Active and passive movement are encoded equally by head direction cells in the anterodorsal thalamus

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Shinder ME, Taube JS. Active and passive movement are encoded equally by head direction cells in the anterodorsal thalamus. J Neurophysiol 106: 788–800, 2011. First published May 25, 2011; doi:10.1152/jn.01098.2010.—The head direction (HD) system is composed of cells that represent the direction in which the animal’s head is facing. Each HD cell responds optimally when the head is pointing in a particular, or preferred, direction. Although vestibular system input is necessary to generate the directional signal, motor/proprioceptive inputs can also influence HD cell responses. Previous studies comparing active and passive movement have reported significant suppression of the HD signal during passive restraint. However, in each of these studies there was considerable variability across cells, and the animal’s head was never completely fixed. To address these issues, we developed a passive restraint system that more fully prevented head and body movement. HD cell responses in the anterodorsal thalamus (ADN) were evaluated during active and passive movement with this new system. Contrary to previous reports, HD cell responses were not affected by passive restraint. Both head-fixed and hand-held restraint failed to produce significant inhibition of the active HD cell response. Furthermore, direction-specific firing was maintained regardless of 1) the animal’s previous experience with restraint, 2) whether it was tested in the light or dark, or 3) the position of the animal relative to the axis of rotation. The maintenance of a stable directional signal without appropriate motor, proprioceptive, or visual input indicates that vestibular input is necessary and sufficient for the generation of the HD signal. Motor and proprioceptive influences may therefore be important for the control of the preferred firing direction of HD cells, but not the generation of the signal itself.

HEAD DIRECTION (HD) cells are neurons that fire when the animal is facing a particular direction relative to a fixed location or landmark in the environment and are believed to represent the animal’s perceived directional heading in its environment (Taube et al. 1990a, 1990b). The responses of HD cells can correlate well with the animal’s behavioral choices in spatial tasks, particularly when the tasks require the use of path integration or utilization of the animal’s perceived directional heading (Dudchenko and Taube 1997; Valerio et al. 2010; van der Meer et al. 2010), and are believed to represent the animal’s perceived directional heading in its environment. The exact mechanism by which the HD signal is generated remains unknown, but it is clearly dependent upon vestibular input (Muir et al. 2009; Stackman and Taube 1997) and brain stem circuitry that includes the dorsal tegmental and supragenual nuclei, as well as the lateral mamillary nuclei (Bassett et al. 2007; Biazoli et al. 2006; Blair et al. 1998; see Shinder and Taube 2010a for review).

One core issue that remains unresolved is the extent to which motor and proprioceptive stimuli contribute to the HD signal. Specifically, does passive motion of the animal elicit normal directional responses? Previous studies have not been consistent in their answers to this question, with early studies showing that motor and proprioceptive information play a significant role in the generation of the HD signal. For example, in their initial study on HD cells in the postsubiculum Taube et al. (1990b) reported a reduction in peak firing rates of ~50% in seven of nine cells, in response to passive rotation of the animal through the cell’s preferred direction. Recording from HD cells in the anterodorsal thalamus (ADN), Taube (1995) and Knierim et al. (1995) both reported substantial reductions in firing rates with passive rotation that produced near or complete suppression of the HD response when the animal was tightly restrained. Similarly, Chen et al. (1994) reported that 9 of 10 HD cells in the retrosplenial cortex displayed suppression of their directional responding to passive movement while the animal was standing still drinking, but unrestrained. Golob et al. (1998) found that responses were suppressed for about half of the HD cells in the postsubiculum during passive restraint and found reduced firing rates in the remaining HD cells. In reviewing these studies, the most marked reductions usually occurred when the animals were tightly restrained, and less decreased firing occurred when the animals were loosely restrained. Passive movement induced-suppression also appeared to extend to many angular head velocity cells in the dorsal tegmental nuclei, which provide critical vestibular information to the HD system (Sharp et al. 2001). Stackman et al. (2003) showed the importance that motor/proprioceptive information plays in maintaining a stable signal by demonstrating that passive transportation of an animal into a novel environment disrupted the ability of HD cells to maintain a stable preferred direction, despite the presence of vestibular and visual cues throughout the route.

In contrast to the above findings, other studies have reported less severe disruption to the HD signal in response to passive rotation. Zugaro et al. (2001) evaluated ADN HD cells during unrestrained passive movement and found only mild inhibition of cell firing, with peak firing rates reduced by only 23% with no loss of directional responding. Furthermore, Bassett et al. (2005) found only a 24% reduction in the peak firing rates of 21 ADN HD cells when the animals were passively moved while being loosely restrained. On the basis of these findings, it was postulated that the previous passive response suppression may be due in part to the degree of restraint used during passive stimulation. These studies argued that the strong re-
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reduced firing during passive rotation. More, we offer suggestions as to why earlier studies reported responses to passive manipulations have used some form of limited restraint, and no study has formally locked the head including the temporal and most lateral parts of the occipital plates. To anchor the head restraint post, which is placed rostral to the electrode array, screws are placed in the frontal skull plate just in front of the restraint post. In addition, further modification was made for sealing the hole around where the electrode array entered the skull. Instead of drilling a 2- to 3-mm hole in the skull above the ADN, a smaller hole (~1 mm) was drilled and a guide tube with an inner diameter slightly larger than the outer diameter of the electrode cannula was lowered through the hole and affixed to the skull. The exposed gap around the electrode array hole is then limited to the space between the wall of the guide tube and the electrode cannula, which can be sealed with petroleum jelly at the outer exposure. Electrode arrays were implanted relative to bregma with the use of the coordinate atlas of Paxinos and Watson (1998): anterior-posterior: ~1.8 mm, medial-lateral: 1.3 mm, dorsal-ventral from the cortical surface: 3.7 mm.

The issue surrounding differential responding in the HD system during active and passive movement was presumed to arise from how vestibular information is processed within and beyond the vestibular nuclei. It is well known that disruption of the vestibular system at the level of the labyrinth interferes with direction-specific firing throughout the HD system (Muir et al. 2009; Stackman and Taube 1997; Stackman et al. 2002). In monkeys, angular head velocity sensitivity, which is believed to form the basis for the HD signal, is suppressed in the vestibular nuclei during active movement (McCrea and Gdowski 2003; McCrea et al. 1999; Roy and Cullen 2004). In turn, this suppression could be reversed downstream by way of an intervening inhibitory neuron, and lead to cells in the HD cell network that respond only during active movement. Although no such active-only cells have been found in the vestibular nuclei, such cells could be postulated to be present downstream of the vestibular nuclei [e.g., in the nucleus prepositus or supragenual nucleus (Brown and Taube 2007)] and have been observed in the dorsal tegmental nuclei (Sharp et al. 2001). Currently, all the studies on HD cells that have explored responses to passive manipulations have used some form of limited restraint, and no study has formally locked the head into a fixed position. Thus the purpose of the present study was to test HD cell responses under conditions in which both the head and body were fixated to an apparatus, similar to testing conditions under which monkey vestibular cells have been recorded. We report that under such conditions HD cells discharged normally to passive rotation and that their responses were similar to those under freely moving conditions. Furthermore, we offer suggestions as to why earlier studies reported reduced firing during passive rotation.

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Animals. Twelve female Long-Evans rats were used in this study. All procedures were approved by the institutional care and use committee at Dartmouth College and adhered to the standards outlined by the National Institutes of Health Guide for the Care and Use of Laboratory Animals and the Society for Neuroscience. Presurgical weights ranged from 300 to 495 g (mean ± SE 352 ± 18 g). Rats were individually housed, maintained on a 12:12-h light-dark cycle, and provided with water ad libitum. After postoperative recovery, animals were food restricted in order to lower their current weight by 10% of their presurgical weight (9.9 ± 1.3% actual reduction).

Surgery. The animals were implanted with a head restraint post and an electrode array positioned above the ADN. The electrode array and its implantation have been described in detail elsewhere (Kubie 1984; Taube 1995). However, some minor modifications to that method were developed in order to improve electrophysiological stability as well as implant integrity during the active-to-passive transitions. This modification included extending skull screws to the lateral parts of the skull including the temporal and most lateral parts of the occipital plates. To anchor the head restraint post, which is placed rostral to the electrode array, screws are placed in the frontal skull plate just in front of the restraint post. In addition, further modification was made for sealing the hole around where the electrode array entered the skull. Instead of drilling a 2- to 3-mm hole in the skull above the ADN, a smaller hole (~1 mm) was drilled and a guide tube with an inner diameter slightly larger than the outer diameter of the electrode cannula was lowered through the hole and affixed to the skull. The exposed gap around the electrode array hole is then limited to the space between the wall of the guide tube and the electrode cannula, which can be sealed with petroleum jelly at the outer exposure. Electrode arrays were implanted relative to bregma with the use of the coordinate atlas of Paxinos and Watson (1998): anterior-posterior: ~1.8 mm, medial-lateral: 1.3 mm, dorsal-ventral from the cortical surface: 3.7 mm.

Behavioral protocols. HD cells were identified while the animal freely foraged in an open cylindrical enclosure (76-cm diameter) and surrounded by a floor-to-ceiling black curtain (2.44-m diameter). Food pellets (20 mg; Bioserve, no. F0071) were automatically dropped to a semirandom location in the enclosure every 30 s. After food restriction, the rats generally foraged throughout the experimental session. A white cue card subtending an ~100° arc was attached to the inside cylinder wall and was used as a visual and tactile landmark. It was not moved from its relative position throughout the experiments. The movement of the rats was tracked via two colored light-emitting diodes (LEDs: 1 red, 1 green) that were positioned 11 cm apart over the rat’s nose and back. These LEDs were tracked at 60 Hz with an automated video monitoring system that has been described previously (Taube et al. 1990a).

A series of sessions were recorded that alternated between active and passive sessions. All series began with a 16-min active foraging session, after which different types of passive sessions were conducted as described further below. Multiple passive sessions were run that involved several types of passive manipulations. Each passive session was 8 min long, and there were an average of 2.07 passive sessions per HD cell. In between each of the active and passive sessions, the animal was removed from the cylinder and placed in a cage adjacent to the cylinder while the floor paper was changed and the animal was prepared for the next session. A final 16-min active foraging session was conducted after completion of all passive sessions. There was no attempt to disorient the animal before any session.

Two forms of passive restraint were tested: hand-held and head-fixed restraint. Hand-held restraint followed the same procedures used previously by Taube (1995). Briefly, the animal was wrapped in a cloth towel and held by the experimenter so as to restrict head and body movement. To cover the neck, the towel was typically wrapped as far forward on the body as possible. However, the towel did not contact the head at any point in front of the ears. Neither the towel nor the experimenter’s hands were in contact with the rat’s head rostral to the intra-axial axis. While this procedure does not involve complete immobility of the head, it is generally effective in restraining the rat and limiting active head movement.

To immobilize the head more effectively during head-fixed restraint, we designed a restraint device that effectively reduced all active head movement. The device consisted of a small platform mounted on a circular bearing, forming a lazy Susan turntable (Fig. 1). The platform extended ~25 cm from the axis of rotation and was ~20 cm high. The size of the restraint device allowed it to be placed within the cylindrical enclosure such that a restrained animal could see the same visual surround within the enclosure that it had experienced while actively foraging. Head restraint was accomplished through a
restraint device for 8 min/day for 5–10 days. During hand-held was hand-held for 10 min each day for 3 days and then placed in the electrophysiological recording, creating three groups. The first group experiments. Each animal received only one type of training prior to restraint training, the animal was held in a position similar to what would later be used during electrophysiological testing—low to the ground and held out in front of and facing away from the experimenter. However, the animal was not moved during restraint training and was not in the cylindrical enclosure that would be used during active and passive sessions. The second group received similar hand-held restraint for 3 days but did not experience restraint in the device prior to electrophysiological recording. Finally, the third group was not restrained either by hand or in the device prior to electrophysiological recording.

**Electrophysiology.** Most of the electrophysiology has been described previously (Taube et al. 1990a). Briefly, the electrophysiological signal was amplified (10,000–50,000 times) and band-pass filtered (300–10,000 Hz, ≥3 dB/octave) and triggered a TTL pulse (dual-window discriminator) that was time-stamped and sent to a computer. The video tracking position information was simultaneously digitized and also stored on a computer for later off-line analyses. Electrodes were evaluated for the presence of HD cell activity while the animal actively foraged around the enclosure. If a cell that contained direction-specific firing was identified, the cell was recorded for 16 min in order to define an initial active baseline condition. After this baseline session, the animal was placed into the restraint device with the animal’s head positioned in the axis of rotation. A passive rotation session was then conducted, in which the animal was rotated back and forth in sweeps of ~150°/s that contained a large range of movement velocities (see RESULTS). During these sweeps the rat was gradually turned through 360° in order to sample all directional orientations. After the initial active and passive sessions, one or more of the other types of passive sessions were also performed. Each passive condition was 8 min, and the animal was restrained for a total of no more than 30 min. Initial passive sessions usually included passive rotations in the light with the head centered on the axis of rotation. One of the passive conditions included repeating these same passive rotations with the exception that they were conducted in the dark in order to remove visual input. Another passive condition replicated the hand-held, towel-wrapped restraint. Finally, the last condition involved passive rotations in the light, but with the head pointed nose-out at ~24 cm from the axis of rotation (referred to as off-axis rotation). After passive sessions were completed a second active foraging session was conducted to evaluate intertrial variance and ensure that cell isolation had not changed during the passive manipulations. Any recording that did not contain a second active session in which the cell’s tuning curve was similar to that of the first active session was not considered for further analysis (see below).

**Analyses.** The rat’s directional heading was determined based on the positions of the two LEDs relative to each other. HDs were divided into sixty 6° bins, and the number of spikes that occurred in each bin was recorded. Firing rates for each directional bin were obtained by dividing the number of spikes in each bin by the time spent in that directional bin. From this data, firing rate vs. HD tuning curves were plotted for each session. From these tuning curves, comparisons were performed on the peak firing rate, preferred firing direction, directional firing range, and anticipatory time interval (ATT) for each HD cell and session. The peak firing rate was the maximal neural response when the animal’s head was pointing in the cell’s preferred direction. The directional firing range was the range of directions around the preferred firing direction in which the neuron’s firing rate was elevated above background levels. We also monitored the cell’s response to different angular head velocities, which was measured by using the HD vs. time plot and computing the slope of the best-fit line from five successive intervals centered around each sampled HD (Taube 1995). The resulting angular head velocities were then sorted into 6°/s bins. Then a regression slope or correlation coefficient was computed for cell firing relative to angular head velocity for HDs within the cell’s directional firing range. All analyses used LabView software.
Angular head velocity was analyzed by measuring the amount of time the animal spent turning its head at different velocities. This analysis was conducted by computing the angular head velocity for each data sample (1/60th s) and then summed across all data samples for a particular session. To measure the angular head velocity for each data point, we constructed a plot of five data points that included the HD of the sample at t₀ along with the HDs from the two previous samples (t₋₁, t₋₂) and two successive samples (t₊₁, t₊₂). The slope of the best-fit line through these five data points was defined as the angular head velocity at t₀. This process was repeated for each data sample recorded during a session. The angular head velocity was then rounded to the nearest 5°/s with clockwise rotations represented as negative values and counterclockwise rotations represented as positive values. The number of samples at each of the binned velocities was then computed and converted into seconds. Then the number of seconds spent at each velocity was computed for each animal during each session, enabling comparisons across conditions.

The peak firing rate of each HD cell was measured by two procedures. The first method used the maximal firing rate value obtained from the cell’s firing rate vs. HD tuning curve and included all samples. This value is referred to as the original peak firing rate (PFRorig). The second method involved computing an adjusted peak firing rate (PFRadj) based on removing the influence that angular head velocity may have on the cell’s firing rate, since previous studies have shown that firing rates of ADN cells also have a small, but significant, correlation with angular head velocity (Taube 1995). Removal of the angular head velocity effects was accomplished by computing each cell’s horizontal angular head velocity sensitivity and then adjusting the peak firing rate by the number of spikes that were produced by the behavioral velocities experienced at the time the cell was recorded. The number of spikes at each velocity (Δspikesvel) was computed by multiplying the angular velocity sensitivity (m) by the head velocity (v) and the amount of time spent moving at that velocity (tvel):

$$\text{Δspikes}_{vel} = m \cdot v \cdot t_{vel}$$

A worst-case scenario was assumed in which every head movement was made facing the cell’s preferred firing direction and influenced the peak firing rate maximally. Therefore, the adjustment to the peak firing rate was computed by summing the number of spikes across the angular head velocity histogram and dividing by the total time period of rotational motion (Δspikevel = Δspikesvel/Δtvel). This calculation yielded a firing rate (Δspikevel) that was dependent on the cell’s angular velocity sensitivity and the angular velocities experienced during the recording session. This angular velocity firing rate was then removed from the original peak firing rate (PFRorig) to determine the cell’s adjusted peak firing rate (PFRadj) with the angular velocity sensitivity removed:

$$PFR_{adj} = PFR_{orig} - \Delta \text{spike}_{rate}$$

The ATI is a measure of the amount of time before or after when a cell fires that best predicts the animal’s HD. Procedures for the computation of the ATI have been described previously (Blair and Sharp 1995), and only a brief description of the procedure is provided here. First, the cell’s directional tuning curve (firing rate vs. HD) was constructed for rightward (CW) and leftward (CCW) head turns, and the mean preferred firing direction was determined for both curves. The difference in the two mean preferred directions, referred to as the separation angle, was computed. Then the spike time series was shifted forward (and backward) in ±16.6-ms increments up to 160 ms. The new separation angle was then recomputed for each incremental shift. A plot was constructed based on the separation angle and its corresponding time series shift. A best-fit linear regression was fit through the data, and the x-intercept (the point where the separation angle equals 0) was defined as the ATI. A positive ATI value indicates that cell firing leads HD, while a negative ATI value indicates that cell firing lags HD. The HD cells discussed below from the ADN had, in general, larger ATIs (~75 ms) than values reported in previous studies (25–50 ms; Bassett et al. 2005; Blair and Sharp 1995; Taube and Muller 1998). The reasons for this difference are unclear, as all the cells were well-isolated and recorded with the same procedures and data analysis programs as in previous studies. Furthermore, other cell properties, such as peak firing rate, directional firing range, and angular head velocity histogram, were similar to those of previous studies.

When determining how HD cells respond to passive rotation it is imperative that a second active session following the passive session(s) shows that the cell response remained stable throughout the series of recordings. To determine whether the response for a particular session was stable, HD cell characteristics for that session were always referenced to the first active foraging session. For most properties (i.e., ATI, preferred firing direction, and directional firing range) a simple subtraction method that calculated the difference between the values from both sessions was used.

Table 1. Mean firing properties for HDs recorded under different conditions

<table>
<thead>
<tr>
<th>n</th>
<th>Peak Firing Rate, spikes/s</th>
<th>ATI, ms</th>
<th>Directional Firing Range, °</th>
</tr>
</thead>
<tbody>
<tr>
<td>First active</td>
<td>44</td>
<td>32.4±3.9</td>
<td>75.2±7.1</td>
</tr>
<tr>
<td>Last active</td>
<td>44</td>
<td>30.4±3.1</td>
<td>74.4±7.8</td>
</tr>
<tr>
<td>Passive</td>
<td>43</td>
<td>37.6±4.3</td>
<td>67.1±5.4</td>
</tr>
<tr>
<td>Dark passive</td>
<td>16</td>
<td>25.8±4.6</td>
<td>54.5±15.8</td>
</tr>
<tr>
<td>Off-axis passive</td>
<td>11</td>
<td>36.3±8.0</td>
<td>68.4±13.7</td>
</tr>
<tr>
<td>Hand-held passive</td>
<td>16</td>
<td>26.9±4.4</td>
<td>46.7±6.0</td>
</tr>
<tr>
<td>MANOVA P</td>
<td>0.55</td>
<td>0.73</td>
<td>&lt;0.01*</td>
</tr>
</tbody>
</table>

Mean ± SE is shown for each parameter, with the range shown underneath. Passive, dark passive, and off-axis passive conditions were all conducted with the head-fixed restraint device. Towel-wrapped, hand-held restraint was used for the hand-held passive condition. P values from a multivariate ANOVA (MANOVA) shown at bottom indicate which head direction (HD) cell properties were statistically different across the 6 conditions. ATI, anticipatory time interval. *P < 0.05.
where $PFR_{\text{first active}}$ is the cell’s peak firing rate in the first session (usually the first active foraging session) and $PFR_{\text{current}}$ is the peak firing rate in the second session (usually the passive session). In Table 2, $\Delta PFR$ is presented alongside $siPFR$ for comparison and is computed as the difference between the $PFR$ in the current session versus the $PFR$ in the first active session ($PFR_{\text{current}} - PFR_{\text{first active}}$).

For the purposes of defining any effects of passive restraint on the stability of the second active session, a meta-analysis of previously published responses of HD cells was performed [total $n = 48$ HD cells obtained from Taube 1995 ($n = 14$); from Clark et al. 2010 ($n = 13$); from Calton et al. 2008 ($n = 7$); and from Bassett et al. 2005 ($n = 14$)]. Each of these HD cells was from intact rats that were tested with an initial active foraging session, a noninvasive, intervening test that did not involve restraining the animal (usually an environmental manipulation like cue rotation), and then a subsequent active foraging session similar to the first active session. These previously published data were compared with the active responses from HD cells recorded in this study that contained an intervening restraint session as well as 25 cells that were recorded with an intervening session that did not include restraint. The previously published data had a first active session-to-last active session peak firing $siPFR = -0.02 \pm 0.02$, while the 25 cells recorded in this study without restraint had a first-to-last active session $siPFR = -0.02 \pm 0.03$, and the 54 HD cells with intervening restraint sessions had a first-to-last session $siPFR = -0.05 \pm 0.03$. For a cell to be included in our analyses, the $siPFR$ had to be within 1.96 standard deviations of the mean $siPFR$ determined from the published data (i.e., between $siPFR$ values of $-0.32$ and $0.28$). Using the 25 cells recorded without restraint sessions would have produced a similar range ($-0.36$ to $0.32$). This range encompasses 94% (45 of 48) of the previously published HD cells and 92% (23 of 25) of the HD cells recorded without restraint sessions in this study; 81% (44 of 54) of the HD cells recorded with restraint sessions were therefore considered for further analysis. Of the 10 outlier cells removed, 3 cells had high $siPFR$ values and 7 cells had low $siPFR$ values. Reanalyzing the data with these outliers included did not alter the results.

To determine the effects of passive rotation on HD cell responses we compared four measures between active and passive sessions: 1) peak firing rate, 2) directional firing range, 3) ATI, and 4) preferred firing direction. For comparisons of peak firing rate we used the $siPFR$ as described above. In the results, this index was used to compare any two experimental sessions, including both active and passive sessions, as well as comparisons of the two active sessions (see above). In contrast, for all other HD cell parameters we computed the difference of that parameter between the two sessions. Changes in ATI were measured as the difference in ATI from the first session to the second session ($\Delta ATI$) in milliseconds. Changes in the preferred firing direction ($\Delta PFD$) and directional firing range ($\Delta Range$) across sessions were measured in degrees. For statistical comparison, a one-way ANOVA was used to evaluate whether passive sessions differed from active sessions. The differences or ratio of HD cell properties from each passive session relative to the first active session were compared with the differences found in the second active session relative to the first active session. This comparison determined whether there was more deviation in the responses during passive stimulation than would be expected given the variation seen in repeated active sessions.

Comparison of the changes in HD cell properties across experimental conditions was quantified with ANOVA tests. Because each cell is defined not only by the condition of the session in which it was recorded (session condition) but also by the restraint experience that the animal received prior to the recording (training condition), a multivariate ANOVA (MANOVA) was performed using both session and training conditions as factors. Two separate MANOVAs were performed—one on the raw data presented in Table 1 and the second on the difference data presented in Table 2. For both of these analyses ATI, peak firing rate, preferred firing direction, and directional firing range properties were used as dependent variables. A Levene test of the data found reasonable equivalence of the variances for all measured HD cell properties and most of the computed comparative properties (Levene $P$ values: peak firing rate: 0.39, ATI: 0.08, preferred firing direction: 0.98, directional firing range: 0.33, $siPFR$: 0.05, $\Delta ATI$: 0.46, $\Delta PFD$: $<0.01$, $\Delta Range$: 0.03). The multivariate $P$ values displayed in Tables 1 and 2 were obtained from the main effect of the session condition factor. The $P$ values displayed in Table 3 were obtained from the analysis of the difference data used in Table 2, which included all session conditions, and represent the main effect of training condition for each HD cell property.

For all univariate ANOVAs noted in the text, unless otherwise specified, the comparison included data only from the cells recorded during the session of interest. The comparison was therefore conducted between the data recorded during the session of interest and the data from those same cells recorded during the initial active session. If a cell was not recorded during that session type, then the initial active data were removed from that particular analysis. Only the data specified by the comparison under consideration was included in the analysis. HD cell properties not specifically referenced in the text as part of a comparison were not included. Therefore, a univariate ANOVA of the $\Delta PFD$ response during head-fixed passive rotation in the light includes only the $\Delta PFD$ data from cells that were tested during head-fixed passive rotation in the light and the $\Delta PFD$ data from those same cells during the first active session.

**Histology.** After the conclusion of testing, animals were euthanized with pentobarbital sodium. Electrolytic lesions were made on one of the electrode wires (20 $\mu A$ for 10 s) in order to aid the localization of specific electrode tracks. Animals were perfused transcardially with 10% formalin in 0.9% saline, followed by removal of the brain. The brains were placed in 10% formalin solution for at least 3 days and then placed in a 10% formalin solution containing 2% potassium ferrocyanide to enable a Prussian blue reaction. After the brains were placed in 20% sucrose solution for a minimum of 1 day the brains were sliced in 40-$\mu$m coronal sections with a cryostat and the tissue sections were processed for thionin staining. Coronal sections were

### Table 2. Mean change in HD cell properties relative to the first active session

<table>
<thead>
<tr>
<th>Condition</th>
<th>$n$</th>
<th>$siPFR$</th>
<th>$PFR$</th>
<th>$\Delta ATI$, ms</th>
<th>$\Delta PFD$, °</th>
<th>$\Delta Range$, °</th>
</tr>
</thead>
<tbody>
<tr>
<td>Second active-first active</td>
<td>44</td>
<td>$-0.01 \pm 0.03$</td>
<td>$-1.92 \pm 1.84$</td>
<td>$-0.8 \pm 8.2$</td>
<td>$-1.0 \pm 2.5$</td>
<td>1.4 ± 4.0</td>
</tr>
<tr>
<td>Passive-first active</td>
<td>43</td>
<td>$0.06 \pm 0.03$</td>
<td>$4.62 \pm 2.74$</td>
<td>$-8.2 \pm 8.9$</td>
<td>$-1.2 \pm 4.1$</td>
<td>20.1 ± 0.8</td>
</tr>
<tr>
<td>Passive dark-first active</td>
<td>16</td>
<td>$-0.08 \pm 0.06$</td>
<td>$-4.25 \pm 4.55$</td>
<td>$-13.7 \pm 19.0$</td>
<td>$-17.9 \pm 10.3$</td>
<td>54.1 ± 12.8</td>
</tr>
<tr>
<td>Off-axis-first active</td>
<td>11</td>
<td>$-0.07 \pm 0.10$</td>
<td>$-9.32 \pm 10.70$</td>
<td>$-15.3 \pm 15.2$</td>
<td>$-12.8 \pm 13.4$</td>
<td>18.0 ± 7.6</td>
</tr>
<tr>
<td>Hand-held-first active</td>
<td>16</td>
<td>$-0.18 \pm 0.08$</td>
<td>$-12.30 \pm 6.82$</td>
<td>$-37.7 \pm 9.5$</td>
<td>$-10.5 \pm 9.4$</td>
<td>25.8 ± 6.2</td>
</tr>
<tr>
<td>MANOVA $P$ value</td>
<td>0.14</td>
<td>0.87</td>
<td>0.42</td>
<td>$&lt;0.01$</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Comparison between the first and second active sessions and responses to different passive conditions compared with the first active session are shown. $PFR$, peak firing rate; $siPFR$, standard index of $PFR$; $PFD$, preferred firing direction. $P$ values from MANOVA at bottom indicate which HD cell properties were significantly different across conditions. *$P < 0.05$ MANOVA; †$P < 0.05$, ‡$P < 0.01$ for individual least significant difference (LSD) post hoc tests comparing changes in the passive condition to changes across the 2 active conditions.

\[
siPFR = \frac{(PFR_{\text{current}} - PFR_{\text{first active}})}{(PFR_{\text{current}} + PFR_{\text{first active}})}
\]
later examined with a microscope to reconstruct the location of the electrode tracks. All of the HD cells reported below were localized to the ADN.

RESULTS

Responses to passive movement in the restraint device. In these experiments, 44 HD cells were recorded from the ADN in 11 rats. The results are summarized in Tables 1 and 2. Overall, both types of passive restraint, head-fixed and hand-held, had little impact on HD cell responses. Tuning curves from five representative HD cells during active foraging and head-fixed passive movement sessions in the restraint device are presented in Fig. 2. For each of the passive sessions the rat was restrained in the restraint device and rotated on the turntable around a rotational axis centered through its head. Compared with both active conditions, each of these cells failed to display any suppression of directional sensitivity.

<table>
<thead>
<tr>
<th>Hand and device restraint (n = 20)</th>
<th align="right">siPFR</th>
<th align="right">ΔPFR</th>
<th align="right">ΔATI</th>
<th align="right">ΔPFD</th>
<th align="right">ΔRange</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active</td>
<td align="right">-0.04±0.04</td>
<td align="right">-3.48±1.98</td>
<td align="right">12.9±15.5</td>
<td align="right">2.0±2.5</td>
<td align="right">4.3±4.5</td>
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<tr>
<td>Passive</td>
<td align="right">0.05±0.04</td>
<td align="right">2.91±1.79</td>
<td align="right">3.8±14.1</td>
<td align="right">-1.2±3.3</td>
<td align="right">17.1±5.2</td>
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<tr>
<td>Hand restraint only (n = 16)</td>
<td align="right"></td>
<td align="right"></td>
<td align="right"></td>
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</tr>
<tr>
<td>Active</td>
<td align="right">-0.02±0.05</td>
<td align="right">-3.40±4.14</td>
<td align="right">-3.5±6.8</td>
<td align="right">-1.7±5.1</td>
<td align="right">5.5±4.1</td>
</tr>
<tr>
<td>Passive</td>
<td align="right">0.08±0.06</td>
<td align="right">6.51±6.70</td>
<td align="right">-21.6±9.0</td>
<td align="right">-4.3±9.7</td>
<td align="right">14.2±5.6</td>
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<tr>
<td>No prior restraint (n = 7)</td>
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<tr>
<td>Active</td>
<td align="right">0.12±0.04</td>
<td align="right">5.70±2.66</td>
<td align="right">-38.0±16.2</td>
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<tr>
<td>Passive</td>
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<td align="right">5.20±5.83</td>
<td align="right">-11.9±32.2</td>
<td align="right">6.1±8.5</td>
<td align="right">42.0±20.2</td>
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<td>MANOVA P value</td>
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<td align="right">0.44</td>
<td align="right">0.75</td>
<td align="right">&lt;0.01*</td>
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</tr>
</tbody>
</table>

Active and passive session responses are compared with the response in the first active session. Format and abbreviations are as outlined in Table 2. Data have been separated according to the type of restraint experience given to the animal prior to recording: 1) exposed to hand-held restraint and the restraint device, 2) received hand-held restraint only, 3) no restraint exposure prior to recording. *Significant difference by behavioral training condition.

Fig. 2. Head direction (HD) cell tuning curves for 5 representative cells. A–E: each plot displays the cell’s firing rate as a function of the animal’s HD. Blue and green traces represent the responses to the first and second active foraging sessions, respectively. Red trace represents the cell’s response for the intervening passive session in which the animal was restrained in the light with the restraint device and rotated about the horizontal axis through its head. Cells A and B were taken from the same animal but from different electrodes on different days. Cells C, D, and E were each recorded from different animals. F: histogram displaying the distribution of the siPFRs for the passive session relative to the first active session (red bars) and the second active session to the first active session (blue bars).

Table 3. Mean change in HD cell properties across behavioral training conditions
during the passive session (red traces in Fig. 2). Indeed, across the population 63% of the cells had an increase in peak firing rate during the passive session (Fig. 2F). Statistically, however, there was no significant difference in the peak firing rate between the active foraging and restraint conditions. This result was true whether the overall mean firing rates were compared [MANOVA values presented in Table 1; separate univariate ANOVA of the 43 HD cells tested under active and head-fixed passive conditions \( F(1,84) = 1.519, P = 0.22 \)] or the comparison was conducted for individual cells using the siPFR index [Table 2 MANOVA; univariate ANOVA \( F(1,84) = 2.487, P = 0.12 \)]. Therefore, there did not appear to be any sign of inhibition or suppression in the firing rate of HD cells during head-fixed restraint.

Concerning other parameters, there was no effect of head-fixed passive restraint on the preferred firing direction or the ATI. There was, however, a significant and consistent effect on the directional firing range (Tables 1 and 2), where passive restraint on the preferred firing direction or the head-fixed restraint.

Movement parameters. The above results differ from previous findings, which found decreased responses during passive rotations—particularly in regard to peak firing rate (Chen et al. 1994; Golob et al. 1998; Knierim et al. 1995; Taube 1995; Taube et al. 1990b). One possible explanation for this difference is that there were various procedural factors influencing or contributing to the inhibition of HD cell responses in previous experiments. Therefore, we evaluated several factors that might account for these differences including 1) the amount of movement, 2) the previous experience of the animal with restraint, and 3) the type of restraint.

We first evaluated differences in the amount of movement between active and passive sessions. While passive movement speeds were used that were within the behavioral range of movement during active foraging, there were differences in the distribution of horizontal angular head velocities. Figure 3A displays the amount of time spent at different angular head velocities as a percentage of the overall session time. This plot reveals that there is a significant difference in the angular head velocities experienced by the animals between active and passive conditions [univariate ANOVAs: first active vs. second active session: \( F(1,408) = 0.01, P = 0.92 \); first active vs. passive session \( F(1,408) = 4.92, P = 0.03 \)]. The actively foraging animals spent the majority of their time at lower velocities compared with passively rotated animals. Behaviorally, the active animals usually foraged for a time and then paused for a moment before resuming foraging activity, which resulted in an increase in the number of low angular head velocity values. Figure 3 shows that actively foraging animals spent 85.0% of their time at angular head velocities \( \leq 83.8^\circ/s \) compared with only 26.8% of their time at or below this value during passive restraint sessions. Therefore, during passive sessions animals spent more time being rotated and less time at lower angular velocities.

To determine whether this difference in angular head velocity influenced the peak firing rate findings, each cell’s sensitivity to horizontal angular head velocity was determined and then used to remove the effects of the different motion profiles across conditions. Previous studies have reported a small, but significant, correlation between HD cell firing rate and angular head velocity (Blair and Sharp 1995; Blair et al. 1997, 1998; Stackman and Taube 1997; Taube 1995). The present results replicated these findings, with the mean correlation (\( r \)) between firing rate and angular head velocity (within the cell’s directional firing range) for the first active session \( r = 0.102 \pm 0.009 \) (\( n = 44 \)); for the second active session \( r = 0.089 \pm 0.008 \) (\( n = 44 \)); and for the passive session \( r = 0.123 \pm 0.018 \) (\( n = 39 \)). Four cells were removed from the passive analysis of angular head velocity because the angular head velocity was undersampled at the lower velocities during these sessions. For this analysis, the mean horizontal angular head velocity sensi-

![Fig. 3](http://jn.physiology.org/)

**A**

![Horizontal Angular Head Velocity](http://jn.physiology.org/)

**B**

![Horizontal Angular Head Velocity](http://jn.physiology.org/)
tivity (slope of the best-fit line, m) was 0.030 ± 0.004, 0.028 ± 0.006, and 0.029 ± 0.003 spikes·s⁻¹·deg⁻¹·s⁻¹ (which is equivalent to spikes/deg) for the first active, passive, and second active sessions, respectively. To compensate for this velocity correlation, each cell's horizontal angular head velocity sensitivity was computed and then used to adjust the peak firing rate by the number of spikes that were produced by the behavioral velocities experienced at the time the cell was recorded (see METHODS). This method assumes a worst-case scenario, where every movement is through the preferred firing direction. Therefore the interpreted reduction overestimates the actual reduction so that if computed peak firing rate adjustments fail to display a difference, then the smaller actual adjusted peak firing rate will also fail to display a difference. For example, if a cell had a peak firing rate of 100 spikes/s recorded over a 10-s interval, and had an angular head velocity sensitivity of 0.01 spikes·deg⁻¹ and a constant head rotation of 100°/s, then the peak firing rate was reduced to 99 spikes/s.

Removing the angular head velocity firing from the peak firing rate values and recalculating the change in peak firing rate between active and passive sessions changed the siPFR from 0.05 ± 0.03 to 0.02 ± 0.03 (n = 39). Thus the adjusted siPFR still did not differentiate active and head-fixed passive sessions [ANOVA: F(1,76) = 0.59, P = 0.45]. In sum, while the amount and type of motion used during passive restraint may have influenced the peak firing rate, it was not masking a significant source of inhibition or suppression of the HD signal during restraint.

**Behavioral experience.** Another possibility that might account for the differences between our results and those of previous studies is the amount of stress that is present during the restraint session. For example, in the previous restraint studies animals were often hand-held or towel-wrapped, and this procedure could have provided a different stress level than was present in the head-restrained conditions, even though the animals in the present study were habituated to the restraint device before cell recording commenced. To address this possibility, some animals were not habituated to restraint before recording and were compared with the passive sessions from animals that received restraint acclimation pretraining. Of the animals that were habituated to restraint, one group experienced both hand-held and device restraint, while the other group was pretrained with only hand-held conditions (see METHODS). The data were then separated according to their pretraining behavioral experience and plotted according to the various firing properties. Figure 4 shows that the training experience did not suppress or inhibit HD cell responses during active and passive sessions. The data from each group of animals overlapped and had a similar distribution. Table 3 presents the statistical analysis of the data, a MANOVA performed comparing HD cell properties for cells that were tested with both active and head-fixed passive sessions, and includes the data shown in Fig. 4. This analysis found no significant differences in peak firing rate, ΔPFD, or ATI due to training condition. In contrast, significant differences were found for ΔRange [F(1,85) = 10.707, P = 0.002], which became slightly broader under passive conditions in animals that were habituated. This difference was most pronounced in animals that were naive.

Another issue to address was whether the amount of experience in the restraint device influenced cell properties. It is possible that the repetition of being restrained for the passive sessions provided a mechanism of habituation to the restraint procedure. We therefore analyzed the first and last cells from animals that had at least four separately recorded passive sessions (n = 9 animals, 26 cells: 13 initial, 13 final). As the first and last cells recorded from an animal are different cells, analyses were conducted on the differences between active and head-fixed passive sessions as outlined in Tables 2 and 3. A MANOVA using session condition and cell order as factors failed to find a significant difference in any of the firing parameters between the active and passive sessions, as well as between the first and last active sessions (all Ps > 0.10). There were also no significant differences for the main effect of restraint condition on any HD cell characteristic, and no significant interaction effects between the two factors. In sum, these results indicate that any inhibition or suppression of the HD cell response is not being masked by the training experience received by the animal prior to being restrained during the passive session.

**Hand-held restraint.** Some of the previous studies that reported reduced firing properties under restraint conditions involved using towel-wrapping for restraint during the passive rotations (Golob et al. 1998; Sharp et al. 2001; Taube 1995). It was therefore important to compare the present method of head and body restraint with the towel-wrapping method. Using the same procedures for the passive session reported above, we tested 16 cells in animals that were towel-wrapped, hand-held, and then rotated back and forth in the cylinder in order to sample different HDs. These procedures were similar to those used previously for hand-held passive rotations (Bassett et al. 2005; Taube 1995). The tuning curves for the active and towel-wrapped passive sessions contained considerable overlap (Fig. 5A), and there was no significant difference in the mean peak firing rate values shown in Table 1. In contrast,
Table 2 shows that while there was no main effect for siPFR [MANOVA: $F(4, 129) = 1.781, P = 0.137$], a downward trend in the peak firing rate was observed as reflected by the mean $\Delta PFR$ value $= -12.30 \pm 6.82$ spikes/s. While a least significant difference (LSD) test on the siPFR values used in the MANOVA above found a significant difference between hand-held and active sessions ($P = 0.028$), this significance was not found when Scheffé, Duncan, or Bonferroni post hoc tests were used ($P = 0.302, P = 0.087$, and $P = 0.284$, respectively). The noted trend was much smaller than the inhibition and suppression found previously, and no cell’s activity was completely suppressed by hand-held restraint. For comparison, Taube (1995) and Knierim et al. (1995) reported near-complete suppression in the firing of 10 cells and all 6 cells recorded, respectively, and Taube et al. (1990b) reported peak firing rate decreases of 69.1% in hand-held sessions compared with active sessions.

The decrease in the ATI contrasts with the increase in ATI observed in previous hand-held restraint sessions (Bassett et al. 2005). Similar to the other passive restraint sessions, directional firing range values increased significantly during the hand-held passive session [MANOVA: $F(4,129) = 7.223, P < 0.001$].

The methods used here differed from those used in earlier studies (Bassett et al. 2005; Taube 1995), which used shorter recording sessions (1–2 min), and thus had less sampling time compared with the head-fixed restraint sessions. Therefore, we conducted an analysis on the present hand-held data using sampling times similar to those in the previous studies (comparing the first 2 min of hand-held restraint to the first 8 min of active foraging). A MANOVA using the recording duration and restraint condition as factors indicated that the only variable to be influenced by shortening the recording period was the $\Delta Range$ [MANOVA main effect for recording duration: siPFR: $F(2,30) = 0.32, P = 0.73$; $\Delta ATI$: $F(2,30) = 0.52, P = 0.95$; $\Delta PFD$: $F(2,30) = 1.75, P = 0.18$; $\Delta Range$: $F(2,30) = 13.65, P < 0.001$]. Using the entire 8-min session, the directional firing range increased by 25.76 $\pm$ 6.24° during passive hand-held restraint compared with active foraging (Table 2) but decreased by 23.05 $\pm$ 9.32° compared with active foraging when only considering the first 2 min of the hand-held session. It should be noted, however, that siPFR, $\Delta ATI$, $\Delta PFD$, and $\Delta Range$ were not significantly different across the first 2, 4, 8, and 16 min of active foraging [univariate ANOVA: siPFR: $F(3,60) = 0.20, P = 0.90$; $\Delta ATI$: $F(3,60) = 0.58, P = 0.63$; $\Delta PFD$: $F(3,60) = 0.46, P = 0.71$; $\Delta Range$: $F(3,60) = 0.25, P = 0.86$].

Similar to the head-fixed passive sessions, the hand-held passive sessions resulted in a similar angular head velocity profile (Fig. 3B). An ANOVA comparing the angular head velocity histograms of head-fixed and hand-held restraint (comparing the amount of time spent at each angular head velocity across all rotational velocities) found no significant difference head velocities experienced under each condition [$F(1,408) = 2.88, P = 0.09$]. It should also be noted that the small, but significant, correlation seen between horizontal angular head velocity and firing rate during the active foraging and head-fixed passive restraint sessions was absent during the hand-held session [the $0.028 \pm 0.006$ spikes·s$^{-1}$·deg$^{-1}$·s$^{-1}$ (spikes/deg) found during the head-fixed passive restraint sessions decreased to $-0.004 \pm 0.002$ spikes/deg during hand-held restraint; $n = 16$]. Hand-held and head-fixed restraint did not differ on any of the other HD cell properties (Tables 1 and 2).

Off-axis rotation. Further effort was directed at what might be influencing the inconsistent responses in directional activity during hand-held restraint. We therefore compared the movement produced during hand-held restraint to the movement produced by the restraint device. While the restraint device produces a horizontal rotation around a vertical axis through the animal’s head, hand-held towel-wrapped restraint produces a mixture of movements that includes both rotation and translation. The animal spends more time being moved with the head at a distance from the axis of rotation because the rotational axis is closer to the animal’s tail. To determine
whether the axis of rotation influenced the responses, the restraint device was adjusted so that the animal faced away from the axis of rotation in order for the rotational axis to be behind the animal by at least one full body length. Tables 1 and 2 compare the results from different axes of rotation and reveal that there was little difference in the responses based on this factor [univariate ANOVA comparing off-axis restraint to on-axis restraint: siPFR: $F(1,20) = 2.99$, $P = 0.10$; $\Delta$ATI: $F(1,20) = 0.33$, $P = 0.57$; $\Delta$PFD: $F(1,20) = 0.13$, $P = 0.72$; $\Delta$Range: $F(1,20) = 0.09$, $P = 0.76$]. The off-axis rotation with the restraint device and hand-held restraint manipulations produced the same pattern of differences relative to active foraging [univariate ANOVA comparing off-axis restraint to hand-held restraint: siPFR: $F(1,16) = 1.13$, $P = 0.30$; $\Delta$ATI: $F(1,16) = 0.93$, $P = 0.35$; $\Delta$PFD: $F(1,16) = 0.13$, $P = 0.71$; $\Delta$Range: $F(1,16) = 0.02$, $P = 0.89$]. Figure 5, A and B, illustrates the responses from representative cells for off-axis rotation and hand-held restraint. Similar to hand-held restraint, the angular head velocity sensitivity during off-axis rotation was also decreased compared with on-axis rotation [mean sensitivity $= 0.004 \pm 0.004$ spikes/deg vs. $0.028 \pm 0.006$ spikes/deg for on-axis rotation (see above)].

**Passive rotation in the dark.** To determine the role of visual cues during the passive sessions, animals were tested under head-fixed passive restraint in the dark. Figure 5C illustrates the response to passive rotation in the dark for a representative cell. Overall, there was little difference between cell responses in the dark compared with passive or active sessions in the light (Tables 1 and 2). Furthermore, the HD cells’ angular head velocity sensitivity was also similar to that seen during active sessions ($0.02 \pm 0.01$ spikes/deg). The one measure that was significantly different for the dark passive sessions was again the directional firing range, which appeared to be increased compared with values observed during the other passive sessions in the light. However, for the 15 cells tested under head-fixed restraint in both light and dark sessions, the increase in the directional firing range in the passive sessions was not significant [univariate ANOVA comparing head-fixed restraint in the light and dark: siPFR: $F(1,28) = 4.03$, $P = 0.06$; $\Delta$ATI: $F(1,28) = 0.13$, $P = 0.72$; $\Delta$PFD: $F(1,28) = 0.36$, $P = 0.55$; $\Delta$Range: $F(1,28) = 1.58$, $P = 0.22$]. There was a trend for lower peak firing rate values during passive rotation in the dark, but as noted by the multivariate tests (Tables 1 and 2) and the univariate ANOVA above, the reduction was not significant.

**DISCUSSION**

Previous studies of HD cell responses during passive movement found at least some degree of reduction in cell firing (Chen et al. 1994; Golob et al. 1998; Knierim et al. 1995; Sharp et al. 2001; Taube et al. 1990b; Taube 1995; Zugaro et al. 2001). In general, those studies that used some form of restraint (Golob et al. 1998; Sharp et al. 2001; Taube et al. 1990b; Taube 1995) found stronger reductions in cell activity than those studies that passively moved an unrestrained subject (Bassett et al. 2005; Zugaro et al. 2001; for an exception see Chen et al. 1994) and found the most marked reductions when the animals were tightly restrained. The conclusion from these findings was that motor and proprioceptive input played an important role in the generation and updating of the HD signal. In contrast to these previous reports, the present study found that passive movement during head-fixed restraint produced no significant reduction in HD cell firing. In addition, our finding that HD cell responses were also maintained during passive movement in the dark implies that visual, motor, and proprioceptive inputs are unnecessary to generate direction-specific responses in HD cells. Our results strongly suggest that the HD signal is more dependent on vestibular information for its generation and is consistent with studies demonstrating that interruption of the vestibular signal leads to the complete loss of the direction signal (Muir et al. 2009; Stackman and Taube 1997; Stackman et al. 2000).

The presence of a stable response in the passive session in our study cannot be explained by differences in experimental design compared with the earlier reports because we used procedures similar to those used in our laboratory previously, which showed significant reduction in cell firing (Bassett et al. 2005; Golob et al. 1998; Taube 1995). Although the previous studies used electrode implant techniques that were initially developed for neuronal recordings during active foraging, these techniques were generally not designed for passive restraint. Because transitioning from active to passive conditions imposes many mechanical and physiological stressors on an animal, the implant used in our study was redesigned for head-fixed passive restraint. Furthermore, the duration of the passive restraint session was expanded from 2 to 8 min, in order to better measure angular head velocity sensitivity. However, the expansion of the recording duration did not mask any early inhibition or suppression of the HD cell response. Thus there were no significant experimental or procedural differences between the hand-held passive restraint testing used here and those used previously (Taube 1995).

Some of the difference seen between the present and previous findings might be attributed to the nature of the hand-held testing procedure—specifically, the head movements imposed by this procedure are a mixture of angular and translational movement in three-dimensional space. Off-axis rotational testing replicated part of the hand-held head motion profile by introducing translational movements, which resulted in similar HD cell properties coupled with a similar loss of angular head velocity sensitivity. Hand-held restraint and off-axis passive rotation were the only conditions in which angular head velocity sensitivity was effectively lost. This finding suggests that the vestibular otoliths, which detect the translational movement and tilt of the head, may play a role in the generation of the HD signal (Calton and Taube 2005; Yoder and Taube 2009). It is possible that otolith input influences HD cell responses to maintain head direction accuracy during three-dimensional movements, depending on the availability of unambiguous otolith input (Shinder and Taube 2010b). The loss of angular sensitivity for off-axis rotations may be due to a rebalancing of contributions from the semicircular canals and the otoliths, such that there is less reliance on input from the rotationally sensitive vestibular canals.

The one property that appeared consistently affected by passive restraint was the increase in the directional firing range. Normally, such an effect would be attributed to instability in the cell’s preferred firing direction, and this change would also be accompanied by a decrease in the cell’s peak firing rate. Neither of these effects, however, was observed in the results. In addition, when evaluating the effects of hand-held restraint,
there was a decreased directional firing range for the first 2 min of a cell’s recording compared with the entire 8-min session. This effect was not seen during the active foraging sessions, and again, this change in the directional firing range was not accompanied either by a shift in the cell’s preferred firing direction or by a decrease in the cell’s peak firing rate. Without additional experiments to explore these issues, we do not have a reasonable explanation to account for these effects.

Experience with restraint was used as a proxy to evaluate stress effects on HD cell responses. While no effects were found, it should be noted that resistance to the restraint and struggle were diminished but not completely abolished even in animals with extensive experience in the restraint device. While the hand-held restraint procedures were replicated from previous studies, they were performed on animals that had previous experience with head-fixed restraint. Therefore, it is important to note that the hand-held restraint used in this study differed from previous studies by the prior experience with the restraint device. However, the present evidence suggests that the small difference that we found in the responses between head-fixed and hand-held restraint does not relate to the animal’s previous experience to restraint.

The generation of the HD signal is thought to be dependent on vestibular input (Muir et al. 2009; Stackman and Taube 1997; Stackman et al. 2002). However, passive movement has been shown to impair the ability of HD cells to maintain a stable preferred firing direction when traveling from a familiar to a novel environment (Stackman et al. 2003). Our findings remain consistent with this view—specifically, that multiple sources of information control the HD signal. The HD signal can be separated into two components—one that generates the signal and a second process that controls the cell’s preferred firing direction (Goodridge and Taube 1997). Our findings relate more to the former process—its generation, and not the mechanisms that control the cell’s preferred firing direction. Thus, while visual, proprioceptive, and motor information are not necessary for the generation of the HD signal, these systems appear important in controlling the preferred firing direction.

Our results indicate that the generation of the HD signal reflects sensory influences that are exclusively vestibular in origin. If the HD signal were to be accurate and consistent, then vestibular inputs to the HD cell circuit may also be expected to represent all head movements accurately and consistently. However, many vestibular nucleus neurons have motor components related to reflexes (e.g., cervical, eye movements) or display different responses to active and passive head movement (McCrea and Gdowski 2003; McCrea et al. 1999; Roy and Cullen 2004). Given our findings, this motor-related activity implies either that the vestibular signals projecting to the HD cell circuit are independent of motor and proprioceptive input or that the motor information is removed prior to being conveyed to the HD circuit. For all sessions, the passive or active nature of the angular head velocity the animal experienced did not significantly alter the peak firing rate of HD cells. However, it should be noted that while the response to passive rotation in the dark was not significantly different from that seen during active foraging sessions, passive rotation in the dark appeared to have peak firing rate responses that were lower and directional firing ranges that were higher than those observed in the light. Such a trend may be due to the loss of optokinetic visual input to the vestibular system, which is known to improve the accuracy of the vestibular signal when head movements are poorly resolved by the vestibular sensory apparatus (Beranec and Cullen 2007; Bryan and Angelaki 2009; Cazin et al. 1980).

Angular head velocity information arising from the vestibular labyrinth requires modification to accurately represent head movement across all conditions. Additional processing is required because the vestibular signal in the vestibular nuclei is not a linear representation of head movement (Fuchs and Kimm 1975; Jones and Milsum 1971; Shinoda and Yoshida 1974). The decreasing sensitivity for angular velocity found in vestibular nuclei neurons with increasing movement velocity (Marlinski and McCrea 2009; Newlands et al. 2009) was not observed in the angular head velocity sensitivity of HD cells in this study. Similar nonlinear head motion representation is found in the nucleus prepositus hypoglossi (Kaufman et al. 2000), a vestibular relay point for angular head velocity information projecting to the HD system (see Shinder and Taube 2010a for review).

To produce responses that accurately reflect the orientation of the head in space, the angular head velocity signal in the vestibular nuclei must be converted into a representation of head position relative to the environment that is minimally influenced by the animal’s head velocity and represents that position even when the animal stops moving, since HD cells maintain their firing when the animal is motionless (see above; Blair and Sharp 1995; Blair et al. 1997, 1998; Stackman and Taube 1997; Taube 1995). Creating an accurate environmentally referenced directional signal from a nonlinear, vestibular input-dependent movement signal requires either that the HD system compensates for nonlinearities in converting angular head velocity into a directional signal, or that the nonlinearity is removed, or is absent prior to the conversion. Some researchers have postulated that a nonlinear transformation of angular head velocity to HD signals is due to nonlinearities in the vestibular signal, and that this nonlinear conversion is responsible for the ATI phenomena (van der Meer et al. 2007). Alternatively, some other information that is nonvestibular in origin, such as proprioceptive feedback or motor efference copy, would be required on a continuous basis to help generate an accurate representation of perceived directional heading.

The HD system has been viewed as dependent upon both sensory and motor information (Taube 2007), and has led to the assumption that the HD signal is the composite of multimodal self-motion information (Angelaki and Cullen 2008; Bassett et al. 2007; Clark et al. 2009). Accurate representation of HD was found in the absence of appropriate motor, proprioceptive, or visual input. Furthermore, behavioral experience was unable to significantly influence HD cell responses to passive movement. In this regard, we could view the HD signal as purely perceptual in nature, a unimodal construct of vestibular sensory information that is referenced to environmental cues by mechanisms that are affected by cognitive, motor, and multisensory information. Through this perspective we can better understand not only the phenomena of the HD signal but also the systems of spatial orientation and navigation that depend upon HD system input. The demonstration that normal HD cell responses are maintained after head-fixed restraint provides an excellent opportunity for future experiments to test their
properties in different dimensional planes, such as the vertical plane or when inverted. In addition, this approach will enable researchers to test HD cells using techniques that are common to the vestibular field. For example, it would be interesting to determine how angular and translational vestibular information is combined for the HD system and whether this produces a representation of HD that is two- or three-dimensional. In addition, this approach also makes it possible to record from HD cells intracellularly and relate the cell’s level of depolarization to the animal’s behavior, as was recently demonstrated for hippocampal place cells (Harvey et al. 2009).

REFERENCES


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