



## Research report

## Recordings of postsubiculum head direction cells following lesions of the laterodorsal thalamic nucleus

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**Abstract**

Areas of the rodent limbic system are important for solving spatial tasks and accurate navigation. Previous studies have identified cells in the postsubiculum (PoS) and the lateral dorsal thalamus (LDN) which discharge as a function of the animal's head direction in the horizontal plane. These two brain areas are reciprocally connected with one another. To determine the contribution of the LDN to the functioning of PoS head direction cells, we lesioned the LDN and recorded single units in the PoS. We report here that lesions of the LDN had little effect upon the firing properties of PoS HD cells. In addition, HD cells from lesioned animals showed normal responses to two environmental manipulations: (1) when the salient visual cue was rotated the preferred firing directions of PoS HD cells shifted a similar amount and (2) cells frequently ceased firing, or had reductions in their peak firing rate, when the animal was restrained and passively rotated through the preferred firing direction. These results indicate that the LDN does not play a substantive role in either the generation or the stability of the HD cell signal in the PoS. © 1998 Elsevier Science B.V.

*Keywords:* Head direction cell; Laterodorsal thalamus; Postsubiculum; Spatial; Navigation

**1. Introduction**

Numerous studies have shown that the hippocampal formation is important for the accurate performance of spatial tasks [17,20]. Lesions of the hippocampus proper, as well as retrohippocampal areas, lead to impaired performance on various spatial tasks [17,18,30]. Single unit studies have provided important information regarding the specific operations that may be implemented within this region. Most notably, recordings from the hippocampal formation have revealed correlations between the firing of neurons and the animal's spatial relationship with its surroundings. Two main types of spatial correlates have been identified. Place cells discharge maximally when the animal is in a particular subregion of its environment and discharge infrequently in other areas of the environment [19]. A second cell type, known as head direction (HD) cells, discharge maximally when the animal's head is

pointing in a particular direction, irrespective of its location within the environment [22,32]. Although HD cells were first identified in the postsubiculum (PoS), they have also been recorded in the anterior thalamic nucleus (ATN) [27], lateral dorsal thalamic nucleus (LDN) [16], retrosplenial cortex [3], and striatum [41].

HD cells are responsive to both salient visual cues as well as internally generated idiothetic cues, such as vestibular, proprioceptive, and motor efference copy [2,7,28,33]. For example, rotation of a large, polarizing visual cue along the walls of a cylindrical arena leads to a similar shift in the preferred firing direction of HD cells. However, the cue card is not necessary for cell firing, since HD cells continue to exhibit directional discharge following removal of the cue card [7,33], when the animal is blindfolded [9], or in a darkened room [16]. Restraining the movement of an animal and slowly rotating it back-and-forth horizontally through the environment has been shown to disrupt directional firing of HD cells in the ATN [27], and to attenuate their activity within the PoS [33]. Finally, it has recently been shown that an intact vestibular system is necessary for the presence of HD cell activity in the ATN [26].

Although HD cells have been identified in several brain areas, how the brain constructs this representation remains

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unclear. Most of the brain areas known to contain HD cells are anatomically interconnected to varying degrees with one another. Prominent reciprocal connections exist between the PoS and both the ATN and LDN [25,36–38]. Reciprocal connections have also been demonstrated between the LDN and the retrosplenial cortex [35,43]. The importance of the LDN for normal spatial learning and place cell discharge was demonstrated by Mizumori et al. [15] who showed that temporary inactivation of the LDN led to impairments on a working memory version of the radial arm maze task. These authors also showed that LDN inactivation led to significant disruptions in hippocampal place cell activity. For example, some place cells completely ceased discharging in a location-specific manner, and other cells had decreased place-specificity scores. Because the PoS and LDN are reciprocally connected and both contain HD cells, the following study examined the functional relationship between these two areas by lesioning the LDN and recording neurons within the PoS. Specifically, we were interested in determining whether an intact LDN is necessary for HD cell activity within the PoS, and if not, whether LDN lesions affect the firing characteristics of PoS HD cells and their responses to environmental manipulations. Some of these results have been reported previously in preliminary form [5,34].

## 2. Materials and methods

### 2.1. Training and apparatus

Seven female Long-Evans rats (4–10 months old) were placed on a food restricted diet ( $\sim 15$  g/d) and maintained at  $\sim 85\%$  normal body weight. Prior to surgery, the animals were trained over several weeks to forage for food pellets thrown randomly inside a gray cylindrical apparatus (76 cm diameter, 51 cm height). This task resulted in a near uniform sampling of locations and directions within the cylinder by the rat. The cylinder contained a large white cue card ( $73 \times 51$  cm) occupying  $100^\circ$  of arc along the entire height of the cylinder wall. This card served as the most prominent visual cue within the cylinder and remained in the same place throughout training. The apparatus was surrounded by floor-to-ceiling black curtains arranged in a 2 m diameter circle. Gray photographic backdrop paper served as the floor of the cylinder. This floor paper was changed prior to all recording sessions to prevent the use of olfactory cues across sessions. Four DC powered lights were symmetrically arranged 2.3 m above the cylinder, and a Sony XC-711 color video camera was vertically aligned above the center of the cylinder. For most of the cells a white noise generator, connected to a black speaker placed in the rafters of the ceiling above the center of the apparatus, was used to mask extraneous sounds.

### 2.2. Electrodes and surgical procedures

The recording electrode consisted of an array of 10 nichrome wires,  $25 \mu\text{m}$  in diameter, which were insulated except at the tips. These wires were contained within a steel cannula affixed to a vertically movable assembly that was embedded in dental acrylic [11].

The animals were anesthetized with intraperitoneal injections of pentobarbital (45 mg/kg) followed by 0.1 ml of atropine sulfate to prevent respiratory complications. Bilateral lesions were made in the LDN of each rat using Bregma coordinates [21]. Two of the animals received electrolytic lesions (2 mA for 15 s) at the coordinates anterior/posterior (AP):  $-2.65$  mm, medial/lateral (ML):  $\pm 2.2$  mm, and dorsal/ventral (DV) from the cortical surface:  $-4.4$  mm. The remaining 5 rats were given neurotoxic lesions using ibotenic acid (10 mg/ml dissolved in  $\text{K}^+$  phosphate buffered saline) injected through a glass micropipette. Four of the five rats received single injections of  $0.1 \mu\text{l}$ /side at the coordinates AP:  $-2.65$  mm and ML:  $\pm 2.2$  mm. The DV coordinates varied between  $-4.2$  and  $-4.6$  mm from the cortical surface. The remaining rat was given two  $0.1 \mu\text{l}$  injections of ibotenic acid/hemisphere at: AP:  $-2.5$  or  $-3.2$  mm, ML:  $\pm 2.2$  mm, and DV from the cortical surface:  $-4.3$  or  $-4.4$  mm, respectively. The neurotoxin was administered over approximately 30 s/injection, and after each injection the micropipette was left in place for at least 10 min.

In addition, recording electrodes were implanted just above the PoS using the coordinates AP:  $-6.6$  mm, ML:  $+2.8$  mm, and DV from the cortical surface:  $-1.6$  mm. All surgeries were performed under sterile conditions. The animals were allowed a minimum of 7 days to recover from surgery before cell screening commenced.

### 2.3. Cell screening and data acquisition

Single units were screened while the animal performed the foraging task in the cylinder as described above. Cell isolation and single unit recording procedures have been described elsewhere [32]. In order to determine the animal's head direction two light emitting diodes (LED's) were attached to the animal's headstage in fixed positions above the animal's midline axis. A red colored LED was positioned above the snout, and a green LED was located 10 cm away over the animal's back. An automated video-computer tracking system, sampling at 60 Hz, recorded the number of cell spikes occurring during each sample period while simultaneously recording the  $x, y$  positions of each LED. Each recording session usually lasted 8 min.

### 2.4. Experimental manipulations

A series of recording sessions was performed for each identified HD cell. In a standard session the environmental

conditions were identical to the training conditions described above. The cue card was positioned at the 3:00 position (defined as 0°) when viewed from the overhead video camera. Following this standard session, several other recording sessions were conducted. First, a cue card rotation session was conducted in order to determine if the cue card influenced the HD cell's preferred direction. With the rat out of view, the cue card was rotated either 90° or 270° from its initial position in the standard session. For the purposes of comparison, the rotation session was always preceded and followed by a standard session. The rat was removed from the cylinder and returned to a cardboard box between sessions. Before each recording session the box containing the animal was rotated slowly back-and-forth for at least 1 min to discourage the use of idiothetic cues.

Other manipulations were conducted in which the rat was restrained and passively rotated through the environment. In these restraint sessions the animal was wrapped tightly inside a towel up to its neck, allowing for an unobstructed view of its surroundings. The animal's head was pointed away from the experimenter and toward the cylinder wall. The animal was held near the center of the cylinder and rotated back and forth in approximately 45° arcs at a velocity ranging from 50 to 100°/s. The experimenter gradually rotated himself around the periphery of the cylinder such that all 360° head directions were sampled. Usually two 1 min sessions were recorded in the handheld condition for each cell. Unrestrained standard sessions were always conducted before and after each handheld session for comparison and to ensure that isolation of the cell was not lost during the restraint session. Results were compared to an additional group of non-lesioned rats ( $n = 4$ ) which had electrodes implanted into the PoS and were tested under similar restraint conditions.

### 2.5. Data analysis

Head direction and spike count per 1/60th s were sorted into one of sixty 6° bins. The firing rate for each bin was determined by summing the total number of spikes and dividing by the total amount of time the animal's head pointed in that particular bin's direction. Graphs were constructed plotting the cell's firing rate as a function of head direction. The firing rate vs. head direction graphs for each cell were characterized by the following four parameters based on a triangular best-fit model: (1) preferred firing direction, (2) peak firing rate, (3) directional firing range, (4) background firing rate [32]. The similarity of the firing rate by head direction curves between sessions was assessed by a best-fit cross-correlation algorithm [33]. This analysis was used to determine the angular shift in the cell's preferred firing direction in the cue rotation sessions and to examine the consistency of the cell's preferred firing direction both within and between standard sessions. Head direction angles were defined using a polar coordi-

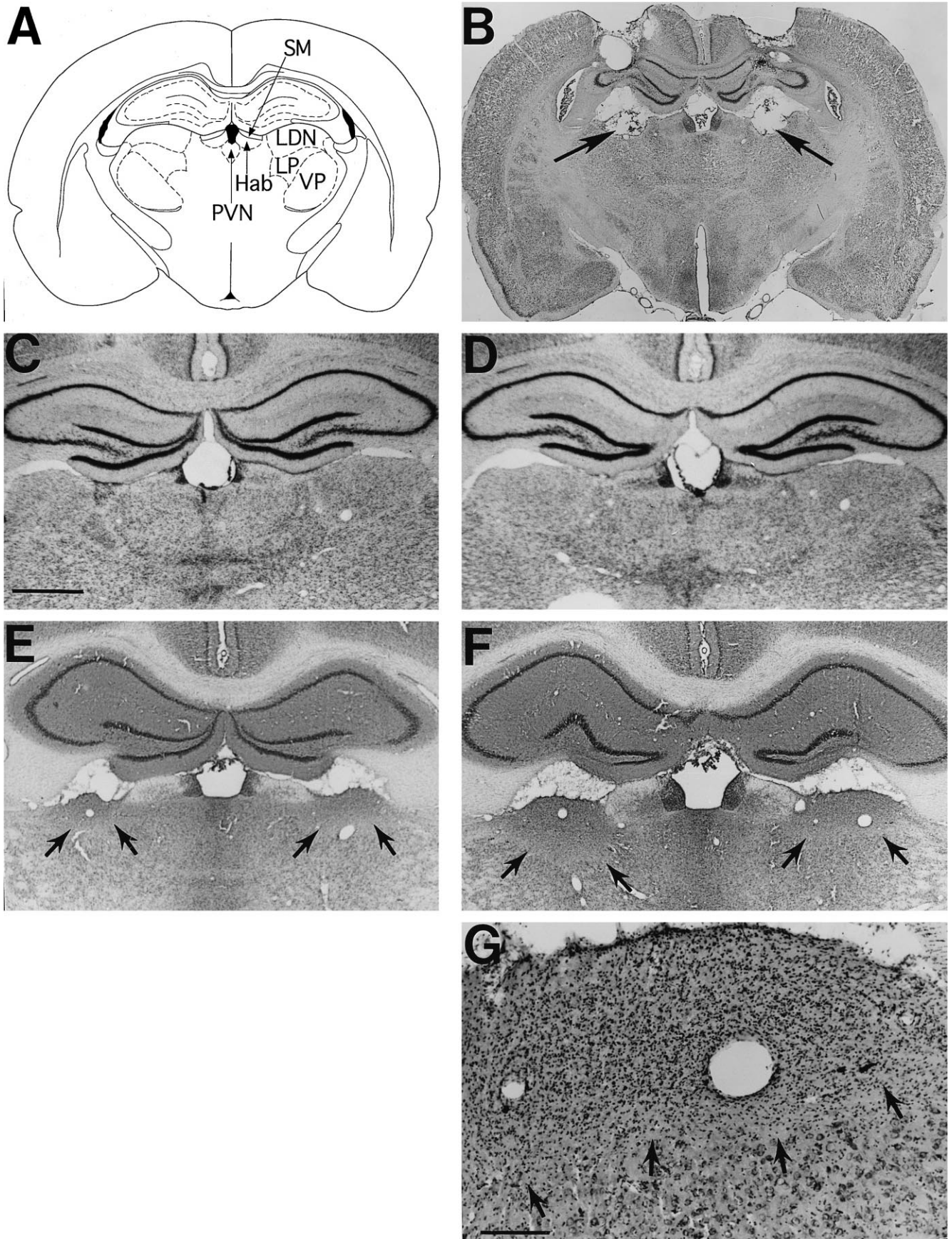
nate system, such that positive angles indicate counter-clockwise (CCW) turns and negative angles indicate clockwise (CW) turns.

Recent studies have examined the moment-to-moment relationship between HD cell discharge and the animal's head direction [1,31]. Both reports have shown that the firing of ATN HD cells anticipates the animal's head direction by about 25 ms (this value has been corrected for the video delay inherent in a charge-coupled-device camera). Furthermore, ATN HD cell firing appears to lead the discharge of PoS HD cells by approximately 30–40 ms. Several measures have been developed to quantify this relationship. As described above, the computer samples the LED positions and the number of spikes every 16.67 ms. Under normal conditions, analyses are conducted where the spike series and head direction series are synchronized with one another. By conducting time shift analyses, where the spike series is shifted forward or backward in time relative to the head direction series, one can determine the optimal time shift which leads to the maximum peak firing rate and information content (for details, see Ref. [31]).

In addition to these parameters, we analyzed the difference in preferred firing direction between CW and CCW head turns ( $\geq 90^\circ/s$ ) (for details, see [1]). The angular difference between the CW and CCW functions for firing rate vs. HD plots is referred to as the shift angle. If cell firing is leading the animal's head direction, then the two types of head turns will have different preferred firing directions, where the firing rate vs. HD function for CCW head turns is shifted to the right of the function for CW head turns and the shift angle is positive. Conversely, if cell firing is lagging head direction, then the firing rate vs. HD function for CCW head turns is shifted to the left of the function for CW head turns and the shift angle is negative. Previous studies from intact animals have shown that for PoS HD cells, the CW and CCW 'tuning curves' generally overlap, indicating that PoS activity encodes the animal's current directional heading [1]. The shift angle for each cell was defined as the difference in preferred firing directions determined from the firing rate vs. HD functions for CW and CCW head turns. Using the cross-correlation method, the CW function was shifted in 6° steps along the HD axis and cross-correlated with the CCW function. The angular shift which yielded the maximal cross-correlation between the two functions (Pearson's  $r$ ) was defined as the shift angle for that cell. To determine whether the optimal time shift parameters were affected by LDN lesions, similar analyses were conducted on the HD cells recorded in lesioned animals and the optimal values were compared to data obtained from intact animals. The level for statistical significance was defined as  $P < 0.05$ .

### 2.6. Histology

The electrodes were advanced 1.25–2.5 mm over the course of 3 months. At the completion of the experiments



the rats were perfused with 10% formalin in saline. Prior to perfusion the animal was deeply anesthetized with sodium pentobarbital and an anodal DC current (15  $\mu$ A for 10 s) was passed through one of the wires in which a HD cell was recorded in order to provide an estimate of the wire's location using a Prussian blue reaction. Following perfusion the brains were placed in 10% formalin for at least 24 h and then placed in 10% formalin + 2% potassium ferrocyanide solution for 24 h before reimmersing them in 10% formalin for 24 h. The brains were then placed in 20% sucrose for 24 h and quick frozen the next day for sectioning. Brain sections were mounted on slides, stained with cresyl violet, and later examined microscopically for lesion damage and electrode localization.

### 3. Results

#### 3.1. Evaluation of the lesions and recording sites

Fig. 1 shows representative coronal sections from rats with bilateral electrolytic and ibotenic acid lesions. Both animals with electrolytic lesions were judged to have complete lesions of the LDN. In addition, there was extensive damage to the overlying cortex and portions of the dorsal hippocampus due to the track made by the lesioning electrode. There was also some damage to adjacent thalamic nuclei, especially the lateral posterior thalamic nucleus (Fig. 1A,B). The anterior dorsal thalamic nuclei (AD), where HD cells have been identified [27], were spared in both animals.

The extent of the lesions using ibotenic acid was more variable than the electrolytic lesions and the data reported below were only from animals judged to have complete bilateral lesions of the LDN ( $n = 5$ ). In addition to the LDN, these animals sometimes had damage to adjacent structures, including the lateral posterior thalamic nucleus and portions of the dentate gyrus (see Fig. 1E,F). Both groups of lesioned animals had complete damage to the dorsal-caudal portion of the LDN where HD cells have been previously identified [16]. Because there were no apparent differences between the animals with electrolytic and neurotoxic lesions, all lesioned animals were pooled

together in one group ( $n = 7$ ). Based on the recording electrode tracts and Prussian blue reaction, all of the recording electrodes were accurately positioned in the PoS.

#### 3.2. Qualitative analysis

A total of 141 cells were sufficiently isolated above background noise to assess their firing properties and behavioral correlates. Each cell was classified into one of four categories: HD cell, place cell, theta cell, or other. Units were classified as HD cells if they fired maximally when the animal was pointing its head in a specific direction (the preferred firing direction) independent of its location in the cylinder, as well as other ongoing behaviors such as grooming or eating.

A total of 15 HD cells (10.6%) were identified and recorded in 6 out of 7 rats. In the one rat where HD cells were not identified, portions of the anterodorsal and anteroventral thalamic nuclei were also lesioned bilaterally. This result is consistent with earlier findings which demonstrated that an intact ATN was necessary for the generation of HD cell activity in the PoS [8] (see Section 4). The number of HD cells per animal ranged from 1 to 7 in the 6 rats where HD cells were identified. The percentage of cells classified as HD cells was lower than the percentages reported in previous studies on non-lesioned animals (25.5% in Ref. [32]; 36.6% in Ref. [23]). However, the value of 10.6% was similar to the percentage obtained in the four non-lesioned rats (11.9%) reported below in the restraint experiments.

Twenty-six cells (18.4%) were classified as theta cells. A unit was classified as a theta cell if it fired spike bursts in the EEG theta frequency range (5–12 Hz). Place cells were infrequently encountered in recording from the PoS. Only 1 of the 141 cells was classified as a place cell. The percentage of cells classified as theta or place cells was similar to values reported for non-lesioned animals [32]. The remaining cells which could not be classified as either directional, theta, or place cells were designated as 'other cells.' This group of 99 cells comprised the majority of cells found in the PoS (70.2%). No apparent behavioral or spatial correlate could be identified for this group of cells.

Fig. 1. Coronal brain sections stained with cresyl violet shown from representative LDN lesioned animals. (A) Schematic diagram of the LDN and nearby structures. (B) Section from an electrolytically lesioned animal, approximately  $-3.2$  mm posterior to bregma. In addition to lesions of the LDN, indicated by the arrows, other areas which received damage included the hippocampus, lateral posterior thalamus, and overlying cortex. (C,D) Zoomed in view of sections from a non-lesioned control animal. Sections (C) and (D) are approximately  $-2.4$  and  $-3.2$  mm posterior to bregma, respectively. Scale bar = 1.0 mm. (E,F) Zoomed in view of two sections at similar anterior/posterior coordinates as (C) and (D) from an animal lesioned using ibotenic acid. Arrows indicate the ventral and lateral borders of the lesion. The lesion shown is mostly confined to the LDN, with some damage to the hippocampus and adjacent thalamic areas (mediodorsal, lateral posterior, and posterior thalamus). Scale is the same as shown in (C). (G) Magnified view of the right LDN lesion area from the section shown in (F). The arrows indicate the transition between the lesioned and non-lesioned regions. The LDN region above the arrows is characterized by an absence of neurons and the presence of microglia cells. The area below this region contains neurons of the posterior thalamic nuclei. Scale bar = 200  $\mu$ m. Abbreviations: Hab, habenula; LDN, lateral dorsal thalamus; LP, lateral posterior thalamus; PVN, paraventricular thalamus; SM, stria medullaris; VP, ventral posterior thalamus.

### 3.3. Quantitative analysis

Fig. 2 illustrates two representative HD cells recorded from different animals with LDN lesions. Each graph plots the cell's firing rate as a function of the animal's head direction averaged over the 8 min recording session. Fig. 2a shows a cell recorded from an animal whose LDN was lesioned electrolytically, while Fig. 2b illustrates a cell from an animal with a neurotoxic lesion.

#### 3.3.1. Cellular properties

The mean background firing rate across all HD cells was  $1.66 \pm 0.57$  spikes/s (range: 0.005–7.8 spikes/s). The mean peak firing rate was  $29.9 \pm 6.4$  spikes/sec (range: 5.0–89.8 spikes/s). The peak firing rate of cells did not systematically vary across animals, and cells with high and low peak firing rates were found within the same animal. The peak firing rate was negligibly correlated with the background firing rate ( $r = -0.14$ ). The preferred firing directions across all the HD cells were evenly distributed throughout  $360^\circ$  and the mean directional firing range was  $98.1 \pm 8.1^\circ$  (range:  $50.1$ – $149.2^\circ$ ). Unpaired  $t$ -tests showed that each of these firing properties was not significantly different from values reported in non-lesioned animals [32] ( $P > 0.10$ ;  $df = 38$  for all tests). The absence of a significant difference in the directional firing range suggests that the HD cells in lesioned animals were able to maintain a stable preferred firing direction within a recording session.

#### 3.3.2. Correlation of firing rates with linear and angular head velocity

Previous studies have suggested that HD cells are influenced by idiothetic inputs, i.e. vestibular, proprioceptive, and motor efference copy [13,24,28]. Other potential indi-

cators of idiothetic influences on HD cells include the findings that restraint of the animal affects HD cell discharge and that the cell's firing rate can be affected by either the animal's linear or angular head velocity ([1,27,33], also see below). Consequently, we assessed the relationship between the animal's linear and angular head velocity with HD cell discharge in LDN-lesioned animals and compared these results with intact rats.

The animal's linear velocity and mean firing rate were computed for each 100 ms episode where the rat's head pointed within  $\pm 6^\circ$  of the cell's preferred firing direction. Across lesioned animals, there was a mean of  $33.7 \pm 4.5$  episodes (range: 9–67 episodes) analyzed during the recording session. The mean correlation coefficient ( $r$ ) between the cell's firing rate and the animal's linear velocity for the 15 cells was  $+0.21 \pm 0.06$  (range:  $-0.26$  to  $+0.53$ ). This value is comparable to the value reported in intact animals ( $r = 0.18$ ) [32].

The correlation between the rat's angular head velocity (independent of turning direction) and the cell's firing rate was also computed for head directions within  $6^\circ$  of the cell's preferred firing direction. The mean correlation was  $0.085 \pm 0.021$  (range: 0.011–0.307). This  $r$  value is also comparable to the value previously reported for PoS HD cells in intact animals ( $r = 0.045$ ) [31].

#### 3.3.3. Analysis of spike timing and directional movement bias

Previous studies have demonstrated that ATN HD cells tend to anticipate the animal's future head direction by about 25 ms and that PoS HD cells lag ATN cell activity by approximately 30–40 ms [1,31]. To determine whether the time lag observed in PoS HD cells in LDN-lesioned animals was different compared to intact animals, we computed the optimal time shift which yielded the maxi-

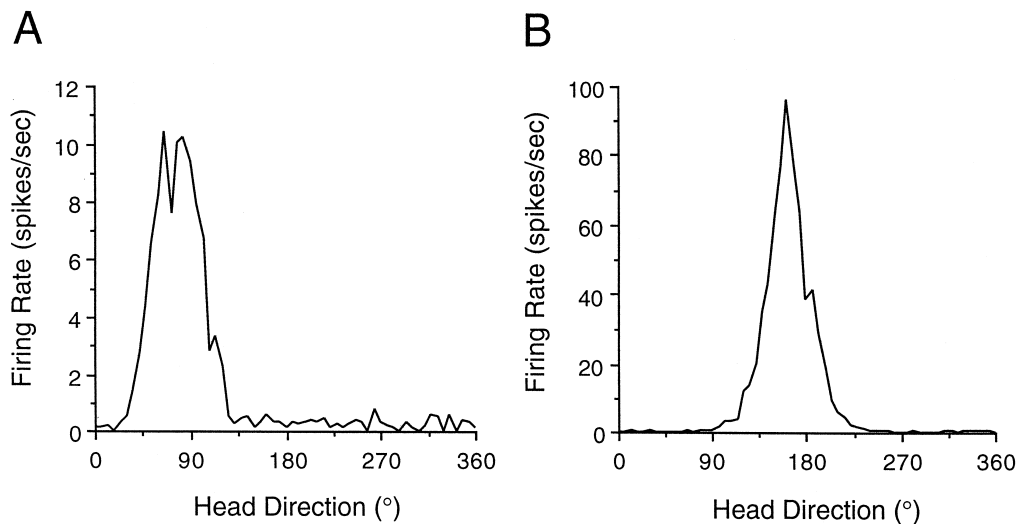


Fig. 2. Head direction versus firing rate plots for 8 min recording sessions. (A) PoS HD cell recorded from the animal with an electrolytic lesion shown in Fig. 1B. (B) PoS HD cell recorded from the animal with a neurotoxic lesion of the LDN depicted in Fig. 1E–G.

imum peak firing rate and information content. In addition, we also computed the angular difference (shift angle) between the firing rate vs. HD functions for CW and CCW head turns (for details, see Ref. [31]). The mean optimal time shift for the 15 cells was  $-0.47 \pm 0.74$  samples (range:  $-7$  to  $+4$  samples) for peak firing rate and  $0.40 \pm 0.83$  samples (range:  $-8$  to  $+4$  samples) for information content. Since each sample corresponds to 16.67 ms, the firing of PoS HD cells lagged the animal's current head direction by  $-7.83$  ms for the maximum peak firing rate measure and led the animal's current head direction by 6.67 ms for the information content measure. These values are not significantly different from values reported from intact animals (peak firing rate:  $t = 0.31$ ,  $df = 44$ ,  $P > 0.05$ ; information content:  $t = 0.83$ ,  $df = 44$ ,  $P > 0.05$ ). The mean shift angle in lesioned animals was  $2.40 \pm 2.40^\circ$  (range:  $-12.0$  to  $+18.0^\circ$ ) and also was not significantly different from values in intact animals ( $t = 1.97$ ,  $df = 44$ ,  $P > 0.05$ ).

### 3.3.4. Peak firing direction stability

To more directly assess the stability of the preferred firing direction over time, we examined each cell's preferred firing direction both within and between recording sessions. Because the LDN receives a prominent input from visual areas [36], it is possible that the utilization of visual landmark cues might be impaired in LDN-lesioned animals. This possibility could be manifested by a drift in the cell's preferred firing direction over time because landmark cues are thought to be important for maintaining a stable preferred firing direction [7,12,33]. Stability of the preferred firing direction within a session was assessed by dividing the 8 min recording session into two 4 min blocks and determining the difference in the preferred firing direction for the two blocks. The mean absolute shift between the two 4 min segments was  $0.0 \pm 1.7^\circ$  (range  $-6$  to  $+12^\circ$ ;  $n = 14$ ). With the exception of 1 cell, the difference in the preferred firing direction between the two segments was  $\leq 6^\circ$ . These results indicate that there was little drift in the cell's preferred firing directions, on the order of minutes, within the recording sessions.

The stability of the preferred firing direction between two different recording sessions was also assessed by comparing the preferred firing direction between two standard sessions which anchored the beginning and end of the cue rotation series. Approximately 10–15 min elapsed between the end of the first standard session and the beginning of the second standard session. The mean difference in the preferred firing direction between standard sessions was  $4.0 \pm 2.8^\circ$  (range  $-12^\circ$  to  $+18^\circ$ ;  $n = 12$ ). In addition, for 3 cells in 2 animals the preferred firing direction was compared in standard sessions recorded across two days. The mean absolute difference in the preferred firing direction between these sessions was  $14.0 \pm 4.0^\circ$  (range:  $6$ – $18^\circ$ ). This difference is similar to findings from non-lesioned animals [33]. In sum, the cells

preferred firing directions remained stable both within and between standard recording sessions.

### 3.4. Cue card rotations

To assess the cue card's influence on the cells preferred firing directions, we monitored the cells responses to  $90^\circ$  rotations of the cue card. Fig. 3 illustrates the firing rate vs. HD profiles for a HD cell recorded in a standard, rotation, standard session sequence. The  $90^\circ$  CCW rotation of the cue card led to an  $84^\circ$  shift in the cell's preferred direction. The cell's preferred direction then returned to its original orientation when the cue was returned to its initial position in the second standard session. A total of 12 cells from 6 animals were tested in the cue rotation series. The cue card was rotated  $90^\circ$  CCW for each series. The mean shift in preferred firing direction for the rotation sessions was  $78.0 \pm 6.0$  (range:  $18$ – $96^\circ$ ). This mean value was diminished somewhat due to one cell whose preferred firing direction shifted by only  $18.0^\circ$ . Ten out of 12 cells shifted their preferred firing directions between  $78^\circ$  and  $96^\circ$ . The mean absolute deviation from the expected rotation ( $90^\circ$ ), if the cell's preferred firing direction followed the cue card perfectly, was  $13.0 \pm 5.8^\circ$ , and was not significantly different from the value reported for non-lesioned animals ( $t = -1.0$ ,  $df = 11$ ,  $P > 0.30$ ) [32]. Therefore, the preferred firing directions of HD cells in LDN-lesioned animals followed the cue card as accurately as non-lesioned animals.

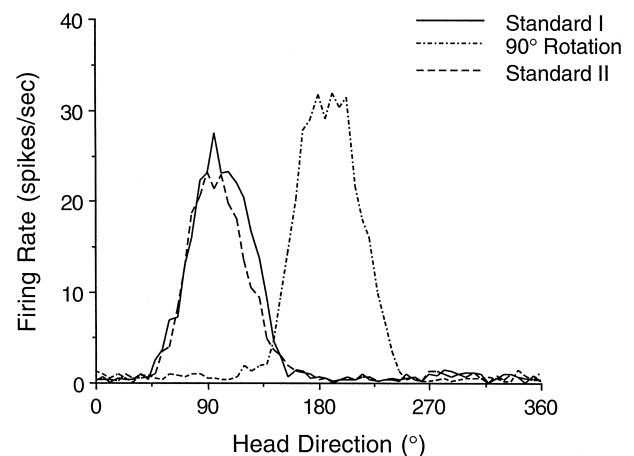


Fig. 3. Graph of an HD cell recorded in a cue card rotation series. The sessions were recorded sequentially: Standard I, Card Rotation, Standard II. For the Standard I and II sessions the cue card was centered at  $0^\circ$ . For the Card Rotation session, the cue card was rotated  $90^\circ$  CCW along the cylinder wall. In Standard I the cell's preferred firing direction was  $96^\circ$ . Following a  $90^\circ$  CCW rotation of the cue card the cell's preferred firing direction shifted  $96^\circ$  CCW to  $192^\circ$ . When the cue card was returned to its initial position, the cell's preferred firing direction shifted back to near its original position in Standard I. The small increase in the cell's peak firing rate in the Card Rotation session was attributed to a change in the isolation of the cell's waveform during recording.

### 3.5. Restraint conditions

#### 3.5.1. Peak firing rate

For 6 of the 13 PoS HD cells (from 5 animals) recorded under restraint conditions, the cell's discharge at all head directions was reduced to a level near background activity. Thus, these cells ceased to show direction specific firing. For brevity, these cell responses will be referred to as 'off' HD cells. In order to ensure that the cessation of cell discharge during restraint testing was not due to the isolation loss of the cell's waveform, a second standard session (freely moving) was conducted immediately following the restraint session. A paired *t*-test between the peak firing rates for the standard sessions pre- and post-restraint revealed that the values were not significantly different ( $t = 0.16$ ,  $df = 10$ ,  $P = 0.87$ ), indicating that the reduction in the cell's peak firing rate during the restraint session was not attributable to a lasting disruption in the ability to record the cell. Of the 7 remaining cells which continued to show directional activity when the animal was restrained, referred to as 'on' HD cells, the mean peak firing rate during restraint was reduced to  $73.2 \pm 10.4\%$  (range: 42.4–123.2%) of the cell's peak firing rate during the initial standard session. There was no propensity for a cell to be categorized as 'on' or 'off' based on the extent of its correlation between firing rate and angular head velocity.

Fig. 4 illustrates the responses from a restraint session for an off HD cell (Fig. 4A) and from an on HD cell (Fig. 4B). In all instances when more than one HD cell was recorded in an animal there was at least one cell from each category. For example, among two HD cells in one animal the peak firing rate during the restraint session was reduced to 12 and 45% of the peak firing rates in the standard session.

An earlier study on PoS HD cells reported that the cell's peak firing rates were frequently reduced during restraint sessions, but the complete cessation of cell activity was not observed [33]. Because the animals in the current study were more tightly restrained than the earlier study (i.e., wrapped snugly in a towel compared to being hand-held), we conducted a series of restraint sessions on 12 HD cells in four additional non-lesioned animals using identical testing conditions as the animals which had LDN lesions. Results showed that 6 cells were classified as off cells, while 6 cells were classified as on cells. Similar to the earlier study, the on cells tended to have lower peak firing rates during the restraint sessions (3 out of 6 cells had a peak firing rate  $< 80\%$  of the standard session). The mean percentage change in peak firing rate across the six on-cells during the restraint sessions was  $89.3 \pm 21.4\%$  (range: 26.2–164.2%). In sum, the distribution of 'on' and 'off' responses in the non-lesioned group was similar to the distribution observed in the LDN-lesioned group.

For the HD cells which remained active during restraint, a repeated measures analysis of variance comparing the peak firing rates across sessions and groups showed a significant effect for session ( $F = 6.00$ ,  $df = 1,10$ ,  $P < 0.05$ ), but no significant effects for group ( $F = 1.30$ ,  $df = 1,10$ ,  $P > 0.25$ ) or session by group interaction ( $F = 1.26$ ,  $df = 10$ ,  $P > 0.25$ ). In addition, the preferred firing directions of cells in both the lesioned and non-lesioned groups remained stable across the Standard-Restraint-Standard sessions. Taken together, these findings indicate that the peak firing rates of cells in both the lesioned and intact animals responded similarly when the animal was restrained.

On one occasion two HD cells were recorded simultaneously in a lesioned animal during restraint. Both of these

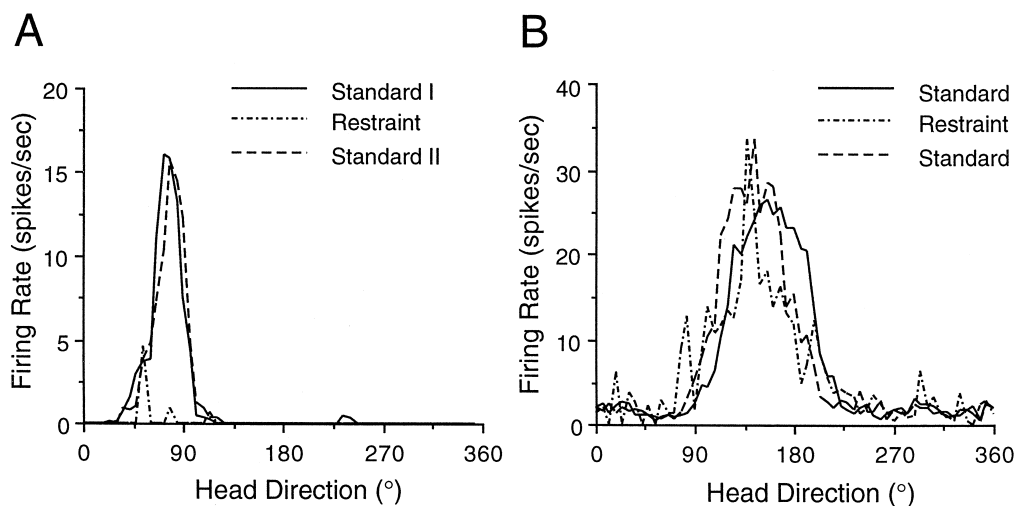


Fig. 4. Response of two HD cells during the series of restraint experiments. The sessions were recorded in the order Standard I, Restraint, Standard II. The Restraint sessions consisted of two collated 1 min sessions where the rat was gently rotated back and forth through  $60^\circ$  sweeps while the experimenter moved the rat around the cylinder sampling all  $360^\circ$ . (A) Example of a cell which ceased firing when the animal was restrained. This cell was classified as an 'off' HD cell. (B) Example of an 'on' HD cell which maintained direction-specific discharge during restraint.

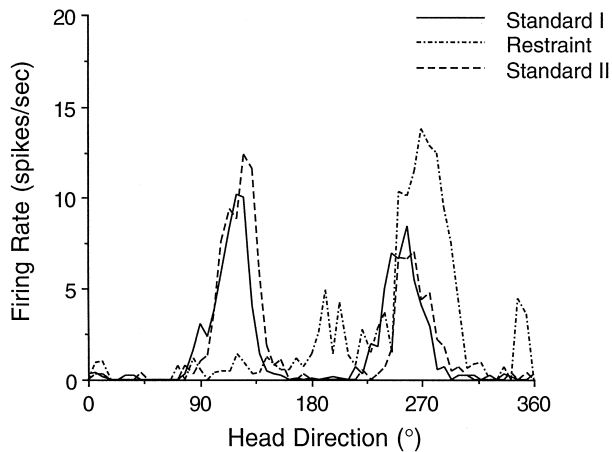


Fig. 5. Example of two HD cells that were recorded simultaneously during Standard and Restraint sessions. The cell on the left reduced its firing rate to near background levels during the Restraint session, and was categorized as an off cell. In contrast, the cell on the right increased its firing rate during the Restraint session and was categorized as on cell. The Standard sessions were 8 min, while the data from the Restraint session were collected from 3 recording sessions totaling 2 min 24 s. The greater variability observed in the responses during the Restraint session was probably due to the lower total sampling time compared to the Standard sessions. The elevated firing at approximately 190° and 350° was due to transient bursts of activity that did *not* occur consistently throughout the recording session. The reason for this activity was unclear.

cells were classified as off-cells. On four occasions in three control animals more than one HD cell was recorded during a restraint session. For two of these sessions both of the HD cells responded similarly to restraint. In one session they both ceased discharging while in the other session there was a reduction in their peak firing rates. In the other two cases there was a mixture of 'on' and 'off' responses. An example from one of these latter sessions is illustrated in Fig. 5. The finding of mixed response types within the same session suggests that the cessation of cell discharge under restraint is specific to particular cells and is not simultaneously found in all HD cells.

#### 4. Discussion

Our results showed that HD cells in the PoS were generally unaffected by the loss of input from the LDN. There were no significant differences in HD cell firing properties recorded from animals with LDN lesions when compared to control animals. Our findings also demonstrated that HD cells in the LDN-lesioned animals retained a small correlation with linear velocity, indicating the continued modulation of HD cell firing patterns by idiothetic cues. In addition, no abnormalities in the response of HD cells to environmental manipulations, such as restraint and cue rotation, were noted in the lesioned animals. Interestingly, the percentage of units classified as HD cells was less than in previously published reports [23,32]. This result may indicate a reduction in the total number of cells

which exhibit direction-specific firing in the PoS of lesioned animals. The remaining subgroup of HD cells that do not require an intact LDN would retain their normal firing patterns, as described above. Although this interpretation would have important implications for processing the HD cell signal, it is also possible that the decrease in the percentage of HD cells is due to differences in the way individual investigators isolate and classify units. For example, in another study using non-lesioned animals, the percentage of HD cells in the PoS was 17.7% (Goodridge and Taube, unpublished observations), a value which is closer to the percentage observed in the LDN-lesioned animals (10.6%). Similarly, in the four non-lesioned animals reported for the restraint experiments, only 11.9% of cells recorded in the PoS were classified as HD cells. Thus, although there were fewer PoS HD cells in LDN-lesioned animals, we cannot rule out interexperimenter differences in contributing to this result.

##### 4.1. Generation of the HD cell signal

Several studies have been conducted in order to determine the importance of various brain regions in the generation of HD cell activity [29]. HD cells have been identified within the ATN after ablation of the PoS [8]. Although we did not record from the ATN in the LDN-lesioned animals, we can predict on the basis of anatomical studies that lesions of the LDN are unlikely to disrupt the generation of the ATN HD cell signal because there are no direct connections between the LDN and ATN [38,39]. However, it is possible that the LDN could still influence the ATN HD signal through its connections with the retrosplenial cortex [38]. In contrast to these findings, the ATN is necessary for HD cell activity in the PoS [8], and it is noteworthy that we were unable to identify HD cells in the one animal which sustained damage to the right ATN, a result that is consistent with this earlier finding. Taken together, our results indicate that the LDN is not critical for the generation of the HD cell signal.

##### 4.2. Landmark control over the preferred firing direction

The LDN receives sensory information from the superior colliculus, pretectum, ventral lateral geniculate nucleus, and to a lesser degree, primary and secondary striate cortex [36]. Vision appears to be the primary modality conveyed by these pathways, although cells in the deep layers of the superior colliculus respond to auditory, somatosensory, or multimodal stimulus presentations as well [14,42]. The dorsal lateral portion of the LDN, the area where Mizumori and Williams [16] reported directionally tuned cells, receives strong afferents from visual areas and also sends a direct projection to the PoS [35,36,38]. Taken together, these findings suggest that the dorsal LDN should play an important role in the normal functioning of PoS HD cells. Our results indicate, however, that whatever

functions the LDN serves, it is neither essential for the generation of PoS HD cell activity, nor for the control of visual cues over the HD cell's preferred firing direction.

The above findings do not preclude a role for the LDN in the normal functioning of PoS HD cells, and there may be other aspects of HD cell activity or other spatial processes which are impaired following LDN lesions. For example, Mizumori et al. [15] reported that hippocampal place cells from animals in which the LDN were temporarily inactivated showed lower place specificity scores. Furthermore, these animals were impaired on a working memory spatial task. Taken together, because permanent lesions of the LDN did not lead to pronounced effects on PoS HD cell activity, these findings indicate that the LDN may have a stronger functional association with the hippocampal place cell system than with HD cells in the retrohippocampal region. Moreover, a recent study has indicated that the generation of directional activity in the PoS and ATN, and the ability of salient visual cues to exert control over HD cell preferred firing directions is not dependent on an intact hippocampus [6]. This finding implies that the LDN is unlikely to substantially influence the preferred firing directions of PoS HD cells via the hippocampus.

Our results show that despite the loss of LDN inputs, the preferred firing directions of PoS HD cells were consistent across recording sessions and shifted their preferred firing directions following rotations of the cue card. These findings indicate that at least some visual information must be reaching the PoS in LDN-lesioned animals and that the HD cells are still capable of utilizing landmark cues to establish their preferred orientations. Thus, other sources besides the LDN must be providing visual information to the PoS. The best candidate regions for providing visual input are direct projections from striate areas [40], or indirect pathways via the retrosplenial cortex and ATN [10,39,43]. Although it is possible that the hippocampus could convey some visual information to the PoS, this notion appears unlikely since hippocampal lesions do not abolish the cue card's control over the preferred firing direction of PoS or ATN HD cells [6].

#### 4.3. Restraint

When the animals were restrained no differences were observed in the firing properties of cells from the lesioned and control animals. The firing rate of neurons from both groups showed a variety of responses under the restraint condition. Importantly, when more than one cell was simultaneously recorded in restrained control animals both 'on' and 'off' cells were observed. This finding suggests that HD cells in the PoS network appear to exhibit a heterogeneous response to the restraint condition, where some cells ceased directional discharge, while others remain directional and only exhibit an attenuation in their peak firing rate. Restraint may disrupt the firing of some HD cells by interfering with motor efference or proprio-

ceptive cues, since vestibular inputs are not directly affected by restraint. However, HD cells appear to continually fire in the absence of movement in unrestrained animals [32]. Thus, the critical variable for cell firing may be the animal's ability to freely locomote, rather than actual movement. Similar findings have also been reported for hippocampal place cells following restraint [4]. More detailed analyses of HD cell discharge when the animal is not moving would help clarify the roles of motor efferent copy and proprioceptive cues in HD cell firing.

In summary, HD cells in the PoS are not markedly affected by the loss of input from the LDN. The characteristic firing properties of HD cells in the PoS, as well as their response to alteration in environmental cues and the restraint condition, were preserved in LDN-lesioned animals.

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