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Rat Head Direction Cell Responses in Zero-Gravity Parabolic Flight

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Taube, Jeffrey S., Robert W. Stackman, Jeffrey L. Calton, and Charles M. Oman. Rat head direction cell responses in zero-gravity parabolic flight. *J Neurophysiol* 92: 2887–2997, 2004. First published June 22, 2004; 10.1152/jn.00887.2003. Astronauts working in zero-gravity (0-G) often experience visual reorientation illusions (VRIs). For example, when floating upside down, they commonly misperceive the spacecraft floor as a ceiling and have a reversed sense of direction. Previous studies have identified a population of neurons in the rat's brain that discharge as a function of the rat's head direction (HD) in a gravitationally horizontal plane and is dependent on an intact vestibular system. Our goal was to characterize HD cell discharge under conditions of acute weightlessness. Seven HD cells in the anterior dorsal thalamus were monitored from rats aboard an aircraft in 0-G parabolic flight. Unrestrained rats locomoted in a clear plexiglas rectangular chamber that had wire mesh covering the floor, ceiling, and one wall. The chamber and surrounding visual environment were relatively up-down symmetrical. Each HD cell was recorded across forty 20-s episodes of 0-G. All HD cells maintained a significant direction-specific discharge when the rat was on the chamber floor during the 0-G and also during the hypergravity pull-out periods. Three of five cells also showed direction-specific responses on the wall in 1-G. In contrast, direction-specific discharge was usually not maintained when the rat locomoted on the vertical wall or ceiling in 0-G. The loss of direction-specific firing was accompanied by an overall increase in background firing. However, while the rat was on the ceiling, some cells showed occasional bursts of firing when the rat's head was oriented in directions that were flipped relative to the long axis of symmetry of the chamber compared with the cell's preferred firing direction on the floor. This finding is consistent with what might be expected if the rat had experienced a VRI. These responses indicate that rats maintain a normal allocentric frame of reference in 0-G and 1-G when on the floor, but may lose their sense of directional heading when placed on a wall or ceiling during acute exposures to 0-G.

INTRODUCTION

Astronauts working in 0-G often experience different types of spatial orientation illusions (Mittelstaedt 1987; Oman 2003; Oman et al. 1986, 1990). Visual reorientation illusions (VRIs) are the most common. During space flight, an astronaut's sense of direction becomes quite labile and dependent on visual factors, since the "down" reference provided by gravity is absent. After a lifetime of upright living on earth, there is a natural tendency for astronauts to perceive that whatever interior surface of the spacecraft appears beneath their feet is a "floor." Hence, when astronauts turn upside down in their cabins, surrounding surfaces often seem to exchange subjective identities. Even when truly upright in the cabin, simply seeing

another crew member floating inverted can make an upright observer believe they are upside down, since the second crew member is assumed to be upright. VRIs in 0-G are similar to the directional reorientations we occasionally experience on earth as when, for example, we leave a subway station, recognize a familiar landmark, and realize we are facing east, not west. The important difference is that, in 0-G, the axis of directional reorientation is not anchored by gravity. VRIs can trigger bouts of space sickness and lead to reaching and navigation errors when moving about. Finding ways to make orientation and navigation easier in 0-G is important for successful spaceflight operations. Understanding the physiological mechanisms that underlie human orientation in 0-G is important for the development of effective countermeasures to prevent (or alleviate) disorientation.

Previous studies have identified a population of neurons in the rat brain that discharge as a function of the animal's head direction (HD) in a gravitationally horizontal reference plane, regardless of head pitch or roll (Taube et al. 1990a; for reviews, see Sharp et al. 2001; Taube 1998). The direction at which the cell discharges maximally is referred to as the cell's preferred direction. These cells were originally identified within the rat dorsal presubiculum (postsubiculum), but have now been identified in several brain areas, mostly within the limbic system. HD cells are particularly abundant within the anterior dorsal thalamic nucleus (ADN) (Taube 1995) and have been identified in the primate presubiculum (Robertson et al. 1999). HD cells respond to a variety of external sensory stimuli, including visual and olfactory landmarks (Goodridge et al. 1998; Taube et al. 1990b). They also respond to internal information about head movements using vestibular, proprioceptive, and/or motor cues (Blair and Sharp 1996; Stackman et al. 2003; Taube and Burton 1995). Neurophysiological correlates that are consistent with a visual reorientation have been observed in HD cells when a rat moves between two familiar environments in which the preferred firing direction of the cell is different in each environment (Taube and Burton 1995). The direction-specific activity of ADN and postsubicular HD cells are abolished by bilateral neurotoxic lesions of the vestibular labyrinth (Stackman and Taube 1997; Stackman et al. 2002). When rats move up or down a gravitationally vertical ladder, HD cell firing is generally dependent on the directional orientation of the rat when it left the horizontal plane (Stackman et al. 2000). If the rat is facing in the cell's preferred firing direction, cell firing continues as the rat moves onto and traverses the ladder. Conversely, if the cell was inactive, it remains inactive while

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the rat traverses the ladder. However, if the rat happens to turn its head while on the ladder so that it is aligned with the normal preferred direction when in a horizontal plane, cell firing recommences. These results indicate that, in 1-G, the reference plane for HD cell responses is aligned with the gravitational horizontal, and cells maintain their preferred direction in three dimensions in consistent ways when animals climb on gravitationally vertical surfaces.

The goal of this study was to characterize HD cell discharge in rats under conditions of weightlessness and hypergravity achieved during parabolic flights in a NASA aircraft. Each cell was recorded during 40–50 parabolic flight cycles, with 0-G phases lasting ≤ 20 s. During each episode, cell responses were monitored when the rat locomoted on the floor, wall, or ceiling of a rectangular test chamber. The issues addressed were as follows. 1) When animals move on the “floor” of the test chamber in 0-G, do their HD cells continue to respond in a clearly directional way? Is the HD cell tuning curve altered? 2) What happens when the animals move onto the “walls” or the “ceiling” of the visually up-down (vertically) symmetrical test chamber? 3) Does the reference plane of HD cell firing ever shift to the wall or ceiling as might be expected if the animal experienced a VRI? We report that HD cells continued to fire normally (i.e., similar to 1-G conditions) when the rat locomoted on the floor in 0-G, but direction-specific firing was either absent or markedly attenuated when the rat locomoted on the wall or ceiling in 0-G.

A preliminary report of this research was presented at the 29th Annual Society for Neuroscience meeting in Miami, FL in 1999.

METHODS

Subjects

Subjects were six female Long-Evans rats, aged 4–8 mo at the time of testing. Animals were individually housed and kept on a 12:12-h light/dark cycle. Two of the animals were initially placed on water-restricted diets but had full access to water on the day of testing. All animals had ad libitum access to food at the time of testing.

Electrode and surgical techniques

The electrodes consisted of a bundle of ten 25- μ m-diam nichrome wires that were insulated except at the tips. The wires were threaded through a stainless steel cannula and attached to a modified 11-pin Augat connector, which in turn was embedded in dental acrylic and made moveable through the use of three screws (for details on electrode construction, see Kubie 1984). Animals were anesthetized with pentobarbital sodium (45 mg/kg, ip) and also were injected with 0.1 ml of atropine sulfate (25 mg/ml) to reduce respiratory problems. Using stereotaxic techniques and Bregma coordinates, the electrode array was implanted just dorsal to the ADN: anterior-posterior, 1.35 mm posterior; medial-lateral, 1.40 mm right; dorsal-ventral, 4.0 mm from the cortical surface (Paxinos and Watson 1998). All surgery was conducted under sterile conditions, and animals were allowed to recover for 7 days before cell screening commenced. All procedures were conducted according to institutionally approved animal care protocols (Dartmouth College and NASA IACUCs) and were in accordance with the American Physiological Society's Guiding Principles in the Care and Use of Animals.

Ground procedures before parabolic flights

One to 3 wk following surgery, animals were flown to a NASA laboratory in Houston, TX. Each animal was screened several times a

day for HD cells in a clear plastic cage (45 \times 20 \times 20 cm) identical in size to their home cage. The recording equipment and screening cage were located in a small, well-lit laboratory room. The rats had full visual access to the laboratory room while inside the screening cage. If an HD cell was not identified on any of the recording wires, the electrode array was advanced 15–50 μ m. When an HD cell was identified, we tried to maintain its isolation until the day of flight (1–7 days later). During the week preceding the parabolic flights, all animals were acclimated to a clear, plexiglas rectangular test chamber (1.2 m wide \times 0.6 m high \times 0.6 m deep) that was used later on the parabolic flights. The chamber contained a wire screen grid (1/4-in square) on three sides (floor, ceiling, and the wall on one end) to allow the rats to grasp and move on three surfaces during the 0-G intervals. The experimenters could reach in and manipulate the animals through a 0.3-m-diam hole located in the center of each of the long sides of the chamber (Fig. 1A). The chamber was mounted onto a 0.6-m-high metal pedestal.

Flight procedures

Before the flights commenced, the rectangular chamber was moved onto the aircraft, and the pedestal was bolted down to the deck of the airplane. To block the animals' view of the rest of the cabin, black cotton curtains were hung floor-to-ceiling across the aircraft in front and behind the chamber area. The two curtains were separated by about 3.5 m. The chamber area in between the two curtains was illuminated by fluorescent lights on both walls of the aircraft. The

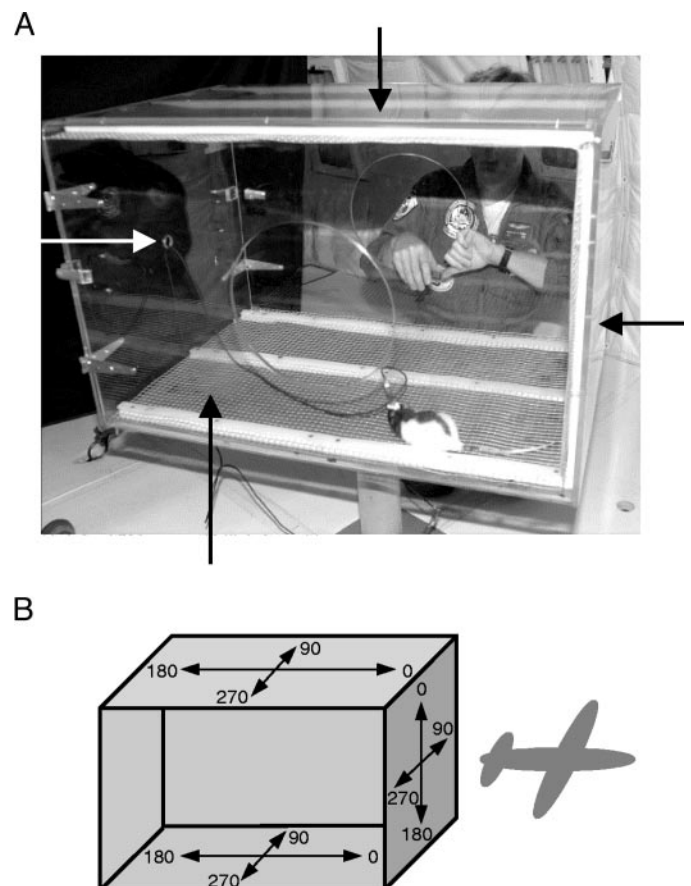


FIG. 1. A: Apparatus. Black arrows point to the 3 sides of the plexiglas cage that contained a wire mesh attached to its surface. The 0.3-m holes on the 2 long sides provided access to interior of the cage. The recording cable passed through a small hole on the left wall (white arrow). A rat is visible in the foreground for scale. B: reference frame used to define the animal's head direction (HD) for each of the different mesh surfaces.

deck and interior fuselage were covered with white foam padding. Except for a tripod mounted video camera, a few other small equipment items, and the two experimenters, the visual environment the animals could see beyond the chamber was largely up-down symmetrical.

We recorded from a different HD cell in a different animal each day. On the day of the flight, animals with one or more previously isolated HD cells were brought onboard the aircraft, attached to the recording cable one at a time, placed in the chamber, and checked for the presence of HD cell responses. If an HD cell was not present in the first rat, another rat was checked. When an HD cell was present, we monitored it for ~2–4 min while the rat locomoted on the floor or wall to determine its approximate preferred firing direction. We also placed the rat on the ceiling and monitored activity, but the rat was usually reluctant to remain hanging inverted on the ceiling for any length of time during this normal 1-G period. The animal was then disconnected and kept in a small cage until after takeoff. As the plane entered the test area, we reattached the rat to the cable, placed it in the rectangular chamber, and monitored the HD cell for several more minutes until the start of the parabolic trajectories. Each trajectory consisted of a hypergravity (~1.8-G) entry phase lasting ~20–30 s, followed by a 0-G parabolic weightless phase lasting ~20 s, and a 20- to 30-s hypergravic pullout phase. Forty to 50 parabolic flight cycles were flown each day. The trajectories were usually repeated without interruption, but occasionally the pullouts and entries were separated by a normal gravity interval of up to several minutes. The aircraft pitch rate during the 0-G phase of each flight cycle was 2–3°/s aircraft nose downward and was imperceptible to the experimenters. Linear accelerations varied by approximately ± 10 milli-G during the weightless phase. On one flight, we recorded two HD cells simultaneously from the same animal. We report data from seven cells recorded from six animals during a total of six flights. Two experimenters were normally positioned by the access holes of the test chamber throughout each flight. One experimenter was responsible for moving the rat to different surfaces during the 0-G periods and also for ensuring the rat was on the floor during the transition period back to 1.8-G. The second experimenter recorded the session using a handheld video camera. A third experimenter beyond the curtain monitored the recording equipment and made sure adequate cell isolation was maintained.

During the hypergravic periods, the rats usually rested motionless on the floor, although there were a few episodes during the initial parabolas where the rat moved around on the chamber floor during this period. During the 0-G periods, the rat was free to move about on any of the three wire mesh surfaces. Typically, the rats moved about the chamber slowly while clinging to the wire mesh during the 0-G portion of the cycle. Because the 0-G episodes were short duration and we wanted to sample as many different directions as possible, one of the experimenters frequently nudged the rat lightly along one of the surfaces. This nudging usually did not involve grasping the rat but rather gently pushing it forward or to the side until it usually moved under its own volition. In addition, to obtain sufficient sampling time on the wall and ceiling, one of the experimenters usually had to grasp the rat at the start of a 0-G phase while on the floor and gently place it on one of the two surfaces. How the rat was oriented as the experimenter placed it on the ceiling in 0-G varied between different flight cycles. Sometimes the rat was rotated about its roll axis onto the ceiling; other times it was rotated about its pitch axis onto the ceiling. At the end of each 0-G period, the rat was grasped and gently placed back onto the floor of the chamber. All the animals tolerated the gravity level changes well, became comfortable with the manipulations, seemed relatively relaxed, and frequently moved on their own volition.

A typical flight consisted of three to four initial flight cycles recording the rat moving on the floor during the 0-G phase, followed by cycles with the animal either on the wall or the ceiling during 0-G. Interspersed throughout the wall and ceiling episodes were occasional

episodes on the floor in 0-G. These episodes served as important controls to verify that cell isolation and activity remained relatively unchanged throughout the flight. Thus whatever changes we observed in cell activity when the animal was on the wall or ceiling could be attributed to the particular gravity level and surface currently being experienced. Most flights included about 10 episodes on the floor, 10 episodes on the wall, and 15–20 episodes on the ceiling. There were many occasions where we were able to monitor cell activity as the rat locomoted between two different surfaces—either floor/wall or ceiling/wall. For the majority of flight cycles, the two experimenters stationed inside the curtained enclosure remained upright with respect to the floor of the aircraft. However, there were a few cycles each day where both experimenters deliberately rolled 90° to one side or 180° upside down.

Recording and data acquisition

For unit screening and recording, the animal was attached to a 13-wire recording cable that was connected on one end to the recording rack and to the animal's headstage on the other end. Electrical signals were passed through a field-effect transistor in a source-follower configuration, amplified (Grass Instruments P511), band-passed filtered (300–30,000 Hz, 3 dB/octave; Peavey Electronics PME8), and sent through a dual window discriminator (Bak Electronics DDIS-1) before being displayed on an oscilloscope (Tektronix 2214). The window discriminator emitted a 10-ms square wave pulse when the cell fired, and this signal was conveyed to audio speakers and to the audio channels of two video cameras. One video camera was permanently positioned on a tripod in one corner of the curtained area and pointed at the apparatus. A second video camera was held by one of the experimenters and was pointed at the rat to monitor its orientation. All data analysis was performed off-line at a later time using LabView 5 software.

Data analysis

After the flights, HD cell activity was correlated with the rat's head direction. Three independent human scorers viewed the videotapes and set a pointer on a computer screen using a mouse indicating the instantaneous head direction relative to the locomotion surface as the tape was played back. Head direction was defined relative to the aircraft as indicated in Fig. 1B. Zero degrees was defined as the direction toward the front of the aircraft, and angles increased progressively in a counterclockwise (CCW) manner. Thus 180° represented a direction toward the rear of the plane; facing toward the front of the aircraft, 90 and 270° represented directions toward the left and right aircraft wings, respectively. This same coordinate frame was used for the ceiling. For the wall, the coordinate frame was rotated 90° along the horizontal wing-to-wing axis such that 0° was pointing up, 180° was pointing down, and 90° and 270° remained pointing toward the left and right aircraft wings, respectively. A computer's data acquisition system (National Instruments DIO-96) sampled each scorer's head direction estimate and the spike data from the videotape's audio channel at a rate of 60 Hz. The rat's HD data were sorted into 1 of 16 (22.5°) bins. The corresponding firing rate for each head direction bin was computed based on the total number of spikes divided by the total time in that bin. Firing rate versus HD bin data were then obtained for each cell, locomotion surface, and phase of the trajectory for each scorer. To minimize the effect of any geometric or personal angle estimation errors by individual scorers, tuning curves were averaged across scorers, and relatively wide 22.5° bins were used. Due to reaction time, scorers' estimates of the rat's HD doubtlessly lagged behind the true directional heading when the rat moved its head quickly, but these errors likely canceled out over time, since the rat moved its head approximately equally in both directions. Thus the estimate of the cell's preferred firing direction should remain relatively accurate. However, the overall peak firing rate of the HD

cell, as determined from the firing rate versus HD plot, will be less than the value obtained using a more accurate automated overhead tracking system and using smaller HD bin widths (Taube et al. 1990a).

From the average tuning curves for each condition, the values for six parameters were determined: 1) preferred firing direction, 2) peak firing rate, 3) background firing rate, 4) signal-to-noise ratio, 5) the correlation value (r) to a best-fit Gaussian curve, and 6) information content. Preferred firing direction was defined as the head direction bin containing the highest firing rate; the firing rate within this bin was defined as the peak firing rate. The background firing rate was defined as the average firing rate of the three lowest HD bins. The signal-to-noise ratio was calculated by dividing the average firing rate of the three highest firing rate HD bins by the background firing rate. Using a least squares method a Gaussian distribution function was optimally fit to each tuning curve, and the Pearson product-moment correlation coefficient (r) was defined as the Gaussian r . This measure represents how well the tuning curve fits a normal distribution. Information content was derived as previously described (Taube and Muller 1998) using

$$IC = \sum p_i(\lambda_i/\lambda) \log_2(\lambda_i/\lambda)$$

where p_i = the time spent with the head pointing in the i th bin divided by the total time (probability that the head pointed in the i th bin), λ_i = the mean firing rate of the cell in the i th bin, and λ = the overall firing rate of the cell for the entire recording session. Information content measures the extent to which the firing of a spike by a cell reduces the uncertainty of the rat's directional heading. Higher values indicate a greater degree of certainty in predicting the animal's directional heading.

Many HD cells lost their direction-specific firing in some of the 0-G conditions. To quantitatively assess the degree of directional firing for each condition, we determined an ad hoc directionality score for each session based on three parameters: 1) information content, 2) signal-to-noise ratio, and 3) Gaussian r . The directionality score was calculated by normalizing the values for each of the three parameters according to the maximal value observed over all the tuning curves and then defining the directionality score as

$$\text{Directionality score} = \frac{nIC + nr + n \log_{10} S/N}{3}$$

where nIC is the normalized information content, nr is the normalized best-fit Gaussian r , and $n \log_{10} S/N$ is the normalized logarithm of the signal-to-noise ratio. Defined this way, directionality scores could range between 0 and 1, with a score approaching 1 indicating a high degree of directionality and a score approaching 0 indicating a low degree of directionality. In addition, we tested whether each session contained a uniform (random) firing distribution over the 16 directional bins using a Rayleigh test (Batschelet 1981). For this test, we used $n = 100$ to determine the probability level.

Doubly multivariate ANOVAs (MANOVAs) with repeated measures were used to assess differences across conditions (1-G vs. 0-G) and surfaces (floor, wall, ceiling). Statistical significance was defined at 0.05.

Histology

At the completion of the experiment, all animals were flown back to Hanover, NH. After several days, animals were anesthetized deeply, and a small anodal current (10–20 μ A for 10 s) was passed through one of the recording wires to later conduct a Prussian blue reaction. The animals were perfused transcardially with 10% formalin (in saline), and the brains were removed and placed in 10% formalin for ≥ 48 h. The brains were placed in a 10% formalin solution containing 2% potassium ferrocyanide for 24 h and reimmersed in 10% formalin (24 h) before being placed in 20% sucrose for 24 h. They were then frozen, sectioned (40 μ m) in the coronal plane,

stained with cresyl violet, and examined microscopically for localization of the recording sites. All recording electrodes ($n = 6$) were localized to the ADN.

RESULTS

Qualitative observations

Seven HD cells were recorded from six rats on six different parabolic flights; two of the seven cells were recorded simultaneously. Results were generally consistent across each HD cell, although there were a few individual differences as described below. All HD cells maintained their direction-specific activity at about the same firing rate in both the 0-G and hypergravic phases when the rat locomoted on the floor. In contrast, when the rat traversed the wall in 0-G or locomoted upside-down on the ceiling in 0-G, HD cells usually lost their direction-specific firing and simultaneously had an increase in baseline firing rate.

Five cells (4 recording sessions) were tested to determine what coordinate reference frame was used by the cells. Before take-off, we initially monitored cell activity and noted the preferred firing direction of the cell with respect to the chamber and airplane. Then, either before take-off or during the flight before the initiation of the first parabolic flight cycle, we rotated the rectangular chamber by 90° in either the clockwise (CW) or CCW direction with the rat in the chamber while noting the cell's response. In three of four sessions, rotations of the chamber did not lead to a shift in the cells preferred firing direction, indicating that the cells were using the coordinate frame of the aircraft as the allocentric reference frame. In the remaining session, the HD cell's preferred firing direction remained stable in the coordinate frame of the aircraft, but after 3–4 s, shifted $\sim 90^\circ$ to align itself with the reference frame of the chamber. In summary, these results indicate that the cells were usually using the interior of the aircraft as their reference frame rather than the rectangular chamber.

Quantitative analyses

The overall repeated measures MANOVA (Floor 1-G, Floor 0-G, Wall 0-G, Ceiling 0-G) revealed significant effects across the complex of three independent measures: signal-to-noise ratio, Gaussian r , and information content [$F(9,54) = 4.387$, $P < 0.001$]. The univariate analyses using Greenhouse-Geisser corrected F s showed that there were significant effects for all three measures: signal-to-noise [$F(1.025,6.148) = 17.358$, $P < 0.005$], Gaussian r [$F(1.797,10.785) = 8.754$, $P < 0.01$], and information content [$F(1.608,9.651) = 25.406$, $P < 0.001$]. To determine where the effect in signal-to-noise was coming from, we examined the two individual components—peak firing rate and background firing rate. These analyses revealed that the effect was accounted for by changes in background firing rate [$F(1.453,8.719) = 6.530$, $P < 0.05$], rather than peak firing rate [$F(1.371,8.227) = 2.289$, not significant (NS)]. As expected, there was also an overall significant effect for the one dependent measure of directionality score [$F(2.166,12.995) = 49.081$, $P < 0.001$].

HD CELL RESPONSES WHEN THE RAT IS ON THE FLOOR. Firing rate versus HD tuning curves for four of the seven cells when the animal was moving on the floor are depicted in Fig. 2, with the two cells recorded simultaneously in one rat shown in the *right*

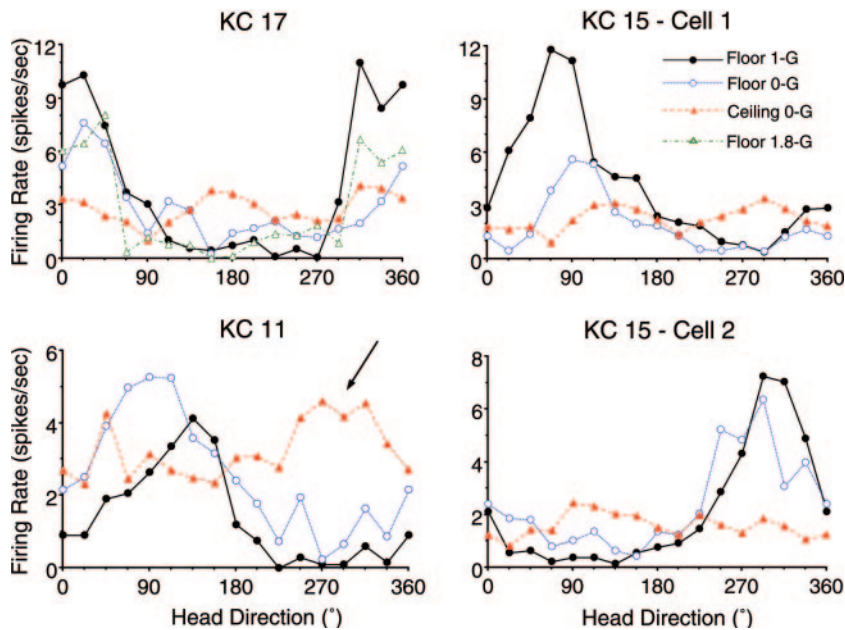


FIG. 2. Firing rate vs. HD tuning curves for 4 HD cells recorded on the floor in 1-G (black traces, filled circles), on the floor in 0-G (blue traces, open circles), and on the ceiling in 0-G (red traces, filled triangles). Plots indicate that direction-specific firing was maintained on the floor in 0-G, but not on the ceiling. When on the ceiling, occasional bursts of activity in directions 180° opposite that of the cell's preferred firing direction on the floor can be seen for cell KC 11 (arrow). Plot for cell KC 17 also shows the responses during the ~1.8-G pullout periods of the aircraft (green trace, open triangles). Direction-specific firing was maintained on the floor during the 1.8-G condition.

column. All cells showed similar tuning curves between 1-G and 0-G conditions, with no appreciable shifts in the cells preferred firing directions. Table 1 shows the mean values for the various tuning curve parameters. The univariate contrast analyses between 1-G and 0-G on the floor showed that there was no effect for peak firing rate [$F(1,6) = 0.749$, NS], background firing rate [$F(1,6) = 3.002$, NS], and Gaussian r [$F(1,6) = 0.227$, NS], but significant effects for signal-to-noise [$F(1,6) = 13.746$, $P < 0.05$] and information content [$F(1,6) = 16.176$, $P < 0.01$]. Comparing individual values between 0-G and 1-G within rats, the signal-to-noise ratio and information content values were decreased across all seven HD cells in the 0-G conditions. These decreases could be partly attributed to the increase in background firing rate that occurred in six of seven cells in the 0-G condition relative to the 1-G condition. Finally, as expected, these changes were associated with a significantly decreased directionality score in the 0-G condition [$F(1,6) = 40.044$, $P < 0.001$]. Directionality scores decreased across all seven HD cells in the 0-G condition (mean percent decrease = 25.8%). Although the directionality scores were reduced for all cells, it is important to note that direction-specific firing continued across all cells in the 0-G condition. Rayleigh tests for a random distribution of directional firing showed that all seven cells had $P < 0.005$, indicating the presence of directional tuning.

In general, the rats did not move around the chamber floor very much during the hypergravic periods; consequently, there was not sufficient sampling for all head directions to construct comparable tuning curves for all cells. However, we were able to plot complete tuning curves for three cells comparing hypergravic to 1-G and 0-G activity on the floor. These tuning curves showed that qualitatively similar results were obtained during the hypergravic phases of the trajectory. One representative example (KC 17, green trace) is shown in Fig. 2.

HD CELL RESPONSES WHEN THE RAT IS ON THE WALL. Five cells were tested under 1-G conditions when the rat locomoted on the wall. Of these five cells, three cells showed robust and consistent direction-specific firing on the wall, while the other

two cells showed weak direction-specific firing on the wall. Treating the wall as an extension of the floor and rotating the reference frame by 90° (Fig. 1B), the preferred firing direction for all the cells on the wall was similar to that on the floor, which is consistent with findings from our previous study with HD cells in the vertical plane (Stackman et al. 2000). Figure 3 depicts three HD cells recorded when the rat was on the wall in 1-G and 0-G conditions. The cell shown in Fig. 3A showed strong directional tuning on the wall in 1-G, while the cell shown in Fig. 3B showed weak direction-specific activity on the wall in 1-G. Both cells showed little, if any, directional tuning in 0-G conditions.

In addition to the five cells tested in both 0-G and 1-G conditions, two other HD cells were recorded on the wall in 0-G conditions, but were not tested on the wall under 1-G conditions. Of the seven cells recorded on the wall in 0-G conditions, only one cell was considered to exhibit a modest degree of direction-specific activity (Rayleigh test: $r = 0.177$, $n = 112$, $P < 0.05$; Fig. 3C). There was little evidence for clear direction-specific firing in the remaining six cells (all Rayleigh P values testing for a nonrandom distribution were NS). The mean values for the various directional parameters in Table 1 support this conclusion. For purposes of statistical analyses, the wall 0-G sessions were compared with the floor 0-G sessions instead of the wall 1-G sessions, primarily because of the insufficient n for the wall 1-G sessions.

Comparing the 0-G responses on the wall with those on the floor, the univariate contrast analyses showed there was a significant decrease in signal-to-noise ratio [$F(1,6) = 35.688$, $P < 0.001$], information content [$F(1,6) = 5.927$, $P = 0.05$], Gaussian r [$F(1,6) = 21.039$, $P < 0.005$], and directionality scores [$F(1,6) = 25.605$, $P < 0.005$]. Signal-to-noise ratio, Gaussian r , and directionality scores decreased across all seven cells in the wall 0-G sessions, while information content decreased in five of seven cells. Although peak firing rate [$F(1,6) = 3.988$, NS] and background firing rate [$F(1,6) = 0.393$, NS] were not significantly different between the floor and wall 0-G conditions, peak firing rate decreased across all

TABLE 1. Mean parameter values for HD cells across different conditions

Condition	Peak Firing Rate (spikes/s)	Background Firing Rate (spikes/s)	Signal/Noise Ratio	Gaussian <i>r</i>	Information Content (bits/spike)	Directionality Score
I-G conditions						
Floor I-G	8.60 ± 1.10 (4.13–11.81)	0.32 ± 0.09 (0.07–0.71)	34.23 ± 7.14 (14.56–63.22)	0.917 ± 0.042 (0.670–0.990)	0.640 ± 0.069 (0.422–0.925)	0.748 ± 0.038 (0.582–0.859)
Wall I-G	9.03 ± 4.06 (2.00–24.36)	0.10 ± 0.03 (0.00–0.19)	129.73 ± 94.51 (22.29–507.94)	0.768 ± 0.101 (0.520–0.980)	0.975 ± 0.112 (0.597–1.288)	0.649 ± 0.077 (0.449–0.846)
0-G conditions						
Floor 0-G	11.66 ± 3.79 (5.27–33.40)	1.85 ± 0.91 (0.45–7.11)	7.52 ± 0.92* (3.67–10.96)	0.896 ± 0.037 (0.700–0.960)	0.372 ± 0.100* (0.078–0.661)	0.556 ± 0.043* (0.390–0.670)
Wall 0-G	6.30 ± 1.27 (2.27–12.66)	2.23 ± 0.51 (0.42–4.32)	2.97 ± 0.43† (1.96–4.66)	0.653 ± 0.054† (0.470–0.820)	0.1140 ± 0.030† (0.080–0.302)	0.339 ± 0.026† (0.248–0.432)
Ceiling 0-G	6.55 ± 1.47 (2.47–12.44)	2.98 ± 0.79† (1.05–7.11)	2.18 ± 0.13† (1.58–2.59)	0.681 ± 0.042† (0.500–0.780)	0.067 ± 0.009† (0.033–0.110)	0.308 ± 0.016† (0.254–0.355)

All values are mean ± SE. Values within parentheses denote range, $n = 7$ for all conditions, except Wall 1-G where $n = 5$. * $P < 0.05$ compared with Floor 1-G condition, MANOVA contrasts test. † $P < 0.05$ compared with Floor 0-G condition, MANOVA contrasts test.

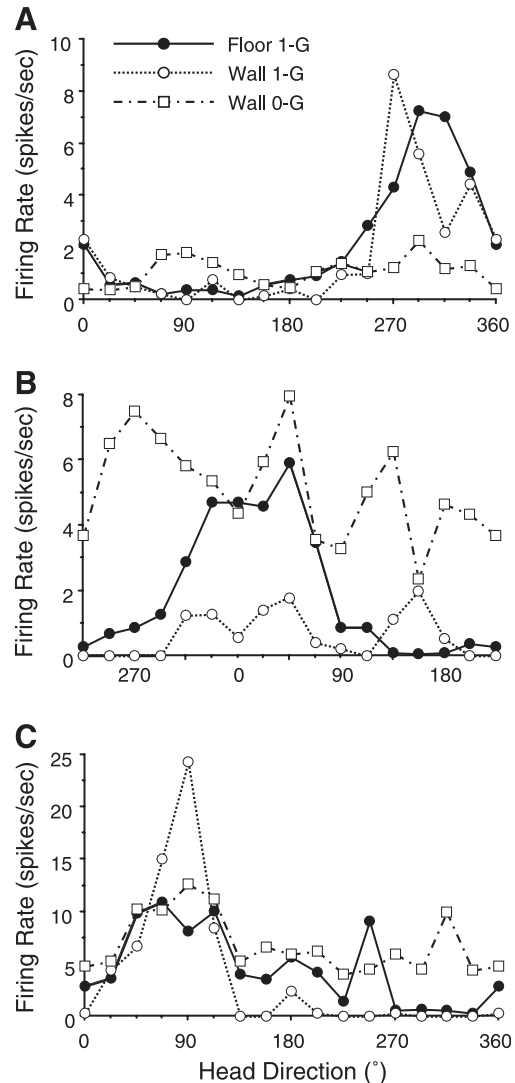


FIG. 3. Firing rate vs. HD tuning curves for 3 HD cells recorded on the floor and wall in 1-G and 0-G conditions. *A*: cell that showed robust direction-specific firing on the wall in 1-G. *B*: cell that showed weak direction-specific firing on the wall in 1-G. To better view the cell's turning curve, the HD axis for this plot was shifted by 135° counterclockwise (CCW). Cells in *A* and *B* showed little direction-specific firing when the animal was on the wall in 0-G. *C*: HD cell that showed moderate direction-specific firing on the wall in both 1-G and 0-G.

seven cells (mean percent change = 36.1% decrease), and background firing rate increased in five of seven cells (mean percent change = 89.3% increase) when the rat was on the wall. Collectively, these findings indicate that directionality decreased when the animal was on the wall in 0-G compared with the floor in 0-G.

HD CELL RESPONSES WHEN THE RAT IS ON THE CEILING. All seven HD cells substantially lost their direction-specific firing characteristics when the rats locomoted on the ceiling in 0-G compared with 0-G on the floor. Because of the limited 0-G exposure time available, it was too far for them to crawl from the floor across the wall onto the ceiling at the start of the 0-G phase. Therefore we manually picked up the animals from the chamber at the start of the 0-G phase and placed them on the ceiling. We deliberately used two different methods to manually convey the animals to the ceiling—rolling them sideways

180° or pitching them nose upward 180°. Note that when the rat was rolled onto the ceiling, its directional heading with respect to the aircraft did not change. In contrast, when the rat was pitched nose upward, its directional heading when placed on the ceiling was 180° opposite from that when on the chamber floor. Using either method, cells appeared to increase their tonic discharge rate, and some activity usually occurred in several different directions. There were instances when a cell would discharge several spikes in a burst, but the direction of the rat's head was variable during these bursts—sometimes pointing in the cell's preferred firing direction, while at other times, the rat's head pointed in a different direction (see next section). Because neither of the manual transport methods resulted in the maintenance of direction-specific firing when the rat was on the ceiling, the data from both methods were grouped together. Figure 2 (red traces) depicts the ceiling responses from four HD cells compared with responses on the floor in 0-G.

Rayleigh tests showed that none of the cells had a nonrandom distribution in their firing rate versus HD tuning curve (all P values were NS). Although the HD cells did not show consistent directional tuning on the ceiling, the firing rate versus HD responses could still be fit to Gaussian functions, and Table 1 summarizes the results from the various parameters. The univariate contrast analyses showed that, compared with 0-G responses on the floor, there was a significant decrease in signal-to-noise ratio [$F(1,6) = 35.935$, $P < 0.001$], Gaussian r [$F(1,6) = 11.267$, $P < 0.05$], information content [$F(1,6) = 10.389$, $P < 0.05$], and directionality score [$F(1,6) = 27.469$, $P < 0.005$]. Further analyses revealed that the signal-to-noise effect was due to a significant increase in background firing rate [$F(1,6) = 13.287$, $P < 0.05$] and not due to a change in peak firing rate [$F(1,6) = 3.662$, NS]. Responses for signal-to-noise ratio (mean percent change: -68.5%), information content (mean percent change: -69.3%), and directionality score (mean percent change: -43.2%) decreased, and background firing rates increased (mean percent change: 41.4%) across all seven HD cells recorded on the ceiling. Gaussian r values decreased in six of seven cells (mean percent change: -22.6%) when the rat was on the ceiling.

Were rats disoriented when on the ceiling or wall in 0-G? Although we cannot answer this question definitively (because we never conducted a behavioral task to assess spatial performance), we noted that some animals occasionally moved from the ceiling to the wall and down to the floor, all in 0-G; thus appearing to prefer to be on the floor rather than the ceiling. This behavior suggests an awareness of where the floor was relative to the ceiling, even in 0-G. Because the end of the 0-G episode culminated in the rapid onset of gravity forces that would propel the rat back to the floor abruptly had we not returned it to the floor at the end of the 0-G period, it was natural for the rats to quickly learn that the floor surface was a safer place than the wall or ceiling. Whether the rats knew where to go based on spatial awareness or they were moving toward a location (beacon navigation) associated with certain odors (e.g., urine or feces odors) is unclear.

EPISODES OF BURST FIRING IN OTHER HEAD DIRECTIONS AND POSSIBLE EVIDENCE OF VRIS ON THE CEILING. Although cells were not directionally tuned when the rat was on the ceiling, there were several instances when a cell discharged with a burst of spikes

when the rat's directional heading was momentarily pointing in a direction other than the cell's preferred direction on the floor. Figure 4 shows representative examples of responses for one cell that had a preferred firing direction of $\sim 90^\circ$ on the floor in 0-G. Figure 4A shows responses on the floor in 0-G, while Fig. 4, B–D, shows responses from three episodes on the ceiling in 0-G. In the plot on the floor, as expected, the HD cell fired regularly in the cell's preferred firing direction (open bars) and was relatively silent at other head directions. In Fig. 4, B and D, the animal was placed on the ceiling facing the opposite direction (e.g., 270°), and there was an increase in firing (black bars), which remained present until the animal turned its head away CCW. In Fig. 4B, when the head pointed 90° (the preferred firing direction on the floor), cell firing was relatively quiet from 11 to 19 s and then fired a short burst from 19 to 21 s. When the animal turned its head away, cell firing was again low (21–22 s). A high firing rate occurred again at 22 s as the head returned toward the 270° direction. In Fig. 4C, the animal was initially facing the 90° preferred direction on the floor for several seconds (i.e., times 0–3 and 4–8 s), but the cell did not fire at a high rate. The firing rate then increased as the animal turned CW to face 270° (times 10–16 s). Thus the preferred firing direction on the ceiling for this cell was $\sim 180^\circ$ opposite to the direction on the floor. Firing rates in the OFF directions were generally higher than those in 1-G, which may explain the partially elevated firing rates observed when the head was in intermediate directions (e.g., Fig. 4D, times 12–16 s). Also, ON responses (when the head was in the floor or ceiling preferred direction) tended to have a less stable, bursty character. Bursts were typically 0.5–2 s long (Fig. 4, B–D, black and cross-hatched bars) and were followed by periods of inactivity for 2–3 s (e.g., Fig. 4D, times 0–3 and 5–7 s) or periods of increased firing with single spikes (e.g., Fig. 4C, times 10–11 s).

For each cell and episode on the ceiling, we counted the frequency of bursts (1) in the cell's floor-preferred firing direction, 2) in ceiling-preferred directions that were reversed along the long axis of symmetry of the chamber, and 3) in other directions. Burst responses in directions other than the preferred firing direction occurred most frequently in three rats and occurred concurrently with a general increase in background firing. The occurrence of this burst activity was independent of the type of rotation the rat underwent when moved to the ceiling. Although bursts sometimes occurred at the cell's floor preferred firing direction while on the ceiling (e.g., Fig. 4B, 19–21 s), they occurred two to three times more often in the ceiling-preferred directions. One of these cells (KC 11) is depicted in Figs. 2 and 4. For this cell, bursts were most common at 270° (arrow in Fig. 2, black bars in Fig. 4), although they occasionally occurred at other directions (Fig. 4, B and C, cross-hatched bars). These bursts were unlikely to be attributed to a second cell that was near the recording electrode tip because unit isolation of the first cell did not change throughout the recordings, and there was no indication of a second cell being present on the electrode wire when the rat was returned to the floor. Finally, it should be noted that burst firing was observed at the 180° opposite direction, but not at the normal direction, for one rat during several flight cycles when it was on the wall in 0-G.

What change in HD cell response would be expected if the rat had experienced a VRI while on the ceiling in 0-G? The

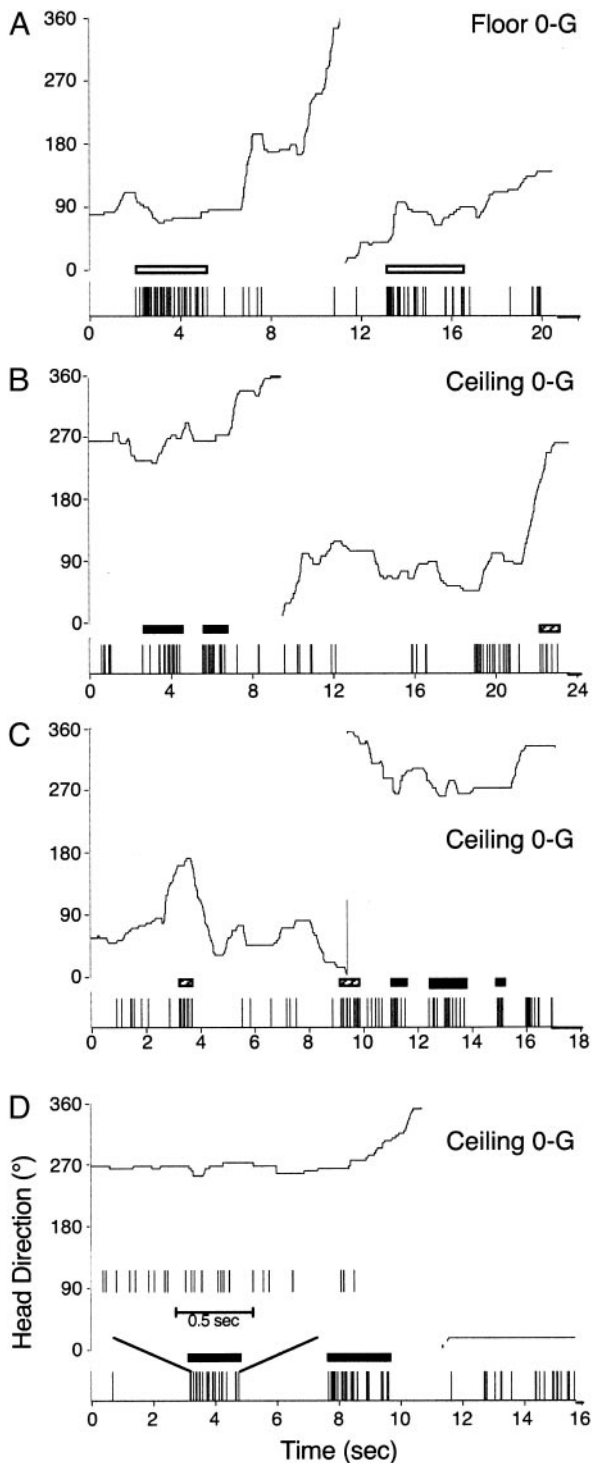


FIG. 4. Examples of HD cell responses from one animal recorded on the floor (A) and ceiling (B-D) in 0-G. Each panel plots head direction and firing rate vs. time. All plots have axis labels as shown in D. In B and D, the rat made a CCW head turn, while in C, the rat made a clockwise (CW) head turn. In A and C, the animal was initially facing in the cell's 90° preferred firing direction on the floor. In B and D, the animal was initially facing 270° , which is opposite to the preferred firing direction on the floor. Open bars show epochs where a floor-oriented response would be expected. Black bars show epochs where burst firing occurred when a ceiling-oriented response was anticipated. Cross-hatched bars show periods of increased firing at head directions other than 90° or 270° . Inset in D shows one burst expanded in time.

chamber has two visual planes of symmetry, one horizontal and another vertical, aligned with the long axis of the chamber. There are two possibilities for how the orientation of the preferred firing direction could respond: 1) based simply on cage symmetry, it might maintain the prior (floor) preferred firing direction, or 2) if the rat used the mesh wall at the end of the chamber as the primary directional reference, we would expect that the preferred firing direction would shift across the long axis of symmetry of the chamber to the corresponding reverse angle on the other side (ceiling-preferred firing direction). For this case, a cell with a preferred firing direction of 90° would shift $180\text{--}270^\circ$, while a cell with a preferred firing direction of 45° would shift 90° about the long axis to 315° .

We presume that, in the absence of gravity, the reference plane of HD cell response would be that of the ceiling grid, and the rat would have the sensation of walking on the floor. The mesh wall at one end of the cage probably provided the principal visual landmark. Therefore the preferred direction would be expected to shift across the long axis of the chamber as discussed above. For the three cells that showed frequent burst activity in directions other than the cell's preferred firing direction, each cell had a preferred firing direction of $\sim 90^\circ$ on the floor (e.g., cell KC 11; Figs. 2 and 4). When on the ceiling in 0-G, bursts occurred at about 270° for the three cells. Thus these bursts occurred at preferred firing directions that were flipped with respect to the long axis of symmetry in the chamber and resemble qualitatively what one would expect if an animal experienced a VRI. However, a cautious interpretation here is appropriate. Cell responses generally had a bursty character and did not last for >2 s, even when the rat maintained its directional heading in an orientation that was flipped from the cell's preferred firing direction on the floor (Fig. 4D, times 0–9 s), although multiple bursts in the same direction on the ceiling were often observed. In humans, VRIs are labile, but usually last for seconds to many minutes (Oman et al. 1986). In 1-G, HD cell activity normally is not bursty and shows little adaptation when an animal maintains a constant directional heading in a cell's preferred firing direction, whether it is moving or still (Taube and Muller 1998). Although cell firing was frequently observed to remain tonically on when the rat was in the proper directional heading on the floor in 0-G, this pattern of activity was never observed in the three rats that showed burst activity when they were on the ceiling. If a rat were to experience a VRI in a way similar to a human astronaut, we might expect HD cell firing to occur continuously at a preferred firing direction flipped with respect to that on the floor for longer periods of time than we observed. Of course, VRIs in rats could simply be very labile under our test conditions where the visual directional cues were deliberately made ambiguous and also could be different from those in humans. Without a behavioral test paradigm to evaluate the rats' perceptions, we cannot definitively know whether or not these burst responses were associated with a VRI-like orientation illusion.

CELL RESPONSES WHEN EXPERIMENTERS WERE INVERTED. Four cells were monitored in 0-G when the rat was on the ceiling with the human experimenters inverted, so that their feet pointed toward the aircraft ceiling and the top of their heads toward the aircraft floor as they faced the apparatus. In this position, the experimenters' faces were upright with respect to

the animals. Cell responses were similar to those on the ceiling when the experimenters were in an upright position and there was no clear evidence for an increase in direction-specific activity in this condition. We saw no trend to observe more burst activity when the rat's head was facing in a particular direction.

Two cells were tested when the rat was on the floor and the experimenters were inverted. This manipulation had no clear effect on HD cell firing, and both cells maintained their direction-specific firing similar to that when the experimenters were in a normal upright position with respect to the aircraft. There was also no change in the preferred firing direction of the cells in this condition. Thus the up-down orientation of the two experimenters had no observable effect on HD cell activity, suggesting that the rats probably used visual landmarks other than the human experimenters to maintain their allocentric reference frame in 0-G.

DISCUSSION

Our results show that, in general, HD cells maintained their direction-specific firing properties in 0-G when the rat locomoted on the floor, the surface plane that it was accustomed to moving on during 1-G. In contrast, a consistent direction-specific discharge in 0-G was rarely seen when the rat was on the vertical wall or upside-down on the ceiling. Although there was a significant decrease in signal-to-noise and information content on the floor in 0-G, the cells still maintained strong directional firing that was readily apparent to anyone observing the animal and monitoring cell firing. The loss of direction-specific activity on the wall and ceiling was usually accompanied by an increase in general background firing, particularly when on the ceiling.

Directional activity on the wall

Only three of five cells tested showed strong direction-specific firing on the wall in 1-G. This result is surprising and stands in contrast to the 1-G findings of Stackman et al. (2000), who showed that all recorded HD cells maintained their directional activity on a vertical surface when the rat locomoted up and down a wall that was situated between two horizontal surfaces placed at different heights. HD cells were never found to cease directional firing on the vertical wall in this earlier study. Given this premise, it was equally surprising to find that directional activity was not maintained on the wall in 0-G. In the previous 1-G study, the rat actively moved itself onto and off the vertical wall. In contrast, in the 0-G experiments, the rats were usually placed onto the wall by the experimenter and therefore did not actively engage in the movements required to change planes. This procedure may have reduced the rats' ability to keep track of and update its directional orientation. It is possible that the cells may have regained their direction-specific firing on the wall with increased familiarity of the wall in the 0-G environment, or better yet, if the rat had been able to move itself from the floor to the wall during the 0-G periods. Support for this notion was recently shown by Stackman et al. (2003), who showed that, when animals were deprived of their normal locomotor cues by passively transporting them via a mobile cart into a novel environ-

ment, the preferred firing direction of HD cells shifted a significant amount compared with animals that self-locomoted into the novel environment. Behavioral studies in hamsters also support this view. Etienne et al. (1986, 1988) showed that animals that were passively transported to a food source in a novel location were impaired at finding the way back to their home nest.

Changes in directional response on the ceiling

Loss of stable directional firing on the ceiling in 0-G was also a surprise. Unfortunately, HD cell responses in animals moving upside-down in 1-G have not been well-studied. In a preliminary experiment, Calton et al. (2000) trained rats to ascend a wire mesh wall and move upside-down across a ceiling to a second wall, where it then descended into a goal box on the floor. Under these conditions, the authors reported two categories of responses. Some HD cells showed robust direction-specific firing while the animal locomoted upright on the floor and walls, but the cells lost their directional tuning when the animal locomoted in an inverted orientation on the ceiling. For other HD cells, the directional tuning was maintained in a world-centered reference frame when the animal locomoted upside-down, although the tuning curves showed much distortion from the typical Gaussian shape. Thus these cells continued to discharge in the same preferred direction with respect to the room as they discharged when the rat was on the floor. As with the wall experiments of Stackman et al. (2000) discussed above, these rats traversed onto and off the different planar surfaces under their own volition. This fact differs from the present parabolic flight experiments where the rats had to be passively transported from the floor to the ceiling because of time constraints. It should be noted that how the rats were passively rotated and placed on the ceiling (i.e., roll or pitch) made little difference in terms of whether or not direction-specific firing continued.

Interestingly, when activity from hippocampal place cells was recorded in prolonged 0-G while traversing a three-dimensional track during the Neurolab Space Shuttle mission (Knierim et al. 2000, 2003), location-specific firing was initially abnormal or poor in two of three animals when recorded on the fourth day of flight. One animal showed similar place responses on each of the three surfaces it locomoted on, as if it had experienced a VRI when changing surfaces. By the ninth flight day, cells from these two animals responded only on a single surface, even though the animal moved in three dimensions.

Again, as with the wall 0-G results, it is possible that with increased exposure time on the ceiling or with opportunities to actively move onto the ceiling surface, HD cells might have regained their directional firing properties. Although the rats were acclimated to the chamber in the week before the flights, they were mostly familiar with the floor surface and spent little time climbing upside-down on the ceiling. During the acclimation period, however, we did spend time passively moving the rat from the floor onto the ceiling, but they only remained suspended for a second before jumping back into the experimenter's hands. Had the experiments been conducted in an orbiting spacecraft, the animals would likely be more adapted to the 0-G environment, and different results might have been obtained. Experiments on hu-

mans during long duration spaceflights have shown improved performance on spatial tasks over time, indicating that adaptation to the 0-G environment does occur (e.g., Glasauer and Mittelstaedt 1998; Oman 2003).

Recent experiments conducted on humans in furnished rooms tilted 90 or 180° from the vertical show that visual scenes exert a powerful influence on the direction of the perceived gravitational vertical, particularly when the subject is not in a gravitationally erect position. The relative orientation of walls, ceiling, and floors, chairs, desks, shelves, and other objects that are normally encountered in a consistent orientation with respect to gravity strongly influence orientation perception, and the effect increases with age (Howard and Hu 2001). Rats also rely heavily on visual landmarks for determining their perceived directional headings (Goodridge and Taube 1995; Taube and Burton 1995; Taube et al. 1990b; Zugaro et al. 2003). Although the up-down cues of the recording environment in the aircraft were made as ambiguous as possible, our rats still had several visual cues available that could have aided them in determining their orientation, such as the relative orientation of the two human experimenters, the camera tripod, and some details visible in the aircraft's cabin. Furthermore, as described in RESULTS, when on the ceiling in 0-G, they often appeared aware of where the chamber floor was and sometimes tried to move to this surface. Thus it is surprising that HD cells did not retain some form of direction-specific firing, at least on the walls.

Loss of directional firing and disorientation

Three forms of spatial disorientation have been identified in humans (Gillingham and Previc 1996). Type I spatial disorientation involves a misperception of one's orientation and is unrecognized by the observer. Assuming the activity of HD cells reflects the sense of direction of the animal, direction-specific firing of HD cells would presumably be maintained in this situation, albeit at an incorrect orientation with respect to the environment. Type II spatial disorientation entails a conscious recognition by the subject that he/she is disoriented, and attempts are made to become oriented by using any available information. Type III spatial disorientation occurs when the subject becomes so disoriented that they are incapacitated. This type of spatial disorientation can occur when the subject is experiencing rapid and continual rotations that lead to confusion or when the subject experiences severe motion sickness or oscillopsia that makes it difficult to override these compelling conditions. In contrast to type I spatial disorientation, types II and III spatial disorientation would be expected to lead to a disruption of normal HD cell discharge. Indeed, type I spatial disorientation would more appropriately be labeled a "misorientation" rather than "disorientation."

With this framework in mind, an important issue is whether the rats were disoriented when on the wall and ceiling in 0-G. Unfortunately, how HD cells respond when a rat is truly disoriented is not known. However, a few studies provide some insight into this issue. Stackman and Taube (1997) and Stackman et al. (2002) reported that direction-specific firing was abolished following either permanent (neurotoxic) or reversible (tetrodotoxin) lesions of the vestibular labyrinth. In both situations, HD cell discharge did not cease and there was a twofold increase in the tonic firing rates of the cells. Although

burst activity was observed in the anterior thalamus following vestibular lesions, the authors reported that this burst activity was only observed from a population of cells that were not thought to be HD cells. In a second study, rats were blindfolded and spun either back-and-forth quickly (Steven and Taube 2002) or continuously (LK Rosow and JS Taube, unpublished observations) on a turntable for 1–2 min while recording HD cells. These procedures should have temporarily disoriented them. After a few seconds of spinning, direction-specific firing ceased and the cell's baseline firing rate increased to 3–5 spikes/s compared with levels of <1 spike/s in freely moving sessions. Similarly, in the 1-G ceiling locomotion experiments by Calton et al. (2000) described above, the cells that did not show direction-specific discharge had a twofold increase in baseline firing rate when the rat locomoted upside-down across the ceiling. In the present experiments, there was an average 60% increase in the background firing rate between the floor and ceiling 0-G sessions (Table 1). Thus comparable changes in baseline firing rate of HD cells are observed in each of these situations. These findings indicate that when direction-specific firing breaks down in HD cells due to circumstances where the rat may be disoriented, there is usually a concomitant increase in baseline firing rather than a complete cessation of activity. This pattern of firing is similar to that observed for HD cells on the wall or ceiling in 0-G and suggests that the rats may have been disoriented (type II or III) when on these surfaces. However, a spatial test would be necessary to definitively prove the rats were disoriented in these circumstances. In contrast to these situations, other experiments have shown that HD cells remain directionally tuned in situations where the rat is misoriented and would be considered to have type I disorientation (Blair and Sharp 1996; Goodridge and Taube 1995; Taube and Burton 1995; Zugaro et al. 2000).

Conclusions and implications

The primary purpose of this study was to develop a neurophysiological understanding of the mechanisms supporting three-dimensional spatial orientation and navigation in pilots and astronauts and an understanding of the physiological correlates of spatial disorientation and VRIs. Our study confirmed that rodent HD cells continue to show strong directional properties in 0-G and ~1.8-G hypergravity while the animal is on the floor, but if the animal is passively moved to a surface oriented in a different spatial plane in the absence of gravity, the directional response of individual cells is greatly attenuated or absent, and the baseline firing rate increases. We suggest that the disappearance of a directional response and the increase in background firing rate of individual cells may be the physiological correlate of type II/III spatial disorientation in humans. Three of the seven cells monitored in 0-G while the animal was on the chamber ceiling occasionally showed increased bursting activity in a direction symmetrically opposite to that observed on the floor of the cage. Although the responses were relatively brief, they could be explained if the animal momentarily perceived the chamber ceiling as the "floor"—the equivalent of experiencing a VRI.

These experiments also provide information on understanding the role of the vestibular system in generating the HD cell signal in both 1-G and 0-G. Loss of direction-specific firing in animals on the wall and ceiling in 0-G, but not on the floor,

indicates that the effect is not simply due to the loss of the gravity vector. We propose that the HD signal loss occurs due to the combination of a sudden disparity between the remembered and seen locations of visual landmarks in azimuth and the absence of a gravity vector that could provide a reference to reorient the animal to the change in visual surroundings. The animal may feel somehow transposed to a new place and direction, but without a concomitant vestibular cue to indicate how it got there. In contrast, loss of direction-specific firing in animals with vestibular lesions is most likely attributed to the absence of a normal semicircular canal signal, rather than the loss of the otolith signal, because the loss of a normal otolith signal in parabolic flight did not abolish direction-specific firing when animals were on the floor.

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GRANTS

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