td>
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<tr>
<td>Rat 2</td>
<td></td>
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<tr>
<td>Preferred firing direction in standard trials vs. baseline session</td>
<td>342</td>
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<td>168</td>
<td>168</td>
<td>168</td>
<td>168</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>0</td>
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<td>12</td>
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<tr>
<td>Corner choice vs. correct corner</td>
<td>0</td>
<td>0</td>
<td>180</td>
<td>0</td>
<td>0</td>
<td>180</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Box location</td>
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<td>N</td>
<td>E</td>
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<td>E</td>
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<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>W</td>
<td>W</td>
</tr>
</tbody>
</table>

Note. For comparison of the preferred firing direction between standard trials and baseline session, the values shown are the preferred firing direction in the standard trials relative to 0° in the baseline session. For the corner choice versus correct corner comparison, the behavioral choice responses are shown in degrees relative to the location of the correct corner (e.g., correct behavioral response = 0°). W = west; N = north; S = south; E = east.

it was on correct trials. These findings suggest that the rats did not exhibit performance decrements when HD cells shifted their preferred directions.

As would be expected, similar results were obtained when performance was analyzed in terms of preferred direction consistency. When the preferred direction was consistent with the baseline session, the rats approached the correct corner on 78% of trials (87/112 trials). When the preferred direction shifted, the rats chose the correct corner 73% of the time (24/33 trials). Thus, rats chose the correct corner at about the same rate whether the preferred direction changed or not. None of the individual rats demonstrated substantial differences in performance as a function of HD cell consistency.

**Discussion**

The primary goal of Experiment 1 was to test the hypothesis that HD cell activity reflects directional heading information that is used by rats when performing a spatial memory task. The main findings were (a) rats accurately performed the reference memory task even though HD cells sometimes shifted their preferred direction; (b) the magnitudes of the shifts in preferred direction were always in multiples of ~90°; and (c) when the preferred direction was induced to shift by conducting the task in a rectangular arena, the rats still performed the task surprisingly accurately by rapidly generalizing from their earlier training. These results do not support the hypothesis that the directional information supplied by ADN HD cells is consistently used by the rats when they generate a behavioral response in a well-learned spatial reference memory task. If the rat’s spatial behavior reflected information provided by HD cells, we would have expected (a) the percentage of concordant trials to be greater than expected by chance conjunctions of behavior choice and preferred direction, (b) a difference in preferred direction consistency for correct versus error trials, and (c) systematic shifts in corner choices when the preferred direction shifted in the rectangle and standard sessions.

Three lines of evidence indicate a dissociation between the rat’s behavioral choice and HD cell activity in the spatial reference memory task. First, in the square arena, HD cell activity and behavioral choice were concordant on only about 60% of the trials. The strongest evidence for the lack of a functional association was provided by 24 trials in the standard sessions in the square, in which the directional system changed its orientation and the rat still chose the correct corner. On the basis of the most frequently approached incorrect corner (11% of trials), the percentage of correct choices when the preferred direction had shifted was nearly twice this value (21.6%). Thus, discrepancies in these trials cannot be fully accounted for by choices that were correct by chance (Figure 5A). Second, rats did not exhibit performance benefits by having a consistent preferred direction. A comparison between trials on which the preferred direction shifted or did not shift showed that the percentage of correct corner choices was about the same (74% vs. 78% respectively). Third, the rectangle sessions usually induced the HD cells to adopt a new preferred firing direction in the rectangular environment. Despite this change in preferred direction, the rats performed the task as well, if not better, than in the square. If the behavioral response were dependent on ADN HD cells activity, we would have expected poorer performance in the rectangle than in the square, especially during the initial trials. Taken together, these findings do not support the notion that HD cell activity provides information that the rat uses to solve this type of spatial memory task.

The standard and cue rotation phases of this study were modeled after the procedures from an earlier study by Dud-
chenko and Taebe (1997). This study showed that the preferred direction of HD cells was highly correlated with the rat’s behavioral choice on an eight-arm radial maze. Across trials, the preferred direction of HD cells was consistent over time (discussed in greater detail below), and the rats usually chose the correct reward location. An important consideration of this experiment was that HD cell activity and the rat’s arm choice were both determined with respect to the same cue, thus preventing the interpretation of a direct link between behavior and HD cell activity. The authors proposed that the HD cell system serves to guide the rat’s performance in the memory task once it has learned to link information from the directional system with the location of the reward. Thus, over the course of acquisition, the rat would increasingly rely on the directional information provided by HD cells to guide its behavioral response. The present results are not consistent with this hypothesis because the rats were able to perform a very similar task at asymptotic levels even after changes in the directional orientation of HD cells. If they were using HD cell information to guide their responses in the present experiment, changes in preferred direction would be associated with incorrect corner choices, a result that was not observed very often.

Experiment 2

The purpose of Experiment 2 was to determine which cues the rats used to define the correct corner on the reference memory task in Experiment 1. In a series of test sessions, we assessed the rats’ use of intra-arena or idiothetic (i.e., vestibular, proprioceptive, motor efference copy) cue sources to solve the task.

Method

Subjects

Five female Long-Evans rats were used in the experiment. Four rats were 3 months old at the beginning of training and were not implanted with recording electrodes. The 5th rat participated in Experiment 1 prior to being used in Experiment 2 and was 8 months old at the time of testing. This subject is included because its results were consistent with the findings from the other subjects that were not used in Experiment 1.

Apparatus and Training Procedures

The square apparatus and testing room were the same as described in Experiment 1 with one exception. Instead of floor paper, a black Plexiglas floor was installed, which allowed a consistent relationship between the floor and the arena walls to be maintained during arena rotation sessions (see below). During training, the rats were given between 32 and 80 trials per day in one or two sessions; procedures were the same as described in Experiment 1. For each trial, the rats were placed into the center of the arena and released. The Plexiglas corral was not used in Experiment 2 because HD cells were not recorded. During training and testing, rats were placed on a water deprivation schedule, with water available for 10–30 min after training or testing sessions. Testing commenced once the subjects achieved a criterion of initially approaching the correct corner on over 80% of the trials on 2 consecutive days.

Experimental Design

Five types of sessions were conducted to define the importance of the cue card, other intra-arena cues, and idiothetic cues: (a) standard, (b) no-cue, (c) arena rotation, (d) slow rotation, and (e) fast rotation. No-cue sessions tested whether the cue card was necessary for accurate performance. Arena rotation sessions examined the influence of intra-arena cues other than the cue card (such as olfactory cues). The slow rotation and fast rotation sessions were designed to assess the importance of idiothetic cues. Each session consisted of an eight-trial sequence with ~10–20 s between trials. The location of the holding box was not changed between sessions. In the standard session, the holding box was placed at one of four locations outside the arena with the cue card at the 3:00 position, as during training. In no-cue sessions, the cue card was removed from the arena while the rat was inside the holding box with the lid closed. For the arena rotation sessions, the cue card was removed, and the floor and arena were rotated together ±90°. The no-cue and arena rotation sessions were preceded and followed by standard sessions. In slow rotation sessions, the cue card was removed from the arena and the holding box with the rat inside was slowly rotated ±90° over 2 min (~0.75°/s). Fast rotation sessions were used as the comparison session for slow rotation sessions. In fast rotation sessions, the holding box was rotated ±90° over ~5 s (~18°/s), followed by a delay of ~115 s before the first trial of the session. Fast rotation sessions always preceded slow rotation sessions, as the slow
rotation session often disrupted the rat’s performance on subsequent sessions (see Results).

The data presented are from the first five sessions (40 trials) of the no-cue, arena rotation, and slow rotation sessions. The typical sequence of sessions (4/5 rats) consisted of completing the five no-cue sessions before starting the arena rotation and slow rotation sessions. The arena rotation sessions were usually completed before starting the slow rotation sessions. In 1 rat, we alternated the no-cue and arena rotation sessions. The findings from the rat given the alternating sequence were similar to the results of the other 4 subjects, and all the data were therefore grouped together. The five sessions took 4–6 days to complete.

Data Analysis

As in Experiment 1, a correct response was defined as an approach to the corner containing the baited water cup. Responses were usually rapid and direct, permitting the unambiguous scoring of correct and error responses. The number of correct responses during the first 40 trials in each test and its comparison session were recorded and converted to a percentage. The percentages of correct responses were analyzed with repeated measures ANOVAs.

Results

The 4 rats given 32–80 trials per day required a mean of 365 ± 72 trials (range: 248–496) to perform at the criterion level. These values are somewhat larger than the values in Experiment 1 and are presumably due to the greater number of trials within the training sessions (16 trials for Experiment 1 vs. 32–80 trials for Experiment 2). A 5th rat that was trained in Experiment 1 required 250 trials before achieving the criterion performance level. Figure 6A shows the percentage of correct responses in the standard, no-cue, and arena rotation sessions after acquisition. In the arena rotation sessions, correct choices were scored when the rat approached the same position relative to the room. Because the arena was rotated, the correct position was now occupied by a different corner of the arena than on previous sessions. The mean percentages of correct choices in the standard, no-cue, and arena rotation sessions were 89 ± 3, 84 ± 4, and 82 ± 4, respectively. A repeated measures ANOVA showed no significant differences across session type, F(2, 8) = 0.68, p > .50. Thus, although Experiment 1 showed that the rats’ choices were dependent on the position of the cue card, the results from Experiment 2 indicate that neither the cue card nor cues on the arena walls (e.g., tactile, olfactory, or visual) were required for accurate performance. When the cue card was removed or the arena without the cue card was rotated, the rats still chose the correct corner relative to the room. How the findings from Experiments 1 and 2 can be reconciled is considered in the Discussion section.

Figure 6B illustrates the percentages of correct choices in the fast and slow rotation sessions. In contrast to the sessions in which arena cues were manipulated, slowly rotating the rat inside the holding box before the test session sharply reduced the number of correct responses. The percentages of correct choices in the fast and slow rotation sessions were 95 ± 3 and 52 ± 9, respectively. These values indicate that slow, but not fast, rotations of the holding box disrupted performance. A repeated measures ANOVA showed a significant difference between the percentages of correct responses in the fast rotation versus slow rotation conditions, F(1, 3) = 47.77, p < .01. It is interesting that on 31% ± 7% of the slow rotation trials, the rats approached the corner that would have been correct had the box not been rotated (e.g., 90° CW rotation of the box led to the rat choosing the corner 90° CW from the correct corner). Because the rats presumably detected the fast rotations, but not the slow rotations, these data indicate that the behavioral response was predominantly determined by idiothetic cues after the rat experienced an initial standard session.

Discussion

In Experiment 2, we assessed the degree to which accurate performance was dependent on the cue card, other intra-arena cues, or internal cues. The findings show that (a) the cue card could be removed without affecting performance accuracy, (b) changing the location of the remaining intra-arena cues did not disrupt performance, and (c) slowly rotating the holding box before a series of trials led to decrements in accuracy. In the slow rotation sessions, erroneous corner choices often reflected the direction of box rotation.

Experiment 2 demonstrated that the cue card was not necessary for successful performance after an initial standard session. The preceding standard session may be an important factor for supporting accurate performance in the no-cue sessions. Preliminary findings suggest that rats initially rely on the cue card to determine
the correct corner because rapidly spinning the rat inside the box usually disrupts performance only when the cue card is removed from the arena (Golob, Stackman, & Taube, 1998). Rotation of the intra-arena cues with the cue card removed also did not prevent the rat from approaching the correct corner (as defined by the room frame of reference). These findings indicate that intra-arena cues are not necessarily a strong determinant of the rat's behavioral response once it has been exposed to the arena in the preceding standard session. Thus, the influence of the cue card on behavioral choice in Experiment 1 may also have been transient.

In contrast, during slow rotation sessions accuracy was nearly half that of fast rotation sessions (52% vs. 95% respectively). This finding has two implications. First, rats were not likely to have used uncontrolled room cues to determine the correct corner, as these cues would have been available in both the fast and slow rotation sessions. Second, the rotation speed in the slow rotation sessions was intended to be near, or below, the threshold for vestibular activation. Impaired performance on the slow rotation sessions suggests that the detection of movement using vestibular cues is necessary for accurate performance. Reliance on vestibular information is further supported by the systematic errors observed in the slow rotation sessions. In ~30% of the slow rotation trials, angular rotation of the holding box led to a corresponding angular shift in corner choice.

In Experiment 2, the test sessions were preceded and followed by standard sessions, allowing for the detection of carryover effects between sessions. No carryover effects were observed in the no-cue and arena rotation sessions because performance was unaffected in the following standard sessions (data not shown). In the slow rotation sessions, however, rats were typically impaired in the subsequent standard session. Thus, the slow and fast rotation sessions were conducted after the no-cue and arena rotation sessions. Although carryover effects were not observed in the no-cue and arena rotation sessions, experience in the no-cue condition could have influenced performance in the following arena rotation sessions by reducing the rat's use of arena cues in general and/or encouraging the use of internal cues to determine which corner to approach. Less reliance on a visual cue for orientation has also been reported for hippocampal place cells after several exposures to an environment in which the visual cue was not a reliable predictor of the animal's spatial orientation because it was placed in conflict with spatial information derived from the animal's internal idiothetic cues (Jeffery & O'Keefe, 1999).

Taken together, what appears to happen is that when the cue card is available in the arena, the rats initially rely on it to determine their corner choice. If the cue card is then removed, the rats are able to rely on internal cues to approach the correct corner. Transfer to reliance on internal cues was rapid in the no-cue sessions, a finding that contrasts with the fact that hundreds of trials were required to achieve accurate performance in the initial training sessions. Rapid transfer in the no-cue sessions suggests that even when the cue card was present, the rats may have relied on internal cues to approach the correct corner. Thus, the cue card might have been used to infer the correct corner only under conditions in which internal cues were unreliable, such as when the rat was first introduced into the arena or after it had been disoriented. Support for this concept also comes from experiments in which the rats often fail to use the cue card to determine corner choice on trials when the cue card is placed at a rotated position in the arena after several no-cue sessions (Golob, Stackman, & Taube, 1998). The difference between this preliminary experiment and the cue rotation sessions in Experiment 1 is that the subjects were not disoriented before the card was placed at the rotated position in the arena.

The percentage of correct choices was somewhat greater in Experiment 2 than in Experiment 1 (~90% vs. ~80%). This difference could be due to several differences between the experiments. The intertrial interval was much shorter in Experiment 2, and the rats were more intensively trained than in Experiment 1. In addition, greater handling and the use of the recording cable and Plexiglas enclosure in Experiment 1 probably caused more distractions than in Experiment 2. Any of these factors could be related to the greater accuracy observed in Experiment 2.

Experiment 3

An important aspect of navigation is the ability to orient oneself with respect to surrounding landmarks. Reorientation is often needed after periods when an animal relies on idiothetic cues for orientation or when perceptual access to the surrounding environment is restricted (Gallistel, 1990; Hermer & Spelke, 1996). Researchers have proposed that the reorientation process often depends on the macroscopic shape of the environment (Gallistel). Under most conditions environmental shape is irregular, thus permitting the unambiguous determination of location and directional heading. The conclusion that reorientation is frequently based on environmental shape follows from studies showing that rats (Cheng, 1986; Margules & Gallistel, 1988), toddlers (Hermer & Spelke, 1994), and to a lesser degree birds (Vallortigara, Zanforlin, & Pasti, 1990) are unable to distinguish the diagonally opposite corners inside a rectangular arena after various forms of disorientation. That is, subjects were equally likely to search for a reward at the correct or diagonally opposite corner in the rectangular environment. These corners are indistinguishable on the basis of shape and are congruent after a 180° rotation of the arena. Subjects exhibit this rotational confusion pattern despite the presence of a salient cue that breaks the symmetry of the two corners (Margules & Gallistel).

In novel environments (Taube & Burton, 1995) or arenas devoid of salient cues (Goodridge, Dudchenko, Worboys, Golob, & Taube, 1998; Goodridge & Taube, 1995), HD cells are thought to rely on idiothetic cues to maintain a consistent preferred firing direction. These same studies showed that when salient landmark cues are available, the preferred firing direction is recalibrated with respect to the landmark cues even when they conflict with the orientation derived from idiothetic cues. As such, resetting the preferred direction of HD cells on the basis of landmark cues may be an aspect of the reorientation process. One goal in the following experiment was to examine the role of HD cells in reorientation by characterizing the relationship between the preferred direction of HD cells and the rat's orientation as inferred by behavioral measures.

Experiment 1 tested the hypothesis that HD cells influence an animal's behavioral response by monitoring HD cell activity while rats performed a spatial reference memory task. The behavioral task was a reference memory paradigm that did not explicitly require the rat to reorient itself. Although a reorientation process may have been operative in that task, the behavioral paradigm is
also consistent with the notion that the rat was continuously oriented with respect to the goal location throughout the task. In Experiment 3, we examined the relationship between HD cell activity and spatial behavior along two additional dimensions, by adding a working memory demand to the task and by requiring the rat to reorient itself before making its choice. The working memory aspect may be an important consideration because lesions of the anterior thalamus disrupt performance on a spatial working memory task (Aggleton, Hunt, Nagle, & Neave, 1996). We chose to use a modification of the paradigm developed by Gallistel and colleagues (Cheng, 1986; Margules & Gallistel, 1988) because working memory and reorientation are features of this task. If HD cells provide a sense of direction and the rats use this information when performing the task, then they would tend to change their preferred directions by \( \pm 180^\circ \) in accordance with searches at the correct or diagonally opposite corners of the rectangle. An important feature of this task is that it introduces variability in spatial behavior that is a consequence of learning. This condition provides an opportunity to assess the relationship between HD cell activity and the animal’s behavior choice if such a relationship is created during the acquisition process.

Method

Subjects

Five female Long-Evans rats between 4 and 9 months old were used. They were housed individually and maintained on a 14:10-hr light–dark cycle. For the training and data collection portions of the experiments, the rats were water restricted, receiving water ad lib for 10–30 min/day. During the cell screening and data collection phases (detailed below) they were also mildly food restricted (10–15 g/day). Rats were examined regularly for signs of dehydration and/or weight loss. Occasionally, ad-lib food and water were reinstated for 1–2 days.

Apparatus

Training and experiments were conducted inside a gray rectangular arena (60 cm \( \times \) 120 cm, 35 cm high) with a gray wooden floor. As in the previous experiments, the arena was surrounded by circular black curtains, and a speaker emitting white noise was hidden in the rafters above the apparatus. The rat’s behavior was videotaped with an overhead video camera. Four DC lights were symmetrically arranged above the curtained area but were not illuminated during the experiments.

Inside the apparatus, four identical opaque water cups (4.5 \( \times \) 4.5 \( \times \) 1.0 cm) were placed in the corners (one per corner). The rectangle contained a prominent white cue card along one of the short walls. The inside of the apparatus was illuminated by four DC xenon light bulbs, one positioned above each corner. Care was taken to ensure that the lights did not project nonuniform shadows onto the surrounding black curtain.

Training Procedures

The environment was designed to isolate the rat from the larger framework of the room, forcing it to orient itself with respect to the arena environment. Consequently, measures were taken to prevent the rat from utilizing stable extra-arena cues. In addition to the circular curtain, white noise, and intra-arena lighting described above, the location and direction of the arena within the room was varied across training days. Water-restricted rats were removed from their cages in the animal colony and placed into a cardboard holding box. As the experimenter walked from the colony to the test room, the box was spun slowly (\( \pm 3–6^\circ/\text{s} \)) in the CW or CCW direction (randomized across blocks) in order to disorient the rat with respect to the surrounding environment. The box containing the rat was brought into the darkened testing room and randomly placed at one of four symmetrical locations outside the arena.

The rats were trained with a modified version of a spatial working memory task first described by Cheng (1986; see Figure 7A, present study). Each block of trials consisted of two sample-test trial pairs, with the reward at the same location for both trials. The baited corner was pseudorandomly varied across blocks, with the restriction that the same corner could not be baited on consecutive blocks. During the sample trial, rats searched all four corners and consumed the water reward. In the test trial, the rats were allowed to approach one corner in an attempt to obtain the water reward.

![Figure 7](image_url)

**Figure 7.** A: Schematic diagram of an overhead view of the apparatus illustrating a typical two-trial sequence in a test block. The corner that contained the water reward is shown by the filled circle in the upper right-hand corner. Unbaited cups in the remaining corners are indicated by open circles. The holding box is shown at the 12:00 position in the sample trial; it was moved 270°, with the rat inside the box, to the 3:00 position before the next test trial. The direction that the rat was facing when released, shown by the arrows, was changed by 180° across trials. B: Diagram illustrating the categorical labels for each corner choice. Because the corner that contained the reward varied across blocks, the label for each corner also changed across blocks. Note that the correct and rotational corners are always 0° and 180°, respectively, using the angular corner labels. The near and far corners could be either 90° or 270° depending on the position of the correct corner.
Initially, rats were given 1–6 sessions in which they were allowed to explore the arena with water available in one corner and empty cups in the other corners. Next, rats were tested with one or two blocks of trials each day according to the following procedures. For the sample trial, one corner was baited and the rats were initially placed inside a clear, rectangular Plexiglas corral (22 cm × 32 cm; 28 cm height) situated in the center of the arena. The purpose of confining the rat inside the corral was to provide sufficient sampling time for HD cell activity at all lead directions before allowing the rat to make its corner choice. After ~1 min, the rat was briefly lifted straight up ~15 cm and the corral was removed. The rat was then placed at its original position and permitted to explore the test environment. After the rat had searched all four corners and consumed the water reward, it was returned to the holding box. The box was then moved to one of the three remaining peripheral locations and slowly rotated by hand (3–6/s) a random amount between 90° and 270° in the CW or CCW direction. The floor of the test arena was wiped clean with a damp sponge, and the corner that previously contained the reward was rebaited. The corral was rotated about the x, y, and z axes to prevent it from serving as a reliable cue for the reward location. The rat was then reintroduced into the corral for ~1 min before the corral was removed. The total amount of time between removal from the arena in the sample phase and release in the test phase was ~90 s. After release, the rat was permitted to approach one corner before being removed from the arena. If the rat chose the correct corner it consumed the water reward. The rat was then placed into the holding box and spun slowly before being returned to its cage.

Records of box locations, the baited corner, and corner choice during the test trial were noted for each rat. Each training block (consisting of two sample–test trials) was separated by at least 4 hr to minimize the effect of proactive interference. One or two blocks of trials were conducted each day. The experimenter stayed inside the dark curtains during the sample and test trials, varying his location with respect to the holding box across each trial. Even though the rats tended to disregard the experimenter once they were familiar with the task, the experimenter carefully avoided cueing the rat by standing at the periphery and adopting a neutral stance during all trials.

Surgical Procedures, Cell Screening, and Data Acquisition

Electrodes were implanted after the rats had achieved a criterion of directing >70% of their test trial choices toward the correct corner or the diagonally opposite corner, with an approximately equal proportion of correct and diagonal corner choices. Electrodes were implanted in the ADN. Surgical procedures, cell screening, and data acquisition were as described in Experiment 1.

Data Analysis

HD cells were recorded while the rats performed the working memory task described above. Of central importance was the neuron’s preferred firing direction on the sample and test trials in a block, and how these results compared with the rat’s behavioral choice on the test trial. Data were continuously collected during each block of trials, and graphs of firing rate versus HD were created by dividing HD into sixty 6° bins and plotting HD against each bin’s mean firing rate, as described above. Shifts in preferred direction were quantified with the cross-correlation algorithm described in Experiment 1.

Behavioral measures were analyzed in two forms: either by descriptive categories or by assigning a degree value to each corner of the apparatus (see Figure 7B). The four possible categorical corner choices within a test trial are defined as follows: correct choice, rotational (choosing the corner diagonally opposite the one that contained the reinforcer); near error (corner adjacent to the correct corner along a short wall); and far error (corner adjacent to the correct corner along a long wall). In accordance with the nomenclature of Margules and Gallistel (1988), near errors and far errors are collectively referred to as misses. The diagonally opposite corner is labeled “rotational” because this location would be congruent with the correct corner after a 180° rotation of the arena. Angular values for each corner were assigned relative to the location of correct corner, which was denoted by “0°”. As with the calculation of angular shift in preferred direction, angular values increase in the CCW direction. Note that the corner labels change across blocks as a function of reward location. Correct and rotational errors are always labeled 0° and 180°, respectively. Near and far errors can either be 90° or 270° depending on the relationship between the baited corner and the rat’s erroneous choice.

Binomial and chi square tests were used to compare relative frequencies of categorical data. ANOVA and t tests were also used for noncategorical data. For all statistical tests, significance was set at the .05 level.

Histology

Histological procedures were the same as those described in Experiment 1.

Results

Behavioral Results

When the rats were permitted to walk throughout the arena on sample trials, they searched all four corners, usually by moving CW or CCW along the walls. On some occasions, the rats would make trips through the center of the arena toward the diagonally opposite corner. The rats did not favor the baited corner after release (data not shown), indicating that they were not using the water as a beacon cue. For test trials, a corner choice usually consisted of a rapid movement toward one of the corners that usually did not terminate until the rat had reached the water cup. Thus, the behavioral assessment of corner choice was nearly always unambiguous. Some rats would pause briefly (<2 s) before initiating a corner approach, but most rats ran toward a corner immediately after being released.

Table 3 shows the behavioral data after the electrodes were implanted for all 5 rats. This table includes not only trial blocks when HD cells were recorded, but also the training blocks between recording sessions. The number of trials to criterion before surgery (>70% corner choices toward the correct or rotational corner) ranged from 12 to 52 (M = 28.0). Table 1 shows a small, but statistically significant, difference in the percentage of correct versus rotational corner choices, t(4) = 4.62, p < .05. The search frequencies at near and far corners (misses) were identical (14%). With the corner choices expressed in angular terms, all but 1 of the rats divided their error choices approximately evenly between the 90° and 270° corners.

The same general pattern of results was also found within each rat. Binomial tests showed a significant bias for correct and rotational choices (ps < .05), with no significant differences between the number of correct and rotational choices (ps > .20). These findings replicate earlier reports that used a similar behavioral paradigm (Cheng, 1986; Margules & Gallistel, 1988). HD cells were recorded from a subset of the trials described above. Table 1 shows that the behavioral results from trials for which HD cells were simultaneously recorded were similar to the results found across all trials.

We also observed an interesting behavior pattern in 2 additional rats that were trained to criterion levels but are not included in the
data set above. After additional training, these rats usually chose one of two diagonally opposite corners of the arena in the test session (37/43 and 29/36 trials). This finding suggests the use of a reference memory strategy based on geometric shape.

Finally, it should be noted that not all of the rats that were trained adopted a strategy based on arena shape. There were 3 rats that never achieved criterion levels during training (data not shown) and were not implanted with electrodes. These subjects distributed their choices evenly among the four corners. Training was terminated in these rats after approximately 40 trials; thus, it is possible that with further training the rats would have also demonstrated a response pattern based on arena shape. Importantly, none of these rats restricted their searches to the correct corner, indicating that they were unable to use the cue card, or any other means, to reliably determine which corner contained the water reward. These 3 rats are not included in the analyses below.

**Consistency of Preferred Firing Direction Across Trials**

HD cells were first recorded for 4 min in the cylindrical enclosure used for screening, before the rat was placed into the rectangular arena. Before the sample phase, a 2-min recording session was conducted with the rat inside the corral to ensure that the LEDs were being tracked accurately. A total of 16 HD cells were recorded from 5 rats (range: 2 to 4 per rat). Examples from two cells recorded during sample-trial blocks are shown in Figure 8. Figure 8A illustrates a block in which the HD cell did not change its preferred direction between the sample and test trials; this trial was therefore classified as consistent. A cell that changed its preferred direction between the sample and test trial, and was thus classified as inconsistent, is shown in Figure 8B. In this instance, the angular shift in preferred direction was 168°. In 39 of 70 trials (56%) the preferred direction was consistent between the sample and test trials. In the inconsistent trials, the preferred direction shifted in multiples of ±90° (± 18°). Consistency or inconsistency was a property of the network because the preferred direction of simultaneously recorded HD cells shifted by an equivalent amount (data not shown) and individual cells were consistent on some blocks and inconsistent on others. Differences in preferred direction across trials for each rat are shown in Table 4. The individual rats appeared to adopt a stereotypical pattern of shift magnitudes: either 180° or 90° and 270° (± 90°) for each rat. We did not observe any instances in which the preferred direction of an HD cell in a given rat shifted by 180° and ±90° on different occasions.

We then determined whether the amount of preferred direction inconsistency between trials was similar to the level of inconsistency between blocks or, instead, was the inconsistency specific to performing the task. The preferred direction for a cell was compared between the two sample sessions performed in the rectangle just before the test trials on the same day, but separated by >4 hr. The preferred direction was consistent between blocks in 17 out of 25 cases (68%), a value that was similar to that found within blocks (56%). This finding indicates that a similar level of instability was present across blocks even before the working memory task was initiated.

To determine whether the shifts were specific to the rectangular arena, we examined the consistency of preferred directions across sessions in the cylinder. A total of 33 comparisons of adjacent sessions were conducted, 18 within a day and 15 across days. The preferred direction shifted by (a) <18° on 28 occasions (85%), (b) 24° on 2 occasions, and (c) ≥24° on 3 occasions. The greater consistency in the cylinder as compared to the rectangle (85% vs. 56% and 68%) suggests that the directional instabilities observed in the rectangle are specific to the context and may not represent a general loss of environmental cue control over HD cell activity.

We next examined the influence of slowly rotating the box and changing its location between trials on the preferred direction shifts. A chi square analysis of box rotation magnitude (90°, 180°, and 270°) by preferred direction change (90°, 180°, 270°) was not significant, $\chi^2(4, N = 70) = 5.41, p > .20$. The magnitude of preferred direction shift was similarly unrelated to particular changes in box location, $\chi^2(4, N = 70) = 5.80, p > .20$. These results indicate that the propensity for directional shifts did not appear to be related to specific angular values of the box rotation or changes in box location.

**Comparison Between HD Cell Activity and Behavioral Responses**

In contrast to the finding that HD cells maintained their preferred direction across 56% of the sample and test trials, the rats chose the correct corner on only 36% of the trials. Thus, neural
Table 4
Preferred Firing Direction Comparisons (Sample vs. Test Trials) Across Rats

<table>
<thead>
<tr>
<th>Rat</th>
<th>0°</th>
<th>90°</th>
<th>180°</th>
<th>270°</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>3</td>
<td>14</td>
<td>0</td>
<td>7</td>
<td>0</td>
<td>21</td>
</tr>
<tr>
<td>4</td>
<td>11</td>
<td>0</td>
<td>9</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td>5</td>
<td>8</td>
<td>6</td>
<td>0</td>
<td>2</td>
<td>16</td>
</tr>
<tr>
<td>Total</td>
<td>39</td>
<td>8</td>
<td>18</td>
<td>5</td>
<td>70</td>
</tr>
</tbody>
</table>

Note. Values represent the number of times the preferred firing direction shifted a given amount (0°, 90°, 180°, 270°) between the sample and test trials.

Table 5 illustrates the rats' behavioral choices on the test trial (expressed in degrees) versus the amount the preferred firing direction shifted between the sample and test trials (categorized in 90° increments). If the rat's corner choices were related to the preferred direction of HD cells, then most of the trial results should fall along the diagonal formed by matching values for behavioral choice and preferred direction shift (i.e., from the upper left to lower right hand corners of Table 5). Table 5 shows that this situation was not the case. Indeed, the difference in preferred direction between the sample and test trials matched the rat's corner choice on only 23 of 70 trials, indicating that, on the majority of trials, the preferred firing direction and behavioral choice were not in register. The data from individual rats were also consistent with this finding (data not shown). Furthermore, even when the preferred firing direction was similar between the sample and test trials, the rats were equally likely to choose the correct (n = 16) or rotational (n = 16) corner. These results demonstrate that the rats were unlikely to have determined their corner choice on the basis of directional information provided by HD cells in the

Figure 8. Examples of head direction cell activity during sample and test trials within blocks. A: Example of a consistent preferred firing direction between trials. B: Example of a block in which the preferred firing direction shifted between the sample and test trials. The difference in preferred direction was 168°.

Figure 9. Example of a head direction cell recorded during two test trials from adjacent sample-test blocks. The cell fired at about the same preferred firing direction on both trials, and the rat chose the same corner (rotational) relative to the baited corner. The trials are also an example of two blocks in which the preferred firing direction was consistent with the rat's behavioral choice because, on the test trials, the rat approached the rotational corner and the preferred firing direction shifted by ~180° from the previous sample sessions.
the arena shape, resulting in either a correct choice or a systematic error directed toward the rotational equivalent corner. The percentage of correct and rotational choices was similar (40% vs. 32%, respectively), and suggests that the rat’s choice was based primarily on the geometric shape of the arena. There apparently are other important influences over performance as well, because 28% of the choices were directed toward the near or far corners (misses), locations that were not geometrically consistent with the location of the reward in the sample trial. This value is similar to the percentage of misses (33%) observed by Margules and Gallistel (1988).

There was little evidence for a direct relationship between the directional orientation of ADN HD cells and the direction of the rat’s behavioral response, even though the task can, in principle, be solved by using a directional strategy (i.e., the reward is in the northwest corner). The data, examined as a group or within each subject, fail to suggest a strong relationship between preferred direction value and corner choice, both of which varied across trials. The finding that rats with $\geq 90^\circ$ shifts in preferred direction preferentially chose the correct or rotational corners, separated by $180^\circ$, also suggests the absence of a simple relationship between preferred direction and response direction. Thus, knowing the orientation of the directional system before the rat was allowed to approach a corner was not predictive of the specific corner the rat would choose.

**HD Cells and Reorientation**

The reorientation process occurs under natural circumstances when animals periodically refer to landmark cues to determine their position (Etienne, Maurer, & Seguinot, 1996; Gallistel, 1990). Landmark cues can exert control over the preferred firing directions of HD cells, with information from landmark cues usually overriding information from internal cues when the two cue sources are in conflict (Goodridge & Taube, 1995). Moreover, HD cells also respond to arena shape, independent of the landmark cue card’s position (Golob & Taube, 1997; Taube et al., 1990b). Thus, there were a priori reasons to suspect a strong relationship between the information provided by HD cells and the rat’s corner choice. The results from our experiment, however, do not support the notion that a strong link exists between the two processes, at least for the spatial working memory task we used. This finding

![Figure 10. Examples of head direction cells recorded during two test trials from adjacent sample-test blocks. As shown in Panels A and B, the rat’s corner choice was inconsistent with the preferred firing direction across blocks. A: The preferred firing direction was approximately the same for both test blocks, but the rat chose either the correct or rotational corners. B: The rat chose the correct corner on both test trials, but the preferred direction was shifted by $174^\circ$ between test trials.](image)

ADN. Thus, the preferred direction of HD cells before the rat’s choice on test trials was not strongly predictive of the rat’s subsequent behavioral response.

**Discussion**

There were three main findings in Experiment 3. First, we replicated the behavioral finding that rats preferentially determined their corner choices with respect to arena shape and did not use a prominent intra-arena cue that reliably specified which corner contained the reward. Second, the preferred firing direction of HD cells was often unstable between trials in the rectangle and did not appear to be directly related to the amount of rotation of the holding box or its location. Third, the preferred firing direction of HD cells was unrelated to the rat’s corner choice, demonstrating that the rats were unlikely to have used the directional signal conveyed by HD cells in the ADN to determine which corner to approach.

The behavioral findings replicated the results of Gallistel et al. (Cheng, 1986; Margules & Gallistel, 1988). In test trials, the rats were unable to use the cue card to determine which corner was baited and usually approached a corner that was correct relative to

<table>
<thead>
<tr>
<th>Corner choice</th>
<th>$0^\circ$</th>
<th>$90^\circ$</th>
<th>$180^\circ$</th>
<th>$270^\circ$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$0^\circ$</td>
<td>16</td>
<td>3</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>$90^\circ$</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>$180^\circ$</td>
<td>16</td>
<td>3</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>$270^\circ$</td>
<td>5</td>
<td>2</td>
<td>8</td>
<td>1</td>
</tr>
</tbody>
</table>

Note. Values indicate the number of times the preferred firing direction of a cell shifted $0, 90, 180$, or $270^\circ$ when the rat made a behavioral response on the test trial (corner choice) that was shifted $0, 90, 180$, or $270^\circ$ relative to the reward location on the sample trial.
has several implications for the reorientation process and HD cell activity.

The finding that rats based their corner choices on the shape of the apparatus led Cheng to postulate the existence of a geometric module for reorientation (Cheng, 1986) that was dedicated to the solution of a particular type of problem and was selectively responsive to specific classes of information (Fodor, 1983). Our findings extend this conception by demonstrating that the geometric module is unresponsive to task-relevant data conveyed by HD cells at the neurophysiological level. Had the rats used the information provided by HD cells when selecting a corner, they would have increased their chances of success from 36% to 56% correct (the percentage of trials with a consistent preferred direction between sample and test trials).

Although HD cells may not contribute to the geometric module, processes associated with the geometric module may influence HD cell activity. Studies have shown that a simple association between the HD cell system and a prominent cue is an insufficient explanation for cue control because changing the arena shape led to preferred direction shifts even when the cue card’s position remained constant (Taube et al., 1990b). The influence of environmental shape over HD cell activity may currently be underestimated because most studies record HD cells when an animal is inside a cylinder, a shape that is incapable of providing any polarizing directional information, or in symmetrical environments (e.g., a square) that provide a set of potential directional orientations (Golob & Taube, 1997; Taube et al., 1990b). Experiments in an environment that is richer in terms of shape would help to determine whether a hierarchical structure exists between environmental shape and individual landmark cues. Perhaps the HD cell system is initially oriented by the macroscopic shape of the apparatus and the cue’s location is then taken into account within the larger context. The properties of cognitive maps in rats may operate according to this type of design (Cheng, 1987; Gallistel, 1990). Nongeometric cues, such as the cue card, would be represented as addresses within the larger framework provided by the macroscopic shape. Alternatively, the HD cell system could independently take into account local cues such as the cue card, geometric information, and cues from other sources, and then determine its orientation by combining these pieces of information.

We observed differences in preferred direction between trials and between test sessions. As in Experiment 1, the magnitude of preferred direction shifts appeared to be constrained by the shape of the apparatus because the preferred direction shifts were always a multiple of \(-90^\circ\). Furthermore, because the shifts within an individual rat were never \(+90^\circ\) and \(+180^\circ\) (i.e., when shifts in the preferred direction occurred, they were either \(-90^\circ\) or \(+180^\circ\) for each rat), it is likely that the rectangular shape, which has \(+180^\circ\) symmetry, also constrained the preferred direction values within individual rats.

General Discussion

**Preferred Firing Direction and Behavioral Response**

The findings from Experiments 1 and 3 demonstrate that there is not a straightforward relationship between the preferred firing direction of ADN HD cells and the behavioral response in these spatial tasks. Because the recordings were limited to HD cells in the ADN we cannot generalize the current results to HD cells in other brain regions.

It may be surprising that the spatial information conveyed by HD cells was not obviously related to performance on a well-learned spatial task. However, other studies have shown that neuronal correlates do not imply a necessary role in the generation of the behavior with which they correlate (Halgren, Marinkovic, & Chauvel, 1998; Schmaltz & Theios, 1972; Sobotka & Ringo, 1996). The apparent independence between HD cell activity and behavioral response is not necessarily a product of the "high-level," abstract nature of the directional correlate. For example, in delayed response working memory studies, cells in the prefrontal cortex exhibit mnemonic firing correlates that are disrupted during occasional error trials (Funahashi, Bruce, & Goldman-Rakic, 1989; Fuster, 1973; Watanabe, 1986a, 1986b), showing that this type of neural activity can correlate well with the animal’s behavior.

Several investigators have proposed that HD cell activity may provide the animal a sense of direction (McNaughton, Chen, & Markus, 1991; Redish & Touretzky, 1997; Skaggs, Knierim, Kudrimoti, & McNaughton, 1995; Taube et al., 1990a, 1990b). Our results are not necessarily inconsistent with this view, but the findings do question the notion that information from HD cells is always engaged on all spatial tasks: The subjects in Experiment 1 performed well, independent of the orientation of HD cells, and those in Experiment 3 frequently selected a corner that was incongruent with the spatial information from HD cells. We defined the rat’s perceived directional heading (i.e., its sense of direction) in terms of which corner it approached for a reward. Therefore, if the behavioral response indicates a sense of direction, and the preferred direction of HD cells on many trials does not correspond with the behavioral choice, then the preferred direction of HD cells may not reflect the rat’s sense of direction. Although this explanation is not very compelling, given the strong relationship between HD cell activity and the rat’s HD, we cannot exclude it. Alternatively, the rat may maintain its sense of direction by means of HD cells but may not use them for guiding its behavior on the spatial tasks used in the present experiments. An ideal test to distinguish between these possibilities would be to record HD cells while rats perform a task that is known to require directional information for accurate performance, but, unfortunately, existing spatial memory tasks can usually be solved by several strategies.

There are two interpretations for how these findings might relate to the function of HD cells that are consistent with a relationship between a sense of direction and the preferred firing direction of HD cells. Both of the following interpretations question the suggestion that directional sense can be expressed by the animals’ behavior in the current behavioral paradigms.

One consideration is that the memory tasks used in the present study might not be allocentric memory tasks. Thus, HD cell activity may not be used in these tasks, even though HD cells, which use an allocentric coordinate frame, would be required for spatial tasks that are dependent on allocentric spatial information. Lesion studies of areas containing HD cells have shown deficits during the acquisition of various allocentric spatial tasks (Aggleton et al., 1996; Sutherland & Rodriguez, 1989; Taube, Kesslak, & Cotman, 1992). Although the arenas used in the current experiments were fairly large, the rats did not have visual access to distal room cues. An environment with richer cue sources could help to
address the question of whether a task is allocentric. A second, but
related, possibility is that HD cells do provide the rat with a sense
of direction, but either the tasks we used do not require a sense of
direction for successful performance or the rats solved the task by
using a nonspatial strategy. Thus, HD cells would not have been
engaged for their solution. Testing the relevance of HD cells to a
sense of direction would require a behavioral task that requires
directional information to be competently performed and cannot
potentially be solved by using other strategies.

In Experiments 1 and 3, HD cells were recorded from rats that
had already acquired the memory task. We note two possibilities
involving the training period that are relevant to the interpretation
of the experimental findings. First, HD cell activity may have been
essential for the rat to acquire the task, but may not have been
necessary once it became proficient. Previous studies have shown
that rats performing a spatial task adopt different strategies de-
pending on how many trials they have experienced (Packard &
McGaugh, 1996). Similarly, HD cells could have initially assisted
the rat in determining which subset of cues indicated the direction
of the correct corner, but were not used at a later time when HD
cell activity was monitored. Second, for both the reference
memory and working memory tasks, it is possible that during the
course of training the rats learned not to rely on the directional signal to
determine the correct corner because the directional information
was unreliable across trials, and instead relied on associations with
specific landmark cues that did not exert control over the preferred
directions of HD cells. The results do not rule out this possibility,
and additional studies would be required to test this hypothesis.

The allocentric, “compaslike” firing correlate shown by HD
cells suggests a functional role as an “on-line” indicator of
moment-to-moment directional heading during navigation. Ac-
cording to this view, the preferred firing direction of HD cells
would be correlated with the rat’s behavioral response in familiar
tasks as well as in novel situations. The absence of a direct
relationship between the preferred firing direction and behavioral
response may be related to the rat’s extensive experience in per-
forming the tasks within an environment containing familiar land-
mark cues. Perhaps the directional information provided by HD
cells is used more in unfamiliar contexts, especially when behavior
is strongly influenced by idiosyncratic cues rather than landmark cues.
According to this conception, HD cells would be constantly active
in preparation for responding to novel situations, such as exploring
new terrain (Renner, 1990), or between episodic “fixes” based on
environmental landmarks (Gallistel, 1990). Once cue control is
established in a given environment, HD cell activity may not be
directly associated with the rat’s behavioral response. The use of
directional information in novel situations or between episodic
fixes are two examples of a possible role for HD cells in navigation
by a path integration strategy (Blair, Lipscomb & Sharp, 1997;
Taube & Burton, 1995). Path integration can be considered a type
of working memory that is dedicated to navigation and is based
exclusively on internally generated, idiosyncratic cues (Mittelstaedt
& Mittelstaedt, 1989). Studies have shown that the anterior thalamus
is important for acquisition of spatial working memory tasks
(Aggleton et al., 1996), but not for the retention of a place response
(Sutherland & Rodriguez, 1989). The anterior thalamus has also
been shown to be selectively active in the primate during several
varieties of working memory tasks (Friedman, Janas, & Goldman-
Rakic, 1990). Taken together, these findings suggest that it would
be worthwhile to monitor HD cell activity during a task that
unambiguously requires path integration or in which performance
has been shown to require an intact anterior thalamus, such as
certain spatial working memory tasks.

Implications for HD and Place Cell Interactions

It would be interesting to monitor place and HD cells simulta-
neously on the tasks used in Experiments 1–3, as a previous study
showed that place cell activity is consistent with a rat’s behavioral
choice, even when the rat searches at an incorrect location
(O’Keefe & Speakman, 1987). A second study, however, showed
that when the preferred direction of HD cells drifts, place cells
change their firing frequency by the same angular value (Knierim,
Kudrimoti, & McNaughton, 1995). In addition, both the preferred
firing direction of HD cells and the place field of place cells are
sensitive to changes in arena shape (Muller & Kubie, 1987;
O’Keefe & Burgess, 1996; Taube et al., 1990b). Thus, our results
do not appear to be consistent with both sets of findings. If HD and
place cells operate in register during the reference memory task,
our results imply that place cell activity would not be predictive
of the rat’s behavioral choice. If, however, place cell activity does
reflect the rat’s corner choice, then HD cell and place cell activity
would not be in register. Alternatively, one could speculate that
discrete shifts in preferred direction are accompanied by place cell
remapping, which may be correlated with the rat’s corner choice,
whereas HD and place cells are coupled when drifting occurs
within a session. This possibility is supported by the findings of
Knierim et al. (1995), who showed that place cells often remap
when the preferred firing direction of HD cells changes.

We also note that the interaction between HD cells and place
cells may be relevant to the large number of training trials required
in Experiments 1 and 2. Perhaps shifts in the preferred firing
direction of HD cells adversely affected place cell representations.

Decrement in Cue Control

Cue control over HD cell activity in Experiments 1 and 3 was
unexpectedly weak compared with previous studies in which HD
cells exhibited a stable preferred firing direction across daily
recording sessions for up to several weeks (Golob & Taube, 1997;
Taube, 1995; Taube et al., 1990b). In Experiment 1, HD cells
changed their preferred directions throughout the sequence of
trials, with the preferred firing direction for a cell being reestab-
lished after changing the holding box’s location. Shifts in preferred
direction between trials were even more common in Experiment 2,
even though the intertrial interval was only ~1 min.

Although systematic studies are required to determine the prin-
ciples that govern preferred firing direction stability, our results
suggest several factors that are related to cue control over preferred
firing direction in HD cells. On the basis of findings from Exper-
niment 1, it appears that shifts in preferred direction can be induced
simply by moving the rat back and forth between the holding box
and the arena, especially after changing the location of the holding
box. Although other studies have found HD cells to be quite stable
within a given environment (Golob & Taube, 1997; Taube et al.,
1990b; cf. Knierim et al., 1995), this experiment was the first time
the subjects had been placed into and removed from the apparatus
so frequently.
Another difference between the present experiment and previous studies concerns the shape of the arenas. In other experiments, HD cells were often recorded with the animal inside a cylinder, a shape that does not provide geometric information that could compete with the cue card (Blair & Sharp, 1996; Knierim et al., 1995; Taube, 1995). On the basis of shape alone, a square can reduce the possible orientations for HD cells to four orthogonal directions, and a rectangle can further reduce the number of directional orientations to two possibilities. The HD cell system may incorporate geometric information from the square to partially determine its directional orientation and be correspondingly less reliant on the cue card. In response to changing the box’s location in Experiment 1, the HD cell system may have been induced to recalibrate its orientation, a process that may be related to episodic reorientation by landmark cues during navigation (Gallistel, 1990). Most of the time, the preferred firing direction was maintained across trials, suggesting that the cue card was a major factor in determining firing direction. If HD cells use the arena’s geometric shape as well as cue location to determine the preferred direction, then occasional changes in preferred direction may reflect the influence of arena shape and not the landmark cue’s location. The incorporation of geometric information may explain why the HD cells shifted in ~90° increments in Experiments 1 and 3. The orthogonal shifts in preferred direction matched the arena’s four-fold symmetry. This hypothesis may be further tested by repeatedly introducing the subjects into an arena without four right corner angles, such as a triangle, and observing the magnitude of directional shifts. Arena shape has also been shown to affect the firing fields of place cells in the hippocampus (Muller & Kubie, 1987).

The results from Experiment 3 show that instability in preferred direction was context specific, because the preferred firing direction of HD cells across sessions in the cylinder was much more consistent than in the rectangle. Note that there are several differences between the recording sessions in the cylinder and those in the rectangle, such as the lack of disorientation procedures, overhead lighting rather than intra-arena sources, and the absence of useful geometric information for orientation in the cylinder. Any of these factors could have served to improve cue control.

Finally, it is worth commenting on the extent to which inconsistent cue control by landmarks could have led to disorientation of the rat. The observation that the rats performed the tasks fairly well suggests that they were probably not disoriented most of the time. However, overall performance levels were not as accurate (~80%) as they could have been, and it is possible that suboptimal performance levels were due to disorientation of the rats on some occasions. Nonetheless, it is important to note that performance was still accurate in cases in which the HD cell’s preferred direction was not stable, and that the HD cell’s preferred firing direction was usually stable in cases when the rat made a response error.

Conclusions

In summary, the main findings showed that the preferred firing direction of HD cells was often unrelated to the direction of the subject’s behavioral response in two types of spatial memory tasks. These findings suggest that the orientation of ADN HD cells was not obviously related to the subject’s behavioral response in these spatial memory tasks.

References


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