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Passive Transport Disrupts Directional Path Integration by Rat Head Direction Cells

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Stackman, Robert W., Edward J. Golob, Joshua P. Bassett, and Jeffrey S. Taube. Passive transport disrupts directional path integration by rat head direction cells. *J Neurophysiol* 90: 2862–2874, 2003. First published July 30, 2003; 10.1152/jn.00346.2003. A subset of neurons in the rat limbic system encodes head direction (HD) by selectively discharging when the rat points its head in a preferred direction in the horizontal plane. The preferred firing direction is sensitive to the location of landmark cues, as well as idiothetic or self-motion cues (i.e., vestibular, motor efference copy, proprioception, and optic flow). Previous studies have shown that the preferred firing direction remains relatively stable (average shift ± 18°) after the rat walks from a familiar environment into a novel one, suggesting that without familiar landmarks, the preferred firing direction can be maintained using idiothetic cues, a process called directional path integration. This study repeated this experiment and manipulated the idiothetic cues available to the rat as it moved between the familiar and novel environment. Motor efference copy/proprioceptive cues were disrupted by passively transporting the animal between the familiar and novel environment. Darkening the room as the animal moved to the novel environment eliminated optic flow cues. HD cell preferred firing directions shifted in the novel environment by an average of 30° after locomotion from the familiar environment with the room lights off; by an average of 70° after passive transport from the familiar environment with the room lights on; and by an average of 67° after passive transport with the room lights off. These findings are consistent with the view that motor efference copy/proprioception cues are important for maintaining the preferred firing direction of HD cells under conditions requiring path integration.

INTRODUCTION

Accurate navigation requires an organism to estimate its current location and directional heading in three-dimensional space. Navigation is thought to involve the continual monitoring of internal, or idiothetic, cues (e.g., consequences of self-motion: vestibular, motor efference copy, optic/auditory flow, or proprioceptive), together with occasional reference to familiar external cues, or landmarks, to correct for error accumulation over time (Barlow 1964; Berthoz et al. 1995; Gallistel 1990). Neural representations of spatial location and direction are likely processed by an integrated multimodal limbic circuit (McNaughton et al. 1995; Taube 1998). Two neurophysiological correlates of allocentric space are found in limbic brain regions of the freely moving rat. Principal neurons of hippocampus discharge in accordance with the spatial location of the rat independent of directional heading in open fields (O'Keefe 1976; Muller et al. 1987), while head direction (HD) cells discharge as a function of the rat's HD in the horizontal plane, independent of current location (Taube 1998; Taube et al. 1990a). The head orientation at which a HD cell exhibits maximal firing is referred to as the preferred firing direction. The activity of the entire HD cell population is believed to represent the perceived moment-to-moment directional heading of the animal.

Manipulation of environmental or idiothetic cues influences the preferred firing directions of HD cells, with familiar landmark cues usually exerting preferential control over HD cell activity (Blair 1996; Goodridge and Taube 1995; Knierim et al. 1995; Taube and Burton 1995; Taube et al. 1990b, 1996). Taube and Burton (1995) demonstrated that when a rat self-locomotes into a novel environment, preferred firing directions of HD cells remained within approximately ±30° of their respective original orientations. The stability of HD cell preferred firing direction in the absence of *familiar* external cues requires the involvement of self-motion or idiothetic cues. Self-motion information is conveyed to the hippocampal formation by several sensory and motor systems. First, as an organism moves through an environment containing various stationary landmarks, these objects appear to stream across the retina, giving rise to optic flow (Gibson 1954). The avian hippocampal formation receives optic flow input from the accessory optic system (Wylie et al. 1999). Second, motor efference copy signals are likely conveyed to hippocampal formation by way of projections arising from the ventral tegmental nucleus (Gasbarri et al. 1994; Vertes and Kocsis 1997) or from motor pathways projecting to the postsubiculum via retrosplenial cortex (Vogt and Miller 1983). The arrival of self-motion signals via these projections may be responsible for the modulatory influence of active movement on limbic HD cells (Taube 1995; Taube et al. 1990b; Zugaro et al. 2001) and hippocampal place cells (Foster et al. 1989). Third, HD cells are also sensitive to angular head velocity information, which may be conveyed by the vestibular system (Blair 1996; McNaughton et al. 1995; Taube 1995), because lesions of the vestibular apparatus disrupt direction-specific firing of HD cells firing in the anterior thalamus (Stackman and Taube 1997) and postsubiculum (Stackman et al. 2002), regardless of

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the presence of the familiar landmark cue. Fourth, proprioceptive information from muscle and bone joints, along with somatic receptors, may also be used for determining how the head and body move through space, although direct evidence for their use in determining and updating directional heading is limited. Thus four sources of idiothetic information can influence HD cell firing and are potential cues for maintaining spatial orientation under conditions that require path integration, such as when landmarks are not available.

Here we examined the contribution of several idiothetic cues to HD cell firing under conditions that require path integration. Specifically, we repeated the experiment of Taube and Burton (1995), but this time, we tested whether preferred firing directions were maintained when visual and/or motor efference copy/proprioceptive cues were manipulated during the animal's translation from a familiar environment to a novel one. Visual cues were manipulated by running the experiment under dark or lit conditions while motor and proprioceptive cues were manipulated by having the animal move between the familiar and novel environment, actively or passively on a cart. We report that the maintenance of preferred firing directions of HD cells was profoundly disrupted if the animal was passively transported into the novel environment whether in darkness or with the room lights on. HD cell preferred firing directions underwent small shifts when the animal walked into the novel environment in darkness. These findings suggest that self-motion cues, such as motor efference copy and proprioception, exert an important influence on directional path integration.

METHODS

Subjects

Subjects were 32 female Long-Evans rats, weighing 250–300 g at the beginning of the experiment. Rats were maintained on a food-restricted diet (15–20 g/d) and housed separately in suspended wire mesh cages. Tap water was available ad libitum. Prior to electrode implantation surgery, all rats were acclimated to a cylindrical apparatus (51 cm high, 76 cm diam) constructed of gray painted plywood. A sheet of white cardboard attached to the inside wall of the cylinder, extending from the floor to the top of the cylinder, served as the only polarizing, directional visual landmark. The cue card was positioned at 3 o'clock and occupied ~100° of arc. Black floor-to-ceiling curtains formed a featureless circular enclosure (2 m diam) surrounding the cylinder. Four uniformly arranged overhead DC lamps provided room illumination. A color video camera (Sony XC-711) was centered above the cylinder 3 m from the floor surface. The cylinder was placed on a sheet of gray photographic backdrop paper that was replaced after each recording session. A stool was placed inside the curtain enclosure, which the experimenter used while connecting and disconnecting the rat from the recording cable. The stool was out of the rat's view while in the cylinder. The position of the stool within the curtained enclosure varied, as an attempt was made to vary the position at which the rat was released into the cylinder during each recording session.

For 5 consecutive days, rats were handled for 10 min and introduced into the cylinder in pairs for a 10-min acclimation session each day. During all habituation and recording sessions, rats were brought from the colony room into the curtained enclosure in a covered corrugated cardboard box (~30 cm × ~30 cm box); no attempt was made to disorient the rats prior to their release into the cylinder. Rats were placed into the cylinder in pairs and received ≥5 habituation trials (1 trial/d) during which food pellets (20 mg, PJ Noyes, Lancaster, NH) were thrown randomly into the cylinder from outside the black curtain. By the completion of training, rats engaged in nearly continuous food pellet search behavior over the entire floor of the cylinder. On completion of training, each rat was implanted with a driveable 10-wire microelectrode array directed at the anterior dorsal thalamus or postsubiculum.

Electrode implantation

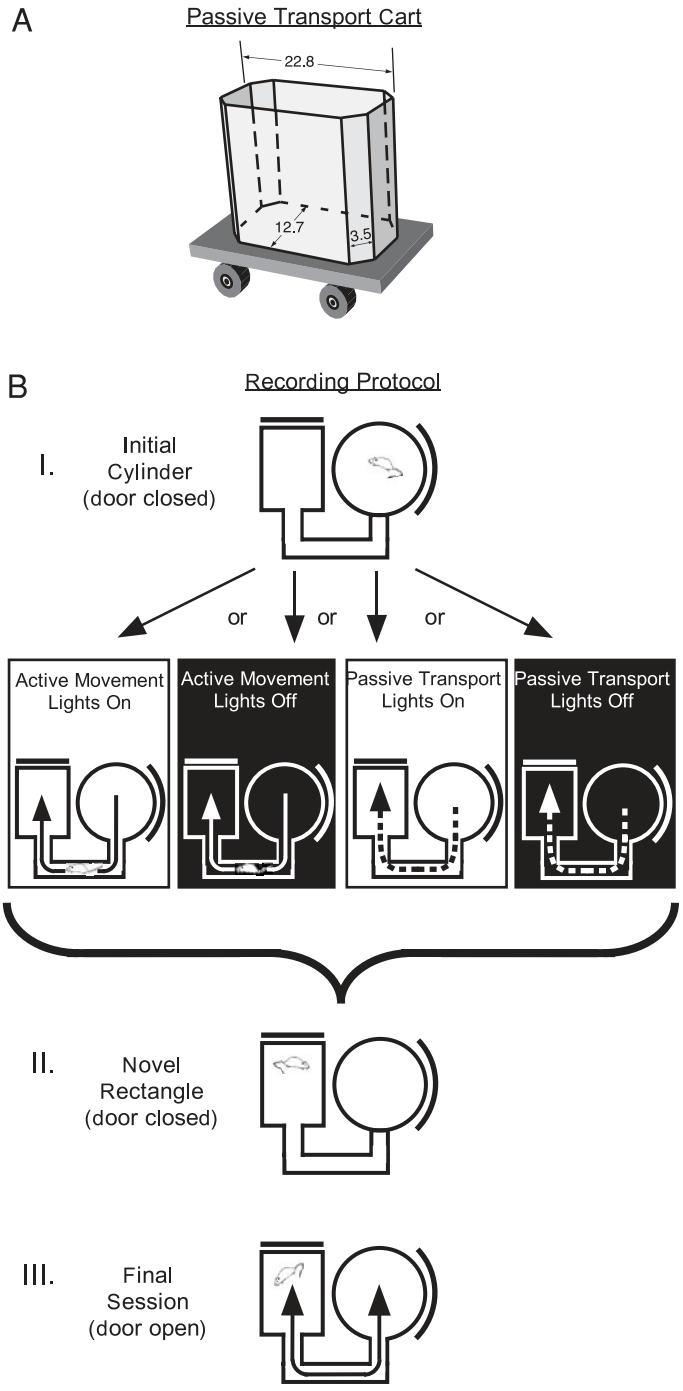
Electrode construction and implantation techniques used were similar to that described previously (Taube 1995). Briefly, each electrode array consisted of a bundle of 10 25-μm diam nichrome wires (California Fine Wire, Grover City, CA) insulated except at the tips. The wire bundle was passed through a 26-gauge stainless steel cannula, and each wire attached to a modified 11-pin Augat connector. The electrode array could be advanced in the dorsoventral plane with three screws attached to the electrode's acrylic base (Kubie 1984). On habituation to the cylindrical apparatus and adequate foraging behavior, each rat was anesthetized with a ketamine-xylazine mixture (2 ml/kg im) and stereotaxically implanted with an electrode array directed at either the PoS or ADN. Electrode coordinates, with respect to bregma, were as follows: PoS, anterior/posterior −6.6 mm; medial/lateral +2.8 mm; ventral 1.6 mm from the cortical surface; ADN, anterior/posterior −1.4 mm; medial/lateral +1.3 mm; ventral 3.7 mm from the cortical surface (Paxinos and Watson 1998). The electrode assembly was attached to the skull using dental cement, with the attachment aided by the placement of four stainless steel, self-tapping screws, placed in the skull plates over the cerebellar cortex, parietal cortex, and frontal cortex. All procedures were conducted according to an institutionally approved animal care protocol. All surgical procedures were conducted under sterile conditions that were approved by the Dartmouth College IACUC and were in accordance with the American Physiological Society's Guiding Principles in the Care and Use of Animals. The rats were allowed a 1-wk postoperative recovery period before single-unit screening commenced.

Isolation and recording of HD cell activity

Following recovery from surgery, the activity from each microelectrode wire was assessed during daily unit screening sessions while the rat foraged for food pellets in the cylinder. The electrode wires were advanced over several weeks while screening for HD cell waveforms that were of acceptable amplitude for isolation from background electrical noise. Each rat was transported into the screening area from the animal colony room in the corrugated cardboard enclosure; again, no attempt was made to disorient the rats prior to their release into the cylinder. The cardboard box was placed on the floor inside the curtained enclosure next to the cylinder. A recording cable was attached to the implanted electrode while the rat was held gently in a towel. The cable included a red light-emitting diode (LED) and a green LED oriented over the rat's snout and back, respectively. The LEDs were spaced 10 cm apart along the rostral/caudal axis of the rat, as described previously (Taube et al. 1990a). Once the cable was attached, the rat was released into the cylinder apparatus from a start position that varied daily in a pseudorandom manner. Unit activity was analyzed using procedures similar to those previously described (Taube 1995; Taube et al. 1990a). Briefly, electrical signals were passed through a field-effect transistor (FET; 1 FET/electrode) in a source-follower configuration, through an overhead commutator (Biela Idea Development, Anaheim, CA), amplified (Grass Instruments P5 Series, West Warwick, RI), band-passed filtered (300–10,000 Hz, ≥3 dB/octave; Peavey Electronics PME8, Meridian, MS), and passed through a series of window discriminators (Model DDIS-1, BAK Electronics, Germantown, MD). The resultant signal was then displayed on an oscilloscope (Model 2214, Tektronix, Beaverton, OR). Electrode activity was monitored while observing the rat's behavior on a video monitor with a camera mounted 3 m above the cylinder floor. If HD cell activity was not found, the electrodes

were advanced 25–50 μm further into the ADN or PoS, and the activity was monitored again the next day. Screening for HD cells occurred over the course of several weeks.

When the waveform of an HD cell could be sufficiently resolved from the background noise by the window discriminators, spike and head direction data were acquired in the following manner. The window discriminators generated a square wave "acceptance pulse" for each waveform that exceeded the discriminator threshold. Each acceptance pulse corresponded to the occurrence of an action potential and was sent to a processor that time-stamped the spikes. During recording sessions, neuronal discharges and the LED coordinates were sampled at 60 Hz by a video-tracking system (Ebtronics, Brooklyn, NY) and acquired by a data acquisition interface board (National Instruments DIO-96, Austin, TX) in a personal computer (Quadra 840AV, Apple Macintosh, Cupertino, CA). Data were stored for subsequent off-line analyses using programs written with LabView software (National Instruments). During recording sessions, white noise was broadcast through a ceiling speaker centered above the cylinder to mask uncontrolled auditory cues.



Behavioral testing/recording procedures

DUAL CHAMBER APPARATUS. The present experiment was essentially an extension of an earlier study by Taube and Burton (1995). In their study, they used a dual chamber apparatus to examine the responses of HD cells under conditions that encouraged path integration to maintain spatial orientation. In the present study, we used the same dual chamber apparatus as diagrammed in Fig. 1*B* and described previously (Taube and Burton 1995). Explicit effort was expended to ensure that the present data were collected using the exact same apparatus, same recording rooms, and same basic training procedures as used previously. The dual chamber apparatus, painted entirely of flat gray, consisted of a cylindrical arena (76 cm diam and 50 cm high) and a rectangular arena (51 cm wide, 68.5 cm long, and 50 cm high), interconnected by a narrow U-shaped passageway (15 cm wide, 40.5 cm long, and 50 cm high) that contained three sloped sides (approximately 10°) so that the overhead video camera could view the entire floor unobstructed by the sides of the passageway. A door in the wall of the cylinder could be removed to grant access to the passageway. A white cardboard cue card was affixed to the wall of the cylinder (at 3 o'clock as viewed by the overhead camera) and to the wall of the rectangle (at 12 o'clock as viewed by the overhead camera). Gray photographic backdrop paper served as the floor of both the cylinder and rectangle; the passageway contained a gray-painted wood floor. A wheeled-cart equipped with a tall, narrow, clear plexiglas rectangular-shaped container was used to passively transport some of the rats from the cylinder to the rectangle (see Fig. 1*A* for a drawing of the cart with dimensions as indicated). The container on the cart was open at the

FIG. 1. *A*: drawing of the wheeled-cart and clear Plexiglas container used to passively transport rats from the cylinder through the passageway and into the novel rectangle. All dimensions are in centimeters. The 3.5 refers to the length of the 45° corners of the container. *B*: recording protocol schematic: each figure represents an overhead view of the configuration of the apparatus during each of the three recording sessions. Cue cards present in the cylinder and in the rectangle are shown as an arc or straight line along the walls of the respective environments. *BI*: Initial Cylinder refers to the initial 8-min session in which the rat was confined to the familiar cylinder environment and head direction (HD) cell activity was monitored as the rat foraged for food pellets on the cylinder floor. With the door closed, this session allowed for a determination of each cell's baseline preferred firing direction and firing properties. Experimental Manipulation depicts each of the four possible conditions imposed immediately following the Initial Cylinder session. Rats were assigned to one of the four conditions: Active Movement/Lights On; Active Movement/Lights Off; Passive Transport/Lights On; or Passive Transport/Lights Off. The respective experimental manipulation was imposed during the rats' initial journey through the passageway into the rectangle. The cylinder door was removed and rats of the Active Movement/Lights On group walked through the passageway into the rectangle with the room lights illuminated; these data comprised that of 4 rats plus the data from Taube and Burton (1995). The rats of the Active Movement/Lights Off group walked into the rectangle in darkness. The room lights were illuminated once the rat reached the center of the rectangular environment. Rats of the Passive Transport groups were placed into the wheeled-cart in the cylinder and then passively transported aboard the cart into the novel rectangle either in darkness or with the room lights illuminated. Dashed line illustrates the path taken by the cart. *BII*: Novel Rectangle recording session began immediately after the completion of the respective experimental manipulation. For rats in the Passive Transport groups, this recording session began after the rat was removed from the cart. During the Novel Rectangle session, HD cell activity was monitored while the rat was confined to the rectangle and passageway for 16 min. *BIII*: Final Session illustrates the last recording session in which the cylinder door was removed, and the rat was allowed to freely shuttle back and forth between the two environments of the dual chamber apparatus.

top, allowing rats to be easily placed into and removed from the cart. Although the rats were confined to a small space when inside the container, the rats were not restrained from moving around. All rats in the two experimental conditions involving passive transport (Passive Transport/Lights Off and Passive Transport/Lights On) were acclimated to the wheeled-cart during several 2- to 3-min sessions to reduce any stress associated with being inside the wheeled-cart. These sessions were conducted following screening for units in the days leading up to isolating HD cells. Cart acclimatization took place in the experimental room inside the cylindrical arena. During each cart acclimation session, the wheeled-cart was placed into the cylinder, the rat was placed inside the plexiglas container, and the cart wheeled slowly around the inside of the cylinder. During the first exposure to the cart, most rats would readily attempt to climb out of the plexiglas container as well as to repeatedly turn around inside the container. After four or five exposures to the wheeled-cart across several days, all rats would sit or rear in the cart while it was being moved about, without attempting to climb out.

PROTOCOL AND EXPERIMENTAL CONDITIONS. On isolation of an HD cell from a given animal, the activity of the cell was recorded in the standard cylinder apparatus during an 8-min session. Following this recording session, the rat was returned to the cardboard box, and the standard cylinder was replaced with the dual chamber apparatus. The dual chamber apparatus recording protocol consisted of three sessions: an Initial Cylinder session during which the rat was restricted to the cylinder (door closed), a Novel Rectangle session (door closed) in which the rat was restricted to the novel rectangle and passageway areas, and a final session (Return Cylinder) in which the rat was allowed free access to all areas of the dual chamber apparatus (door opened) as described previously (Golob and Taube 1999; Taube and Burton 1995). During all sessions, food pellets were tossed into the apparatus at an approximate rate of 3–4/min. Just prior to the Novel Rectangle session, food pellets were scattered in the passageway and rectangular arena. The Initial Cylinder session entailed removing the rat from the cardboard box, attaching the recording cable and placing the rat into the cylinder of the dual chamber apparatus. Previous studies have demonstrated that the preferred firing directions of HD cells are consistent between the standard cylinder used for unit screening and the cylinder of the dual chamber apparatus (Golob and Taube 1999; Taube and Burton 1995). Thus we concluded that the rats likely considered the cylindrical arena a familiar environment.

Consistent with theories of path integration, the preferred firing directions of HD cells recorded from intact rats were maintained when the rats actively walked from the cylinder through the passageway into the novel rectangle arena (Taube and Burton 1995). This finding suggests that the availability of idiothetic cues during the initial movement through the novel environment were sufficient to support HD cell firing and the animal's spatial orientation. We tested this prediction in the present experiment by exposing each rat to one of three experimental manipulations designed to disrupt the rats' use of one or more sources of idiothetic cue information during the initial translation from the cylinder to the rectangle (see Fig. 1*B* for a schematic diagram of the recording protocol). The three experimental conditions were as follows: *1*) Active Movement/Lights Off; *2*) Passive Transport/Lights On; or *3*) Passive Transport/Lights Off; and the control condition was referred to as Active Movement/Lights On. Each rat was assigned to one of these conditions after completion of the Initial Cylinder session in the dual chamber apparatus. Each rat was only tested once in the dual chamber apparatus since after the initial visit to the rectangle this environment was no longer considered novel to that rat. Therefore these data were collected over a protracted period of time by all four authors. The exact procedures for each condition are outlined below.

ACTIVE MOVEMENT/LIGHTS OFF. For this condition, on starting a 16-min recording session, the door was opened, approximately 3 s later the room lights were turned off, and the rat was allowed to actively walk from the cylinder through the novel passageway and into the rectangle in the dark. Once the rat passed the threshold from cylinder into passageway, the door was replaced to prevent the animal from returning to the cylinder. During the initial walk into the novel rectangle, the green LED was turned off and the red LED was dimmed to reduce the remaining light available to the rat. This condition was considered dark because rats are blind to light in the red spectrum (Birch and Jacobs 1975). One experimenter monitored the progress of the rat on a video monitor. When the rat crossed the threshold between the passageway and the rectangle, the room lights were turned back on, and the LEDs adjusted to their normal brightness. Although most rats displayed some hesitation in leaving the cylinder in the dark, all rats completed the initial walk into the novel environment in 5 min or less. On completion of the 16-min recording session, the cylinder door was removed, and a second 16-min recording session was begun during which the rat could freely move between all areas of the dual chamber apparatus (Return Cylinder session). This experimental condition was designed to interfere with the rats' use of optic flow during their initial walk through the passageway into the rectangle. Arguably, this manipulation should not have altered the rats' access to or use of vestibular and motor efference copy/proprioceptive cues.

PASSIVE TRANSPORT/LIGHTS ON. For this condition, the wheeled-cart was placed inside the cylinder, and the rat was gently placed inside the Plexiglas box. A 16-min recording session was begun, the cylinder door was removed, and the cart and rat were gently wheeled from the cylinder, through the passageway, and into the center of the rectangle. The cylinder door was replaced, and the rat was lifted from the cart and released into the rectangle. The passive transport of the rats aboard the cart was accomplished at speeds that were well above the vestibular threshold for detecting movement of the cart. As indicated by the dashed lines in the two rightmost diagrams of Fig. 1*B*I, the path of the cart during the passive transport was generally a *U*-shaped path consisting of a straight line with two 90° turns; one after leaving the cylinder and a second to enter the rectangle. Food pellets were not provided to the rat while aboard the cart. On completion of the 16-min recording session, the cylinder door was removed, and a second 16-min recording session was begun during which the rat could freely move between all areas of the dual chamber apparatus (Return Cylinder session). This experimental condition was designed to interfere with the rats' use of motor efference copy/proprioceptive cues during their initial translation through the passageway into the rectangle. As designed, this condition (and the following condition) was expected to disrupt the normal reliability of motor efference copy/proprioceptive cues. That is, passive transport may have created a conflict between the rat's motor efference copy/proprioceptive cues and the physical angular and linear movement of the rat. This manipulation should not have altered the rats' access to or use of vestibular cues and optic flow.

PASSIVE TRANSPORT/LIGHTS OFF. For this condition, the wheeled-cart was placed inside the cylinder, and the rat was gently placed inside the plexiglas box. The room lights were turned off, a 16-min recording session was begun, the cylinder door was removed, and the cart and rat were gently wheeled from the cylinder, through the passageway and into the center of the rectangle. The cylinder door was replaced and the rat was lifted from the cart and released into the rectangle. Food pellets were not provided to the rat while aboard the cart. On release of the rat into the rectangle, the room lights were turned on. All attempts were made to execute the passive transport of the rats aboard the cart in the dark in a manner identical to that used for the Passive Transport/Lights On condition. On completion of the 16-min recording session, the cylinder door was removed, and a second 16-min recording session was begun during which the rat could freely move between all areas of the dual chamber apparatus (Return Cylinder session). This experimental condition was designed to disrupt the reliability of both motor efference copy/proprioceptive

cues and optic flow during the initial translation through the passageway into the rectangle. As before, this manipulation should not have altered the rats' access to or use of vestibular cues.

ACTIVE MOVEMENT/LIGHTS ON. This control condition comprised the original data of Taube and Burton (1995) plus data from four additional rats collected over the same time period that data from the above experimental conditions were collected. The data from these four additional HD cells were not different from those of Taube and Burton (1995). For this condition, the cylinder door was removed, and a recording session was begun. The cylinder door was replaced after the rat passed the threshold of the cylinder and entered the passageway. On completion of ≥8 min of recording the cell in the rectangle/passageway, a second 16-min recording session was conducted during which the rat could freely move between all areas of the apparatus (Return Cylinder session).

In summary, the experiment consisted of a 2 × 2 design in which motor efference copy/proprioceptive cues (Active Movement vs. Passive Transport) and access to optic flow (Lights On vs. Lights Off) were the manipulated variables. The purpose of each of the experimental conditions was to examine how interfering with specific idiothetic cues affected the stability of HD cell preferred firing directions as the animal traveled from the familiar environment into a novel one.

Data analysis

The rat's head direction was determined from the relative positions of the two LEDs using procedures defined previously (Taube 1995; Taube et al. 1990a). The rat's location was defined as the point one-quarter the distance from the red LED to the green LED. Head direction and unit data for each trial were sorted according to the animal's location (cylinder vs. rectangle/passageway). Data were further sorted to extract data acquired while the rat was passively transported aboard the cart. Plots of firing rate as a function of HD were constructed by expressing HD in sixty 6° bins and plotting the mean firing rate for each HD bin. Consistent with previous studies (Taube 1995; Taube et al. 1990a), each HD cell was analyzed to determine the basic firing properties: *1*) preferred firing direction; *2*) peak firing rate; and *3*) background firing rate.

To quantify the degree of shift in the preferred firing direction between the two discrete compartments of the dual chamber apparatus, we used a cross-correlation method described in detail previously (Taube et al. 1990a). Briefly, the firing rate versus HD tuning function of data from one session was shifted in 6° increments and cross-correlated with the tuning function of the same cell recorded during another session (i.e., Initial Cylinder session vs. Novel Rectangle session). The degree of shift necessary to maximize the correlation between the tuning functions of the two sessions was considered the amount of shift in the preferred firing direction. A positive shift reflects a counterclockwise shift, while a negative shift reflects a clockwise shift. To further examine the dynamics of the shift in the preferred firing direction during the initial visit to the novel environment, we divided the Novel Rectangle/passageway session data into eight 1-min blocks. Shifts of HD cell preferred firing direction were defined again using the cross-correlation method. Shifts are reported as mean ± SD and were computed using circular statistics (Batschelet 1981).

Histology

At the conclusion of unit screening, rats were overdosed with sodium pentobarbital (100 mg/kg; ip). Weak anodal current (15 μA for 10 s) was passed through one of the electrode wires to mark the wire location by the deposition of iron (Prussian blue reaction). The rats were perfused transcardially with 0.9% saline followed by 10% formalin, and the brains were removed and placed into 10% formalin for ≥48 h. The brains were then transferred to a 10% formalin solution containing 2% potassium ferrocyanide for 24 h and returned to a 10% formalin solution for 24 h, after which the brains were placed into a 20% sucrose solution for ≥48 h. The brains were then blocked and frozen on dry ice, and coronal sections were cut at 25 μm on a cryostat and mounted on to microscope slides. The sections were stained with cresyl violet and examined under light microscopy to determine the location of recording sites.

RESULTS

Histological analyses verified that electrodes passed through the respective recording site (ADN or PoS). There were no differences in the responses to the manipulations recorded from HD cells in the ADN ($n = 25$) and PoS ($n = 9$), and data from all cells were grouped together according to experimental condition. Thirty-four HD cells were recorded from 34 rats with the number of HD cells per experimental group as follows: Active Movement/Lights Off ($n = 13$); Passive Transport/Lights On ($n = 9$); and Passive Transport/Lights Off ($n = 12$). Data from these experimental groups were compared against the control condition Active Movement/Lights On ($n = 21$). There were two occasions where two HD cells were recorded simultaneously. The data from only one HD cell in each case was included in the analyses below.

HD cell activity in the novel environment

After the initial recording session in the cylinder portion of the dual chamber apparatus, the door was opened, and the rats either walked, or were transported passively on the cart, into the novel rectangular environment with the room lights either on or off. After this manipulation, HD cell activity was recorded while the rats were confined to the novel environment. Consistent with previous studies (Golob and Taube 1999; Taube and Burton 1995), all HD cells continued to discharge after entry into the novel rectangle environment. Figure 2, *A–D* illustrates a representative firing rate by HD plot of one HD cell from the control condition and from each of the three experimentally manipulated groups. The HD tuning curves demonstrate that the primary influence of the novel environment on HD cells was to shift the preferred firing direction between the familiar and novel environment. Other firing properties of HD cells (background firing rate, peak firing rate, and range of directional firing) were consistent between the two environments.

HD cells of those rats that were passively transported into the novel rectangle exhibited substantial shifts of their preferred firing direction when compared with their preferred firing direction in the familiar cylinder. The mean ± SD absolute shifts of the preferred firing direction in the novel rectangle for the two passively transported groups were Passive Transport/Lights On, 69.5 ± 26.3°; Passive Transport/Lights Off, 66.7 ± 24.9°. In contrast, the mean ± SD absolute shift in the preferred firing direction for the Active Movement/Lights Off rats was considerably lower than that for the passively transported rats: 28.8 ± 16.9°. The mean ± SD absolute shift value for the Active Movement/Lights Off rats was larger than the value for the Active Movement/Lights On rats: 17.2 ± 7.4°. For the purposes of satisfying assumptions of the ANOVA model, heterogeneity of the variance between groups

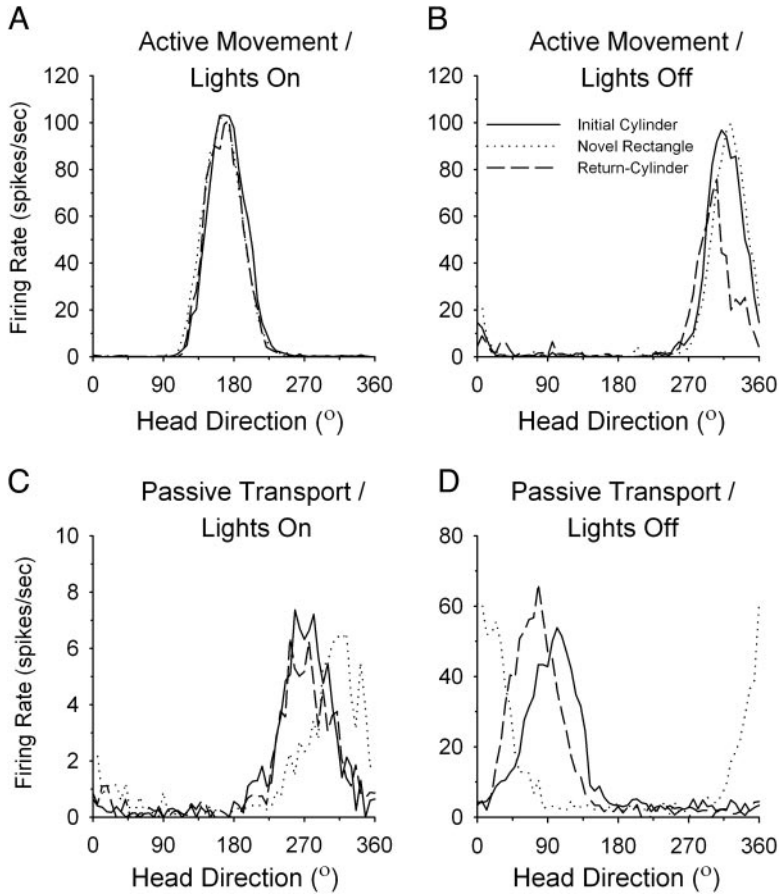


FIG. 2. Representative HD cell responses during the three phases of the recording protocol. Each graph depicts the cell's firing rate as a function of head direction during the Initial Cylinder session (solid line), during the Novel Rectangle session (dotted line), and during the segment of the final session in which the rat first returned to the cylinder (dashed line). *A* and *B*: HD cell tuning functions for rats that actively walked into the novel environment with the room lights on (*A*) or in the dark (*B*). *C* and *D*: tuning functions for rats that were passively transported into the novel rectangle either with the room lights on (*C*) or in darkness (*D*). Respective shifts in HD cell preferred firing direction between the Initial Cylinder and Novel Rectangle session were 0° (*A*, Active Movement/Lights On), 6° (*B*, Active Movement/Lights Off), 42° (*C*, Passive Transport/Lights On), and −84° (*D*, Passive Transport/Lights Off).

was alleviated by square root transformation of the directional shift values. A two-factor (cart, room lights) repeated measures ANOVA comparing the transformed directional shift values for the four groups yielded a significant effect of cart [$F(1,51) = 36.05$, $P < 0.001$], but a nonsignificant effect of room lights [$F(1,51) = 0.10$, n.s.] and a nonsignificant interaction of cart × room lights [$F(1,51) = 1.18$, n.s.]. The distributions of preferred firing direction shifts between the familiar cylinder and the novel rectangle for each of the four groups are depicted in polar coordinate plots in Fig. 3. HD cells of the Active Movement/Lights On rats exhibited a bias toward a clockwise shift in preferred firing direction between the Initial Cylinder session and the Novel Rectangle session (15 of 21 cells, see the *leftmost plot* of Fig. 3). This bias may be attributed to the rat making overall two clockwise turns in walking from the familiar cylinder to the novel rectangle. As the remaining plots of Fig. 3 illustrate, a similar trend toward clockwise shifts in preferred firing direction in the novel rectangle was observed for both groups of passively transported rats (Passive Transport/Lights On: 7 of 9 cells; Passive Transport/Lights On: 9 of 12 cells). However, the Active Movement/Lights Off rats had preferred firing direction shifts that were evenly distributed, with five cells shifting their preferred firing direction clockwise, seven cells shifting their preferred firing direction counterclockwise, and one cell that did not shift its preferred firing direction between the two environments. Ray-

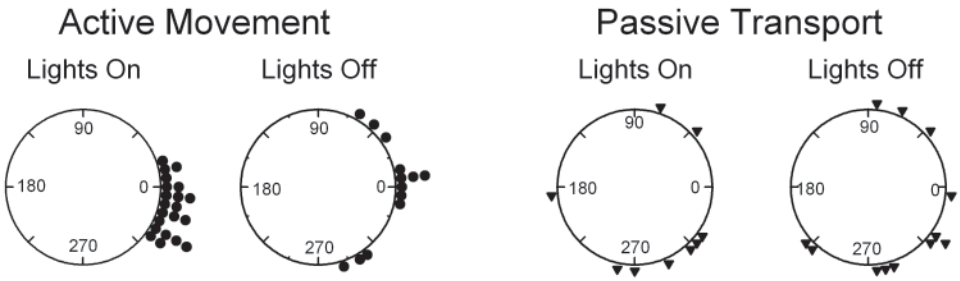


FIG. 3. Distribution of HD cell preferred firing direction angular shifts between the Initial Cylinder and Novel Rectangle session plotted in polar coordinates for the control group and each of the three experimental groups. In general, HD cells in rats that actively walked into the novel environment (●) with the room lights on maintained their preferred firing direction, with all cells exhibiting a mean shift in preferred firing direction between the two environments of 17.1°; while rats that walked into the novel environment with the room lights off exhibited a mean shift of 28.8°. HD cells of rats that were passively transported into the novel environment (▲) exhibited a shift of preferred firing direction that averaged 69.5° for the Lights On condition and 66.7° for the Lights Off condition.

leigh tests were conducted for each condition to determine whether these shifts were randomly distributed. Analyses revealed that the Passive Movement/Lights On distribution was randomly distributed ($R = 0.46$; $P = 0.14$), while the remaining three distributions were not randomly distributed: Active Movement/Lights On ($R = 0.98$; $P < 0.001$); Active Movement/Lights Off ($R = 0.79$; $P < 0.001$); and Passive Movement/Lights Off ($R = 0.59$; $P = 0.012$). The fact that the shift distribution for the Passive Movement/Lights Off condition was not random suggests that other cues available during the passive transport, such as vestibular, may have influenced the preferred firing direction in the novel environment for these rats.

Other firing properties of HD cells were not significantly different between the familiar and novel environments. Measures of HD cell peak firing rate, directional firing range, and background firing rate during the Novel Rectangle session were expressed as a percentage of the value for the Initial Cylinder session. Two-factor ANOVAs indicated no significant effects of cart, room lights, or cart × room lights interaction for peak firing rate, directional firing range, or background firing rates [$F(1,51) < 3.10$, n.s.]. Thus as a consequence of either active or passive movement into a novel environment in the dark, HD cells respond by shifting their preferred firing direction, while all other firing parameters remain unchanged.

In summary, in traveling from the familiar environment to the novel environment, the passive transport condition led to the largest shifts in the cells' preferred firing directions, and although there was a noticeably larger shift value for the Active Movement/Lights Off group compared with the Active Movement/Lights On group, this difference did not reach significance. These data suggest that self-motion cues available to the rats of the Active Movement conditions allowed the rats to maintain their spatial orientation during movement through the novel passageway.

Drift in preferred firing direction during first exposure to novel environment

The large shifts in the preferred firing direction after passive transport were determined from examining HD cell activity averaged over recording sessions that were approximately 10–14 min in length. It was of interest to determine when the shift in preferred firing direction occurred, i.e., at the beginning or at the end of the novel environment session, or gradually over the course of the session. To more closely examine the temporal dynamic of the development of this shift, the first 4 min of data from the first visit to the novel rectangle were subdivided into four successive 1-min epochs. This period represents the first 4 min of time in the novel environment, which began on the rats' entry into the rectangle or removal from the cart. Preferred firing direction values were then defined for each 1-min epoch for each HD cell and compared with the preferred firing direction of the Initial Cylinder session to determine the amount of shift. Each shift value was plotted over the 1-min epoch to graphically represent the amount of shift induced by the respective experimental condition. Figure 4 illustrates these plots for each of the four groups of rats. The plots indicate that the shift in preferred firing direction developed primarily over the first 2 min after entry into the novel rectangle, and the preferred firing direction was then generally stable for the remaining time. Indeed, for cells that were recorded for longer than 4 min in the rectangle, the preferred firing direction of all cells remained stable from 4 min onward (data not shown). A similar analysis was performed on the initial 1-min epoch by subdividing it into four 15-s bins. This analysis revealed a similar pattern as that above; there was considerably greater variability of shifts in the preferred firing direction shift in the Passive Transport rats than in the Active Movement rats during the first 15-s bin in the novel environment.

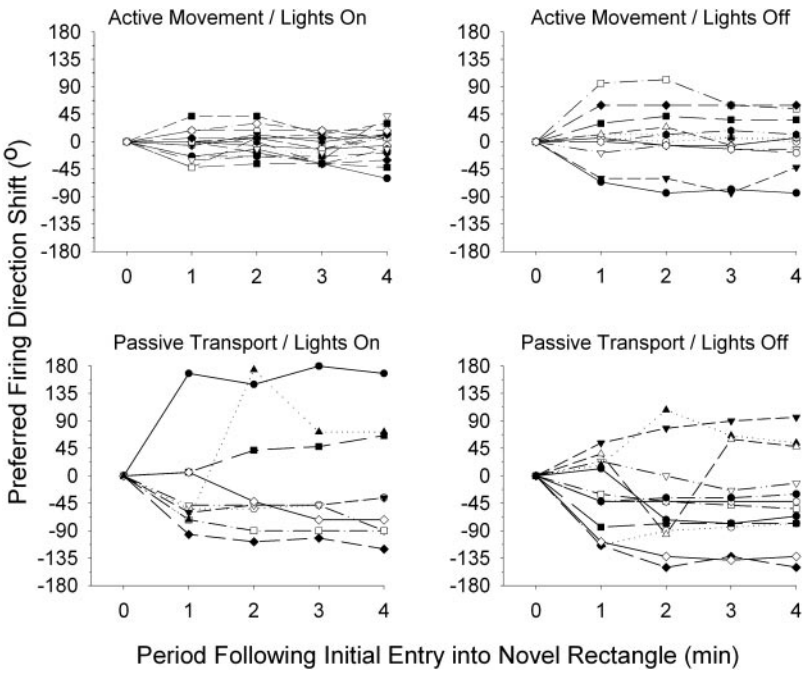


FIG. 4. Temporal dynamics of HD cell preferred firing direction shift during the first 8 min of the Novel Rectangle recording session for each respective group. For each cell, the shift in preferred firing direction for each 1-min epoch was compared with that of the Initial Cylinder session. Each shift was plotted according to the respective 1-min epoch for each of the rats of the 4 groups. Plots depict shift data for each cell of each respective group and reveal a tendency for the preferred firing direction shift to develop over the course of the first 3–4 min of the Novel Rectangle session and remain stable for the remaining duration of the recording session.

Return visit to the cylinder and to the rectangle

Following the initial recording session in the rectangle, the cylinder door was removed, and HD cells were recorded as the animal freely moved between the two compartments. Therefore we were able to examine whether experience in the novel rectangle influenced HD cell preferred firing directions when the rat actively returned to the cylinder. Second, we were able to examine the amount of shift in the preferred firing direction as the animal left the cylinder, walked through the passageway, and returned to the now familiar rectangle. We refer to this recording segment as the Return Rectangle session.

Return Cylinder session

For the Return Cylinder sessions, preferred firing directions were maintained in rats of the Active Movement groups. The mean absolute shifts between the Return Cylinder and the Initial Cylinder sessions were 15.0 ± 10.0° and 5.4 ± 3.0° for the Active Movement/Lights Off and Active Movement/Lights On conditions, respectively. In contrast, the mean absolute shifts for the Passive Transport/Lights On and Passive Transport/Lights Off groups were 29.9 ± 26.4° and 27.0 ± 22.8°, respectively, and were significantly higher than both Active groups, indicating that the experience of being passively transported led to the adoption of a new preferred firing direction in the cylinder that was significantly different from that of the Initial Cylinder session. The distributions of preferred firing direction shifts between the Initial Cylinder and the Return Cylinder sessions for each of the four groups are depicted in polar coordinate plots in Fig. 5*A*. A two-factor (room lights, cart) ANOVA on the absolute shift in preferred firing direction revealed a significant effect of cart [$F(1,51) = 11.64$, $P < 0.001$], but nonsignificant effects of room lights [$F(1,51) = 0.002$, n.s.] and of lights × cart interaction [$F(1,51) = 1.19$, n.s.].

To examine whether other firing properties of HD cells (peak firing rate, directional firing range, and background firing rate) were different between the Initial Cylinder and the Return Cylinder sessions, we expressed the values for each of these measures as a percent of the respective Initial Cylinder session measure. Two-factor ANOVAs conducted on the peak firing rate, directional firing range, and background firing rate measures revealed no significant effects of either cart or lighting condition [$F(1,51) < 3.21$, n.s.].

In summary, the degree to which HD cells fired consistently in the cylinder, before and after exposure to the novel environment, was dependent on whether the rats were passively transported or actively walked into the novel environment—importantly, the mode of transport only affected the cell's preferred firing direction and did not influence other firing properties. Figure 5*B* illustrates the mean preferred firing direction shift angles for each of the four experimental groups (Initial Cylinder, closed circle; Rectangle/Passageway, thin vector; Return-Cylinder, thick vector). The length of these vector lines corresponds to the variability in the mean shift angles for each respective group. As these plots indicate, HD cells of rats passively transported into the novel environment adopt a new preferred firing direction on returning to the

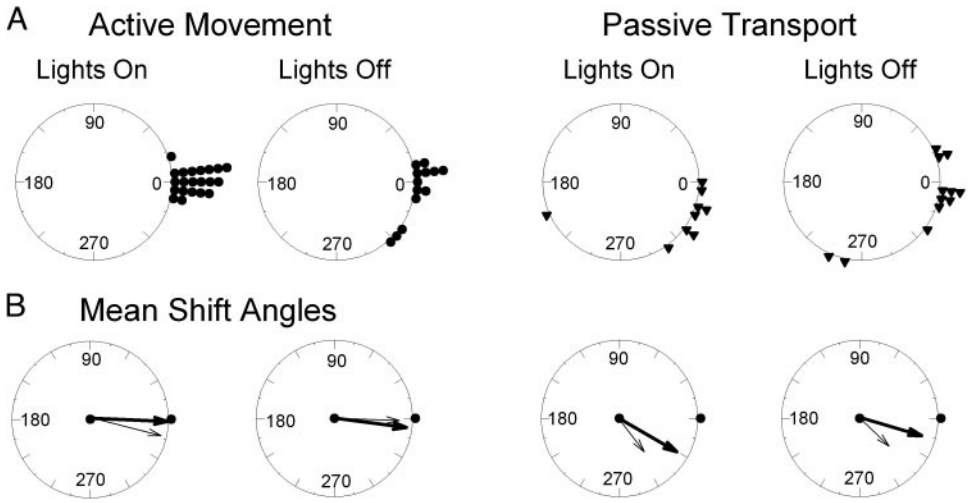


FIG. 5. *A*: distribution of HD cell preferred firing direction angular shifts between the Initial Cylinder and Return Cylinder session plotted in polar coordinates for each of the 4 groups. In general, HD cell preferred firing direction shifts of rats that actively walked into the novel environment (●) were quite similar between the Initial Cylinder and Return Cylinder sessions. In contrast, HD cells of rats that were passively transported into the novel environment (▼) often exhibited a shift in preferred firing direction between the Initial Cylinder and Return Cylinder sessions. The lack of consistency in HD cell firing between the Initial Cylinder session and the Return Cylinder session suggests that, in some cases, the passive transport manipulation caused a lasting reorientation of the preferred firing direction of HD cells in the familiar cylinder environment. *B*: summary plots depicting the mean preferred firing direction shift angles for each experimental group. To simplify these plots, the mean preferred firing direction during Initial Cylinder sessions are all aligned at 0° (●); the mean preferred firing direction during Novel Rectangle sessions are presented by the thin vector; and the mean preferred firing direction during Return-Cylinder sessions are presented by the thick vector. Length of each vector line depicts the variability in the mean shift angle for each group. As these plots illustrate, HD cells of rats that actively walked from the cylinder into the novel environment exhibited minimal shifts in their preferred firing direction between the Initial Cylinder and Return-Cylinder sessions (HD cells shifted back to their original preferred direction; the mean preferred firing direction of the Return-Cylinder session is approximately equivalent to that of the Initial Cylinder session). In contrast, HD cells of rats passively transported into the novel environment exhibited a preferred firing direction in the Return-Cylinder session that was approximately an average of that of the Initial Cylinder and Rectangle/Passageway preferred directions.

familiar cylinder that is approximately midway between the original preferred direction and the direction in the novel environment. This new preferred firing direction during the Return Cylinder session suggests that HD cells undergo a type of remapping because of the passive transport manipulation. It is possible that HD cells can account for small shifts in preferred firing direction between two environments, but large shifts (i.e., ≥45°) cause HD cells to adopt a new preferred direction in the familiar environment that is an average of the original preferred direction and the direction in the novel environment.

Return Rectangle session

When comparing the Return Rectangle and Novel Rectangle sessions, HD cells generally maintained their initial rectangle preferred firing directions during the return visit to the rectangle. The mean shifts in preferred direction for each of the conditions were Active Movement/Lights Off, 2.8 ± 2.5°; Active Movement/Lights On, 2.8 ± 2.6°; Passive Transport/Lights Off, 5.0 ± 3.9°; and Passive Transport/Lights On, 3.3 ± 2.7°. A two-factor (cart, lights) ANOVA on preferred firing direction absolute shift between Novel Rectangle and Return Rectangle revealed no significant effects of cart, lights, or cart × lights interaction [$F(1,51) < 1.18$, n.s.]. Similarly, there were no significant differences for the other firing properties of HD cells (peak firing rate, directional firing range, and background firing rate) between the Novel Rectangle and Return Rectangle sessions [2-factor ANOVA (cart, lights), $F(1,51) < 1.19$, n.s.). Taken together, these data indicate that HD cell firing properties were stable between the novel visit and subsequent visits to the rectangle, and the amount of shift in preferred firing direction exhibited during the Novel Rectangle session was preserved during the subsequent return visits to the rectangle.

HD cell activity and behavior during passive transport

HD CELL ACTIVITY ON THE CART. The goal of the present study was to examine how passive transport of a rat into a novel environment affected the preferred firing direction of HD cells in the novel environment. However, it is also of interest to examine HD cell activity during the actual passive transport manipulation, since passive transport itself may have affected the preferred firing direction of HD cells. Unfortunately, it was not possible to monitor HD cell activity when the rat was transported on the cart in the passageway because either the green LED was turned off (Lights Off conditions) or during the Lights On cart condition one or both of the LEDs was often obscured as the experimenter moved the cart through the apparatus. Consequently, accurate sampling of HD was not possible during passive transport in the passageway. However, to address this issue, 18 HD cells were monitored while the rat was passively transported on the cart in the cylinder for approximately 30 s, just prior to moving the cart through the passageway. Under this condition, most HD cells exhibited preferred firing directions that were similar to that of the Initial Cylinder session (absolute shift = 14.3 ± 15.2°; 14 of 18 cells exhibited shifts in their preferred firing direction ≤ 24°). The large variability is primarily accounted for by one cell that exhibited a 108° shift. Interestingly, this HD cell shifted its preferred firing direction by −84° after passive transport, suggesting that the shift on the cart while in the cylinder was independent of that after passive transport into the novel environment. Furthermore, the shift in preferred firing direction on the cart was not predictive of the subsequent shift exhibited in the novel environment ($r = 0.16$, not significant). Analyses of the 18 HD cells recorded on the cart revealed no significant difference in HD cell peak firing rates between the Initial Cylinder session and that aboard the cart, independent of whether the room lights were on or off [$t(17) = 0.16$; not significant]. These results indicate that HD cells maintained their discharge properties while the rats were passively transported aboard the cart inside the familiar cylinder.

BEHAVIORAL DIFFERENCES. Differences in the number, frequency, or speed of head movements during the passive or active conditions could influence the subsequent HD cell preferred firing directions in the novel environment. For example, an increase in back-and-forth head turning onboard the cart might increase the accumulating error in the directional path integration system and thus contribute to a large shift in preferred firing direction in the novel environment. Since head movements during passive transport or active movement in the dark cannot be resolved, the contribution of head movements cannot be discounted. However, our observations indicated that the rats on the cart generally stood on the floor of the cart or periodically reared during the passive translation into the novel environment. Occasionally the rat would turn around while the cart was moved through the apparatus, although this behavior was not consistent across all passively transported rats and did not appear to influence the amount of shift in the preferred firing direction. We observed rats making fewer 360° turns onboard the cart than those rats that actively walked into the novel environment. In fact, once inside the passageway, the rats in the active movement groups were observed to often turn around and return to the closed door leading into the cylinder before completing the movement through the passageway. Thus if any group was making more frequent or larger head turns during its time in the passageway, it was the active movement group rather than the passive transport group. This finding discounts the possibility that differences in head movement behavior between the active and passive groups accounted for the different amounts of shift in the cells preferred firing directions.

TIME TO COMPLETE MOVEMENT FROM FAMILIAR TO NOVEL ENVIRONMENT. The elapsed time to complete the initial walk or passive transport from the cylinder into the rectangle was calculated to determine whether the larger shifts in the Passive Transport rats could be accounted for by a longer duration of the translation from familiar to novel environment. Some rats completed the movement within 30 s or less, while others took several minutes, since they would often walk a few inches into the passageway and then retreat back to the cylinder. However, once in the passageway and the door leading to the cylinder closed, the rats usually completed the path into the rectangle within 30 s. There was no significant difference in translation time between active and passive movement conditions [regardless of lighting conditions; $t(47) = -0.43$, n.s.]; rats that walked into the novel environment required an average of 53 ± 10 s to complete the movement, while the duration of the passive transport averaged 60 ± 2 s. The average time required to complete the movement into the novel environment for rats

in the Active Movement/Lights On condition was 40 ± 6 s and 80 ± 26 s for the Active Movement/Lights Off condition. Although these values are quite different, they were not significantly different [$t(26) = -1.99$, $P = 0.06$], primarily due to the substantial variability in these values. Thus differences in duration of the initial movement from the familiar to the novel environment are unlikely to account for the magnitude of the directional shifts.

USE OF THE CART AS A REFERENCE FRAME. HD cells did not appear to switch reference frames from the cylinder cues to the longitudinal axis of the cart container while the rat was in the cart in the cylinder. Therefore we presume that directional activity continued as the rats were transported through the passageway. However, it is possible that the subsequent shift in preferred direction in the novel environment was the result of the rats using the cart as a reference frame. In this case, HD cell preferred firing directions might have shifted along with rotations of the cart as it was wheeled into the novel rectangle. Thus when the cart moved through a +90° turn, the preferred firing direction also underwent a +90° shift relative to the room. A HD cell that used the cart as its reference frame would be expected to undergo a considerable shift in preferred firing direction when the rat was removed from the cart after passive transport. Indeed, because the cart always traversed the same route, a route that contained two 90° clockwise turns, we might expect a cell's preferred firing direction to shift 180° when the rats were released from the cart in the rectangle. To test this possibility, we examined HD cell activity during the first 15 s after the rat was released from the cart. Only three HD cells out of 21 exhibited large shifts in preferred firing direction that approximated 180° within the first 15 s after release from the cart (shift values of 144, 180, and 216°). Interestingly, five cells out of 30 cells recorded from the Active Movement rats also exhibited large shifts in preferred firing direction within the first 15 s after arriving in the rectangle. These results indicate that 180° shifts of the preferred firing directions were not common to HD cells of passively transported rats and suggest that these rats were not consistently using the cart as a reference frame, or if they were, they quickly abandoned its use as soon as they left the cart.

DISCUSSION

An organism can reliably maintain its spatial orientation as it moves through an unfamiliar environment by continually monitoring its movements with respect to a known reference or start point. A key issue is what cues enable this ability? Are they vestibular, proprioceptive, motor efference copy, optic flow, or some combination of these cues? Experiments across different species have failed to provide a clear answer to this question. For example, in terms of motor/proprioceptive cues, behavioral studies have demonstrated that hamsters can return directly to their nest site after a circuitous outward journey to a food source and this ability is impaired if the hamster is passively transported to the food source in a novel location (Etienne et al. 1986, 1988; Mittelstaedt and Mittelstaedt 1980). Similar experiments conducted with geese, albeit a nonmammalian species, have shown that homing is accurate after passive transport aboard a wheeled cart provided the birds have access to visual cues during the passive translation (Saint Paul 1982). In humans that were subjected to optic flow, locomotion, or vestibular stimulation, estimates of directional heading were most accurate after stimulation that included self-locomotion by the subject (Telford et al. 1995). In the absence of visual input, the information provided by active locomotion led to more accurate heading estimates than estimates given after vestibular stimulation. Collectively, these findings point to the importance of motor efference copy and proprioception in path integration. On the other hand, studies of human navigation have found that when people are subjected to passive translation aboard a robotic cart along a straight path in darkness, subjects can reliably replicate the velocity and distance of the translation (Berthoz et al. 1995; Grasso et al. 1999; Israel et al. 1997). These studies suggest a capacity for storing a spatial memory of dynamic movement profiles without the use of motor efference copy. Moreover, humans with bilateral vestibular system damage have difficulty navigating in the dark, despite the availability of information from intact motor and proprioceptive systems (Glasauer et al. 2002). These data highlight an important role for vestibular cues in updating ones spatial orientation. Taken together, a clear picture for the role of motor efference cues and proprioception in path integration and navigation cannot be gleaned from these studies.

The present experiment was designed to examine the influence of motor efference copy, proprioception, and optic flow in maintaining a stable preferred firing direction in HD cells when rats move from a familiar to a novel environment. The primary finding was that passive transport of rats from the familiar to the novel environment caused a substantial shift in HD cell preferred firing direction between the familiar cylinder and novel rectangle environment. Removal of visual cues and optic flow by turning off the overhead room lights during the initial journey into the novel environment induced a moderate, but nonsignificant, shift in preferred firing direction compared with rats that walked into the novel environment with the room lights illuminated. Passive transport of rats into the novel environment in the dark, a combined manipulation of motor efference copy/proprioception and optic flow, resulted in a degree of preferred firing direction shift that was not different from that of rats passively transported with the room lights illuminated.

Converging evidence indicates that HD cell activity is influenced by the ongoing motor behavior of the animal. For example, most HD cell firing rates are decreased when the rat is securely restrained and passively rotated through the cell's preferred firing direction (Knierim et al. 1995; Golob et al. 1998; Taube 1995; Taube et al. 1990b). Zugaro et al. (2001) also found that when unrestrained *motionless* rats were passively rotated, HD cell peak firing rates decreased, but preferred firing directions remained stable. In the present study, HD cell firing properties were maintained while the rat was passively transported in the familiar cylinder aboard a wheeled-cart that confined, but did not restrain the rat from moving. In cases in which sufficient HD sampling was achieved, the preferred firing directions of HD cells were found to be similar (i.e., within ±14°) to those observed during the prior freely moving sessions and there was no change in peak firing. From these observations and those of Zugaro et al. (2001), it is unlikely that active or passive movement aboard the cart was the primary cause of the shift in the preferred firing direction of HD cells after passive transport into the novel environment. Rather, the large shifts are more likely a conse-

quence of poor spatial updating in the rat, and represent a type of spatial disorientation where the rat perceives it is oriented correctly, but this orientation is incorrect. This form of disorientation is categorized as type I spatial disorientation (Gillingham and Previc 1996) and can be considered more of a "misorientation." This condition contrasts with type II spatial disorientation, where subjects are consciously aware of their disorientation and attempt to become reoriented by using any available information.

Landmarks versus idiothetic cues

Most theories of navigation contend that animals exhibit a hierarchical use of external landmark cues over idiothetic or self-motion cues. A number of studies have demonstrated that rodents will navigate by idiothetic cues when visual landmarks are not available, such as in darkness (Etienne et al. 1995; Mittelstaedt and Mittelstaedt 1980). The cue card of our "standard" cylinder acts as a landmark by exerting stimulus control over the firing of HD cells (Taube et al. 1990b). The maintenance of directional firing in the absence of the cue card (Goodridge and Taube 1995; Goodridge et al. 1998) has been attributed to information provided from idiothetic cues. In this study, when the rats leave the familiar cylinder and enter the novel passageway and rectangle, there are no familiar cues available that can serve as landmarks. Therefore during the initial journey through the passageway and into the rectangle, the rat must rely on idiothetic cues to maintain its spatial orientation. Taube et al. (1990) reported that when rats were removed from a familiar cylindrical environment and placed into a novel rectangular environment, HD cells underwent a significant shift in preferred firing direction (8 of 10 cells shifted their preferred firing direction by $\geq$48°) (Taube et al. 1990b). However, as illustrated in Fig. 3, when the rats actively walked from the familiar cylinder environment to the novel rectangle, HD cells were observed to undergo preferred firing direction shifts that averaged $\pm$18°, with no cells shifting their preferred firing direction more than $\pm$36°. These data suggest that access to and the use of idiothetic cues during the initial journey into the novel environment enabled the rat to maintain its spatial orientation, thus the preferred firing direction of HD cells was generally similar between the familiar and novel environments.

Passive transportation of the rat aboard a wheeled cart enabled us to test the accuracy of HD cell path integration during a manipulation designed to disrupt the reliability of motor efference copy/proprioceptive cues. Passive transport caused a substantial shift in the preferred firing direction that averaged 67.8°, suggesting that motor efference copy and/or proprioception are important signals for accurately updating the animal's perceived HD under conditions in which familiar landmarks are not available.

While our results may be accounted for by the absence of a reliable motor efference copy/proprioceptive signal, a number of other explanations need to be considered. These possibilities include differences in the number of head movements, elapsed time in the passageway, or use of the cart as a reference frame. We analyzed our video and spike data to test each of these possibilities, but a clear association between these influences and the resulting shifts in HD cell preferred firing direction could not be made. Another important difference between the Active Movement and Passive Transport conditions was the availability of tactile information. Transportation aboard the cart interfered with the rats' ability to acquire information about the tactile and geometric features of the passageway during their initial movement through this section. Taube and Burton (1995) suggested that, during their initial trip through the passageway, the rats might learn about such features of the apparatus. This information could then be readily integrated into the HD cell system and reduce the ultimate shift in preferred firing direction between the cylinder and novel environment. Thus animals on the cart would have been deprived of tactile cues that could have aided them in updating their directional heading as they were moved to the novel environment. We have no data to exclude this possibility, and it would be of interest to further test this notion in future experiments.

It is also possible that the shift in preferred firing direction was induced by stress or by the rat not attending to idiothetic cues while on the cart. The rats were, however, acclimated to the wheeled cart for several days prior to the experimental manipulation to reduce the likelihood that stress contributed significantly to the shift in preferred direction. Although we have no data to indicate that rats were, or were not, attending to idiothetic cues while aboard the cart, Gavrilov et al. (1996, 1998) have shown that passive rotation of rats aboard a robotic cart influenced the activity of hippocampal place cells and theta rhythm. These results suggest that rats remained responsive to vestibular signals in the absence of active movement. In light of these data, we anticipated that disrupting the reliability of motor efference copy/proprioceptive cues might have led the Passive Transport rats to rely more heavily on vestibular cues for maintaining the preferred firing directions of HD cells. Thus it is noteworthy that vestibular information, which was available to the rats aboard the cart, was not sufficient to track the rat's movements and update the preferred firing direction of HD cells. This finding is at odds with recent studies demonstrating that vestibular information was required to accurately perform a spatial task involving path integration in rats (Stackman and Herbert 2002; Wallace et al. 2002). Our previous studies have demonstrated the importance of vestibular cues for the generation of directional firing of HD cells (Stackman and Taube 1997; Stackman et al. 2002). Given that the sodium arsanilate-induced vestibular lesions of Stackman and Herbert (2002) and Wallace et al. (2002) would have abolished HD cell activity and perhaps place cell firing also, it is unclear whether the path integration deficits that they report are due to loss of vestibular cues or loss of HD and place cell information. Although we have no evidence to indicate that the rats' vestibular system adequately monitored the right angle turns of the cart, the passive movement of the rats aboard the cart was well-above vestibular threshold. Furthermore, the cart's path was not particularly lengthy or circuitous and involved only two 90° right angle turns with each turn completed over the course of 2–3 s.

To further test the differential involvement of vestibular and motor efference copy/proprioceptive cues in directional path integration, it would be interesting to devise an additional experimental condition (e.g., Active Movement/No Vestibular) that disrupts vestibular cues but spares motor efference copy. This possibility would be difficult, however, because the disruption of vestibular cues abolishes direction-specific firing altogether in rats (Stackman and Taube 1997; Stackman et al.

2002). The nonrandom distribution of shifts in the preferred firing direction in rats of the Passive Transport/Lights Off condition support a role for vestibular and optic flow (and other) cues in influencing HD cell firing in the novel environment. Given the relatively short time constant of the brain stem vestibular nuclei (approximately 6 s) (Cohen et al. 1981), the vestibular system probably evolved to detect head motion over relatively short time periods, and therefore would not be suited for certain aspects of path integration where accurate tracking of long, slow head movements over a long path, which are typical of locomotion, are necessary. To further differentiate the roles of motor efferent copy signals and proprioceptive cues, lesions of the dorsal columns and spino-cerebellar tracts could be used to eliminate proprioceptive information.

The minimal preferred firing direction shifts we observed for Active Movement/Lights Off rats might indicate maintained spatial orientation due to the redundancy of idiothetic cues available to these rats as compared with the passively transported rats. Similarly, the relative paucity of idiothetic cues available to the Passive Transport/Lights Off rats would be expected to lead to difficulties in maintaining one's orientation, and the rats may have quickly become misoriented. From our data, we cannot determine whether this misorientation resulted from the length of the passive transport path, the number of 90° turns that occurred, or the sheer duration of time spent aboard the cart. These concerns raise interesting questions for future experimentation regarding the relevance of discrete idiothetic cues for path integration. For example, would the preferred firing direction shift be of lesser magnitude if the passive transport path were shorter? If the passageway was longer, and comprised more 90° turns, one might expect greater shifts in HD cell preferred firing direction of rats walking into the novel environment with room lights illuminated. The considerable shifts in preferred firing direction after passive transport to a novel environment may represent one neurobiological consequence of passive transport that may explain the disruptive effect that manipulating motor efference copy/proprioceptive cues has on path integration (Etienne et al. 1986, 1988; Mittelstaedt and Mittelstaedt 1980).

We found no additional influence of lighting condition on the resulting shift in HD cell preferred firing direction of passively transported rats. This result suggests a minimal influence of optic flow cues for directional path integration by rats. The contribution that optic flow may contribute to perceived spatial orientation, and hence to accurate path integration was recently demonstrated by Froehler and Duffy (2002), who showed that neurons in the medial superior temporal cortex of nonhuman primates responded to the directional movement of the animal when transported on a cart, or when stationary and shown a simulated view of the moving visual field on video. However, these neurons cannot by themselves be responsible for accurate path integration because many animal species are capable of accurate path integration over long distances in the dark, a condition that would preclude the use of optic flow cues.

Experience-dependent alteration in HD cell-preferred firing direction

Following the Novel Rectangle session, the cylinder door was removed and the rats were allowed to shuttle back-and-forth between the two environments. HD cells of rats that actively walked into the novel environment (whether the room lights were on or off) shifted back to their original preferred firing directions when the rat returned to the cylinder. However, HD cells from rats that were passively transported to the novel environment, exhibited significant shifts in their preferred firing direction between the Initial Cylinder and Return Cylinder sessions (see Fig. 5). This shift in the preferred firing direction in the cylinder suggests that passive transport into a novel environment resulted in a persistent change in the HD cell's preferred firing between the Initial Cylinder and Return Cylinder session. These shifts in the preferred firing direction were stable despite multiple return visits to the rectangle. Thus, whatever new preferred firing direction that was adopted by the HD cells in the novel environment, HD cells maintained this new preferred firing direction on active locomotion back to the cylinder in the Return Cylinder sessions. HD cells of rats that had been passively transported were observed to 'flip' between their two orientations according to whether the animal was in the Cylinder or Rectangle/Passageway during the third recording session. It is important to note that in cases where a large shift in the preferred firing direction occurred when the rat was initially passively transported into the rectangle, the shift was preserved when the rat actively walked back through the passageway into the rectangle on its return visit. The preservation of the preferred firing direction shift even after the rat had experience *actively moving* from the cylinder into the rectangle suggests that during the Novel Rectangle session, the rats likely learned about the new environment and incorporated information about the geometry of the rectangle and passageway, along with spatial information about the cue card in the rectangle. These cues were then used subsequently as landmarks. When the rats returned to the cylinder from the rectangle, HD cells shifted their directional firing as the rat reoriented itself with respect to the cylinder landmark. On leaving the cylinder and entering the passageway, HD cells again shifted their preferred firing direction as the rat oriented itself with the learned landmarks of the passageway and rectangle. In essence, this new firing orientation was maintained over the remainder of the recording protocol throughout the entire apparatus and suggests that the passive transport event alters the directional firing of HD cells in both a novel environment and one that is familiar to the animal.

In summary, this study suggests the importance of motor efference copy/proprioceptive cues for maintaining the preferred firing direction of HD cells during an initial journey into a novel environment. HD cells of rats that underwent a passive transport manipulation designed to disrupt the use of and reliability of motor efference copy/proprioceptive cues exhibited large shifts in preferred firing direction during the initial journey from a familiar environment to a novel one. The present findings also indicate that providing optic flow cues to passively transported rats did not improve directional path integration by HD cells.

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DISCLOSURES

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