HEAD DIRECTION CELL NETWORK AND SPATIAL NAVIGATION: EFFECTS OF SILENCING ANTERODORSAL THALAMIC NEURONS USING DREADDS

by

Brittany Nicole Crafton

A Thesis Submitted to the Faculty of

The Charles E. Schmidt College of Science

in Partial Fulfilment of the Requirements for the Degree of

Master of Arts

Florida Atlantic University

Boca Raton, FL

July 2023

HEAD DIRECTION CELL NETWORK AND SPATIAL NAVIGATION: EFFECTS OF SILENCING ANTERODORSAL THALAMIC NEURONS USING DREADDS

by

Brittany Crafton

This thesis was prepared under the direction of the candidate's thesis advisor, Dr. Robert W. Stackman Jr., Department of Psychology, and has been approved by all members of the supervisory committee. It was submitted to the faculty of the Charles E. Schmidt College of Science and was accepted in partial fulfillment of the requirements for the degree of Master of Arts.

SUPERVISORY COMMITTEE:

Robert W. Stackman Jr., Ph.D
Thesis Advisor

Carmen Varela, Ph.D.

Henriette van Praag, Ph.D.

Alan Kersten, Ph.D.
Chair, Department of Psychology

Valery E. Forbes, Ph.D.
Dean, Charles E. Schmidt College of Science

Robert W. Stackman Jr., Ph.D.
Dean, Graduate College

July 18, 2023
Date

ACKNOWLEDGEMENTS

I would like to offer my appreciation to my committee members for all of their guidance and support, and a special thanks to my advisor, Dr. Robert Stackman Jr. for his role in my research career. His encouragement, attention to detail, and incredible knowledge has shaped me beyond what I could have imagined possible. I am grateful to Charles E. Schmidt College of Science for providing the research contract to conduct my study. Finally, I would like to thank my lab mates for all their support.

ABSTRACT

Author: Brittany Nicole Crafton

Title: HEAD DIRECTION CELL NETWORK AND SPATIAL NAVIGATION: EFFECTS OF SILENCING ANTERODORSAL THALAMIC NEURONS USING DREADDS

Institution: Florida Atlantic University

Thesis Advisor: Dr. Robert W. Stackman Jr.

Degree: Master of Art

Year: 2023

While the thalamus and hippocampus are generally understood to contribute to mammalian spatial navigation, the degree to which thalamic input contributes to representations of space during navigation remains unclear. Specifically, anterior dorsal thalamic nuclei (ADN) provide a relational or directional framework known as the head direction (HD) network, which is hypothesized to play a significant role in guiding hippocampal-dependent navigation. The current study focuses on the contribution of the ADN to direction and place-dependent spatial navigation in adult male C57BL6J mice. An inhibitory chemogenetic (hM4Di) receptor was bilaterally expressed in the ADN after viral stereotaxic injection. Mice were trained in a spatially focused task, the Morris water maze (MWM), and after systemic administration of the hM4Di agonist, clozapine-N-oxide (CNO) at 5mg/kg, demonstrated equivalent preference for using directional or place-based search behavior. These results suggest that the selective silencing of ADN at

5mg/kg CNO does not negatively affect spatial navigation in mice.

HEAD DIRECTION CELL NETWORK AND SPATIAL NAVIGATION: EFFECTS OF SILENCING ANTERODORSAL THALAMIC NEURONS USING DREADDS

LIST OF TABLES

LIST OF FIGURES

GENERAL INTRODUCTION

Features of Navigation-Based Processing

The significance of the hippocampal formation for spatial navigation and cognitive mapping has been widely demonstrated, although a great deal of recent research emphasizes the importance of connecting structures which support processing during navigation. When encountering a novel environment, the brain rapidly 'maps' various key details of the surroundings (e.g., buildings, the color of walls, a large tree) and their spatial relationships to one another. Landmarks serve as 'cues' during navigation mapped during initial habituation to and encoding of a location; landmarks also facilitate recognition of a familiar place previously visited. The brain utilizes several aspects of somatosensory input during navigation to not only plan routes during movement, but to memorize key features of an environment for future reference. It is important to understand how the brain processes features of an environment and how navigation is influenced by such features. Understanding the underlying mechanisms involved in destination or goal-driven navigation may help to develop potential therapeutic approaches for pathologies like Alzheimer's Disease and dementia. An individual's ability to navigate through space depends upon spatial memory processing (Sorrentino et al., 2019), where the ability to utilize and process spatial cues allows for meaningful associations to be made with our surroundings as we orient and move to reach our destination. To better dissect the many types of memory required for

accurate navigation, procedural and declarative memory are two subdivisions that should be considered (Morellini, 2013; Squire and Zola, 1996; Squire and Dede, 2015; Whitaker, 2010). Declarative (explicit) memory permits one the ability to recollect autobiographical information, experiences, and truths; such abilities rely on complex neural connections within the medial temporal lobe. When navigating to, or within a previously visited place (declarative spatial processing), cognitive maps of the spatial relationships amongst cues and landmarks experienced during movement through the environment are encoded within declarative or explicit memory circuits and subsequently retrieved. The formation and successful maintenance of these declarative memories permits information to be stored long-term and retrieved relatively quickly for later use, known as procedural memory. Procedural memory involves components of long-term implicit memory, knowing *how* to do day-to-day tasks — e.g., how to tie your shoes, lock a door, or brush your teeth. Procedural memory is useful when encoding the surroundings of a place, or the 'rules' of how to move about in a space (e.g., the knowledge of knowing you need to open a door to enter a building). Understanding both short and long-term associations of memory is important when studying the processes of spatial navigation; how we familiarize and interact with a novel environment, as well as how we are able to revisit a space to more efficiently navigate to a goal destination.

In terms of spatial processing, an important feature includes spatial working memory. Spatial working memory is a form of declarative memory, which contributes to the ability to retain and process visuospatial information (Baddeley, 1986; Fenner et al., 2000). While procedural memory is important for long-term spatial representations as highlighted, more attention needs to be given to these early declarative memory processes

when studying spatial navigation: how mental maps or associations form during initial environmental exposure, the circuitry that is involved when encoding spatial information, and the underlying mechanisms that support continual updating of spatial information throughout the brain to guide successful navigation.

Spatial Processing: Involved Circuitry

The neural circuits that are essential for spatial navigation and path integration include the hippocampus, entorhinal cortex, subicular complex, retrosplenial cortex, subiculum, lateral mammillary nuclei (LMN), and the limbic thalamus (McNaughton et al., 2006). In-vivo recording studies have determined that components of the circuitry tuned to spatial navigation-based input provide neuronal signals representing spatial location (hippocampus), directional heading (post-subiculum, anterior thalamus), and a coordinate system for computing metric navigation (entorhinal cortex) (Ainge and Langston, 2012; Aronov and Tank, 2014; Blair and Sharp, 1995; Blair et al., 1998; Bolding et al., 2019; Fiáth et al., 2016; Sasaki et al., 2015; Han et al., 1993; Harvey et al., 2009; Knierim et al., 2014; Nagai et al., 2019; Vantomme et al., 2020; Wang and Cai, 2006; Xiang and Brown, 2004; Xiao and Barbas, 2002). Taken together, these spatially modulated neuronal signals are theorized to be integrated to support self-location and spatial navigation (Moser et al., 2008; 2017; Jacobs et al., 2013). While the CA1 pyramidal cell layer of the hippocampus is known to be a primary structure involved in spatial navigation and spatial memory, the degree to which these hippocampal functions are influenced by head direction (HD) signals from the anterior thalamus and post-subiculum are not well represented. Moreover, it has been previously established that the limbic thalamus sends projections to the hippocampus, but the volume of projections per

thalamic subdivision associated with spatial processing is unclear, and little is known about their overall physiological impact on the CA1 (Bertram and Zhang, 1999).

Previous Approaches to Studying the Place-Head Direction Relationship

As previously demonstrated by Calton et al. (2003), lesions to brain regions associated with HD signaling such as the ADN or postsubiculum (PoS) resulted in a degradation of CA1 place fields and increased head-direction sensitivity during an open field task. Further, CA1 place fields recorded in ADN-lesioned subjects were more sensitive to heading direction, and place cells recorded from PoS-lesioned rats in a cylindrical open-field arena, failed to undergo the expected angular shift in place field position when the prominent polarizing landmark cue was rotated 90°. This evidence supports the importance of ADN-HD cell input to the hippocampal place cell network, and its role in spatial tuning to influence appropriate ego- and allocentric representations of space during navigation.

Although overall HD cell volume per thalamic region of the rodent brain has not yet been fully quantified, the anterior dorsal thalamus has the highest density of HD cells, suggesting the relevance of the ADN for spatial navigation (Viejo & Peyrache, 2020; Clark and Taube, 2012). Neurons tuned to heading direction in the ADN appear to have a greater influence on hippocampal CA1 place cell representations compared to more medial and lateral thalamic areas (Taube, 1995, 2007, 2009; Yoganarasimha et al., 2006). While some studies have attempted permanent lesioning of the ADN to investigate its role in navigation (Aggleton et al., 1996; Béracochéa and Jaffard, 1994; Mitchell and Dalrymple-Alford, 2006; Morris et al.,1982; van Groen et al., 2002; Wolff et al., 2008), such lesioning has not only compromised allocentric cue-based navigation in rodents

during spatial tasks but, disrupted a notable degree of signaling processes beyond the targeted area of interest. Particularly, a previous study by Poirier et al. (2008) identified that post-ADN lesioning, the retrosplenial cortex displayed notable cellular transcriptome changes, such as a decrease in the relative levels of specific mRNAs that support energy metabolism and neuronal plasticity. These changes in functional gene expression may be guided mainly by the decline in expression of gene-encoding transcription factors including brd8, c-fos, fra-2, klf5, nfix, nr4a1, smad3, smarcc2 and zfp9, as well as considerably less (nfat5, neuroD1 and RXRγ) displaying increased expression. The concern however with the permanent lesioning approach, is the potential long-term effects of, for example, alterations to transcription factor expression. Moreover, the long-term impact this may have on other brain areas beyond those relevant for spatial processing is not well-defined. Therefore, this increases the difficulty in which results may be interpreted. An additional example of unanticipated physiological alterations due to permanent lesioning was demonstrated by Vann and Albasser (2009), where permanent lesions of the ADN were found to contribute to a selective loss of synaptic transmission, revealed as a widespread decrease in neuronal activity (as shown by a decline in c-*fos*) across the retrosplenial cortex. Laminae of the retrosplenial cortex (superficial layer II and III, and deep-lower III to VI) revealed a significant decrease in activity, or c-*fos* counts post-ADN lesioning. The previous findings provide convincing evidence that excitotoxic lesioning of the ADN results in distal pathological changes, such as hypoactivity in the retrosplenial cortex which may ultimately negatively affect normal spatial learning processes (Aggleton and Nelson, 2015; Poirier et al., 2008). Therefore, although permanent lesioning of brain regions is a traditional approach to

testing brain-behavior relationships, the interpretation of behavior changes after the lesion of a given structure is complicated by potential compensatory changes in neural circuits, distal changes in signaling, or even the fact that some lesions may promote neural plasticity (e.g. paradoxical lesioning). Using a temporary and region-, or cell type-specific approach to neuronal silencing with a rodent model can overcome some of these concerns since neural circuits are not permanently altered. Newer chemogenetic approaches using designer receptors exclusively activated by designer drugs (DREADDs) permits cell type selective manipulations of brain circuits (Smith et al., 2016; Urban and Roth, 2015). The present study used inhibitory DREADDs receptor (hM4Di)-induced silencing of ADN neurons to investigate their contribution to the encoding of location and spatial navigation.

Defining HPC and ADN: Contributions to Spatial Representations

Successful spatial navigation requires accurate knowledge of one's current location in space and current directional heading. Hippocampal CA1 neurons, in particular, place cells, encode the allocentric/surrounding spatial location information of an animal in an environment (O'Keefe and Nadel, 1978; O'Keefe et al., 1998), while complementary HD cells in the anterodorsal (Taube, 1995), anteroventral (Yoganarasimha et al., 2006; Tsanov et al., 2011) thalamic nuclei, and post subiculum encode egocentric/relative directional heading information of the animal. As we turn our heads to a different angular position in space, the HD cell network activity 'updates' place representations: our intrinsic reference frames (Mou et al., 2006); the integration of allocentric direction and place information together with egocentric information is considered to support spatial problem solving and navigation. Thus, integrated place and

direction information streams permit accurate mental representations of moment-to-moment spatial location information, which is essential for successful navigation overall. Pathways that are tuned to place and directional heading are driven by both external and internal cues, which are vital information streams that support spatial navigation (Moser et al., 2008). Nonetheless, the extent to which certain substructures support neuronal representations of space and general navigation requires further examination, in particular, the interactions between HD and place cell populations.

Spatial Testing: Morris Water Maze

The MWM task has been widely implemented to evaluate neurobiological mechanisms of navigation in rodents, where the target location is defined by its absolute position with respect to distal cues (Morris et al., 1982). It has been previously demonstrated that male C57BL/6J mice exhibit a preference for relative responding in the MWM, similar to that found in laboratory rats (Hamilton et al., 2007). However, relative responding in MWM was disrupted when the ADN neuronal activity was temporarily silenced by local infusion of muscimol, a GABA-A agonist (Stackman et al., 2012). Such findings support previous findings that the ADN plays a meaningful role in directional and place input during navigation. However, since local infusion of muscimol likely suppressed activity of all neurons, within the vicinity of the infusion, expressing GABA-A receptors, that is, excitatory and inhibitory neurons, projection neurons as well as intrinsic neurons, it is not possible to relate the loss of relative responding in the mice receiving intra-ADN muscimol to the selective suppression of ADN projection neuron activity alone. Thus, to test the selective contribution of the anterior thalamic-

hippocampal circuit to navigation, it is necessary to utilize an approach that permits a cell-type specific effect.

Hypotheses

In the current study, it was hypothesized that ADN neurons projecting to the hippocampal CA1 place cell network serve a key role in successful navigation, by updating place information with heading input during exploration, and that inhibitory DREADDs-induced silencing of such ADN neurons projecting to the CA1 will affect hippocampal-dependent spatial navigation. It was predicted that after DREADDs-induced silencing of the ADN, mice would display a reversal of directional preference— as reported previously by Stackman et al. (2012) after muscimol-induced silencing of anterior thalamic neuronal activity. Inhibition of directional preference as demonstrated by silencing ADN strengthens previous evidence supporting the critical role of ADN neurons in guiding spatial navigation. It was also predicted that average locomotor behavior (such as distance traveled and velocity) in both the Open Field and Linear Track control settings would not be significantly affected before or after the administration of the DREADDs receptor agonist, CNO.

Summary of Experimental Procedures

Mice received bilateral injections of viral vector to express the inhibitory hM4Di DREADD receptor or a fluorescent reporter eGFP control into the ADN. Following postoperative recovery mice were administered the hM4Di receptor agonist, CNO (5 mg/kg) or vehicle (0.9% saline) and placed in an Open Field arena and Linear Track. Results showed that CNO silencing of ADN neuronal activity did not significantly impact exploratory locomotor behavior. Next, mice were trained in the MWM for 7-10 d

followed by probe testing after treatment of CNO, or vehicle. Results indicated that hM4Di- and eGFP-expressing mice acquired the MWM task equivalently. Neither vehicle- or CNO-treated hM4Di-expressing mice displayed a significant preference for relational or direction platform search behavior during post-training probe testing, in contrast to previous reports. Results of this study suggest that unlike reports of rodents with lesions of the anterior thalamus, DREADDs-mediated silencing ADN neuronal activity does not compromise spatial navigation in male C57BL/6J mice. However, in the current study, region-specific modulation using DREADDS permitted a more targeted approach to investigate the role that HD cells in the ADN play in modulating spatial processing by hippocampal CA1 place cells during navigation.

Testing hM4Di-expressing mice under the influence of the DREADDs receptor agonist after acquisition of spatial memory in the MWM, permitted an analysis of the degree to which navigation, where the subject must rely on relative and distal cues to reach a goal destination, is dependent upon input from the ADN head direction cell network. Specifically, MWM probe testing allowed for the direct observation of explorative behavior and locomotion before and during ADN silencing, and whether subsequent chemogenetic inhibition significantly affected goal-driven spatial navigation.

MATERIALS AND METHODS

Subjects

Male C57BL/6J mice (7-8-week-old; Jackson Labs, Bar Harbor, ME) were group-housed initially with 2-4 per cage pre-surgery (*n*=16), with *ad libitum* access to food and water. Room temperature was maintained at 22 ± 4°C and humidity at 50 ± 5%. A 12-h light/dark cycle was maintained with lights on beginning at 7:00 AM. All experimental procedures were conducted during the light period following NIH guidelines; procedures were reviewed and approved by the Florida Atlantic University's Institutional Animal Care and Use Committee before the initiation of experiments.

Mice in this study underwent surgery after at least 1-week of vivarium acclimatization. Testing began 7 days post-operatively, to allow for sufficient recovery from surgical procedures, and when mice were at least 10-weeks old. Prior to the critical stage of memory testing (described below), each subject was administered a 5 mg/kg injection of the DREADDs receptor agonist, Clozapine N-oxide (CNO, HelloBio, Princeton, NJ) or 0.9% saline (as vehicle) intraperitoneally.

Surgical Procedures

Stereotaxic surgery was conducted at least 2 weeks prior to the start of behavioral testing. Mice were anesthetized with isoflurane (5% for induction, 1.5% for maintenance) using an induction chamber (SomnoSuite, Kent Scientific, Torrington, CT) and were

placed in a stereotaxic apparatus (Kopf Instruments, Tujunga, CA) after scalp shaving. Ears were tagged once under anesthesia for individual identification. Mice received a bilateral infusion of either a DREADDs virus carrying the inhibitory DREADDs receptor and fluorescent reporter (AAV5-hSyn-hM4D(Gi)-mCherry; *n* = 9), or a control virus carrying a fluorescent reporter (AAV5-hSyn-eGFP; *n* = 7) (AddGene, Watertown, MA). Each virus was aliquoted to a volume of 1.5 μl and stored at -80° C until the surgical procedure.

Each subject was weighed and inspected for any abnormalities/injuries prior to surgery. Sterile Vaseline was used to cover and protect the eyes during the surgery. Prior to the scalp incision, 0.1mL Lidocaine (2-8 mg/kg/0.1 mL) was injected subcutaneously at the base of the skull and massaged using a disposable Q-tip applicator, followed by Betadine solution, 70% alcohol, and Betadine scrub to clean the scalp. An incision was made in the scalp to expose the skull, and small burr holes were made in the skull with a #70 drill (0.028 mm cutting diameter, Kyocera) to create an opening for the bilateral infusion of virus into anterodorsal thalamus (A/P – 0.82 mm, M/L ±0.75 mm, D/V – 2.50 mm from Bregma, Franklin and Paxinos, 2008) using a 10μl NanoFil syringe and metal needle (2", 33 GA, World Precision Instruments (WPI), Sarasota, FL). A micro-infusion pump and controller (UMP3 and MICRO2T, World Precision Instruments, Sarasota, FL) attached to the stereotaxic manipulator arm held the WPI syringe. For each bilateral injection, the tip of the syringe was lowered to the ventral coordinate corresponding to the anterior dorsal thalamus (-2.50 mm). After the target depth was reached, a wait time of 3 minutes was implemented to allow tissue to reassimilate around the inserted needle. The pump then delivered 50 nl of virus at a rate of 0.28 nl/s (50 nl/ 3 min). After the viral

injection was complete, the syringe remained in place for 10 min to ensure the virus diffused within the ADN. The syringe was then retracted by just 0.1000 mm, and another wait time of 5 minutes was implemented to reduce the risk of virus dragging upward upon removal of the needle. The syringe needle was then fully, and slowly retracted thereafter. The incision was closed by VetBond (3M, Saint Paul, MN) and each mouse received postoperative analgesia. Postoperative monitoring included daily assessment of body weight and well-being of each subject. Behavioral training was initiated at least 10 days after surgery to allow for proper viral uptake in tissue.

CNO Preparation

A stock solution of the DREADDs agonist, Clozapine N-oxide (CNO) was dissolved in 100% dimethyl sulfoxide (DMSO; Fisher Scientific, Pittsburgh, PA) at a concentration of 100 mg/ml, immediately prior to injections. The CNO stock was diluted to a concentration of 0.5 mg/ml in a solution of sterile 0.9% saline containing 2% DMSO (Tuscher et al., 2018). During testing, mice were individually held in otherwise empty polycarbonate mouse cages for 30 min after drug or vehicle administration. A within-subjects cross-over design was used where each subject was tested in each behavioral task under the influence of vehicle, and the DREADDs agonist.

Locomotor Testing

Open-Field. The Open-Field arena consisted of an open-topped high-walled cylindrical enclosure made of white high-density non-porous plastic (36.27 cm height, 45.72 cm diameter) and was surrounded by black curtains (floor to ceiling) to minimize extra-maze cues. Each subjects' weight was recorded during habituation, and prior to testing. Handling and room habituation occurred for two days total prior to testing to minimize stress during behavior testing. Subjects were weighed, and then transported from the vivarium in their home cages to the room in the laboratory for open-field testing. Each subject was transferred individually into separate polycarbonate holding cages, given mock injections/needle pricks, and then returned to their respective holding cages and kept in the procedure room for about an hour. Testing occurred for two days, one trial per day. There was a 2-day interval between testing days to permit sufficient time for drug elimination prior to the next administration. Each subject received a systemic injection of either 5 mg/kg CNO or vehicle 30 min prior to testing. Each subject was placed in the center of the arena facing away from the experimenter at the beginning of each trial, where the distance from the start position to the arena wall was equal. Behavior was recorded using EthoVision XT 16 (Noldus, 2021). Recording began <1 s upon placing the subject in the arena with EthoVision initiating the 30 min trial upon detecting the mouse inside the arena. Average velocity, cumulative distance travelled and thigmotaxis were the main behavioral measures used for analysis. Each subject was returned to their home cage after each trial. Another 2-day delay was implemented prior to testing on the linear track under the opposite drug condition to avoid any carry-over effects of the first drug application.

Linear Track. The linear track was constructed of white acrylonitrile-butadine-styrene (ABS, 102 cm x 122 cm), with the sides angled to create a 46 cm base enclosure. The apparatus was enclosed following the same surroundings as the Open Field task, including black curtains surrounding and simple distal cues present (i.e., high-contrast shapes hung on the inside of the black curtain enclosure). Handling and room habituation was not reimplemented as the procedure room was the same as in the Open Field task. During testing, each subject received either a systemic injection of CNO or vehicle 30 minutes prior to testing. Subjects were then placed in the 'start position' on one end of the linear track facing away from the experimenter. Trials consisted of each subject freely shuttling along the track from one end to the other during a 10-min session, 1x/day for two days. Again, behavior was recorded using EthoVision XT 16 (Noldus, 2021). Subjects were then returned to their home cages at the conclusion of the 10-min trial. Average distance and velocity were the main behavioral measures used for analysis, along with total number of shuttles across the track, or full transitions from one end of the linear track to the other, and latency to reach the end of the track on the first shuttle behavior. A two-day delay was implemented prior to water maze room habituation to avoid any carry-over effects of prior drug administration.

Morris Water Maze

Room Configuration. Two days after the conclusion of linear track testing, mice began training in the Morris Water Maze (MWM). The MWM procedure utilized followed the dimension and training parameters used previously (Stackman, Lora, and Williams, 2012). The pool (109 cm diameter, 65 cm high), constructed of seamless white polyethylene, was placed on a wheeled base for ease of linear translocation. A clear Plexiglas platform (8 cm diameter, 31 cm high) was positioned in either the center, or east within the pool. The pool was filled to 1 cm above the platform with the water made opaque by stirring in nontoxic white tempura paint. The water temperature was maintained at a range of 22–24°C. A circular black curtain surrounded the pool to eliminate competing environmental cues. Behavior of the mice in the pool was recorded by a video camera positioned on the ceiling in the center of the testing room, where the camera was interfaced with the EthoVision XT 16 video-tracking system (Noldus Information Technologies), used to acquire all behavior of the mice in the water maze.

Habituation. During habituation, mice were removed from the vivarium in their respective home cages, weighed, and transported into the testing room inside separate polycarbonate cages for 1-hour; for each of two consecutive days. Mice then received 2 days of nonspatial training to acclimate to the pool and the submerged platform, and then received hidden platform training (four trials per day) to examine hippocampal-dependent spatial learning and memory (Morris et al., 1982). During nonspatial training, the pool was positioned at the center of the testing room, the platform positioned in the center of the pool, and no spatial cues were present on the curtain enclosure surrounding the pool. During nonspatial training each mouse was transported from the holding cage, carried inside the curtained area, and placed onto the platform where it remained for 60 s total prior to being returned to its separate polycarbonate container to dry, and then returned to its respective home cage. Each mouse experienced two 60-s duration stays on the platform with at least a 5 min intertrial interval between two days. In between trials, each subject was placed in a holding cage under a warm air stream. Next, each mouse was released into the pool at four locations immediately adjacent to the platform at the north, south, east, and west positions to acclimate the mouse to swimming and mounting the platform. Afterward, each mouse was again returned to the holding cage with the warm air stream where the subject remained for ~5 min before being returned to its home cage.

Hidden Platform Training. Mice were trained for 10 days (4 trials/day) to learn the location of a hidden platform within the pool. The pool was moved to the west location of the testing room, with four prominent distal visual cues present on the inside of the curtained enclosure, equidistant from one-another. Further, for this first day of hidden platform training in the MWM, the platform was moved from the center of the pool to the center of the east quadrant of the pool; the novel pool and platform location implemented during training promotes mice to use internal and external cues to learn how to escape to the escape platform. All mice were trained drug-free to learn the location of the hidden platform. Each training trial involved carrying the mouse inside the curtained area and releasing the mouse into the pool (facing the wall) at one of the start points (NE, SE, NW, or SW); each subject was permitted 60 s to locate the platform. If the mouse found the platform within 60 s, it was allowed to remain on the platform for 30 s to acclimate with the surrounding distal cues. If the mouse failed to find the platform within 60 s, it was guided to the platform where it remained for 30 s. Probe testing began once mice were performing consistently at an asymptotic level. Measures of escape latency (s) and cumulative distance to the platform center (in cm) were determined from each training trial for each mouse.

Probe Testing. Probe testing was utilized to assess the strength of the spatial memory for platform location, and to determine the preferred search strategy used by each mouse, whether a hippocampal-dependent place response, or an ADN dependent relative or directional response (see *Figure 7*). The probe test was conducted after the platform was removed from the pool, and the pool translated to the eastern location of the testing room. With the pool translated to the east position in the room, the absolute location where the platform was located during training, as defined by the extra-maze cues, was now in the opposite position of the pool relative to the pool wall. Each mouse received a systemic injection of CNO (5 mg/kg) or vehicle 30 min prior to probe testing. The drug- or vehicle-treated mouse was carried into the curtained enclosure and then released into the pool at either the North or South starting location and permitted to swim freely for a 30-s probe test. After 30 s, the mouse was removed from the pool and returned to the holding cage and placed under the warm air stream. On the day following the probe test, the pool was returned to the West position of the room, and all mice received 4 re-training trials for four days, as described above. After completing 4 consecutive days of this re-training, the procedures for the probe test were repeated with the drug assignments reversed to further evaluate any effects on place navigation of ADN silencing. During the probe test, if the mouse swam directly to the previous place in the room where the platform was located during training, as defined by the distal room cues, then the mouse was defined to have navigated in a direction opposite to that used to locate the platform during training. Such behavior was scored as a place, or absolute response (hippocampal dependent). Alternatively, if a subject chose to swim in the same relative direction from the starting location in the translated pool, as it did during training, then the mouse would arrive in a

location opposite used during the training trials. Such behavior was then scored as a directional, or relative response (ADN dependent) (Stackman et al., 2012).

Histology

To verify proper placement of virus and adequate expression of hM4Di and GFP in ADN, after the completion of behavioral data collection all subjects were deeply anesthetized using Euthasol (Virbac AH, Inc) and transcardially perfused with 0.1 M phosphate buffer (PB), followed by 4% paraformaldehyde (PFA) in 1x phosphate-buffered saline (PBS). Brains were then removed and post-fixed in 1x PBS/4% PFA overnight, followed by 2 days in 30% sucrose solution in 4% PFA, and 2 days in 20% sucrose. Tissue was then sectioned on a microtome (50 μm) and mounted onto microscope slides using DAPI Fluoromount-G mounting medium (Southern Biotech). Fluorescent images were captured using a Nikon E600 Fluorescence microscope. Expression of hM4Di and GFP in ADN were confirmed using these standard histological methods.

Statistical Methods

Open Field. Data from the Open Field task was analyzed in using three 10-min trial blocks, with the first 10 minutes being the primary focus to compare any significant differences in locomotor behavior with initial arena exposure. Distance moved (cm) and velocity (cm/s) were used to assess locomotor activity with drug on- and off-board. Three-factor (viral group, treatment, and time bin) ANOVAs were run on measures of velocity and distance with the trial time bins as the repeated measure to test the influence of ADN silencing on locomotor activity. Time spent in the border zone (area of the

surrounding wall) was analyzed using a repeated-measures ANOVA to assess treatment effects on thigmotaxic behavior.

Linear Track. Similar procedures were used to assess linear track data, with distance traveled and velocity as factors, in five 2-min time bins per 10 min trial. Number of full tracks (fully traversing from the start to the end of the track) were accounted for, as well as incomplete tracks (stopping and turning around before the end of the track). A within-subject two-factor (treatment vs distance traveled) ANOVA was conducted again with the trial time bins as the repeated measure to assess locomotor activity with drug on and off-board. Time spent (s) in each respective zone (start, midline, and end zone) was compared across subjects between trials using a two-way repeated measure ANOVA to further investigate whether the presence of CNO will affect velocity, distance travelled, or, for example, increased freezing behavior at various time points.

Morris Water Maze. Data from water maze training trials was analyzed in four-trial block means with a two-factor (viral group and trial block) ANOVA, with trial block as the repeated measure. For probe test data, three-factor between-subjects ANOVAs were conducted to assess spatial search strategy preference, with viral type (eGFP vs hM4Di), treatment (Veh vs CNO), and search zone (absolute vs relative) as factors. Similar analyses were used to investigate the mean distance travelled (in cm) from the center of the two search zones during the probe tests. Bonferroni tests were used for *post hoc* comparisons provided the overall ANOVA yielded a significant main effect or a significant interaction. Follow-up analysis to further define the degree of relative or absolute preference was compared using heading error (the deviation from a direct swim

path to the relative search zone using latency to first entry). Differences for all tests were considered significant at $p < 0.05$.

RESULTS

Histology

Histological analysis confirmed accurate placement of the bilateral micro infusion of virus within ADN, based on detection of the respective fluorescent reporter (*Figure 1*, eGFP; *Figure 2*, hM4Di) within the ADN of the 18 mice. In cases where viral infusion was not localized to ADN in hM4Di-expressing mice, these subjects were excluded from behavioral analysis (n_{hM4Di} = 6 and n_{eGFP} = 8 excluded). Expression of hM4Di beyond the ADN, such as into surrounding regions of the hippocampus and/or anteroventral or anteromedial thalamic nuclei could potentially confound navigational behavior associated with silencing off-target structures. Two eGFP mice were included in the analyses despite some off-target eGFP expression, as these subjects did not exhibit any spatial deficit or behavioral alteration, as well as to balance group size for power of analysis across all conditions (see *Figure 3a* & *b*).

Open Field

To investigate whether the expression of virus in ADN, or DREADD-agonist treated mice resulted in locomotor impairments, mice were exposed to an Open Field arena (Cinalli et al., 2022) for two 30-min trials, with either CNO or vehicle administered 30 min prior to behavioral testing followed by a 2-day inter-trial interval where treatments were then reversed. The total distance travelled (cm) per 10-min time bin was analyzed across viral condition and drug treatment to assess whether ADN silencing had

a significant effect on locomotor activity. The data was analyzed as a three-way ANOVA with viral type (AAV5-hSyn-eGFP or AAV5-hSyn-hM4D(Gi)-mCherry), treatment (vehicle or CNO), and 10-min time bin as factors. There was a significant main effect of time bin, ($F_{(2, 84)} = 10.737$, $p < 0.001$; *Figure 4*). There was no significant main effect of viral type ($F_{(1, 84)} = 1.961$, *n.s.*), nor any significant main effect of treatment type ($F_{(1, 84)} = 0.137$, *n.s.*). A *post-hoc* analysis for time bin revealed significant differences between time bin 1 vs time bin 3 ($t_{(15)} = 4.573$, $p < 0.05$) and time in 1 vs 2 (3 ($t_{(15)} = 2.933$, $p < 0.05$). However, there was no significant difference between time bin 2 and 3 ($t_{(15)} = 1.641$, *n.s.*). These results indicate that silencing ADN did not result in any significant effects on locomotor activity with respect to total distance traveled per trial.

Linear Track

Following Open Field testing and a 2d delay post CNO or vehicle administration, locomotor activity was assessed in a linear track. Similar measures were used to assess locomotor activity both with and without drug on-board. Distance travelled (cm) was a primary measure to assess activity during each trial day. A 3-way (viral group, drug administered, and the five 2-min time bins) ANOVA was conducted on distance travelled (cm) measures. There was a significant main effect of time bin, where subjects explored less the *more* time they spent in the linear track ($F_{(4, 159)} = 74.315$, $p < 0.001$; see *Figure 5*). There was no significant main effect of viral group ($F_{(1, 159)} = 1.805$, *n.s.*), drug treatment ($F_{(1, 159)} = 0.220$, *n.s.*), nor any significant interaction effects (all p-values > 0.05).

Morris Water Maze

Hidden Platform Training. Mice were trained in the MWM to learn the location of a hidden platform. For training data, measures of escape latency and cumulative distance to platform center (cm) were analyzed in four-trial blocks with a two-factor (viral group and trial block) repeated-measures ANOVA, with trial block as the repeated measure. There was a significant main effect of trial block on measures of escape latency ($F_{(9,135)} = 15.536$, $p < 0.05$; see *Figure 6*). There was no significant main effect of viral type ($F_{(1,135)} = 1.452$, *n.s.*), nor significant interaction effect of viral type vs trial block ($F_{(9,135)} = 1.273$, *n.s.*). For cumulative distance to platform center, there was a significant main effect of trial block ($F_{(9,135)} = 10.021$, $p < 0.05$). There was no significant main effect of viral type ($F_{(1,135)} = 0.727$, *n.s.*), nor a significant interaction effect of viral type vs trial block ($F_{(9,135)} = 1.003$, *n.s.*). These results indicate that mice progressively learned to locate the hidden platform prior to probe testing; all subjects were matched for spatial performance regardless of viral type prior to spatial probe testing. After acquiring asymptotic performance in the MWM, the influence of ADN silencing via systemic CNO administration was tested during probe tests with the platform removed from the MWM. Effects on spatial navigation were measured by search zone preference (relative or absolute) during the 30-s probe tests.

Probe. All 16 mice were administered either CNO or Vehicle immediately following their final hidden platform training trial. A 30-min delay was implemented to allow for adequate time of CNO binding within hM4Di-expressing mice (Jendryka et al., 2019; Martinez et al., 2019; Smith et al., 2021; Cinalli et al., 2022) prior to probe testing. Each mouse received a 30-s probe test with the pool shifted to the east location of the testing room. As described in the Methods section, shifting the pool position permitted testing whether the mice exhibit a place-based, hippocampal search strategy or a directional, anterior thalamic search strategy during the probe test. Strategy choice was determined by the latency to enter a circular zone around where the platform had been during training as defined by the distal room cues (place or absolute strategy), or a circular zone around the relative location where the platform would have been in the pool independent of the pool position shift (direction or relative strategy). Other measures of spatial search behavior included mean distance to the relative or absolute zone, and the heading error or deviation of the mouse's initial path from a direct path to the relative zone.

A three-way ANOVA on latency first entry to absolute vs relative platform zone (*s*) revealed a significant three-way interaction between viral type, treatment, and search zone ($F_{(1,61)} = 4.331$, $p < 0.05$). There was no significant main effect of treatment ($F_{(1,61)} = 0.0855$, *n.s.*), viral type ($F_{(1,61)} = 1.013$, *n.s.*), or search zone ($F_{(1,61)} = 0.932$, *n.s.*); nor any significant interaction effects between viral type and treatment, viral type and search zone, nor treatment and search zone (all *p*-values > 0.05). To evaluate the effect of whether sample size contributed to any significant variability with the current study, a Student's 2-tailed *t*-test revealed no significant difference of mean values between viral groups ($t_{(14)} = 0.216$, *n.s.*).

Heading Error

eGFP+Veh (*Figure 9a, dark blue*). The total mean heading error for the eGFP+Veh condition was 24.7°±5.4, where 0° indicates no error, or a direct route to the relative search zone with no deviation in path (length of the heading error vector, $r = 0.93$).

eGFP+CNO (*Figure 9a, light blue*)**.** Mean heading error for the eGFP+CNO condition was 18.3°± 7.8°, (length of the heading error vector, $r = 0.95$).

*eGFP subjects who preferred the relative search zone *regardless* of treatment type had a heading error of 2.8°±0.6°, where heading error for subjects that displayed an absolute response preference regardless of treatment was 41.2°±2.2, where 32° was determined as the angle opposite to the relative search zone.

hM4Di+Veh (*Figure 9b, dark red*). The overall mean heading error for the hM4Di+Veh condition was 23.6°± 6.1°(length of the heading error vector, $r = 0.89$).

hM4Di+CNO (*Figure 9b, light red*). Mean heading error for the hM4Di+CNO condition was 21.7°± 5.7°(length of the heading error vector, $r = 0.94$).

*hM4Di subjects who preferred the relative search zone *regardless* of treatment type had a heading error of 6.7°±1.9°, where heading error for subjects that displayed an absolute response preference regardless of treatment was 38.7°±1.6, again, where 32° was determined as the angle opposite to the relative search zone.

DISCUSSION

Converging evidence indicates that the hippocampus is essential for fast encoding and storage of new episodic memories. The involvement of the hippocampus is particularly important when forming associations between landmarks during navigation tasks, as well as reaching a goal location or object (O'Keefe and Nadel 1978; Morris et al. 1982, Maguire et al. 1998; Teng and Squire 1999; Ekstrom et al. 2003). Structures relative to the hippocampal region such as the entorhinal cortex, thalamus, retrosplenial cortex, post subiculum, and striatum all play a role in spatial navigation, such as processing surrounding cues for later reference, fine-tuning paths to optimally reach a goal location, and so on. Theoretical models of spatial navigation have suggested that the integration of directional information within the hippocampal region stem from post subiculum input to location-specific neuronal codes in hippocampus (Burgess et al., 1994; McNaughton et al., 1996; Redish & Touretzky, 1997; Whishaw & Jarrard, 1996; Poulter et al., 2021; Kay et al., 2016) where postsubicular HD coding has been thought to critically dependent on projections from the anterior thalamic nuclei (Goodridge & Taube, 1997). The head direction cell signal is hypothesized to stem from the pathway between dorsal tegmental nucleus and the lateral mammillary nucleus (LMN; Bassett & Taube, 2001; Sharp et al., 2001). A method of interrupting the HD system is the use of lesioning, or silencing of LMN (Vann, 2005, 2011), where directional signaling is thereby ousted in ADN (Goodridge & Taube, 1997; Blair et al., 1998, 1999), which in turn is necessary for normal hippocampal representations of space during movement.

However, the mechanisms behind the transmittance of HD signal from ADN to forming spatial representations in the hippocampus is still only marginally understood (Frost et al., 2020).

The current study investigated the importance of ADN signaling during navigation in a goal-directed spatial task in male C57BL/6J mice. The experiment implemented bilateral expression of an inhibitory DREADD (AAV5-hSyn-hM4D(Gi)-mCherry), or a control viral construct (AAV5-hSyn-eGFP) in the ADN, and then systemic administration of vehicle control or the DREADDs receptor agonist, CNO to evaluate spatial performance in the MWM task with intact ADN input versus temporary silencing in CNO-treated DREADDs expressing mice.

Exploratory behavior was analyzed in the open field and on a linear track in CNO and vehicle treated eGFP and hM4Di-expressing mice prior to MWM testing, which confirmed that ADN silencing caused no anomalous locomotor differences between viral groups and treatment type. It was important to establish that mice were able to move about an environment normally regardless of systemic treatment or viral condition prior to testing in a spatial navigation task, so that all results during spatial testing would be properly interpretable. Thereafter, training in the MWM task was introduced. Mice were trained for 10 days (4 trials/day) in the MWM to locate the hidden platform. eGFP and hM4Di-expressing mice acquired the hidden platform MWM task in an equivalent manner, indicating that the bilateral expression of the inhibitory DREADDs receptor did not influence the contribution of the limbic thalamus to spatial behavior. Following the final training trial on the 10th day, probe tests were given to determine whether ADN silencing with 5 mg/kg of CNO administered 30 min prior significantly affected search

behavior in hM4di-expressing subjects, as compared to controls. Although all subjects (regardless of viral type) learned the hidden platform location, as determined by escape latency and cumulative distance to platform analyses, there were inconsistencies observed during the probe tests that contradicted hypothesized search behavior. Based on prior reports from our lab (Stackman et al., 2012; Zhang et al., 2017) and others (Hamilton et al., 2007; 2008) indicating that rodents exhibit a preference for relative or direction search behavior in the MWM, it was expected that control mice (i.e., eGFP+Veh/CNO; hM4Di+Veh) would show a preference for using a relative or directional search strategy during the probe tests. However, control mice in the present study exhibited equal preference for relative and absolute search strategies. This lack of preference in one search strategy over another was also identified for the experimental mice (i.e., hM4Di+CNO). The overall ANOVA on overall search preference yielded a significant three-way interaction between drug treatment, viral type and search zone, yet the main effects of each of the three factors were non-significant. Therefore, the results of the post-hoc analyses were difficult to interpret. The significant three-way interaction term is difficult to interpret due to the continual issue of random search preference regardless of treatment day or viral type. While effect size for between group sample means was medium to low, it would be interesting to see if a significant three-way interaction persists with a larger sample size; might a greater number of subjects potentially abolish this phenomenon, or highlight something more meaningful? Would less trials have made a difference, despite unevenly distributed performance during training? Despite tests for whether there was a large effect size due to the current sample

size, it was not determined that the current sample sizes per viral condition significantly contributed to the variability observed during probe testing.

Several other factors may have contributed to differences in the platform search behavior of the mice from the present study compared to that of previous reports. For example, the mice may have been overtrained as a result of the number of trials required for the mice to achieve asymptotic performance in the hidden platform MWM task. It has been previously observed that rats trained for more than 4 days in the hidden platform MWM task (12 d prior to initial probe day) showed no preference for the trained search quadrant, or opposite search quadrant (Kealy et al., 2008), yet this was not observed for male C57BL/6J mice in prior studies from our lab. Further, it had been hypothesized that subjects exposed for extended training trials, regardless of individual performance in spatial tasks, may develop a heightened dependence on distal cues (place) rather than a relative (directional) search strategy. Kealy et al. (2008) had postulated that extended training in spatial tasks may result in haphazard search behavior re-emerging in later training trials, which may be attributed to a random 'switching' effect between brain regions which rely heavily on absolute room cues, versus those which are tuned more specifically to relative heading in an environment. It is important to note that all mice in the current study were trained for the same amount of time until all subjects were able to successfully locate the hidden platform with a relatively direct swim path (roughly in 10s or less). Ideally, subjects should have been probed based on *individual* performance, that is, upon reaching some acquisition criterion rather than after completing a set number of training trials. Matching mice for acquisition or spatial memory encoding, then permits subsequent testing of the retention and retrieval of that spatial memory. Of course, one

subject may learn to locate the hidden platform in 4 d of training, while another might not learn until 8d of training in MWM. Collectively probing subjects in this manner certainly invites issues associated with overtraining, particularly, the inability to effectively assess *true* search preference/behavior between treatment type and viral group upon probe days. This re-emergence of varied swim behavior where swim patterns appeared less direct in later trials may likely be attributed to extended training.

Regardless, temporary bilateral DREADDS-induced inactivation of ADN did not consistently alter search preference in hM4Di treated mice, as expected. Apart from the issue of over-training, the administration of CNO at 5 mg/kg failed to influence spatial search strategy selection or the accuracy of platform search behavior during the probe tests. It would have been of interest to test whether a higher dose of 10 mg/kg CNO may have produced more robust silencing in ADN and a notable alteration in search preference in hM4Di-treated mice (Cinalli et al., 2022). Mice expressing hM4Di in hippocampal CA1 neurons and given CNO at 5 mg/kg exhibited altered performance in an object recognition memory task, but 10 mg/kg CNO was required to produce a significant impairment of spatial navigation in the MWM (Cinalli et al., 2022). These findings suggest that in order to successfully repeat previous findings, the training protocol must be fine-tuned to each subject, and evaluation of the dosage of CNO must be considered.

To better understand how neuronal signaling had differed between controls and hM4Di subjects with, and without CNO administered, recording studies should be implemented in future approaches to more closely observe neuronal activity in ADN and CA1 with intact signaling, and after different doses of CNO to inhibit neuronal activity in

mice expressing hM4Di in the ADN. In this way, it will be possible to better understand and evaluate ADN heading direction cells, place firing/firing fields in CA1, and how ADN silencing using the DREADDS approach ultimately affects overall spatial representations between the anterodorsal-hippocampal circuit during a navigation-based task. Previous approaches to ADN silencing have been attempted in order to examine its function during navigationally driven tasks. As previously executed by Stackman et al. (2012), mice received an intracranial infusion prior to probe testing, either 0.25 µl of artificial CSF as a control, or the GABA-A agonist, muscimol (Tocris Bioscience) for silencing. Cannulations were placed in the above dorsal CA1 of the hippocampus, or just above ADN. In the relative vs absolute condition, the pool was moved linearly to the location opposite to that during training. In this condition, which emulates the current approach, mice displayed a significant preference for the relative search zone, as well as visits to the relative search quadrant. Mice administered muscimol into the ADN prior to probe displayed an abolished relative response preference, instead seeking the absolute search zone. To validate placement and better visualize the nature of muscimol spread in tissue, a separate cohort of mice underwent cannulation surgeries, and were run similarly to the testing group. The separate cohort was given infusions of fluorophore-conjugated muscimol (FCM) to do this. The FCM group received a micro infusion into the ADN, where the fluorescence showed clear spread within the ADN. However, histological analysis revealed that there was some spread in the lateral and ventral directions to the dorsomedial anterior ventral thalamic nuclei, ventrolateral anterior ventral thalamic nuclei, and anteromedial thalamic nuclei, and minimal spread in the medial direction to the stria medullaris of the thalamus (Stackman et al., 2012). Mice in this previous study

with muscimol-induced silencing of ADN exhibited a change in swim patterns, such as swimming in tight circles during testing, which was interpreted as disorientation. Thus, it is possible that disruption of relative responding after muscimol infusions previously reported reflects the suppression of neuronal activity within the ADN as well as the surrounding nuclei of the anterior thalamus.

However, despite all mice included in the current study with confirmed ADN placement in the hM4Di condition, regardless of CNO present per probe day, did not display a significant preference for either search zone, or a difference in swim pattern (e.g., tight circling) as previously seen by Stackman et al. (2012) using the local muscimol infusion approach. The more drastic impact of muscimol induced silencing may have been due to muscimol spreading to surrounding thalamic regions. Given the more selective expression of hM4Di within the ADN in the current study, it was not anticipated that a drastic change in swim pattern would be observed in the current study. A greater degree of silencing across multiple thalamic regions invites the issue of impacting a subjects' ability to compensate for lack of HD signaling via ADN, hence, abnormal or disoriented swim behavior such as that previously observed.

As found in the 2012 study, it was expected that with no drug present, mice would prefer the trained relative search zone, where hM4Di mice administered CNO 30-min before probe testing would demonstrate a 'switching' of their initial search strategy with ADN activity silenced. Additionally, it was expected that subjects would display *less* of a disoriented swim behavior with ADN silencing, in contrast to the previous study as previously mentioned. This hypothesized result was based on findings described by Stackman et al. (2012), in the relative vs. absolute probe condition with an improved

approach to targeted silencing. However, results of search preference or a significant effect of viral type and treatment were inconclusive, where neither control eGFP nor hM4Di subjects displayed a significant preference for either search zone, regardless of vehicle or 5 mg/kg CNO administration prior to testing. The current study must be reassessed not only in the determining criterion for a subject to graduate from hidden water maze trials to probe testing to circumvent overtraining, but it will also be important to include a relative-only condition to evaluate whether pool location exerts control over directional responding (Stackman et al., 2012), and even CNO dosage once baseline preference can be established.

In the current study, the ability to localize neuronal silencing to ADN may have allowed for a greater ability for subjects to utilize compensatory search behavior, and thus, no disorientation in swim behavior. Despite converging evidence from previous lesioning studies where notable search behavior changes were reported with loss of input from ADN (Aggelton & Nelson, 2015; Mitchell & Dalrymple-Alford, 2006; Calton et al., 2003; Stackman & Taube, 1997), it is important to reiterate the difference of the current, less invasive approach where ADN remains in-tact. It is possible the current approach did not fully silence the ADN, or not all ADN-specific neurons were infected by virus. Further, it must be considered that there are several other HD-containing regions such as entorhinal cortex, retrosplenial cortex, subiculum, lateral thalamus, lateral mammillary nuclei of the hypothalamus, and so on, that were left in-tact in the current study. While the focus was on ADN for evaluating its contribution to spatial navigation in the current study, HD input from surrounding HD-containing regions very likely allowed for

compensatory signaling to occur, where alterations to search behavior during ADN-silencing could vary from subject to subject.

For the purpose of the current study, aside from determining the impact of ADN silencing on search behavior, it was important to verify the relationship between ADN and hippocampus (particularly CA1) histologically. Utilizing DREADDS paired with florescent tagging permitted this, where very clearly infected cells via ADN were present in hippocampus (see *Figures 1 & 2*). This histological confirmation justifies the importance of isolating ADN via DREADDS to explore navigational learning and memory with respect to CA1, but further investigation will be required to better understand this relationship using the DREADDS approach. However, a method of quantifying average total number of neurons in the ADN needs to be devised for a few reasons. First, quantification of ADN population permits the ability to analyze the ratio of successfully targeted neurons in the structure using the current infusion protocol, vs uninfected ADN. However, there has been little data on this with a mouse model, currently. It has been postulated that the mouse lateral posterior nucleus contains roughly ~31,000 neurons (Seecharan et al., 2003 & Evangelio et al., 2018), but little is presented for anterior nuclei. Secondly, quantification of ADN leads to the question: *Of the percentage of successfully hM4Di-infected cells within ADN, what is the ratio of successful CNO binding post-IP injection?* Some previous studies mention that CNO does not fully cross the blood-brain-barrier (BBB), moreover, some potency may be lost during this process and metabolizing the ligand (Raper et al., 2018). Converging evidence refutes this idea of BBB being an issue for CNO reaching and binding to DREADDS-targeted cells, however, this must be considered especially when targeting such a small

region, where, successful ligand binding becomes even more critical. Further, as previously discussed, the current dose of CNO is a consideration as to whether 5 mg/kg is sufficient for successful binding hM4Di-expressing neurons in ADN. However, defining neuronal population in ADN will allow for a more informed approach when determining appropriate dosage of CNO. Exploring a confirmation method to determine a success ratio of CNO binding to the current DREADDS construct used, should certainly be implemented in future approaches.

Overall, it will be important to continue investigating the contribution of thalamic input to hippocampal functionality during navigation. Heading direction information and how it modulates place cell firing when forming mental maps permits the ability to traverse a space from point A to B, as well as maintain these reference frames, and store for later reference during re-exposure to an environment. Future thalamic exploration using targeted approaches such as DREADDS, must consider methods to define how to target head direction related neurons, if possible. The current approach does not define which populations of neurons targeted were, in fact, specific to both processing and relaying of head direction information via ADN to relevant structures.

Trial Block	eGFP	hM4Di
1	38.51 ± 3.56	38.67 ± 4.10
2	20.86 ± 5.98	29.24 ± 4.68
3	20.10 ± 3.15	28.16 ± 4.94
4	15.95 ± 2.51	28.88 ± 3.96
5	19.67 ± 4.46	20.89 ± 3.18
6	15.06 ± 2.40	18.25 ± 1.64
7	15.77 ± 2.71	16.07 ± 1.78
8	9.91 ± 1.56	13.04 ± 1.93
9	11.05 ± 1.63	11.49 ± 1.52
10	7.62 ± 1.83	8.92 ± 1.71

Table 1. **Mean Latency to 1st ± S.E.M Per Trial Block.** Values of mean latency to first arrive to the hidden platform (in *s*) for all 10 days of training for both eGFP and hM4di mice.

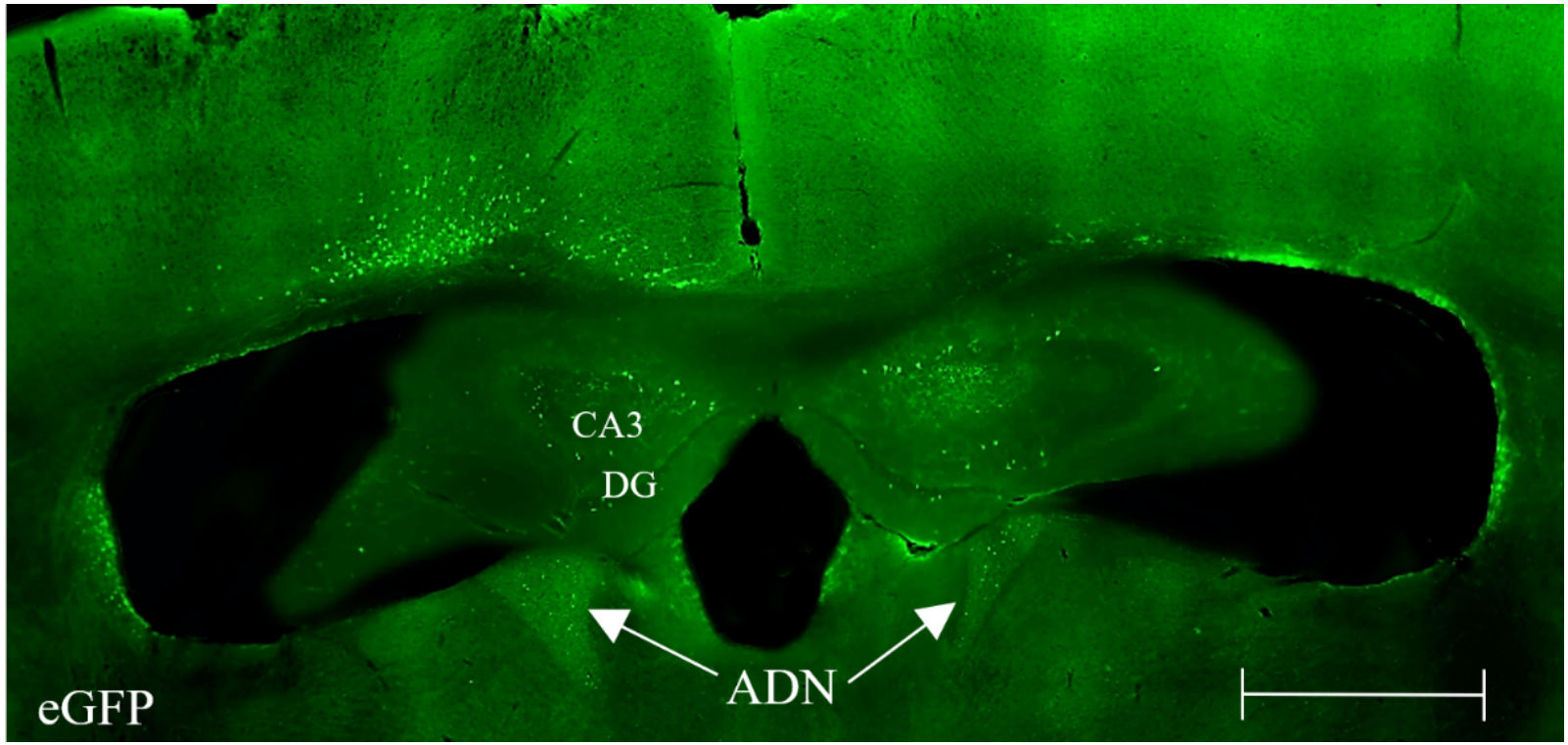


Figure 1. **Expression of Fluorescent Reporter in Anterior Thalamic Nuclei (ADN): eGFP Localization.** Histological verification of bilateral infusion of control AAV5-hSyn-eGFP placement into the anterior thalamic nuclei. Arrowheads indicate the bi-lateral localization of the fluorescent tag in the anterior dorsal region of the thalamus (-2.50mm V). 50 µm slice, -0.98mm.

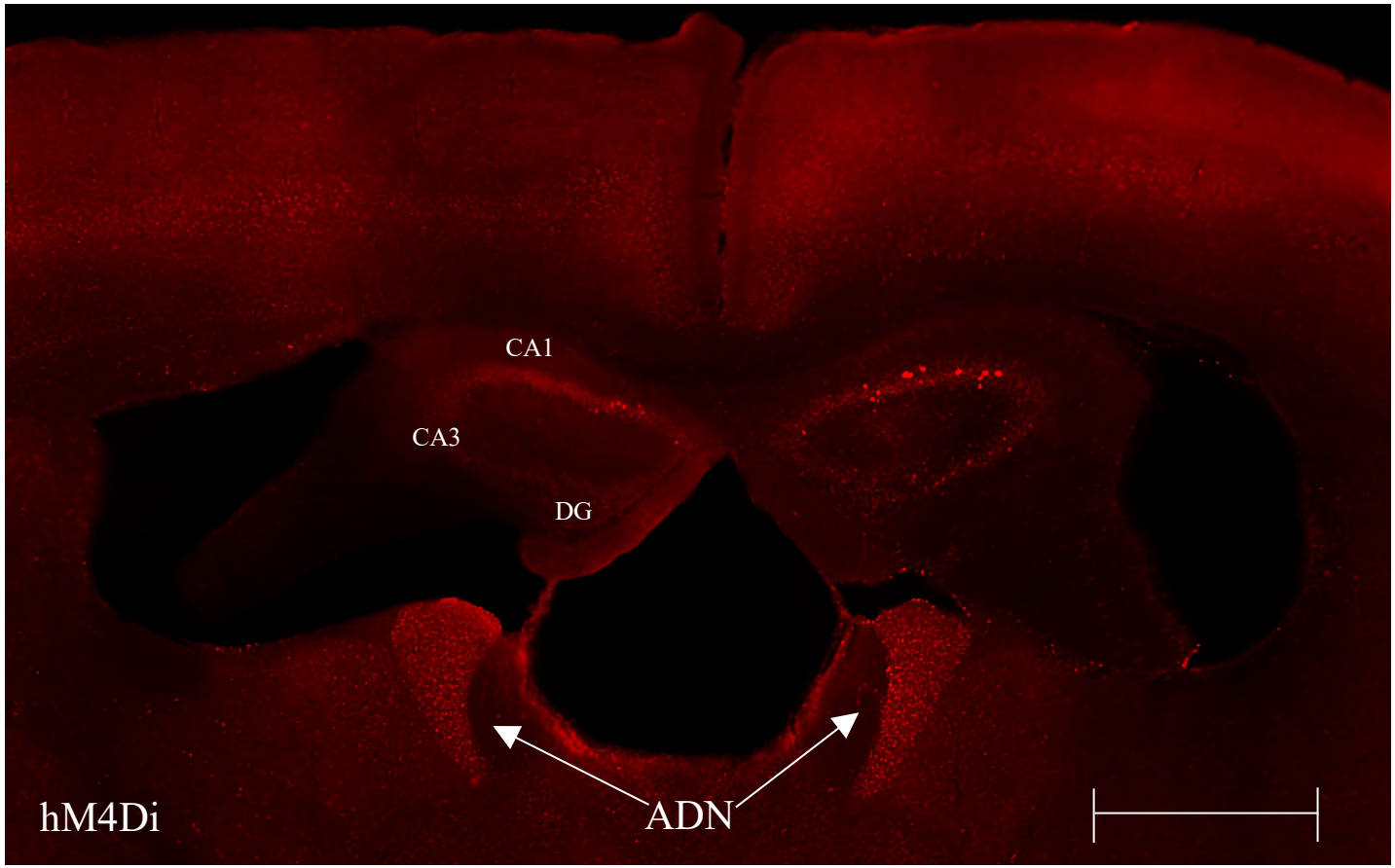


Figure 2. **Expression of Fluorescent Reporter in Anterior Thalamic Nuclei (ADN): hM4Di Localization.** Histological verification of bilateral infusion of experimental DREADD virus AAV5-hSyn-hM4D(Gi)-hM4Di placement into the anterior thalamic nuclei. Arrowheads indicate the bi-lateral localization of the fluorescent tag in the anterior dorsal region of the thalamus (-2.50mm V). 50 µm slice, -1.01mm.

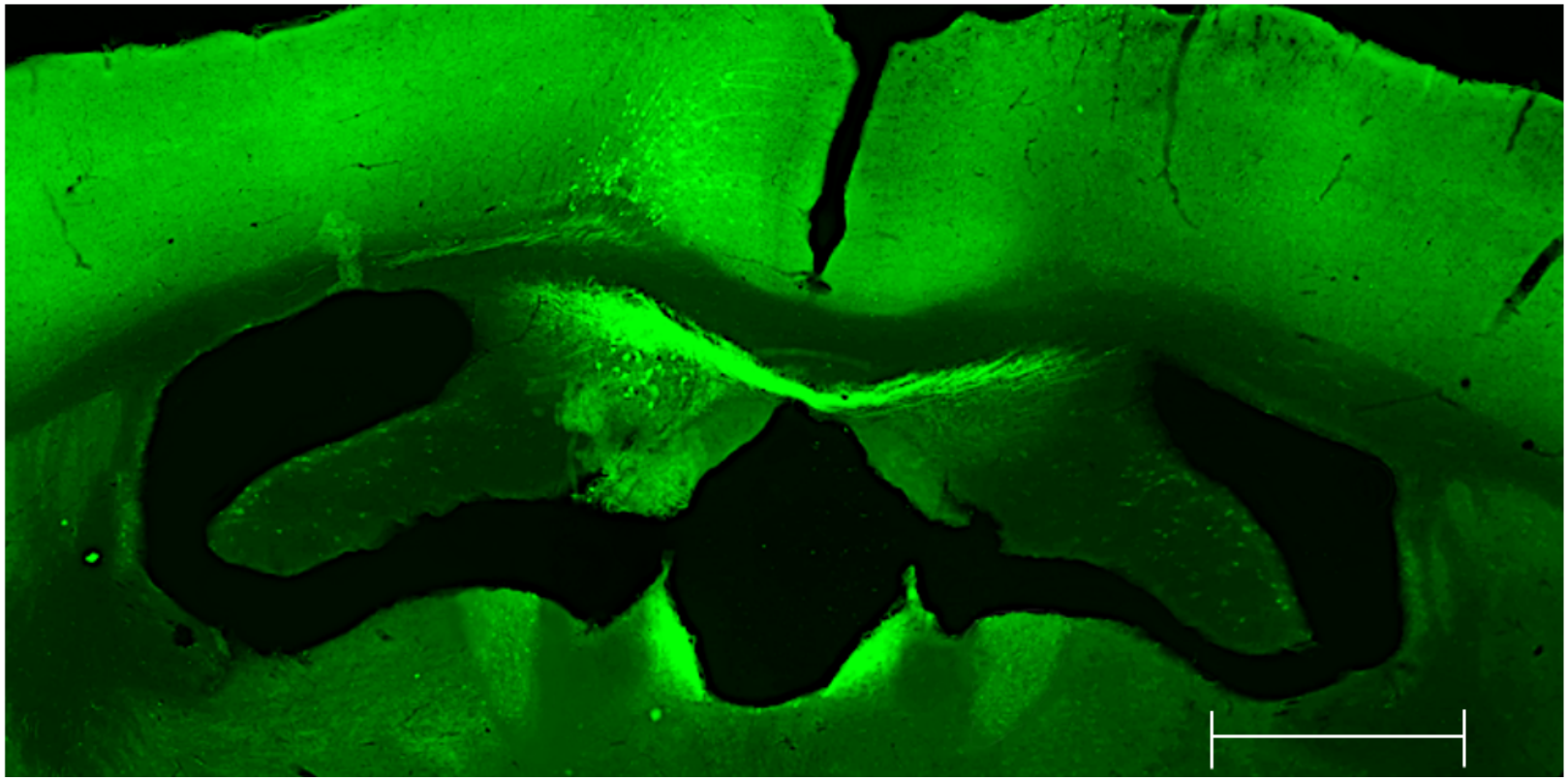


Figure 3 a. **Off-Target eGFP Expression of Fluorescent Reporter in Anterior Thalamic Nuclei.** 1/2 eGFP subject that - was included in the study displaying viral leakage during histological analysis. Occurring in the hippocampal region, leakage likely occurred during removal of the infusion needle during surgery. While anterior dorsal thalamus had been targeted, a majority of control virus had dispensed superiorly into hippocampus. 50 µm*;* -0.92mm.

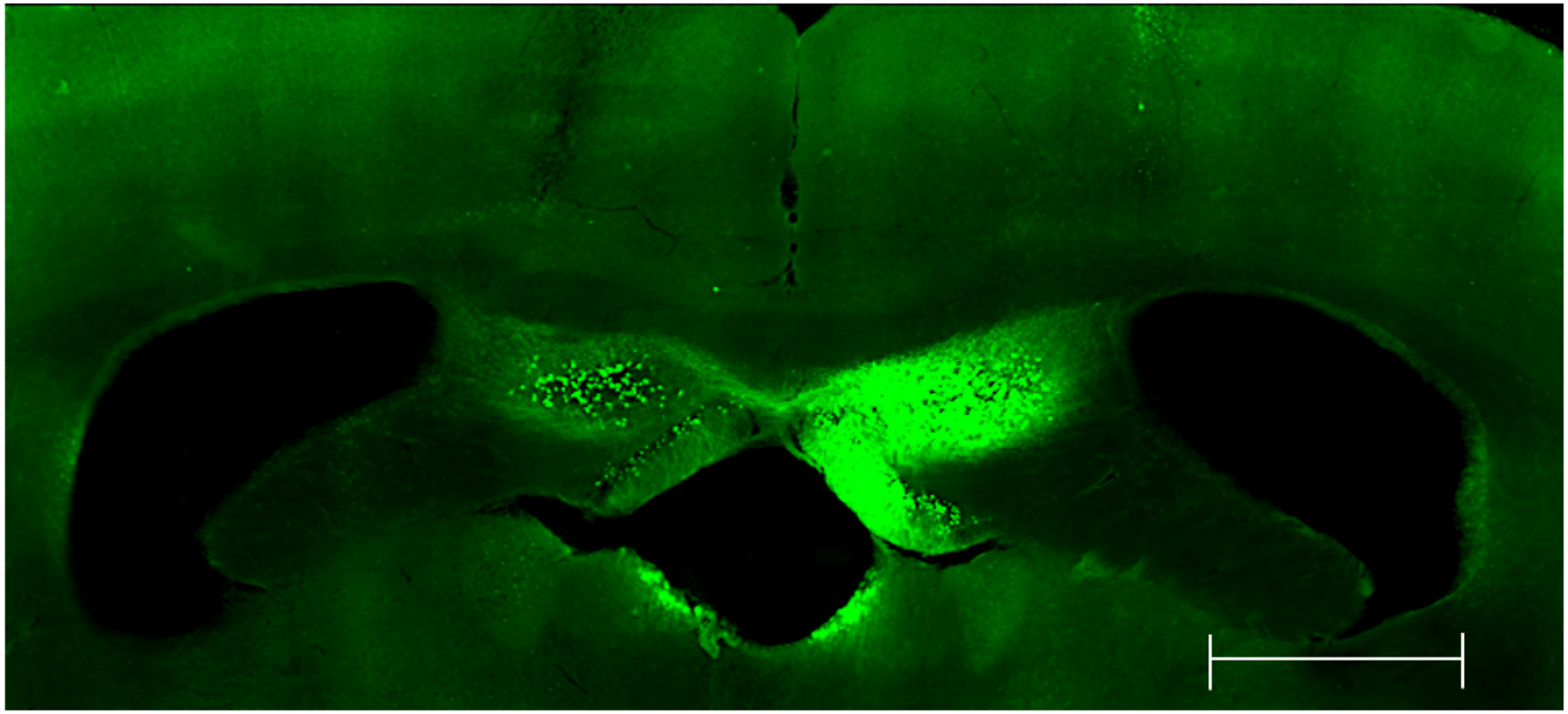


Figure 3 a. **Off-Target eGFP Expression of Fluorescent Reporter in Anterior Thalamic Nuclei**. 2/2 eGFP subject that was included in the study displaying viral leakage during histological analysis. Occurring in the hippocampal region, leakage likely occurred during removal of the infusion needle during surgery. While anterior dorsal thalamus had been targeted, a majority of control virus had dispensed superiorly into hippocampus. 50 µm; -0.94mm.

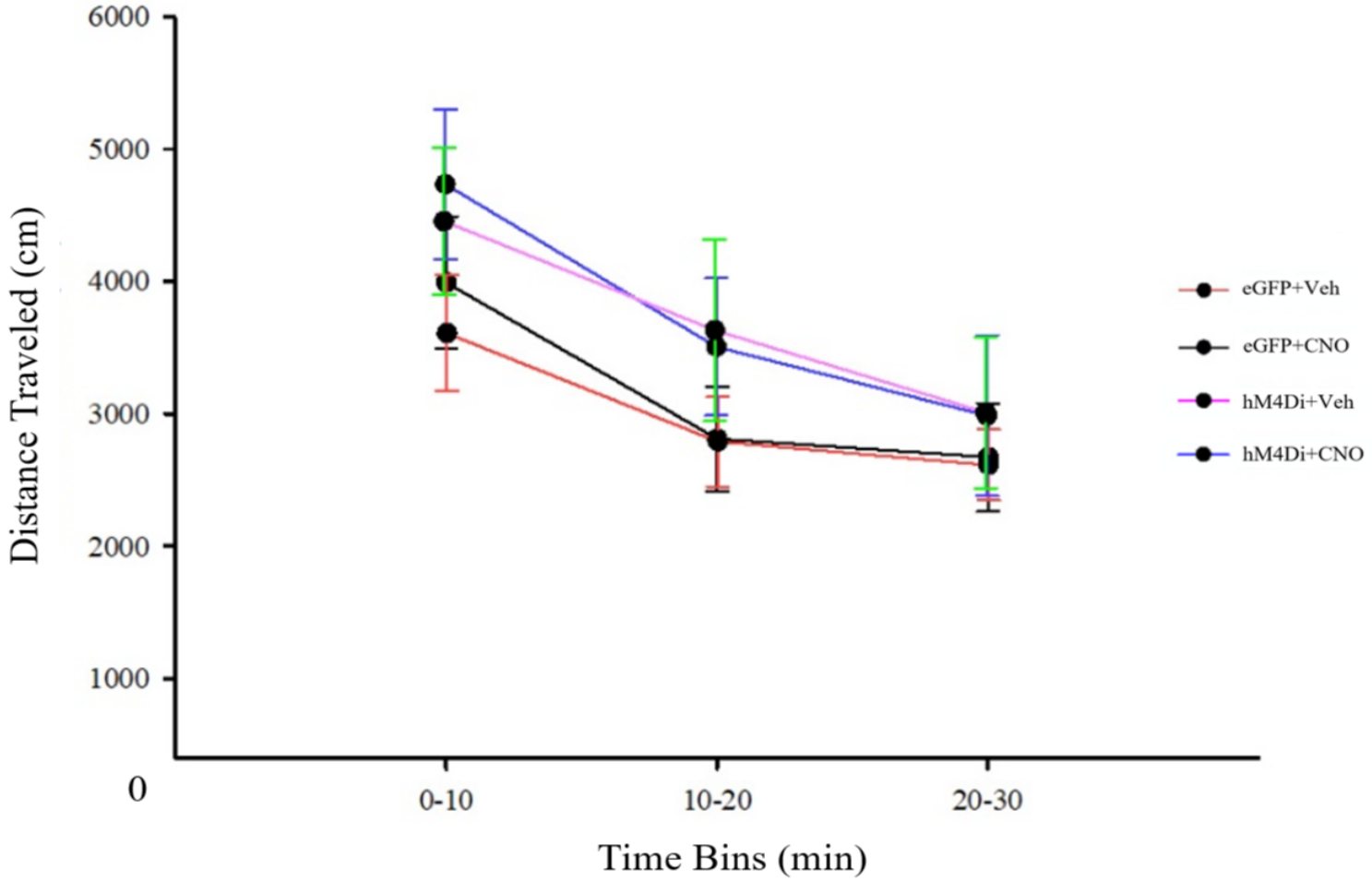


Figure 4. **Distance Traveled – Open Field.** Depicts mean (± S.E.M) distance moved (cm) across three 10-min time bins for each viral condition and treatment in the Open Field arena.

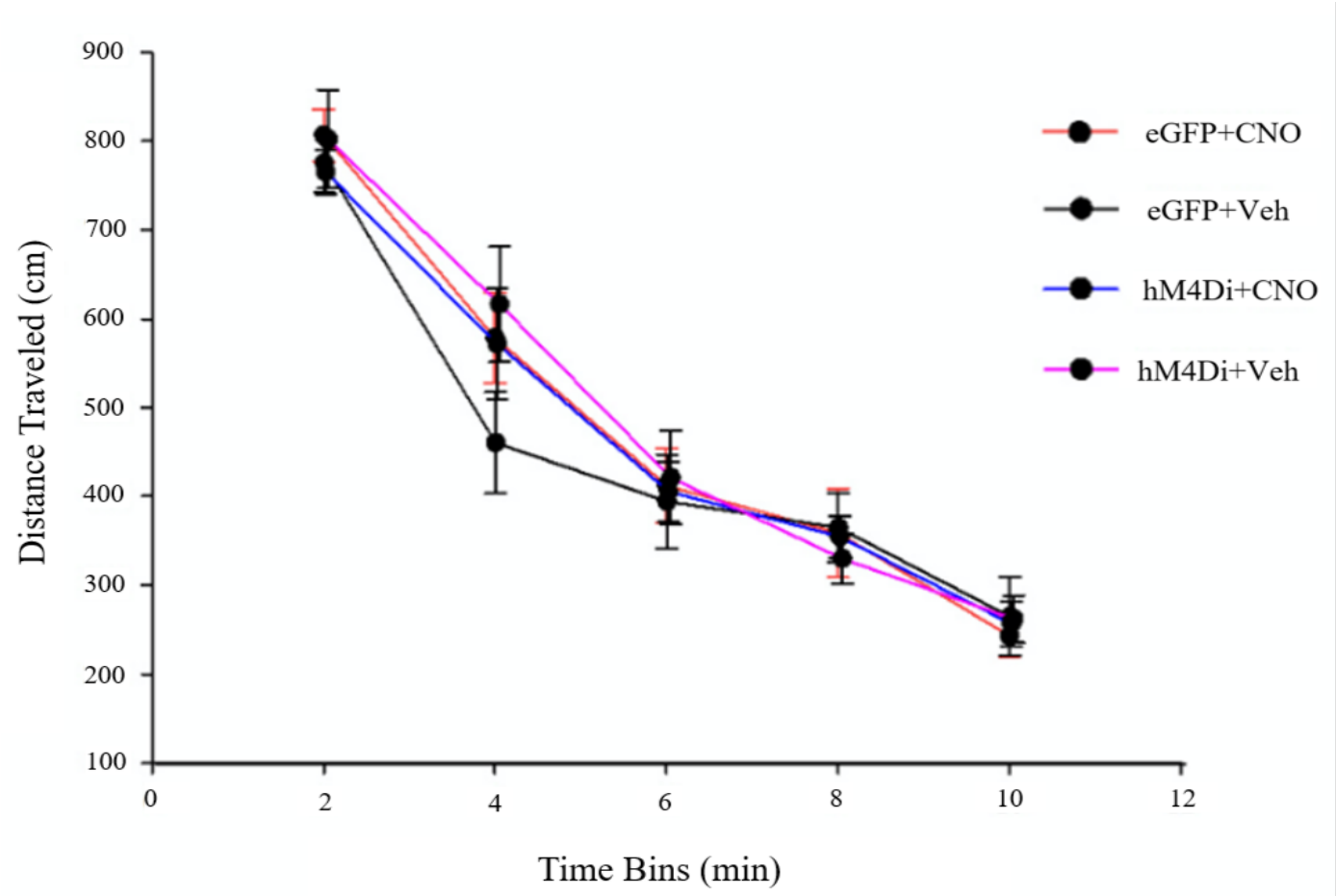


Figure 5. **Distance Traveled – Linear Track.** Depicts mean (± S.E.M) distance moved (cm) across five 2-min time bins for each viral condition and treatment in the Linear Track arena.

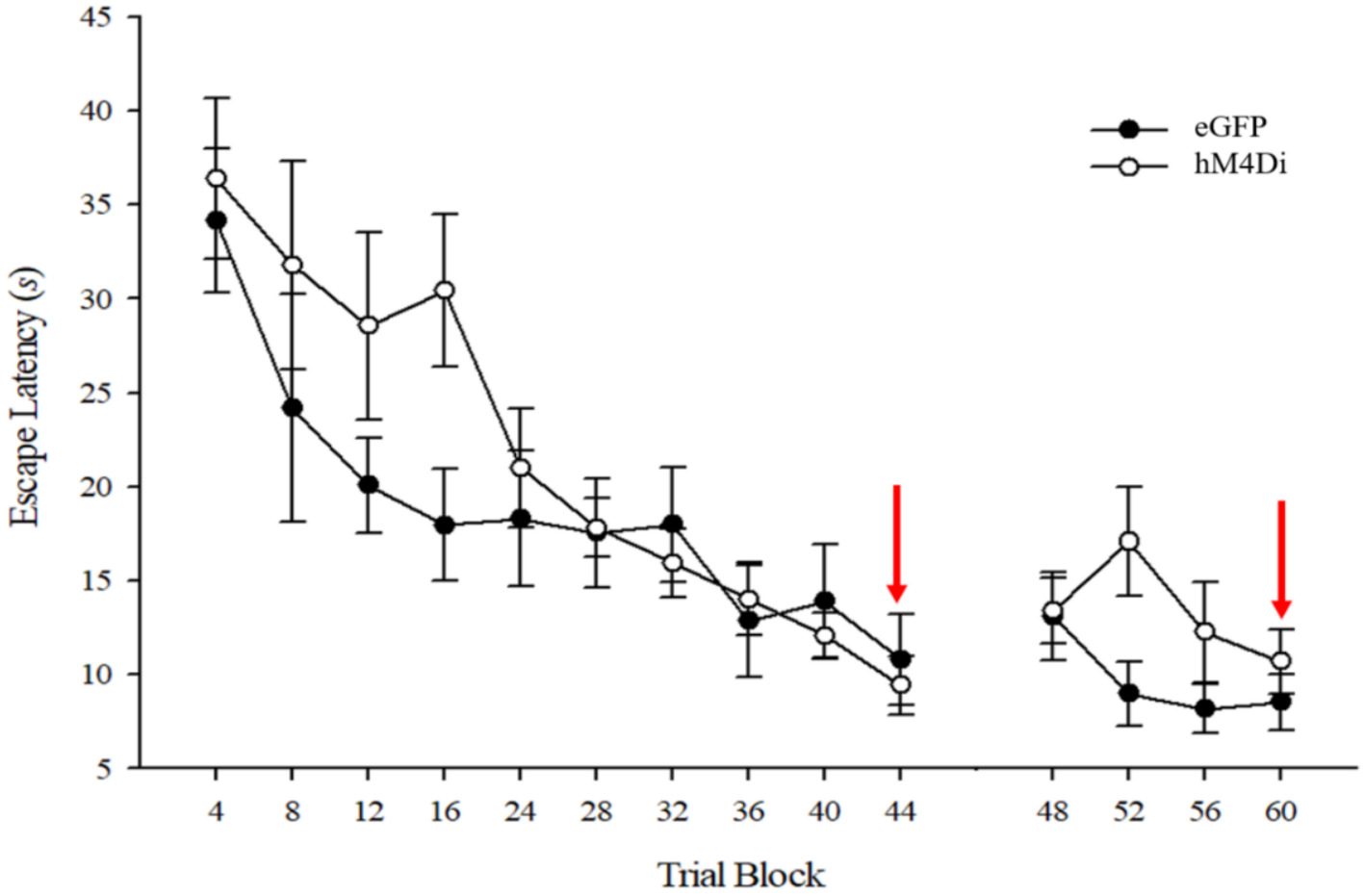


Figure 6. **MWM: Hidden Platform Training.** Depiction of average latency to first reach the hidden platform (in *s*) by four-trial blocks per day, between eGFP and hM4Di viral groups. Arrows indicate when each probe test was presented. Four-days of re-training was implemented prior to the 2nd probe test. Error bars represent ± S.E.M.

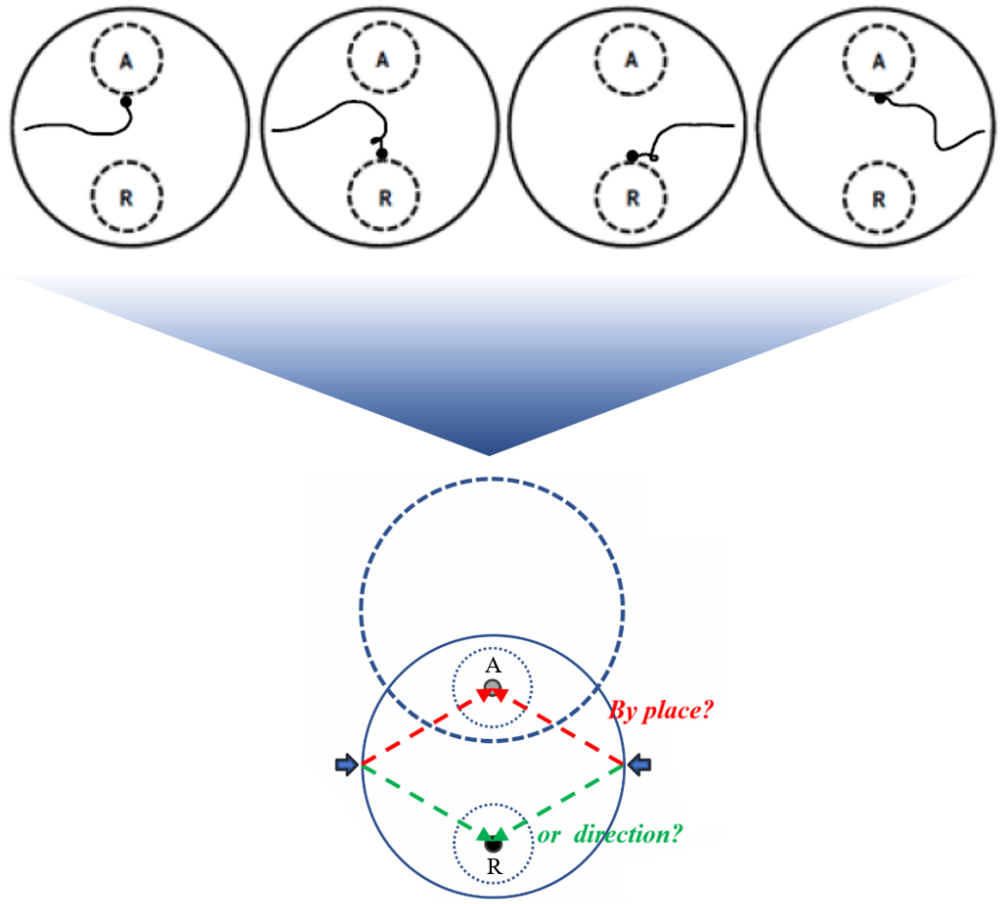


Figure 7. **Relative vs Absolute Swim Paths.** Representative probe test swim paths from release to arrival at the preferred search zone for four mice. Paths depict the swim path to either the relative (direction) or absolute (place) search zones.

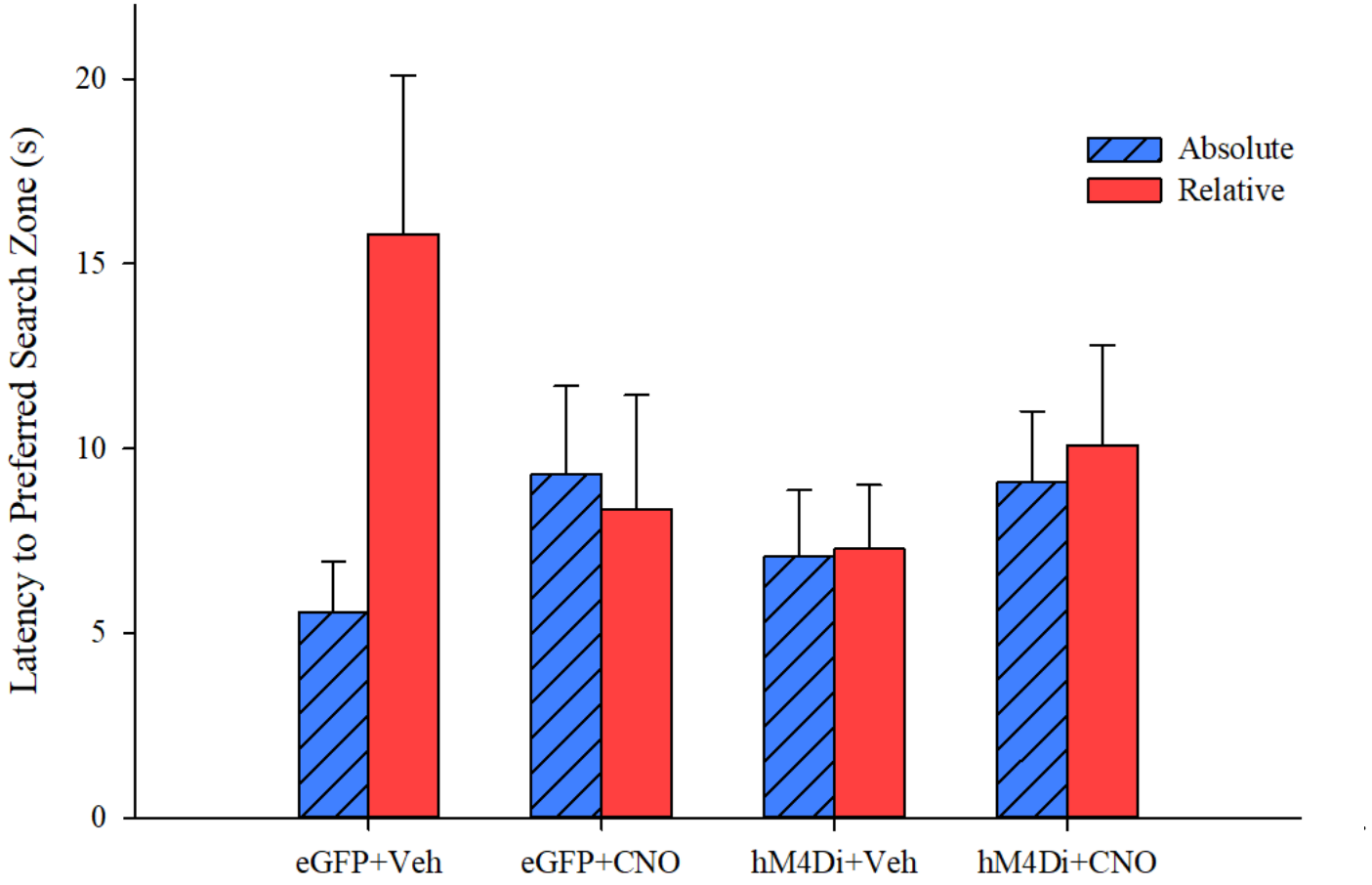
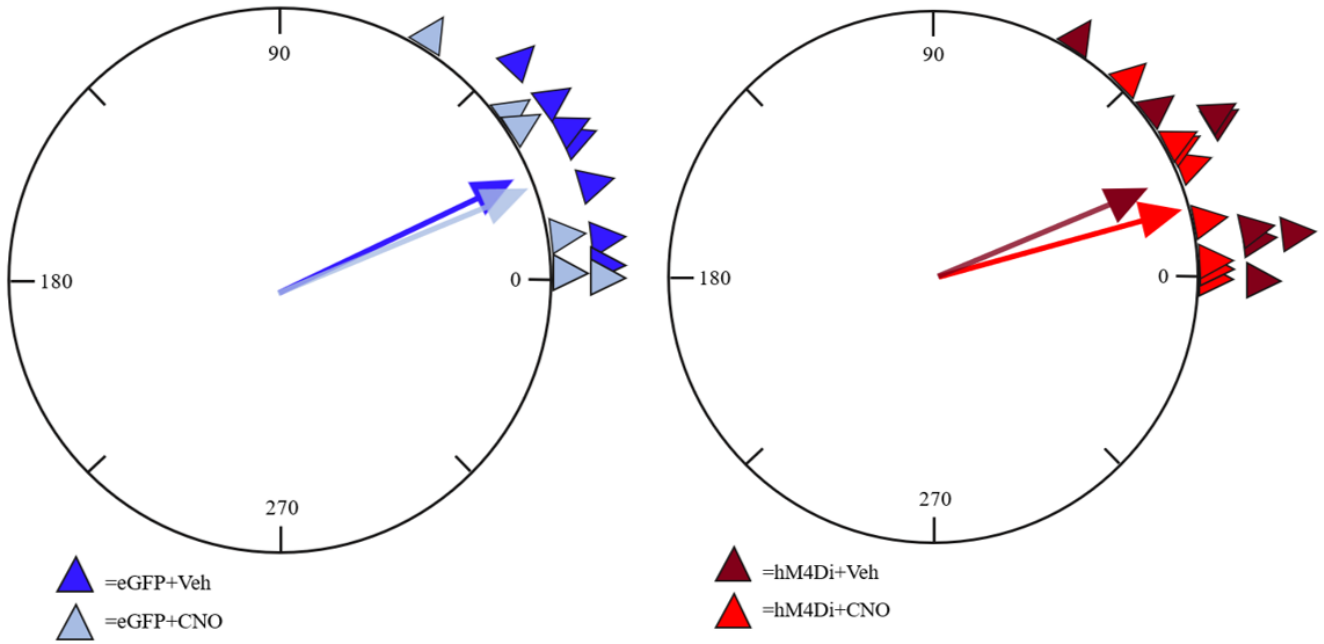


Figure 8. **MWM: Search Preference – Probe.** Average latency to first entry (in *s*) to relative or absolute search zone. Each subject per viral group was evaluated on first entry into either the relative or absolute search zone across probe 1 and 2.



a. *b.*

Figure 9. **Heading Error: eGFP (a) & hM4Di (b).** The polar plots of the distributions of individual heading error measures for the eGFP control viral group per treatment day across both probes (left), and for the hM4Di experimental viral group per treatment day across both probes (right).

REFERENCES

Aggleton, J. P., Hunt, P. R., Nagle, S., & Neave, N. (1996). The effects of selective lesions within the anterior thalamic nuclei on spatial memory in the rat. *Behavioural Brain Research*, *81*(1-2), 189–198. https://doi.org/10.1016/s0166-4328(96)89080-2

Aggleton, J. P., & Nelson, A. J. D. (2015). Why do lesions in the rodent anterior thalamic nuclei cause such severe spatial deficits? *Neuroscience & Biobehavioral Reviews*, *54*, 131–144. https://doi.org/10.1016/j.neubiorev.2014.08.013

Ainge, J. A., & Langston, R. F. (2012). Ontogeny of neural circuits underlying spatial memory in the rat. *Frontiers in Neural Circuits*, *6*. https://doi.org/10.3389/fncir.2012.00008

Aronov, D., & Tank, D. W. (2014). Engagement of neural circuits underlying 2D spatial navigation in a rodent virtual reality system. *Neuron*, *84*(2), 442–456. https://doi.org/10.1016/j.neuron.2014.08.042

Baddeley, A. (1992). Working memory. *Science*, *255*(5044), 556–559. https://doi.org/10.1126/science.1736359

Bassett, J.P., & Taube, J.S. (2001). Neural correlates for angular head velocity in the rat dorsal tegmental nucleus. *Journal of Neuroscience, 21*(15):5740-51. doi: 10.1523/JNEUROSCI.21-15-05740.2001

Béracochéa, D. J., & Jaffard, R. (1994). Effects of anterior thalamic lesions on spatial memory in mice. *NeuroReport*, *5*(8), 917–920. https://doi.org/10.1097/00001756-199404000-00016

Bertram, E. H., & Zhang, D. X. (1999). Thalamic excitation of hippocampal CA1 neurons: a comparison with the effects of CA3 stimulation. *Neuroscience*, *92*(1), 15–26. https://doi.org/10.1016/s0306-4522(98)00712-x

Blair, H. T., Cho, J., & Sharp, P. E. (1998). Role of the lateral mammillary nucleus in the Rat Head Direction Circuit. *Neuron*, *21*(6), 1387–1397. https://doi.org/10.1016/s0896-6273(00)80657-1

Blair, H. T., Cho, J., & Sharp, P. E. (1999). The anterior thalamic head-direction signal is abolished by bilateral but not unilateral lesions of the lateral mammillary nucleus. *The Journal of Neuroscience: the official journal of the Society for Neuroscience*, *19*(15), 6673–6683. https://doi.org/10.1523/JNEUROSCI.19-15-06673.1999

Blair, H. T., & Sharp, P. E. (1995). Anticipatory head direction signals in anterior thalamus: Evidence for a thalamocortical circuit that integrates angular head motion to compute head direction. *The Journal of Neuroscience*, *15*(9), 6260–6270. https://doi.org/10.1523/jneurosci.15-09-06260.1995

Bolding, K. A., Ferbinteanu, J., Fox, S. E., & Muller, R. U. (2019). Place cell firing cannot support navigation without intact septal circuits. *Hippocampus*, *30*(3), 175–191. https://doi.org/10.1002/hipo.23136

Burgess, N., Recce, M., & O'Keefe, J. (1994). A Model of Hippocampal Function. *Neural Networks, 7*, 1065-1081. https://doi.org/10.1016/S0893-6080(05)80159-5

Calton, J. L., Stackman, R. W., Goodridge, J. P., Archey, W. B., Dudchenko, P. A., & Taube, J. S. (2003). Hippocampal place cell instability after lesions of the head direction Cell Network. *The Journal of Neuroscience*, *23*(30), 9719–9731. https://doi.org/10.1523/jneurosci.23-30-09719.2003

Cinalli, D. A., Cohen, S. J., Calubag, M., Oz, G., Zhou, L., & Stackman, R. W. (2022). DREADD-inactivation of dorsal CA1 pyramidal neurons in mice impairs retrieval of object and spatial memories. *Hippocampus*, 33(1), 6– 17. https://doi.org/10.1002/hipo.23484

Clark, B. J., and Taube, J. S. (2012). Vestibular and attractor network basis of the head direction cell signal in subcortical circuits. *Front. Neural Circuits,* 6:7. doi: 10.3389/fncir.2012.00007

Cohen, S. J., & Stackman Jr., R. W. (2015). Assessing rodent hippocampal involvement in the novel object recognition task. a review. *Behavioural Brain Research*, *285*, 105–117. https://doi.org/10.1016/j.bbr.2014.08.002

Ekstrom, A. D., Kahana, M. J., Caplan, J. B., Fields, T. A., Isham, E. A., Newman, E. L., & Fried, I. (2003). Cellular networks underlying human spatial navigation. *Nature, 425*(6954),184-188. doi: 10.1038/nature01964

Evangelio, M., García-Amado, M., & Clascá, F. (2018). Thalamocortical Projection Neuron and Interneuron Numbers in the Visual Thalamic Nuclei of the Adult C57BL/6 Mouse. *Frontiers in neuroanatomy*, *12*, 27. https://doi.org/10.3389/fnana.2018.00027

Fenner, J., Heathcote, D., & Jerrams-Smith, J. (2000). The development of wayfinding competency: Asymmetrical effects of visuo-spatial and verbal ability. *Journal of Environmental Psychology*, *20*(2), 165–175. https://doi.org/10.1006/jevp.1999.0162

Fiáth, R., Beregszászi, P., Horváth, D., Wittner, L., Aarts, A. A., Ruther, P., Neves, H. P., Bokor, H., Acsády, L., & Ulbert, I. (2016). Large-scale recording of thalamocortical circuits: In vivo electrophysiology with the two-dimensional electronic depth control silicon probe. *Journal of Neurophysiology*, *116*(5), 2312–2330. https://doi.org/10.1152/jn.00318.2016

Frost, B. E., Martin, S. K., Cafalchio, M., Islam, M. N., Aggleton, J. P., & O'Mara, S. M. Anterior thalamic function is required for spatial coding in the subiculum and is necessary for spatial memory. *Journal of Neuroscience, 41*(30), 6511-6525. https://doi.org/10.1523/JNEUROSCI.2868-20.2021

Garden, D. L., Massey, P. V., Caruana, D. A., Johnson, B., Warburton, E. C., Aggleton, J. P., & Bashir, Z. I. (2009). Anterior thalamic lesions stop synaptic plasticity in

retrosplenial cortex slices: Expanding the pathology of Diencephalic Amnesia. *Brain*, *132*(7), 1847–1857. https://doi.org/10.1093/brain/awp090

Goodridge, J. P., & Taube, J. S. (1997). Interaction between the Postsubiculum and Anterior Thalamus in the Generation of Head Direction Cell Activity. *Journal of Neuroscience*, *17*(23), 9315-9330. https://doi.org/10.1523/JNEUROSCI.17-23-09315.1997

Hamilton, D. A., Akers, K. G., Weisend, M. P., & Sutherland, R. J. (2007). How do room and apparatus cues control navigation in the Morris water task? evidence for distinct contributions to a movement vector. *Journal of Experimental Psychology: Animal Behavior Processes*, *33*(2), 100–114. https://doi.org/10.1037/0097-7403.33.2.100

Han, Z.-S., Buhl, E. H., Lörinczi, Z., & Somogyi, P. (1993). A high degree of spatial selectivity in the axonal and dendritic domains of physiologically identified local-circuit neurons in the dentate gyms of the Rat Hippocampus. *European Journal of Neuroscience*, *5*(5), 395–410. https://doi.org/10.1111/j.1460-9568.1993.tb00507.x

Harvey, C. D., Coen, P. & Tank, D. W. (2012). Choice-specific sequences in parietal cortex during a virtual-navigation decision task. *Nature, 484,* 62–68. https://doi.org/10.1038/nature10918

Harvey, C. D., Collman, F., Dombeck, D. A., & Tank, D. W. (2009). Intracellular dynamics of hippocampal place cells during virtual navigation. *Nature*, *461*(7266), 941–946. https://doi.org/10.1038/nature08499

Jacobs, J., Weidemann, C. T., Miller, J. F., Solway, A., Burke, J. F., Wei, X. X., Suthana, N., Sperling, M. R., Sharan, A. D., Fried, I., & Kahana, M. J. (2013). Direct recordings of grid-like neuronal activity in human spatial navigation. *Nature Neuroscience, 16*(9), 1188–90. doi:10.1038/nn.3466

Jendryka, M., Palchaudhuri, M., Ursu, D., van der Veen, B., Liss, B., Kätzel, D., Nissen, W., & Pekcec, A. (2019). Pharmacokinetic and pharmacodynamic actions of clozapine-N-oxide, clozapine, and compound 21 in DREADD-based chemogenetics in mice. *Scientific reports*, *9*(1), 4522. https://doi.org/10.1038/s41598-019-41088-2

Kay, K., Sosa, M., Chung, J.E., Karlsson, M.P., Larkin, M.C., & Frank, L.M. (2016). A hippocampal network for spatial coding during immobility and sleep. *Nature*, *531*(7593),185-90. doi: 10.1038/nature17144

Kealy, J., Diviney, M., Kehoe, E., McGonagle, V., O'Shea, A., Harvey, D., & Commins, S. (2008). The effects of overtraining in the Morris water maze on allocentric and egocentric learning strategies in rats. *Behavioural Brain Research, 192*(2), 259–263. https://doi.org/10.1016/j.bbr.2008.04.009

Lisa Kinnavane, L., Vann, S.D., Nelson, A.J.D., O'Mara, S.M., & Aggleton, J.P. (2018). Collateral Projections Innervate the Mammillary Bodies and Retrosplenial Cortex: A New Category of Hippocampal Cells. *eNeuro, 5*(1), ENEURO.0383-17.2018; DOI: https://doi.org/10.1523/ENEURO.0383-17.2018

Knierim, J. J., Neunuebel, J. P., & Deshmukh, S. S. (2014). Functional correlates of the lateral and medial entorhinal cortex: Objects, path integration and local–global reference frames. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *369*(1635), 20130369. https://doi.org/10.1098/rstb.2013.0369

Maguire, E. A., Burgess, N., Donnett, J. G., Frackowiak, R. S., Frith, C. D., & O'Keefe, J. (1998). Knowing where and getting there: a human navigation network. *Science*, *280*(5365), 921-924. doi: 10.1126/science.280.5365.921.

McNaughton, B. L., Barnes, C. A., Gerrard, J. L., Gothard, K., Jung, M. W., Knierim, J. J., Kudrimoti, H., Qin, Y., Skaggs, W. E., Suster, M., & Weaver, K. L. (1996). Deciphering The Hippocampal Polyglot: the Hippocampus as a Path Integration System. *Journal of Experimental Biology*, *199*(1), 173–185. doi: https://doi.org/10.1242/jeb.199.1.173

McNaughton, B. L., Battaglia, F. P., Jensen, O., Moser, E. I., & Moser, M.-B. (2006). Path integration and the neural basis of the 'cognitive map'. *Nature Reviews Neuroscience*, *7*(8), 663–678. https://doi.org/10.1038/nrn1932

Mitchell, A. S., & Dalrymple-Alford, J. C. (2006). Lateral and anterior thalamic lesions impair independent memory systems. *Learning & Memory*, *13*(3), 388–396. https://doi.org/10.1101/lm.122206

Morellini, F. (2013). Spatial memory tasks in rodents: What do they model? *Cell and Tissue Research*, *354*(1), 273–286. https://doi.org/10.1007/s00441-013-1668-9

Morris, R. G., Garrud, P., Rawlins, J. N., & O'Keefe, J. (1982). Place navigation impaired in rats with hippocampal lesions. *Nature*, *297*(5868), 681–683. https://doi.org/10.1038/297681a0

Moser, E. I., Kropff, E., & Moser, M.-B. (2008). Place cells, grid cells, and the brain's spatial representation system. *Annual Review of Neuroscience*, *31*(1), 69–89. https://doi.org/10.1146/annurev.neuro.31.061307.090723

Mou, W., McNamara, T. P., Rump, B., & Xiao, C. (2006). Roles of egocentric and allocentric spatial representations in locomotion and reorientation. *Journal of Experimental Psychology: Learning, Memory, and Cognition*, *32*(6), 1274–1290. https://doi.org/10.1037/0278-7393.32.6.1274

O'Keefe, J., Burgess, N., Donnett, J. G., Jeffery, K. J., & Maguire, E. A. (1998). Place cells, navigational accuracy, and the human hippocampus. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, *353*(1373), 1333–1340. https://doi.org/10.1098/rstb.1998.0287

O'Keefe, J., & Nadel, L. (1978). 4.8.1 (b). The CA3 field in *The hippocampus as a cognitive map* (pp. 226–229). Clarendon & Oxford University Press.

Poirier, G. L., Shires, K. L., Sugden, D., Amin, E., Thomas, K. L., Carter, D. A., & Aggleton, J. P. (2008). Anterior thalamic lesions produce chronic and profuse transcriptional deregulation in retrosplenial cortex: A model of retrosplenial hypoactivity and covert pathology. *Thalamus & Related Systems*, *4*(01). https://doi.org/10.1017/s1472928808000368

Poulter, S., Lee, S.A., Dachtler, J., Wills, T.J., & Lever, C. (2021). Vector trace cells in the subiculum of the hippocampal formation. *Nat Neurosci*, *24*(2): 266-275. doi: 10.1038/s41593-020-00761-w.

Redish, A.D., & Touretzky, D.S. (1997). Cognitive maps beyond the hippocampus. *Hippocampus, 7*(1), 15-35. doi: 10.1002/(SICI)1098-1063(1997)7:1<15: AID-HIPO3>3.0.CO;2-6

Sasaki, T., Leutgeb, S., & Leutgeb, J. K. (2015). Spatial and memory circuits in the medial entorhinal cortex. *Current Opinion in Neurobiology*, *32*, 16–23. https://doi.org/10.1016/j.conb.2014.10.008

Seecharan, D. J., Kulkarni, A. L., Lu, L., Rosen, G. D., & Williams, R. W. (2003). Genetic control of interconnected neuronal populations in the mouse primary visual system. *The Journal of Neuroscience: the official journal of the Society for Neuroscience*, *23*(35), 11178–11188. https://doi.org/10.1523/JNEUROSCI.23-35-11178.2003

Sharp, P.E., Tinkelman, A., & Cho, J. (2001). Angular velocity and head direction signals recorded from the dorsal tegmental nucleus of gudden in the rat: implications for path integration in the head direction cell circuit. *Behavioral Neuroscience,115*(3):571-88. https://pubmed.ncbi.nlm.nih.gov/11439447/

Smith, K. S., Bucci, D. J., Luikart, B. W., & Mahler, S. V. (2021). Dreadds: Use and application in behavioral neuroscience. *Behavioral Neuroscience*, *135*(2), 89–107. https://doi.org/10.1037/bne0000433

Sorrentino, P., Lardone, A., Pesoli, M., Liparoti, M., Montuori, S., Curcio, G., Sorrentino, G., Mandolesi, L., & Foti, F. (2019). The development of spatial memory analyzed by means of Ecological Walking Task. *Frontiers in Psychology*, *10*. https://doi.org/10.3389/fpsyg.2019.00728

Squire, L. R., & Dede, A. J. O. (2015). Conscious and unconscious Memory Systems. *Cold Spring Harbor Perspectives in Biology*, *7*(3). https://doi.org/10.1101/cshperspect.a021667

Squire, L. R., & Zola, S. M. (1996). Structure and function of declarative and nondeclarative memory systems. *Proceedings of the National Academy of Sciences*, *93*(24), 13515–13522. https://doi.org/10.1073/pnas.93.24.13515

Stackman, R. W., Lora, J. C., & Williams, S. B. (2012). Directional responding of C57BL/6J mice in the Morris water maze is influenced by visual and vestibular cues and is dependent on the anterior thalamic nuclei. *Journal of Neuroscience*, *32*(30), 10211–10225. https://doi.org/10.1523/jneurosci.4868-11.2012

Taube, J. S. (1995). Head direction cells recorded in the anterior thalamic nuclei of freely moving rats. *The Journal of Neuroscience*, *15*(1), 70–86. https://doi.org/10.1523/jneurosci.15-01-00070.1995

Taube, J. S. (2007). The head direction signal: Origins and sensory-motor integration. *Annual Review of Neuroscience*, *30*(1), 181–207. https://doi.org/10.1146/annurev.neuro.29.051605.112854

Taube, J. (2009). Head direction cells. *Scholarpedia*, *4*(12), 1787. https://doi.org/10.4249/scholarpedia.1787

Teng, E., & Squire, L. R. (1999). Memory for places learned long ago is intact after hippocampal damage. *Nature, 400*(6745), 675-677. doi: 10.1038/23276.

Tsanov, M., Chah, E., Vann, S. D., Reilly, R. B., Erichsen, J. T., Aggleton, J. P., et al. (2011a). Theta-modulated head direction cells in the rat anterior thalamus. *J. Neurosci*. 31, 9489–9502. doi: 10.1523/JNEUROSCI.0353-11.2011

Tuscher, J. J., Taxier, L. R., Fortress, A. M., & Frick, K. M. (2018). Chemogenetic inactivation of the dorsal hippocampus and medial prefrontal cortex, individually and concurrently, impairs object recognition and spatial memory consolidation in female mice. *Neurobiology of Learning and Memory*, *156*, 103–116. https://doi.org/10.1016/j.nlm.2018.11.002

Urban, D. J., & Roth, B. L. (2015). DREADDs (designer receptors exclusively activated by designer drugs): Chemogenetic Tools with therapeutic utility. *Annual Review of Pharmacology and Toxicology*, *55*(1), 399–417. https://doi.org/10.1146/annurev-pharmtox-010814-124803

van Groen, T., Kadish, I., & Michael Wyss, J. (2002). Role of the anterodorsal and anteroventral nuclei of the thalamus in spatial memory in the rat. *Behavioural Brain Research*, *132*(1), 19–28. https://doi.org/10.1016/s0166-4328(01)00390-4

Vann S. D. (2005). Transient spatial deficit associated with bilateral lesions of the lateral mammillary nuclei. *The European journal of neuroscience*, *21*(3), 820–824. https://doi.org/10.1111/j.1460-9568.2005.03896.x

Vann S. D. (2011). A role for the head-direction system in geometric learning. *Behavioural brain research*, *224*(1), 201–206. https://doi.org/10.1016/j.bbr.2011.05.033

Vann, S. D., & Albasser, M. M. (2009). Hippocampal, retrosplenial, and prefrontal hypoactivity in a model of diencephalic amnesia: Evidence towards an interdependent subcortical-cortical memory network. *Hippocampus*, *19*(11), 1090–1102. https://doi.org/10.1002/hipo.20574

Vantomme, G., Rovó, Z., Cardis, R., Béard, E., Katsioudi, G., Guadagno, A., Perrenoud, V., Fernandez, L. M. J., & Lüthi, A. (2019). A thalamic reticular circuit for head direction cell tuning and Spatial Navigation. *Cell Reports*, *31*(10). https://doi.org/10.1101/804575

Viejo, G., Peyrache, A. (2020). Precise coupling of the thalamic head-direction system to hippocampal ripples. *Nature Communications,* 11, (2524). https://doi.org/10.1038/s41467-020-15842-4

Wang, G.-W., & Cai, J.-X. (2006). Disconnection of the hippocampal–prefrontal cortical circuits impairs spatial working memory performance in rats. *Behavioural Brain Research*, *175*(2), 329–336. https://doi.org/10.1016/j.bbr.2006.09.002

Whitaker, H. A. (Ed.). (2016). *Concise encyclopedia of brain and language* (1st ed.). ELSEVIER.

Wolff, M., Gibb, S. J., Cassel, J.-C., & Dalrymple-Alford, J. C. (2008). Anterior but not intralaminar thalamic nuclei support allocentric spatial memory. *Neurobiology of Learning and Memory*, *90*(1), 71–80. https://doi.org/10.1016/j.nlm.2008.01.007

Wolff, M., Loukavenko, E. A., Will, B. E., & Dalrymple-Alford, J. C. (2008). The extended hippocampal-diencephalic memory system: Enriched housing promotes recovery of the flexible use of spatial representations after anterior thalamic lesions. *Hippocampus*, *18*(10), 996–1007. https://doi.org/10.1002/hipo.20457

Whishaw, I.Q., & Jarrard, L.E. (1996). Evidence for extrahippocampal involvement in place learning and hippocampal involvement in path integration. *Hippocampus, 6*(5), 513-524. doi: 10.1002/(SICI)1098-1063(1996)6:5<513: AID-HIPO4>3.0.CO;2-J

Xiang, JZ, Brown, MW (2004) Neuronal responses related to long-term recognition memory processes in prefrontal cortex. *Neuron*, 42(5): 817–829.

Xiao, D, Barbas, H (2002) Pathways for emotions and memory: II. Afferent input to the anterior thalamic nuclei from prefrontal, temporal, hypothalamic areas and the basal ganglia in the rhesus monkey. *Thalamus & Related Systems,* 2(1): 33–48.

Yoganarasimha, D. (2006). Head direction cell representations maintain internal coherence

during conflicting proximal and distal cue rotations: Comparison with hippocampal place cells. *Journal of Neuroscience*, *26*(2), 622–631. https://doi.org/10.1523/jneurosci.3885-05.2006