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The Head-Direction Signal Plays a Functional Role as a Neural Compass during Navigation

Highlights

- Inhibition of prepositus→dorsal tegmental pathway impairs head direction (HD) cell stability
- This instability is consistent with a disruption of angular head velocity inputs
- Influencing HD cell stability during navigation impairs homing behavior

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In Brief

Butler et al. used optogenetically targeted inhibition of the brainstem to induce instability in the head-direction network without affecting locomotor behavior. These induced directional shifts in head-direction cell tuning caused the animals to make equivalent homing errors, providing evidence that this system plays a causal role in navigation.

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The Head-Direction Signal Plays a Functional Role as a Neural Compass during Navigation

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SUMMARY

The rat limbic system contains head direction (HD) cells that fire according to heading in the horizontal plane, and these cells are thought to provide animals with an internal compass. Previous work has found that HD cell tuning correlates with behavior on navigational tasks, but a direct, causal link between HD cells and navigation has not been demonstrated. Here, we show that pathway-specific optogenetic inhibition of the nucleus prepositus caused HD cells to become directionally unstable under dark conditions without affecting the animals' locomotion. Then, using the same technique, we found that this decoupling of the HD signal in the absence of visual cues caused the animals to make directional homing errors and that the magnitude and direction of these errors were in a range that corresponded to the degree of instability observed in the HD signal. These results provide evidence that the HD signal plays a causal role as a neural compass in navigation.

INTRODUCTION

Navigation is a complex process requiring the integration of multimodal internal and external cues in order to guide behavior [1, 2]. Decades of research have shown that a network of spatially tuned neurons exist within the brain [3], including place cells [4], head direction (HD) cells [5], and grid cells [6]. HD cells are the most basic of these representations and fire according to animals' heading in the horizontal plane [7]. The firing of these HD cells is thought to be an important component of the more complex spatial tuning of grid cells and place cells [8, 9]. Due to their directional representation, the signal formed by the firing of a population of HD cells has been conceived of as a "neural compass" that can guide an animal's behavior, and previous work has shown that an animal's heading over time can be reliably reconstructed from the firing of an ensemble of HD cells [10]. However, attempts to relate the firing of these cells to behavior have yielded mixed results, depending on the type of spatial task used [11–13]. The most convincing evidence that HD cells may provide a directional signal for guiding behavior has come from recording studies using a path integration task, which found that HD cell tuning strongly correlates with the direction of animals' behavioral choices in two variations of a homing task [14, 15]. Correlations between place cell firing and spatial behavior, especially in relation to goal-driven behavior [16], have prompted recent work showing that there is a causal link between place cells and an animal's sense of location [17]. However, despite numerous lesion studies showing indirect evidence that the HD circuit might be involved broadly in spatial processing [18–21], a direct causal role of the HD signal as a neural compass that guides directional behavior has not been demonstrated yet.

The intrinsic organization of the HD signal has been conceptualized as a ring attractor, wherein the activity of the network is shifted to the proper population of HD cells by angular head velocity inputs to the network [22]. This ring attractor organization is thought to be generated by reciprocal activity between the lateral mammillary nucleus and dorsal tegmental nucleus (DTN) [23, 24]. Damage to areas upstream of these nuclei produces a bursting pattern of firing in anterodorsal nucleus of the thalamus (ADN) cells that resembles HD cells that are disconnected from the animal's heading [25, 26]. This tegmento-mammillary circuit receives its inputs from two brainstem nuclei: the supragenual nucleus and the nucleus prepositus hypoglossi (NPH) (see Figure 7C), which are believed to provide an angular head velocity (AHV) signal to the DTN that is integrated over time in order to form and update the HD signal [27, 28]. To address the role of the HD signal in navigation, we unilaterally targeted the NPH inputs into the network in order to impair HD cell stability without affecting the ring attractor organization of the network and to minimize disruption of the vestibular system and overall locomotor behavior. Collectively, we used a dual virus optogenetic approach that disrupted a single pathway, which provides inputs into the attractor network, in order to selectively control the stability of the HD signal.

RESULTS

We injected a retrogradely transported canine adenovirus carrying Cre recombinase (CAV2-Cre) into the DTN unilaterally (Figure 1A). We then infused the ipsilateral NPH with an adeno-associated viral vector carrying either halorhodopsin (eNpHR3.0-eYFP) or a no-opsin control (eYFP only), which were both expressed in a Cre-dependent manner using the double-floxed inverted open reading frame vector approach (Figures 1B and S1). We recorded HD cells several synapses downstream in the ADN, an area containing a high proportion of HD cells [29] that transmits the HD signal to the cortex [26] while animals freely foraged for randomly scattered sucrose pellets in a circular environment (Figure 1C).

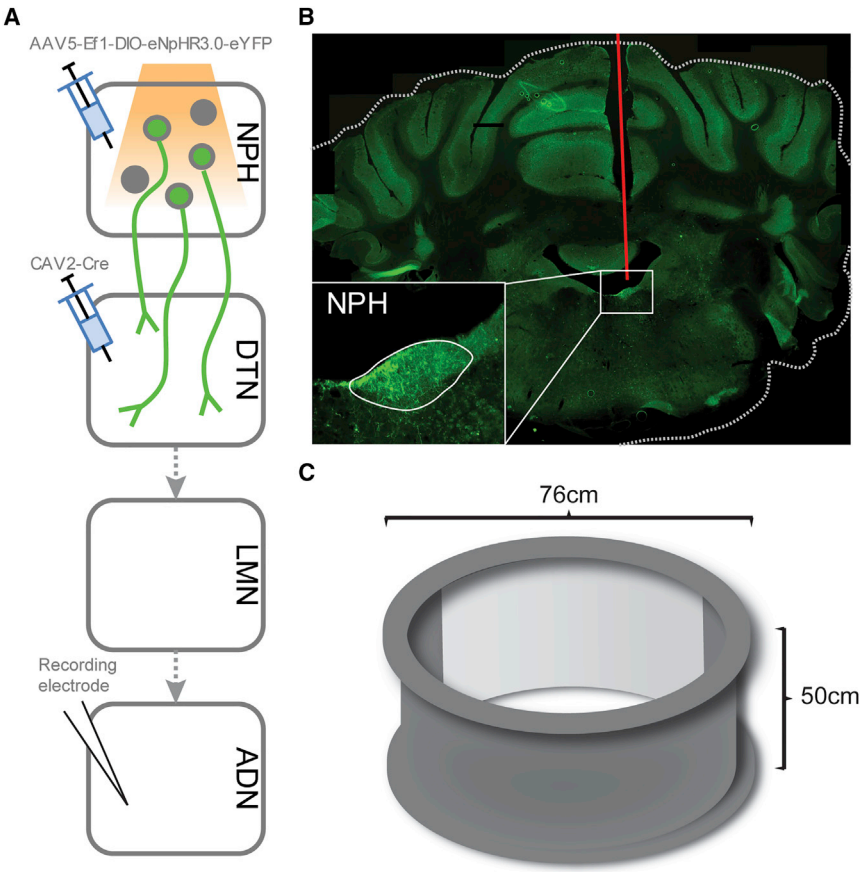

Figure 1. Optogenetic Targeting of Inputs to the HD Network

(A) Cells in the NPH with projections to the HD circuit were infected with Cre-dependent halorhodopsin via retrogradely transported Cre-recombinase that was injected into the dorsal tegmental nucleus (DTN). HD cells were recorded downstream in the anterodorsal nucleus of the thalamus (ADN).

(B) Expression of halorhodopsin was confined to the NPH. These NPH→DTN cells were inhibited by 593.5 nm yellow-orange light delivered through a fiber implant; red line indicates the track of the fiber implant.

(C) After viral infection, animals were screened and recorded in a cylindrical environment under standard (with visual landmark) and dark conditions. See also Figure S1.

Selective Manipulation of the Stability of the HD Cell Signal

Photoinhibition of NPH→DTN neurons resulted in ADN HD cell instability only under dark conditions when no visual cues were available to the animal (Figures 2 and S2). Under standard conditions, HD cells maintained stable directional tuning during laser illumination, as measured by their preferred firing direction (PFD) (mean cumulative PFD shift from the start to end of the session: 6.91°). The cumulative PFD shift during laser illumination did not differ significantly from the standard laser off sessions ($t(75) = -0.567$; $p = 0.57$), nor did the HD cells' overall Rayleigh values (Wilcoxon rank-sum test; $p = 0.07$). In addition, HD cells' general firing properties, including peak firing rate ($t(75) = 0.396$; $p = 0.69$), directional firing range ($t(75) = 0.973$; $p = 0.33$), and background firing rate (Wilcoxon rank-sum test, $p = 0.26$) were also unaffected. Further, the anticipatory time interval (ATI) of HD cells also did not change between standard laser off and standard laser on conditions in the halorhodopsin group (paired $t(7) = 0.402$; $p = 0.70$).

In contrast, during laser illumination under dark conditions, rather than firing to a single consistent direction in the environment, the PFDs of the HD cells were unstable and shifted constantly over time in a continuous manner, in some cases rotating a full 360° over the course of the 8 min recording session (mean absolute shift: 137.30°). Post hoc testing revealed a significant increase in the absolute cumulative PFD rotation between dark laser off and dark laser on sessions ($t(93) = -4.294$; $p = 0.0004$). Unsurprisingly, this increase in directional instability was also associated with a significant decrease in Rayleigh value ($t(93) = 3.859$; $p = 0.0002$) and a significant increase in directional firing range ($t(93) = 2.607$; $p = 0.011$). These results are similar to the effects of a partial lesion of the NPH, which similarly caused HD cell instability only under dark conditions [28].

Though HD cell PFDs changed continuously over time under dark laser on conditions, the rate of shift in the PFD was not constant throughout each session. For example, over a 10 s segment, the PFD sometimes shifted quickly (>6°/s), whereas at other times shifted slowly or not at all (<0.01°/s). Further, the PFD shifts could occur in one direction for a period a time (e.g., clockwise) and then switch to the opposite direction (e.g., counter-clockwise; e.g., see Figures 3A and S3). Correlations between the animals' cumulative head turns and the cumulative changes in PFD were often significant (87% of dark laser on sessions). In this subset of sessions, instead of a constant shift over time, the changes in the PFD were either significantly correlated (61.5% of sessions; Figure 3A, left) or significantly anti-correlated (38.5% of sessions; Figure 3A, right) with the animals' cumulative head turns over the course of the session. Accordingly, the Pearson's r values for these correlations followed a bimodal distribution (Figure S3). The proportion of sessions in which the HD cell's PFD shifted in the same direction as the animals' cumulative head turns was similar between sessions where the animal turned cumulatively in a counter-clockwise (CCW) direction (69%) or cumulatively in a CW direction (60%), even though the inhibition was always on the same side of the brain. Importantly, despite this instability, the angular spacing between the PFDs of co-recorded pairs of HD cells (n = 13 cell pairs, recorded from three eNpHR3.0 animals) remained constant (Figure 3B) and HD cells' maximum firing rates did not change across conditions ($F_{1,349} = 0.60$; $p = 0.44$). We inferred from these data that the organization of the HD network remained intact during this instability and

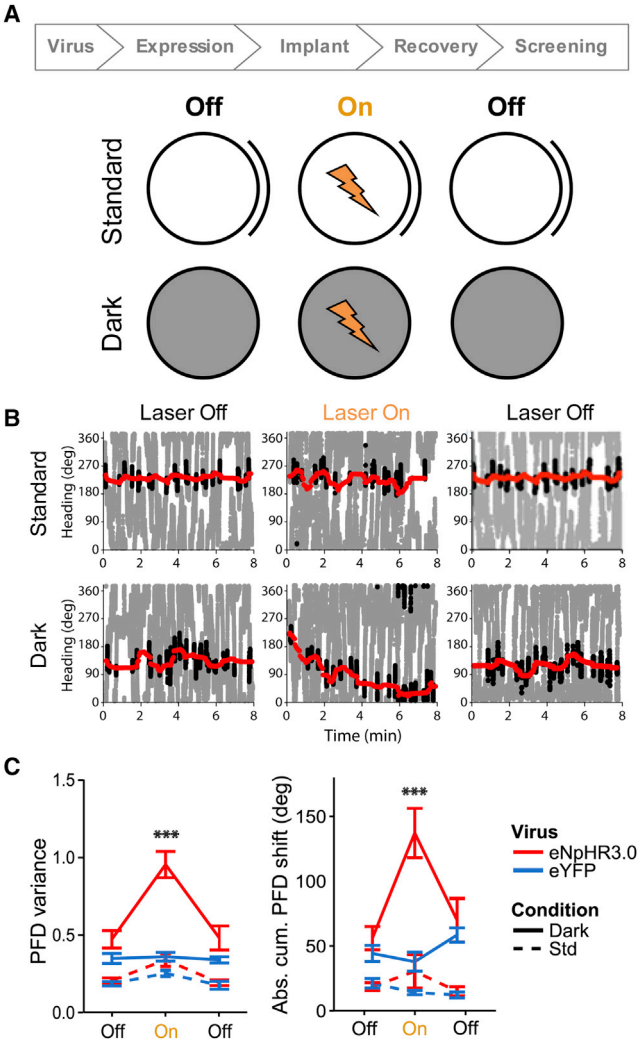

Figure 2. Projection-Specific Inhibition of NPH Neurons Causes HD Cell Instability under Dark Conditions

(A) Outline of the experimental timeline. HD cell activity was monitored with and without laser illumination under either standard or dark conditions. For the dark condition sessions, there was no landmark cue present. Table S1 describes how many observations were made under each of these conditions.

(B) Two HD cells recorded across three sequential 8 min sessions under either standard (top) or dark conditions (bottom). The cell recorded under dark conditions displayed a dramatic shift in directional tuning (~200°) over the course of the photoinhibition session. Black, headings in which the cell fired at a rate >70% of its peak firing rate; gray, animal's heading; red, the calculated preferred firing direction (PFD) of the cell.

(C) Optogenetic disruption of the NPH→DTN inputs causes significant HD cell instability only under dark conditions in eNpHR3.0 rats (n = 5) relative to eYFP control rats (n = 4) (interaction, three-way ANOVA, virus × laser × condition: $F_{1,353} = 40.04$; $p < 0.001$). Values are shown as mean ± SEM.

See also Figures S2 and S7.

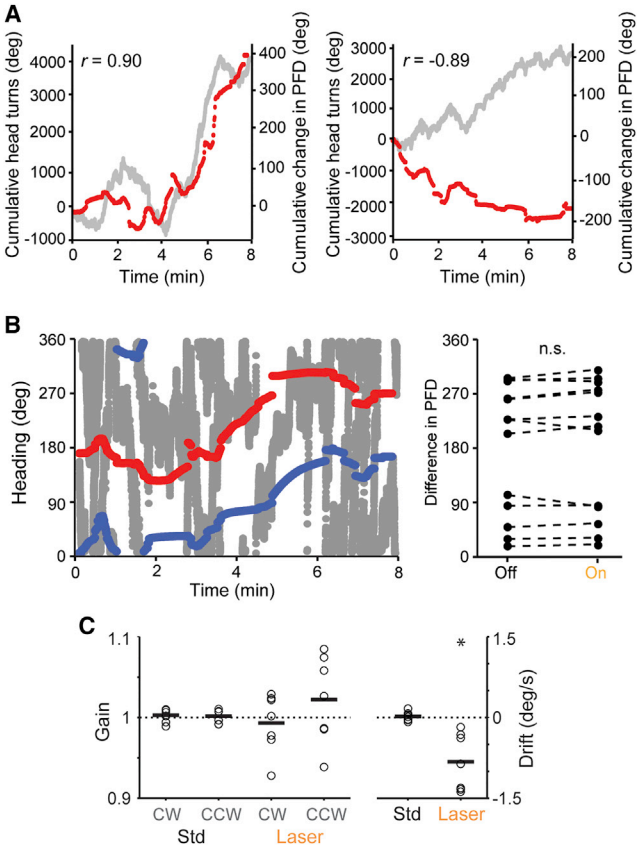

Figure 3. HD Cell Network Organization Is Maintained during Directional Instability

(A) The direction of "drift" in HD cells' PFD tended to correlate significantly either positively (left) or negatively (right) with the animals' head turns (mean $r^2 = 0.49 \pm 0.05$). Gray: the animal's cumulative head turns; red: the cumulative change in the HD cell's PFD.

(B) HD cell pairs maintain a constant orientation relative to one another, despite their overall preferred firing direction (PFD) instability. The difference in directional tuning between co-recorded HD cell pairs (n = 13) did not change between dark laser off and dark laser on sessions ($F_{1,12} = 0.85$; $p = 0.38$). Blue, the calculated PFD of another simultaneously recorded HD cell; gray, animal's heading; red, the calculated PFD of one HD cell.

(C) The instability observed in the HD signal during dark laser on sessions is best modeled by a combination of a gain change in the inputs to the HD network relative to animals' actual head turns (in both the CW and CCW direction) and a constant drift term that results in the signal accumulating error at a constant rate. The individual gain (left) and drift (right) parameters obtained for each session (standard [Std] or dark laser on) are indicated by open circles. Dark lines, means of each group. Dashed lines indicate the values corresponding to a perfectly stable HD signal.

See also Figures S3–S5.

that the amount of excitatory drive provided to downstream structures, such as the parahippocampal and entorhinal cortices, was not diminished. Further, these results support the view that the shifts in the PFD were caused by a disruption of head movement inputs into the postulated ring attractor network.

Computational Modeling of the HD Signal's Instability

Models of the HD circuit have identified multiple distinct components that, when disrupted, could result in HD tracking error. These errors include incorrect integration of AHV inputs to the HD network (as characterized by a gain parameter; deviations from a gain of 1 lead to error proportional to the animal's AHV) and drift (which leads to error independent of the animal's head movements). We fitted several HD models to the spiking data from the dark laser on session in order to infer the

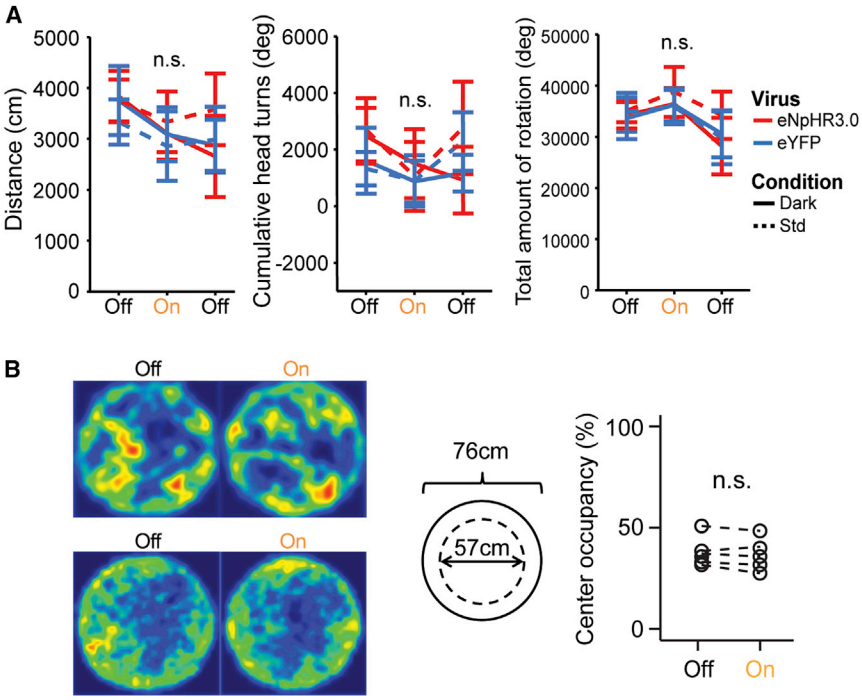

Figure 4. Locomotor Activity Is Not Affected by Inducing HD Cell Instability

(A) Animals' locomotion is not affected by the optogenetic manipulation (three-way interactions of virus × condition × laser for each measure: $F_{1,163} = 0.016$, $p = 0.90$; $F_{1,163} = 0.22$, $p = 0.64$; $F_{1,163} = 0.09$, $p = 0.77$). Values are shown as mean ± SEM.

(B) Experimental (eNpHR3.0) animals' sampling of the environment did not vary between dark laser off and dark laser on recording sessions (left; representative place/time heatmaps of sessions from two different experimental animals). The proportion of the session that eNpHR3.0 animals (n = 5) spent in the center portion (radius = 75% of total radius) of the apparatus relative to the periphery did not significantly change between dark laser off and dark laser on sessions (right; paired $t_{(4)} = -2.02$; $p = 0.11$).

underlying deficit most compatible with the observed tracking error (Figure S4). The unstable HD cell data were best explained by a combination of both (1) changes in the clockwise (CW; toward the side of the opto-inhibition) and CCW gains of the angular inputs to the network and (2) varying degrees of CW drift (Figures 3C and S5). However, the change in the gain parameter was relatively small compared to the large change in the drift parameter. This result is similar to findings in monkeys where unilateral neurotoxic lesions of the NPH produce (1) a gaze holding failure in both eyes and eye position drifts back to a central position after a saccade and (2) only minor changes in gain for the horizontal vestibulo-ocular reflex [30, 31]. Although only the estimated drift parameter had a mean that was statistically different from the ideal value (0; $p = 0.016$; Wilcoxon rank-sum test; CW and CCW gain both non-significant), there were significant differences in the variance of the estimated gain parameters between standard (Std) and dark laser on conditions (Levene test; CW: $F_{1,12} = 5.97$, $p = 0.03$; CCW: $F_{1,12} = 12.83$, $p = 0.004$), supporting our interpretation that animals' head turns contributed to the instability of the HD cells. In summary, we found that the changes in HD cells' PFDs observed during NPH illumination were consistent with the disruption of angular head velocity inputs to the HD network.

Locomotion Is Not Affected by Disruption of the NPH→DTN Pathway

Importantly, animals' locomotor behavior was not altered during this optogenetically induced HD cell instability. The total linear distance traveled, the cumulative head turns, and the total amount of absolute head rotation were all not affected by the optogenetic manipulation (Figure 4A). Further, the ratio of time spent in the center of the arena relative to time spent in the periphery was also equivalent between experimental conditions (Figure 4B), indicating that animals did not resort to a thigmotaxic pattern of movement during HD cell instability. Together, these results suggest that the instability of the HD signal did not produce any changes to the animals' normal locomotor behavior during these free-foraging recording conditions. Co-recorded theta-modulated firing in the thalamus (quantified by analyzing theta-related spike autocorrelograms) was also not affected by the optogenetic-induced instability (Table S2). This finding further indicates that the experimental manipulation was specific to the inputs to the HD network and not to the vestibular system in general, because vestibular system lesions lead to reduced power and frequency in the hippocampal theta rhythm without affecting the relationship between firing rate and linear velocity [32].

Manipulation of Homing Behavior via HD Cell Instability

We then tested animals' directional behavior on a food-carrying task [33] both with and without this manipulation of HD cell tuning. The food-carrying task has been used previously as a reliable method of dissociating the contributions of internal and external cues to navigation [34] and takes advantage of the natural proclivity for rodents to compute a return path to a home base after foraging for food in the environment [35]. In this experiment, animals were trained to retrieve food pellets from random locations on a featureless circular platform and bring them back to a home refuge (Figure 5A); the HD signal was not manipulated during training. As in the recording experiment, animals were trained and tested under both standard (with a prominent visual cue) and dark conditions; these conditions were maintained over the entire duration of each training or testing session. As reported previously [34, 36], animals were less accurate at homing under dark conditions compared to light conditions (ANOVA, main effect of condition; homing error: $F_{1,540} = 11.224$, $p = 0.0009$; homing circuity: $F_{1,539} = 31.463$, $p < 10^{-7}$). During optogenetic testing, NPH→DTN photoinhibition (on a 10 s on/1 s off cycle throughout the entire session) did not affect animals' ability to accurately direct their homing paths toward

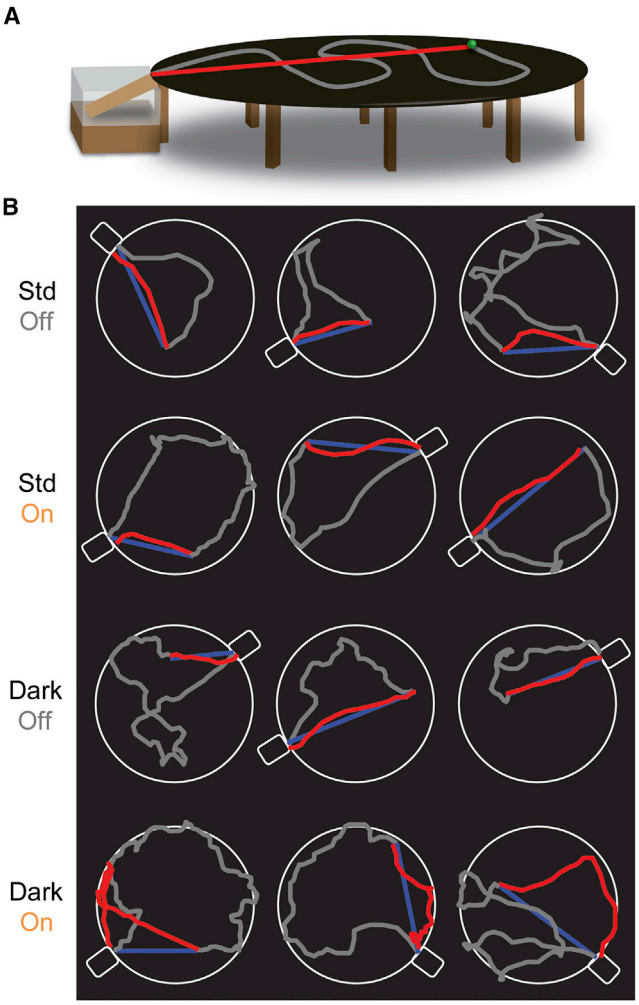

Figure 5. Testing Path Integration through a Food-Carrying Task

(A) The food-carrying apparatus was composed of a circular platform (182 cm diameter) with a home refuge placed just below the edge of the platform at one of four possible locations on its periphery. Animals were trained to forage (gray) for a food pellet (green) and carry the food pellet back to the refuge along a homing path (red) for consumption.

(B) Examples of eNpHR3.0 animals' foraging (gray) and homing paths (red) under each experimental condition. The blue line indicates the most direct path between the food pellet and the refuge for each trial.

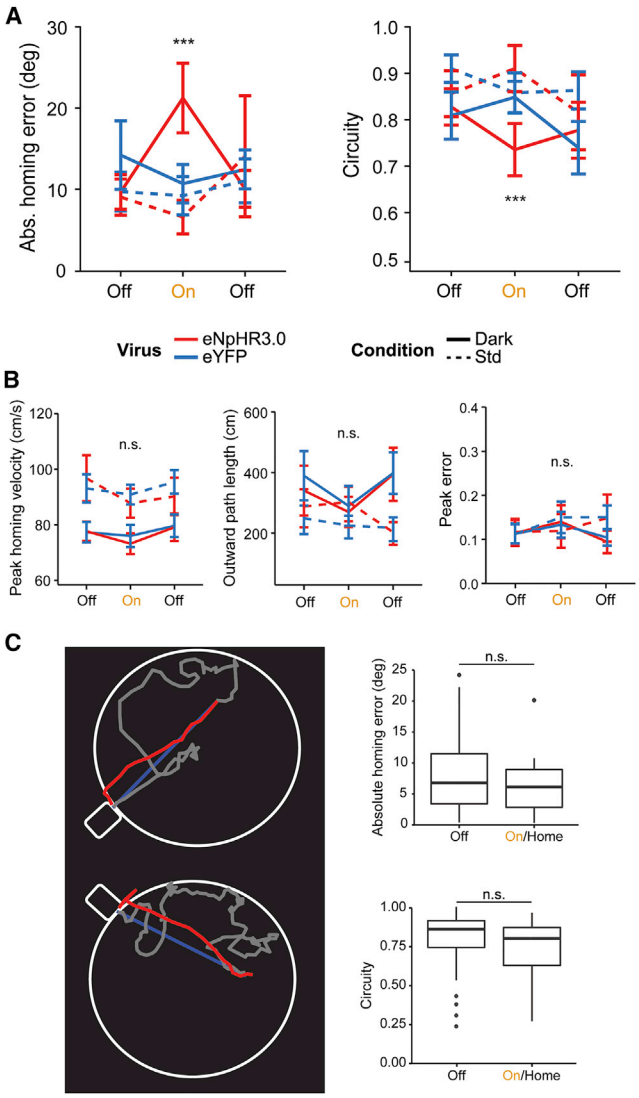

Figure 6. Inducing Instability in the HD Signal Results in Inaccurate Homing

(A) Photoinhibition of NPH caused inaccurate (left; $F_{1,536} = 19.76$; $p < 0.0001$) and less efficient (more circuitous) homing paths (right; $F_{1,535} = 15.51$; $p < 0.0001$) under dark conditions in eNpHR3.0 rats (n = 8) relative to eYFP controls (n = 8). Values are shown as mean ± SEM.

(B) Left: animals still displayed a kinematically typical increase in speed and reached a similar peak velocity on their homeward trip, despite their inaccuracy during homing ($F_{1,536} = 0.89$; $p = 0.35$). Middle: animals' impairments in homing were not due to longer foraging paths ($F_{1,536} = 0.39$; $p = 0.53$). Right: animals' peak error, a measure of their innate ability to estimate the upcoming length of their homing path and modulate their speed accordingly, also was not disrupted ($F_{1,526} = 1.057$; $p = 0.30$). Values are shown as mean ± SEM.

(C) Animals' behavior is unaffected by manipulating the HD signal after path integration. Left: examples of homing paths under dark conditions when laser illumination is confined to the homing path alone. Right: under dark conditions, eNpHR3.0 animals' (n = 2) homing is just as accurate as laser off conditions when laser illumination is confined to the homing path alone (homing error: unpaired $t_{(94)} = 0.83$, $p = 0.41$; homing circuity: unpaired $t_{(93)} = 1.54$, $p = 0.13$).

the refuge under standard, well-illuminated conditions but impaired their homing in the absence of visual cues (Figure 5B). Under dark conditions with laser illumination, animals' directional homing error and the total distance traveled when attempting to return to the refuge were both significantly increased (Figure 6A). Despite these impairments, there were no significant differences in animals' peak homing speed or in the length of animals' outward paths while searching for the food pellet (Figure 6B), suggesting that the manipulation of the HD signal stability was selective to the angular component of their navigational response. Previous work in the food-carrying task has also shown that rats are able to modulate their homing path speeds as a function of the distance they must travel, such that the point of greatest velocity falls near the midpoint of their homing path

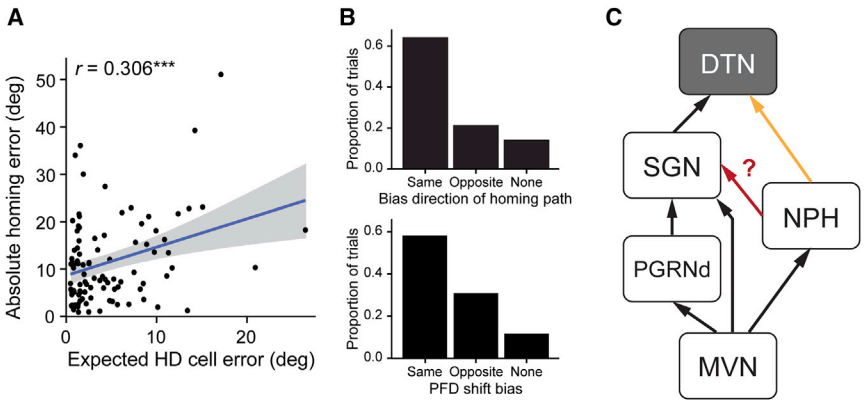

Figure 7. The Degree and Directionality of Navigational Error Matches the Instability Observed in the HD Network

(A) The degree to which individual animals (n = 4) were impaired in the food-carrying task correlated with the degree of instability observed in their HD cells (under the same recording conditions) and the length of time spent searching for the food pellet on each trial (r = 0.306; p < 0.0001). Blue, fit line; shaded gray area, SEM.

(B) The direction of eNpHR3.0 animals' (n = 8) homing paths in the food-carrying task relative to the direction of their outward path (top) matches the direction of drift in the HD signal relative to the direction eNpHR3.0 animals' (n = 5) head turns in the recording experiment (bottom; χ^2 = 2.18; p = 0.34).

(C) Alternative sources of self-movement information for the HD network are not directly disrupted by the optogenetic methods. The medial vestibular nucleus (MVN) is a likely source for self-movement inputs to the HD circuit and has projections to not only the NPH but also the dorsal paragigantocellular reticular nucleus (PGRNd) and supragenual nucleus (SGN), which also most likely contribute to HD cell stability. The NPH→DTN pathway (orange) is the primary pathway targeted directly in the current approach; it is unknown whether the NPH→SGN pathway (red) is also affected, because retrogradely infected NPH cells may also send collaterals to SGN.

See also Figure S6.

[36]. We therefore performed a kinematic analysis on the rats' return trips to the refuge in order to assess their ability to perform linear distance estimation. We compared the ideal midpoint of their return trip path to the actual point that contained the greatest linear velocity. Further supporting our interpretation of the impaired behavior, this measure of animals' distance estimation was also not affected by our manipulation (Figure 6B, right). In other words, the animals were still capable of foraging for the food pellets and initiating homing paths at the correct velocity and distance, but these paths were no longer accurately directed toward the refuge.

In contrast to these impairments, NPH→DTN inhibition that was initiated when the animal reached the food pellet did not affect the subsequent homing path (Figure 6C, left). Neither the degree of angular error in the return path nor the length of the homing path varied from control conditions when photoinhibition was confined to the homing path alone (Figure 6C, right). These results support the view that photoinhibition over the whole session caused the HD signal (PFD) to gradually rotate or drift during the animal's outward foraging path and that the animal then took a directional bearing from this inaccurate HD signal in order to determine its homing path.

Comparing Behavioral Impairments to HD Cell Instability

In order to further investigate the possible causal link between the instability of the HD signal and the instability in animals' homing behavior, we performed two additional analyses. First, four animals (two experimental and two control) were run in both the recording and behavioral experiments. Within this group, we first determined the average rate of instability in the HD signal for each animal under each type of experimental condition (standard/laser off, standard/laser on, dark/laser off, and dark/laser on). An assumption of this analysis is that the degree of instability is largely consistent within a session; this assumption was supported by comparing the PFD shift in the first half of each session to the PFD shift in the second half of the session (Figure S6A). Therefore, as a substitute for directly recording during this homing task, we instead calculated each animal's average rate of instability for each session type. Although the drift rate of each cell's PFD was somewhat variable over time and the timescale for each homing trial (~20 s) was markedly shorter than the timescale for HD cell recording (8 min), this drift rate represented the best estimate for each cell's instability. This average rate was then multiplied by the length of time the animal spent foraging on each trial to predict the amount of shift in the HD signal on that trial. We then could compare animals' actual behavior to this predicted amount of HD signal error on each trial. Despite the indirect nature of this approach, we found that there was a significant correlation between the amount of homing error on each behavioral trial and the predicted amount of shift in the HD signal during the foraging path on that trial (Figure 7A). We further confirmed that this correlation was not simply due to the non-normality of our data by showing that a log transformation of the data also yielded a significant correlation (r = 0.254; p = 0.003; Figure S6B).

Additionally, we compared the direction of shift in the HD signal relative to animals' head turns in the first experiment and the direction of homing error relative to animals' head turns in the second experiment. We found that the proportion of trials in which HD cells were correlated or anti-correlated with the animal's head turns was comparable to the proportion of trials in which animals made errors in the same or opposite direction of their overall head turns during the outward foraging path (Figure 7B). In other words, the direction of animals' homing error, relative to the direction of their outward paths, was comparable to the direction of shift in the HD signal observed in the recording experiment. Taken together, these results further support the claim that animals' behavioral impairments on the food-carrying task were due to their taking a directional bearing from an unstable HD signal.

DISCUSSION

In summary, we found that optogenetic manipulation of the subset of NPH neurons projecting to the HD circuit produced instability in downstream HD cells in the absence of visual

cues. By creating an artificial disconnect between the neural representation of direction and the animals' actual heading, we were then able to demonstrate that animals' directional homing behavior depends upon the directionality of the HD signal, providing confirmation of the long-hypothesized role of HD cells as a neural compass in navigation. Although we did not combine all of our experimental techniques into a single unified experiment (due to the technical difficulty of simultaneously implementing electrophysiology, optogenetics, and trained navigational behavior), this study provides additional evidence of a causal relationship between HD cell firing and behavior. This additional evidence critically supplements and expands upon previous studies that have found correlational evidence of this relationship [11–15]. Specifically, this study is the first one to directly and selectively manipulate HD cell firing and subsequently show that this manipulation has a significant effect on behavioral performance.

Previous studies examining the possible importance of the HD signal in behavior have generally fallen into two research strategies. One approach has attempted to show a relationship between HD cell tuning and animals' directional behavior by recording cells during behavior. The results from these studies have been mixed; different studies have shown either correlation or lack of correlation between HD cell activity and spatial responding depending on the behavioral task used [11–13]. More recently, two studies have had success demonstrating a significant correlation between HD cells and navigation in the form of homing behavior [14, 15]. However, one limitation of these experiments is that they did not manipulate neural circuits in a way that would allow them to conclude that there is a causal relationship between HD cell firing and navigation. In the same way that showing correlations between place cells and goal behavior does not necessarily demonstrate that the animals use place cells as a "cognitive map," these studies were not able to show that animals actually used the HD signal as a neural compass.

The other approach to explore the role of HD cells in behavior has shown that lesions of areas involved in the HD signal leads to various deficits in spatial ability [18–21]. However, a major limitation to interpreting these studies as demonstrating that HD cells are used in navigation comes from the absence of specificity afforded by a lesion. For example, lesions to areas such as the mammillary bodies [21] or anterior thalamus [18, 20] most likely have broader effects on learning and memory in addition to their effects on HD cells [37, 38]. In addition, if the effects that these lesions have on behavior are due to knocking out the HD signal, it is unclear why lesions further upstream in the network have greater effects than lesions in, for example, downstream cortical regions [20]. In contrast to this previous work, by using the temporal and anatomically specific inactivation afforded by optogenetics, we were able to address the role of HD cells in behavior much more directly by targeting a well-defined input to the HD network.

The NPH is thought to be important for integrating angular information about an animal's head turns to subserve both the oculomotor [30] and HD circuits [28]. Our results in both experiments are consistent with a selective disruption of angular head turn inputs to the HD network. During recording, only the angular stability of the HD signal was affected by optogenetically silencing the NPH; additionally, computational modeling of the HD signal's instability showed that inaccurate angular head turn inputs (both constant drift as well as small changes in CW and CCW gain) significantly explained the shifts in PFD observed in the HD cells. In the food-carrying task, animals' behavioral impairments were confined to the angular direction of their homing paths and were consistent with the expected drift of the HD network, further supporting our interpretation that these shifts in navigational behavior were caused by the animals' reliance on an unstable neural compass.

Our finding that the instability of the HD network was related to the animals' head turns is also noteworthy. An important caveat of our methods is that they do not disrupt all of the proprioceptive and vestibular-derived inputs into the network (Figure 7C). Instead, we interpret our effects as being due to the introduction of error into a subset of these inputs, causing a gradual drift of the HD signal over time. Other possible routes of proprioceptive information, such as the dorsal paragigantocellular reticular nucleus (PGRNd) [39], are not directly affected. Inputs from the supragenual nucleus (SGN) to the DTN are also not directly inhibited, although it is not known the extent to which the SGN→DTN projection is affected by the inhibition of the NPH→DTN pathway (Figure 7C, red arrow), because the same NPH cells that are infected with the virus could theoretically also project to SGN. Regardless, it is likely that a subset of the inputs to the DTN, primarily from the SGN, is unaffected by the photoinhibition of NPH. This point helps to explain the difference between our results and the results of previous work with lesions or knockouts of vestibular inputs, which recorded only non-directional bursts of firing instead of HD cells [26, 40]. Because of the subtler optogenetic methods employed in this study, we also were able to examine the gradual accumulation of error in the HD network more closely.

Both grid cells and place cells utilize the firing of HD cells in the anterior thalamus to varying degrees [8, 9]. Unlike previous studies that used inactivation to separate the contributions of theta rhythmicity [41] and HD cell firing [9] to the grid signal, the current experimental manipulation did not reduce excitatory drive to downstream regions but instead only altered the directional accuracy of the HD signal. Because excitatory drive has also been shown to play a critical part in grid cell firing [42], the current manipulation may be especially useful in examining the nature of the information the HD network provides to grid cells more closely. If the spatial periodicity of grid cells depends on a directional tuning signal coming from HD cell inputs [43], selectively manipulating the directionality of HD cells while recording grid cells could provide a more direct test of this hypothesis. This type of manipulation might also provide greater insight into how heading information is integrated into grid cell firing, beyond the binary distinction of whether or not it plays any role at all [9]. One might expect that using the same optogenetic manipulation to cause HD cells to become unstable would also cause a gradual rotation of grid cell firing nodes, in unison with the gradual circular drift of the HD signal observed in the current experiments. Although measuring drift in grid cells is likely more difficult than in HD cells, researchers have recently had success in estimating cumulative error and consequent correction in grid cell populations [44].

Similarly, manipulating HD cell stability may also affect downstream place cells, though the contributions of the HD signal to

place cell firing is less clear [8] and, perhaps, less direct. Previous studies have found a tight coupling between co-recorded HD cells and place cells under cue conflict conditions [45], implying that there is some functional relationship between the two signals. HD cells appear to play a direct role in generating grid cell firing [9], and grid cells in turn most likely provide critical information to the place cell network [46]. Although layer III of the entorhinal cortex, which contains HD cells [47], also has direct projections to the hippocampus [48], the HD network's contribution to place cells may be mediated by the grid cell network. Moreover, engaging this optogenetic manipulation while recording in the hippocampus could further clarify the importance of the HD signal for place cell firing. If the HD signal contributes angular information to the place signal, place cells might similarly rotate over time during this manipulation; over the course of a long enough recording session, place fields might resemble an annulus shape, an outcome originally hypothesized by McNaughton et al. [49], but not found after lesions of the ADN or postsubiculum [8]. However, because place cell firing is not as dependent on the HD signal as grid cells are, place cells may be able to maintain their stability through other inputs despite this manipulation, strengthening previous conclusions that the HD signal does not directly contribute to place cells' spatial firing [8].

Although these experiments focused solely on the HD signal, many other neural processes contribute to navigation. The use of simultaneous circuit-specific manipulation and recording during behavior could be a powerful approach for resolving the precise contributions of HD cells to other spatial signals. Finally, although this work only studied animals' spatial behavior in terms of the relatively simple task of food retrieval and homing, similar approaches may also prove useful for understanding how these spatial signals combine to produce complex navigational behavior [50].

EXPERIMENTAL PROCEDURES

All experimental procedures conformed to the NIH Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee at Dartmouth College. Please see the Supplemental Information for details of the methods.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, seven figures, and two tables and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2017.03.033.

AUTHOR CONTRIBUTIONS

W.N.B., K.S.S., and J.S.T. planned the experiments and analyses. W.N.B. performed the experiments. W.N.B. analyzed the data. M.A.A.v.d.M. performed the statistical modeling. W.N.B., K.S.S., M.A.A.v.d.M., and J.S.T. discussed the results and wrote the manuscript.

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REFERENCES

1. Gallistel, C.R. (1990). The Organization of Learning (MIT Press).
2. Etienne, A.S., and Jeffery, K.J. (2004). Path integration in mammals. Hippocampus *14*, 180–192.
3. Moser, E.I., Kropff, E., and Moser, M.B. (2008). Place cells, grid cells, and the brain's spatial representation system. Annu. Rev. Neurosci. *31*, 69–89.
4. O'Keefe, J., and Nadel, L. (1978). The Hippocampus as a Cognitive Map (Oxford University Press).
5. Taube, J.S., Muller, R.U., and Ranck, J.B., Jr. (1990). Head-direction cells recorded from the postsubiculum in freely moving rats. I. Description and quantitative analysis. J. Neurosci. *10*, 420–435.
6. Hafting, T., Fyhn, M., Molden, S., Moser, M.B., and Moser, E.I. (2005). Microstructure of a spatial map in the entorhinal cortex. Nature *436*, 801–806.
7. Taube, J.S. (2007). The head direction signal: origins and sensory-motor integration. Annu. Rev. Neurosci. *30*, 181–207.
8. Calton, J.L., Stackman, R.W., Goodridge, J.P., Archey, W.B., Dudchenko, P.A., and Taube, J.S. (2003). Hippocampal place cell instability after lesions of the head direction cell network. J. Neurosci. *23*, 9719–9731.
9. Winter, S.S., Clark, B.J., and Taube, J.S. (2015). Spatial navigation. Disruption of the head direction cell network impairs the parahippocampal grid cell signal. Science *347*, 870–874.
10. Johnson, A., Seeland, K., and Redish, A.D. (2005). Reconstruction of the postsubiculum head direction signal from neural ensembles. Hippocampus *15*, 86–96.
11. Dudchenko, P.A., and Taube, J.S. (1997). Correlation between head direction cell activity and spatial behavior on a radial arm maze. Behav. Neurosci. *111*, 3–19.
12. Golob, E.J., Stackman, R.W., Wong, A.C., and Taube, J.S. (2001). On the behavioral significance of head direction cells: neural and behavioral dynamics during spatial memory tasks. Behav. Neurosci. *115*, 285–304.
13. Muir, G.M., and Taube, J.S. (2004). Head direction cell activity and behavior in a navigation task requiring a cognitive mapping strategy. Behav. Brain Res. *153*, 249–253.
14. van der Meer, M.A., Richmond, Z., Braga, R.M., Wood, E.R., and Dudchenko, P.A. (2010). Evidence for the use of an internal sense of direction in homing. Behav. Neurosci. *124*, 164–169.
15. Valerio, S., and Taube, J.S. (2012). Path integration: how the head direction signal maintains and corrects spatial orientation. Nat. Neurosci. *15*, 1445–1453.
16. Hok, V., Lenck-Santini, P.-P., Roux, S., Save, E., Muller, R.U., and Poucet, B. (2007). Goal-related activity in hippocampal place cells. J. Neurosci. *27*, 472–482.
17. de Lavilléon, G., Lacroix, M.M., Rondi-Reig, L., and Benchenane, K. (2015). Explicit memory creation during sleep demonstrates a causal role of place cells in navigation. Nat. Neurosci. *18*, 493–495.
18. Frohardt, R.J., Bassett, J.P., and Taube, J.S. (2006). Path integration and lesions within the head direction cell circuit: comparison between the roles of the anterodorsal thalamus and dorsal tegmental nucleus. Behav. Neurosci. *120*, 135–149.
19. Clark, B.J., Rice, J.P., Akers, K.G., Candelaria-Cook, F.T., Taube, J.S., and Hamilton, D.A. (2013). Lesions of the dorsal tegmental nuclei disrupt control of navigation by distal landmarks in cued, directional, and place variants of the Morris water task. Behav. Neurosci. *127*, 566–581.
20. Peckford, G., Dwyer, J.A., Snow, A.C., Thorpe, C.M., Martin, G.M., and Skinner, D.M. (2014). The effects of lesions to the postsubiculum or the anterior dorsal nucleus of the thalamus on spatial learning in rats. Behav. Neurosci. *128*, 654–665.

21. Harland, B., Wood, E.R., and Dudchenko, P.A. (2015). The head direction cell system and behavior: The effects of lesions to the lateral mammillary bodies on spatial memory in a novel landmark task and in the water maze. Behav. Neurosci. *129*, 709–719.
22. Zhang, K. (1996). Representation of spatial orientation by the intrinsic dynamics of the head-direction cell ensemble: a theory. J. Neurosci. *16*, 2112–2126.
23. Sharp, P.E., Blair, H.T., and Cho, J. (2001). The anatomical and computational basis of the rat head-direction cell signal. Trends Neurosci. *24*, 289–294.
24. Bassett, J.P., Tullman, M.L., and Taube, J.S. (2007). Lesions of the tegmentomammillary circuit in the head direction system disrupt the head direction signal in the anterior thalamus. J. Neurosci. *27*, 7564–7577.
25. Stackman, R.W., and Taube, J.S. (1997). Firing properties of head direction cells in the rat anterior thalamic nucleus: dependence on vestibular input. J. Neurosci. *17*, 4349–4358.
26. Clark, B.J., and Taube, J.S. (2012). Vestibular and attractor network basis of the head direction cell signal in subcortical circuits. Front. Neural Circuits *6*, 7.
27. Clark, B.J., Brown, J.E., and Taube, J.S. (2012). Head direction cell activity in the anterodorsal thalamus requires intact supragenual nuclei. J. Neurophysiol. *108*, 2767–2784.
28. Butler, W.N., and Taube, J.S. (2015). The nucleus prepositus hypoglossi contributes to head direction cell stability in rats. J. Neurosci. *35*, 2547–2558.
29. Taube, J.S. (1995). Head direction cells recorded in the anterior thalamic nuclei of freely moving rats. J. Neurosci. *15*, 70–86.
30. Cannon, S.C., and Robinson, D.A. (1987). Loss of the neural integrator of the oculomotor system from brain stem lesions in monkey. J. Neurophysiol. *57*, 1383–1409.
31. Kaneko, C.R.S. (1999). Eye movement deficits following ibotenic acid lesions of the nucleus prepositus hypoglossi in monkeys II. Pursuit, vestibular, and optokinetic responses. J. Neurophysiol. *81*, 668–681.
32. Russell, N.A., Horii, A., Smith, P.F., Darlington, C.L., and Bilkey, D.K. (2006). Lesions of the vestibular system disrupt hippocampal theta rhythm in the rat. J. Neurophysiol. *96*, 4–14.
33. Whishaw, I.Q., and Tomie, J. (1997). Piloting and dead reckoning dissociated by fimbria-fornix lesions in a rat food carrying task. Behav. Brain Res. *89*, 87–97.
34. Wallace, D.G., Hines, D.J., Pellis, S.M., and Whishaw, I.Q. (2002). Vestibular information is required for dead reckoning in the rat. J. Neurosci. *22*, 10009–10017.
35. Mittelstaedt, M.-L., and Mittelstaedt, H. (1980). Homing by path integration in a mammal. Naturwissenschaften *67*, 566–567.
36. Wallace, D.G., Hamilton, D.A., and Whishaw, I.Q. (2006). Movement characteristics support a role for dead reckoning in organizing exploratory behavior. Anim. Cogn. *9*, 219–228.
37. Vann, S.D., and Aggleton, J.P. (2004). The mammillary bodies: two memory systems in one? Nat. Rev. Neurosci. *5*, 35–44.
38. Aggleton, J.P., and Nelson, A.J.D. (2015). Why do lesions in the rodent anterior thalamic nuclei cause such severe spatial deficits? Neurosci. Biobehav. Rev. *54*, 131–144.
39. Biazoli, C.E., Jr., Goto, M., Campos, A.M., and Canteras, N.S. (2006). The supragenual nucleus: a putative relay station for ascending vestibular signs to head direction cells. Brain Res. *1094*, 138–148.
40. Muir, G.M., Brown, J.E., Carey, J.P., Hirvonen, T.P., Della Santina, C.C., Minor, L.B., and Taube, J.S. (2009). Disruption of the head direction cell signal after occlusion of the semicircular canals in the freely moving chinchilla. J. Neurosci. *29*, 14521–14533.
41. Brandon, M.P., Bogaard, A.R., Libby, C.P., Connerney, M.A., Gupta, K., and Hasselmo, M.E. (2011). Reduction of theta rhythm dissociates grid cell spatial periodicity from directional tuning. Science *332*, 595–599.
42. Bonnevie, T., Dunn, B., Fyhn, M., Hafting, T., Derdikman, D., Kubie, J.L., Roudi, Y., Moser, E.I., and Moser, M.B. (2013). Grid cells require excitatory drive from the hippocampus. Nat. Neurosci. *16*, 309–317.
43. Burgess, N., Barry, C., and O'Keefe, J. (2007). An oscillatory interference model of grid cell firing. Hippocampus *17*, 801–812.
44. Hardcastle, K., Ganguli, S., and Giocomo, L.M. (2015). Environmental boundaries as an error correction mechanism for grid cells. Neuron *86*, 827–839.
45. Knierim, J.J., Kudrimoti, H.S., and McNaughton, B.L. (1995). Place cells, head direction cells, and the learning of landmark stability. J. Neurosci. *15*, 1648–1659.
46. Brun, V.H., Leutgeb, S., Wu, H.Q., Schwarcz, R., Witter, M.P., Moser, E.I., and Moser, M.B. (2008). Impaired spatial representation in CA1 after lesion of direct input from entorhinal cortex. Neuron *57*, 290–302.
47. Sargolini, F., Fyhn, M., Hafting, T., McNaughton, B.L., Witter, M.P., Moser, M.B., and Moser, E.I. (2006). Conjunctive representation of position, direction, and velocity in entorhinal cortex. Science *312*, 758–762.
48. Kitamura, T., Pignatelli, M., Suh, J., Kohara, K., Yoshiki, A., Abe, K., and Tonegawa, S. (2014). Island cells control temporal association memory. Science *343*, 896–901.
49. McNaughton, B.L., Knierim, J.J., and Wilson, M.A. (1995). Vector encoding and the vestibular foundations of spatial cognition: neurophysiological and computational mechanisms. In The Cognitive Neurosciences, M. Gazzaniga, ed. (MIT Press), pp. 585–595.
50. McNaughton, B.L., Battaglia, F.P., Jensen, O., Moser, E.I., and Moser, M.B. (2006). Path integration and the neural basis of the 'cognitive map'. Nat. Rev. Neurosci. *7*, 663–678.