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by

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ABSTRACT

Previous studies have shown that the anterior thalamic nuclei (ATN) contain a large population of head direction cells, which fire as a function of an animal's directional orientation in an environment, thereby providing a compass-like representation guiding navigation. Recent work has suggested that directional orientation information stemming from the ATN is critical for the generation of hippocampal and parahippocampal spatial representations, and may contribute to the establishment of unique spatial representations in radially oriented tasks such as the radial arm maze. While studies have confirmed that ATN lesions impair the acquisition of new spatial information in variants of the radial maze, few have attempted to dissociate its unique contributions to acquisition vs. retrieval and spatial reference vs working memory in radial tasks. Here, we addressed these questions by training rats in a radial arm maze procedure to asymptotic levels, and after 24hrs, animals were administered muscimol inactivation of the ATN before a 4 trial probe test. We report impairments in retrieval of both spatial reference and working memory, suggesting a general absence of improved navigation across post-inactivation training trials. Taken together, the results above suggest that the ATN modulates the retrieval of

previously acquired allocentric spatial information in the radial-arm maze, but also suggests a critical role in the online guidance of accurate spatial behavior. The results are discussed in relation to the thalamo-cortical circuits involved in spatial information processing.

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Introduction

The ability to navigate depends on neural systems involved in tracking an animal's moment-to-moment changes in directional orientation and spatial location when moving from one place to another (McNaughton et al., 2006; Moser et al., 2008; Taube, 2007). Previous studies have shown that a critical component of the neurobiology of spatial orientation is the anterior thalamic nuclei (ATN), which is embedded within the classic Papez circuit and has strong interconnections with diencephalic subcortical nuclei as well as hippocampal, parahippocampal, parietal, and retrosplenial cortices (Aggleton et al., 2010; Clark & Harvey, 2016; Wilber et al., 2015). The ATN contain a large proportion of neurons that are modulated by an animal's directional heading, called "head direction cells" (Jankowski et al., 2015; Mizumori & Williams, 1993; Taube, 2007; Tsanov et al., 2011). The directional orientation of ATN head direction cells are strongly influenced by allothetic spatial stimuli such as environmental landmarks, in the absence of stable environmental features, the orientation of head direction cells can be maintained by self-movement cues such as vestibular, proprioceptive, and motor stimuli (Clark & Taube, 2009; Clark et al., 2012; Shinder & Taube, 2011; Yoder et al., 2011).

The role of the ATN and head direction cell activity in spatial memory has received considerable attention, but its precise functions are poorly understood (Aggleton et al., 2010; Clark & Harvey, 2016). One hypothesis suggests that directional signals conveyed via the ATN may influence the generation and/or stability of hippocampal and parahippocampal spatial representations (McNaughton et al., 1991; Yoganarasimha et al., 2006). Supporting this hypothesis, experiments have shown that inactivation or lesions of the ATN abolish parahippocampal grid cell activity (Winter et al., 2015), and lesions of the ATN reduce the

spatial specificity of hippocampal place cells (Calton et al., 2003). Further, recent studies have demonstrated a tendency for place cells and grid cells to establish unique spatial firing patterns in radially oriented environments or in environments with opposed directions, but form similar firing patterns in parallel environments with similar directional orientations (Derdikman et al., 2009; Fuhs et al., 2005; Grieves et al., 2016; Speirs et al., 2015). This observation points to the possibility that representations of directional orientation, possibly based on ascending thalamic head direction signals to the hippocampal formation, might facilitate the generation of distinct spatial representations in radial environments (Grieves et al., 2016; Sanchez et al., 2016).

Whether the ATN and head direction cell activity is involved in disambiguating locations in radially oriented environments has been investigated in recent studies (reviewed in Clark & Harvey, 2016). For instance, Yoder and Kirby (2014) measured the spatial behavior of vestibular deficient (otoconia knockout) mice, an animal model previously shown to impair ATN head direction cell signals (Yoder & Taube, 2009), in a reference memory variant of the radial arm and open field (Barnes maze) tasks. While the authors reported that the vestibular mice exhibited spatial memory deficits in the radial arm maze, animals were unimpaired in the open field task. Experiments in which the ATN has been specifically targeted by neurotoxic lesions and behavior subsequently monitored in radial maze tasks are generally consistent with the results of Yoder & Kirby (Aggleton et al., 1996; Beracochea et al., 1989; Mair et al., 2003; Mitchell, & Dalrymple-Alford, 2005; 2006; Wolff et al., 2008; for review, see Yoder & Taube, 2014). Nevertheless, many of these studies have utilized procedures in which working memory and the acquisition of non-spatial strategies are favored over spatial reference memory (Dubreuil et al., 2003; Olton & Samuelson, 1976). Further, the role of the ATN in the retrieval of previously learned spatial information has received limited attention, and is complicated by the fact that while some studies

have reported retrieval deficits in animals after post-acquisition lesions of the ATN (Alexinsky, 2001; Warburton et al., 1999), others have reported that the expression of reference memory for spatial locations can be maintained after ATN disruption (Stackman et al., 2012; Sutherland & Rodriguez, 1989).

In the present study we aimed to evaluate the role of the ATN in spatial behavior in the radial arm maze. We adopted a radial maze procedure in which two maze arms were consistently baited, thus allowing evaluation of both working and reference memory. To determine whether the ATN contributes to the retrieval of spatial memory in radial environments, we first pre-trained rats in the radial arm maze, and then inactivated the ATN with a local infusion of muscimol. Here, we report that inactivation of the ATN causes deficits in the expression of a previously acquired spatial representation in the radial arm maze, and produces impairments in both working and reference memory. The results suggest a critical role for the ATN in the retrieval and online guidance of spatial behavior in radially oriented environments.

Methods

Subjects. Subjects were 12 male hooded Long-Evans rats (Harlan, Indianapolis, IN) that were approximately 160 days of age at the beginning of the experiments. All animals were pair-housed in plastic cages on a reverse 12 h light:dark cycle with food and water available *ad libitum*. During habituation-training and experiments, rats were placed on a restricted food diet to maintain 90% of their *ad libitum* weight. Rats were given access to water *ad libitum*. The Institutional Animal Care and Use Committee at the University of New Mexico approved all procedures for the studies reported here.

Surgery. Twelve rats were surgically implanted with custom fabricated bilateral cannula that targeted the anterior thalamus. The custom cannulas were made of two 26-gauge stainless steel outer cannula and 33-gauge inner dummy cannula. Animals were anesthetized with isoflurane and placed in a stereotaxic frame with atraumatic ear bars. The head was adjusted in the frame to achieve flat skull coordinates. Anesthesia was maintained via an inhalation nose cone affixed to the mouth bar on the frame. Lidocaine (2%) was used as a local anesthetic underneath the skin above the skull. Under sterile conditions, a midline incision was made, and the skull exposed. The outer cannula were targeted just above the ATN such that the inner infusion cannula, which protruded ~1mm below the outer cannula, would be centrally placed within the ATN at the following coordinates relative to bregma: anterior-posterior -1.74 mm, medial-lateral 1.25 mm (2.48 mm between two cannula), dorsal-ventral (DV) -5.23 mm (DV coordinate measured from skull surface) and were held in place using dental acrylic. Coordinates were based on plates from Paxinos & Watson (1998) and previous histological assessment. After completion of the implantations, the skin was cleaned and sutured. The rats were given subcutaneous injections of buprenex (0.03 mg/ml concentration and a 0.1mg/kg dosage) right after surgery and once a day for two days. Following surgery, rats were single-housed to prevent damage to the implant. All rats were given 7 days to recover with unlimited access to food and water, followed by 7 days of food restriction prior to returning to the experiment.

Radial Arm Maze. The radial arm maze consisted of eight black Plexiglas arms (each 40.1cm × 9.30cm, separated by 45° from each other) that radiated out from a center platform (25 cm in diameter). One recessed reward cup was located on a platform (20cm x 30cm) at the distal end of each arms. The maze was located near a corner of a testing room with many extra-maze cues, including a sink, filing cabinet, chair, and wall posters. A transparent plastic cylinder (25

cm in diameter) located in the center of the maze was used to restrict the rats to that region of the maze before the initiation of a training trial. A camera was positioned above the maze and digital videos were obtained for each testing session for off-line analysis.

Habituation Trials. Rats first underwent 10 min habituation training trials on the radial arm maze over 3 consecutive days. During habituation, all of the recessed cups were baited with a food reward (quarter piece of dry cereal). In a trial, rats were first placed in the transparent cylinder located at the center of the maze for 15-30 seconds, followed by the removal of the cylinder, thereby allowing the rat to explore the maze and consume food from the food cups for the remaining time.

Acquisition Trials. Acquisition training occurred over 11 days in blocks of four trials per day. In this phase of training, only two of the eight arms (separated by 135°) were baited with a food reward. The spatial relationship between the baited maze arms and the room cues was maintained throughout training and the baited/un-baited arm configuration was counterbalanced between rats. At the beginning of each trial, the rat was placed in the cylinder in the center of the maze, where it remained for 15-30 seconds before starting the trial. To discourage the use of the experimenter as a cue, the direction in which the rat was placed in the cylinder was counterbalanced over trials. After the 15-30 seconds had elapsed, the rat was released and allowed to freely investigate the maze and search for the baited arms. Trials were terminated when the animal either located the baited arms and consumed the food reward, or after 5 minutes had elapsed. Once rats located and consumed the reward, they were returned to a transport cage for ~1 minute while the entire maze was cleaned with a non-toxic cleaning solution. The maze was cleaned and rotated 180° at the end of each day to discourage the use of intra-maze cues (e.g., local features and/or odors) between days.

Inactivation Probe. After acquisition, rats completed two probe trial blocks on the RAM using an un-blinded within-subjects cross design (Law & Smith 2012; Stackman et al., 2012). On day twelve, half of the rats received bilateral intracranial infusion of muscimol into the ATN (0.2-0.3ul at a concentration of 0.25ug/ul; Tocaris Bioscience), while the remaining rats received infusions of saline (0.2-0.3ul at a concentration of 0.9%). Infusions were administered by first gently restraining the rats, removing the dummy cannula, and then inserting the bilateral 33-gauge infusion cannula. Infusions were performed through two 10uL Hamilton syringes held in a Harvard Apparatus '22' syringe pump (Harvard Apparatus, MA). The infusions were delivered at a rate of 0.167/min for 1.5 min, infusion cannula remained in place for 30 sec after the infusions, and dummy cannula were then re-inserted. Each rat was returned to a transport cage for 30 min before being transported to the radial arm maze for the probe test. On day 13, all rats completed another trial block of radial arm maze testing to act as a recovery day, and on day 14, rats received a second intracranial infusion of muscimol or saline followed by a trial block in the radial arm maze using the same methods described above. On day 14, treatment conditions were reversed such that all rats received both muscimol and saline infusions.

Scoring and data analysis. Performance measures included the percentage of correct trials, the number of errors, and search latency calculated for each animal during acquisition and probe testing, as previously described by Yoder & Kirby (2014). Briefly, an arm choice was counted when all four of the rat's paws crossed the threshold of an arm. A correct choice was counted only if the rat approached and ate from a baited food cup. Error trials were categories into three subtypes: reference memory errors, working memory-correct errors, and working memory-incorrect errors. Reference memory errors occurred when a rat entered an unbaited arm or if they entered a baited arm without approaching and eating from the food cup. This partial

entry was classified as a reference memory error because the rat had made a choice (arm entry) that did not meet the criteria to be classified as a correct choice. Working memory-correct errors occurred when a rat re-entered an arm that had been previously baited. Working memory-incorrect errors occurred when a rat re-entered an arm that never contained a reward. Latency was measured as the time elapsed from the beginning to the end of each trial. All measures were averaged across each trial block.

Video records were evaluated for the search strategy used by rats during acquisition and probe testing. Three strategies were identified based on previous descriptions: spatial, serial, and mixed subtypes (Hodges, 1996; O'Leary and Brown, 2012; Yoder & Kirby, 2014). A search path was spatial if an animal's last two arm choices were baited. A serial strategy occurred when an animal first visited a baited arm and then subsequently visited arms in a clockwise or counter clockwise fashion until they reached the second baited arm. A mixed strategy was used when animals searched arms in a nonsystematic pattern. Finally, video records from probe tests were scored for behaviors reflecting the horizontal head scanning movements previously described as "vicarious trial-and-error" (VTE). VTEs are characterized as side-to-side head movements directed toward the entry point of adjacent maze arms, but occur without an explicit arm choice (Bett et al., 2015; Bimonte & Denenberg, 2000; Brown & Cook, 1986; Redish, 2016). VTEs were scored when animals paused near the entry of a maze arm and appeared to investigate with at least their nose passing within ~2.5cm of the threshold of the arm. If an animal crossed the threshold with all four paws, a VTE was not counted.

Acquisition measures were subjected to repeated measures analysis of variance (ANOVA) with trial block as within subject factors. A multivariate repeated measures ANOVA was used to test search strategy performance with strategy as between subject factors and trial

block as within subject factors. For the probe test, behavioral measures were subjected to paired t-tests (two-tailed). ANOVAs and t-tests were conducted using SPSS (23.0, SPSS Inc., Chicago, IL). Effect sizes for ANOVAs and t-tests were calculated using Cohen's d (d) and partial eta squared (η^2), respectively.

Histology. At the completion of testing, rats were deeply anesthetized with sodium pentobarbital and were then transcardially perfused with saline, followed by a 4% formalin solution. The brains were removed from the skull and were post-fixed in 4% formalin for 24 hours. The brains were then cryoprotected in a 30% sucrose solution for at least 24 hrs. A cryostat was used to cut 40um coronal sections through the ATN. Each section was mounted on glass microscope slides, dried, and stained with crystal violet before being cover-slipped. Bilateral placement of infusion cannula was examined under light microscopy.

Results

Histology. Histological analysis confirmed that the majority of cannula were placed within the ATN, particularly the anterodorsal and anteroventral subnuclei (n = 10). In one case, however, cannula placement was observed in the habenula, and in a second case, bilateral cannula were located within the boundaries of the mediodorsal thalamus. Because infusions in the latter two rats included adjacent subcortical regions, the data from these animals were excluded from further analysis. Figure 1 shows the results of histological analysis from the remaining 10 rats included in the behavioral analyses below.

Acquisition. Figure 2 plots the percentage of correct trials, reference memory errors, working memory errors, and latency across radial arm maze training. A repeated measures ANOVA on pre-inactivation performance indicated that rats showed increasing measures of

percent correct, ($F(10,90)=27.65$, $p<.001$, $\eta^2=0.75$), reduced reference memory errors, ($F(10,90)=27.08$, $p<.001$, $\eta^2=0.71$), reduced working memory errors, ($F(10,90)=22.23$, $p<.001$, $\eta^2=0.54$), and decreasing measures of latency, ($F(10,90)=17.158$, $p<.001$, $\eta^2=0.66$), suggesting that by the end of training animals learned the task. Indeed, measures of the percentage of correct trails were significantly above chance performance (25%) on the final day of training (Mean $\pm$ SEM: 68.92 $\pm$ 3.77%; $t(9)=11.67$, $p<.001$). To evaluate the effects of surgical implantation on task performance, we compared the percent correct of our current group to the percent correct a previous preliminary group of non-implanted animals as a sham control and found no significant differences over days ($F(5,10)=2.137$, $p=.208$, $\eta^2=.810$), suggesting that cannulation of the ATN did not cause deficits in spatial navigation on the radial arm maze.

Inactivation Probe Trials. Figure 3 plots the percentage of correct trials, reference memory errors, working memory-correct errors, working memory-incorrect errors, latency, and VTEs following the inactivation of ATN. In the probe trials following intracranial infusions, animals that received muscimol treatment demonstrated decreases in the accuracy of selecting the correct maze arms. This observation was confirmed by a significant reduction in the overall percentage of correct trials ($t(9)=4.57$, $p=0.001$, $d=1.65$), an increase in the number of reference memory errors ($t(9)=-2.31$, $p=0.046$, $d=-1.04$), and an increase in search latency ($t(9)=-4.31$, $p=0.002$, $d=-1.26$). Further, percent correct from the last day of acquisition and percent correct from the probe recovery day did not differ, $t(9)=-.179$,$p=.862$, $d=0.088$ suggesting infusions did not have long lasting effects on spatial navigation. We also observed a tendency for animals with muscimol infusions to perseverative their searches toward previously visited arms. Notably, in some cases, animals would alternate between two maze arms for up to 19 consecutive choices. The adoption of perseverative behavior by muscimol infused animals is captured by measures of

the number of working memory errors, which show a significant increase in the muscimol group compared to controls (working memory-incorrect: t(9)=-2.72, p=0.024, d=-1.15). On average, muscimol infusions tended to increase the number of working memory-correct errors (0.55 $\pm$ 0.26 errors/trial) compared to saline infusions (0.05 $\pm$ 0.03 errors/trial), however, this difference failed to reach significance (t(9)=-1.84, p=0.098). It is noteworthy that animals in the muscimol group showed a greater tendency to perseverate choices toward incorrect arms (1.30 $\pm$ 0.43 errors/trial) compared to correct arms (0.55 $\pm$ 0.26 errors/trial), further indicating that muscimol administration to the ATN resulted in a general failure in reference memory. Analysis of the number of reference memory errors across the 4 post-inactivation trials also failed to indicate a reduction in the number of errors (muscimol: F(3,27)=0.21, p=0.89; control: F(3,27)=0.16, p=.22), indicating a persistent impairment across probe testing.

We also addressed the possibility that the impaired spatial performance by muscimol infused rats described above was due to an inability to execute the appropriate movements to guide behavior. We therefore quantified the number of VTE head movements made by rats after intracranial infusions. On average, we observed that control (1.85 $\pm$ 2.34 VTEs/trial) and muscimol (2.00 $\pm$ 4.65 VTEs/trial) animals performed a similar rate of VTEs per trial, and the similarity between the two groups was confirmed by a non-significant t-test (t(9)=-0.12, p=0.91). We reasoned, however, that a general measure of VTE performance by trial might be confounded by the fact that muscimol animals spent significantly more time on the maze per trial than control rats (see Fig. 3E). A disproportionate amount of time on the maze would possibly allow additional time to perform VTEs. We therefore normalized the number of VTEs for each rat by the time spent in the center of the maze (the only region of the maze that a VTE can be performed). This analysis revealed that muscimol animals made a slightly greater number of

VTEs/sec compared to controls (muscimol: 1.17 $\pm$ 0.46; controls: 0.71 $\pm$ 0.19); however, this mean difference failed to reach statistical significance ($t(9)=-1.00$, $p=0.34$).

We also quantified the number of spatial, serial, and mixed search strategies expressed by rats during task acquisition as well as in the probe trials (Fig. 4). As expected, during training, we observed a significant interaction between strategies ($F(1,9)=15.00$, $p=0.004$, $\eta^2=0.63$) with an increase in the number of spatial searches performed by rats ($F(10,90)=13.27$, $p<0.001$, $\eta^2=0.60$), and a corresponding decrease in mixed strategies ($F(10,90)=4.36$, $p=<0.001$, $\eta^2=0.33$). It was notable, however, that we failed to observe the use of serial strategies throughout testing, suggesting that the task demands in the present study favored spatial solutions rather than non-spatial serial behavior. Muscimol infusions resulted in a significant reduction in the percentage of spatial strategies performed by rats ($F(1,39)=10.19$, $p=0.003$, $\eta^2=0.22$), suggesting that the use of a spatial search strategy involves signals processed by ATN. A corresponding increase in the number of mixed behavioral search strategies was therefore observed after muscimol infusions (muscimol 35.0 $\pm$ 8.97; control 22.5 + 6.03), however this mean difference failed to reach significance ($t(9)=-0.832$, $p=4.27$).

Discussion

The results of the present study support three novel conclusions regarding the role of the ATN in radially arranged environments. First, our findings demonstrated clear deficits in both spatial reference and working memory following inactivation of the ATN (see Fig. 3). Specifically, animals treated with muscimol failed to accurately select the two arms of the radial maze that were consistently rewarded over 11 days of pre-training, as indicated by a significant increase in the number of reference memory errors and decrease in the percentage of correct

trials during the probe test (see Fig. 3A and 3B). Further, muscimol inactivation produced a greater number of working memory errors which appear to be due, in part, to a large increase in the number of perseverative entries into incorrect arms (see Fig. 3D). Collectively these observations suggest that inactivated animals tended to make errors toward non-rewarded arms (i.e., reference memory errors), but also perseverated in making these errors throughout probe testing (i.e., working memory errors).

A number of previous studies have reported spatial working memory deficits in the radial maze after ATN lesions (e.g., Aggleton et al., 1996; Beracochea et al., 1989; Mitchell, & Dalrymple-Alford, 2005), but the role of the ATN in reference memory in the radial maze has not received similar attention. Indeed, much of the previous work investigating the relationships above have utilized procedures that favor the acquisition of non-spatial, investigatory behaviors and working memory strategies. In these studies, animals were typically exposed to a maze in which all of the arms were baited, and spatial memory errors were simply scored as returns to previously visited arms within a given session. For example, Dubreuil and colleagues (2003) reported a tendency for rats to serially sample maze arms (e.g., visit arm 1, then arm 2, then arm 3, etc.), suggesting that non-spatial strategies might be favored in this radial maze variant. In the present study, we utilized a radial maze procedure in which in which two maze arms were consistently baited in each daily training session; thus, the animal was required to learn a consistent relationship between spatial cues and the reward locations (Yoder & Kirby, 2014). The results of the present study support the conclusion that animals in this radial maze variant learned these spatial relationships by demonstrating a significant tendency to direct movements toward the reward arms by the end of training (i.e., a spatial strategy; see Fig. 4A). In contrast, the occurrence of serial search strategies was virtually non-existent throughout acquisition and

probe trials further suggesting that the current radial arm maze task promotes spatial search strategies. Additionally, ATN inactivated animals used a spatial search strategy at a lower rate than control animals.

A second general conclusion of the present study is that extensive pre-training in the radial arm maze failed to ameliorate the effects of ATN inactivation on subsequent spatial performance. Previous studies assessing spatial reference memory in the Morris water task have been less clear regarding the influence of pre-surgical task acquisition. For instance, Sutherland and Rodriguez (1989) showed that large lesions of the ATN failed to impair spatial memory after extensive pre-lesion training, whereas Warburton et al (1999) reported the opposite pattern of results. Further, Stackman et al (2012) reported that some forms of spatial memory are retained in the water maze after post-acquisition muscimol inactivation of the ATN. Specifically, impairments were observed in the use of spatial information to guide swim trajectories toward specific directions in the pool, but swim paths toward absolute spatial locations within pool coordinates were spared. This finding suggests that pre-training may spare particular forms of spatial processing, but not spatial strategies reliant on generating a directional trajectory toward a goal location. Alternatively, circular arena findings from Stackman et al. (2012), Sutherland and Rodriquez (1989), and Yoder and Kirby (2014) that show a lack of deficits in spatial reference memory following impairment to the head direction signal might be due in part to the circular environment itself in contrast to radial environments. Because discrete directional decisions are not required to complete circular arena tasks, such as in radial tasks, spatial reference memory about goal locations might be spared. This speculation is supported in place cell literature where lesions of the ATN reduce the spatial specificity of hippocampal place cells but do not completely admonish their signal (Calton et al., 2003), suggesting that reference memory of

locations but not directions might be intact following head direction signal degradation. In contrast to studies conducted using the Morris water maze, Alexinsky (2001) reported that animals with sham and neurotoxic lesions of the ATN were impaired in radial arm maze navigation after extensive pre-lesion training on the task. It is notable, however, that in this study the post-surgical performance of control animals was relatively poor compared to pre-surgical performance. A possible explanation for this finding is that the delay between pre and post-surgery, which was 2 weeks in duration, was sufficient to induce reference memory impairments. Thus, in the present study, we avoided this potential confound by surgically cannulating animals before training in the radial maze and using muscimol infusion procedures which allowed rapid inactivation of the ATN shortly (24hrs) after pre-training. Further, because there was no surgical recovery interval between acquisition and retention testing, the effects of neural compensation and covert pathological changes in other regions of the limbic system are limited (Dumont et al., 2012; Jenkins et al., 2004).

The mechanism by which the ATN may serve a role in spatial memory in the radial arm maze is poorly understood, but one long standing hypothesis has argued that the ATN and head direction cell activity plays a role in the establishment of spatial representations in the hippocampal formation (McNaughton et al., 1991; Sharp et al., 2001; Taube, 2007; Yoganarasimha et al., 2006). Certainly the fact that lesions of the ATN reduce the spatial specificity of hippocampal place cells (Calton et al., 2003), and abolish parahippocampal grid cells (Winter et al., 2015) seems confirmatory. In recent work, the ATN and head direction cell activity has also been linked to the fact that place cells and grid cells form unique spatial firing patterns in radially oriented environments (Derdikman et al., 2009; Fuhs et al., 2005; Grieves et al., 2016; Speirs et al., 2015). Specifically, the ATN may play a central role in disambiguating

spatial locations based on directional orientation (Clark et al., 2015; Grieves et al., 2016; Sanchez et al., 2016; Stackman et al., 2012), which would ultimately influence the accuracy of reference and working memory in the radial maze. Observations of increased activity dependent gene expression in anterior thalamic nuclei following training in the radial arm maze supports this hypothesis (Vann et al., 2000), but the effects of direct manipulations of the ATN on hippocampal spatial representation in radial environments is presently unknown.

A final conclusion relates to the observation that rats with muscimol inactivation of the ATN continued to exhibit investigatory behaviors (Fig. 3F), in particular, arm checking behaviors in which animals direct their head movements towards the entrance of maze arms, and produce scanning head movements between adjacent maze arms. This behavior, also referred to as vicarious trial-and-error or VTEs (Brown & Cook, 1986; Redish, 2016; Tolman, 1939), has long been argued to serve a role in gathering environmental information, perhaps about the locations of relevant landmarks, and the establishment of spatial representations (O'Keefe & Nadel, 1978). Support for this notion comes from studies demonstrating that these head movements can be altered in rats after hippocampal lesions (Bett et al., 2015; Clark et al., 2005), and that declines in head movements after hippocampal damage can be correlated with spatial learning impairments (Hu & Amsel, 1995). Because the ATN has large reciprocal connections with the hippocampal formation, and contributes to the processing of spatial representations within the hippocampus, a reasonable hypothesis would be that the ATN may also contribute to the guidance of investigatory movements. Nonetheless, the lack of significant changes in VTEs after ATN inactivation in the present study fails to confirm this hypothesis. Further, our findings suggest that deficits in spatial reference memory and working memory after ATN disruption are not explained by alterations in head scanning behaviors and point to a potential functional

dissociation between the hippocampal formation and ATN.

To summarize, the results of the present study indicate that the ATN are necessary for the expression of spatial reference memory, spatial working memory, and performance of spatial strategies in the radial arm maze. Further, the present study demonstrated that VTE behaviors are relatively intact after ATN inactivation, strongly suggesting that declines in reference memory and working memory after ATN inactivation are not explained by deficits in investigatory behaviors. Together, the results suggest that the ATN modulates not only the online guidance of accurate spatial behavior, but is also necessary for the expression of a previously acquired spatial representations in the radial arm maze.

Figures & Captions

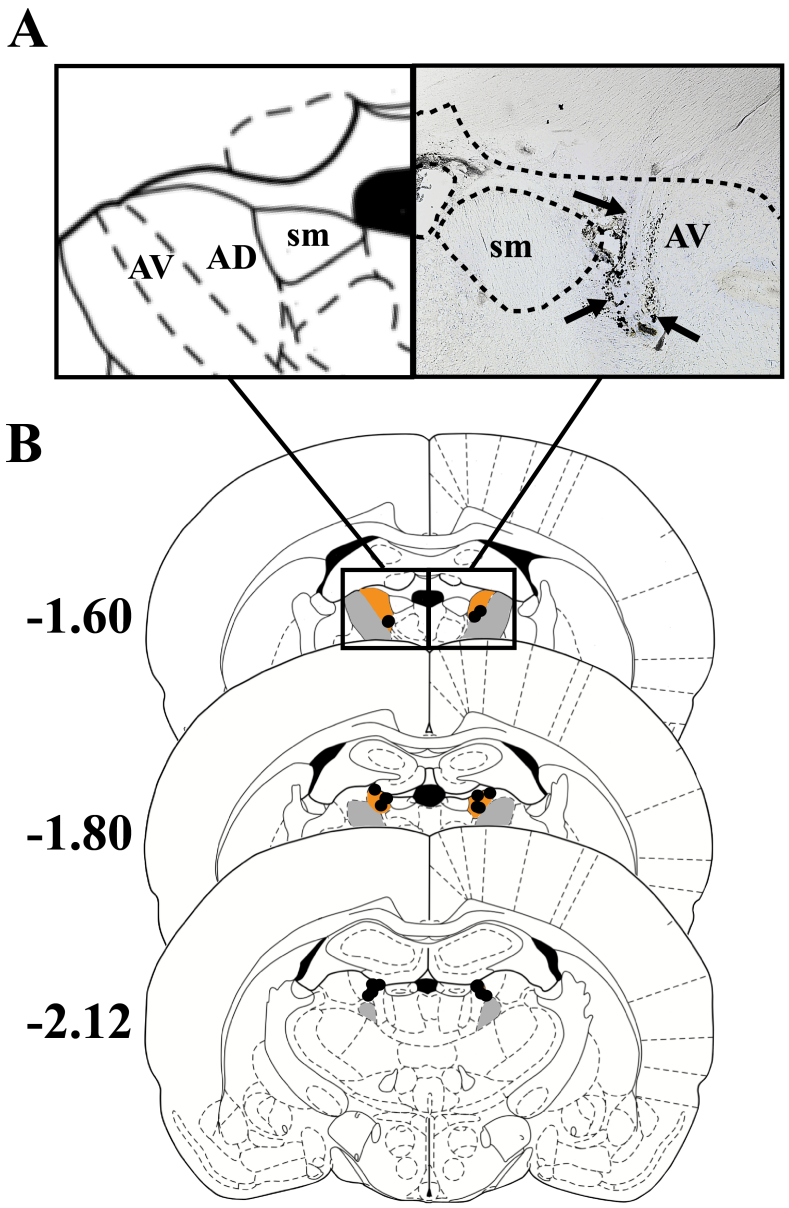

Figure 1. A: Left: The anterodorsal thalamic nuclei (AD), anteroventral thalamic nuclei (AV), and stria medullaris (SM) are identified in an atlas plate from Paxinos & Watson, 1998. Right: Representative coronal section depicting bilateral infusion tracks through the ATN. Black arrowheads indicate track of infusion cannula. **B:** The individual placements of infusion sites are

indicated with black circles for 10 rats are presented against the atlas plates from -1.60 to -2.12mm from bregma (Paxinos & Watson, 1998). Orange represents AD and grey represents AV.

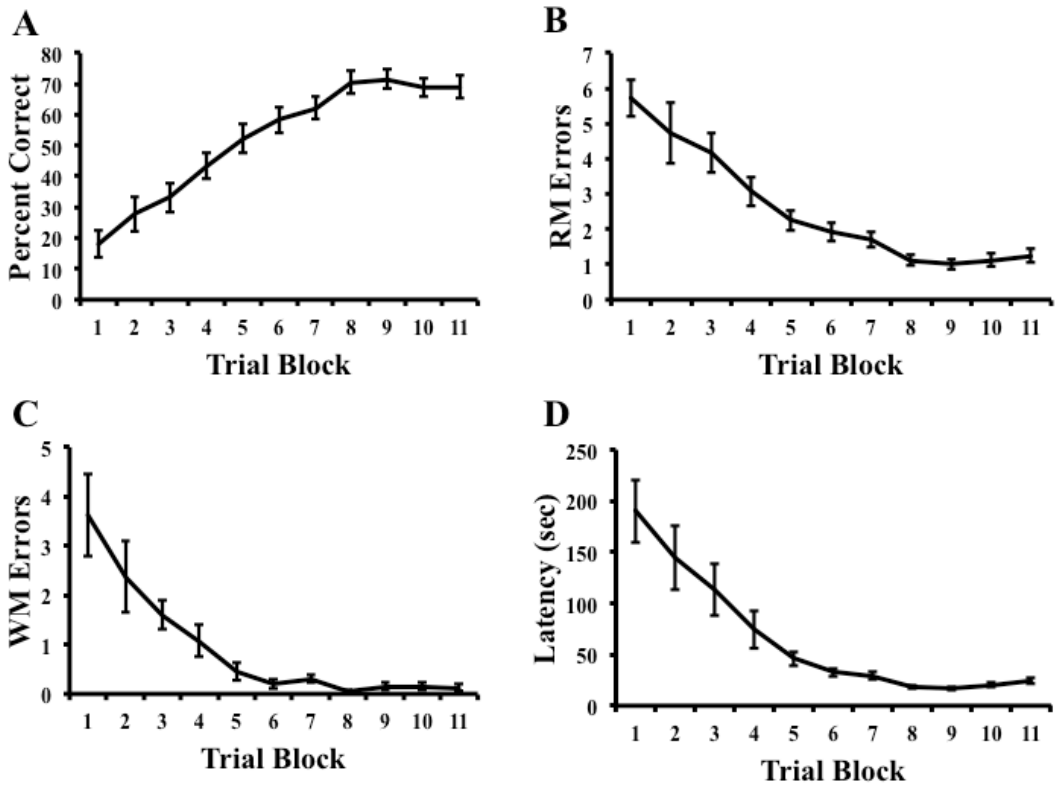

Figure 2. Results of radial arm maze task acquisition. A: Percentage of correct arm choices increased over trial blocks. B-C: Reference memory (RM) and working memory (WM) errors decreased across trial blocks. D: Latency to complete the task decreased across trial blocks. Mean ± SEM.

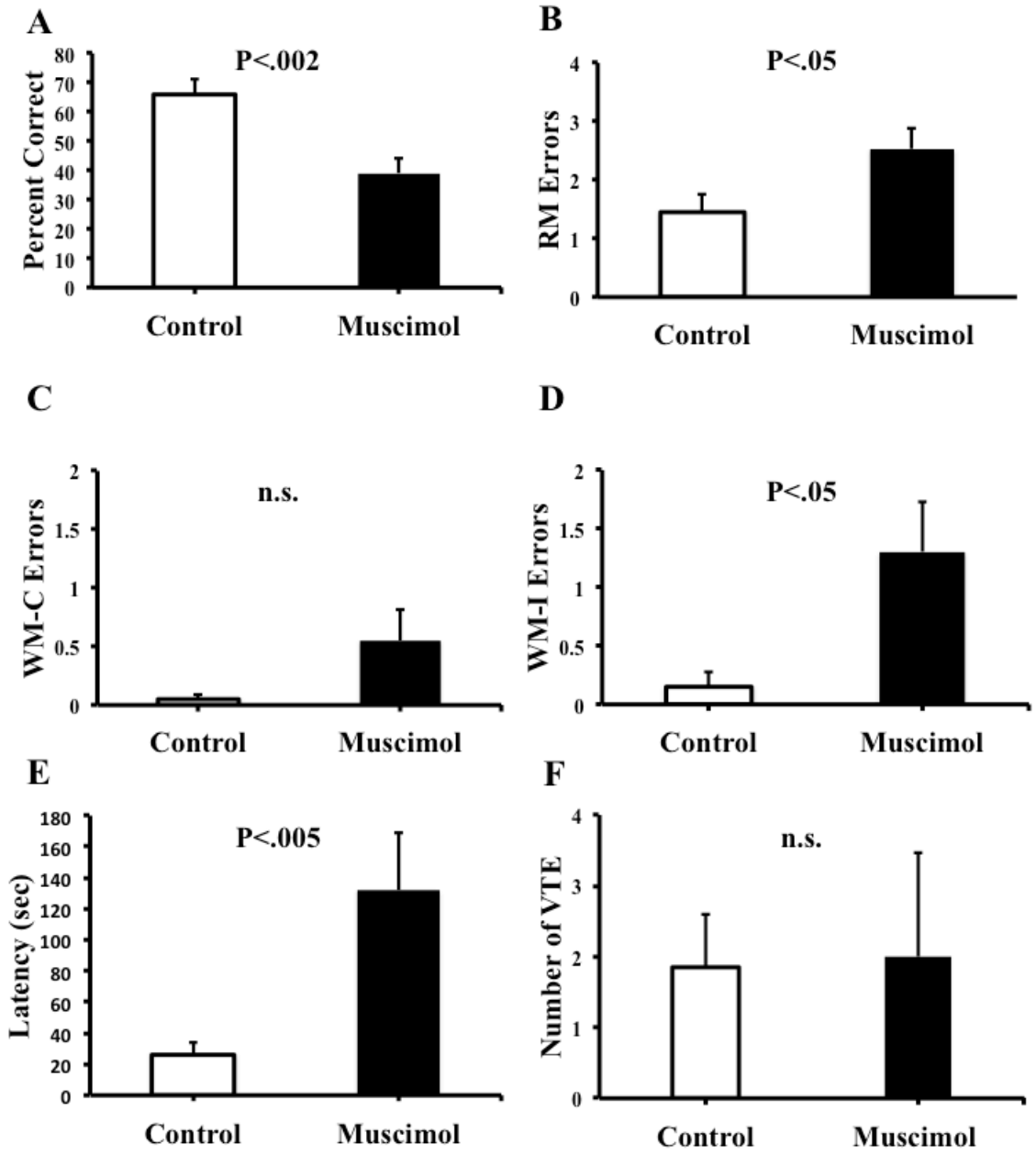

Figure 3. A: Percentage of correct arm choices was higher in control animals than in muscimol animals. B: RM error were significantly greater in muscimol animals than in control animals. C: Working memory-correct (WM-C) errors did not significantly differ, but note that on average muscimol animals made greater WM-C errors compared to controls. D: Working memory-incorrect (WM-I) errors were significantly greater in muscimol animals than in control animals.

E: Latency to complete the task was significantly greater in muscimol animals than in control animals. F: Muscimol and control animals made a similar total number of VTEs per trial. Mean + SEM.

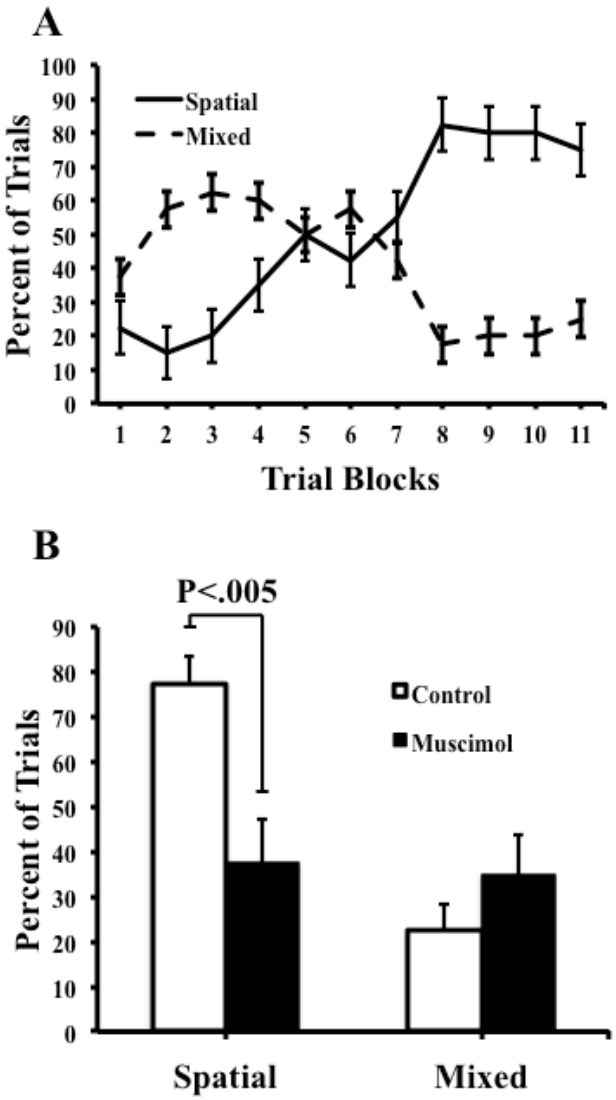

Figure 4. Control and muscimol animals favored different search strategies during probe sessions. A: Animals favored a mixed search strategy during the first trial blocks, but ultimately favored a spatial search strategy by the end of acquisition. B: During probe trials, control animals favored a spatial search strategy while muscimol animals had no preferred search strategy. Mean + SEM.

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