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ABSTRACT

THE ROLE OF MEDIAL SEPTUM CHOLINERGIC FUNCTION IN PROCESSING SELF-MOVEMENT CUES TO MAINTAIN SPATIAL ORIENTATION

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Wandering is the most life-threatening and commonly reported symptom of Alzheimer's Disease (AD), a neurodegenerative disorder characterized by an inability to maintain spatial orientation. The often deadly consequences of wandering are projected to rise in the coming decades due to the advancing aged population. These upcoming challenges necessitate a more comprehensive understanding of the neurobiology of spatial orientation in order to evaluate novel therapeutic techniques for symptoms such as wandering. The inability to maintain spatial orientation in AD may be due to the pathological degeneration of the hippocampal cholinergic system. This neurological system is conserved across species for its function in processing self-movement cues to maintain spatial orientation. The current study investigated the role of medial septum cholinergic projections to the hippocampus in processing self-movement cues in female and male rats. Additionally, this study evaluated sequential exploration under dark conditions as a novel behavioral assessment for hippocampal cholinergic function. Rats aged between 90-143 days received selective lesions to the medial septum cholinergic system with the immunotoxin 192 IgG-saporin or a sham lesion using saline. Two weeks following the operation, rats explored a completely dark environment for offline analysis of topographic and kinematic movement

organization. Immunotoxin lesions resulted in significantly decreased hippocampal cholinergic density; however, movement organization was not disrupted. The results of this study suggest that sequential exploration under dark conditions is not sensitive in assessing hippocampal cholinergic function.

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THE ROLE OF MEDIAL SEPTUM CHOLINERGIC FUNCTION IN PROCESSING SELF-MOVEMENT CUES TO MAINTAIN SPATIAL ORIENTATION

BY

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I am forever grateful for the support of Jon, the encouragement of my parents, the guidance of Ashley, and the mentorship, lessons, and opportunity gifted from Dr. Doug Wallace.

DEDICATION

To my Grandpa Richard, who gave me the career advice, “You have a brain. Use it.”

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CHAPTER 1

INTRODUCTION

Wandering is the most life-threatening and commonly reported symptom of Alzheimer's Disease (AD), affecting approximately 70% of patients (Silverstein, Flaherty, & Tobin, 2002). This impaired ability to maintain spatial orientation has devastating consequences. Wandering is a leading cause of hospitalization (Sanford, 1975), a determining factor in nursing home placement (Young, Muir-Nash, & Ninos, 1988), and a significant threat to personal autonomy and quality of life for both patients and caretakers (Cushman, Stein, & Duffy, 2008). After 24 hours of wandering and becoming lost, patients suffer a 46% mortality rate (Koester & Stooksbury, 1995). Wandering is significantly more likely to occur in AD than in any other dementia diagnosis (Klein et al., 1999), suggesting that AD is comprised of neuropathology that specifically affects systems and structures that support spatial orientation.

Currently, 5.4 million Americans have an AD diagnosis. With no known cure and an advancing aged population, diagnoses are expected to triple by 2050 (Brookmeyer et al., 2007). Though a cure for AD has been heavily pursued, treating and delaying symptoms is also of imminent importance. It is predicted that if the onset and progression of AD could be delayed by five years the diagnosis rate would be reduced to half (Khachaturian, 1998). Consequently,

dangerous outcomes from wandering may be delayed or prevented. Developing and evaluating treatments for symptoms such as wandering require a comprehensive understanding of the neurobiological mechanisms mediating spatial orientation.

Neurobiology of Alzheimer's Disease

A definitive diagnosis of AD requires the presence of neuritic plaques, neurofibrillary tangles, and cholinergic depletion. Several genetic changes are proposed to precede the neuropathology of AD, including mutated coding for Amyloid Precursor Protein, Presenilin-1, Presenilin-2, and Apolipoprotein E. These genetic mutations are associated with deficits in regulation of lipid metabolism, cell proliferation, adhesion for nerve growth factor, amyloid beta peptide length, and neural development. The resulting cellular deficits in AD occur earliest and most predominantly in cholinergic cells of the hippocampal formation (Braak & Braak, 1991).

A hallmark feature of AD is the presence of neuritic plaques that consist of neurotoxic insoluble proteins composed from amyloid beta peptide. Neuritic plaques are formed when amyloid precursor protein is abnormally cleaved by the enzymes beta secretase and gamma secretase. The resulting amyloid beta misfold and clump together to form oligomers which may aggregate into large fibrils and deposit as neuritic plaques. Aggregates of neuritic plaques weaken the synapse and make neural communication increasingly difficult. Neuritic plaques are dispersed throughout the brain in AD neuropathology but are predominantly located in the hippocampal formation, resulting in a disruption of choline acetyltransferase activity

(Khachaturian, 1985). This disruption in neural communication can result in neuronal atrophy; however, the amount of neuritic plaques is not significantly correlated with changes in hippocampal volume. This suggests that factors outside of neuritic plaques contribute to the observed neuronal loss.

Neurofibrillary tangles are significantly correlated with neuronal atrophy in AD. These tangles lead to decreased communication that is associated with observed cognitive and behavioral symptoms in AD. Neurofibrillary tangles result from misfolded tau protein. In a healthy brain, tau is responsible for maintaining microtubules that traffic proteins. In AD, tau dissociates from the microtubules, misshapes, and moves from the axon into the cell body of neurons. Misfolded tau can become insoluble and form tangles that spread among neurons, damage neurons, and advance AD neuropathology throughout the brain. Neurofibrillary tangles originate in the hippocampus during the course of AD and largely affect the entorhinal cortex, nucleus basalis, and neocortex (Khachaturian, 1985). Neurofibrillary tangles are significantly associated with hippocampal formation atrophy (Bobinski et al., 1996), resulting in an average 60% decrease in hippocampal volume throughout the course of AD.

The majority of cholinergic neurons are located in the hippocampal formation (Mosconi, 2005) and are particularly vulnerable to tau pathology (López & DeKosky, 2003). Post-mortem AD patients show a 60-90% decrease in acetyltransferase, the enzyme responsible for synthesizing acetylcholine. The combination of the role of acetylcholine in memory and cognition, along with its degeneration in AD, provides the foundation for the cholinergic hypothesis of AD. The cholinergic hypothesis posits that the depletion of cholinergic neurons in

the hippocampal formation largely contributes to the symptoms observed in AD (Perry et al., 1992).

The hippocampal formation consists of the hippocampus and its surrounding basal forebrain structures, including the medial septum. The medial septum provides the majority of cholinergic innervation to the hippocampus. The hippocampal formation is highly implicated in spatial orientation (Teles-Grilo Ruvio, & Mellor, 2013). Investigating the contribution of medial septum cholinergic projections to the hippocampus in spatial orientation may elucidate the neuropathology contributing to wandering in AD.

Neuropsychology of Alzheimer's Disease

Neuropathological markers of AD precede many symptoms by several years (Hof, Glannakopoulos, & Bouras, 1996). These symptoms are consistent with the function of neural systems and structures affected by AD neuropathology that leads to a predictable and sequential loss of function. Early AD neuropathology affects temporal lobe structures such as the hippocampus and entorhinal cortex, which corresponds with memory deficits. Episodic memory and semantic memory are often first impaired. The loss of memory for events, general facts, concepts, and word meaning is often observed in verbal fluency impairments in patients with AD. Deficits in the ability to learn and retain new information is reflected in the inability to effectively consolidate and store new information (Weintraub, Wicklund, & Salmon, 2012). In addition to memory deficits, AD neuropathology results in impairments in executive function,

which often lead to difficulties in problem solving and mental manipulation. Patients with AD are impaired in focusing attention to hold information in a temporary state (Baddeley, 2003) which may further contribute to memory deficits. Neuropsychological evaluation of memory and executive function is administered for a clinical diagnosis of AD (Albert, 1996); however, a definitive diagnosis can only be affirmed post-mortem.

Additional neurological functions are impaired during the course of AD and may be beneficial to assess when considering a diagnosis. Identifying deficits in the preclinical stage of AD is important for providing a diagnosis that allows for early disease treatment. Visuospatial abilities are highly impacted during the progression of AD and may even be impaired in the preclinical disease phase (Johnson et al., 2009). Visuospatial abilities allow an animal to identify spatial relationships that can be used to guide navigation through an environment. During the course of AD, a large number of visual inputs that project to the hippocampus are degenerated in AD neuropathology (Hüfner et al., 2011).

In a healthy brain, visuospatial abilities enable optic flow. Optic flow provides an animal with information about the apparent movement of stimuli in an environment generated from an animal's own movement. By displacing stimuli across the retina in real time, optic flow helps contribute to detecting velocity and distance traveled (Gibson, 1966). Additionally, optic flow provides information about direction moved. Optic flow is impaired in patients with AD (Mapstone & Duffy, 2010; Tetwesky & Duffy, 1999). During a real-life navigation test, patients with AD were impaired in judging direction moved. When participants were guided on a walk throughout a hospital lobby and instructed that they would be questioned about the path layout, length of corridors, direction of turns, and pictures of landmarks, participants with AD

demonstrated poor performance compared to healthy young and elderly controls. To demonstrate that optic flow impairment contributed to these deficits, participants were presented with visual digital information that simulated movement of environmental stimuli. Participants with AD were unable to accurately judge what their own movement would be in relation to environmental stimuli motion. Additionally, when participants were presented with digital simulated self-movement along with environmental simulated motion, participants with AD were unable to adjust their perceived self-motion in relation to the environmental motion, consistently reporting self-motion to be toward a fixation point regardless of environmental motion. The results of these studies indicate that patients with AD have impaired optic flow. The inability to integrate visuospatial information to accurately judge direction moved may contribute to spatial disorientation observed in AD.

The vestibular system is also highly implicated in spatial orientation. In vivo electrophysiologic and caloric stimulation studies have observed a vestibular signal that contributes to hippocampal cholinergic system function (Horii et al., 1994). Stimulation of the vestibular system resulted in a 152% increase in acetylcholine levels in the hippocampus. The vestibular system's multisynaptic projections to the hippocampal cholinergic system are affected by and associated with AD neuropathology (Previc, 2013). Neurodegeneration of this system is evident in heightened rates of falling and hip fractures in AD patients compared to healthy elderly populations (Buchner & Larson, 1987). Additionally, patients with AD are less capable of relying on their vestibular system to maintain balance when presented with incongruent visual information (Chong, Horak, Frank, & Kaye, 1999). AD neuropathology affecting the vestibular system may contribute to spatial disorientation.

The vestibular system contributes to spatial orientation. Damage to this system results in deficits in direction estimation when visual information is not available during navigation (Zheng, Darlington, & Smith, 2005; Zheng, Goddard, Darlington, & Smith, 2009). The vestibular system provides information about spatial orientation through a collection of inner ear structures. Endolymph fluid within the semicircular canals and otolith organs displaces in response to an animal's movement, triggering hair cells that project signals to the hippocampus. Semicircular canals respond to angular acceleration within pitch, roll, and yaw planes of motion. Otolith organs respond to linear acceleration and direction of gravity. Additionally, otolith organs assist in eye movements that help maintain a stable plane of vision while an animal is in motion. Vestibular inputs project to the hippocampus, which integrates these movement signals to provide an animal with a sense of direction (Barlow, 1964; Yoder et al., 2011). The ability to integrate information from one's movement in space is critical to maintaining spatial orientation. Evidence from bilateral labyrinthectomy studies have demonstrated the importance of vestibular information in maintaining spatial orientation. Rats with bilateral labyrinthectomies exhibited movement consistent with disorientation during a navigational task when environmental cues were not available (Wallace, Hines, Pellis, & Whishaw, 2002; Zheng, Goddard, Darlington, Smith, 2009). Additionally, mice with congenital vestibular deficits exhibited movement consistent with disorientation during a navigational task when environmental cues were not available (Blankenship et al., 2017; Donaldson et al., 2018). Degeneration of the vestibular system, along with its effects on multisynaptic afferent projections to the hippocampal formation, likely contributes to the spatial disorientation observed during the progression of AD.

AD neuropathology affects systems and structures implicated in spatial orientation. The degeneration of cholinergic projections to the hippocampus in AD may contribute to the spatial disorientation observed in patients. The ability to process visuospatial and vestibular information is crucial for maintaining spatial orientation. Degeneration of the systems that support the processing of this information may lead to an inability to maintain spatial orientation during the course of AD.

Navigational Cues and Strategies

The ability for animals to successfully navigate throughout an environment is essential to successfully forage for food, locate mates, and avoid predators (Gallistel, 1990). Due to frequent and unpredictable environmental changes that can occur, animals have developed flexible navigation strategies. For example, polar bears navigating vast Arctic environments may experience unpredictable changes such as moving sea ice, snowstorms, or complete darkness that preclude the visibility of cues in the environment. Changing environmental conditions necessitate the ability for animals to use multiple sources of information to maintain spatial orientation. Two dominant sources of information animals use to maintain spatial orientation are environmental and self-movement cues (Gallistel, 1990). Environmental cues are external sources of information, such as a polar bear's footprints or bodies of water. Self-movement cues are internally generated sources of information, such as vestibular signals generated from a polar bear's movement. Environmental and self-movement cues can be used in combination or

independently from each other. Self-movement cues provide animals with the most flexible source of information to maintain spatial orientation.

The ability for animals to flexibly navigate environments is commonly proposed to be due to a cognitive map (Tolman, 1948). The cognitive map theory states that animals encode a mental representation of space and their position within it. From this representation, animals are capable of flexible navigation throughout an environment. Animals can use several different strategies to navigate, including beacon homing, piloting, and dead reckoning. While beacon homing and piloting rely on environmental and self-movement cues, dead reckoning relies specifically on self-movement cues, which demands a higher cognitive load. Evaluating the ability for animals to use flexible navigation strategies is critical to understanding how animals maintain spatial orientation.

Beacon homing is a navigational strategy that relies on the presence of a stable environmental cue. Animals can use a stable environmental cue to guide navigation toward a goal (Gallistel, 1990). For example, an animal can retain the stimulus characteristics of a tree where they cached a food item to later return to the same tree and recover the food item. However, if the stimulus characteristics of the tree are changed, an animal can no longer use this environmental cue to guide navigation. The visible platform condition of the Morris water maze (MWM) allows animals to use a beacon homing strategy to escape the water (Morris, 1981). Upon being placed in the MWM, animals will search for a visible platform to escape the water. After several trials, animals will locate the platform faster, swim shorter distances to reach the platform, and take more direct paths toward the platform. These efficient paths toward the platform occur even when animals start their search from novel locations along the perimeter of

the MWM. Improvement in water maze performance, regardless of the start location, demonstrates that animals are able to encode an escape response using the platform as a visual cue. From this association, animals are able to use beacon-homing-based navigation. Animals can also use stable odor cues for beacon homing. For example, rats have been trained to associate varying scented strings with food items (Wallace, Gorny, & Whishaw, 2002). The requirement for environmental cues to remain stable limits the advantages of beacon homing. For example, when rats were trained to associate landmarks with a food item and associate different landmarks with no food item, rats only successfully reached the food item when the landmarks remained in stable positions (Biegler & Morris, 1993). Though beacon homing is advantageous in that it requires a low cognitive load, dynamic environments make beacon homing a less optimal navigational strategy.

Piloting is a more flexible and adaptive navigational strategy but demands a higher cognitive load compared to beacon homing. Piloting consists of an animal forming associations between one or more environmental cues to navigate toward a goal (Gallistel, 1990). The non-visible platform condition of the MWM allows animals to use a piloting strategy. By forming associations between multiple environmental cues and the platform location, animals are able to pilot to a non-visible platform from multiple different starting points along the perimeter of the MWM. This demonstrates that animals are able to form flexible associations between a goal and environmental cues. Though piloting is more flexible than beacon homing, it requires an animal to be capable of identifying environmental cues to access formed associations in order to navigate toward a goal (Epstein & Vass, 2014).

Identifiable or available environmental cues are not always accessible to an animal. To effectively navigate without access to environmental cues, animals can use dead reckoning. Dead reckoning is a cognitively demanding but flexible navigational strategy that does not rely on environmental cues, but instead it relies on self-movement cues generated from an animal's own movement (Barlow, 1964; Darwin, 1873; Gallistel, 1990; Maaswinkel & Whishaw, 1999; Mittelstaedt & Mittelstaedt, 1980). As an animal moves, self-movement cues are generated through the vestibular system, optic flow, proprioception, and motor efferent commands. Self-movement cues provide an animal with information to maintain a continuously updated representation of current position in space. This representation can be used to estimate distance and direction moved from the point where movement originated. Self-movement cues can be used alone or in conjunction with environmental cues; however, self-movement cues are alone sufficient to enable an animal to return to their point of movement origination (Wallace, Hamilton, & Whishaw, 2006). Dead reckoning is conserved across both vertebrates and invertebrates ranging from ants (Wehner & Flatt, 1972) to rats (Mittelstaedt & Mittelstaedt, 1980; Whishaw & Wallace, 2003) to humans (Loomis et al., 1993). Although these animals have different sensory and nervous systems, they are all capable of relying on self-movement cues to effectively dead reckon (Wallace, Martin, & Winter, 2008). The conservation of dead reckoning across a large range of species is consistent with the strong adaptive value of this navigational strategy.

Several computational theories have attempted to explain the process of dead reckoning. An initial theory predicted that an animal computes the average time taken to travel varying directional path segments; however, this would imply that an animal is moving at a constant

speed throughout time (Jander, 1957). To address this issue, a different theory suggested that animals record the average length of each path segment and its directional heading. From the resulting x- and y- coordinates, animals trigonometrically calculate a vector to determine their position in space (Mittelstaedt, 1985). Another theory has asserted that the length of each path segment and changes in directional heading between path segments are calculated separately throughout an entire navigational trip (Benhamou, Sauvé, & Bovet, 1990). Even more frequent calculations are theorized to occur between unit-sized steps of movement in a path segment and directional heading compared to previous movements to calculate a vector (Müller & Wehner, 1988). A more recent theory indicates that the computational processing which enables dead reckoning occurs through parallel distributed neural processing. Elements of movement, such as path length, time, and change in heading, are processed neurologically in parallel, producing a representation of current position in space (Maurer & Séguinot, 1995). Lesion and electrophysiological studies paired with a behavioral paradigm that dissociate navigational strategies are critical to better understand the systems and structures that support dead reckoning.

The ability for animals to use multiple navigational strategies is essential for the success of species. When the ability to use flexible navigational strategies is impaired, animals can easily become disoriented. This disorientation may preclude an animal's ability to maintain spatial orientation and accurately return after an excursion in their environment. A comprehensive understanding of the neurobiology supporting different navigational strategies is needed to better understand spatial orientation. Particularly, identifying the systems and structures that support self-movement cue processing may elucidate mechanisms that underly deficits in spatial orientation.

Neurobiology of Spatial Orientation

The role of neural structures mediating spatial orientation has seen considerable debate. An impaired place response in the MWM following hippocampal lesions has provided support for the role of the hippocampus as a cognitive map (O'Keefe & Dostrovsky, 1971; O'Keefe & Nadel, 1978). The hippocampus has been proposed to underly a cognitive map by forming associations between environmental cues. These associations are proposed to allow animals to maintain spatial orientation even when taking novel paths throughout an environment; however, the role of the hippocampus in spatial orientation has changed as navigational tasks have evolved. Navigational tasks that dissociate environmental and self-movement cue availability have demonstrated differential performance in animals with hippocampal lesions. Evidence from these studies suggest that the role of the hippocampus in spatial orientation is to process self-movement cues (Gorny, Gorny, Wallace, & Whishaw, 2002; Martin et al., 2007; Martin & Wallace, 2007; Whishaw & Maaswinkel, 1998; Whishaw, Hines, & Wallace, 2001). Dissociation of environmental and self-movement cues in navigational tasks have been critical in evaluating the role of the hippocampus in spatial orientation. Dissociation of cues may provide insight into potential mechanisms mediating wandering in AD, as the disease neuropathology largely affects systems and structures that support self-movement cue processing (Delpolyi, Rankin, Mucke, Miller, & Gorno-Tempini, 2007).

The role of the hippocampus in spatial orientation was first introduced following patient H.M.'s temporal lobectomy. After suffering with epilepsy for several years, H.M. underwent surgery to remove portions of his temporal lobes. Though removal largely alleviated his seizures,

H.M. developed anterograde and partial retrograde amnesia. The role of the hippocampus in memory was evident. Researchers aimed to further understand the mechanism of the hippocampus in memory by developing animal models. By selectively removing medial temporal lobe structures including the hippocampal formation, researchers have been able to demonstrate that there are differential roles for this structure in varying memory task demands. Non-matching to sample tasks have been used to evaluate performance in recognition memory. When monkeys received hippocampal and amygdala aspiration lesions, performance was disrupted in the non-matching to sample task (Mishkin, 1978). However, more selective lesions to these structures that spared connecting fibers did not disrupt performance in the same task (Murray & Mishkin, 1984). Hippocampal formation lesions also differentially impact spatial memory task performance. The MWM measures a component of spatial memory by evaluating the ability of animals to form a place response. When rats received hippocampal lesions following a learned place response, they spent less time swimming in the quadrant of the previously located platform (Morris et al., 1982). However, extensive training resulted in eventual place learning following hippocampal aspiration lesions (Morris, Schenk, Tweedie, & Jarrard, 1990). The hippocampus is also implicated in working memory. The radial arm maze evaluates working memory by quantifying repeated returns to the same arm. Rats with selective lesions to extrinsic fiber connections of the hippocampal formation made repeated returns to arms baited with a food reward, but not unrewarded arms (Olton, Walker, Gage, & Johnson, 1977). This behavioral performance suggests that selective hippocampal damage spares spatial learning of an environment but impairs working memory. As these studies have indicated, the role of the hippocampus in memory continues to be debated.

The hippocampus as a cognitive map was largely supported through single-cell recordings as animals moved throughout different environments. Recordings of single cells in the CA1 and CA3 subregions of the hippocampus showed distinct firing patterns as rats freely moved throughout a cylindrical arena (O'Keefe & Dostrovsky, 1971). These distinct place cell firing patterns correlated with the location of an animal in the environment, regardless of the heading direction the animal was facing. These place cell fields appear to be distinctly spatial, as firing was not associated with other types of behavior such as locomotion, eating, or drinking (O'Keefe & Dostrovsky, 1971). Place cell firing is largely influenced by environmental cues, as demonstrated by placing rats in a monochromatic gray cylindrical apparatus with a single white cue card located on the wall. When the location of the white cue card changed, place fields changed in congruence with the card's location (Kubie & Muller, 1991). The discovery of place cells and environmental cue control over their firing patterns served as further evidence to support the role of the hippocampus as a cognitive map by forming associations between environmental cues. These associations can be used by an animal to form a layout of their environment for flexible navigation (O'Keefe & Nadel, 1978). However, tasks that have dissociated environmental cue from self-movement cue use have challenged the role of the hippocampus in spatial memory.

In the absence of visual environmental cues, single-cell recordings of hippocampal place cells show distinct firing patterns (Quirk, Muller, & Kubie, 1990). Electrophysiological studies have demonstrated that place cells maintain firing patterns in the absence of environmental cues. This provides evidence that self-movement cues contribute to spatial orientation. Self-movement cues generated from an animal's head movement particularly contribute to place cell activation.

When place cells in the CA1 region of mice were recorded during a virtual reality task, their firing patterns greatly increased when mice were allowed to freely move their heads compared to when their heads were in a fixed position (Chen et al., 2013). When mice heads were fixed, 25% of place cells fired, but free movement of heads regained the remainder of place cell firing. These studies provide evidence that the hippocampus has a role in processing self-movement cues.

Dissociating environmental from self-movement cues in spatial tasks is important for evaluating the role of the hippocampus in spatial orientation. Previous work that asserted the role of the hippocampus as a cognitive map did not sufficiently dissociate environmental and self-movement cues (Alyan & McNaughton, 1999; Shrager, Kirwan, & Squire, 2008). The food hoarding paradigm has been used for over 20 years and consistently has dissociated environmental and self-movement cues (Maaswinkel, Jarrard, & Whishaw, 1999). This task has allowed researchers to evaluate neural structures mediating different navigational strategies. Food hoarding relies upon an animal leaving a refuge, searching a table for a randomly located food pellet, and navigating back to the refuge for consumption of the food pellet. When the experimental room is illuminated, rats can use multiple sources of cues to navigate to the refuge. For example, visual cues associated with the refuge allow for a beacon homing strategy to be used, whereas multiple distal environmental cues allow animals to use a piloting strategy.

Visible environmental cues can be eliminated by creating a completely dark environment. When animals cannot rely on environmental cues, they must rely on self-movement cues to guide navigation, requiring a dead reckoning navigational strategy. Evaluating rats with hippocampal lesions in the food hoarding task under completely dark conditions has

demonstrated a role for the hippocampus in mediating self-movement cue processing (Maaswinkel & Whishaw, 1999). In both light and completely dark conditions, control rats typically will make direct returns to a refuge after searching the table for a food pellet. Rats with hippocampal damage demonstrate these direct returns under light conditions; however, returns are circuitous under dark conditions. For example, rats that received selective ibotenic lesions that selectively destroyed hippocampal cells made direct returns to a refuge under light conditions, but circuitous returns under dark conditions. Similar behavioral patterns were observed in mice under dark conditions that received electrolytic lesions of the fimbria fornix, which eliminates communication between the hippocampus and its connected structures (Gorny, Gorny, Wallace, & Whishaw, 2002). Further, rats that have received selective lesions to the medial septum cholinergic system, which has direct projections to the hippocampus, demonstrated circuitous paths and varied temporal pacing when returning to a refuge under dark but not light conditions (Martin & Wallace, 2007).

The observed movement organization differences under only dark conditions in rodents with hippocampal formation damage challenges the role of the hippocampus as a cognitive map. Under light conditions, both control rodents and rodents with hippocampal damage demonstrate similar movement organization, such as direct returns to a refuge. This performance is consistent with spared environmental cue processing. In contrast, under dark conditions, rodents with hippocampal damage follow a circuitous return path to a refuge. This is consistent with impaired self-movement cue processing. Evaluating the role of selective hippocampal systems in self-movement cue processing will provide a more comprehensive understanding of the role of the hippocampus in spatial orientation.

Advanced molecular lesion techniques have enabled a more selective evaluation of the role of the hippocampus in spatial orientation. The hippocampal formation consists of several different types of cellular projections to the hippocampus, including GABAergic and cholinergic cells. GABAergic fibers synapse on inhibitory interneurons of the hippocampus and, when activated, result in greater inhibition of hippocampal pyramidal neurons (Köppen, Winter, Stuebing, Cheatwood, & Wallace, 2013). Infusion of GAT1-saporin into the medial septum produces selective GABAergic deafferentation of the hippocampus. These lesions have been shown to attenuate performance in food hoarding and MWM tasks under dark but not light conditions. As observed in more gross hippocampal lesion studies, rats with hippocampal GABAergic lesions made more circuitous returns to a refuge compared to control rats under dark conditions, but this performance was spared under light conditions. The dissociation of performance observed under dark and light conditions suggests that the hippocampal GABAergic system is specifically implicated in processing self-movement cues. The hippocampal cholinergic system consists of fibers that synapse onto dense pyramidal neurons of the hippocampus. Evaluating the role of the hippocampal cholinergic system in spatial orientation can be performed with the selective immunotoxin 192 IgG-saporin. 192 IgG-saporin is composed of a monoclonal antibody chemically linked to saporin which binds to the 192 IgG nerve growth factor. Infusion of this neurotoxin results in selectively destroyed cholinergic pathways while sparing other cellular systems. 192 IgG-saporin produces between a 60-90% depletion of cholinergic functional markers in the hippocampus, which can be inferred with acetylcholinesterase histological staining. This selective lesion technique produces a surgical model of AD, as similar patterns of cholinergic loss in the hippocampal formation are observed

in AD neuropathology. Further, this selective lesion technique allows for the evaluation of spatial orientation in AD, as the severe degeneration of the hippocampal cholinergic system may mediate symptoms such as wandering (Rabins, 1982). Similar to full hippocampal lesions, rats with selective lesions to the hippocampal cholinergic system demonstrated impaired navigational performance when limited to dark but not light conditions in the food hoarding task (Martin & Wallace, 2007). For example, rats with selective hippocampal cholinergic lesions under dark conditions had more circuitous paths when returning to a refuge under dark conditions compared to rats with intact hippocampal systems; however, these movements were not observed in either group under light conditions. This demonstrates that the hippocampal cholinergic system is implicated in maintaining spatial orientation by processing self-movement cues.

The hippocampus processes self-movement cues generated from multiple sensory systems, such as the vestibular system. The vestibular system provides information about spatial orientation through a collection of inner ear structures. Endolymph fluid within the semicircular canals and otolith organs displace in response to an animal's movement within pitch, roll, and yaw planes of motion. This displacement of endolymph fluid activates hair cells that project signals to the hippocampus. Vestibular activity directly influences cell firing in the hippocampus. For example, when the vestibular system is inactivated, cell firing patterns in the hippocampus are disrupted (Stackman & Taube, 1997). Additionally, patients with bilateral vestibular loss have significant hippocampal atrophy (Brandt et al., 2005). Disruptions in vestibular activity can be observed in navigational tasks. For example, rats with bilateral labyrinthectomies were unable to accurately return to a refuge under dark conditions, but these differences abated under light conditions. Rodents also demonstrated these disruptions in movement organization when

allowed to freely explore an open table without a refuge. Rats with acquired (Wallace, Hines, Pellis, & Whishaw, 2002) and mice with genetic (Blankenship et al., 2017; Donaldson et al., 2018) vestibular pathology demonstrated disrupted movement patterns in navigational tasks under dark, but not light, conditions. Like rodents with hippocampal damage, those with vestibular pathology demonstrated more circuitous progressions. These movements are consistent with impaired self-movement cue processing. Interestingly, vestibular deficits are often observed in patients with AD (Baloyannis, Manolidis, & Manolidis, 2000; Chong, Horak, Frank, & Kaye, 1999; Mapstone & Duffy, 2010). The ability to detect deficits in self-movement cue processing may provide a novel behavioral assessment for navigational deficits such as wandering in AD. However, previous navigational tasks using a refuge have required extensive shaping, thus limiting the amount of observable movement. Sequential exploration, as used in the aforementioned mouse vestibular studies, has been sensitive in detecting self-movement cue processing deficits in vestibular rodent models. Evaluating rats with selective hippocampal cholinergic damage in sequential exploration under dark conditions may provide both an efficient and novel way to assess self-movement cue processing deficits. Particularly, this may be useful for evaluating wandering in AD.

Exploration

Rodent exploratory behavior occurs spontaneously in novel environments. The behavior during exploration consists of highly organized sequences of movement based around an

animal's home base (Eilam & Golani, 1989). Even in the absence of a refuge, animals establish a home base within the first two minutes of exploring an environment. The presence of a home base is marked by behaviors such as frequent grooming, rearing, and circling. An animal's home base establishment is essential for several aspects of survival, including protection, foraging, social behavior, and the maintenance of spatial orientation (Whishaw, Hines, & Wallace, 2001). Animals use the home base to maintain spatial orientation by making frequent returns and longer stops in its location. Returns to the home base allow an animal to recalibrate its representation of position in space following trips into the environment (Valerio & Taube, 2012; Wallace, Martin, & Winter, 2008). By analyzing these highly organized sequences of movement, researchers have been able to evaluate self-movement cue processing in rats and mice.

Exploratory behavior was first analyzed using single trip exploration that included the use of a refuge. Single trip exploration consists of animals making outward and homeward segments of movement organized around the refuge, which animals adopt as a home base. Animals display topographic and kinematic differences between outward and homeward segments toward the refuge (Tchernichovski & Golani, 1995; Wallace, Hamilton, & Whishaw, 2006). For example, outward segments of movement into the environment consist of slower and more circuitous progressions. Additionally, outward segments consist of more frequent stopping with larger changes in heading direction. In contrast, homeward segments of movement are faster and consist of less circuitous progressions. Additionally, the peak speed of movement occurs in the middle of a progression. These outward and homeward progression differences are consistently observed in control animals under both light and dark conditions. Damage to the hippocampus disrupted this movement organization under dark, but not light, conditions (Winter, et al., 2013).

For example, animals with hippocampal lesions demonstrated greater changes in directional heading under dark conditions when returning to the refuge but performed similar to control animals under light conditions. These results suggest that the hippocampus contributes to processing self-movement cues but does not organize movement under conditions with access to environmental cues. Though single trip exploration is a useful paradigm for evaluating spatial orientation, an animal leaving a refuge requires extensive shaping. Additionally, progressions from the refuge into the environment need to be sufficiently long to analyze movement. A more efficient exploratory paradigm that animals will engage in without training is sequential exploration.

Analysis of sequential exploration is sensitive in detecting the contribution of the vestibular system to spatial orientation (Blankenship et al., 2017; Donaldson et al., 2018). Sequential exploration consists of an animal making several movements organized around an established non-physical home base. These movements are organized into series of stops and progressions. Progressions are relatively fast segments of movements, quantified by periods of movement greater than or equal to the average velocity traveled for more than two frames of recorded movement. On a general level, progressions can be analyzed by measuring distance traveled. Greater distance traveled typically indicates general locomotion factors such as hyperactivity or motivation. On a specific level, progressions can be analyzed to infer an animal's ability to process self-movement cues. Control animals will make non-circuitous progressions throughout an environment. Additionally, control animals will scale their speed with progression length, so that longer progressions are associated with faster speeds. Stops are quantified by relatively slow segments of movement below the animal's average velocity for at

least two frames of recorded movement. On a general level, the frequency of stops typically indicates general locomotion factors such as hyperactivity or motivation. On a specific level, stops can be analyzed to infer an animal's ability to process self-movement cues. Control animals will make more frequent stops for longer durations in consistent locations of an environment, resulting in a clustering of stops. Additionally, control animals typically have smaller changes in heading between stops. This consistent movement organization can provide an analysis of spatial orientation in animals.

The movement organization during sequential exploration under dark conditions has been evaluated across multiple species of animals (Donaldson et al., 2019). Rats and mice demonstrate general locomotive differences in sequential exploration under dark conditions. For example, mice make more frequent progressions that result in longer distances traveled compared to rats with faster peak speeds. Additionally, mice spend less time stopping relative to rats. However, specific aspects of movement organization are similar between the species. Both rats and mice take non-circuitous progressions throughout their environment, have low changes in heading between stops, and cluster their stops similarly throughout sequential exploration under dark conditions. This pattern of movement organization indicates that while differences in hyperactivity may be driving general locomotive differences, self-movement cue processing is similar between species. The ability to compare species in sequential exploration under dark conditions provides high translational value. While this analysis has been previously used to detect self-movement cue processing deficits in mice with vestibular pathology, it may be able to detect similar impairments in rats with damage to the hippocampal cholinergic system.

Analysis of movement organization during sequential exploration under dark conditions has demonstrated that the vestibular system contributes to self-movement cue processing. Rodents with vestibular pathology, such as otoconia-deficient mice, demonstrate more circuitous progressions, larger changes in heading, and less stable stop clustering under dark conditions (Blankenship et al., 2017). Under light conditions, however, these differences in movement organization are not observed. Similarly, harmonin-deficient mice exhibit circuitous progressions under dark but not light conditions (Donaldson et al., 2018). These results demonstrate that the vestibular system is an important source of self-movement cues. Further, these studies demonstrate that analysis of sequential exploration under dark conditions is sensitive in detecting deficits in self-movement cue processing. Research has yet to evaluate neural systems involved in higher level processing of self-movement cues in sequential exploration under dark conditions, such as hippocampal cholinergic function. Hippocampal cholinergic function is highly degenerated in AD neuropathology and can be modeled by infusing the selective immunotoxin 192 IgG-saporin into the medial septum, which provides the majority of cholinergic projections to the hippocampus. Evaluating the effects of this degeneration on sequential exploration under dark conditions provides a novel and efficient behavioral assessment of hippocampal cholinergic function. Further, this may elucidate the role of the hippocampal cholinergic system in self-movement cue processing and its implications for wandering in AD.

CHAPTER 2

CURRENT STUDY

Specific Aims

The current study investigated whether selective lesions of medial septum cholinergic projections to the hippocampus disrupted movement organization in female and male rats during sequential exploration under dark conditions. Additionally, this study evaluated sequential exploration under dark conditions as a novel behavioral assessment for hippocampal cholinergic function. Hippocampal cholinergic degeneration, along with wandering, occurs in both sexes during the course of AD. Most studies have indicated that no sexual dimorphisms exist in wandering behavior (Hope et al., 2001; Jost & Grossberg, 1996; Teri, Larson, & Reifler, 1998); however, a study that observed sexual dimorphisms used measures of agitation rather than spatial orientation (Ott et al., 1996). Previous rodent work has demonstrated that selective lesions of medial septum cholinergic projections to the hippocampus did not result in sexual dimorphisms in place learning in the MWM (Jonasson et al., 2004); however, this task did not dissociate environmental and self-movement cues. Previous navigational work that dissociated environmental and self-movement cues have largely used female rats. However, the limited studies that have used both female and male rats have found differences in general measures of

movement organization, but not specific measures of movement organization sensitive to detecting self-movement cue processing (Köppen et al., 2015). Therefore, it was important to evaluate if sexual dimorphisms would be observed in the ability to process self-movement cues.

It was hypothesized that sexual dimorphisms would not be observed in specific measures of self-movement cue processing in control or lesion rats. Additionally, it was hypothesized that rats with selective lesions of medial septum cholinergic projections to the hippocampus would exhibit disruptions in specific measures of movement organization. Specifically, it was hypothesized that lesion rats would demonstrate increased average path circuity, decreased average movement scaling, less stable stop clustering, and increased average changes in heading. These differences in movement organization are consistent with deficits in self-movement cue processing and are indicative of an impaired ability to maintain spatial orientation.

CHAPTER 3

METHODS

Subjects

Twenty-four female and twenty-six male Long-Evans hooded rats bred at Northern Illinois University from stock purchased at Envigo were used in the current study. Rats were aged between 90-143 days at the time of surgery and maintained at a +2%/-2% of their baseline 90-day body weight throughout the course of the study. Rats were fed with Rodent Breeder Diet food pellets, PMI Nutrition International (Brentwood, MO). Rats were pair housed in groups of two in opaque plastic cages kept in a colony room maintained on a 12-hour light/12-hour dark cycle. Temperature of the colony room was maintained between 20°C and 21°C. All experimental procedures were approved by the Northern Illinois University's Institutional Animal Care and Use Committee (IACUC, LA19-0010), which follows standards set by the National Institutes of Health

Surgery

Rats were deeply anesthetized with a mixture of isoflurane and oxygen before and during surgery. Rats received either medial septum lesions with the immunotoxin 192 IgG-saporin (female n=14, male n=17) or sham lesions with saline (female n=10, male n=9). Medial septum lesions were produced with microinjections of 192 IgG-saporin (lot# 119-8, Advanced Targeting Systems, San Diego, CA). The immunotoxin concentration consisted of 0.50 micrograms per microliter injected into two sites per hemisphere in coordinates relative to bregma and the surface of the dura: AP +0.30, ML ±0.20, DV1 -7.5 (0.40 microliters at each site), DV2 -6.5 (0.30 microliters at each site). Injections were made at the rate of 0.10 microliters per minute. The cannula was maintained in position for three minutes after each injection to prevent diffusion of the solution up the tract of the needle. Rats that received sham surgery were treated identically except saline was infused in place of the immunotoxin. These immunotoxin lesions have previously been shown to produce a near total loss of choline-acetyltransferase-positive neurons in the medial septum and a 40% decrease in cholinergic neurons in the hippocampus (Chang & Gold, 2004). This procedure has previously been shown to effectively produce significant decreases in medial septum cholinergic neurons, decreased acetylcholinesterase fiber staining restricted to the hippocampus, and have minimal effects on immunoreactivity for parvalbumin-positive neurons in the medial septum (Martin & Wallace, 2007).

Apparatus

The exploration arena was a circular table (198cm in diameter and 130cm high) made of wood and painted white. Two experimental rooms were used. Infrared cameras were positioned perpendicular to the surface of the table. Four infrared emitter banks illuminated the room during dark testing conditions to allow the testing room and rats to be visible on camera (see Figure 1). Infrared is a wavelength that rats are unable to visually detect (Neitz & Jacobs, 1986). Night-vision binoculars were used to place the rat on the center of the table, replace the rat after falls, and remove the rat at the conclusion of the session. Sequential exploratory sessions under dark conditions were recorded at five frames per second and transferred to DVDs for offline analysis.

Figure 1: The exploratory apparatus during sequential exploration under dark conditions. Rats individually explored the large, open circular arena without walls. The experimental room was completely dark and only visible on camera with infrared emitter banks.

Procedure

Rats completed one sequential exploration session under completely dark conditions. Sequential exploratory sessions under dark conditions occurred during the light portion of the rat's light-dark cycle. Sequential exploration under dark conditions testing began two weeks after surgery. Rats were removed from the animal colony room and transported to the testing room in an opaque cage covered by a towel in a circuitous path to prevent learning the relationship between the animal colony and testing rooms. Upon entering the completely dark testing room, the cage was placed on a nearby surface separate from the exploration arena. The rat was removed from the cage and placed on the center of the table by the researcher. Each rat was left to explore the arena for 40 minutes. If the rat fell off the table, it was retrieved in completely dark conditions and replaced on the center of the table. Upon completion of the session, the experimenter retrieved the rat, placed it in a transport cage covered by a towel, and left the experimental room in a circuitous path before returning the rat to the animal colony. The arena and all surfaces touched were sanitized between sessions with Glance cleaning solution to eliminate odor cues.

Behavioral Analysis

Ethovision video tracking system (Noldus Information Technology, Lessberg, VA) was used to quantify kinematic and topographic movements during sequential exploration under dark

conditions provided by the video recording. Rat movement was tracked by selecting one pixel every fifth frame that corresponded to the midline of the rat's body. The resulting x- and y-coordinates of the rat's movement on the exploration arena were used to analyze movements. Four consecutive five-minute samples of behavior were used for analysis two minutes after a rat was placed on the center of the table. The two-minute delay was selected based on work demonstrating animals exhibit markers of home base establishment during this time (Blankenship et al., 2017). If a rat fell off the table, the two-minute delay was applied. Measures of movement organization were analyzed across the twenty-minute sample.

General measures of movement evaluated included average velocity, total distance traveled, and total average stop time. Progressions are relatively fast segments of movement greater than the average velocity of the individual rat for greater than two frames. Stops are relatively slow segments of movement less than the average velocity of the individual rat for greater than two samples. These measures are indicative of general locomotion measures such as hyperactivity or motivation and not differences in self-movement cue processing (Briegleb et al., 2004; Christmas & Maxwell, 1970; Donaldson et al., 2019).

Specific measures of movement evaluated kinematic and topographic differences in progressions and stops. Specific progression measures included average path circuity and average movement scaling. Path circuity was calculated by dividing the Euclidean (shortest) distance of a progression by the actual path taken. Path circuity values range from zero to one, with a higher value indicating a more direct progression. Movement scaling was calculated by measuring the correlation of peak speed to Euclidean distance. Values range from zero to one, with higher values indicating higher scaling of peak speeds to progression lengths. Previous

work has demonstrated that path circuity and movement scaling are disrupted in animal models of vestibular pathology (Blankenship et al., 2017; Donaldson et al., 2018). Control animals typically take non-circuitous progressions, with the progression peak speed increasing as the progression becomes longer.

Specific stop measures included stop cluster analysis and average change in heading. Circular statistics were used to quantify density of stop clustering. Each second of a stop represented a single observation and was converted from Cartesian coordinates (x-,y-) into polar coordinates (theta, r) (Figure 2). The heading direction of each stop (theta) relative to the edge of the exploration arena was used for several characteristics of stop cluster analysis to evaluate the stability and location of stops. Average within sample stop clustering analysis was calculated from the average stop frequency across samples. Average within sample stop clustering values range from zero to one, with higher values indicating concentrated stopping within each sample. Between sample stop clustering analysis was calculated from each sample's average heading to indicate home base stability. Between sample stop clustering values range from zero to one, with higher values indicating concentrated stopping across time. Average change in heading was calculated by the supplementary angle subtended by the preceding progression peak speed location, average stop location, and subsequent progression peak speed location. Average change in heading values range from zero to 180, with higher values indicating larger changes in heading. Previous work has demonstrated that stop clustering and change in heading are disrupted in animal models of vestibular pathology (Blankenship et al., 2017; Donaldson et al., 2018). Control animals typically have concentrated stop clustering and small changes in heading.

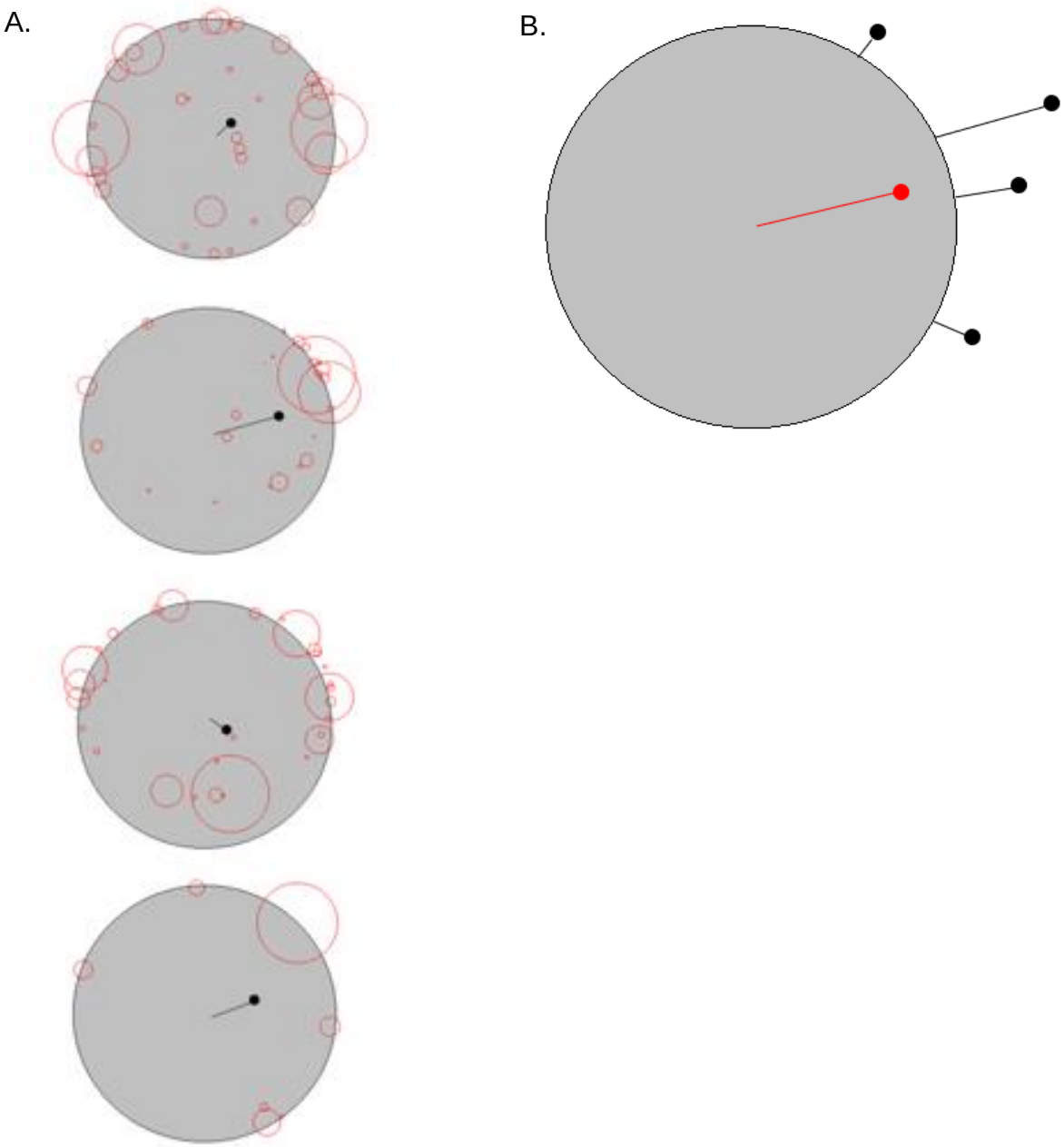

Figure 2: Representative within sample stop clustering plotted across four five-minute samples for a representative sham rat with average polar heading direction indicated (A). Between sample stop clustering is plotted for the same representative sham rat with polar heading direction indicated to illustrate home base establishment (B).

Two-way ANOVAs were used to evaluate the main effects and interactions of lesion and sex with an alpha set at 0.05. Partial eta squared (η^2_p) values were reported for each main effect and interaction effect, as a measure of effect size. Post hoc analyses used the Tukey HSD with Cohen's D as a measure of effect size. JASP 0.12.2 (University of Amsterdam) was used to calculate statistical results.

Histology

After completing behavioral testing, rats were deeply anesthetized with 0.4-0.6mg of phenobarbital. After deep sedation, rats were perfused through the right cardiac ascending aorta with 200ml of phosphate buffered saline followed by infusion of 200ml of 4% paraformaldehyde solution. Brains were extracted and stored in a 30% sucrose solution at approximately -14°C. Brains were frozen at approximately -29°C in a cryostat and coronally sliced into 40 micrometer sections from the emergence of the medial septum to the ventral hippocampus. Every third section was mounted onto chromalum subbed slides. Slices were processed for acetylcholinesterase, a reliable marker to infer hippocampal cholinergic function (Figure 3). (Hoover, Muth, & Jacobowitz, 1978).

Microscopic images of full brains and the left and right dorsal hippocampus and cortex were taken for each rat with a color digital camera (Penguin 600 CL, Pixera Corporation, USA) attached to a microscope (Olympus BH2-RFCA, Olympus America Inc., USA). Color digital photographs were transformed into gray scale. Digital photographic files were opened with Scion

Image for Windows (Scion Corporation, USA, freely available on the internet at http://www.scioncorp.com). Optical density values in both hemispheres of hippocampal areas (CA1, CA3, dorsal dentate gyrus, ventral dentate gyrus) and cortical areas (motor cortex, dysgranular cortex, granular cortex) were obtained. Optical density values were obtained from the corpus callosum to control for background differences in stain density (Figure 4).

Prusky-Sutherland Acetylcholinesterase Stain Protocol

1. Add 6.24mg of Tetraisopropyl pyrophosphoramide in 200ml of distilled water. Stir until dissolved.
2. Place slides in tray in the Tetraisopropyl pyrophosphoramide solution and stir lightly on a belly dancer shaker for 30 minutes.
3. Reaction mixture
    a. Add chemicals to 200ml of distilled water and stir lightly until dissolved:
        i. 5,720mg Trizma maleate buffer
        ii. 2,040mg Trizma base
        iii. 100mg Acetylthiocholine iodide
        iv. 294mg Sodium citrate
        v. 150mg Cupric citrate
        vi. 32.8mg Potassium hexacyanoferrate(III)
4. Remove slides in tray from Tetraisopropyl pyrophosphoramide solution.
5. Gently submerge slides in tray twice in distilled water.
6. Place slides in tray in reaction mixture and stir lightly on a belly dancer shaker for four hours.
7. Gently submerge slides in tray twice in distilled water.
8. Remove slides from tray and lay flat to dry.
9. Coverslip the following day.

Figure 3: The Prusky-Sutherland acetylcholinesterase stain protocol modified for picric-acid-fixed tissue mounted on chromalum slides.

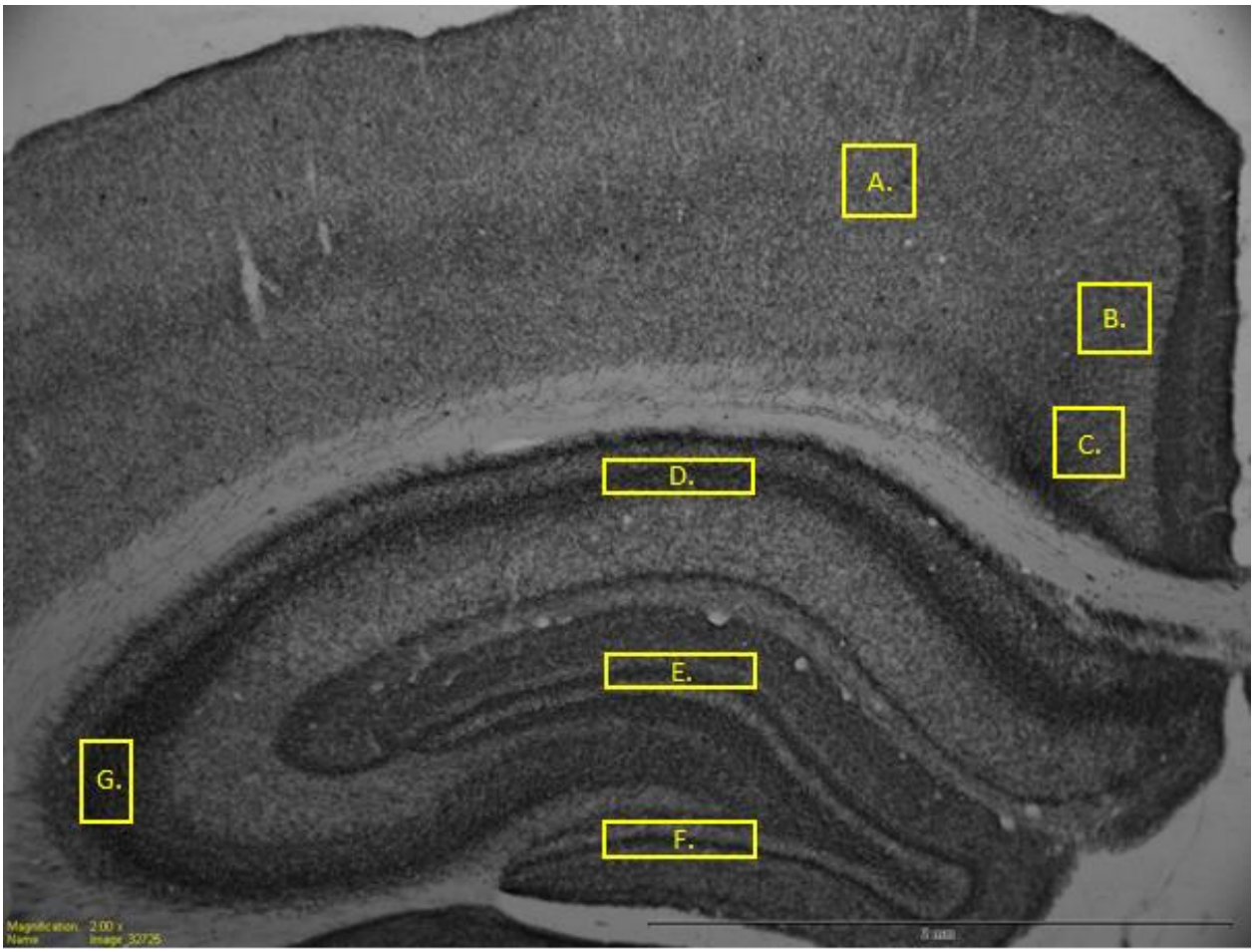

Figure 4: The cortical and hippocampal subregions sampled for optical densities (displayed by yellow boxes). Scion Image for Windows (Scion Corporation, USA) was used to obtain three 40x40 pixel values in each region per hemisphere. (A. motor cortex, B. dysgranular cortex, C. granular cortex, D. CA1, E. dorsal dentate, F. ventral dentate, G. CA3).

Optical density values were measured by taking the average number of three gray-scaled pixel values at 40x40 scale using Scion Image for Windows (Scion Corporation, USA). Scion Image^{TIM} follows IMB PC convention and displays zero pixels as black and those with a value of

255 as white. The average corpus callosum value was subtracted from each hippocampal and cortical area to control for background levels of acetylcholinesterase staining and subtracted from subregion average optical density values. All average optical density values were transformed to 100 above their raw values. Higher optical density values indicate greater cholinergic deafferentation. Lower optical density values indicate greater cholinergic density. Optical density values were analyzed using one-way ANOVAs.

Data Analysis

Two-way ANOVAs were used to evaluate the effects of lesion and sex on movement organization. One-way ANOVAs were used to evaluate the effects of 192 IgG-saporin on cholinergic neurons of the hippocampus and cortex between complete, unilateral, and sham lesions.

CHAPTER 4

RESULTS

Histology

Acetylcholinesterase staining revealed that infusion of 192 IgG-saporin produced complete lesions and unilateral lesions (Figure 5). A unilateral lesion was defined by one hippocampi optical density value falling within ± two standard deviations of sham lesion optical density value, and the other hippocampi optical density value falling within ± two standard deviations of complete lesion optical density values. Therefore, three lesion groups were evaluated: sham (female n=10, male n=9), unilateral (female n=9, male n=11), and complete (female n=5, male n=6). The ANOVA conducted on optical densities revealed significant differences in optical densities between lesion groups in cortical (average cortex, motor cortex, dysgranular cortex, granular cortex) and hippocampal (average hippocampus, CA1, dorsal dentate, ventral dentate) areas (Table 1). In general, the ANOVA conducted on average optical densities from hippocampal and cortical areas revealed a significant effect of Group; however, neither the effect of Sex nor the Group by Sex interaction was significant (Table 1).

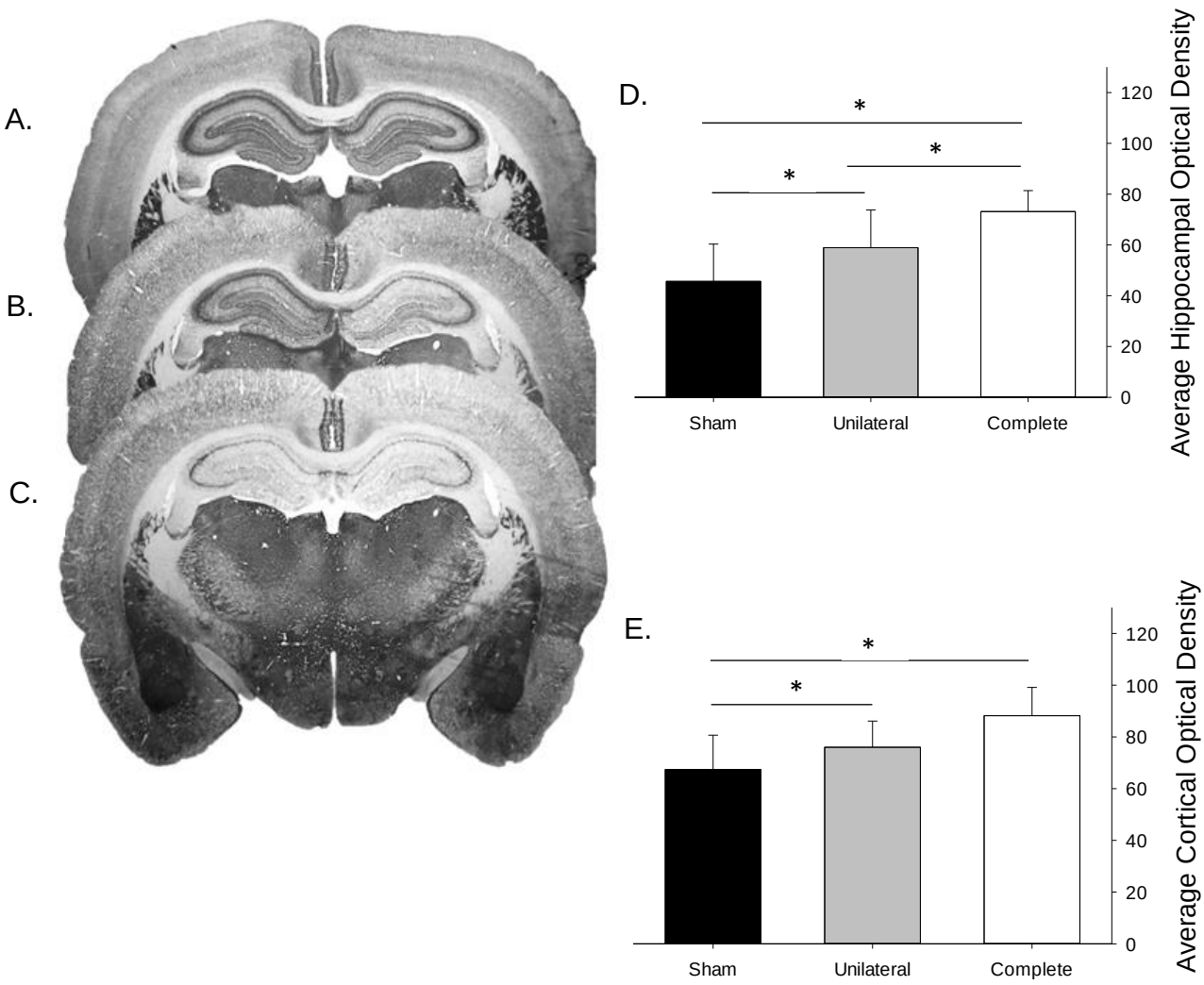

Figure 5: Representative histological photographs for a sham (A), unilateral (B), and complete (C) lesion brain. Average optical density values are plotted for hippocampal subregions (D; CA1, dorsal dentate, ventral dentate), and cortical subregions (E; motor cortex, dysgranular cortex, granular cortex). A scale transformation of 100 was added to all raw values. No sex differences were observed in optical densities. Graphs represent combined female and male values. Higher values indicate greater cholinergic deafferentation.

Table 1: Optical Densities Between Sex and Lesion Group

		df	F	p	η^2p
Average Hippocampus					
	Sex	1,44	0.117	0.734	0.003
	Group	2,44	14.348	<.001	0.395
	Sex x Group	2,44	0.975	0.385	0.042
CA3					
	Sex	1,44	0.341	0.562	0.008
	Group	2,44	1.439	0.248	0.061
	Sex x Group	2,44	0.863	0.429	0.038
CA1					
	Sex	1,44	0.013	0.908	<.001
	Group	2,44	11.621	<.001	0.346
	Sex x Group	2,44	1.299	0.283	0.056
Dorsal Dentate					
	Sex	1,44	0.218	0.643	0.005
	Group	2,44	13.454	<.001	0.379
	Sex x Group	2,44	0.676	0.514	0.030
Ventral Dentate					
	Sex	1,44	0.445	0.508	0.010
	Group	2,44	14.787	<.001	0.402
	Sex x Group	2,44	0.756	0.476	0.033
Average Cortex					
	Sex	1,44	0.054	0.818	0.001
	Group	2,44	10.818	<.001	0.330
	Sex x Group	2,44	0.897	0.415	0.039
Motor Cortex					
	Sex	1,44	0.002	0.967	<.001
	Group	2,44	3.541	0.037	0.139
	Sex x Group	2,44	0.939	0.399	0.041

(Table 1 continued on next page)

(Table 1 continued)

		df	F	p	η^2p
Dysgranular Cortex					
	Sex	1,44	0.560	0.458	0.013
	Group	2,44	12.383	<.001	0.360
	Sex x Group	2,44	0.932	0.401	0.041
Granular Cortex	Sex	1,44	0.002	0.962	<.001
	Group	2,44	8.501	<.001	0.279
	Sex x Group	2,44	0.219	0.804	0.010

The CA3 region of the hippocampus was an exception with no significant effect of Group; however, this is likely due to the large variability in optical density values. Therefore, the CA3 region was not included in average hippocampal analyses. Post hoc analyses revealed that rats with complete lesions had significantly higher optical density values compared to sham lesions. Higher optical density values indicate greater deafferentation. Post hoc analyses revealed that rats with complete hippocampal lesions had significant cholinergic deafferentation (51.795%) compared to sham hippocampal lesions (Tukey HSD, p<.001, *d* = 2.164) and significant cholinergic deafferentation (24.120%) compared to unilateral hippocampal lesions (Tukey HSD, p = .024, *d* = 1.096). Additionally, rats with unilateral hippocampal lesions had significant cholinergic deafferentation (22.297%) compared to sham hippocampal lesions (Tukey HSD, p = 0.11, *d* = -.913). Post hoc analyses revealed that rats with complete cortical lesions had significant cholinergic deafferentation (27.324%) compared to sham cortical lesions (Tukey HSD, p<.001, *d* = 1.659) and significant cholinergic deafferentation (16.020%) compared to unilateral cortical lesions (Tukey HSD, p = .024, *d* = 1.162). Rats with unilateral cortical lesions did not have significantly different cholinergic deafferentation (9.743%) compared to sham

cortical lesions (Tukey HSD, p = 0.70, *d* = -.731). Overall, rats with complete lesions had significantly greater cholinergic deafferentation compared to unilateral and sham rats in average hippocampal and cortical regions (see Figure 5). These results indicate that 192 IgG-saporin produced effective hippocampal cholinergic deafferentation, whereas sham lesions produced with saline spared hippocampal cholinergic function.

Sequential Exploratory Behavior Under Dark Conditions

General Behavior

Several measures were used to characterize general aspects of sequential exploratory behavior under dark conditions (Figure 6). Overall, there were no effects of Group, Sex, or Group by Sex interactions on general behavior. No group differences were observed in average velocity. The ANOVA conducted on average velocity failed to reveal a significant effect of Group, Sex, or Group by Sex interaction (Table 2). Further, no group differences were observed in total distance traveled. The ANOVA conducted on total distance traveled failed to reveal a significant effect of Group, Sex, or Group by Sex interaction (Table 2). Finally, no group differences were observed in total average stop time. The ANOVA conducted on total average stop time failed to reveal a significant effect of Group, Sex, or Group by Sex interaction (Table 2). Finally, no group differences were observed in total average stop time. The ANOVA conducted on total average stop time failed to reveal a significant effect of Group, Sex, or Group

by Sex interaction.(Table2). No group differences or interactions in general measures of behavior indicate that all rats demonstrated similar levels of locomotion.

Specific Behavior

Progressions. Several measures of progressions were used to characterize topographic and kinematic aspects of sequential exploratory behavior under dark conditions (Figure 7). Overall, there were no effects of Group, Sex, or Group by Sex interactions on specific measures of progressions. The ANOVA conducted on average path circuity failed to reveal a significant effect of Group, Sex, or Group by Sex interaction (see Table 2). Further, groups did not differ in average movement scaling. The ANOVA conducted on average movement scaling failed to reveal a significant effect of Group, Sex, or Group by Sex interaction (see Table 2).

Stops. Several measures of stops were used to characterize specific aspects of sequential exploratory behavior under dark conditions (Figure 8). Overall, there were no effects of Group, Sex, or Group by Sex interactions on stopping behavior. First, all rats exhibited stop clustering consistent with home base establishment (see Table 2). The ANOVA conducted on average within sample stop clustering failed to reveal a significant effect of Group, Sex, or Group by Sex interaction. Further, the ANOVA conducted on between samples stop clustering, or home base stability, failed to reveal a significant effect of Group, Sex, or Group by Sex interaction. Finally, the ANOVA conducted on average change in heading failed to reveal a significant effect of Group, Sex, or Group by Sex interaction (see Table 2). The next chapter will discuss potential explanations for the current results.

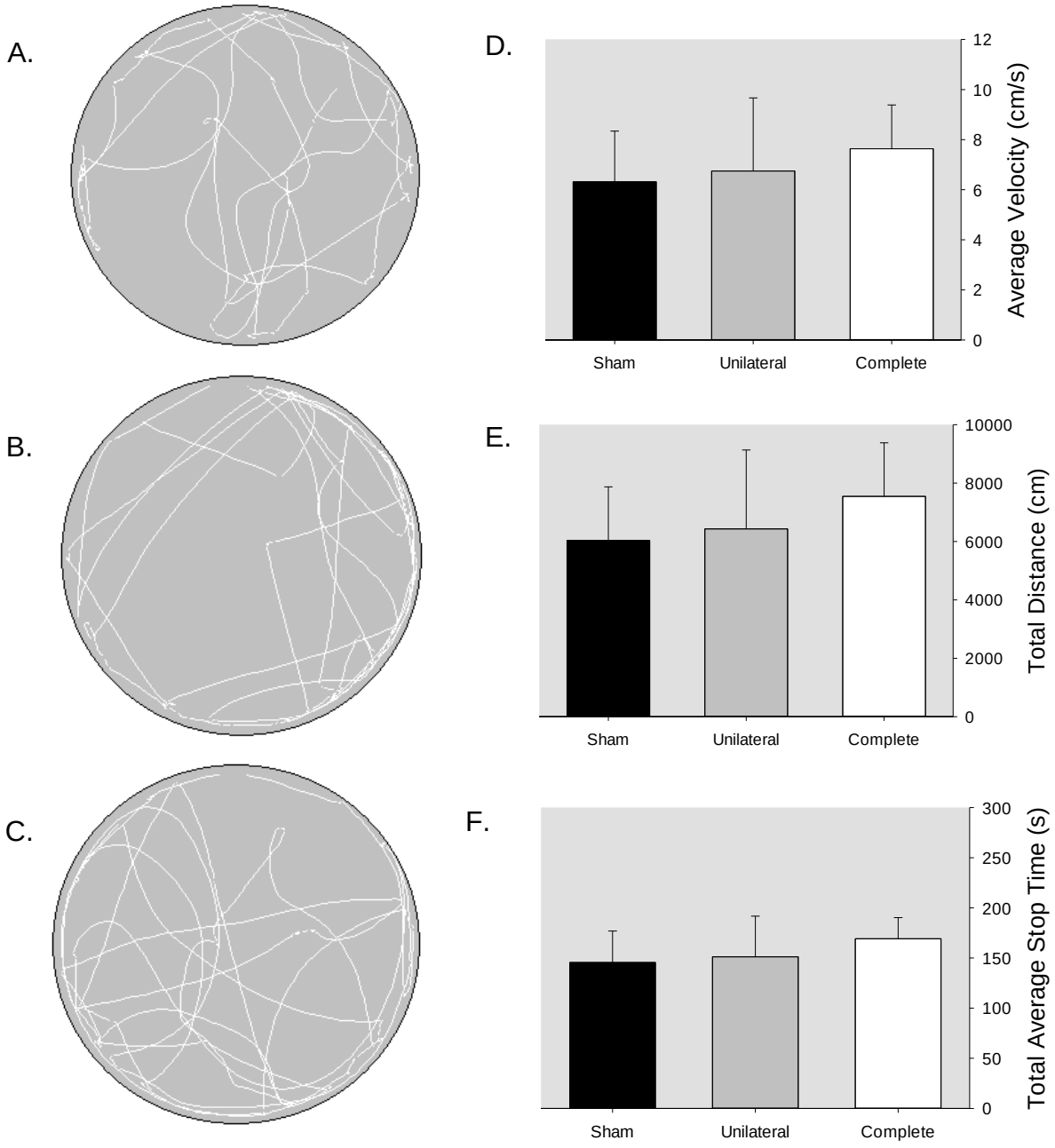

Figure 6: Representative path progressions for a sham (A), unilateral (B), and complete lesion (C) rat across a five-minute sample. General measures of behavior are graphed between lesion groups (C, D, E).

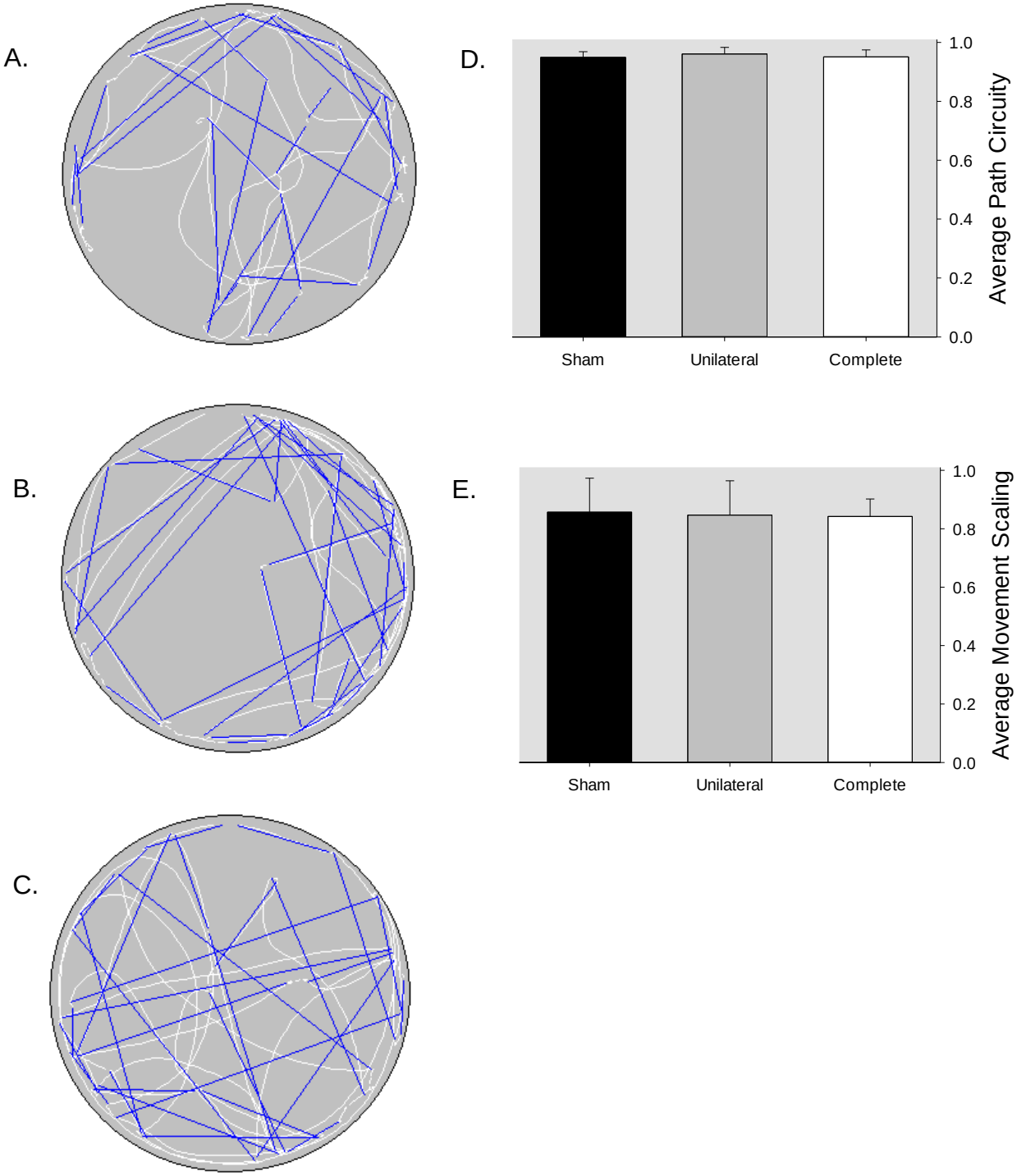

Figure 7: Representative path progressions (white) and Euclidean paths (blue) for a sham (A), unilateral (B), and complete lesion (C) rat across a five-minute sample. Specific measures of progressions are graphed between groups (D, E).

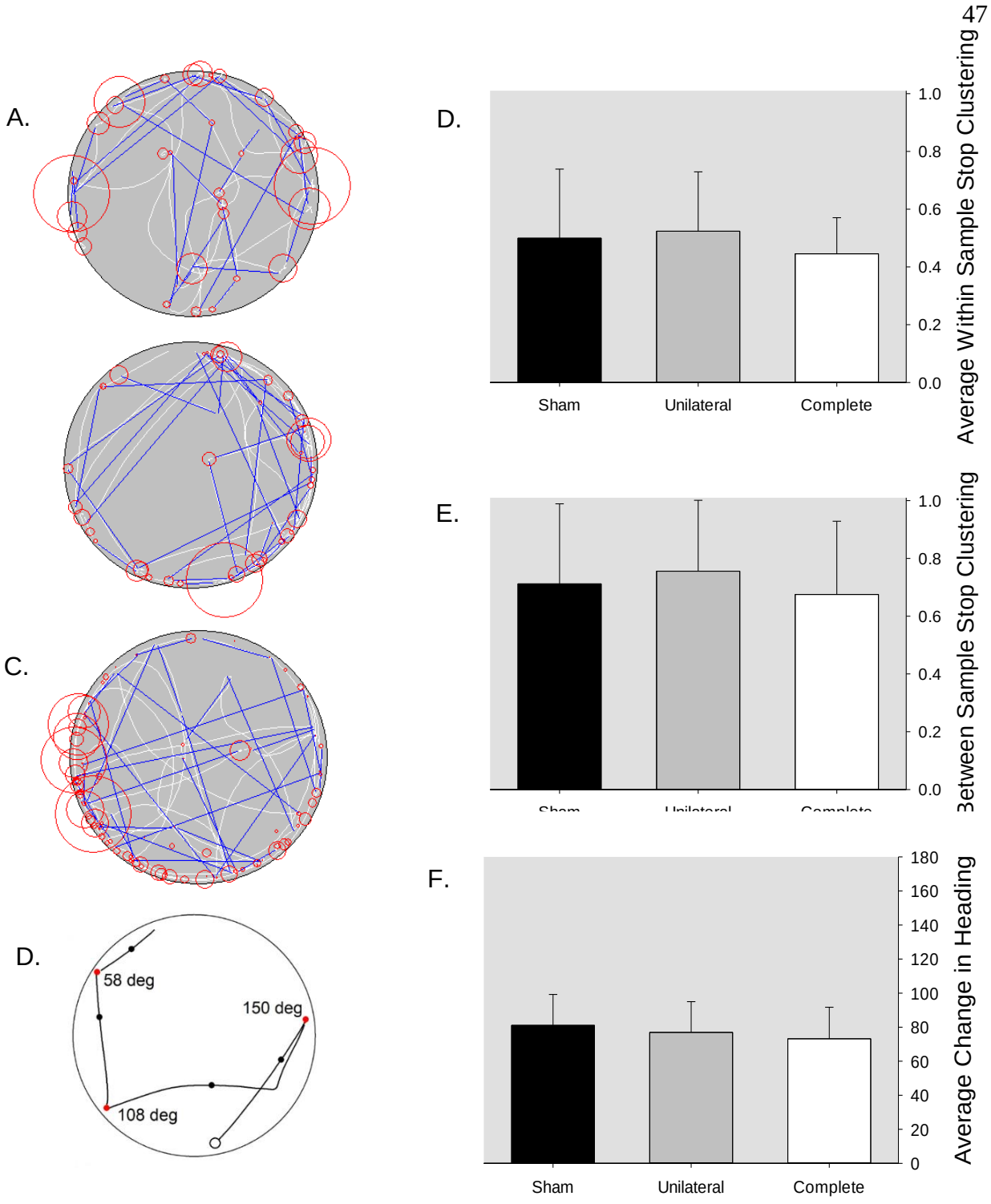

Figure 8: Representative path progressions (white), Euclidean paths (blue), and stops (red) for a sham (A), unilateral (B), and complete lesion (C) rat across a five minute sample. A representation of change in heading is plotted (D). Increased diameter of red circles indicates increased stop time. Specific measures of stops are graphed between groups (D, E, F).

Table 2*:* Sequential Exploratory Behaviors Under Dark Conditions Between Sex and Lesion Group

		df	F	p	η^2p
Average Velocity					
	Sex	1,44	1.260	0.268	0.028
	Group	2,44	1.119	0.336	0.048
	Sex x Group	2,44	1.088	0.346	0.047
Total Distance					
	Sex	1,44	1.276	0.265	0.028
	Group	2,44	1.674	0.199	0.071
	Sex x Group	2,44	1.202	0.310	0.052
Total Avg. Stop Time					
	Sex	1,44	0.077	0.783	0.002
	Group	2,44	1.577	0.218	0.067
	Sex x Group	2,44	0.296	0.745	0.013
Avg. Path Circuity					
	Sex	1,44	0.169	0.683	0.004
	Group	2,44	1.413	0.254	0.060
	Sex x Group	2,44	0.608	0.549	0.027
Avg. Movement Scaling					
	Sex	1,44	0.088	0.769	0.002
	Group	2,44	0.087	0.917	0.004
	Sex x Group	2,44	1.050	0.358	0.046
Avg. Within Sample Stop Clustering					
	Sex	1,44	3.753	0.059	0.079
	Group	2,44	0.524	0.596	0.023
	Sex x Group	2,44	0.608	0.549	0.027
Between Sample Stop Clustering					
	Sex	1,44	3.587	0.065	0.075
	Group	2,44	0.307	0.737	0.014
	Sex x Group	2,44	0.630	0.538	0.028
Avg. Change in Heading					
	Sex	1,44	1.507	0.226	0.033
	Group	2,44	0.845	0.432	0.037
	Sex x Group	2,44	1.591	0.215	0.067

CHAPTER 5

DISCUSSION

The current study investigated the effects of 192 IgG-saporin infused into the medial septum on the sequential organization of exploratory behavior under dark conditions and sexual dimorphisms in movement. Significant variability was observed in optical density values which prompted an analysis of three lesion groups: sham, unilateral, and complete. There was no effect of lesion on sequential exploratory behavior under dark conditions between lesion groups. It is possible that the level of analysis combined with environmental structure may have limited the ability to detect hippocampal cholinergic pathology. Alternatively, it is possible that the sparing of downstream neural structures, such as the vestibular system, may have been sufficient to support the organization of sequential exploratory behavior under dark conditions. Additionally, there was no significant difference in optical density values between female and male rats. Further, there was no effect of lesion on sequential exploratory behavior under dark conditions between female and male rats. These results suggest that factors other than self-movement cue processing may be driving commonly observed superior male performance in spatial tasks. The following sections will evaluate the relationship between sex, cholinergic function, the environmental structure, and neural circuits on exploratory behavior.

Sexual Dimorphisms and Exploratory Behavior

A substantial amount of evidence supports that there are sexual dimorphisms observed in performance on a variety of spatial tasks. For example, male animals ranging from rats to humans consistently demonstrate superior performance in navigational tasks such as the physical and virtual MWM (Andreano & Cahill, 2009). The basis for these sexual dimorphisms in performance remains debated. The possibility of emotion driving sexual dimorphisms in spatial task performance has been evaluated. Compared to males, females have higher basal serum corticosterone levels. Additionally, an inverse relationship between corticosterone levels and MWM performance in female rats has been observed. Studies that have controlled for stress levels in female and male rats through significant pretraining (Anderson et al., 2013) or adrenalectomies (Beiko et al., 2004) have eliminated sexual dimorphisms in spatial task performance in the MWM. This suggests that stress has a more substantial effect on spatial performance in females compared to males. Additionally, spatial learning strategies have been attributed to sexual dimorphisms in spatial task performance. Specifically, females more frequently report using an egocentric strategy (e.g., left or right) when learning an environment, as compared to males who more frequently report using an allocentric strategy (e.g., north, south, east, or west; Lawton et al., 1994). Though there is a sexual dimorphism in strategy preference, female and male rat performance in the MWM is equivalent when trained using an egocentric strategy, but male rats perform superior when using a place, or allocentric strategy (Blokland, Rutten & Prickaerts, 2006). Therefore, it appears that spatial learning strategy and emotional factors influence sexual dimorphisms in spatial task performance; however, limited work has

investigated whether sexual dimorphisms persist when rats are limited to using self-movement cues.

Dissociating environmental from self-movement cues has provided insight into navigational strategies that may mediate sexual dimorphisms in spatial task performance. When rats were limited to using self-movement cues during a food hoarding task, sexually dimorphic performance was no longer observed in measures of spatial orientation (Köppen et al., 2015). Specifically, female and male rats demonstrated no differences in heading error or movement scaling when returning to a refuge, and this performance did not vary across the estrous cycle. However, female rats returned to the refuge faster than male rats upon finding a food pellet. These results indicate that self-movement cue processing may not explain sexual dimorphisms in other spatial task performance, but general locomotion may differ between males and females.

The current study did not provide evidence of sexual dimorphisms in the organization of sequential exploratory behavior under dark conditions. No sexual dimorphisms were observed in general measures of movement organization, such as average velocity, total distance traveled, or total average stop time. This is inconsistent with previous work suggesting that females are typically more active in open-field tasks than males (Blizard, Lippman, & Chen, 1975); however, this increase in female locomotion has been attributed to higher levels of anxiety. Previous work has demonstrated that anxiolytics resulted in decreased general locomotion but did not decrease the amount of time an animal spent exploring a hole board (Crawley, 1985), suggesting that general locomotion can be dissociated from exploratory behavior. It is possible that the lack of aversive light in the current study attenuated the stress response of increased locomotion generally seen in open-field behavior. However, sexual dimorphisms were observed in general

measures of locomotion under dark conditions in the food hoarding task (Köppen et al., 2015). These differences in findings between the current study and previous work using the food hoarding task may be mediated by motivational factors. Specifically, food hoarding uses a refuge and food pellets, but sequential exploration under dark conditions does not include these factors. Previous work has demonstrated that female rats hoard more food than male rats (Marx, 1950) and have greater locomotor activity in the open field relative to male rats (Archer, 1964). Therefore, it is possible that the current study's lack of observed sexual dimorphisms in general measures of movement organization was driven by a lack of food-based motivation. Though the current study did not demonstrate sexual dimorphisms in general behavior, the lack of differences observed in specific behavior is consistent with previous work (Köppen et al., 2015). These results demonstrate that females and males process self-movement cues similarly, thus, self-movement cue processing is likely not driving sexually dimorphic performance observed in other spatial tasks.

Cholinergic Function and Exploratory Behavior

Hippocampal cholinergic function has been implicated in organizing exploratory behavior. Substantial work has demonstrated that damage to this system results in disrupted movement organization when animals rely on self-movement cue processing. Specifically, rats with damage to this system have more circuitous returns to a refuge compared to control rats under dark, but not light, conditions (Martin et al., 2007; Martin & Wallace, 2007; Whishaw &

Wallace, 2003). These studies have provided evidence that the hippocampal cholinergic system is involved in self-movement cue processing. Specifically, the ability to accurately estimate distance and direction to return to a refuge during single trip exploration under dark conditions is impaired in rats with hippocampal cholinergic deafferentation.

Interestingly, the current study did not demonstrate that hippocampal cholinergic deafferentation influenced the organization of sequential exploratory behavior under dark conditions. First, all rats demonstrated organized sequential exploratory behavior. When introduced into the novel environment, all rats spontaneously explored the arena. This sequential exploration was organized into a series of stops and progressions. Analysis of general measures of stops and progressions indicated that rats traveled similar distances and stopped for similar durations. These results demonstrate that rats exhibited similar levels of locomotion. Second, rats in all groups were able to establish a home base. Stop cluster analysis revealed that rats made frequent stops for longer durations in consistent locations. In contrast, other stops in the environment were more disperse and lasted for shorter durations. The consistency and duration of stops in clustered locations across all lesion groups demonstrate that hippocampal cholinergic deafferentation did not disrupt the ability of rats to establish a home base. Additionally, all rats demonstrated low changes in heading between stops. Third, all rats demonstrated similar progression behavior. Analysis of specific progression measures demonstrate that rats exhibited similar path circuity and movement scaling. All rats had high values for path circuity, indicating that rats followed non-circuitous paths throughout their progressions. All rats also had high values for movement scaling, demonstrating that rats scaled their faster progressions with their longer travel distances. Rats in all lesion groups demonstrated high levels of performance in

specific measures of movement organization. These results indicate that all rats were able to accurately estimate distance and direction moved in sequential exploration under dark conditions.

Maintaining spatial orientation during sequential exploration under dark conditions is reliant on the ability to accurately process self-movement cues. Specifically, estimating direction and distance moved allows animals to continuously update their representation of position in space. The cognitive demands imposed by sequential exploration under dark conditions is prone to an accumulation of errors in estimating direction and distance moved. To reduce these errors, animals make frequent returns to the home base in order to reset their representation of position in space (Valerio & Taube, 2012; Wallace, Martin, & Winter, 2008). The ability for rats across all lesion groups in the current study to establish a stable home base may have supported their ability to accurately detect changes in direction and distance moved as they explored the environment.

All groups had relatively direct progressions throughout sequential exploration under dark conditions. Rats across groups had high average path circuity values. As average path circuity values approach 1, the path traveled is more direct. Additionally, all groups had relatively small changes in heading. Rats across groups had low average change in heading values. Values approaching 180 represent a complete reversal in heading direction, and values near 0 represent a maintained heading direction. These observations in behavior are consistent with accurate direction estimation and maintained spatial orientation. Previous work has demonstrated that humans take increasingly circuitous or spiraling paths the longer they had been traveling without access to environmental cues (Schaeffer, 1928). Additionally, humans

exhibited a similar spiraling tendency when they were navigating in deserts and dense forests without access to distinct environmental cues (Souman et al., 2009). Further, humans demonstrated an impaired ability to estimate direction from the point of movement origin when their paths were more circuitous (Wallace et al., 2006). These results indicate that animals across species exhibit similar behavior for direction estimation. All groups accurately scaled their peak speeds with Euclidean distance length traveled. As average movement scaling values approach 1, the scaling of peak speed to Euclidean distance traveled is more consistent. Rats across groups had high average movement scaling values. Parallel results have been observed in human work that demonstrated participants accurately scaled their peak speed to Euclidean distance traveled in planar reaching tasks (Gordon et a., 1994). These observations provide evidence that animals across species demonstrate similar behavior when making distance estimations. Overall, the current study demonstrated that rats in all groups performed well in measures related to direction and distance estimation.

The current study's findings that rats in all groups demonstrated organized sequential exploratory behavior under dark conditions is inconsistent with previous research. Previous work has demonstrated that hippocampal cholinergic deafferentation resulted in disrupted movement organization for direction and distance estimation (Martin & Wallace, 2007; Whishaw & Wallace, 2003). To further investigate the influence of hippocampal cholinergic deafferentation on movement organization, regression analyses were performed. A linear regression analysis revealed that average hippocampal cholinergic optical density values did not predict total distance traveled, average path circuity, average movement scaling, average change in heading, or average within sample stop clustering. However, average hippocampal cholinergic optical

density values did significantly predict between sample stop clustering (Table 3). Specifically, the greater the cholinergic deafferentation, the less stable the home base. The significant result for between sample stop clustering should be qualified with the violation of linear model assumptions, making these results unlikely to be meaningful. Therefore, it is important to consider why hippocampal cholinergic deafferentation did not predict movement organization as observed in previous work. The first plausible explanation may have to do with the environmental structures of the task. Specifically, previous work demonstrating a relationship between hippocampal cholinergic function and exploratory behavior has used the presence of a refuge and/or a food pellet, unlike the current task. A second plausible explanation is that neurological structures involved in spatial orientation differentially contribute to aspects of self-movement cue processing. The following sections will examine these possibilities.

Table 3: Regression Analyses of Hippocampal Optical Density and Sequential Exploratory Behavior Under Dark Conditions

	R	R^2	df	F	p
Total Distance	0.206	0.043	1,49	2.133	0.151
Avg. Path Circuity	0.220	0.049	1,49	2.449	0.124
Avg. Movement Scaling	0.078	0.006	1,49	0.292	0.591
Avg. Change in Heading	0.189	0.036	1,49	1.777	0.189
Avg. Within Sample Stop Clustering	0.190	0.036	1,49	1.797	0.186
Between Sample Stop Clustering	0.294	0.087	1,49	4.548	0.038

Environmental Structure and Exploratory Behavior

The structure of an environment influences exploratory behavior. For example, the size, openness, and stimuli in an environment have differential effects on movement. It is possible that environmental structure may mediate exploratory behavior differentially through motivational factors. The influence of motivation on exploratory behavior is currently unclear; however, the results of the current study compared to previous work may contribute to an improved understanding. Two major differences should be considered between the environmental structure of the current study and previous work using food hoarding or single trip exploration. First, food hoarding incorporates the use of food pellets. Second, both food hoarding and single trip exploration incorporate the use of a refuge. It is possible that these differences in environmental structure may influence the motivational demands of rodents in spatial tasks. Differences in motivational demands may contribute to the ability to detect impaired processing of self-movement cues.

The food motivation component of food hoarding may contribute to the ability of this task to detect hippocampal cholinergic deafferentation. In the food hoarding task, rodents leave a refuge to search an arena for a food pellet, then return with the food pellet to the refuge for consumption. Rats with hippocampal cholinergic deafferentation exhibit increased path circuity when returning to a refuge with a food pellet under dark conditions in the food hoarding task (Martin & Wallace, 2007). This suggests that rats with hippocampal cholinergic deafferentation may not be able to maintain an accurate representation of position in space relative to a home base. Previous work with honeybees has demonstrated that food motivation can reset the

representation of a home base location (Dyer, Gill, & Sharbowski, 2002). When honeybees were captured on an exploratory trip to search for food, then released from a novel location, they accurately flew back to their hive. In contrast, when food-deprived honeybees were released after capture, they did not fly back to their hive. Instead, food-deprived honeybees flew in a path that would be taken to reach a food source as if they had been released from their hive, while seemingly ignoring familiar landmarks. This suggests that food-deprived honeybees had reset the representation of their home base from the hive to the captured location. It is possible that the food motivation component of food hoarding attenuated the ability of rats with hippocampal cholinergic deafferentation to maintain an accurate representation of position in space relative to the refuge. However, this is unlikely, as similar disruptions in performance have been observed in rats with hippocampal cholinergic deafferentation in single trip exploration.

Single trip exploration does not involve food motivation; however, the physical refuge may contribute to detecting hippocampal cholinergic deafferentation. Single trip exploration consists of a rat leaving a refuge, exploring the environment, and returning to the refuge. As observed in food hoarding, rats with hippocampal cholinergic deafferentation exhibited increased path circuity when returning to a refuge under dark conditions (Martin et al., 2007). Motivation to use the refuge for safety may mediate the ability to detect hippocampal cholinergic deafferentation. Previous work has demonstrated that the safety afforded by a refuge influences the likelihood that a rat will explore its environment (Whishaw et al., 2006). For example, rats placed in a small refuge were less likely and more hesitant to leave and explore an open environment compared to rats placed in an open home cage. This pattern of exploratory behavior is dependent on the structure of the refuge and is motivated by the rodent minimizing risk. The

motivation to use the refuge as a home base in food hoarding and single trip exploratory tasks may have contributed to an ability of these tasks to detect impaired self-movement cue processing associated with hippocampal cholinergic deafferentation. However, this explanation for task differences does not fit with the observations of the current study. Specifically, without access to a physical home base, all rats in the current study established stable home bases and organized their movements around these locations. Differences in motivational demands between dead reckoning tasks are not likely limiting factors in detecting impairments in self-movement cue processing.

The accurate performance of rats in all groups in the current study introduces the possibility that sequential exploration under dark conditions is not dependent on self-movement cue processing. Rats across all groups exhibited consistent stop clustering, took non-circuitous progressions, scaled peak speeds to progression lengths, and had low changes in heading. These patterns of movement organization are consistent with the ability to establish a home base and accurately process self-movement cues. Though the current study did not detect deficits in self-movement cue processing in rats with hippocampal cholinergic deafferentation, it is not likely that this task is not dependent on self-movement cue processing. Previous work has demonstrated that this task, including variations of this task involving a tab, is sensitive in detecting genetic and acquired vestibular pathology in mice (Banovetz et al., under review; Blankenship et al., 2017; Donaldson et al., 2018). For example, mice with vestibular pathology exhibited circuitous progressions with large changes in heading during sequential exploration under dark conditions without the use of a refuge. These patterns of movement organization are consistent with impaired self-movement cue processing. It is possible that the ability for this task

to detect deficits in self-movement cue processing depends on general locomotion. Previous work has demonstrated that mice exhibit more progressions than rats in sequential analysis of exploration (Donaldson et al., 2019). Therefore, the current task may be to detect differences in mice due to the ability to analyze a greater amount of progressions than exhibited by rats. It is unlikely that the current task does not depend on the ability to use self-movement cue processing. Instead, it may be possible that the current task is sensitive in detecting different levels of damage that contribute to self-movement cue processing.

Different spatial tasks incorporate the use of environmental structures that may put varying motivational demands on animals. Further work is needed to evaluate how motivation may contribute to sequential exploratory behavior under dark conditions. Additionally, it is important that future work evaluates the sensitivity of specific spatial tasks in detecting different levels of damage to neural systems that support spatial orientation. Understanding how these principles contribute to sequential exploratory behavior under dark conditions will establish a foundation to investigate the neural basis of spatial orientation.

Neural Circuits Supporting Exploratory Behavior

A large network of neural structures have been implicated in self-movement cue processing (Figure 9). Stimulation of the vestibular system results in a significant increase in hippocampal cholinergic activity (Horii et al., 1994). Therefore, damage to the hippocampal cholinergic system was expected to interfere with processing of self-movement cues generated

from the vestibular system. However, measures of self-movement cue processing were spared in the current study. Therefore, it is possible that sequential exploration under dark conditions is sensitive in detecting more downstream systemic disruptions, rather than upstream damage from medial septum cholinergic projections. Downstream regions relative to the medial septum and hippocampus such as the dorsal tegmental nucleus and anterior dorsal nucleus may result in robust self-movement cue processing deficits that otherwise higher levels of damage do not demonstrate. This could be due to the differential contributions to distance and direction estimation provided by varying neural regions. The following section will examine the role of different systems and structures in dead reckoning based navigation.

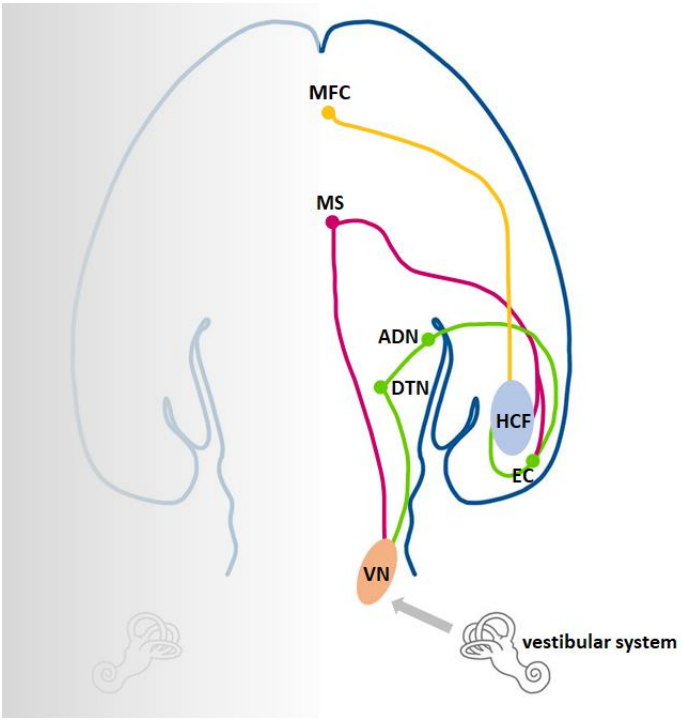

Figure 9: Proposed pathways to the hippocampal formation on a horizontal section of the rat brain. The head direction pathway (green) has connections from the vestibular system to the vestibular nuclei (VN) to the dorsal tegmental nucleus (DTN), anterior dorsal nucleus (ADN), entorhinal cortex (EC), and hippocampal formation (HCF). The medial septum (MS) pathway (pink) has connections to the VN, HCF, and EC. The medial frontal cortex (MFC) has projections (yellow) to the HCF. Figure adapted from Aitken, Zheng, and Smith, (2018).

The vestibular system is highly implicated in spatial orientation. When an animal moves, fluid displaces in a collection of inner ear structures. This displacement triggers cells that provide information to the brain about directional plane and acceleration of movement. When the vestibular system is disrupted through genetic or acquired damage, animals are impaired in receiving information about position in space. Previous work has been able to demonstrate the contribution of the vestibular system to self-movement cue processing. For example, otoconia deficient mice exhibit circuitous progressions and greater variability in stop clustering compared to control mice when evaluated in sequential exploration under dark conditions (Blankenship et al., 2017). Similar findings have been observed in mice with bilateral chemical labyrinthectomies in sequential exploration under dark conditions, specifically, circuitous progressions with large changes in heading (Banovetz et al., under review). Humans with vestibular pathology also exhibit impairments in spatial orientation. Deficits in spatial orientation following vestibular damage may be mediated by the hippocampus, as deficits in the vestibular system disrupt the response of spatially tuned cells in the hippocampus (Stackman & Taube, 1997). However, vestibular damage may result in spatial orientation deficits through more proximal structures to the hippocampus.

Subcortical structures, such as the dorsal tegmental nucleus, are an integral component of the head direction pathway which receives direct projections from the vestibular system. Head direction cells fire in response to the directional heading of an animal. Specifically, the linear and angular velocity produced from head movement triggers the firing of head direction cells (McNaughton et al., 1996). Importantly, head direction cells maintain this firing pattern even when an animal does not have access to environmental cues, demonstrating that head direction

cells are sensitive to self-movement cues. Previous work has demonstrated that *N*-methyl-D-aspartic acid (NMDA) lesions to the dorsal tegmental nucleus resulted in greater heading direction errors when returning to a refuge in a food carrying task under dark conditions compared to conditions where environmental cues were available or restricted (Frohardt, Bassett, & Taube, 2006). These results suggest that the dorsal tegmental nucleus contributes not only to self-movement cue processing, but the ability to compensate with environmental cues due to directional disorientation. Systematically higher lesions to the anterior dorsal nucleus show similar impairment as with dorsal tegmental nucleus lesions; however, these impairments were much more mild. These results suggest that damage to downstream structures from the medial septum with direct projections to the vestibular system result in navigational deficits. Sequential exploration under dark conditions may be specific to detecting self-movement cue processing deficits from more downstream or parallel damage rather than upstream damage (see Figure 8).

Cortical regions that project to the hippocampus, such as the entorhinal cortex, may be sufficient to support self-movement cue processing after infusing 192 IgG-saporin into the medial septum. The entorhinal cortex receives projections from the head direction network and consists of grid cells specifically tuned to environmental and self-movement cues (Moser, Kropff, & Moser, 2008). Grid cells fire in a continuous topographical hexagonal layout as an animal moves throughout an environment, even in the absence of environmental cues (Moser et al., 2017). Previous work has demonstrated that electrolytic lesions to the entorhinal cortex impair measures of distance, but not direction estimation, during a dead reckoning task (Winter et al., 2013). Specifically, rats with electrolytic lesions to the entorhinal cortex did not exhibit their peak speed of movement in the center of a progression as control rats did when returning to

a refuge under dark conditions. However, rats with electrolytic lesions to the entorhinal cortex maintained non-circuitous progressions and low changes in heading when returning to a refuge under dark conditions. These findings demonstrate that there is a dissociation in systems involved in distance and direction estimation when using self-movement cues. Specifically, electrolytic lesions to the entorhinal cortex disrupt distance estimation; NMDA lesions to the hippocampus disrupt direction estimation. These results suggest that damage to higher level neural regions may spare components necessary to maintain spatial orientation when relying on self-movement cues, such as distance or direction estimation. This is further supported by findings that demonstrate damage to neural regions upstream from the hippocampus, such as cortical subregions, appears to spare all aspects of self-movement cue processing.

Cortical areas have been implicated in self-movement cue processing. The medial frontal cortex has several projections to the CA1 region of the hippocampus (Ferino, Thierry, & Glowinski, 1987). Therefore, it is possible that cortical regions contribute to aspects of self-movement cue processing. Imaging work using fMRI has demonstrated that medial frontal cortex and hippocampus activity are associated with performance during a human virtual dead reckoning task (Wolbers et al., 2007). However, aspiration lesion studies in rats have indicated that the medial frontal cortex is not necessary for self-movement cue processing (Blankenship et al., 2016). Specifically, rats with medial frontal cortex aspiration lesions were able to establish a home base and organize their movement around it with similar performance under dark and light conditions. Other cortical lesions studies have also demonstrated that this region is not necessary for self-movement cue processing. Specifically, rats with aspiration lesions to the anterior cingulate cortex were able to make accurate returns to a refuge under dark conditions (Whishaw

& Maaswinkel et al., 2001). Though cortical regions have connections to the hippocampus, it is likely that damage to these areas spare hippocampal input from more downstream neural systems.

It is possible that specific neurotransmitter systems may be involved in and compensate for impaired self-movement cue processing. The septohippocampal GABAergic system has been implicated in self-movement cue processing (Köppen et al., 2013). Septohippocampal GABAergic projections span from the medial septum to the hippocampus and are spared with selective 192 IgG-saporin infusion. However, these projections can be selectively inactivated with GAT1-saporin infusion. Inactivation of these GABAergic neurons with GAT1-saporin lesions leads to greater inhibition of hippocampal pyramidal neurons, and these projections have effects on hippocampal theta (Wu et al., 2000). Previous work has demonstrated that infusing GAT1-saporin into medial septum GABAergic projections to the hippocampus resulted in movement organization differences in food hoarding under dark conditions. Specifically, rats with GABAergic deafferentation had more circuitous paths with larger heading errors when returning with a food pellet to the refuge under dark, but not light, conditions. These results suggest that GABAergic projections are involved in self-movement cue processing. However, it is likely that this lesion model would result in findings similar to the current study, given the similarity in origination from upstream neurological structures into the hippocampus.

It is possible that selective medial septum cholinergic lesions spare specific components necessary for self-movement cue processing. Previous work has demonstrated that inactivating the medial septum cholinergic system with muscimol resulted in grid cells losing their spatial periodicity, whereas head direction cells maintained their selectivity as a rat freely moved in

space (Brandon et al., 2011). The spared head direction cell activation from medial septum deafferentation may provide an animal with enough information to compensate for the loss of accurate grid cell firing. It may be possible that sequential exploration under dark conditions is sensitive in detecting impaired self-movement cue processing when all aspects, such as direction and distance, are disrupted. However, sequential exploration under dark conditions may not be sensitive in detecting navigational impairments when components that support dead reckoning are spared.

CHAPTER 6

CONCLUSION

The current study investigated the effect of 192 IgG-saporin lesions to medial septum cholinergic projections to the hippocampus and sexual dimorphisms on rat sequential exploratory behavior under dark conditions. Neither sex nor hippocampal cholinergic deafferentation had effects on rat sequential exploratory behavior under dark conditions. These results suggest that female and male rats process self-movement cues similarly. Additionally, these results demonstrate that sequential dark exploration may not be sensitive in detecting self-movement cue processing deficits in this lesion model. However, this may be due to a lack of motivational factors or environmental structure during exploration. Future work is needed to investigate how motivational and environmental factors contribute to self-movement cue processing. Additionally, future work is needed to evaluate the neurological levels of damage sequential exploration under dark conditions may be sensitive in detecting. These future directions may help elucidate the role of the medial septum cholinergic projections to the hippocampus in maintaining spatial orientation. Exploring these avenues may contribute to a better understanding of navigational deficits such as wandering in Alzheimer's Disease.

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