Directional Integrity and Landmark Anchoring of the Head Direction Cell System in the TgF344-AD Rat Model of Alzheimer’s Disease

Laura E. Berkowitz
University of New Mexico

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Laura E. Berkowitz

Candidate

Psychology

Department

This dissertation is approved, and it is acceptable in quality and form for publication:

Approved by the Dissertation Committee:

Benjamin J. Clark
Chair

Claudia D. Tesche
Member

Derek A. Hamilton
Member

Jeremy Hogeveen
Member

Samuel A. McKenzie
Member

David N. Linsenbardt
Member
Directional Integrity and Landmark Anchoring of the Head Direction Cell System in the TgF344-AD Rat Model of Alzheimer’s Disease

BY

Laura E. Berkowitz
B.S., Neuroscience, California State University, Sacramento, 2014
M.S., Psychology, University of New Mexico, 2017

DISSERTATION

Submitted in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy

Psychology
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DEDICATION

To all women in science who came before me and will come after me.
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ABSTRACT

Spatial navigation and memory are impaired in the early stages of Alzheimer’s disease (AD) and are prominent behavioral markers of preclinical AD. The TgF344-AD rat model of AD exhibits similar behavioral and pathological features to humans and thus serves as an excellent model to investigate the mechanisms underlying spatial impairment in AD. Brain regions in the parahippocampal cortex contain spatially responsive pyramidal cells that support spatial navigation and memory including place cells, grid cells, and head direction cells. Furthermore, GABAergic interneurons in these regions support precise temporal organization of spike dynamics and may play a critical role in maintaining reliable spatial representations. Both pathological and electrophysiological markers have identified evidence of hyperexciatablility and GABAergic dysfunction across various rodent models of AD-like pathology; consistent with the theoretical framework of excitatory—inhibitory imbalance underlying cognitive decline in AD. Critically, TgF344-AD rats exhibit GABAergic cell loss and epileptiform activity in local field potential traces as early as 9 months of age in areas within the parahippocampal cortex. The presence of GABAergic pathology and epileptiform activity suggests that local microcircuitry may be disrupted. The postsubiculum, a region within the parahippocampal cortex, contains a large population of cells that propagate vestibular driven head direction information to cortical targets. Importantly, the integrity of this circuit relies on several types of GABAergic interneurons to maintain spiking dynamics of head direction cells during fast head turns or sustained head position. Therefore, interneuron dysfunction resulting from AD-like pathology would predict diminished integrity, instability, and poor discrimination of the head direction signal within postsubicular cell populations. The broad goal of
the current research was to establish whether postsubicular head direction cells and GABAergic interneurons show dysfunctional firing dynamics in TgF344-AD rats. The first aim of this research addressed this goal by characterizing the firing characteristics of postsubicular head direction cells and GABAergic interneurons during active exploration. The second aim evaluated the response of postsubicular head direction cells following environmental manipulations, such as cue rotation or environment change. The results indicated that putative interneurons exhibited a higher degree of directional encoding in TgF344-AD rats, with little difference observed in pyramidal head direction cells. Furthermore, a local visual cue controlled the preferred direction of head direction cells in TgF344-AD rats, indicating landmark anchoring is intact in aged TgF344-AD rats. This work indicated alterations of the postsubicular head direction cell circuit in TgF344-Ad rats. More broadly, these findings helped establish whether the altered firing of putative excitatory and inhibitory neurons contributed to the disruption of cellular networks that support spatial navigation and memory; thus, informing targeted treatment development for AD.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>List of Figures</td>
<td>ix</td>
</tr>
<tr>
<td>List of Tables</td>
<td>x</td>
</tr>
<tr>
<td>Introduction</td>
<td>1</td>
</tr>
<tr>
<td>Chapter 1</td>
<td>6</td>
</tr>
<tr>
<td>Chapter 2</td>
<td>33</td>
</tr>
<tr>
<td>Chapter 3</td>
<td>59</td>
</tr>
<tr>
<td>Conclusion</td>
<td>78</td>
</tr>
<tr>
<td>References</td>
<td>83</td>
</tr>
</tbody>
</table>
# List of Figures

1. Example plots for demonstrating HD cell tuning ........................................... 9
2. 3D rendering of HD cell circuit from mouse ............................................. 10
3. Example apparatus used for landmark manipulation experiments ........... 18
4. Simplified HD cell circuit diagram .......................................................... 23

1. Proportional and functional changes of TgF344-AD pyramidal and interneuron populations ................................................................. 37
2. Encoding of Head Direction is increased in TgF344-AD interneurons .......... 39
3. TgF344-AD Interneuron HD cells convey more directional information and are more stable over time ......................................................... 41
4. Increased sensitivity to linear speed in TgF344-AD HD x Speed cells .......... 43
5. Adherence of attractor dynamics in TgF344-AD HD cell pairs ................. 45

1. TgF344-AD HD interneurons have stable, increased directional tuning across all conditions .......................................................... 63
2. Landmark anchoring is intact in TgF344-AD head direction cells .......... 65
3. TgF344-AD HD cells show variable response of preferred direction shifting following an environmental change ................................. 67
4. Example of coherent and non-coherent ensembles in TgF344-AD rats .... 68
# List of Tables

<table>
<thead>
<tr>
<th>S1</th>
<th>Summary of encoding types across group, rat, and cell type</th>
<th>56</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>Summary of head direction cells across group, rat, and cell type used in rotation analysis</td>
<td>77</td>
</tr>
<tr>
<td>S2</td>
<td>Summary of head direction cells across group, rat, and cell type used in box analysis</td>
<td>77</td>
</tr>
</tbody>
</table>
Introduction

Spatial Navigation Deficits and Functional Alterations of Parahippocampal Excitatory—Inhibitory Connectivity

Alzheimer’s disease (AD) is the leading cause of dementia, and AD-related pathology is predicted to initiate as early as 20 years before the onset of symptoms [9]. Spatial deficits in virtual or real-world navigation tasks are among the first symptoms to manifest in individuals with AD [6, 48, 67, 113, 139, 192, 195], may distinguish prodromal AD from other forms of dementia [64, 266, 267], and serve as promising behavioral biomarkers for identifying individuals at risk of developing AD [64]. Characterizations of these deficits have found that individuals with prodromal AD make longer or more meandering paths to a goal location [113, 147] or have trouble remembering the position of landmarks on a previously traversed route [6, 18, 48, 71, 147]. Over time, more severe deficits in spatial navigation emerge and lead to an increased incidence of getting lost or wandering episodes [50, 162, 225, 284]. While the neural basis of spatial deficits in AD is currently unknown, structural and functional evidence suggests pathological processes induce severe changes to local microcircuitry within the parahippocampal cortex.

Parahippocampal regions that succumb to AD-associated pathology, including the entorhinal cortex, hippocampus, and subicular cortex (presubiculum, postsubiculum, parasubiculum, and subiculum proper), contain functional cell types that are thought to assist or support spatial navigation [161, 169, 170, 186]. These cell types fire relative to specific locations [184], at a given distance from a boundary [11, 151], along a tessellating grid pattern [97, 220], or relative to head direction [258, 259]. The temporal precision of spatial firing has been linked to inhibitory interneurons [20, 93, 163, 183, 218, 239]. For example, Grienberger et al (2017) found that silencing interneurons within the hippocampus increases the outfield firing rate of place cells and decreases the range of theta phase precession. Similarly, grid and place cells within the entorhinal cortex show differential responses to the selective silencing of Parvalbumin (PV+) and Somatostatin (SOM+) interneurons [163]. These studies suggest that an inhibitory drive is necessary for stable, precise spatial representation. Critically, AD-like pathology induces GABAergic dysfunction [191], such as cell loss [168, 207, 277] or changes in astrocytic GABAergic processing [128, 181, 198], and such dysfunction has been observed throughout the parahippocampal cortex.

Classic features of AD-associated pathology include toxic amyloid oligomeric
species and amyloid plaques [261], hyperphosphorylated tau [27, 28], inflammation [211], and cell loss [99] occur throughout the parahippocampal cortex in humans [109] and animal models of AD-like pathology [148]. Consistent with the amyloid cascade hypothesis [102], toxic amyloid pathology interacts with intracellular and extracellular processes resulting in excitotoxicity [117], astrocyte activation [153], and apoptosis [176]. Additionally, epileptiform activity is observed in animal models with AD-like pathology [96, 247], which is consistent with the high rate of epilepsy observed in humans with AD [281]. Such activity may be driven by the interaction of amyloid oligomeric species and glutamate receptors [214]. Indeed, animal models of AD-like pathology exhibit hyperexcitability signatures before the observation of cell death among pyramidal and GABAergic interneurons in the parahippocampal cortex [247]. Furthermore, parahippocampal place and grid cells exhibit reduced spatial stability and increased outfield firing rates in several models of AD-like pathology [82, 130, 157], similar to the effects following the loss of inhibitory drive on spatial cell types [163, 183]. While mounting evidence suggests AD-like pathology may lead to severe changes in excitatory—inhibitory transmission [278, 280], there has been no investigation of the functional integrity of parahippocampal microcircuitry.

Investigation of excitatory—inhibitory connectivity of parahippocampal spatial cell types is critical for understanding how spatial navigation deficits may arise in AD. There are several lines of evidence that suggest microcircuitry changes may underlie the instability of spatial cell types in the parahippocampal cortex. First, anatomical and physiological evidence has shown that parahippocampal neurons with spatial firing correlates are often excitatory principal cells [178, 238, 289]. Populations of these cells show monosynaptic connectivity with local inhibitory interneurons [75, 239], and loss of inhibition is observed in the parahippocampal cortex of animal models of AD-like pathology [278, 280]. Following this line of reasoning, the firing rate of excitatory cells should be increased while the firing rate of inhibitory cells should be decreased. Critically, a loss of inhibition through optogenetic silencing leads to increases in outfield firing rates of spatial cell types [163, 183]. Thus, the decreased spatial precision of firing fields observed in animal models of AD-like pathology may be due to GABAergic dysfunction or a loss of inhibition. Additionally, active navigation through an environment requires continuous integration of linear speed and angular velocity [161]. PV+ interneurons are thought to mediate this process given their facilitating dynamics during head turns and depressing dynamics at rest [163, 178]. Thus, the loss of PV+
interneurons observed in animal models of AD-like pathology should impair processes dependent on accurate angular velocity integration, such as accurate heading direction [205]. Overall, burgeoning evidence implicates AD-associated pathologies driving inhibitory dysfunction and degradation of spatial processing in the parahippocampal cortex.

The role of Inhibition in Maintaining a Postsubicular Head Direction Signal

The postsubicular cortex is a critical hub for supplying vestibular-driven head direction information to upstream parahippocampal targets. Connectivity between head direction cells and inhibitory interneurons within the postsubiculum is posited to be critical for maintaining an accurate head direction signal [239]. Thus changes in excitatory—inhibitory cell function secondary to amyloid pathology may lead to a degradation of head direction signaling within the postsubiculum and its downstream targets. Humans with AD and rodents with AD-like pathology develop unique amyloid pathology in the postsubicular cortex (dorsal presubicular cortex in humans) whereby markers of tauopathy are reduced [4, 125, 285]. Consequently, investigations into the functional integrity of postsubicular head direction cells provide a unique opportunity to understand potential microcircuitry changes related to amyloid pathology.

Head direction cells have several defining characteristics. First, the tuning curves of head direction cells resemble a circular Gaussian distribution around a preferred firing direction or the direction of the animal’s head at which the cell fires maximally, with low or no firing around 180 degrees opposite of the preferred direction [255]. Anatomical and functional evidence suggests the generation of the head direction signal arises from neurons in the dorsal tegmental nucleus that respond to the direction of head translation and angular velocity [226]. These neurons inhibit excitatory head direction cells within the lateral mammillary nucleus (LMN) [107]. The precision of the head direction signal, such as tuning curve width, becomes more precise at higher levels of the circuit [54, 255]. Tuning curves of head direction cells in the postsubicular cortex exhibit the most precise tuning [258]. Additionally, Head direction cells tend to fire at a preferred direction relative to a salient and static landmark [307]. For example, head direction cells will maintain a preferred direction in a static environment, but shift their preferred direction following the rotation of a visual cue [90]. In contrast, navigation in
darkness results in preferred directions that drift along the azimuth, as if the landmark acted as an anchor for the HD tuning \[89\]. Finally, head direction cells exhibit persistent firing without habituation \[301\]. Such characteristics of HD cells have spurred computational models to explain how HD cells as a population maintain coherent directional firing.

Most models of the head direction cell system consist of ring attractors that depend on global inhibition to maintain a stable heading signal \[23, 92, 209, 240, 242, 248, 292, 302\]. The main components of these models include an inhibitory ring of clockwise and counterclockwise inputs which converge onto a ring of recurrently connected excitatory head direction cells. The general network connectivity facilitates a hill of activity that is amplified at a given direction along the azimuth. Angular velocity inputs enable the hill of activity to move in the direction of motion, while symmetric connections during rest stabilize the hill of activity in a given direction. The integrity of the head direction signal depends on the amplification of head direction cells within the hill of activity through global inhibition of head direction cells out of phase with the currently activated units. Both postsubiculair and reciprocally connected thalamic head direction cell populations follow attractor dynamics \[136\]; possibly through excitatory—inhibitory connectivity \[239\]. In-vitro studies and juxtacellular recordings in the postsubiculum have found that $SOM^+$ and $PV^+$ interneurons may provide the inhibition required for maintaining attractor dynamics of the head direction signal \[178, 237\]. $SOM^+$ interneurons exhibit facilitating dynamics with sustained stimulation, such as when the head is at rest, and enact lateral inhibition of pyramidal neurons. $PV^+$ interneurons exhibit facilitating dynamics during fast head translation followed by depressive dynamics when the head is at rest \[205\]. Furthermore, $PV^+$ interneurons exert feed-forward inhibition on postsubiculair pyramidal neurons via thalamic head direction cell input \[178\]. The dynamics of these two interneuron types suggest that $SOM^+$ interneurons may provide the inhibition required to maintain a stable head direction signal at rest, whereas $PV^+$ interneurons may facilitate the movement of the head direction signal around the azimuth during head turns. To this end, changes to local interneuron function as a result of AD-like pathology may lead to the instability of head direction cells within the postsubiculair cortex.

The experiments included in this dissertation tested whether postsubiculair head direction cells and interneurons exhibit dysfunction in the TgF344-AD rat model of AD-like pathology. TgF344-AD rats show comparable spatial navigation deficits to individuals with AD \[17, 168\]. The TgF344-AD rat model is also an ideal
model for examining the effects of amyloid pathology on parahippocampal circuitry. First, this model expresses mutated amyloid precursor protein and presenilin 1 proteins, which result in a similar amyloid pathogenesis observed in individuals with AD [58]. Additionally, along with classical features of AD-like pathology, GABAergic dysfunction and epileptiform activity have been observed in the parahippocampal cortex of TgF344-AD rats [168, 247], indicating deleterious alterations in excitatory and inhibitory connectivity are present. Overall, the characterization of postsynaptic head direction cells and interneurons will inform how network alterations to excitatory and inhibitory cell function may underlie the degradation of spatial representations in AD.

**Overview of Chapters**

The general aim of this dissertation sought to determine the functional integrity of postsynaptic head direction cells in an animal model of Alzheimer’s disease. To the authors knowledge, postsynaptic head direction cell function has not previously been examined in an animal model of AD-like pathology. Specifically, this is the first report of changes in intrinsic firing characteristics, directional tuning, attractor dynamics, and landmark anchoring of both pyramidal and putative interneurons that encode head direction in an Alzheimer’s disease rodent model. In line with this goal, the main aim of Chapter 1 was to provide an extensive review of the circuitry underlying the head direction signal. This chapter provides an overview of the anatomy, function, and computational modeling that inform several hypotheses throughout the subsequent chapters. The aim of Chapter 2 was to investigate the intrinsic firing characteristics and directional tuning of postsynaptic head direction cells in a model of Alzheimer’s disease. Chapter 3 further investigates how postsynaptic head direction cells respond to environmental manipulations to address potential underlying mechanisms of landmark processing deficits observed in humans with AD and animal models of AD-like pathology. Finally, the dissertation concludes with a summary of the findings as well as suggestions for future work.
Chapter 1

Examination of the anatomy, function, and modeling of the rodent head direction cell circuit

Abstract

This review will consist of four sections: functional anatomy, landmark manipulation experiments, functional connectivity, and visual components of HD cell modeling. First, the functional anatomy will be comprehensively reviewed. This section is split into two major components: subcortical and cortical circuitry. The influences of the vestibular components of the HD circuitry have been evaluated previously [32, 57]; however, the review here is crucial to understanding how the internally generated directional sense can be influenced by external cues. Evaluation of the functional components of cortical circuitry will reveal how multiplexed representations of spatial features may be used to integrate external information into the subcortical circuit. The second section summarizes the landmark manipulation experiments. By evaluating the response of HD cells in various environmental contexts, the important circuit mechanism will be revealed, leading to a conclusion that both self-motion and visual cues are equally essential to landmark control of the HD cell system. The third section supports these conclusions by evaluating the effects of selective circuit lesions. Finally, the theoretical modeling of the HD cell system is reviewed. Initial models and basic components of these models are briefly described (but are thoroughly reviewed in the literature [136, 229]. An emphasis on visual components is explored and compared to empirically performed landmark manipulations.

Introduction

The ability to navigate is critical for the survival of many mammalian species. Neural systems underlying spatial navigation have been studied extensively, whereby the identification of spatially modulated cell types and their neural firing properties can reliably predict an animal’s location in space [57, 170, 210]. Head direction (HD) cells, discovered by James Ranck in 1984, are posited to provide a directional signal that may be important for estimating one’s position in space. These cells are found throughout the parahippocampal cortex and fire maximally relative to an animal’s head position. Anatomical and functional examination of the circuit revealed how the vestibular drive of thalamic head direction cells is, in turn, propagated to the parahippocampal cortex. Furthermore, the temporal stability of
this signal relies on accurate integration of self-motion and visual inputs, which are likely achieved through multiplexed cell populations in the cortex. In addition, mounting evidence indicates that inhibitory interneurons may subserve the spatial specificity of pyramidal neuron firing [230].

HD cells have several defining characteristics (Fig.1). First, the HD cell firing fields resemble a circular Gaussian distribution around the direction at which the cell maximally fires, termed the preferred direction. In addition, HD cells exhibit low or no firing around 3.14 radians opposite of the preferred direction. The vestibular system supports this directional signal by providing head translation information while tactile, olfactory, and visual cues allow for error correction. Visual information seems to enact the strongest influence on the HD cell circuit [305]. HD cells stabilize their preferred direction relative to a visual landmark, termed landmark anchoring. For example, HD cells will shift their preferred direction to the same degree and direction as the shift of a visual landmark. Such characteristics of HD cells have spurred computational models that may explain how HD cells as a population maintain coherent directional firing.

The most commonly accepted model is in the form of a ring-attractor first proposed by Skaggs, Knierim, Kudrimoti, and McNaughton [240]. With the entire azimuth coded for by the HD cell population, ring attractor models predict recurrent connectivity of HD cells with similar preferred directions leading to an activity bump within the ring. Models have successfully explained the Gaussian-like firing behavior of individual HD cells. Models tend to include a second inhibitory cell layer that acts to laterally inhibit HD cells of sufficiently different preferred directions. With the addition of a velocity parameter, inhibition predicts the translation of the activity bump following simulated head movements. While many models of the HD cell system focus on the generative portion of the circuit [13, 209, 248, 302], few models have addressed how visual input may contribute to the stabilization of the HD cell system [23, 34, 123, 189, 250].

The next sections will thoroughly review the literature surrounding head direction cell circuit function. First anatomical evidence will be described to show how the system is organized in a hierarchical fashion, starting with generative processes in the vestibular system and ending with multiplexed HD cell populations in the cortex. The next section will describe how HD cells throughout the circuit respond to different landmark manipulation experiments. Work that describes HD cell response to visual landmarks will be emphasized. The third section will examine the functional connectivity of the circuit with an emphasis on lesion studies. This
section will provide evidence for a cortical dependence on thalamic HD cell drive. Finally, computational models of the HD cell system will be reviewed, starting with generative models and moving towards modeling the integration of visual information.

Hierarchical organization of the head direction circuit

Head direction cells have been found throughout the Papez’ circuit (Fig.2). Subcortical loci include the dorsal tegmental nucleus (DTN) [13, 226], the lateral mammillary nucleus (LMN) [24, 245], and the Anterior Thalamic Nucleus (ATN) [25, 260] while cortical loci include the postsubiculum (PoS) [156, 254], parasubiculum (PaS) [26, 103], Retrosplenial Cortex (RSC) [49, 68, 156], posterior parietal cortex [46], and Medial entorhinal cortex (MEC) [84, 94, 103]. General connectivity between these areas is illustrated in (Fig.2) & (Fig.4). A convergence of histological and functional assessments of these regions have indicated a hierarchical organization starting at the level of the brainstem [57].

Subcortical Circuit

Convergence of head translation and eye position result in linear and angular velocity signals

Linear and angular velocity signals arise from three main nuclei that contain neurons that respond to head translation; the medial vestibular nuclei (MVN), nucleus prepositus hypoglossi (NPH), and Supragenual Nucleus (SGN). Movement of the head activates hair cells in the horizontal semicircular canals, and these signals converge onto the vestibular nuclei [66, 303]. There are two prominent types of neurons found in in the MVN. Type I neurons that respond to ipsiversive head turns and Type II neurons that respond to contraversive head turns. For example, the firing rate of a Type I neuron recorded in the right hemisphere would increase when the head is turned towards the right, whereas the firing rate of a Type II neuron in the right hemisphere would increase during a head turn to the left. Angelaki and colleagues [7] also found that MVN neurons are modulated by otolith and horizontal canal activation and that the firing patterns of these neurons correspond to a spatial and temporal code of linear acceleration. Additionally, recordings in MVN of monkeys found that vestibular neurons differentially respond to head translation during active versus passive movements [44], indicating that motor efferent copy modulates vestibular processing very early in the circuit. The
Figure 1: Example plots for demonstrating HD cell tuning. A. Tuning curve showing increased firing (y-axis) relative to head direction (x-axis). Dashed vertical red line indicates preferred direction. Half-width and tuning range (horizontal red lines) are sometimes used to describe directionally specificity. B. Head direction cell firing over time. Gray indicates the heading of an animal over time (x-axis). Red dots indicating the firing of the cell overhead direction (y-axis). Note that the firing of this cell only occurs at around 180 degrees. The firing is also relatively stable (no-drift away from the preferred direction (~180 deg) over the course of the session.

MVN projects to the nucleus prepositus hypoglossi (NPH), which also contains Type I and Type II neurons, although these neurons also respond to optokinetic stimulation [149], as well as the supragenual nucleus (SGN), which also receives efferent connections from the NPH [22, 31, 32]. Finally, the NPH and SGN project to the dorsal tegmental nucleus (DTN). Thus, head translation and eye position information is processed within the MVN, NPH, and SGN circuit is sufficient to produce a velocity signal observed in the DTN [13, 226].

The dorsal tegmental nucleus and lateral mammillary nucleus generate the HD signal

Anatomical and functional evidence suggests that the DTN and lateral mammillary nucleus (LMN) drive the head direction signal through the integration of velocity
and head translation.

Skaggs et al. [240] originally proposed a ring of rotation cells that received vestibular input for right and left head turns. These cells would then project onto the HD cell layer, facilitating the translation of the activity bump along the azimuth. Although these predictions have not been fully verified, much evidence suggests velocity signals are relayed from the DTN, which contains an abundance of velocity modulated cells, to the LMN, which contains head direction modulated cells. A large proportion of neurons in the DTN are GABAergic [88, 107] and receive contralateral vestibular input from the SG nucleus as well as ipsilateral input from the NPH. The DTN is also reciprocally connected to the interpeduncular nucleus, which contains cells that are sensitive to movement direction [231]. The firing properties of DTN neurons have been assessed in rats engaging in food foraging tasks [13, 229]. A small proportion of cells in the DTN exhibited functional correlations with head direction. These cells were broadly tuned and their firing rate also correlated with angular velocity. Sharp, Kinkelman & Cho [226] found that the firing rate of these HD cells increased during ipsiversive head turns but exhibited broader tuning curves during contralateral head turns. Most cells recorded in this region were modulated by angular velocity (AV cells). Bassett and
Taube [13] found that AV cells exhibited symmetric or asymmetric linear relationships with angular velocity. Asymmetric cells increase or decrease the firing rate relative to a given head turn whereas symmetric cells will increase or decrease firing rate similarly for either right or left turns. In both studies, AV cells were found to exhibit a dynamic temporal relationship with angular velocity and tended to have a preferred velocity range at which they fired maximally. The DTN, therefore, provides an inhibitory effect relative to head translation and angular velocity to its upstream target, the lateral mammillary nucleus (LMN).

LMN neurons are largely excitatory projection neurons that terminate in the tegmentum and thalamus. Descending projections terminate in the ipsilateral intermediate and proximal dendrites and somata of DTN neurons [107]. Ascending projections include dense bilateral projections to the ADN, in addition to para- and postsubiculum [223, 270, 291], retina [62, 120], anterior cingulate cortex [234], granular Retrosplenial cortex [291], caudal dorsal reticular nucleus [232]. Functional cell types identified in the LMN include cells modulated by head direction, angular velocity, head pitch, and theta-modulated [24, 245]. HD cells in the LMN display lateralized firing with more precise directional tuning and decreased anticipation of future head direction during contraversive head turns. Blair, Cho & Sharp [229] proposed that the firing characteristics observed by LMN HD cells may indicate that type II DTN neurons inhibit LMN HD cells during head turns that allows for lateral inhibition of HD cells during contralateral head turns. Thus, the functional relationship between angular velocity cells in the DTN and HD cells in the LMN may facilitate the transformation of velocity and multiplexed angular velocity by HD information into a stable HD signal found in the anterior dorsal thalamic nucleus (ADN).

**Thalamic HD cell processing**

The ADN is known to contain a homogeneous population of HD cells that exhibit more refined directional tuning relative to HD cells found in upstream areas [24, 260]. The ADN contains the largest relative proportion of HD cells (~60%) compared to the DTN (~13%), LMN (~23%) or postsubiculum (PoS) (~10-24%). First characterized by Taube [260], ADN HD cells exhibit similar characteristics to the first reported HD cells in the PoS, although ADN HD cells exhibit higher firing rates and near-zero baseline firing rates. Importantly, ADN and PoS are reciprocally connected and functional evaluation of these regions show coherent tuning between HD cells recorded simultaneously [203]. ADN neurons project to ipsilateral cortical
regions, including layers I, III (sparse layer V, VI) of the presubiculum and parasubiculum, and postsubiculum layer 1 and III-IV [178, 270, 273], Retrosplenial cortex, granular a and b cortices layer 1 and III-IV (Rga & Rgb, respectively) [233, 270, 274]. Relay neurons in the ADN are excitatory and express both vesicular glutamate transporter isoforms VGluT1 & VGluT2, specifically in the ventral portion of the ADN [182]. The ADN also receives inhibitory feedback from the postsubiculum via the reticular nucleus [276]. Overall, these findings suggest that ADN HD cells provide strong excitatory input throughout the Papez cortical circuit.

HD cells have also been identified in the lateral dorsal thalamic nucleus (LDN) [167] and the anterior ventral thalamic nucleus (AVN) [263, 283]. The LDN is densely connected with visual areas and is reciprocally connected to all RSC sub-regions and the postsubiculum. Specifically, the LDN projects to the Rsd, Rgb and 18b (layers I, III-IV for all areas) [272]. Additionally, LDN projects to PoS layers that contain HD cells (e.g., I, III/VI as well some in superficial layer V). Projections to other subicular regions such as the PreS and PaS are mainly to deep layers and LDN sends sparse projections to the deep layers of the MEC. Few studies have evaluated HD cell activity in the LDN. Mizumori & Williams [167] found evidence of directionally modulated cells as rats performed a radial arm maze task. These neurons resembled classical HD cells during light conditions but became silent after 2-3 min of dark exposure. Given the significant visual input into this region, the potential of these cells to be visually dominated rather than modulated by head direction is an open question. AVN receives dense inputs from the medial mammillary nuclei [232] and sends projections to the RSC, PoS, PreS, and MEC [273]. Cell activity in the AVN exhibits bursty activity within theta frequency (6—8 Hz) [159, 263, 283]. HD cells in these nuclei have also been shown to exhibit theta-modulation in their firing frequency [263, 264]. Movement and theta modulated cells have also been found in the AVN [283]. Simultaneous recording of this cell type along with HD cells indicates coherent directional tuning, meaning the ensembles responded similarly to environmental manipulations [263]. Although the full functional role of the AVN and LDN has yet to be fully elucidated, this evidence suggests possible roles in visual processing and possible propagation of theta modulation into cortical limbic structures.

HD cells have been identified in the subicular cortex, retrosplenial cortex, posterior parietal cortex, and medial entorhinal cortex. The subicular cortex consists of several sub-regions defined by differences in cytoarchitecture, including the postsubiculum (PoS) (also referred to as the dorsal presubiculum), presubiculum
(PreS), parasubiculum (PaS), and subiculum proper [271]. HD cells have largely been characterized in the PoS [91, 156, 227, 235, 254], but have been found in the PreS, PaS [26, 84, 220]. Subregions of the subicular cortex, including the PoS and PaS, are connected to both the Retrosplenial and Posterior Parietal cortices [187, 188]. The medial entorhinal cortex receives projections from the PoS and PreS. Classically defined HD cell firing, such as those cells that fire maximally in one direction independently of place and continue to demonstrate coherent directional firing in the dark, has been found in these cortical regions. However, cortical components of the HD cell system often exhibit a more heterogeneous population of cell activity. For instance, multiplexed cell firing, in which firing correlates with multiple spatial or behaviorally modulated features, is often observed alongside classically defined HD cells [204, 228]. Overall, the firing characteristics and anatomical connectivity within and between the cortical regions of the HD cell circuit implicate a general role in processing high-order sensory information with vestibular driven head direction signals.

**Post-, Pre- and Parasubicular cortices**

The subicular loci of the HD cell system demonstrate varying degrees of HD cell tuning. The most prominent of the loci is the PoS, where HD cells exhibit sharp tuning and fire relative to the current head direction [24]. HD cell firing in the PaS exhibits more broad tuning characteristics but contains a larger proportion of HD cells relative to the PreS [26]. Despite functional differences, all three areas receive input from subcortical HD cell loci [232, 270].

The PoS makes up the dorsal portion of the presubiculum and is distinguished from PreS by the presence of small pyramidal neurons organized into rows in layer III, tight cell clusters in layer II, and significant acetylcholinesterase (AChE) staining in layer I [270]. The PoS receives dense projections from ADN, Rgb, LD, and moderate to sparse inputs arising from CA1, Rga, Entorhinal cortex, lateral mammillary nuclei and AV [270]. Critically, HD cells from ADN and Rgb project densely to PoS layer III, which is known to harbor HD cells [205]. Hippocampal, retrosplenial, and LD also project to layers III-V, layers III & V, and layer III, respectively [205, 270]. Apical dendrites of layer III pyramidal neurons reach layer I, which receives moderately dense input from the subiculum, entorhinal cortex, Rga, Rgb, LD, AD and AV. Two prominent types of GABAergic interneurons may contribute to the directional firing properties of PoS HD cells. Martinotti cells (MC) are identified as somatostatin-positive interneurons that fire regularly with
frequency adaption. MC send axonal collaterals to layer I, indicating they are situated to modulate incoming signals from sensory areas [179, 205, 270]. Fast-spiking parvalbumin (PV) interneurons have also been identified. These neurons tend to exhibit a hyperpolarized resting potential, but can produce high-frequency spiking with little frequency adaption [179]. Simultaneous in-vitro recordings of layer III pyramidal cells and MC indicate an inhibitory feedback of pyramidal cell firing under high frequency stimulation, like that observed when a HD cell is within its preferred firing direction. Computational modeling of these interactions predict that MC neurons may mediate the stability of HD cell tuning [239]. Multiplexed neuronal populations have also been found in the PoS, including place by HD cells [204] and angular velocity by HD cells [205, 227, 238, 268]. Overall, the PoS is anatomically situated to integrate high-order sensory information from the cortex with vestibular head direction information from the thalamus [297].

HD cells in the PreS and PaS are only a small proportion of functional cell types [26]. The PreS is located ventral to the PoS and exhibits significant AChE staining in the third cell layer. Projections from layers III-V of the PreS terminate bilaterally in layers I and III of the PreS and PaS. Reciprocal topographical projections are observed for PreS to MEC. However, the distribution of inputs from MEC varies along the rostrocaudal axis whereby caudal MECto PreS projections arise from layer IV while rostral MEC to PreS arise from layers III & V, both terminate in layer I of the PreS. PreS inputs into the MEC arise from layers II and III [83, 112, 270]. Retrosplenial inputs in the PreS, from Rga, Rgb and Rsd, all emanate from layer V [270]. Thalamic input into the PreS largely arise from the rostromedial aspects of the AV and LD nuclei [270, 273]. The PaS sends projections to ipsilateral CA1 and subiculum in addition to bilateral PoS layer V, PreS layer I & III and MEC layer II [270]. Inputs into the PaS are extensive, including CA1, MEC, subiculum, PreS, PoS, claustrum, basolateral amygdala, nucleus reunions, endopiriform nucleus, supramammillary nucleus, locus coeruleus, and midbrain raphe nuclei [270]. The firing characteristics of these regions have been evaluated in-vitro and in-vivo. In-vitro studies revealed that deep layers exhibited burst-like firing after stimulation of the subiculum, medial entorhinal cortex and deep layers of the PaS [83]. Furthermore, bursting occurred in deep layers when the slices were bathed in NMDA agonist CPP, whereas AMPA agonist preparations prevented firing in deep layers. Boccara et al. [26] assessed the firing characteristics in PreS and PaS superficial (II & III) and deep (V & VI) layers in-vivo while rats explored a square environment. Three major types of cells were identified; grid cells, theta
modulated cells, and head direction cells. Many of these cell types exhibit multiplexed firing properties, for example, a cell may fire in a grid formation but only when the head is at a given heading (i.e., grid by head direction). Grid cells were found to be equally distributed in the superficial and deep layers of the PreS and PaS. Many grid cells were modulated by head direction, although HD cells without multiplexed grid firing were also observed. HD cells in the PreS exhibited sharper tuning relative to HD cells in the PaS. Finally, some HD cells also demonstrated theta modulation. These firing characteristics may be representative of a convergent stream of processing (as reviewed by Brandon et al., [30]).

Medial Entorhinal Cortex

The medial entorhinal cortex became a focus of spatial navigation research after the recent discovery of grid cells [97]. Other spatially modulated cell types have been observed, including speed cells [143] and head direction cells [84, 220]. The functional milieu of the MEC indicates a possible role for integrating self-motion signals relative to landmarks during navigation.

The MEC receives dense projections from various regions within the subiculum cortex [112, 270, 275]. Projections from the PoS (layers II, III) terminate in layer III of the MEC [270], a region where HD cells have been found [84, 94, 220]. The first description of directionally modulated cells in the MEC was by Sargolini et al., [220]. HD cells were found in layers II, III & VI of the MEC. Furthermore, multiplexed HD by grid cells were identified in layers III (60% multiplexed) & V (90% multiplexed). These layers in turn project to the hippocampus, specifically with layers II projections initiating the trisynaptic circuit and while layer III projections terminate in the proximal CA1 [288]. Giocomo et al. [84] assessed the distribution of directionally modulated cells in the MEC. Just as the size of grid fields increase along the dorsal to the ventral axis [97], tuning curves of HD cells were found to become wider. This may correspond to the topographic distribution of inputs from the PoS (dorsal MEC layer III) and PreS (ventral MEC layer II) [112]. Furthermore, although most inputs from PoS and PreS are excitatory, a significant population of GABAergic neurons in the PoS target MEC interneurons [275]. This differential connectivity between regions may drive the differences in HD tuning across the dorsoventral axis of the MEC.

The MEC may play a functional role in the integration of self-motion signals and visual signals [76]. As noted above, MEC neurons exhibit rich complexity in the encoding of several spatial features [101]. Head direction encoding in the MEC may
diverge from HD encoding in upstream areas whereby directional tuning may not be limited to an allocentric, or a viewer-independent, frame of reference (see [173] for review). One example of this was shown by Kornieko et al. [140], where HD cells that were rhythmically modulated in theta frequency responded differently to a visual cue change. Specifically, non-rhythmic HD cells tended to shift their preferred directions following a visual cue change, whereas rhythmic HD cells remained stable across the visually different environments. This suggests that, while both cells exhibit directional tuning, visual inputs differentially influence the state of their respective attractors. In addition, visually responsive neurons have been identified in the MEC [132], and the integration of speed signals is influenced by visual cues [69]. Given that visual information may enable error correction that arises from vestibular-driven self-motion signals [76, 161], it is possible that visual signals correct for error associated with self-motion within the MEC.

Landmark manipulation experiment

Head direction cells orient to environmental landmarks, which was first demonstrated in Taube, Muller, and Ranck [259]. Specifically, the preferred direction of PoS HD cells maintained a stable directional heading relative to a local cue card following a cue rotation [258]. This process, known as landmark anchoring, is strongly modulated by visual landmarks over other types of sensory information [90]. However, in the absence of a polarizing visual cue, other environmental features have been shown to influence the directional tuning of head direction cells [258]. Additionally, HD cells will shift their preferred direction in register with cue shifts, providing evidence that cells may be functionally connected through ring attractor dynamics. Once established, the HD cell network maintains this fixed heading, even when navigating to a novel environment, through path integration.

The evaluation of landmark anchoring was inspired by the cylinder rotation performed by Muller & Kubie [172] that was used to evaluate place cells. Taube [258] used this task to evaluate whether HD cells respond to a landmark shift like that of place cells, given that both cells fire within an allocentric, or viewer-independent, frame of reference. To test this, PoS HD cells were exposed to environments with a shifted visual cue, no visual cue, a rectangular environment, and a square environment. During rotation experiments, the preferred direction of HD cells shifted along with the visual cue in all environmental types, although HD cells tended to under-rotate relative to the degree of cue rotation (e.g. there is some error in the degree of shift observed). When the visual cue was removed, HD cells
shifted unpredictably but retained basic firing characteristics, indicating that the visual cue only influenced the directional tuning of the cell. The geometry of the environment also impacted the directional tuning of HD cells. In square or rectangular environments, HD cells would shift their preferred direction to a new heading, although the rotation of a visual still enacted control of HD cell tuning. Overall, this study provided the first evidence that HD cells responded to visual stimuli and may be influenced by room geometry. Subsequent experiments using different cue manipulations have revealed that the HD cell system will anchor to various cue types relative to task demand (See Fig3 for general paradigm descriptions).

Effects of Visual Cues on Landmark Anchoring

HD cells preferentially to visual landmark cues, although visual input is not the only sensory cue to modulate HD cell tuning [89, 305]. HD cells in the PoS, ATN and LMN are all similarly responsive to cue manipulations in that the preferred direction will maintain a fixed heading relative to a familiar cue and rapidly rotate along with a corresponding cue rotation [244, 254, 260]. During exposure to a novel visual cue, the HD system has shown to reliably rotate with that cue after an 8 min exposure [89]. Taube and Burton [253] evaluated HD tuning during navigation into a novel environment. In this study, animals navigated from a familiar to a novel environment connected by a hallway. HD cells shift in their preferred directions to a small extent (∼17 degrees) indicating that motion cues enabled them to maintain a fixed direction even out of sight from the familiar visual cue. This finding was replicated in a more recent study whereby animals navigated to a different room in both light and dark conditions [295]. Under-rotation of the preferred direction of HD cells has been observed in many landmark rotation or path integration studies [89, 90, 134, 254] and has been proposed to result from “cue averaging”. This theory has been described in detail elsewhere [123]. Briefly, cue averaging reflects the idea that multiple types of sensory information enact partial control over the HD cell system. This may explain why HD cells exhibit under-rotation [90, 254, 260] or more specifically exhibit a preferred direction that reflects an “average” of preferred directions observed in conditions with cue conflicts [135, 138, 253]. In all, HD cells rapidly integrate visual information during navigation.

Many researchers have used paradigms that include conflicts between proximal and distal cue shifts [300, 305, 307, 308] to evaluate HD cell tuning under different visual contexts. Zugaro et al. [308] evaluated the efficacy of proximal maze cue
Figure 3: Example apparatus used for landmark manipulation experiments. The white line indicates the location of the proximal cue card. A. Simple rotation task with no cue variant. Standard sessions are intermixed with manipulations to verify HD cells return to baseline directional firing. B. Dual-chamber experiment. Navigation between environments enables the assessment of angular integration during navigation. C. Apparatus used for testing the influence of Geometry on directional tuning. Rhomboid is from Clark et al. [55] while triangles are two environments used in Knight et al. [137]. D. Generic Proximal-Distal Conflict paradigm. Proximal cue (small black arch) and distal cue (large black arch) are rotated to create a cue conflict. A black curtain surrounds the environment prevents the use of room cues.
shifts on HD cell anchoring within an enclosure or with the enclosure removed allowing curtains located at the periphery to serve as the background. HD cells recorded in the ADN shifted along with proximal cues only in the enclosed environment. The authors indicated this may be due to changes in motion parallax between the two conditions. Similar findings have been observed with proximal cues that included a texture component. Yoganarasimha, Yu, Knierim [300] recorded thalamic HD cells while rats ran clockwise on a circular track. HD cells coherently rotated most prominently to the distal cue, but also to a small extent to proximal cues. Although most of these recordings were in the ADN, some recordings occurred in surrounding nuclei that are connected to visual processing areas including the LDN or reticular nucleus. Recordings in these regions showed similar results to the anchoring preference of ADN HD cells. Additionally, one recording of simultaneously ADN and LDN HD cells indicated their shifts were in register, indicating that at least the LDN, which is implicated in visual processing as well as directional tuning, becomes anchored similarly to that of ADN HD cells.

**Effects of Self-motion on Landmark Anchoring**

The differential use of proximal or distal cues may arise from visual self-motion information. Continuous perturbation of idiothetic cues via disorientation can weaken the effect of a familiar landmark on HD cell tuning [134]. When the conflicts between the idiothetic signals and external cues are large, external cues enact a smaller degree of control over the HD cells [135]. Thus, although external cues stabilize the HD cell network, external cues must be experienced as stable relative to idiothetic signals during navigation. Zugaro et al. [307] sought to evaluate whether motion parallax between the two cue types contributed to cue preference observed in prior studies. ADN HD cells were recorded as rats foraged for food on a small platform with a proximal cue (~36 cm from the platform) and distal cue (~144 cm from the platform). These cues were shifted in opposite directions and during two light conditions (continuous and stroboscopic lighting). The use of stroboscopic lighting was intended to disrupt the typical motion parallax or optic flow by changing the sampling of visual information input during navigation. Under continuous lighting conditions, 57% of HD rotated with the distal cue compared to only 33% in the stroboscopic lighting condition, while proximal cue anchoring increased from 9% to 27%, respectively. Thus, disruption of the motion parallax shifted the bias of HD cells anchoring onto proximal cues, indicating that motion parallax is an important feature for landmark anchoring. Arleo et al. [8] tested the
influence of optic flow on HD cell tuning. HD cells were found to remain stable with projected point fields and exhibited drifting during the rotation of the point fields. Although the extent of the HD cell drifts under-shot the rate of rotation of the point fields, these results indicated that optic flow may subtly contribute to the establishment of HD tuning. More recently, the MEC has been implicated in integrating landmark locations during navigation. Park et al. \cite{193} recorded MEC HD cells under stable and rotating conditions while a rat performed a passive avoidance task. HD cell tuning was unstable during the rotated conditions. However, instability was coherent across HD cell ensembles among all MEC cells recorded. Furthermore, the proximity of the rat to one of the two shock zones indicated that the reference frame of the animal was altered during rotations. This suggests that, when self-motion cues are inconsistent with visual and idiothetic signals, HD cells may register with a task-relevant reference frame. More importantly, a role for path integration in maintaining HD cell preferred direction stability was established. Lastly, Dudchenko & Zinyuk \cite{73} found that if rats were transported to a different room to explore a familiar T-Maze, ADN HD directional tuning anchored relative to the maze instead of maintaining a fixed heading relative to room cues. However, over time and with self-navigation to the adjacent room, the directional heading was observed to stabilize. Overall, these studies suggest the importance of the agreement between visual self-motion cues and idiothetic signals on landmark anchoring.

The hippocampus may play an important role in path integration mechanisms that contribute to directional stability. Two studies have evaluated the effects of large hippocampal lesions on HD cell functioning within a novel environment \cite{85, 86}. The first study evaluated intact versus hippocampal lesioned animals as they foraged for food in several environmental configurations. Landmark-shift experiments indicated that HD cells in both groups could rotate with a visual cue without a hippocampus. HD cells also demonstrated rotations within rectangular or square environments for both groups, indicating that the local geometric cue influenced HD cell tuning independent from the hippocampus. Finally, the stability of HD cells to maintain their preferred direction in novel environments was stable across days. Thus, this study found that landmark anchoring was not dependent on hippocampal function, even under novel conditions. Although the hippocampus may not influence landmark anchoring, it was possible that it could influence path integration mechanisms that are important for maintaining the HD signal under dark exploration \cite{61}. This concept was tested in a follow-up publication by the
same authors [85]. Three experiments were implemented to assess the HD cell system under conditions where path integration mechanisms are necessary to maintain orientation. Importantly, this study included a group to control for incidental damage to overlying cortical tissue that was observed in hippocampal lesioned animals, including frontal areas 1 & 2, hind limb area of the somatosensory cortex, the posterior parietal cortex, and occipital area 2. Several major findings were observed. First, the stability of HD cells in the lesioned groups was impaired during initial navigation into a novel environment, followed by relative stability during a short period. Second, the preferred direction of HD cells may shift after reintroduction to a novel environment, independent of experience. Third, the degree of the shift was significantly influenced under dark conditions. These results suggest that although the mechanisms underlying landmark control were intact, the hippocampus, as well as overlying cortical areas, contributed to the path integration mechanism during navigation.

Effects of Environmental Geometry on Landmark Anchoring

The geometry of an environment influences the behavior of rats [47] and strongly anchors the HD cell system under reorientation conditions. Indeed, although HD tuning exhibits unstable drifting prior to eye-opening [252], recent findings indicate that geometric cues can stabilize the HD cells from pre-visual rat pups [14]. Several studies evaluated HD cell tuning with various environmental manipulations. In a study by Clark, Harris, and Taube [55], rats were disoriented and then exposed to a trapezoid or rectangle. In the first two experiments, these enclosures were rotated 90 degrees with or without a distal cue. Results indicated that HD cells were strongly anchored with the geometry as rotations within the trapezoid always led to rotations of HD cells when a distal cue was absent or present. When no distal cue was present, the rotation of HD cells in the rectangle was somewhat less clear. HD cell tuning showed either no rotation, a ± 90-degree rotation or a 180-degree rotation, indicating that non-controlled room cues or local maze cues enacted control over HD cell tuning. During trials where a distal cue was present, HD cell tuning became more anchored to the distal cue, although this result was highly influenced by cells from one rat. Nevertheless, the saliency of the distal cue seemed to compete with the local geometry. To explore this, the authors progressively increased distal cues and performed the same rotation experiments in the trapezoid. Through each progression, HD cell tuning seemed to average the influence of the geometry and distal cues. Altogether, these results indicated that environmental
geometry may compete with distal cues in anchoring the HD cell system when rats are disoriented prior to experimentation. Indeed, Knight et al. [137] found that geometry influenced HD tuning only after rats were disoriented. Thus, it seems that environmental geometry becomes the most salient cue when an animal becomes disconnected from the external world. If this is true, then the use of geometry cues should decrease with experience as the animal incorporates the environmental geometry with the external world. Only one study has assessed this concept. Gupta, Beer, Keller and Hasselmo [94] recorded MEC HD and grid cells in rats exposed to more recent (visited in the last week) or less recent (not visited in last week) exposures to a shifted T-maze. HD cell tuning was found to maintain heading relative to the external cues during exposures to more recent shifts of the maze while less recent shifts corresponded to a larger shift of the HD cells preferred direction. Grid cells shifted in register with HD cells, indicating that both cell types are similarly anchored to landmarks. Thus, local geometry preferentially anchors the HD cell system when external cues prove unreliable relative to idiothetic signals.

**Functional Connectivity of the HD Circuit**

The functional connectivity between regions within the HD cell circuit has been extensively explored through lesion and inactivation studies. Basic firing characteristics and landmark anchoring are often measured to evaluate how the site of the lesion may functionally contribute to the HD cell circuit. Altogether, lesion studies have provided significant insight into the hierarchical arrangement of the HD cell network (Fig.4).

**Basis of Idiothetic Drive**

Vestibular information has been shown to be integral to the formation of HD cells. When information about head turns is removed, HD cell activity in the ADN becomes disconnected from head direction [171, 269]. Muir et al. [171] was the first study to evaluate HD function after occlusion of the semicircular canals in the chinchilla. While recording in the ADN, no HD cell activity was observed. Instead, cells exhibited burst-like properties like those of HD cells, but importantly there was no correlation between bursting and head direction. The authors concluded that these bursty cells were likely disrupted HD cells based on several factors. First, the firing rate of bursty cells was equally correlated to angular head velocity as observed in HD cells. Second, the inter-spike intervals during burst episodes for bursty cells

![image]
**Figure 4**: Simplified HD cell circuit diagram. Contralateral projections are not shown to limit further complexity. Warm deep colors reflect more idiothetic firing correlates while cool deep colors reflect more allocentric firing correlates. Rga, Retrosplenial granular cortex a; Rgb, Retrosplenial granular cortex b; Rsd, Retrosplenial dysgranular cortex; PPC, posterior parietal cortex; PoS, postsubiculum; PaS, parasubiculum; PreS, presubiculum; ADN, anterior dorsal thalamus; AVN, anterior ventral thalamus; LD, lateral dorsal thalamus; DTN, dorsal tegmental thalamus; LMN, lateral mammillary nucleus; SG, supra genual nucleus; NPH, nucleus prepositus hypoglossi; MVN, Medial vestibular nucleus.
and HD cell were similar indicating the firing properties between these two neuron types were similar. Finally, ensembles of bursty neurons would fire in the same temporal order relative to head turn direction whereby the time interval between cell pair firing was correlated with angular velocity. These findings have most recently been replicated in a mouse model with mutated semicircular canals [269]. In addition, lesions of the medial vestibular nucleus have also resulted in HD cell dysfunction [244]. ADN cells recorded in MVN lesioned animals lose directional firing properties and cells that exhibited bursty activity showed no relationship to angular head velocity nor temporal firing patterns related to head turn. Altogether, head translation information is essential to anchoring the HD cell system to internal heading while the vestibular nuclei have a more critical role in the generation of downstream HD cells.

Idiothetic input into the HD cell system contributes to angular integration during navigation. ADN neurons exhibit bursty yet non-directionally anchored firing characteristics in full (>75% lesioned) bilateral lesions of the NPH [37]. However, in this same study, ADN neurons could rotate with the cue and were directionally anchored in light but not dark conditions with partial NPH lesions (>40 & <75% lesioned). The latter findings are consistent with optogenetically silenced NPH neurons that project to the DTN. Under these conditions, ADN neurons only became unstable during dark conditions and authors found that this was specifically due to dysfunctional angular velocity integration [36]. Therefore, NPH → DTN may facilitate angular velocity integration into the HD cell system, though the role of the NPH may play a larger role in idiothetic processing due to the more severe impact of full relative to partial NPH lesions on ADN HD cells. The integration of angular velocity during navigation may also be processed via the SG nucleus, which sends projections to both the DTN and LMN [22]. Lesions of the SG also result in the appearance of a population of bursty cell activity in the ADN, and HD cells in lesioned animals exhibited transient landmark control deficits as well as preferred direction drifting in the dark. Moreover, SG lesions increased the anticipatory time interval of ADN HD cells, indicating a role of the SG in facilitating the prediction of future head direction. Motor information may enter the HD cell circuit at the level of the DTN via the Interpeduncular nucleus (IP) [52, 231]. Lesions of the IP result in HD tuning drift during the dark sessions or as an animal navigates from a familiar to a novel environment. Furthermore, familiar landmarks do not robustly reset HD tuning to the original orientation after drift occurs, suggesting that the loss of self-motion information into the DTN also
impairs landmark anchoring within a familiar environment. Lesions of the NPH and SG result in similar findings to MVN lesions, whereas lesions of the IP disrupt landmark control. Overall, the integration of velocity signal is required to accurately fire relative to head direction, while self-motion information may be more critical in establishing a stable perception of novel landmarks.

**Generation of the HD Signal**

Lesions of tegmentomamillary components of the HD cell system indicate these regions facilitate the generation of the HD cell signal. Basset, Tullman, and Taube [12] was the first study to lesion the DTN and record ADN cell activity. Directional modulation of cell activity in the ADN was greatly reduced. In addition, a small number of observed HD cells were found to be greatly modulated by angular head velocity. The DTN is reciprocally connected to the ipsilateral LMN and both structures have been implicated in constituting the ring component of the ring attractor theory of HD cell functioning [23]. Whereas lesions of the DTN reduce directional firing in the ADN, bilateral lesions of the LMN completely abolish ADN HD cell signaling [21, 230]. This finding was first demonstrated in Blair et al. [24] in which the LNM was bilaterally ablated. After lesions of the LMN, ADN neurons lose their directional tuning but maintain firing characteristics, such as bursting and firing rate, similar to unaffected ADN head direction cells. Unilateral lesions of the LMN do not abolish HD cell signaling [24], likely considering that the LMN projects bilaterally to the ADN. These results suggest a generative relationship between the DTN and the LMN for several reasons. First, DTN contains a large proportion of cells that code for angular head velocity and is reciprocally connected to the ipsilateral LMN that contains both angular velocity as well as multiplexed angular velocity by head direction cells [13, 226, 245]. DTN neurons provide strong inhibitory input, which is reflected in the sharpening of tuning curves in the LMN during ipsiversive head turns [13, 226]. Thus, by lesioning the DTN, LMN tuning may convey less specific spatial information to the ADN by firing equally relative to ipsiversive and contraversive head turns. The fact that ADN neurons still exhibit some modulation by angular head velocity without the DTN could be resultant from the moderate input to the LMN from the SG nucleus. Nevertheless, this HD cell activity is greatly diminished, indicating that SG inputs into the LMN serve a minor role in the generation of the HD signal. Second, bilateral lesions of the LMN indicate that ADN neurons require an excitatory drive from both nuclei to function as unilateral lesions spared long-term ADN HD cell functioning. The only other
source of vestibular information into the ADN could arise indirectly via the medial longitudinal fasciculus. However, lesions of this area do not seem to impair HD cell function [57]. Thus, DTN and LMN serve as the generative nodes of the HD cell circuit.

Cortical Areas Influence Stability of HD Tuning

ADN HD cells maintain directional firing characteristics after lesions of downstream cortical targets including the PoS, MEC, and RSC, but exhibit variable dysfunction related to landmark processing [56, 91]. Goodridge & Taube [91] found that lesions of the ADN abolish HD signaling in the PoS. In contrast, ADN HD cells are still observable after PoS lesions, though these cells exhibit a broader tuning range, longer anticipatory time interval and are poorly controlled by an intra-maze cue [91]. ADN inactivation has also been shown to decrease gridness of grid cells recorded in the MEC [287]. However, MEC lesions do not fully disrupt ADN HD cell functioning. Indeed, ADN HD cells were found to be relatively intact after MEC lesions during navigation to a novel environment, dark environment exploration and after landmark shift experiments. However, like that of PoS lesions, ADN HD cells recorded from MEC lesioned animals did show an increased anticipatory time interval, indicating the MEC is involved in conveying movement information to the ADN. Clark, Bassett, Wang & Taube [53] lesioned large portions of the caudal Retrosplenial cortex and recorded HD cells in the ADN. Again, HD cells exhibited a larger anticipatory time interval similar to lesions of the PoS and MEC. However, the stability of preferred directions was impacted as indicated by observed preferred direction drift in both light and dark conditions as well as impaired landmark rotation and maintaining a directional heading while navigated to a familiar environment [53]. Although the functional significance of ADN inputs into the RSC is not completely clear, lesions of the ADN have demonstrated changes in immediate early gene expression in the rostral and caudal RSC and hippocampus [104, 124]. These studies demonstrate a dependence of ADN input for generating cortical HD or grid cell activity.

Functional Connectivity Summary

Single unit recordings and landmark manipulation experiments have demonstrated several important characteristics of the HD cell system. One important finding is that HD cells are functionally coherent. This is demonstrated by the observation
that HD cells will shift their preferred directions in register with each other during cue manipulations. Although there has been one recent exception of this in MEC HD cells in mice [140], ensemble recordings between regions largely demonstrate coherent shifts of HD cells or multiplexed HD cells relative to external cues [204, 283, 300]. A second characteristic is that HD cells are driven by idiothetic cues, and maintain their stability through the integration of self-motion, tactile, olfactory, and visual sensory input. Velocity and self-motion signals are processed through brainstem nuclei and this information is critical for the observation of downstream HD cell firing. DTN \(\rightarrow\) LMN circuitry reflects the generation of HD signal in the ADN and LMN neurons may reflect complimentary ipsiversive and contraversive information necessary to facilitate sharp directional tuning in the ADN. Although visual information seems to have the largest impact on the anchoring of the HD cell system, different visual features hold varying modulatory weight over time. The third functional characteristic is that HD cells can rapidly establish landmark anchoring to any task-relevant feature. For instance, local cues such as environmental geometry are robust at anchoring the HD cell system during reorientation states. However, global cues, such as those that are polarizing, stable, and distally placed, seem to anchor the HD cell system more robustly with increased experience. Landmark information is likely conveyed from cortical circuitry as lesions of these regions result in landmark instability. Overall, the HD cell circuit consists of a generative subcortical circuit that requires the cortical drive to stabilize within a given external reference frame.

**Modeling of HD cell system**

Attractor dynamics have been proposed to underlie various neurobiological systems including working memory [222], linguistics [43], motor movements [118], orientation coding in the visual cortex [16, 63, 265], and the head direction cell system [63, 209, 302]. Generally, attractor networks reflect persistent and dynamic systems. Recurrent connections between units within the system enable a graded bump of activity. Translation of the activity bump within the system can occur through the integration of vector-based information, such as velocity. The components of various attractor dynamics and its relation to biological systems [74] and the general role of attractor dynamics in forming spatial representations have been reviewed previously [136]. This section is focused on the use of a continuous ring attractor as a model for HD cell persistent activity. Although many models rightfully focus on the generation of the HD signal, which comprises the ring dynamics, the purpose of this
evaluation is to summarize and assess the mechanisms employed in these models that stabilize the ring relative to the external world.

**HD Circuit as a Continuous Ring Attractor**

The generation of the HD signal was first proposed to consist of a continuous ring attractor by Skaggs, Knierim, Kudrimoti, and McNaughton [240]. The model consisted of cell populations assuming a ring connectivity pattern. These rings included vestibular, rotation, visual feature detectors, and HD cells. Vestibular cells allowed for head movement information to be projected onto rotation cells that became activated by clockwise or counterclockwise head turns. These inhibitory rotation cells would become active via excitatory connections from a given HD cell, allowing a lateral inhibition by the rotation cell on neighboring HD cells. Recurrent connectivity between the HD cell that is most active at a given direction and neighboring HD cells of similar preferred directions enables a hill of activity to emerge at that direction along the azimuth. Visual feature detectors would provide biasing input onto HD cells that would align the HD cell ring to a given orientation relative to visual landmarks. Although solely qualitative, this model predicted several cell types found within the HD cell circuit, including inhibitory angular velocity cells (putative DTN neurons) and excitatory multiplexed angular velocity by head direction cells (putative LMN neurons). The influence of this study on the design of subsequent models is very much evident. Many quantitative models followed [14, 23, 34, 92, 209, 219, 229, 242, 248, 292, 302]. The specific mechanism within each of these models may differ, though there are several consistent components. The first includes a mechanism for the integration of clockwise and counterclockwise angular velocity inputs. These inputs are oftentimes inhibitory as originally predicted, although excitatory inputs have also been explored [92]. The second includes the convergence of these signals onto an HD cell ring. Recurrent connectivity between HD cells within this ring is often proposed. The third component consists of how the HD ring is stabilized relative to the external world. This has been accomplished by setting each cell in the ring with one preferred direction along the azimuth, so that the entire ring will code for all possible headings [92, 209] or through the use of “visual” bias input. Overall, these basic components have proved useful in modeling aspects of the HD cell circuit.
Visual Components of HD Models

Several models have explored how visual information may stabilize the HD network to a given orientation relative to external landmarks. Zhang [302] first described the theoretical characteristics of visual inputs. First, visual inputs were predicted to be persistent and directionally modulated so that HD cells could be set to a specific orientation to a given landmark. Second, a visual input represents a given orientation of a landmark relative to the head. Over time, the visual cues will bias HD cell firing relative to the landmark position. Importantly, landmarks must reactivate the orientation of the HD cell system that was established upon the first view of the landmark. Indeed, visual inputs intended to stabilize the ring attractor often represented as Gaussian functions [98, 242, 248, 250, 302], but may also exhibit a one-to-one ring pattern [23].

Two studies have modeled the mechanisms underlying cue averaging [189, 250]. Page et al. [189] modeled visual input as a ring attractor in which landmarks consist of Gaussian activity bumps that are asymmetrically connected to the HD cell ring. To account for different landmarks or cue conflicts, visual inputs modify the HD cell ring through Hebbian mechanisms. Simulations indicated accurate replication of the cue averaging results reported in Knight et al. [138]. Furthermore, coherent shifting of the entire population of HD cells emerged after adequate sampling of the environment, which is in line with path integration studies that find cue averaging after self-locomotion between two adjacent environments [80, 295]. More recently, Sun et al. [250] proposed a means of updating the HD cell ring by anti-Hebbian visual inputs by implementing a simple ring attractor described by Touretzky [262]. In this model, a single HD ring receives global inhibition and excitatory recurrent feedback from interneurons. Gaussians representing the location of two environmental cues provide excitatory input on the HD cell ring. The strengths of these cues are defined by an error term (high error leads to increased instability i.e., weak control). The model was compared with an expected result calculated through maximum likelihood estimation. Cue conflict experiments indicated that the model performed similarly to experimental results [138]; the cues were averaged for small to moderate conflicts but used the stronger of the two cues during larger conflicts. Models of the HD cell system that incorporate landmark components have been evaluated in the context of landmark anchoring to a distal cue. Hahnloser [98] evaluated visual inputs that represented the distal landmark by setting a Gaussian input at coordinates fixed relative to the rat (i.e. artificial agent) head. This Gaussian supplied bias to a dual-ring system with asymmetric offsets.
receiving either clockwise or counterclockwise input. As the head turns, the rings succumb to repeated excitation from the visual input for that given heading, while the connectivity between the two rings enables amplification of the visual inputs. This model could stabilize after a landmark shift and during the dark (i.e. visual input set to zero). However, the temporal progression of stabilizing the ring was much longer than is biologically observed (3 seconds for shifts and 4 seconds for no cue), while empirically derived stabilization times are estimated on the order of milliseconds. Degris utilized a model that satisfied biologically comparable results. This model incorporates an attractor model predicted to lie within the DTN (inhibitory) and LMN (recurrent excitatory) circuits, while visual feedback is applied through the visual (Gaussian) PoS LMN circuit. The visual inputs, governed by Hebbian mechanisms, consist of a static excitatory Gaussian function (15-degree width) of varying strength (varying from 0—1) to mimic a salience effect. With a 90-degree rotation, the HD cell ring rapidly rotated with the visual cue within 40ms. Although the first model demonstrated the possible value of dual-ring systems for visual information, while the latter model resembled an attractor state consistent with HD circuitry, the influence of idiothetic input on the stability of the HD ring was not established. Stringer, Trappenberg, Rolls & Araujo utilized an associated learning rule to integrate self-motion cues into the attractor through Hebbian mechanisms. Although there was no landmark shift assessment, this model was successful in maintaining a stable heading even after the visual input was removed (i.e. set to zero). Given the influence of self-motion on landmark anchoring (see Effects of Self-motion on Landmark Anchoring section above for further descriptions), the value of the model from Stringer et al., cannot be overlooked.

Self-motion cues have been evaluated by incorporating model parameters that simulate proximal cues. Most models incorporate visual cues that exhibit a finite distance whereby the influence of the motion parallax is not applicable. However, Zugaro et al. found that disruption of visual motion through stroboscopic lighting dynamically changed the types of cues used for landmark anchoring by the HD cell system, indicating that motion parallax signals may play a role in HD cell anchoring. Biacanski & Burgess proposed an attractor model that incorporates the processing of proximal cues by utilizing a visual ring attractor that modulates direction by place cells in the retrosplenial cortex. This model also includes interactions between CA1 pyramidal neurons and retrosplenial cortex to facilitate egocentric by allocentric inputs. Thus, egocentric information combined with directional information could intuitively account for the changing perspective.
of the landmark as the animal moves through the environment. This model successfully enabled the HD cell ring to anchor to a proximal cue. When the authors simulated hippocampal lesions, the HD cell ring exhibited instability when navigating to a novel environment like that observed in prior lesions studies of the hippocampus \[85, 86\]. Furthermore, this model provides evidence of a potential mechanism underlying the HD cell instability that is observed after lesions of the retrosplenial cortex \[53\]. This model was the first to demonstrate how specific cell firing observed in the retrosplenial cortex may contribute to landmark anchoring of the HD cell system. More importantly, the visual input did not simply assume a Gaussian input that biases the HD cell system. The visual cue instead reflects visual receptive fields that are stimulated as the landmark emerges in the visual field of the virtual agent. Moreover, this approach allows for motion parallax signals to contribute to landmark stability, which has proven important for landmark anchoring \[307, 309\].

The combined results of these studies suggest that visual inputs in the form of Gaussian or one-to-one connections with the HD cell ring are successful in replicating some effects of landmark anchoring observed from in-vivo electrophysiological work. The integration of multiple landmark cues resulted in the replication of cue averaging experiments \[138\]. The recent observation of landmark cells in the Rsd \[121\] could serve as a biological substrate of the multiple landmark inputs proposed by Page et al. \[189\] and Sun et al. \[250\]. These cells do convey Gaussian firing patterns relative to visual landmarks. Furthermore, Rsd innervates the PoS, Rga and Rgb, of which head direction cells have been found \[272\], and is consistent with findings that RSC lesions disrupt landmark control processes \[53\]. Although models utilizing a distal landmark demonstrate major features of HD tuning during landmark shift experiments, Biacanski and Burgess \[23\] were the first to provide a biologically feasible model for proximal cue anchoring. Importantly, this work makes use of self-motion cues, which are essential for the integration of angular information during navigation. Despite differences in model parameters, this work provides invaluable information for understanding how visual inputs may be integrated into an attractor system.

**Conclusion**

The purpose of this review was to evaluate landmark anchoring mechanisms of the head direction cell circuit in the context of anatomical, functional, and theoretical frameworks. First, the subcortical components were evaluated to determine how
functional firing correlates and anatomical connectivity could result in HD cell firing patterns. This revealed a substantial role in the generation of the HD signal in brainstem, tegmental, and tectal loci. Next, the assessment of cortical circuitry and function revealed a prominent role in integrating external cues with idiothetically derived heading information. An account of landmark rotation experiments was then reviewed, to reveal how external inputs could influence the directional tuning of HD cells. This review found that the HD cell system rapidly responds to external cues relative to task demands. Experience corresponds to the use of distal cues whereas disorientation states are more indicative of the use of proximal cues. Furthermore, the use of either cue is governed by self-motion information. Importantly, self-motion cues must match expected visual cues for HD cells to stabilize within an environment. Finally, a review of attractor models indicates that visual cues represented as Gaussian functions or one-to-one visual inputs can replicate some major features of landmark control such as cue averaging and self-motion updating. In all, this evidence suggests landmark anchoring is a multi-step process that involves the integration of sensory information, self-motion, and idiothetic derives heading information.
Chapter 2

Increased directional tuning of head direction by postsubicular interneurons in the TgF344-AD rat model of Alzheimer’s disease

Abstract

Spatial navigation and memory are impaired in Alzheimer’s disease (AD). The TgF344-AD rat model of AD has been shown to exhibit similar behavioral and pathological features to humans and thus serves as an excellent model to investigate mechanisms of spatial navigation impairment in AD. Previously, we found that 10-month-old TgF344-AD rats make progressively less spatially precise movements during a reference memory variant of the Morris water maze. In addition, TgF344-AD rats also exhibit progressive pathology in cortical areas involved in the encoding of head direction. Thus, this study sought to characterize directionally modulated cell types in the postsubiculum of 10—20 month female TgF344-AD rats and aged matched F344 controls. Rats performed a pellet chasing task within a cylinder fitted with a white cue card during recording. Putative pyramidal and interneuron proportions were altered in TgF344-AD rats, whereby a lower relative proportion of pyramidal cells was observed. In addition, pyramidal and interneurons were found to encode for head direction, running speed, and angular velocity in both TgF344-AD and control rats, although TgF344-AD rats exhibited a larger proportion of putative interneurons that encode head direction. In addition, TgF344-AD interneurons that encode head directions were found to convey increased directional information, exhibit more stable directional tuning over time, and are more likely to fire in bursts.

Furthermore, TgF344-AD multiplexed head direction and running speed interneurons had an increased sensitivity to linear speed, whereby firing rate increased to a greater degree with running speed. TgF344-AD pyramidal cells that encode the head direction showed no differences in firing characteristics or directional tuning compared with control cells. Head direction cell pairs exhibited spatial and temporal correlations, consistent with attractor dynamics, with no differences between groups. Overall, these results suggest that the postsubicular head direction cell circuit in TgF344-AD rats is comprised of an altered function of putative interneurons that encode head direction with no observable difference in pyramidal cell function. These findings further support hypotheses surrounding altered interneuron function in animal models of AD-like pathology.
Introduction

Rodent models of Alzheimer’s disease (AD) pathology commonly display disruptions to spatial navigation and memory [17, 122, 168]. Pathological features of AD in humans include toxic amyloid beta species, tauopathy, inflammation, and neurovasculature dysfunction [99], and changes associated with these pathological processes are predicted to occur up to 20 years prior to symptom development [119, 152, 279]. AD-like pathology in rodent models is associated with changes in the excitability of neurons [21, 190, 206], disruptions of synaptic plasticity [217], and alterations of excitatory-inhibitory balance [21, 201, 278]. In addition, neurons that encode spatial features, which are thought to support spatial navigation and memory, exhibit altered function in animal models of AD-like pathology. These alterations consist of reductions in firing specificity and stability [41, 51, 157, 294] as well as disruption of mechanisms underlying memory consolidation [40, 142, 180]. Critically, these processes are associated with precise temporal coordination between excitatory and inhibitory cells [163, 218, 239, 246]. Thus, disruption of excitatory and inhibitory networks may underlie memory impairments observed in AD, which has been a recent topic of investigation [40, 108, 129, 175, 191, 194, 277, 293]. While the hippocampus has been the main target of this research [2], extrahippocampal areas, such as the postsubicular cortex, have received less attention.

The postsubiculum is a cortical region located within the parahippocampal cortex [271]. The postsubicular cortex serves as an important target for elucidating the impact of AD-like pathology on spatial memory. In human studies of AD, the postsubiculum (or dorsal presubiculum) develops a unique pathology of lakes of amyloid-beta fibrils [4, 125, 174], and atrophy [154, 199]. Amyloid pathology within the postsubiculum has also been observed in several mouse models of AD-like pathology [285]. In addition, lesions of the postsubiculum lead to similar deficits observed in individuals with AD [140, 147] or animal models of AD-like pathology [17], including imprecise navigation during homing tasks [196] and reduced performance in alternation tasks [19]. This evidence suggests that the functional integrity of the postsubiculum may be disrupted following the development of AD-like pathology.

Head direction (HD) cells are cells that fire maximally around one direction in the horizontal azimuth and were first characterized in the postsubiculum [254]. These cells are thought to be critical for conveying vestibularly driven head direction information to the entorhinal cortex [91, 115, 287]. In addition, the
encoding of velocity signals has also been observed in the postsubiculum [77, 227], and the integration of velocity and head direction has been posited to support the generation of place firing in downstream loci [30, 161]. Additionally, juxtacelluar recording of excitatory postsubicular neurons reveals that HD tuning, while strongly driven by feed-forward excitation from the anterior thalamus [178], may be further supported by local inhibitory feedback [238]. Thus, the changes in excitatory and inhibitory cells that are associated with AD, as described above, may be consequential to the encoding of head direction by cells in the postsubicular cortex.

To explore the consequences of AD-like pathology on the HD cell circuit, we recorded single-units in the postsubiculum of TgF344-AD rats (n = 4) and age-matched littermate F344 controls (n = 4). These rats express the familial mutation associated with AD, including human mutated presenilin 1 and amyloid precursor protein, and develop a comprehensive profile of AD-associated pathology [58]. Cell activity was recorded while rats performed a pellet chasing task in a cylindrical environment. Altered function of inhibitory neurons in the postsubiculum may lead to a degradation of head direction cell circuit. To examine this, four main hypotheses were examined in this chapter. Putative pyramidal and interneurons were identified and the encoding of spatial features including HD, linear speed, and angular velocity were estimated. First, given TgF344-AD rats show evidence of excitatory—inhibitory imbalance in hippocampal and cortical regions [247], putative interneurons and pyramidal cells were examined for evidence of network alterations. Specifically, changes in network function, such as measures of burst and firing regularity, as well as hyperexcitability, are hypothesized to be increased in TgF344-AD postsubicular neurons. Second, alterations of interneuron function may result in reduced specificity of cells that encode spatial features. Thus, we tested the hypothesis that directional tuning would be reduced in TgF344-AD rats, whereby tuning curves should be wider following reductions in lateral inhibition from interneuron function. Third, given interneurons show increased responses during head rotations [205] and are thought to support the integration of speed and head direction, we tested the hypothesis that changes in network function, such as hyperexcitability, would lead to an increase in the sensitivity of angular velocity. Finally, postsubicular head direction cells have been shown to follow attractor dynamics whereby neurons are temporally and spatially correlated relative to their preferred firing directions [203]. We thus tested the hypothesis that head direction cell pairs would have increased temporal coupling due to increased firing rates, and that spatial coupling would be reduced following a deterioration of
the postsubicular attractor state.

## Results

Single units were recorded from the postsubiculum (Fig 1A) of TgF344-AD (N = 4, n = 1719) and F344 control (N = 3, n = 2355) while rats foraged for fruit loops in a cylindrical environment (Fig 1B). Data from one control rat was removed due to a grounding issue. Standard measures of locomotion were examined between groups. No differences in overall angular velocity, linear speed, distance travelled, or the duration of recording session was observed, indicating TgF344-AD rats and controls exhibited similar behaviors during the recording sessions ($ps \geq 0.239$). Putative pyramidal and interneurons were classified by their waveform duration, whereby pyramidal cells exhibited a duration of greater than 0.5 msec (Fig 1C). The proportion of putative pyramidal and interneurons was examined across recording sessions and was weighted by the number of total cells recorded. The weighted average for the proportion of pyramidal cells recorded per session was greater in controls (70.3%) compared to TgF344-AD rats (30.5%), ($b = -2.11, SE = 0.89, p = 0.0183$, Fig 1D). Overall, these results indicate that the proportion of interneurons and pyramidal cells are altered in TgF344-AD rats, which is consistent with previous reports of altered GABAergic and pyramidal cell counts within the parahippocampal cortex of TgF344-AD rats [58, 168].

Neuronal hyperexcitability is a common feature observed in human with Alzheimer’s disease and rodent models of AD-like pathology. To examine whether hyperexcitability is present in postsubicular neurons recorded from TgF344-AD rats, we measured the firing characteristics of all recorded units. Pyramidal and interneurons recorded from TgF344-AD rats exhibited higher but non—significant baseline firing rates compared to controls ($ps > 0.057$, Fig 1G,J). No differences in burst index were identified for pyramidal and interneurons between groups ($ps > 0.41$), although TgF344-AD interneurons exhibited more irregular firing, ($b = -0.771, SE = 0.10, p = 0.0097$) despite no differences in the regularity of firing for pyramidal cells, ($p = 0.21$).

Thus, while the proportion of interneurons and pyramidal cells is altered, there are only subtle changes to intrinsic firing of postsubicular neuron populations in TgF344-AD rats.
Figure 1: Proportional and functional changes of TgF344-AD pyramidal and interneuron populations. A) Coronal depictions of electrode sites, and example image showing electrode traversal through the postsubiculum of one rat (LB03). B) Cartoon layout of the task apparatus, and example path for a single recording session. C) Example average waveforms for all cells classified as interneurons (left, dark grey) and pyramidal cells (right, light grey), and distribution of waveform duration (ms) for all recorded cells. D) Proportion of pyramidal and interneurons across groups. Each observation indicates a proportion for a given session and the size of the observation indicates the total number of cells recorded within that session. Pink horizontal bar indicates the weighted mean. Note that the relative increase in the proportion of interneurons observed in TgF344-AD rats does not relate to the total number of interneurons, as sessions with a higher number of interneurons also show a lower number of total recorded cells. E) Example autocorrelograms for two example neurons. Blue bars indicate the $2\text{ms} - 9\text{ms}$ and $40\text{ms} - 50\text{ms}$ used to calculate burst index (see Methods). Note that the neuron on the left has a strong probability of spiking in short time intervals given the large peak in the $2\text{ms} - 9\text{ms}$ range. F-H) Empirical cumulative density functions for all putative interneurons, with each line depicting cells for a single animal and colors indicating group (TgF344-AD: orange, controls: blue). P-values represent effect of group from linear mixed effects model. I) Cartoon depiction of regularity of inter-spike intervals using the CV2 measurement. Raster plots of simulated data are plotted according to CV2. Note that the regularity of interspike intervals is associated with lower values, whereas values at or greater than 1 resemble a poisson process. J-L) Same as E-G with the exception that cells included were pyramidal. For eCDF plots, each line represents a rat and blue and orange colors represent groups (controls = wt, TgF344-AD = tg)
Proportion of cells encoding of head direction and angular velocity and interneuron head direction encoding is increased in TgF344-AD rats

The postsubicular cortex contains neurons that encode various behavioral features including head direction (HD), linear speed (Speed), angular velocity (AV), and position [26, 87, 156, 204, 227]. Cells that encode HD only or are multiplexed with a combination of HD, speed, or AV were identified using a shuffling procedure (see Methods). We examined whether head direction encoding varied across pyramidal and interneuron populations. Interneurons were evaluated given the structural similarity of postsubicular interneurons and interneurons from neocortex [237] that exhibit shared tuning of local pyramidal cell populations and play a role in shaping the incoming sensory signals [155, 202, 290]. While the average proportion of pyramidal cells encoding HD was increased in TgF344-AD rats compared with controls (14% vs 5.4%, respectively), this difference was not significant ($p = 0.06$). However, interneurons encoding HD was significantly increased, ($b = 1.29, SE = 0.64, p = 0.04$, Fig 2B), with an average 25.8% encoding HD in TgF344-AD rats and 10.3% encoding HD in controls. In addition, pyramidal cells encoding HD x AV were significantly increased ($mean_{Tg} = 2.6\%$ and $mean_{control} = 0.5\%$, $b = 1.52, SE = 0.54, p = 0.0053$, Fig 2A), while differences were not observed for interneurons encoding HD x AV, ($p = 0.091$). No proportional differences were observed for pyramidal or interneurons encoding HD x Speed, ($ps \geq .33$).

Altogether, these results indicate that HD encoding is present in the postsubiculum of TgF344-AD rats. Pyramidal cells encode HD x AV to a greater extent, and the proportion of interneurons encoding HD-only are significantly increased in TgF344-AD rats. Thus, there are proportional differences in the encoding of HD and AV across pyramidal and interneuron populations in the postsubicular head direction circuit.

TgF344-AD interneurons encoding head direction are more bursty

The previous sections indicate changes in the function and encoding of both pyramidal and interneuron populations within the postsubicular cortex of TgF344-AD rats. We next sought to answer whether cells that encode HD only or are multiplexed with HD and Speed or HD and AV show changes in intrinsic firing characteristics. Specifically, head direction cells are observed to exhibit highly irregular inter–spike intervals yet show reliable overall spiking when the head passes
Figure 2: Encoding of Head Direction is increased in TgF344-AD interneurons. A) Stacked bar graphs showing the proportion breakdown of encoding cell types across pyramidal cells (left) and interneurons (right) (#p < 0.10, *p < 0.05, **p < 0.01) B) Example tuning curves and average wave-forms for head directions for controls (top) and TgF344-AD rats (bottom). C) Example tuning curves and average waveforms for pyramidal head direction cells multiplexed with angular velocity (left) and linear speed (right).
through the preferred direction \[256\]. Irregular firing at shorter timescales has been posited to be critical for the integration of sensory inputs [141]. Pyramidal and interneuron HD cell populations showed no difference in baseline firing rates \((ps \geq 0.21)\) nor in the variability of inter–spike intervals \((ps \geq 0.074)\). While pyramidal HD cells showed no difference in burst index between groups \((p = 0.88)\), TgF344-AD interneurons HD cells exhibited a higher burst index, \((b = −1.56, SE = 0.48, p = 0.0018)\). Thus, intrinsic firing characteristics of TgF344-AD pyramidal HD-cells show no differences from controls, while TgF344-AD HD cell interneurons are more bursty.

**Interneurons encoding head direction are more stable, have reduced tuning width, and convey more directional information in TgF344-AD rats**

We next sought to identify whether directional tuning was altered in TgF344-AD rats by inspecting features of head direction tuning curves Fig 3B. The half-width, or the width of the tuning curve at 50% of the relative peak, was \(\sim 120 – 130\) degrees across both groups Fig 3A, and TgF344-AD HD cell interneurons showed a decrease in tuning width compared to controls, \((b = 12.76, SE = 6.086, p = 0.0393)\). TgF344-AD pyramidal HD cells were as stable and exhibited similar peak firing rates compared with pyramidal HD cells recorded from control rats \((p = 0.75)\). However, TgF344-AD pyramidal HD cells conveyed more directional information per spike compared to control HD cells, \((b = −0.62, SE = 0.23, p = 0.01)\). Directional information content was also increased in TgF344-AD interneurons \((b = −0.94, SE = 0.17, p = 0.0037)\). Furthermore, TgF344-AD HD cell interneurons tended to be more stable over time, \((b = −0.12, SE = 0.03, p = 0.021)\), and exhibited increased peak firing rates, \((b = −0.33, SE = 0.09, p = 0.0006)\). These data suggest that directional tuning is intact in TgF344-AD rats with evidence to suggest the directional precision of HD tuning is increased across pyramidal and interneuron HD cells. However, TgF344-AD interneurons tend to be more stable and exhibit reduced half-width compared to HD interneurons recorded from control rats.

**Interneuron head direction cells are more sensitive to linear speed in TgF344-AD rats**

Encoding of linear speed or angular velocity in the postsubiculum is posited to play a role in the generation of place coding in downstream areas such as the medial
Figure 3: TgF344-AD Interneuron HD cells convey more directional information and are more stable over time. A) Empirical cumulative density function for directional information content (dIC), tuning half-width, stability, and peak firing rate. Each line represents a rat and blue and orange colors represent groups (controls = wt, TgF344-AD = tg) B) Example TgF344-AD interneuron HD cells. Directional tuning statistics are plotted above a linearized directional tuning curve, with the red dashed line indicating the degree of the half-width measurement. Heatmaps (bottom panel) show the tuning curves created after the rat successfully samples the entire horizontal azimuth. Dark colors indicate higher firing rates. Note that the first two cells have baseline firing rates above zero, but still maintain stable firing over time as indicated by consistent increases in firing at the preferred direction over time.
entorhinal cortex and hippocampus \textsuperscript{[106, 161]}, and may be critical for maintaining an accurate heading direction \textsuperscript{[226]}. An example of this relationship is depicted in (Fig 4A,B). The slope of the linear relationship between speed or angular velocity and firing rate was examined within multiplexed HD cell populations (Fig 2C). The change in firing rate as a function of speed or angular velocity was similar between groups across all pyramidal multiplexed classes, \((ps \geq 0.07)\), and interneurons HD x AV and HD x Speed x AV, \((ps \geq 0.10)\). However, interneuron HD x Speed cells showed steeper slopes for linear speed compared to control cells \(b = -0.46, SE = 0.20, p = 0.027\) (Fig 4C). Thus, TgF344-AD HD x Speed cells exhibited a stronger increase in firing rate as a result of linear speed. To verify whether this was specifically related to multiplexed HD x Speed cells, we also examined the slopes of non-HD interneurons and pyramidal cells that encode Speed or AV. Curiously, no differences were observed \((ps \geq 0.15)\), suggesting that while non-HD Speed or AV cells show no differences between groups, HD x Speed cells show increased gain of firing rate as a function of running speed.

**Temporal and spatial coupling of HD cell pairs is spared in TgF344-AD rats**

Computational models suggest that postsubicular HD cell dynamics may be dependent on SOM interneuron populations, whereas PV interneurons may be more critical for tuning properties \textsuperscript{[239]}. While these populations were indiscernible based on the waveform characteristics of the current data set, the changes in interneuron firing characteristics and directional tuning properties may suggest changes in attractor dynamics of local HD-cell pairs. To examine attractor dynamics, the spatial and temporal correlations were examined across HD cell pairs (Fig 5A). Spatial coupling was measured by examining the variance of the angular difference of a pair’s tuning curves over time (Fig 5B), while temporal correlations were determined by examining the central one second of the pair’s z-scored crosscorrelogram (Fig 5C). Given the differential role of pyramidal and interneurons in HD cell attractor models \textsuperscript{[179, 239]}, cell pairs were evaluated based on the putative cell class (i.e., pyramidal—pyramidal, interneuron—interneuron, and pyramidal—interneuron).

We first examined if HD-cells pairs showed stable spatial coupling between cell pairs over time, (Fig 5A-B,E). Tuning curves were created in 90 second intervals within a recording epoch, and the angular distance between the preferred directions of these tuning curves was measured. The variance associated with HD-cell pair
Figure 4: Increased sensitivity to linear speed in TgF344-AD HD x Speed cells  

A) Example modulation of firing rate (FR) (left, top panel) by linear speed (Speed) (left, middle panel), and head direction (HD) for control HD x Speed interneuron (left, bottom panel) across 15 seconds. The session tuning curves for linear speed (right, top panel) and head direction (right, bottom panel). This cell fires maximally when the head is facing around 0 radians (red) and the rat is running 20 cm/s. Note the large burst firing when the animal is moving at its peak velocity and is facing near the preferred direction, whereas firing is reduced if the animal is facing the preferred direction but is moving slower. B) Same as A but for TgF344-AD HD x Speed interneuron. The preferred direction of this cell is around 1.7 radians (green). At the start of the epoch, the rat is facing the preferred direction and the cell appears to fire in bursts. However, firing rate increase later in the epoch when the animal is running ~ 25 cm/s and faces the preferred direction. C) Empirical cumulative density functions for HD x Speed and Speed encoding interneurons (left panel) and pyramidal cells (right panel). Each line represents a rat and blue and orange colors represent groups (controls = wt, TgF344-AD = tg).
tuning over time was used to measure whether cells fired at consistent angular distances over time. Across both groups, postsubicular head direction cells showed variable spatial coupling over time, with 50% of the population of HD-cell pairs for both groups showing an angular difference variance of about 0.78 radians, (Fig 5D). Finally, angular variance was not different across groups or pair types, ($p_s \geq 0.44$), indicating that HD-cell pairs maintained comparable, but variable, spatial correlations across TgF344-AD rats and controls.

We next examined if HD-cell pairs maintained temporal coupling associated with the degree of their angular difference, (Fig 5C,D). For example, two HD cells with similar preferred firing directions (Fig 5A,C (top panel)) are more likely to fire together than pairs with opposite preferred firing directions (Fig 5A,C (bottom panel)). TgF344-AD HD-pairs exhibited similar temporal coupling compared to HD-cell pairs from control animals ($p_s \geq 0.57$). Across both groups, HD-cell pairs was negatively associated with the angular difference between their tuning curves across pyramidal-pyramidal, interneuron-interneuron, and pyramidal-interneuron cell pairs, ($b_s \geq -0.34, SE \leq 0.07, p \leq 1.16 e -09$). Thus, HD cell pairs exhibited similar temporal firing patterns as controls. Overall, postsubicular neurons showed adherence to attractor dynamics with HD-cell pairs in both groups showing similar temporal and spatial coupling compared to controls.

**Discussion**

This study examined postsubicular head direction cell populations in aged TgF344-AD rats and litter-mate age-matched controls, and found consistent changes in the function of postsubicular neurons. First, the proportion of interneuron and pyramidal cells observed during the recording sessions was altered, whereby TgF344-AD rats showed a relative decrease in pyramidal cells across sessions. Second, pyramidal and interneurons recorded from TgF344-AD rats exhibited subtle evidence of hyperexcitability as baseline firing rates were increased, although non-significantly different from control cells. Furthermore, TgF344-AD interneurons tended to have more irregular firing patterns at shorter timescales. Third, the proportion of cells encoding head direction was increased among pyramidal cells and putative interneurons in TgF344-AD rats. In addition, while HD by angular velocity encoding was generally low, TgF344-AD rats showed a larger proportion of HD by angular velocity cells compared to controls. Fourth, across all cells that encode HD, TgF344-AD HD interneurons conveyed increased directional information per spike.
Figure 5: Adherence of attractor dynamics in TgF344-AD HD cell pairs. TgF344-AD HD cell pairs demonstrated similar temporal and spatial coupling compared to HD-pairs recorded from control animals. A) Tuning curves for three HD cells recorded in the same session. Comparison shows a cell pair with large angular difference (top) and overlapping angular difference (bottom). B) Tuning curves created over 90 second intervals throughout the session for cell pairs in A. White line indicates the peak of the tuning curve at a given time bin. The angular difference of the peak bins over time is depicted in the last panel (blue line), and the mean differences is plotted in red. Note that these cell pairs maintain fairly stable angular offsets over time. C) Temporal correlation of the cell-pairs indicated in A, with the central second indicated by the vertical blue line. Note that cells with large angular difference have a negative central second, while cells with overlapping tuning have a positive peak at the central second. D) Temporal coupling is negatively associated with angular difference across pyr-pyr, int-int, and pyr-int cell pairs. Scatter plots showing central second magnitude and angular difference for cell pairs (rows) and groups (columns, controls are left panel). E) Empirical cumulative density functions for angular variance (top) and central second (bottom) with each line representing a rat and blue and orange colors represent groups (controls = wt, TgF344-AD = tg).

compared to that of control cells. Furthermore, while directional tuning was equally stable among pyramidal HD cells between groups, TgF344-AD interneuron HD cells
tended to have more similar tuning curves over time compared to control cells. Fifth, while most multiplexed HD cells showed similar modulation by either linear speed or angular velocity, TgF344-AD HD x Speed interneurons showed increased modulation of linear speed. Finally, while the temporal and spatial coordination between HD-cell pairs was generally more noisy in postsubicular HD cell pairs, HD-cell pairs from TgF344-AD rats showed similar temporal and spatial coupling compared to control HD-cell pairs. Overall, these results suggest that the postsubicular cortex of TgF344-AD rats undergoes alterations of intrinsic firing properties and that interneurons in particular exhibit increased directional tuning.

The examination of intrinsic firing characteristics of postsubicular HD cells showed subtle, population-specific alterations. For instance, while putative interneurons, independent of functional encoding classification, exhibited more irregular firing, putative HD interneurons were observed to only have increased burst firing and peak firing rates. Hyperexcitability of GABAergic interneurons is prevalent observation in AD research [277, 293], and may be associated with changes in metabolic function [131, 181]. Critically, hyperexcitability of neurons has been shown to increase with proximity to amyloid pathology [35, 251]. It is possible that these changes are manifested in TgF344-AD rats and are contributing to the increased peak firing rates observed in HD interneurons. Indeed, firing homeostasis is a posited contributor to network dysfunction in AD [81, 249], and is thought to underlie changes in neuronal excitability and excitatory-inhibitory imbalance that correlate with AD-associated pathology [21, 35, 108, 166, 201]. Lastly, the change in burstiness of HD interneurons may be an indication of compensatory mechanisms, either due to increased excitation from pyramidal cells or due to their slight elevation in baseline firing rate. While these results do not provide direct evidence of dysfunction related to amyloid pathology, there is indication that the functional milieu of the postsubiculum in TgF344-AD rats is altered compared to controls.

The increase in directional tuning across interneuron HD-cells may be indicative of an enhancement of excitatory synapses from upstream targets. The proportion of cells encoding of head direction was increased in TgF344-AD interneuron HD cells, and these cells showed stable and sharper directional tuning compared to control cells. The encoding of spatial features in interneuron populations has been identified in hippocampal formation [100, 286], and is posited to arise from interneuron-pyramidal cell connectivity [158]. In the postsubiculum, interneurons have been observed to exhibit weak direction tuning as well as modulation of angular velocity [205]. Directional tuning is thought to arise from
feed-forward excitation from the anterior dorsal thalamic nucleus [178], though local interneuron recruitment by pyramidal HD cells may also lead to interneurons inheriting directional tuning [239]. Thus, the observed increased proportion of TgF344-AD interneurons encoding head direction suggests changes to either local circuitry or changes in excitatory inputs. While this study did not perform a histological examination of local AD-like pathology, the reduced proportion of pyramidal cells observed in TgF344-AD rats suggest structural changes may be occurring and should be a point of future work. An overall reduction of pyramidal cells may lead to a reduction in feedback excitation of local interneurons. Indeed, pyramidal—interneuron connectivity is a potential driver of directional tuning in interneurons, so a reduction of pyramidal cells encoding HD may alter these dynamics. However, cell death has only been reported in this model at 24 months of age [58], which is four months older than the oldest animal included in this study. Another potential contributor of increased directional tuning of interneurons may be related to amyloid pathology, as this been attributed to an enhancement of excitatory synapses [190, 212, 224]. Thus, amyloid-induced changes in excitation may result a more sensitive response from postsubicular cells that are targeted by anterior thalamic inputs. Alternatively, increased directional tuning may result from a reduction of multiplexing. For example, Brandon et al., [29] found that disrupting the medial septum led to a reduction of multiplexed head direction by grid cells in the medial entorhinal cortex. Specifically, these cells maintained their directional tuning despite losing their grid-like firing pattern. Critically, no investigation of amyloid toxicity or examination of pyramidal/interneuron cell counts was performed in this study. While amyloid pathology has been observed in several mouse models of AD-like pathology, no study to date has characterized postsubicular pathology in the TgF344-AD rat model of Alzheimer's disease. Thus, future studies may benefit from examining the integrity of thalamic and postsubicular circuitry and to investigate whether changes to this circuit are contributing to the enhancement of head direction encoding postsubicular interneurons.

Interneuron HD cells also showed an increased gain in firing rate associated with linear speed. Importantly, this effect was only observed in interneuron HD by speed cells, whereby pyramidal or interneurons encoding only linear speed and angular velocity showed no such differences. Changes associated with velocity encoding may have implications for path integration or goal-directed navigation. TgF344-AD rats show reduced ability to move directly to a goal location whereby movement tends to be more circuitous or use local cues consistent with taxon
navigation [17][22]. Multiplexed velocity and head direction encoding has been associated with the support of position encoding in downstream areas [33][106][101]. Thus, it is possible that changes in speed encoding in the postsubiculum may lead to corresponding changes in the estimation of position. While this has yet to be examined in TgF344-AD rats, a recent study identified altered speed encoding in the medial entorhinal cortex of a tauopathy model of AD-like pathology [216]. Another potential implication of altered speed encoding in postsubicular HD cells relates to the processing of visual landmarks. A recent computational model of the postsubicular head direction circuit found that head direction or multiplexed head direction and velocity populations show dynamic decoding of a simulated visual stimuli, whereby pure head direction cells were best for decoding stable stimuli over longer timescales and multiplexed populations were better at decoding dynamic changes in stimuli [77]. Thus, changes in velocity encoding within the postsubiculum may contribute to the differential integration of visual stimuli within a complex environment. Future work is needed to verify whether changes in velocity and head direction encoding contribute to performance in goal direction navigation across dynamic visual environments.

Summary

This is the first characterisation of postsubicular head direction cells that includes examination of directional tuning of interneurons and is the first characterization of postsubicular head direction in an Alzheimer’s rat model. Directional tuning was observed to be intact across pyramidal HD cells in TgF344-AD, although both HD cells from both groups showed evidence of a broad range of tuning width compared to studies in young long-evans rats [156][254][257]. The key finding of this chapter indicates that putative interneurons exhibit stronger directional tuning than interneurons recorded in control animals. Multiplexed HD x Speed interneurons also demonstrated an increased sensitivity to running speed, whereby the firing rate changes more rapidly as a function of running speed. Recently, interneuron function has been a focus of investigation for targeted treatment development in Alzheimer’s disease [191][293], given the metabolic demand of inhibitory interneurons [39]. Overall these findings support the hypothesis of interneuron dysfunction in Alzheimer’s disease [10][277]. Critically, not all TgF344-AD interneurons examined in this study exhibited differential function compared to control cells. This highlights an important point that changes in interneuron function may not be general [290]. Overall, these experiments expand on the current knowledge of
parahippocampal dysfunction in AD.

Materials and methods

Subjects

Female TgF344-AD (n = 4) rats and littermate F344 controls (n = 4) aged 10—20 months were used as subjects. Animals were bred onsite at the University of New Mexico Animal Research Facility by pairing TgF344-AD females with F344 males. TgF344-AD breeders were provided by the Rat Research and Resources Center. Offspring were then genotyped (Transnetyx Inc, Tennessee) to verify the presence of the mutated Amyloid precursor protein Swedish mutation (APPSW) and the Presenilin 1 exon 9 mutation (PS1ΔE9). Females positive and negative for these mutations were reserved for the study and aged a minimum of 9 months before experimentation. Males were excluded from the study given the finding that TgF344-AD females exhibited earlier signs of spatial navigation deficits [17]. All animals received ad-libitum food and water before the commencement of experimentation, after which their weight was restricted to two-standard deviations of the average weight of their strain. All animals were pair housed for the duration of the study to limit behavioral changes associated with isolation. Vivarium lighting was maintained on a 12-hour light-dark cycle with lights on at 0900 hours. All procedures were approved by the Institutional Animal Care and Use Committee at the University of New Mexico.

Recording and Techniques

Electrodes

Two types of electrodes were used to collect neural data for these experiments. Rats were implanted with custom built 32-channel electrodes consisting of 8-tetrode bundles or a four-shank 64-channel Cambridge Neurotech assy-156 probe. The electrodes were grounded by soldering a stainless steel wire to a Jewlers screw that was placed in the frontal or cerebellar plates. Reference for tetrode-based electrodes consisted of wire located within the bundle.

Data Acquisition

Data was acquired using Neuralynx Digitalynx system or Open Ephys Rhythm FPGA acquisition system. For acquisitions using Neuralynx system, neural data
was acquired by connecting the subject’s electrode to a multichannel impedance matching, unity gain preamplifier headstage. The signal was then sent to a data acquisition system with 32 digitally programmable differential amplifiers (Neuralynx, Tucson, AZ, USA). Waveforms with single-unit activity above 20—40\(\mu\text{V}\) were timestamped, digitized at 32 kHz, and stored for offline sorting. For acquisitions using the Open Ephys system, neural data was collected by connecting the subject’s electrode to an RHD recording headstage with 3-axis accelerometer (Intan Technologies, Los Angeles, CA) equipped with an Intan R1264 chip with amplifier and multiplexing capabilities. Neural data was sampled at 30 kHz and stored for offline sorting. Single units were sorted into clusters using klustakwik [http://klustakwik.sourceforge.net/](http://klustakwik.sourceforge.net/), and clusters were manually checked using MClust [https://github.com/adredish/MClust-Spike-Sorting-Toolbox](https://github.com/adredish/MClust-Spike-Sorting-Toolbox) or Phy [https://github.com/cortex-lab/phy](https://github.com/cortex-lab/phy). The rat’s head position was tracked and timestamped (30 Hz) by an overhead camera.

**Screening**

Screening for isolated single units occurred in the standard cylinder condition (Fig 1A). The black curtain remained open and only the room lights remained on during screening. If no identifiable units were observed, the animal’s electrode was lowered 25—75 \(\mu\text{m}\). Screening was repeated up to three times per day, and the rat was allowed a minimum one-hour break between screening sessions to allow the neural tissue to settle.

**Recording**

If identifiable clusters were observed, the room was then prepared for the experiment while the rat rested on an elevated holding pedestal. First, the room lights were turned off and the recording chamber curtain was closed. Recording sessions lasted between 12—30 minutes to allow the animal to traverse the environment at least twice. After the recording completed, the electrode was advanced 25—75 \(\mu\text{m}\) and the animal was returned to the home cage.

**Testing Room**

All experiments were carried out in a 1.2 m x 1.2 m x 2.1 m recording chamber that was enclosed on three sides. The recording chamber was shielded with copper mesh to reduce electrical interference. A black curtain was placed on the open side of the
chamber during recording to restrict visual cues. Eight led lights were placed in a
circle at the top of the recording chamber for illumination during light conditions.

Data Analysis

Pyramidal and Interneuron Classification

Cells were classified into putative pyramidal and interneurons based on their
waveform duration obtained using a wavelet transform [203]. Waveforms were
obtained by first filtering the wideband signal between 600 and 9000 Hz, and then
taking a 1 ms window relative to the spike timestamp. The peak of each waveform
was centered at 0.26 ms. Waveform duration was estimated using the inverse peak
frequency of a wavelet transform of the spike waveform [202]. Plotting the
distribution of waveform duration for all recorded units showed a separation at 0.5
ms, consistent with previous reports showing pyramidal cells having wider duration
at or above this threshold [200, 203].

Measures of Intrinsic Firing Characteristics

Measures of intrinsic cell firing included average firing rate, burst index [218], and
coefficient of variation II (CV2) [110]. Average firing rate was calculated by dividing
the total number of spikes over the session duration. Burst index was calculated
from a neuron’s autocorrelogram (Fig 1E). Briefly, the peak within the 2ms – 9ms
range was divided by the mean of the baseline firing 40ms – 50ms range. This value
was then normalized by the peak when the peak value was greater than the
baseline, or normalized by the baseline for peak values less than the baseline. This
allowed values to range from −1 to 1, with higher positive values indicating higher
burstiness. CV2 was utilized to control for variations in firing rate that might occur
throughout a session. CV2 was defined as follows,

\[
CV2 = \frac{1}{N} \sum_{n=i}^{N} \frac{2 \times |t_{i+1} - t_i|}{t_{i+1} + t_i}
\]

(1)

where \( t \) is the spike time stamp at index \( i \) and \( N \) is the total number of spike
times.

Measures of directional tuning and stability

Directional tuning was measured using directional information content and stability
score computed from head direction tuning curves. Briefly, tuning curves were
obtained by binning spikes across 60 angular bins according to the head direction the animal was facing during which the spike occurred. The data was then normalized by directional occupancy to obtain a firing rate histogram across binned angles and the tuning curves were then smoothed with a Gaussian kernel (s.d. = 18 degrees). Directional information content was computed by measuring the mutual information between head direction and the firing rate \cite{203, 241}. Directional information content was defined as follows,

\[
DI = \sum_{i=1}^{60} \lambda_i \log_2 \left( \frac{\lambda_i}{\lambda} \right) p_i
\] (2)

where \(i\) is the index of the binned angle, \(\lambda_i\) is the firing rate at angle bin \(i\), \(\lambda\) is the overall firing rate of the cell, and \(p_i\) is the probability of the head facing angle \(i\). DI is divided by the overall firing rate to measure directional information in bits per spike.

The stability score reflects the similarity of consecutive tuning curves made within the session. Briefly, tuning curves were created for each full traversal of the horizontal azimuth. Stability score was then computed by obtaining the mean pairwise correlation across angular bins. Stability scores closer to 1 reflected more stable directional tuning across time. Stability was defined as,

\[
\text{stability} = \text{nanmean}(\text{corr}(X^T))
\] (3)

where \text{nanmean} and \text{corr} are Matlab functions and \(X^T\) is the transpose of the matrix consisting of tuning curves over azimuth traversals. Note that on-diagonal correlations were set at NaN prior to computing the mean.

Tuning half-width represents the angular range at which the neuron fires at 50% of the peak above the baseline firing rate. To compute this, we first measured the modulation depth by subtracting the peak from the min of the tuning curve. The relative 50% of the peak firing rate was computed by subtracting the peak firing rate by 50% of the modulation depth:

\[
\text{half}_\text{fr} = \text{max}_{\text{tuning}} - (\text{max}_{\text{tuning}} - \text{min}_{\text{tuning}}) \times 0.5
\] (4)

The number of angular bins with values exceeding the \text{half}_\text{fr} was then summed and multiplied by bin width to obtain an estimated tuning width.
Head direction tuning curve construction

First, position and spiking data were speed filtered; only epochs with instantaneous running speeds of 3 cm/sec or more were included. Speed filtering was employed given the previous finding that TgF344-AD rats have a higher rate of spike and wave discharges [247], and these episodes tend to occur more often when rats are at rest [72]. Then occupancy normalized firing rate maps were constructed using 6 degree angular bins. Maps were then smoothed with a 18 degree standard deviation Gaussian kernel.

Head Direction modulation

Cells were classified as head direction cells based on the extent of their response to head direction. A shuffling procedure was employed repeatedly (N = 1000) to determine the statistical significance of the extent of directional information conveyed by each spike [241]. First, a surrogate distribution was generated by circularly shifting spikes to disconnect cell responses from rodent behavior. Briefly, the spike train of a single cell was circularly shifted by a random increment from 20 seconds to -20 seconds from end of the recording session. A tuning curve was then created from the shifted spike train, and the directional information content was computed from the shuffled tuning curve. Cells passed the shuffling procedure if the computed Monte-Carlo p-value was less than 0.01. To insure more precise directional tuning, the modulation depth was calculated by taking the ratio between the peak and minimum firing rate of the smoothed tuning curve. Cells that exhibited a modulation depth of at least 1.5 and passed the shuffling procedure were classified as head direction cells.

Linear speed and angular velocity modulation

Analysis of speed modulation of firing rate was similar to previous approaches [173], and was adapted from the code located at https://github.com/GiocomoLab/Munn_et_al_2019. Briefly, linear speed and angular velocity was binned in 200 ms bins. Bins that exceeded 100cm/sec for speed and -200 or 200 degrees/sec for angular velocity were excluded to prevent the influence of non-behavioral artifacts in the data. A Spearman correlation was used to evaluate the relationship between binned speed or angular velocity vectors and binned firing rate. The degree of firing rate change across levels of speed or angular velocity was measured by fitting the data with a first-degree polynomial regression
and estimating the slope. Cells were designated as speed or angular velocity modulated if their speed score (Spearman correlation coefficient) surpassed the 95th percentile of a shuffled distribution.

**Multiplexed cell classification**

Cells were designated as multiplexed if their firing rate showed significant modulated of two or more behavioral correlates. Classes were mutually exclusive. For example, a cell that met for head direction and angular velocity must show non-significant modulated to linear speed as determined by the shuffling procedure.

**Temporal and Spatial Correlations**

Temporal and spatial correlations between co-recorded head direction cell pairs were performed using methods performed previously [14]. Head direction cells were only evaluated when at least two head direction cells were recorded in the same session. Furthermore, only cells with tuning curves that met for non-uniformity across the horizontal azimuth were included (Rayleigh test, p < 0.01 and z > 5). Firing rate normalized cross-correlograms consisting of 200 ms bins over -20 to 20 seconds were made for each cell pair. The central second of the cross-correlogram was z-score and used as a measure of temporal coupling between cell pairs. Spatial correlations were estimated by examining the offset between the preferred directions of pairs of HD cells over time. First, smoothed tuning curves were created for consecutive 90 second intervals across the duration of the trial. The duration of these intervals was chosen to insure adequate sampling of the entire azimuth. The difference in the preferred direction between HD cell pair tuning curves was computed, and the variance of this offset vector was taken as a measure of spatial coupling. Lower variance scores indicate the preferred direction offset was consistent across time and represents stronger spatial coupling over time.

This was created by examining the head direction of spikes from the test cell that occurred within a 10 second window following a single spike of the reference cell. Angular offset was determined by calculating the circular distance between the head direction of the reference cell spike and the head direction of the test cell spikes within the time window.
Histological Analysis

Subjects were sacrificed and their brain was collected to verify electrode positions. First, subjects were administered a lethal dose of pentobarbital and perfused transcardially first with 250 ml of 0.9% phosphate buffered saline followed by 250 ml 4% paraformaldehyde solution. The electrode was carefully removed, and the brain was extracted and stored in PFA for 24 hours and stored in PBS. Prior to slicing, fixed brains were submerged in 30% sucrose solution for 2 – 3 days. Brains were then sliced coronally at 30 – 40 µm using a cryostat. Brain regions in close proximity (+/- 1mm) to the electrode site were placed on slides, allowed to dry for at least 24 hours, and stained with cressyl violet.

Statistical Methods

Statistical modelling was carried out in R. Linear models were used to examine group differences. Where appropriate, group differences were determined using mixed effects modelling to control for variance associated within a recording session and within individual rats using lme4 package [15]. Proportional data was evaluated using a binominal generalized linear mixed effects model [144]. The response consisted of proportional data computed within a session with 'group' as the fixed effect and 'rat' as the random effect. Differences in continuous variables were assessed using a linear mixed effects model with 'group' as the fixed effect while 'session' was nested within 'rat'. Backward model selection was performed, and the simplest model that accounted for the most variance was kept for interpretation. Given the multitude of subclasses of neurons evaluated, analyses included a subsample of data from each rat. The number of neurons for each cell type and encoding type across rats are summarized in Table S1.

Software and Code Availability

Python code used for analysis and generating figures in the paper is available at https://github.com/lolaBerkowitz. The codebase relies on the following Python packages: Numpy [105], Matplotlib [116], Seaborn [282], and Pandas [208]. All code is open-source and licensed under the MIT Software License.
# Supplemental Tables

**Table S1:** Summary of encoding types across group, rat, and cell type

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Chapter 3

Landmark Anchoring and directional stability of postsubicular HD cells

Abstract

Landmark processing deficits are commonly observed in humans with Alzheimer’s disease and are thought to contribute to poor performance in landmark-based navigation tasks. Critically, cortical areas, including the postsubiculum, that are posited to support landmark processing, succumb to AD-associated pathology, and lesions of the postsubiculum result in similar behavioral deficits to those observed in humans and animal models of AD. Postsubicular head direction cells will stabilize their tuning to a prominent or visually unambiguous feature in an environment, a process known as landmark anchoring. Given the potential changes to landmark processing in Alzheimer’s disease, an open question remains as to whether landmark anchoring is intact in postsubicular head direction cells. To examine landmark anchoring, head direction cells were evaluated in two paradigms. TgF344-AD (N=3) and F344 controls (N=2) randomly foraged for fruit loop pieces in a cylindrical environment with a prominent cue followed either by a cue rotation or were transferred to a box with no cue. Putative head direction interneurons were observed to convey increased directional information, be more stable within a session, have sharper directional tuning, and have higher peak firing rates compared to control head direction interneurons. Overall, landmark anchoring was intact in TgF344-AD head direction cells, as the pairwise angular difference in the preferred direction between similar and rotated environments was no different than controls. Furthermore, co-recorded head direction ensembles exhibited coherent rotation following a cue rotation, indicating that attractor dynamics are also maintained in TgF344-AD head direction cell populations. In contrast, examination of TgF344-AD head direction tuning in a box without a local cue resulted in random shifts of the preferred direction, whereas the tuning remained stable between cylinder conditions. Overall, the results presented in this chapter provide evidence for intact landmark anchoring of TgF344-AD head direction cells in response to a local cue.

Introduction

The ability to use landmarks for navigation or associate landmarks with a place is disrupted in individuals with prodromal or advanced Alzheimer’s disease [67, 71, 113]. These deficits manifest as an inability to navigate precisely to a goal.
location [113, 139], which is also observed in animal models of AD-like pathology [17, 122]. These similarities suggest that AD-like pathology may disrupt neural processes that support the use of landmarks in navigation. Indeed, cortical areas that succumb to AD-associated pathology, such as retrosplenial cortex, entorhinal cortex, and presubicular cortex, may be critical for landmark processing [10, 78, 121, 296]. Furthermore, these regions contain neurons that encode spatial features, such as place and head direction, which stabilize their firing fields relative to landmarks [11, 121, 132, 145, 213, 307]. Thus, a critical topic of investigation is to determine whether cells that encode spatial features exhibit altered responses to environmental landmarks. To the authors' knowledge, no studies to date have examined whether spatial cell types respond to cue manipulations in an animal model of AD-like pathology, except for place cell remapping as a function to a change in environmental context rather than an environmental shift of local cues [130]. Unlike place cells, head direction cells encode low dimensional information related to head position in the azimuth [45], and have predictable responses to environmental landmark shifts [59, 134, 300]. Therefore, alterations in the processing of landmarks that result from AD-like pathology may be more easily discernible by examining head direction cell responses to landmark rotations.

The extensive investigation into head direction cell response to landmark manipulation has revealed several predictable outcomes. First, head direction cells will rapidly anchor to a landmark within milliseconds of exploring a novel environment, and once established can remain stable even in darkness over longer periods of time [306]. This indicates that the head direction cell system rapidly responds to visual information and that this response is endured even in the absence of visual input. Second, both visual and locomotor cues are shown to support the stability of head direction cell tuning [243, 295]. For example, the tuning of a head direction cell may be different between two separate environments, however; allowing active navigation between these environments results in a stabilization of HD cell tuning [260, 297]. Thus, multiple landmarks may be integrated over time to form a ‘map-like’ representation of the entire environmental landscape. Importantly, prominent cues, which tend to be distant and stable over time, are more likely attributed to landmark anchoring that smaller or proximal objects in the environment [55, 133, 300]. This may be due to factors associated with distance, such as reduced motion parallax for distal landmarks compared to more proximal landmarks [307]. Finally, head direction cells exhibit ring attractor dynamics whereby ensembles of co-recorded head direction cells will shift their preferred
direction coherently with a landmark shift \[23, 209, 262\].

Thus, we sought to determine whether head direction cell response to environmental manipulations remains intact in TgF344-AD rats. TgF344-AD (N=3) and F344 controls (N=2) randomly foraged for fruit loop pieces in a cylindrical environment. While the mechanisms supporting landmark anchoring remain unclear, regions involved in landmark processing exhibit amyloid pathology in TgF344-AD rats \[58\] and project densely to the postsubicular cortex \[271\]. Thus, we tested the hypothesis that landmark anchoring would be altered in head direction cells within TgF344-AD rats. Landmark anchoring was examined by comparing the preferred firing direction of individual HD cells or ensembles of HD cells across a rotation condition or a transition to a box that lacks a local landmark.

**Results**

Head direction cells were recorded in the postsubiculum of TgF344-AD (N=3,n=154 (138 interneurons) and F344 controls (N=2,n=141 (84 interneurons)) while animals foraged for fruit loops across four environmental conditions. No difference in mean angular velocity, linear speed, distance travelled, or duration of recording session was observed between groups, indicating TgF344-AD rats and controls exhibited similar behaviors during the recording session (\(p_s \geq 0.088\)). Only cells that met criteria for encoding head direction across all conditions were kept for the analyses. A minority of cells identified as head direction cells in the first and second sessions, lost their directional tuning following the cue rotation (TgF344-AD n = 7, control n = 10).

**TgF344-AD head direction tuned interneurons exhibit more stable and precise directional tuning across conditions**

We first measured the directional tuning of head direction cells across environmental conditions by examining directional information content, tuning half-width, within-trial stability, and peak firing rate across the four conditions. There were no observed differences between the groups for pyramidal HD cells across any measure of directional tuning, indicating pyramidal HD cells in TgF344-AD rats remain relatively intact (Fig 1B-E, right panel). However, TgF344-AD HD interneurons tended to be more stable (\(b = -0.11, SE = 0.02, p = 5.31e - 05\)), conveyed increased directional information per spike, (\(b = -0.874, SE = 0.12, p = 1.61e - 07\)), and had decreased tuning half-width,\((b = 21.6, SE = 5.89, p = 0.0013)\), compared to HD interneurons recorded from control animals (Fig 1B-D, left panels). In addition, the
peak firing rate of TgF344-AD HD interneurons was increased compared to control HD interneurons \((b = -0.21, SE = 0.10, p = 0.047)\)(Fig 1E, left panel). There were no effects associated with condition except that HD pyramidal and interneurons tended to have increased firing rate in the last condition across both groups, \((bs \geq 0.18, SEs \leq 0.11, ps \leq 0.0204)\). Overall, these results indicate that directional tuning was not disrupted as a function of environmental conditions, and that TgF344-AD HD interneurons show increased directional tuning compared to that of control cells.

**TgF344-AD HD cells show coherent and precise responses to cue rotation**

Given directional tuning remained intact across conditions, we next sought to examine whether the preferred firing directions of HD cells remained stable between similar environments and rotated with the cue card following a cue-rotation. The angular difference was computed between the standard conditions and the preceding/following the rotation condition, and a Bayesian mixed-effects regression model \([65]\) was employed to test whether the circular means of these distributions were equal between groups using. HD cells were observed to maintain relative stability across similar conditions, as the distribution of angular differences was centered around zero degrees (first three panels, Fig 2C). Furthermore, HD cells shifted their preferred direction following a cue rotation (last two panels, Fig 2C), indicating that cue anchoring is intact in aged animals. Overall, TgF344-AD HD cells and control HD cells exhibited similar circular means across all conditions, \((\mu_{tg} - \mu_{wt} = -0.13\text{radian}, 95 \% \text{ HPD} = [-0.83, 0.615])\).

While cue anchoring was intact across the distribution of HD cells, some cells exhibited shifts in their preferred direction outside of the expected angle for the respective conditions. Attractor dynamics predict that HD cell ensembles will rotate coherently or shift their respective preferred directions in the same direction and degree. To test this, we examined sessions with a minimum of 7 co-recorded HD cells for TgF344-AD \((n=11)\) and controls \((n=9)\), and tested whether the distribution of the ensemble angular offsets within a condition qualified as a Von Mises distribution. Ensembles with angular offsets that are uniformly distributed around the azimuth would be indicative of incoherent rotations. The proportion of coherent ensembles ranged from 56% — 68% for control HD cell ensembles, and 61% — 81% for TgF344-AD HD cell ensembles (Fig 2D). Overall, the proportion of ensembles that maintained coherence in their degree of angular differences across all conditions was not significantly different \((p = 0.820)\). Furthermore, the proportion
Figure 1: TgF344-AD HD interneurons have stable, increased directional tuning across all conditions

A) Cartoon depiction of overhead view of testing conditions. White arc indicates the cue card position. B-E) Box plots summarizing measures of directional tuning for interneurons (left panel) and pyramidal cells(right panel) (B,directional information content (Directional IC); C, Tuning half-width; D, stability score; E, peak firing rate. Groups are indicated by color (controls = wt, blue and TgF344-AD = tg, orange). Note that TgF344-AD HD interneurons have increased directional information content, increased stability, decreased tuning width, and increased peak firing rate across all conditions. P-values represent main effect of group.
of ensembles that exhibited preferred directions relative to the expected angle was not different between groups ($p = 0.705$) (Fig 2E), indicating that the ensembles of HD cells maintained coherence and stabilized their preferred directions relative to the cue card in both groups. Additionally, HD cell ensembles from both groups generally had high mean vector lengths, (Fig 2F), indicating that angular differences were more tightly clustered around one direction.

Overall, these analyses indicate that landmark anchoring is intact in TgF344-AD rats, and that ensembles of co-recorded HD cells exhibit coherent tuning across similar conditions and following cue rotation.

TgF344-AD HD cells show variable response of preferred direction shifting following an environmental change

We next examined whether HD cells maintained stable preferred directions across familiar, but geometrically different environment. In the previous section, TgF344-AD HD cells exhibited landmark anchoring in an enclosed environment fitted with a proximal cue card. HD cells have been shown to rotate to proximal cues in the absence of available distal cues [308]. Thus, it is unclear whether HD cells in TgF344-AD animals can maintain stable tuning without access to a proximal visual cue. While a sufficient number of HD cells were recorded from TgF344-AD rats ($N=2$, $n=65$ (57 interneurons)), only nine pyramidal HD cells were observed from one control rat, and only two of these cells met criteria for HD encoding across all conditions within this paradigm. The discrepancy in sample size between the two groups prevents adequate power for comparisons between groups. Thus, this section will examine the stability of directional tuning for HD cells recorded from TgF344-AD rats, however; no inferences can be made about the influence of group identity on HD cell tuning in this paradigm.

The majority of HD cells in the recorded population were interneurons ($57/65$, 87.5%). Measures of directional tuning or peak firing rate of HD cells were not observed to change as a function of environment across interneurons or pyramidal cells. Given that directional tuning remained intact, we next tested whether HD cells maintained a stable preferred direction across environments (Fig 3A). Rats were allowed access to all room cues during the transition between environments, with average inter-trial intervals of $\sim 120 - 180$ seconds. The angular difference in the preferred direction of HD cells was examined across environments, and the preferred directions were predicted to be stable across the difference environments given no disorientation treatment was performed. Example tuning
**Figure 2:** Landmark anchoring is intact in TgF344-AD head direction cells. A) Heat maps of 1D tuning curves organized by peak bin from Standard 1 condition, for controls (top) and TgF344-AD rats (bottom). Darker colors indicate higher normalized firing rate. Cartoon depictions of recording environment across conditions are indicated in grey. B) Linearized head direction tuning curves for individual cells. Colors indicate condition. Note that these cell shift their tuning along with the cue shift (green line). C) Histograms showing the pairwise angular difference of preferred direction across condition comparison. Between standard conditions, both TgF344-AD and controls have similar preferred directions as indicated by the distributions concentrated around zero radians. Distributions for angular difference are concentrated around 1.57 and -1.57 radians for rotated vs. standard conditions, indicating preferred directions shifted as expected. D) Overall proportion of ensembles (≥ 6 co-recorded HD cells) that maintained similar angular differences across conditions. E) Circular mean of ensemble angular differences across comparisons. Each observation represents an ensemble, while grey lines indicate expected angular difference for comparison. F) Mean vector length of ensemble distributions. Values closer to 1 are indicative of a leptokurtic distribution. Note that ensembles maintained fairly leptokurtic distributions with mean vector lengths ranging from 0.5 to 1 across all comparisons.
curves are shown in (Fig 3B,D). Most TgF344-AD HD cells exhibited variable shifts in their preferred direction after being placed within the box environment, (Fig 3C). In contrast, the preferred direction was stable between cylinder conditions as the distribution of angular difference between these conditions centered around 0 radians (Fig 3C, rightmost panel). Only four recording sessions contained an adequate number of HD cells (≥ 6) to examine ensemble coherence, and these sessions were recorded from two rats. The four ensembles showed inconsistent response in preferred direction shifts during the box condition (Fig 4). Specifically, the angular differences of 2/4 HD ensembles were not coherent for box conditions (Fig 4A). Furthermore, for the 2 ensembles that did exhibit coherence, the preferred directions shifted when in the box condition (Fig 4B). Nevertheless, all ensembles showed strong stability in their preferred firing directions across cylinder environments, as indicated by a significant Rayleigh V test indicating the angular difference was centered around 0 radians (ps < 0.05).

Overall, examination of TgF344-AD HD cells suggests that HD tuning may not remain stable across different environments, given cells may shift their preferred directions following placement in a box yet maintain stable tuning across a similar environment. Critically, given the lack of a control group, it is unclear whether this response is due to aging or is influenced by the rat’s genotype.

**Discussion**

The results of these experiments provided novel insights into the landmark anchoring of HD cells in the TgF344-AD rat model of AD-like pathology. First, TgF344-AD HD cells maintained similar preferred directions between similar environments and rotated their preferred direction to the same degree and direction as a cue rotation, indicating landmark anchoring is intact in aged TgF344-AD animals. Second, TgF344-AD HD cell ensembles rotated coherently following a cue rotation. Third, while the box experiment did not have an adequate control group, TgF344-AD HD cells shifted their preferred direction by varying degrees in the box condition but maintained precise anchoring to the cue card in the cylinder conditions. This provides preliminary evidence that either the age of the rats or an Alzheimer’s genotype may alter stable tuning across different environments. Overall, TgF344-AD HD cells show intact stability and landmark anchoring in a familiar environment fitted with a proximal cue.

The first finding identified that TgF344-AD HD cells showed no change in their
Figure 3: TgF344-AD HD cells show variable response of preferred direction shifting following an environmental change

A) Cartoon depiction of environmental conditions. B) Two control cells that met for HD tuning across all conditions. C) Histograms showing the pairwise angular difference of preferred direction across condition comparison for HD cells recorded in TgF344-AD rats. Interneurons are indicated in grey and pyramidal cells in red. Note that HD cells maintain a similar preferred direction across cylinder conditions, but generally shift unpredictably between box conditions. D) Example tuning curves for HD cells across the three conditions. While most cells demonstrated unpredictable shifting of their preferred direction when placed in the box condition (left panel), some cells maintained a similar preferred direction (right panel). Note that the cells in the preferred direction (PD) stable column were co-recorded and maintained coherent tuning across all environments.
Figure 4: Example of coherent and non-coherent ensembles in TgF344-AD rats

A) Two ensembles that showed incoherent rotation following placement in the box. Note the angular differences are widely distributed between standard and box conditions (ss1 vs. box and box vs. ss2). B) Two ensembles that show coherent responses as indicated by the alignment of angular differences around one direction. Note that these ensembles shift their preferred direction following box placement. All ensembles exhibit coherence between standard sessions (ss1 vs. ss2). Angular differences reflect the circular difference between conditions. Circular mean of the distribution of angular differences is indicated by a vertical blue line.
directional tuning over time. Specifically, there was no change in tuning width, directional information content, or within session stability across conditions, suggesting that the integrity of head direction encoding is robust to AD-like pathology. This finding is in contrast to studies examining hippocampal place cells in other animal models of AD-like pathology. While place cells and head direction cells are fundamentally different, the comparison of the current findings and examination of place cells in Alzheimer’s disease models is warranted given prior literature showing that these two systems interact \[42, 299\]. Generally, hippocampal place cells show reductions in the spatial specificity of their tuning over sessions. For example, Zhao \[304\] found that place cells reduced their tuning width and were more stable after repeated visits to a novel environment in wild-type mice, whereas APP mice showed no such change in the specificity of place cells. More recently, Takamura and colleagues \[251\] found a reduced proportion of place cells that exhibited stable mapping on a virtual linear track over sessions. These studies suggest that, at least in the hippocampus, AD-like pathology results in reductions in the spatial tuning of hippocampal neurons. Critically, the hippocampus is not limited to the encoding of spatial information \[185\], but is more generally associated with the consolidation and retrieval of memory (discussed in detail by Buzsaki \[38\]). Likely, changes in the spatial specificity of hippocampal place cells in animal models of AD-like pathology may instead be resultant from disruptions of the encoding of hippocampal sequences \[236\]. As such, the hippocampal mechanism of memory are altered in AD models \[40, 129, 180\].

This is the first implementation of landmark rotation manipulation in an animal model of AD-like pathology, and indicates that postsubicular HD cells show intact landmark anchoring to a proximal cue in a familiar environment. Specifically, TgF344-AD HD cells exhibited similar preferred directions in the cylinder environment and shifted their preferred direction along with the cue shift. The specific mechanisms underlying landmark anchoring in the head direction cell system are not known, although lesion studies have suggested that the retrosplenial cortex may contribute to landmark anchoring in upstream HD cell loci \[53, 296\]. Pathologic inflammatory processes have been observed in the retrosplenial cortex of TgF344-AD rats as early as 6 months, while plaque pathology emerges at 16 months of age \[58\]. It is possible that the severity of pathology in the retrosplenial cortex of the TgF344-AD rat model is not sufficient to induce changes in landmark anchoring in postsubicular HD cells. Indeed, it is possible that a significant amount of disruption may be required to alter the landmark anchoring processes of
postsubicular HD cells. One study supports this notion given strong and sustained stimulation of juxtacellularly recorded postsubicular HD cells failed to alter the cell’s preferred direction in a cue-rich environment, and only a resulted in a small bias when environmental cues were lacking [59]. Thus, its possible that lesions of the retrosplenial only alter landmark anchoring given the extent of the damage. A alternative consideration is that the retrosplenial cortex is not the only input necessary for landmark anchoring of postsubicular HD cells. There anatomical evidence is support of this as visual information is conveyed to the postsubiculum through other thalamic and cortical inputs (see Chapter 1 for details of anatomy). Furthermore, given the complexity of encoding observed by retrosplenial neurons 5, 164, 165, its possible that changes in retrosplenal function were not detectable in the rotation paradigm but led to the preferred direction shifts observed in the box paradigm.

Most TgF344-AD HD cells shifted their preferred direction after being placed in the box. Critically, the the rats were not disoriented between cylinder and box transitions, and instead were placed on a pedestal with access to visual cues around the entire room. The procedure was similar to Knight et al. 137, which found that HD cells in non-disoriented rats did not shift their preferred direction in environments of varying geometry. Thus, the observation that HD cells recorded from TgF344-AD rats exhibited shifts in their preferred direction without undergoing disorientation treatment suggests potential dysfunction. Clark and colleagues 55 found that a trapezoidal environment provoked a shift in the preferred direction of thalamic head direction cells, however; these rats were disoriented prior to being placed in the trapezoid. Thus, its possible that under a disoriented state, environmental geometry may influence the preferred direction of HD cell populations. The pellet chasing task used in this study is not suitable for the assessment of disorientation, although navigation deficits consistent with spatial disorientation have been observed in TgF344-AD rats as early as 10 months of age 17. Thus, its possible spatial disorientation may be contributing to the observed changes in the preferred direction in the box condition. Future work should consider behavioral paradigms that measure spatial disorientation or include more precise disorientation procedures to examine the influence of disorientation on landmark anchoring of HD cells in TgF344-AD animals. Lastly, given the lack of control group, it is possible that the shifting of HD cells in the box condition is related to normal aging 150, although age-related changes in vestibular function observed in Fischer-344 rats 60, 114 may not be a factor here given HD cells show remarkable
stability within an environment.

**Conclusion**

This was the first study to examine landmark anchoring of head direction cells in an animal model of AD-like pathology. TgF344-AD HD interneurons show more precise directional tuning over time, while pyramidal HD cells show no differences compared to control cells. Aged TgF344-AD rats and controls show intact landmark anchoring of postsubicular HD cells following the rotation of a proximal cue, however; TgF344-AD HD cells show variable preferred direction shifts after being placed in a box environment. The results of these experiments indicate that landmark anchoring in postsubicular HD cell populations are intact in aged TgF344-AD rats and controls. The behavioral paradigm used in this chapter limits the inferences of landmark anchoring to that of proximal cues. Its unclear how TgF344-AD HD cells may respond to a more complex environment or when placed in a novel environment. Specifically, it is possible that HD cells anchor to proximal cues even in the presence of prominent distal cues, which have been previously shown to exhibit stronger anchoring on HD cells [299, 300]. Examination of HD cells in more complex landmark manipulation experiments is warranted to address these unknowns.

**Materials and methods**

**Subjects**

Female TgF344-AD (n = 3) rats and littermate F344 controls (n = 2) aged 10—20 months were used as subjects. Animals were bred onsite at the University of New Mexico Animal Research Facility by pairing TgF344-AD females with F344 males. TgF344-AD breeders were provided by the Rat Research and Resources Center. Offspring were then genotyped (Transnetxy Inc, Tennessee) to verify the presence of the mutated Amyloid precursor protein Swedish mutation (APPsW) and the Presenilin 1 exon 9 mutation (PS1∆E9). Females positive and negative for these mutations were reserved for the study and aged a minimum of 9 months before experimentation. Males were excluded from the study given the finding that TgF344-AD females exhibited earlier signs of spatial navigation deficits [17]. All animals received ad-libitum food and water before the commencement of experimentation, after which their weight was restricted to two-standard deviations of the average weight of their strain. All animals were pair housed for the duration
of the study to prevent behavioral changes associated with isolation. Vivarium lighting was maintained on a 12-hour light-dark cycle with lights on at 0900 hours. All procedures were approved by the Institutional Animal Care and Use Committee at the University of New Mexico.

**Testing Room**

All experiments were carried out in a 1.2 m x 1.2 m x 2.1 m recording chamber that was enclosed on three sides. The recording chamber was shielded with copper mesh to reduce electrical interference. A black curtain was placed on the open side of the chamber during recording to restrict visual cues. Eight led lights were placed in a circle at the top of the recording chamber for illumination during light conditions.

**Screening**

Screening only occurred in the cylinder condition. The black curtain remained open and only the room lights remained on during screening. If no identifiable clusters were observed, the animal’s electrode was lowered 25 – 50 µm. Screening was repeated up to three times per day, and the rat was allowed a minimum one-hour break between screening sessions.

**Recording**

If identifiable clusters were observed, the room was then prepared for the experiment while the rat rested on the pedestal. First, the room lights were then turned off and the recording chamber curtain was closed. Two experimental paradigms were used to test the effects of different cue rotation or environment changes on spatial tuning. Rotation experiments consisted of four conditions performed in a plastic cylindrical environment (76.5cm diameter) that was fitted with a white paper cue card. The cue card extended the height of the cylinder and covered a 1.76 radian arch. The cylinder was maintained in the relative north position for all Standard conditions, but the entire cylinder was rotated 1.57 radians in the Rotation condition. A wooden 1mX1mX0.20m box painted light grey was used for the box sessions. Recording sessions lasted between 45 – 120 minutes with each environmental condition consisting of 12 – 30-minute recordings. Between environmental conditions, the rat was placed in a flowerpot covered with a towel to limit visual cues (rotation paradigm) or placed on the pedestal in full access of all visual cues (environment change). The floor and walls of the environment were cleaned with
40% ethanol solution to disrupt the use of olfactory cues. Preceding and following cue manipulation, the rats underwent a disorientation procedure \[89, 90\] to disrupt the internal orientation sense and increase the reliance on the proximal cue for reorienting. Briefly, the animal was gently spun in random directions along the perimeter of the cylinder for about 1 minute. After the recording was completed, the electrode was advanced 50-75 µm and the animal was returned to the home cage.

**Pyramidal and Interneuron Classification**

Cells were classified into putative pyramidal and interneurons based on their waveform duration obtained using a wavelet transform \[203\]. Waveforms were obtained by first filtering the wide band signal between 600 and 9000 Hz, and then taking a 1 ms window relative to the spike timestamp. The peak of each waveform was centered at 0.26 ms. Waveform duration was estimated using the inverse peak frequency of a wavelet transform of the spike waveform \[202\]. Plotting the distribution of waveform duration for all recorded units showed a separation at around 0.5 ms, consistent with previous reports showing pyramidal cells having wider duration at or above this threshold \[200, 203\].

**Measures of directional tuning and stability**

Directional tuning was measured using directional information content and stability score computed from head direction tuning curves. Briefly, tuning curves were obtained by binning spikes across 60 angular bins according to the head direction the animal was facing during which the spike occurred. The data was then normalized by directional occupancy to obtain a firing rate histogram across binned angles and the tuning curves were then smoothed with a gaussian kernel (s.d. = 18 degrees). Directional information content was computed by measuring the mutual information between head direction and the firing rate \[203, 241\]. Directional information content was defined as follows,

\[
DI = \sum_{n=1}^{60} \lambda_n \log_2 \left( \frac{\lambda_n}{\lambda} \right) p_i \tag{5}
\]

where \(i\) is the index of the binned angle, \(\lambda(i)\) is the firing rate at angle bin \(i\), \(\lambda\) is the overall firing rate of the cell, and \(p_i\) is the probability of the head facing angle \(i\). DI is divided by the overall firing rate to measure directional information in bits per spike.
The stability score reflects the similarity of consecutive tuning curves made within the session. Briefly, tuning curves were created for each full traversal of the horizontal azimuth. Stability score was then computed by obtaining the mean pairwise correlation across angular bins. A stability score closer to 1 reflected more stable directional tuning across time and was defined as follows,

\[ \text{stability} = \text{nanmean}(\text{corr}(X^T)) \] (6)

where nanmean and corr are Matlab functions and \( X^T \) is the transpose of the matrix consisting of tuning curves over azimuth traversals. Note that on-diagonal correlations were set at NaN prior to computing the mean.

Tuning half-width represents the angular range at which the neuron fires at 50% of the peak above the baseline firing rate. To compute this, we first measured the modulation depth by subtracting the peak from the min of the tuning curve. The relative 50% of the peak firing rate was computed by subtracting the peak firing rate by 50% of the modulation depth:

\[ \text{half\_fr} = \max_{\text{tuning}} - (\max_{\text{tuning}} - \min_{\text{tuning}}) \times 0.5 \] (7)

The number of angular bins with values exceeding the half\_fr was then summed and multiplied by bin width to obtain an estimated tuning width.

**Head direction tuning curve construction**

First, position and spiking data were speed filtered; only epochs with instantaneous running speeds of 3 cm/sec or more were included. Speed filtering was employed given the previous finding that TgF344-AD rats have a higher rate of spike and wave discharges [247], and these episodes tend to occur more often when rats are at rest [72]. Then occupancy normalized firing rate maps were constructed using 6 degree angular bins. Maps were then smoothed with a 18 degree standard deviation Gaussian kernel.

**Head Direction Cell Classification**

Cells were classified as head direction cells based on the extent of their response to head direction. A shuffling procedure was employed repeatedly (\( N = 1000 \)) to determine the statistical significance of the extent of directional information conveyed by each spike [241]. First, a surrogate distribution was generated by
circularly shifting spikes to disconnect cell responses from rodent behavior. Briefly, the spike train of a single cell was circularly shifted by a random increment from 20 seconds to -20 seconds from end of the recording session. A tuning curve was then created from the shifted spike train, and the directional information content was computed from the shuffled tuning curve. Cells passed the shuffling procedure if the computed monte-carlo p-value was less than 0.01. To insure more precise directional tuning, the modulation depth was calculated by taking the ratio between the peak and minimum firing rate of the smoothed tuning curve. Cells that exhibited a modulation depth of at least 1.5 and passed the shuffling procedure were classified as head direction cells.

**Rotation Analysis**

The preferred direction of single units was examined over each condition to determine whether HD tuning remained stable in similar environmental conditions (i.e. Standard 1, Standard 2, Standard 3) and shifted with the cue following cue rotation (i.e. Rotation). The angular difference of the preferred direction was calculated between Standard 1 and Standard 2, Standard 1 and Standard 3, Standard 2 and Standard 3, Standard 2 and Rotation using the circ_dist function in Matlab (CircStat 2012a, [https://www.mathworks.com/matlabcentral/fileexchange/10676-circular-statistics-toolbox-directional-statistics](https://www.mathworks.com/matlabcentral/fileexchange/10676-circular-statistics-toolbox-directional-statistics)) or python ([https://github.com/circstat/pycircstat](https://github.com/circstat/pycircstat)), and Rotation and Standard 3. The expected angular difference was 0 radians for standard session comparison and 1.57 or -1.57 for rotation comparisons. The distribution of angular differences across pairwise comparison conditions was examined using circular mixed modelling (see Statistical Analysis for details) to determine if groups varied in the circular mean across conditions.

**Ensemble Analysis**

Sessions that contained (≥ 6) co-recorded HD cells were included in the ensemble analysis. The threshold for inclusion was chosen as this is the minimum sample size required to examine circular features [79]. Ensemble coherence was concluded if the angular difference distribution that was significantly nonuniformly distributed around the azimuth, as determined by a Rayleigh test (p < 0.05). Features of the angular difference distributions for coherent ensembles included the circular mean and the mean vector length were evaluated.
Histological Analysis

Subjects were sacrificed, and their brain was collected to verify electrode position. First, subjects were administered a lethal dose of pentobarbital and perfused transcardially first with 250 ml of 0.9% phosphate buffered saline followed by 250 ml 4% paraformaldehyde solution. The electrode was carefully removed, and the brain was extracted and stored in PFA for 24 hours and stored in PBS. Prior to slicing, fixed brains were submerged in 30% sucrose solution for 2 – 3 days. Brains were then sliced coronally at 30 – 40 µm using a cryostat. Brain regions in proximity (+/- 1mm) to the electrode site were placed on slides, allowed to dry for at least 24 hours, and stained with cressyl violet.

Statistical Analysis

Linear models were used to examine group differences. Where appropriate, group differences were determined using mixed effects modelling to control for variance associated within a recording session or within individual rats using lme4 package [15]. Proportional data was evaluated using a binomial generalized linear mixed effects model [144]. The response consisted of proportional data computed within a session with 'group' as the fixed effect and 'rat' as the random effect. Differences in continuous variables that were assessed using a linear mixed effects model used 'group' as the fixed effect while 'session' was nested within 'rat'. Backward model selection was performed, and the simplest model that accounted for the most variance was kept for interpretation. Circular descriptive statistics were computed using the package Circular [3], and circular mixed effects modelling was achieved using the bpnme function from bpnreg package [65]. All cells that met criteria for encoding head direction across both interneuron and pyramidal cell types were included in the analyses Table S1 and Table S2.

Supplemental Tables
**Table S1:** Summary of head direction cells across group, rat, and cell type used in rotation analysis

<table>
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<th>cell_type</th>
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</tr>
</thead>
<tbody>
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<td>tg</td>
<td>LB04</td>
<td>int 384</td>
</tr>
<tr>
<td>2</td>
<td>tg</td>
<td>LB04</td>
<td>pyr   2</td>
</tr>
<tr>
<td>3</td>
<td>tg</td>
<td>LB07</td>
<td>int   25</td>
</tr>
<tr>
<td>4</td>
<td>tg</td>
<td>LB07</td>
<td>pyr   3</td>
</tr>
<tr>
<td>5</td>
<td>tg</td>
<td>LB10</td>
<td>int   17</td>
</tr>
<tr>
<td>6</td>
<td>tg</td>
<td>LB10</td>
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<td>7</td>
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<tr>
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<td>wt</td>
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<td>pyr   9</td>
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</table>

**Table S2:** Summary of head direction cells across group, rat, and cell type used in box analysis

<table>
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<tbody>
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<td>int 37</td>
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<tr>
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</tr>
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<td>pyr   5</td>
</tr>
<tr>
<td>5</td>
<td>wt</td>
<td>PoS4</td>
<td>pyr   2</td>
</tr>
</tbody>
</table>
Conclusion

The broad aim of this dissertation was to examine the integrity of postsubicular neuronal circuitry, with an emphasis on functional properties of pyramidal cells and interneurons, and how these neuron classes encode the sense of direction in a TgF344-AD rat model of Alzheimer’s disease. To do this, this dissertation is broken up into three parts. Chapter 1 provides a detailed account of head direction cell circuitry throughout the brain. Chapter 2 focuses on identifying the function integrity of postsubicular neurons with an emphasis on head direction cells and highlights the intrinsic firing properties as well as the precision of directional tuning of multiple cell populations. This is done to examine how neuronal firing is altered by AD-like pathology. Finally, chapter 3 explores how head direction cells orient relative to a proximal cue over several recording sessions to assess how landmark anchoring is affected by AD-like pathology.

Chapter 1 consists of a review in four parts: functional anatomy, landmark manipulation experiments, functional connectivity, and visual components of head direction cell modelling. First, the functional anatomy was comprehensively reviewed and split into two major components; subcortical and cortical circuitry. The influences of the vestibular components of the HD circuitry have been evaluated previously [32, 57]; however, the review here is crucial to understanding how the internally generated directional sense can be influenced by external cues. Evaluation of the functional components of cortical circuitry revealed how multiplexed representations of spatial features may be used to integrate external information into the subcortical circuit. This concept of multiplexed representation informs the direction of chapter 2. The second section summarized landmark manipulation experiments and led to the conclusion that both self-motion and visual cues are equally essential to landmark control of the HD cell system. This section informs the landmark control experiments in chapter 3. The third section supports the landmark control conclusions by evaluating the effects of selective circuit lesions. Finally, the theoretical modelling of the HD cell system was reviewed. Initial models and basic components of these models were described. An emphasis on visual components is explored and compared to empirically performed landmark manipulations.

Chapter 2 sought to investigate the functional integrity of postsubicular neurons. Spatial navigation and memory are impaired in Alzheimer’s disease (AD). The TgF344-AD rat model of AD has been shown to exhibit similar behavioral and
pathological features to humans and thus serves as an excellent model to investigate mechanisms of spatial navigation impairment in AD. Previously, we found that 10-month-old TgF344-AD rats make progressively less spatially precise movements during a reference memory variant of the Morris water maze. In addition, TgF344-AD rats also exhibit progressive pathology in cortical areas involved in the encoding of head direction. Thus, this study sought to characterize directionally modulated cell types in the postsubiculum of 10—20 month female TgF344-AD rats and aged matched F344 controls. Rats performed a pellet chasing task within a cylinder fitted with a white cue card during recording. Putative pyramidal and interneuron proportions were altered in TgF344-AD rats, whereby a lower relative proportion of pyramidal cells was observed. In addition, pyramidal and interneurons were found to encode for head direction, running speed, and angular velocity in both TgF344-AD and control rats, although TgF344-AD rats exhibited a larger proportion of putative interneurons that encode head direction. In addition, TgF344-AD interneurons that encode head directions were found to convey increased directional information, exhibit more stable directional tuning over time, and are more likely to fire in bursts. Furthermore, TgF344-AD multiplexed head direction and running speed interneurons had an increased sensitivity to linear speed, whereby firing rate increased to a greater degree with running speed. TgF344-AD pyramidal cells that encode the head direction showed no differences in firing characteristics or directional tuning compared with control cells. Head direction cell pairs exhibited spatial and temporal correlations, consistent with attractor dynamics, with no differences between groups. Overall, these results suggest that the postsubicular head direction cell circuit in TgF344-AD rats is comprised of an altered function of putative interneurons that encode head direction with no observable difference in pyramidal cell function. These findings further support hypotheses surrounding altered interneuron function in animal models of AD-like pathology.

Landmark processing deficits are commonly observed in humans with Alzheimer’s disease and are thought to contribute to poor performance in landmark-based navigation tasks. Critically, cortical areas, including the postsubiculum, that are posited to support landmark processing, succumb to AD-associated pathology, and lesions of the postsubiculum result in similar behavioral deficits to those observed in humans and animal models of AD. Postsubicular head direction cells will stabilize their tuning to a prominent or visually unambiguous feature in an environment, a process known as landmark
anchoring. Given the potential changes to landmark processing in Alzheimer’s disease, an open question remains as to whether landmark anchoring is intact in postsubicular head direction cells. To examine landmark anchoring, head direction cells were evaluated in two paradigms. TgF344-AD (N=3) and F344 controls (N=2) randomly foraged for fruit loop pieces in a cylindrical environment with a prominent cue followed either by a cue rotation or were transferred to a box with no cue. Putative head direction interneurons were observed to convey increased directional information, be more stable within a session, have sharper directional tuning, and have higher peak firing rates compared to control head direction interneurons. Overall, landmark anchoring was intact in TgF344-AD head direction cells, as the pairwise angular difference in the preferred direction between similar and rotated environments was no different than controls. Furthermore, co-recorded head direction ensembles exhibited coherent rotation following a cue rotation, indicating that attractor dynamics are also maintained in TgF344-AD head direction cell populations. In contrast, examination of TgF344-AD head direction tuning in a box without a local cue resulted in random shifts of the preferred direction, whereas the tuning remained stable between cylinder conditions. Overall, the results presented in this chapter provide evidence for intact landmark anchoring of TgF344-AD head direction cells in response to a local cue.

The overall findings of this work provoke several questions related to the observed increased directional tuning of postsubicular interneurons. First, postsubicular interneurons have been noted to exhibit mild directional tuning properties [202, 205]. Increased directional tuning suggests either an enhancement of excitatory synapses from thalamic head direction cells or reduction in synaptic input from local pyramidal cells. To address these possibilities, it is critical that a thorough examination of the postsubicular connectivity in TgF344-AD rats is carried out. Specifically, it is important to determine whether there is evidence of up–regulation of glutamatergic synapses on layer III and layer IV neurons, as these postsubicular layers are known to contain head direction cells [237]. Second, the connectivity of postsubicular pyramidal and interneurons as well as the projections of these neuronal populations has been well characterized [111, 112, 115, 179, 197]. Thus, the examination of pyramidal and interneuron connectivity in TgF344-AD rats may inform whether the functional changes observed in this dissertation are due to local reorganization of postsubicular neurons. Third, directional tuning of postsubicular neurons is driven by anterior thalamic head direction cell populations [91, 177], and dual recordings reveal the precise temporal coupling of these two
regions \[203\]. Thus, a functional examination of these loci may reveal whether interneurons that encode HD show altered temporal organization in thalamic HD cells.

Another important avenue of investigation is whether the increased incidence of spike and wave discharges (SWDs) associated with absence seizures, observed in TgF344-AD rats \[247\] and other mouse models of AD-like pathology \[95, 126, 127, 215\], results in acute disruption of postsubicular head direction cells. SWDs are often cortical in origin and frequently arise in the somatosensory cortex \[72\]. During SWDs, activity in the thalamus is depressed as a result of feed-forward inhibition by thalamic reticular neurons via a cortico-thalamic drive \[160\]. Curiously, we noticed SWDs in two rats while the animal was resting on a pedestal before or after a recording session. It is unclear whether the seizure was local to the postsubiculum or was resultant from a generalized seizure event. Nevertheless, SWDs in the postsubiculum may induce inhibition of thalamic neurons via feed-forward inhibition by the reticular nucleus \[276\] and could disrupt the thalamic drive of cortical head direction cells. The neural data examined in this dissertation excluded epochs when the rats were moving less than 3 cm/s, such as when the rat paused to eat a fruit loop piece, given that SWDs occur when a rat is at rest. Thus, it is unlikely SWDs related activity was included in these analyses. Given the particular role the anterior thalamus plays in supporting cortical head direction function \[91\], future studies may examine the impact of SWDs on thalmocortical head direction circuits.

Finally, an integral follow-up to this work would be to examine whether the changes observed in postsubicular head direction cells correspond to the precision of goal-directed navigation. TgF344-AD rats have exhibited reduced precision in navigating to a hidden platform in the water maze \[17\] as well as during a homing task in the barnes maze \[168\]. Berkowitz et al., \[17\] found that 10 month TgF344-AD rats showed similar learning rates and learning magnitude as controls despite making fewer direct paths towards the hidden platform. This suggests that navigational impairment is observed before the onset of spatial learning deficits. In line with this, it is possible TgF344-AD rats exhibit deficits in estimating their position over time. Recently, Yoder et al., \[298\] found that bilateral postsubiculum lesions resulted in rats making more circuitous paths when returning to an escape refuge. Importantly, the postsubiculum is thought to support landmark-based navigation \[296\] and also receives input from deep brain structures involved in self-motion processing \[271\]. Thus, it is possible that altered postsubicular function
observed in this dissertation may be contributing to poor performance of
goal-directed navigation observed in previous studies. However, future work is
needed to clarify whether deficits in self-motion or using landmarks for navigation,
or both are disrupted in TgF344-AD rats.

Through these chapters, this dissertation sought to broaden our understanding
of the AD-like pathology through the investigation of the postsubiculum and
broader circuitry as these networks are thought to critically support spatial
navigation. This dissertation shows that, throughout the previous literature,
interneurons play an integral role in supporting head direction cell tuning within
cortical regions. Based on the findings in chapter 2, a subpopulation of interneurons
show altered function with enhanced directional tuning and changes in intrinsic
firing characteristic suggesting extra-hippocampal regions undergo changes in
function that may contribute to the overall network dysfunction observed in AD-like
pathology. An investigation into how stable head direction cell populations are over
time revealed that despite changes in interneuron function, directional tuning was
stable and rotated in conjunction with a proximal cue, suggesting that landmark
anchoring is robust to the subtle network changes discussed above. With the
progressive nature of AD pathology, these findings may represent a habituation of
functional circuitry resultant from the burgeoning pathological processes. All of
these findings and details discussed throughout this dissertation provide a critical
step in the characterization of postsubicular neural dynamics in AD, which furthers
our understanding of the effects of neurodegeneration on spatial navigation.
Following this work, future studies should be newly equipped to further investigate
the circuitry underlying spatial navigation in AD.
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