Exp Brain Res (2000) 133:198–208
Digital Object Identifier (DOI) 10.1007/s002210000365

RESEARCH ARTICLE

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Influence of conflicting visual, inertial and substratal cues on head direction cell activity

Received: 20 July 1999 / Accepted: 28 January 2000 / Published online: 30 March 2000

Abstract In order to navigate efficiently, animals can benefit from internal representations of their moment-to-moment orientation. Head-direction (HD) cells are neurons that discharge maximally when the head of a rat is oriented in a specific ("preferred") direction in the horizontal plane, independently from position or ongoing behavior. This directional selectivity depends on environmental and inertial cues. However, the mechanisms by which these cues are integrated remain unknown. This study examines the relative influence of visual, inertial and substratal cues on the preferred directions of HD cells when cue conflicts are produced *in the presence of the rats*. Twenty-nine anterior dorsal thalamic (ATN) and 19 postsubicular (PoS) HD cells were recorded from 7 rats performing a foraging task in a cylinder (76 cm in diameter, 60 cm high) with a white card attached to its inner wall. Changes in preferred directions were measured after the wall or the floor of the cylinder was rotated separately or together in the same direction by 45°, 90° or 180°, either clockwise or counterclockwise. Linear regression analyses showed that the preferred directions of the HD cells in both structures shifted by ≈90% of the angle of rotation of the wall, whether rotated alone or together with the floor (r^2>0.87, P<0.001). Rotations of the floor alone did not trigger significant shifts in preferred directions. These results indicate that visual cues exerted a strong but incomplete control over the preferred directions of the neurons, while inertial cues had a small but significant influence, and substratal cues were of no consequence.

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Key words Spatial orientation · Anterodorsal thalamic nucleus · Postsubiculum · Passive rotation · Multisensory integration

Introduction

In rats, head-direction (HD) cells are a possible substrate for an internal representation of the momentary orientation in the horizontal plane (Ranck 1984). These neurons have been found in many different areas of the rat brain, such as the postsubiculum (PoS) (Taube et al. 1990a), the anterodorsal thalamic nucleus (ATN) (Taube 1995), the dorsal striatum (Wiener 1993), the lateral dorsal thalamic nucleus (LDN) (Mizumori and Williams 1993), the lateral mammillary nucleus (LMN) (Stackman and Taube 1998; Blair et al. 1998), and certain areas of parietal and retrosplenial cerebral cortices (Chen et al. 1994). They discharge selectively when the head of the animal is oriented in a specific direction, the *preferred direction*, independently of position or ongoing behavior (Ranck 1984). Salient visual cues exert a strong influence on the preferred directions of the HD cells (Taube et al. 1990a; Goodridge and Taube 1995; Dudchenko et al. 1997), while olfactory and tactile cues exert a much smaller influence, and auditory cues do not appear to exert any influence at all (Goodridge et al. 1998). However, directional selectivity persists in total darkness (Chen et al. 1994; Mizumori and Williams 1993; Blair and Sharp 1996), and is abolished after lesions of the vestibular apparatus even when visual cues are available (Stackman and Taube 1997). Taken together, these results indicate that HD cells are influenced by a combination of environmental and self-movement cues.

It is of particular interest to understand how these diverse cues are integrated to produce HD cell responses, and this could shed light on the problem of multisensory fusion. However, in previous studies addressing this question, mixed results have been found (Goodridge and Taube 1995; Blair and Sharp 1996; Knierim et al. 1998). Although visual cues have been shown to exert a strong

influence on the preferred direction of ATN and PoS HD cells (Taube et al. 1990a; Taube 1995), this was found in conditions where the influences of inertial cues and substratal cues (such as tactile and olfactory cues on the floor) were minimized: between the control and test sessions, the rats were removed from the recording cylinder and disoriented, and paper on the floor was changed to remove potential cues. In a different study, Blair and Sharp (1996) addressed the question of the influence of inertial cues on the preferred direction of ATN HD cells directly by rotating the rats passively, but this was done in the absence of polarizing visual cues. Knierim and colleagues (1998) showed that the preferred directions of ATN HD cells follow the visual cues when the whole recording apparatus is rotated in the presence of the rat, except for fast rotations by a large angle (135° or 180°), for which the preferred directions shifted only half of the time. However, the respective influence of visual and substratal cues could not be distinguished under these conditions, because both wall and floor cues could have triggered the shifts.

Here, we examined the responses of ATN and PoS HD cells after experimental manipulations of visual, inertial and substratal cues *in the presence of the rats*. Since the animals remained in the cylinder and were not disoriented during cue manipulations, the influence of inertial cues could be tested directly. The experimental apparatus consisted of a black cylinder with a white card attached to its inner wall. The wall and floor of this cylinder could each be rotated independently (Blair and Sharp 1996). By rotating the wall or the floor separately, or both the wall and floor together in the same direction while the rat remained in the cylinder, we induced several types of conflicts between visual, inertial and substratal cues. By comparing the effects of these three types of manipulations, we were able to estimate the relative influence of the cues upon the preferred directions of the HD cells. Some of this work has been presented previously in abstract form (Zugaro et al. 1999).

Materials and methods

Experimental subjects

The subjects were seven male Long-Evans rats, weighing 200–250 g upon arrival (CERJ, Le Genest-St-Isle). They were housed in pairs until the time of surgery, and then kept in separate cages. After recovery they were placed on a food restriction diet keeping them at approximately 85% of their normal weight. Water was freely available. The animals were maintained on a 12 h light/12 h dark cycle. All animal care and experimental protocols were in accord with institutional and international standards and legal regulations ("Principles of laboratory animal care", NIH publication No. 86–23, revised 1985, as well as specific national laws where applicable).

Electrode implantation

Three rats were implanted with tetrodes – groups of four twisted nichrome wires (Recce and O'Keefe 1989), diameter 13 µm or 25 µm. Four rats were implanted with bundle electrodes. All electrodes had gold-plated tips (impedance 200–700 kΩ). Four rats were implanted both in ATN and PoS, and three rats received bilateral ATN implantation. Before surgery, the electrodes were inserted in 30-gauge stainless steel cannulas. Cannulas were mounted on two connectors that could be advanced independently via screws attached to a common base (Wiener 1993). For surgery, the animals were tranquillized with xylazine, then deeply anesthetized with pentobarbital (40 mg/kg). The tetrodes were implanted above the PoS (AP –6.5 mm to –7.3 mm, ML ±2.8 mm to ±3.5 mm relative to bregma) and above the ATN (AP –1.4 mm to –1.8 mm, ML ±1.2 mm to ±1.5 mm relative to bregma) using conventional surgical techniques. The headstage was permanently fixed to the skull with dental acrylic and seven tiny screws.

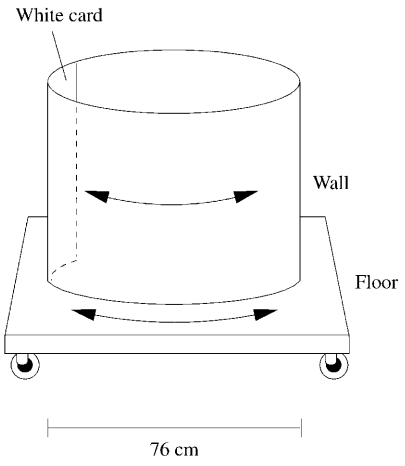

Fig. 1 The experimental apparatus. The cylinder wall and floor could be rotated independently

Behavioral apparatus

The square recording room was enclosed by black curtains suspended from the ceiling along four walls. Illumination was provided by a 40-W overhead lamp which diffused light evenly within the cylinder. The brightness of the lamp masked possible cues outside the cylinder. The experimental apparatus (Fig. 1) consisted of a black cylinder (60 cm high, 76 cm in diameter). A white card (50 cm wide, covering 75°) attached on the inner wall served as a salient visual landmark, referred to as the "cue card." The wall and the floor of the cylinder could be rotated independently (Blair and Sharp 1996). In order to ensure precise rotation angles, the floor was rotated manually with a pulley system calibrated for 45° steps. The wall had an angular graduation drawn on its outer side for calibration. All electronic instruments and computers were situated outside the curtains, and the entire experimental room was phonically isolated from the rest of the building.

Behavioral task

Before each recording session, the wall and floor were first rotated to a reference position, with the cue card center at 0°. The animals had been trained to retrieve small food pellets (5 mg chocolate sprinkles) thrown manually into the cylinder at pseudorandom locations (Muller et al. 1987). This kept the rats moving throughout the session, and resulted in visits to most of the floor surface and a fairly uniform distribution of head orientations over time. Each session lasted 20–25 min, and included three or four environmental manipulations (rotation of the wall, rotation of the floor, and rotation of both at ~10°/s) in the presence of the rat. These manipulations were made in a pseudorandom sequence. Wall and floor rotation angles included –180°, –90°, –45°, +45°, +90° and +180°.

The final configuration of the experimental apparatus was identical to the initial one. The following data compare preferred directions in recordings immediately prior to and after such manipulations.

Unit isolation and data collection

Animals were brought into the recording room in a transparent plastic cage; then the recording cable was attached to the electrode assembly, and the rat was placed in the cylinder without any attempt to disorient it. The electrode channels were screened while the rat performed the foraging task in the cylinder. If no suprathreshold HD cell activity was present, the electrodes were slightly advanced (50 µm, each pair of tetrodes being independently driveable), and the animal was checked again 4–96 h later. If cells were present, the floor was cleaned again (if necessary) and the curtains were closed before the recording session began. Note that since screening was conducted every working day, the rats were rather familiar with the environment.

During the recording sessions, electrode signals passed through FETs and were differentially amplified (×10,000) and filtered (300 Hz to 5 kHz, notch at 50 Hz). The signal was then passed to a computer for automatic data collection. The acquisition software (DataWave Discovery) digitized and collected 32 data points (at 20 kHz) for each signal that crossed a user-set threshold. In most cases, activity of individual neurons appeared on only one of the four twisted wires. Single unit activity was discriminated post hoc using "cluster cutting" techniques based on a maximum of eight different waveform parameters.

Prior to recordings, a support with two small lamps (10 cm separation) was mounted above the headstage. Reflectors were attached to the lamp in the rostral position to make it appear larger than the caudal lamp. The positions of the two lamps were detected by a video camera mounted above the platform (using the DataWave video tracking system) and sampled at a rate of 60 Hz. The heading direction of the animal was later computed using the positions of the two lamps. Inversions of the lamps due to tracking errors were corrected with our own interactive software. Counterclockwise rotations are considered positive here.

In order to build tuning curves for the HD cells, our software counted the number of spikes for each position sampling interval (16.6 ms), and associated the resulting frequency with the corresponding head angle. This was used to compute a histogram, for which each bin height was the average of all the frequencies associated with head angles within the range of the bin. Analyses were carried out on sessions where the rat spent a minimum of 2 s per 6° bin. Note that our software corrects for the delay between video and cell signal processing times.

To calculate HD cell parameters (preferred direction, peak firing rate, firing range, baseline firing rate), we used a discrete adaptation of the Gaussian-like fit employed by Zhang (1996):

$$f(\theta)=A+B.e^{K.\cos(\theta-\theta_0)}$$

where $f(\theta)$ is the firing rate, θ_0 the preferred direction, $B.e^K$ the peak firing rate, $230°/\sqrt{K}$ the firing range (width of the curve at the baseline level, computed using the two tangent lines at the inflexion points of the Gaussian curve), and A the baseline firing rate. A best-fit approximation to this curve was obtained via Matlab (The MathWorks, Natick, MA) software (least squares distance obtained with a Nelder-Mead type simplex search method).

Shifts in preferred directions were computed for the two successive preferred directions measured before and after each environmental manipulation. Statistics were performed using Statistica (StatSoft Inc., Tulsa, OK) software.

Histology

At the end of the experiments, a small electrolytic lesion was made by passing a small cathodal DC current (20 µA, 10 s) through one of the recording electrodes to mark the location of its tip. The rats were then deeply anesthetized with pentobarbital. Intracardial perfusion with saline was followed by 10% formalin-saline. Histological sections were stained with cresyl violet. Recording sites were reconstructed by detecting the small lesion and the track of the 30-gauge cannula, taking into account the distance that the microelectrode driver had been advanced from the point of stereotaxic placement of the electrodes. The recording sites were calculated by interpolation along the electrode track between the lesion site and the implantation site.

Experiment 1: rotation of the wall only

Manipulation

In this experiment, we recorded the HD cells for 5 min, rotated only the wall of the cylinder, and recorded for 5 more min (angles of rotation included –180°, –90°, –45°, +45°, +90°, and +180°).

Results

For this experiment, 29 ATN and 18 PoS HD cells were recorded in the 7 rats (in a total of 21 and 17 recording sessions respectively). The main characteristics of the directional tuning curves of these cells are displayed in the first two rows of Table 1.

The effect of rotating the wall of the cylinder upon the preferred directions of two HD cells is displayed in Fig. 2. The preferred directions of these HD cells shifted after the cylinder wall was rotated: they followed the wall cues, but shifted by a smaller angle. This will be referred to here as an "underrotation."

In order to examine the effect of wall rotations on the preferred directions, shifts in preferred directions were plotted against angles of rotation of the wall. This showed a linear relation (Fig. 3), except for two sessions (ATN cells recorded from the same animal, rotations of the wall

Table 1 HD cell firing properties for the three experiments. The same cells were often recorded in more than one experiment. These values are similar to those reported in previous studies (values are means ± standard errors of the mean)

Rotation	Structure	Peak firing rate (spikes/s)	Firing range (°)	Baseline firing rate (spikes/s)
Wall rotations	ATN	32.2±3.5	100.9±5.2	0.6±0.3
	PoS	20.7±3.1	105.3±11.0	5.5±1.6
Wall and floor rotations	ATN	31.1±3.4	98.1±5.0	0.8±0.3
	PoS	17.0±3.3	91.0±8.8	3.4±1.6
Floor rotations	ATN	32.9±3.4	94.1±4.0	0.5±0.3
	PoS	18.0±2.4	95.8±13.6	3.6±2.0

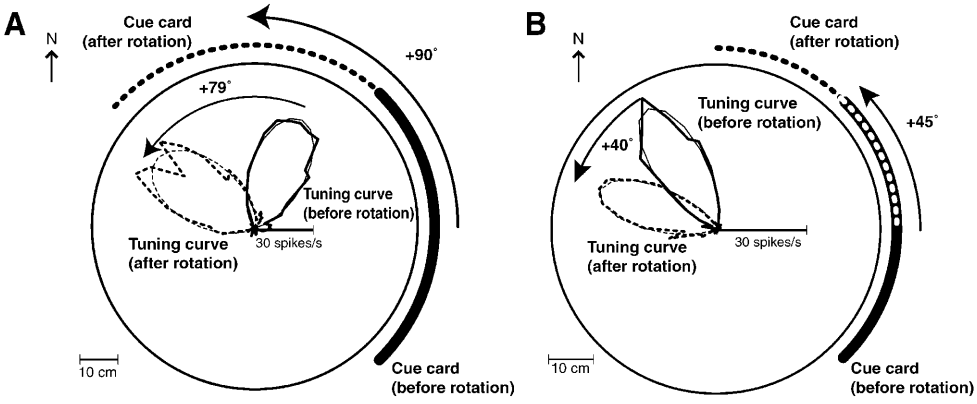

Fig. 2A,B Examples of underrotation of the preferred direction of two ATN HD cells after rotation of the cylinder wall only. The firing rate is plotted as a function of the head direction in polar coordinates (bin size: 6°). **A** While the cue card was centered at 0° (starting position, continuous arc), the preferred direction of this HD cell was 68° (*thick continuous line*; the pseudo-Gaussian approximation of this tuning curve is shown by *the thin continuous line*). After the cue card was rotated by 90° (*dotted arc*), the preferred direction rotated by only 79° (*thick dotted line*; the pseudo-Gaussian approximation of this tuning curve is shown by *the thin dotted line*). This is an underrotation of 12% (11°/90°). **B** After the cue card was rotated by 45°, the preferred direction of this cell rotated by only 40° (same conventions as in **A**). This is an underrotation of 11% (5°/45°)

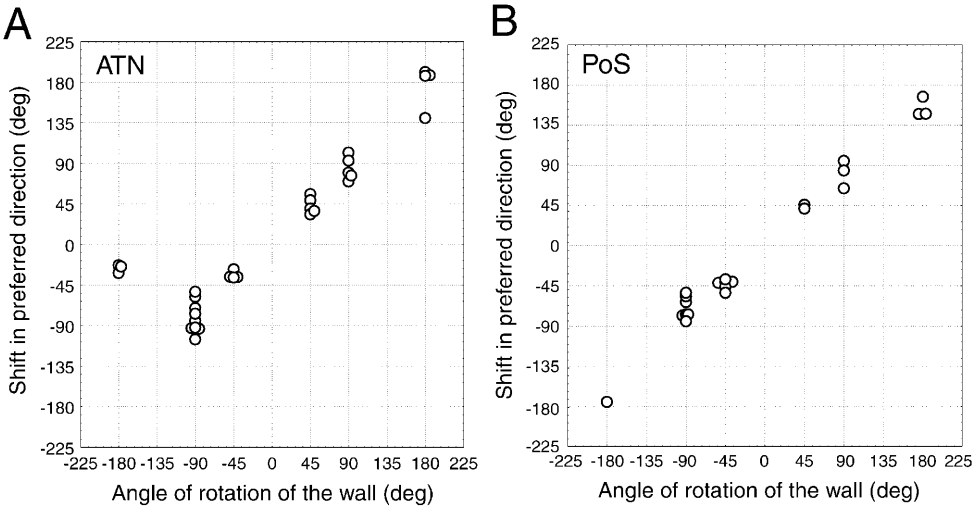

Fig. 3 The complete data set for shifts in preferred directions of all cells recorded after rotations of the wall only (**A** ATN cells, **B** PoS cells). Overlapping points are displaced laterally

by –180°) where the preferred directions shifted by a small angle. For these two sessions, however, the absence of preferred direction shift was obtained only after the rat had been trained in a different experiment conducted in the dark with asymmetrically distributed olfactory cues.

To quantify this linear relation, data for 180° rotations were excluded because it was not possible to determine whether the corresponding shifts in preferred direction were smaller or greater than wall rotations (for instance, a CW rotation of 170° is equivalent to a CCW rotation of 190°).

Linear regression analyses on the shifts in preferred directions ($\Delta\theta$) against the angles of rotation of the wall ($\Delta\alpha_{wall}$) yielded: $\Delta\theta=0.91\Delta\alpha_{wall}+2.51°$ ($r^2=0.97$) for ATN cells, and $\Delta\theta=0.85\Delta\alpha_{wall}+2.56°$ ($r^2=0.97$) for PoS cells. In both cases, the regression slope was highly significant ($P<0.001$), but the intercept value was not significantly different from zero ($P>0.1$).

From one to three neurons were recorded simultaneously in the sessions. Since the simultaneously recorded cells responded similarly to wall rotations, this could lead to overemphasis of the importance of such sessions, and bias the linear regression analysis (Goodridge et al. 1998). Therefore, in each recording where multiple HD cells were recorded simultaneously, the individual shifts in preferred directions were replaced by their mean. A second set of linear regression analyses confirmed the previous results (Fig. 4).

Blair et al. (1998) found differences in the responses of LMN HD cells during head turns depending on the hemisphere the cells were recorded from. To investigate possible differences between responses of neurons recorded in the left and right hemispheres to CW or CCW wall rotations, in Fig. 4 the data points are shown with different symbols. No obvious relation appears between lateralization and response properties.

In order to test for differences between responses to wall rotations in ATN and PoS recordings, we first conducted a linear regression analysis on the pooled data.

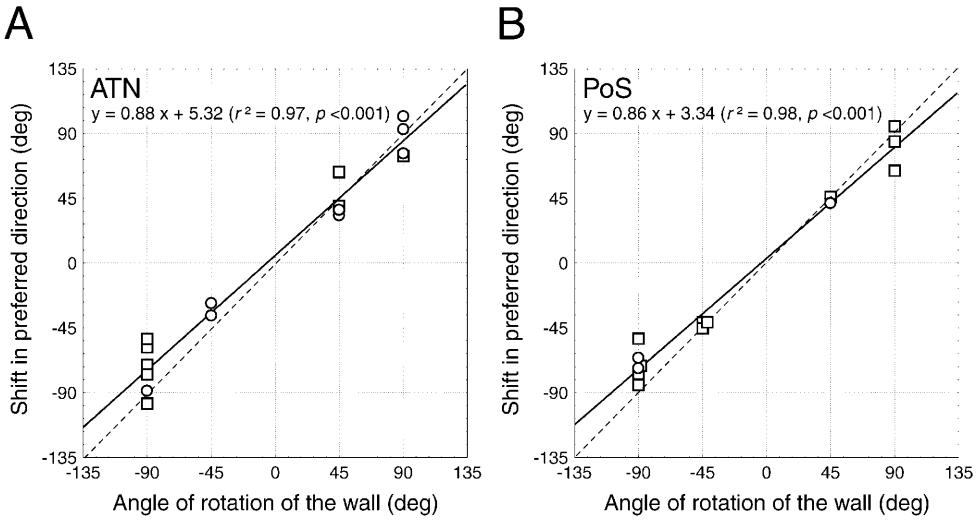

Fig. 4 Linear regression analysis of the effects of rotating only the wall of the cylinder upon the preferred directions of the HD cells (**A** ATN, **B** PoS). Each point is the average shift in the preferred direction of all cells recorded simultaneously in a given session. The data points are plotted along with the regression line (*continuous line; the dashed line* shows where the points would appear if the wall exerted complete control upon the preferred directions). The equations of the regression lines are indicated above. *Symbols* indicate the hemisphere from which the cells were recorded (*circles, left; squares, right*)

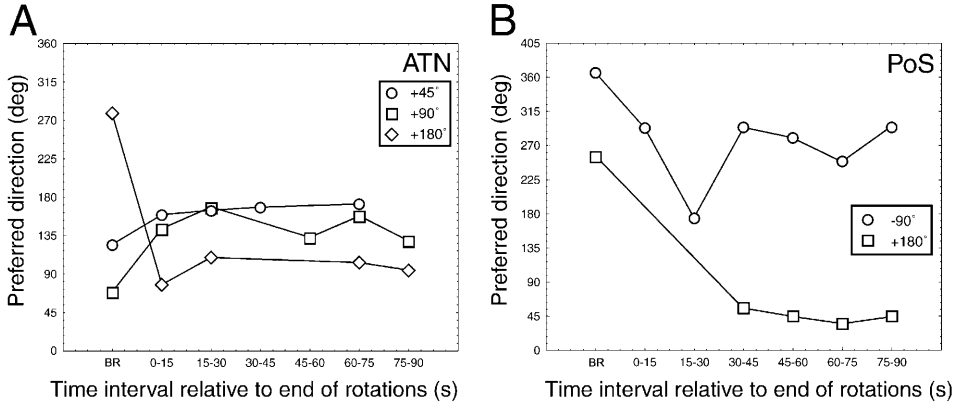

Fig. 5A,B Time course of preferred direction shifts after wall rotations for sessions with sufficient sampling. For each cell, *the first point* shows the preferred direction measured during the 5 min preceding the rotation (*BR* before rotation). Tuning curves are computed during successive 15-s blocks after rotations ended (**A** ATN cells, **B** PoS cells)

Then, residuals were computed for all the data points, and were separated into two groups corresponding to the respective structures. A *t*-test showed no significant difference between the two groups ($P>0.5$). This provides evidence that there was no significant difference between the results from ATN and PoS recordings in this experiment.

In summary, the preferred directions of the HD cells in both structures shifted in register with wall rotations, but by angles about 10% smaller. To test whether shifts in preferred directions were significantly smaller than wall rotation angles, the difference between complete (100%) and observed shifts in preferred directions was examined as a function of wall rotation angles. Linear regression analyses showed that the shifts in preferred directions are significantly different from wall rotation angles (ATN: $r^2=0.32$, $P<0.05$ for slope, $P>0.1$ for intercept offset; PoS: $r^2=0.56$, $P<0.01$ for slope, $P>0.1$ for intercept offset).

To investigate the time course of the shifts in preferred directions after wall rotations, tuning curves were made for six 15-s periods after the rotation ended. Since this reduced the data samples for each interval, recordings where the rat oriented its head a minimum of 100 ms in each 6° bin were selected (Fig. 5). Each tuning curve was treated as a histogram, and the preferred direction during each interval was computed as the mean of the histogram. Figure 5 shows that the preferred directions of the HD cells shifted to their new orientation as rapidly as 15–30 s after wall rotation and showed no apparent tendency for drift afterward.

In order to determine whether the degree of familiarity of the animals with the experiment affected the influence of the wall cues, the normalized shift in preferred directions (shift divided by wall rotation angle) was plotted against session number (Fig. 6). The absence of an obvious trend indicates that the control exerted by the wall cues did not depend on the previous experience of the rats with the wall rotations.

Discussion

The cues on the wall (the most salient of which was the cue card) exert a strong influence on the preferred directions of the HD cells in both structures. Furthermore,

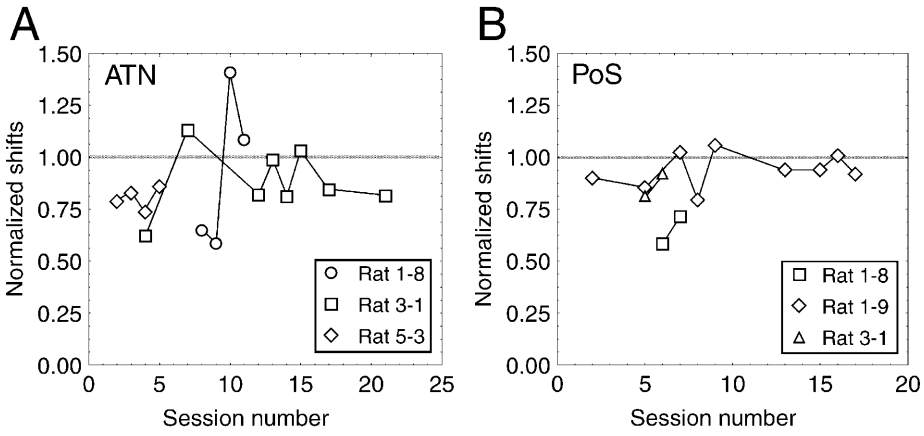

Fig. 6A,B Averaged preferred direction shifts after wall rotations in measurements from successive recording sessions. For each rat, normalized shifts in preferred directions (shifts divided by wall rotation angles) are plotted against session number. Sessions where no cells were isolated are counted. Data have been excluded for rats having only one recording session for a given structure (**A** ATN cells, **B** PoS cells)

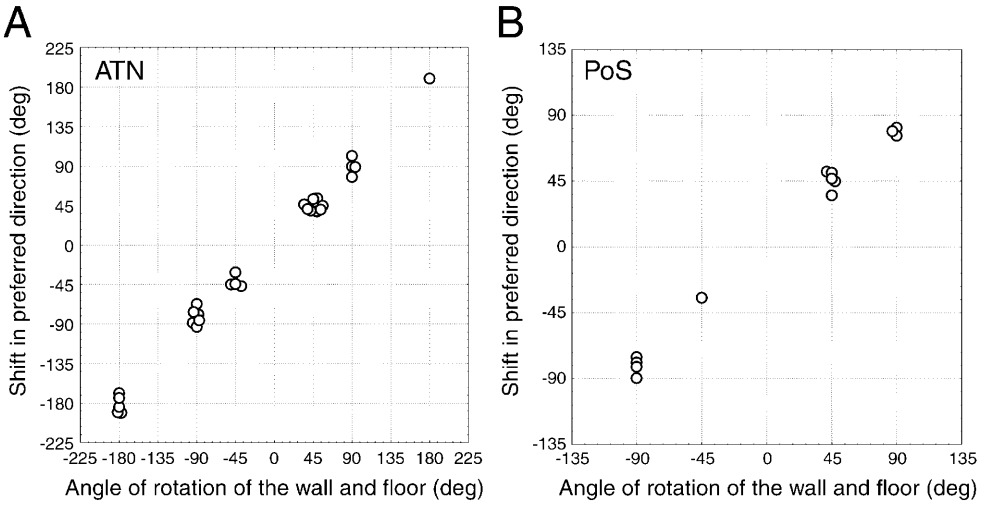

Fig. 7 The complete data set for shifts in preferred directions of all cells recorded after rotations of the wall and floor (**A** ATN cells, **B** PoS cells)

since the preferred directions tend to follow the cue card despite the lack of coherent inertial cues (under normal circumstances when the rat moves about, rotations of the visual cues are produced by self-rotations in the opposite direction), the results show that the visual cues dominate over the inertial cues under these conditions – our pilot experiments indicate that the olfactory cues on the wall exert no reliable effect on the preferred directions (Zugaro, Fouquier, Tabuchi, unpublished observations). However, the preferred directions rotate significantly less than the cue card: this trend to underrotation indicates that the cue card does not exert complete control upon the HD system.

Experiment 2: rotation of the wall and floor

Manipulation

In this experiment, we recorded the HD cells for 5 min, then rotated the wall and floor of the cylinder together, and recorded for 5 more min (angles of rotation included –180°, –90°, –45°, +45°, +90° and +180°).

Results

For this experiment, 28 ATN and 13 PoS HD cells were recorded in the 7 rats (in a total of 18 and 8 recording sessions, respectively). The main characteristics of the tuning curves of these cells are displayed in the middle two rows of Table 1.

The shifts in preferred directions ($\Delta\theta$) were plotted against the angles of rotation of the wall and floor ($\Delta\alpha_{both}$) (Fig. 7). All angles were measured relative to the fixed reference frame of the experimental room. Similar to experiment 1, there was a linear relation between shifts in preferred directions and angles of rotation of the wall and floor.

Linear regression analyses yielded: $\Delta\theta=0.95\Delta\alpha_{both}+2.47°$ ($r^2=0.99$) for ATN cells, and $\Delta\theta=0.91\Delta\alpha_{both}+1.85°$ ($r^2=0.99$) for PoS cells. In both cases, the regression slope was highly significant ($P<0.001$), while the intercept value was not ($P>0.1$). When replacing data obtained for simultaneously recorded cells by their mean, linear regression analyses were similar (Fig. 8).

Data points in Fig. 8 were represented differently depending on the hemisphere the cells were recorded from. This showed no obvious combined effect of sense of rotation and lateralization.

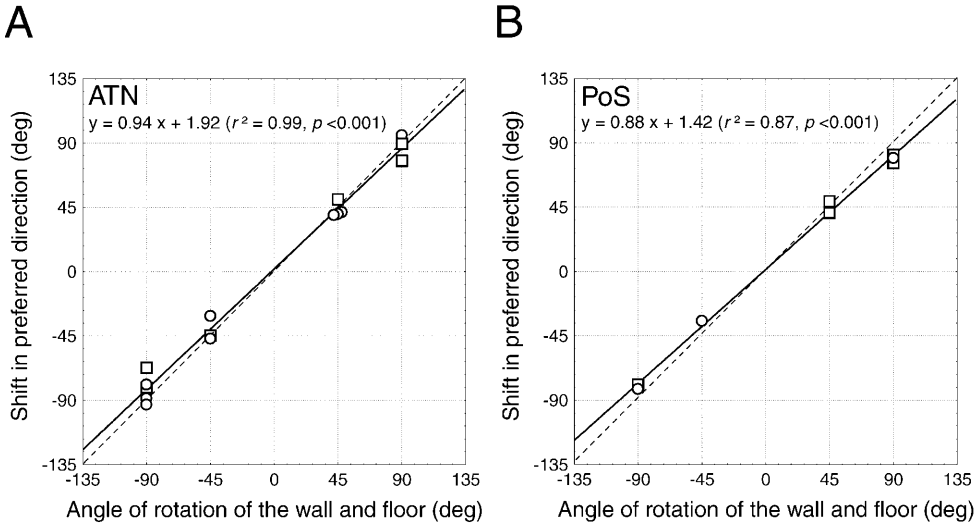

Fig. 8 Linear regression analysis of the effects of rotating the wall and floor of the cylinder upon the preferred directions of the HD cells (**A** ATN, **B** PoS). Each point is the average shift in the preferred directions of all cells recorded simultaneously in a given session. The data points are plotted along with the regression line (*continuous line; the dashed line* shows where the points would appear if the wall and floor exerted complete control upon the preferred directions). The equations of the regression lines are indicated above. *Symbols* indicate the hemisphere from which the cells were recorded (*circles, left; squares, right*)

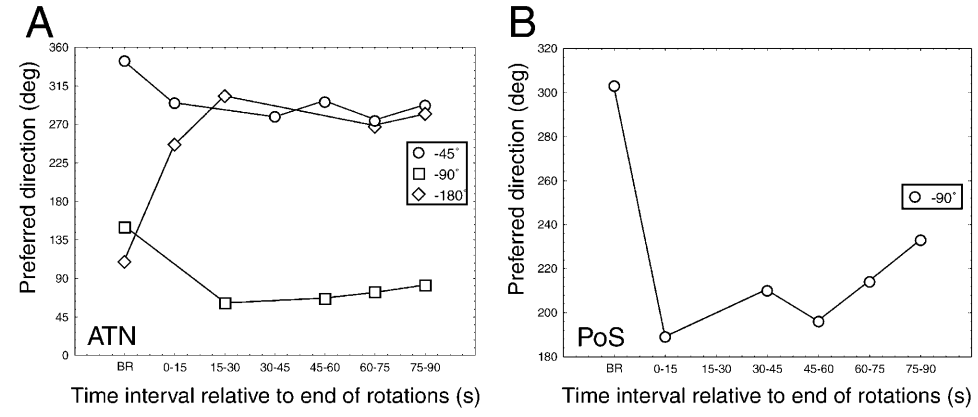

Fig. 9A,B Time course of preferred direction shifts after rotations of both wall and floor. Tuning curves are computed during successive 15-s blocks after rotations ended (**A** ATN cells, **B** PoS cells)

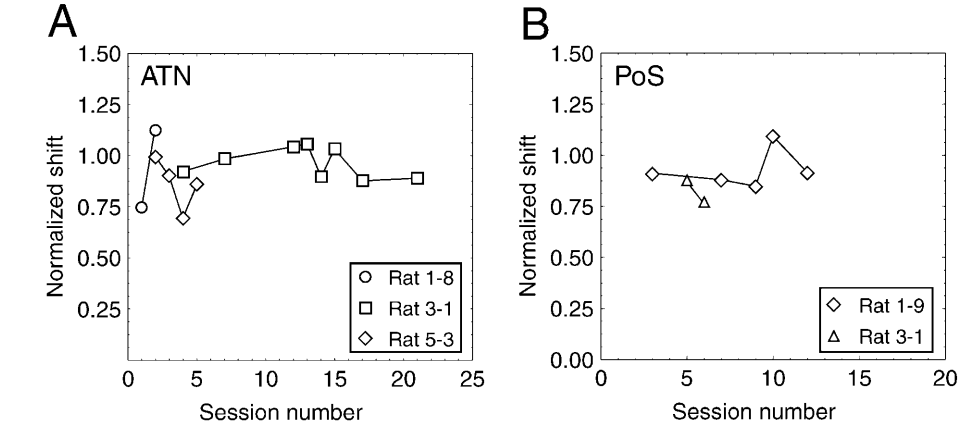

Fig. 10A,B Averaged preferred direction shifts after wall and floor rotations in successive recording sessions. For each rat, normalized shifts in preferred directions (shifts divided by wall rotation angles) are plotted against session number. Sessions where no cells were isolated are counted. Data have been excluded for rats having only one recording session for a given structure (**A** ATN cells, **B** PoS cells)

As in experiment 1, to test for differences between responses to wall and floor rotations in ATN and PoS recordings, linear regression analysis was conducted on the pooled data. A *t*-test on the two groups of residuals showed no significant difference between the two groups ($P>0.1$). This provides evidence that there was no significant difference between the results from ATN and PoS recordings in this experiment.

To test whether the preferred directions shifted significantly less than the wall and floor, the difference between complete (100%) and observed shifts in preferred directions was examined as a function of wall rotation angles. Linear regression analyses showed that the shifts in preferred directions are significantly different from rotation angles of the wall and floor (ATN: $r^2=0.28$, $P<0.05$ for slope, $P>0.1$ for intercept offset;

PoS: r^2=0.83, P<0.01 for slope, P>0.1 for intercept offset).

Similar to experiment 1, Fig. 9 shows that the preferred directions of the HD cells shifted to their new orientation within 15 s after wall and floor rotation and showed no apparent tendency for drift afterward. Similar to experiment 1, the control exerted by the wall and floor cues did not depend on the experience of the rats (Fig. 10).

In order to determine whether the effect of rotating both the wall and floor together was different from the effect of rotating the wall alone, we conducted a *t*-test on the residuals of a pooled regression (using the same methods as described above). This showed no significant difference between the two conditions (P>0.1 for ATN recordings, and P>0.1 for PoS recordings).

Discussion

The results indicate that the ensemble of cues within the cylinder exert a strong but incomplete influence on the preferred directions of both populations of HD cells. However, the shifts in preferred directions are not significantly different from those observed in the previous experiment. This suggests that the influences of wall and floor cues are not combined in a linear manner. Alternatively, floor cues may not exert any influence at all on the preferred directions of the HD cells under these experimental conditions.

Experiment 3: rotation of the floor only

Manipulation

In this experiment, we recorded the HD cells for 5 min, rotated the floor of the cylinder, and recorded for 5 more min (angles of rotation included –180°, –90°, –45°, +45°, +90° and +180°).

Results

A total of 28 ATN and 14 PoS HD cells were recorded in the 7 rats (in a total of 18 and 7 recording sessions, respectively). The main characteristics of the tuning curves of these cells are displayed in the last two rows of Table 1.

The shifts in the preferred directions ($\Delta\theta$) were plotted against the angles of rotation of the floor ($\Delta\alpha_{floor}$). All angles were measured in the fixed reference frame of the experimental room. Similar to previous experiments, there was a linear relation between shifts in preferred directions and floor rotation angles. Linear regression analyses yielded $\Delta\theta=0.04\Delta\alpha_{floor}-0.02°$ (r^2=0.16) for ATN cells, and $\Delta\theta=-0.001\Delta\alpha_{floor}+1.94°$ (r^2=0.002) for PoS cells. The regression slopes were not significant (P>0.05 for ATN and P>0.5 for PoS), and neither were the intercept values (P>0.1 in both cases).

The linear regression analyses for values averaged for each recording were similar (Fig. 11).

Similar to previous experiments, there was no significant difference between the results from ATN and PoS recordings (*t*-test on the two groups of residuals obtained from a linear regression analysis on the pooled data, P>0.5).

Discussion

The results indicate that substratal cues (such as odors or tactile cues on the floor) alone do not exert any significant influence on the preferred direction of the HD cells under these experimental conditions.

General discussion

In this study, we examined the influence of visual, inertial and substratal cues upon the HD cell system. The results show that, in this paradigm, visual cues have a

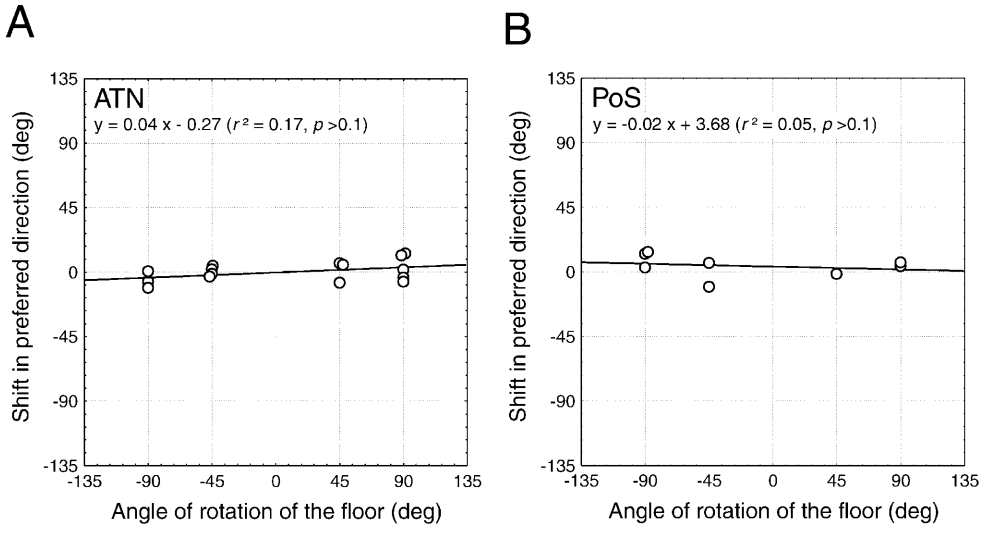

Fig. 11 Absence of effect of rotating only the floor of the cylinder upon the preferred directions of the HD cells (**A** ATN, **B** PoS). Each point is the average shift in the preferred direction of all cells recorded simultaneously in a given session. The data points are plotted along with the regression line (*continuous line*). The equations of the regression lines are indicated *above*

Table 2 Influences of the diverse cues on the preferred directions of the HD cells (measured relative to the experimental room) for the three experiments. As a reminder, the experimental (normalized) shift in preferred directions observed during the recordings is given in the last column. This indicates the relative influence of the cues

Type of rotation	Question 1	Question 2			Observed shift in preferred directions in ATN and PoS cells
	Does the HD change relative to the room?	Does this type of cue indicate that the HD has changed? A conflict with answer to question 1 would provoke a shift in preferred directions (indicated in parentheses)			
		Visual cues	Inertial cues	Substratal cues	
Wall	No	Yes (shift)	No (no shift)	No (no shift)	≈90%
Both	Yes	No (shift)	Yes (no shift)	No (shift)	≈90%
Floor	Yes	Yes (no shift)	Yes (no shift)	No (shift)	≈0%

strong but incomplete influence upon the updating of the preferred directions of the HD cells. In particular, although the preferred directions tend to recalibrate relative to the cue card when it is rotated, the angle of rotation is smaller than that of the cue card. In the following, we suggest that underrotation is due to the influence of inertial cues. Throughout this discussion, the preferred directions are measured in the fixed reference frame of the experimental room.

Resolving multisensory conflicts

In order to determine the respective influences of the diverse cues in our experiments, it will be helpful to answer the following two questions in each experiment for each type of cue: (1) after the cue rotation is performed, is the head of the rat oriented in a different direction *relative to the experimental room*? (2) does this cue indicate to the rat that its head has rotated *relative to its previous orientation*? If the answers to these two questions are different, this conflict could trigger a shift in the preferred directions of the HD cells (measured relative to the experimental room). Since all of our environmental manipulations induced conflicts between the diverse types of cues, the magnitudes of shifts in preferred directions observed above indicate the relative influence of each type of cue.

After rotation of only the wall of the cylinder, the head of the rat does not point to a different direction (relative to the experimental room). However, the new orientation of the cue card relative to the rat indicates that the head of the animal now points in a different direction. If the preferred directions of the HD cells depended solely on visual cues, they would shift after the rotation of the wall. On the other hand, since the rotation of the wall does not provide any inertial stimuli, the inertial cues indicate that the head of the animal has not moved. Similarly, since there is no movement of the floor relative to the rat, the substratal cues also indicate that the head has not moved. Hence, inertial and substratal cues would not tend to provoke a shift in preferred directions after rotation of the wall. This is summarized in the first row of Table 2.

After rotation of both the wall and floor of the cylinder together, the head of the rat points in a different direction (relative to the experimental room). However, the orientation of the cue card relative to the rat does not change, and this visual input indicates that there was no displacement of the head of the animal. Similarly, since the floor is not rotated relative to the rat, the substratal cues also indicate that the head has not moved. If the preferred directions of the HD cells depended solely on visual or substratal cues, they would shift after the rotation of the wall and floor. On the other hand, the inertial cues provided by this passive rotation indicate that the head of the animal now points in a different direction, and would not tend to provoke any shift in preferred directions after the rotation of the wall and floor. This is summarized in the second row of Table 2.

Finally, after rotation of only the floor of the cylinder, the head of the rat points in a different direction (relative to the experimental room). The new orientation of the cue card relative to the rat indicates that the head of the animal now points in a different direction. Similarly, the inertial stimuli provided by the passive rotation also indicate that the head of the animal now points in a different direction. If the preferred directions of the HD cells depended solely on visual or inertial cues, they would not shift after the rotation of the floor. On the other hand, since the floor is not rotated relative to the rat, the substratal cues indicate that the head of the animal points in the same direction, and would tend to provoke a shift in preferred directions after the rotation of the floor. This is summarized in the final row of Table 2.

The results showed that all shifts in preferred directions occurred rapidly and were consistent across recording sessions. This indicates that under these experimental conditions where manipulations were not abrupt (cue rotations typically lasted a few seconds), the HD cell system was able to resolve cue conflicts in an efficient manner.

Note that, during environmental manipulations, the rats often continue moving about, and associated sensorimotor activity also provides orienting cues. However, since there are no conflicts, the normal mechanisms called into play during active movement should make the HD system automatically compensate for these voluntary

movements. Therefore the self-initiated movements of the rats during the experimental manipulations should not affect the shifts in preferred directions.

Relation to previous studies

Our results are consistent with previous studies indicating the strong influence of visual cues on HD cell preferred directions. Taube et al. (1990b) recorded HD cells in the PoS and ATN (Taube 1995; Goodridge and Taube 1995) before and after rotating a cue card by 90°. They observed a similar shift in the preferred directions of the HD cells. This was interpreted as evidence for a control of the cue card over the preferred directions of the HD cells. The mean absolute difference between the angle of rotation of the cue card and the shift in preferred direction was approximately 13° for ATN cells and 20° for PoS cells (10/15 ATN cells underrotated and 3/15 overrotated, while 10/16 PoS cells underrotated and 6/16 overrotated). This was interpreted as indicating that the cue card exerts imperfect control on the preferred directions, but alternate influences could not be tested because, in these experiments, the rat was removed from the experimental cylinder during card rotations, and was disoriented. Also, the floor paper was changed before the rat was reintroduced into the cylinder. Note that in our experiments the mean difference between the angle of rotation of the wall and the shift in preferred direction was only 8° for both structures (data for rotations of the wall by 90°), *but* with a significant trend for underrotations. Such an influence of inertial cues could not be tested in previous experiments where the rat was intentionally disoriented before being returned to the cylinder (Taube et al. 1990b; Taube 1995).

To examine the interactions between visual and inertial cues, Knierim and colleagues (1998) recorded ATN HD cells before and after rotating the whole experimental apparatus (wall and floor). They observed a strong control of the visual cues for small rotation angles (+45°), but not for larger ones (+180°). In particular, three HD cells were tested under conditions comparable to those of our study (rotations of +45° in a familiar cylinder). The results for these cells are not consistent with those reported here: in all three cells, the preferred directions shifted more than the angle of rotation of the cylinder (range of overrotation: +3° to +15°). This may be due to differences in methods and analyses (Fisher-344 rats have poorer vision than the Long-Evans rats used here, the recordings lasted only 2–3 min, the rotations of the apparatus were almost instantaneous, the resolution of the tuning curves was 10°, each bin was averaged with the two closest bins, the preferred direction was defined as the bin with the highest firing rate, etc.), or the small sample of the latter study. Some of these differences may also explain why, contrary to our results, Knierim et al. observed that visual landmark control was delayed after large apparatus rotations (135° or 180°): the HD cells maintained their preferred directions immediately after the rotations, then slowly drifted over the course of a minute or two until they were realigned with the cue card. In our study, the shifts in preferred directions after rotations of the visual landmarks usually occurred in less than 15 s (for large as well as small angles). The model of Zhang (1996) actually predicts that the preferred directions should "jump" to their new orientation after large rotations, whereas transitions for smaller angles should be smooth. This is not inconsistent with our results, because the time course of such "jumps" or smooth transitions is predicted by the model to be on the order of 1 s, too rapid for detection with our techniques.

Blair and Sharp (1996) investigated the respective importance of dynamic visual cues and inertial cues in ATN HD cells: they applied passive rotations of the animal and visual field rotations separately or simultaneously. The visual cues consisted of a series of four vertical black and white stripes taped on the inner wall of the experimental cylinder, which ensured that the visual pattern remained the same after rotation of the wall by an angle of 90°. It must be emphasized that since the four cue cards were symmetrically placed, they did not polarize the environment like the cue cards in other studies, and they could not have served as a landmark cue. When the wall and the floor were rotated together (thus in the absence of any visual field flow), in most cases the preferred directions did not change relative to the room. Since the rat was actually rotated passively at perceptible velocities, and there was no optic flow, the stability of the preferred directions could be provoked only by inertial cues (see row 2, column 4 of Table 2). Moreover, when the wall alone was rotated by multiples of 90°, there were no shifts in the preferred directions (note that this provided no inertial stimulation, simply visual field motion – and, after the rotations, the environment appeared unchanged). This indicates that inertial cues dominate over visual motion cues, and points to the important distinction between optic field flow (not taken into account separately in our treatment above) versus visual landmark cues. This distinction explains why the results of Blair and Sharp (1996) are not inconsistent with those of Taube et al. (1990b; Taube 1995).

In the present study the effect of cue rotations upon the preferred directions of the HD cells did not vary across recording sessions as the rats became more experienced with the experimental conditions. In particular, the cue card continued to exert a strong control on the preferred directions even when the rats had experienced many wall rotations (more than 20 rotations each for 3 animals). Similarly, Knierim et al. (1995) found that the visual landmark cues, provided that they were stable from session to session during training, retained their strong influence on the preferred directions of ATN HD cells even when disorientation procedures repeatedly induced conflicts between visual and inertial cues. In our study, the rats had experienced the cylinder as stable for many days or even weeks before the first experiments were conducted. It is interesting that the influence of the cue card was not altered by the fact that the rats could see it being ro-

tated during the experiments. However, it is noteworthy that one of our rats also showed only a small shift in preferred directions after wall rotations by 180° in two recording sessions. This occurred only after the rat had been trained in the dark with asymmetrically placed olfactory cues. Although previous work suggests that the influence of visual landmark cues becomes stochastic after large rotations (Knierim et al. 1998), in our study this absence of shifts in preferred directions was not observed in the other rats. It is possible that the new experimental conditions trained the rat to use different strategies to orient itself within the cylinder, and this could have weakened the influence of visual cues observed here.

Finally, shifts in the preferred directions did not appear to depend on the hemisphere from which the HD cells were recorded, even when taking into account the sense of rotation of the cues. Together with the finding that the tuning curves of the HD cells in ATN, contrary to those of the HD cells in the lateral mammillary body (LMN), are not different during ipsiversive (toward the hemisphere of the cell) versus contraversive (in the opposite direction) head turns (Blair et al. 1998), this indicates that ATN and PoS HD cells may not have hemispherically lateralized properties. Alternatively, the effects could be very weak, and would require more data to appear.

In summary, under the present experimental conditions (where visual cues are salient), visual and inertial cues have unequal influences on HD signals. This is consistent with the notion that visual cues could be used to stabilize and realign directional responses continuously updated by self-motion cues (Mizumori and Williams 1993; McNaughton et al. 1993).

Acknowledgements We thank Prof. A. Berthoz for support in all aspects of this work; Profs. J.S. Taube and P. Sharp for critical reading and comments on earlier versions of the manuscript; C.F. Fouquier for help with data analysis; M.-A. Thomas and N. Quenech'du for the histology; Dr. J. Droulez for advice on the statistical tests; A. Treffel, M. Ehrette and S. Ilic for the construction of the behavioral apparatus; F. Maloumian for illustrations; and D. Raballand for animal care. This work was supported by CNES, Cogniseine, and GIS.

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