Neuroscience 336 (2016) 1–11

THE MEDIAL FRONTAL CORTEX CONTRIBUTES TO BUT DOES NOT ORGANIZE RAT EXPLORATORY BEHAVIOR

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Abstract—Animals use multiple strategies to maintain spatial orientation. Dead reckoning is a form of spatial navigation that depends on self-movement cue processing. During dead reckoning, the generation of self-movement cues from a starting position to an animal's current position allow for the estimation of direction and distance to the position movement originated. A network of brain structures has been implicated in dead reckoning. Recent work has provided evidence that the medial frontal cortex may contribute to dead reckoning in this network of brain structures. The current study investigated the organization of rat exploratory behavior subsequent to medial frontal cortex aspiration lesions under light and dark conditions. Disruptions in exploratory behavior associated with medial frontal lesions were consistent with impaired motor coordination, response inhibition, or egocentric reference frame. These processes are necessary for spatial orientation; however, they are not sufficient for self-movement cue processing. Therefore it is possible that the medial frontal cortex provides processing resources that support dead reckoning in other brain structures but does not of itself compute the kinematic details of dead reckoning.

Key words: path integration, spatial orientation, dead reckoning, translational neuroscience, medial frontal, *Rattus norvegicus*.

INTRODUCTION

Many neurological disorders are associated with disruptions in spatial orientation (Aguirre and D'Esposito, 1999; Tetewsky and Duffy, 1999; O'Brien et al., 2001). For example, patients that have experienced stroke often exhibit impairments in spatial orientation (Meerwaldt and Van Harskamp, 1982). Specifically, patients with damage to frontal cortical areas have been reported to display lateralized impairments in their ability to process egocentric spatial information, known as spatial neglect (Mesulam, 1990; Heilman et al., 1993). One spatial orientation strategy, dead reckoning, involves the online processing of self-movement cues (i.e., vestibular, proprioception, efferent copies of action commands) to estimate direction and distance to the point that movement originated (Gallistel, 1990). Work on humans (Worsley et al., 2001; Philbeck et al., 2004; Shrager et al., 2008; Kim et al., 2013) and rats (Maaswinkel et al., 1999; Wallace and Whishaw, 2003; Winter et al., 2013; Kim et al., 2013; however, see Alyan and McNaughton, 1999) has provided support for these cues being processed by a network of brain structures that include the hippocampal formation and hippocampal cholinergic (Martin and Wallace, 2007) and GABAergic (Köppen et al., 2013) projections that originate in the medial septum. In addition, several other cortical areas contribute to dead reckoning including the entorhinal (Parron and Save, 2004; Winter et al., 2013; Van Cauter et al., 2012), parietal (Parron and Save, 2004), and retrosplenial (Cooper et al., 2001; Whishaw et al., 2001) cortical areas.

Imaging work has provided support for expanding this network of structures to include the medial frontal cortex (Wolbers et al., 2007). Human participants were given a dead reckoning navigation task during an fMRI scan that required participants to indicate the direction of the start location after traveling a route that involved a change in heading. Hippocampal and medial frontal cortex activity was associated with performance on this virtual dead reckoning task. In contrast, previous rat work has demonstrated that medial frontal cortex lesions spare performance on a task that depends on self-movement cue processing (Whishaw et al., 2001a). In the rat study, the animals were given many trials in which they carried food to a home base and it is possible that the reinforcement contingencies of the task may have allowed the animals to compensate for impairments in self-movement recognition. The goal of the current study, therefore, is to examine the contribution of the medial frontal cortex in a dead

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Abbreviations: ANOVA, analysis of variance; cc, corpus callosum; Cg1, cingulate cortex; fmi, forceps minor; gcc, genu of the corpus callosum.

http://dx.doi.org/10.1016/j.neuroscience.2016.08.041
0306-4522/

reckoning task that involves the computation of spatial position from spontaneous exploratory movement similar to that given to human participants in the virtual dead reckoning task.

Rat exploratory behavior is organized at several levels. At a macro level, rats introduced to a novel environment will first establish a home base or a location associated with stopping behavior, long dwell times, and grooming behavior (Whishaw et al., 1983; Eilam and Golani, 1989; Golani et al., 1993; Clark et al., 2005). At a micro level, rats organize their movements around the home base location (Eilam and Golani, 1989; Drai et al., 2000). Movements away from the home base are typically slow and more circuitous relative to movement sequences directed back to the home base (Tchernichovski et al., 1998). Returns to the home base are characterized by a consistent temporal pacing of moment-to-moment speeds that are scaled to the distance between the rats' current location and their home base (Wallace et al., 2006). This organization of the exploratory trip into outward and homeward segments has been observed independent of environmental cue availability—consistent with self-movement cue processing guiding exploratory behavior. Specifically, self-movement cues generated after departing the home base are processed within the temporal context they occur to update an online representation of the current direction and distance to the home base (Barlow, 1964; Mittelstaedt and Mittelstaedt, 1980; Gallistel, 1990; Biegler, 2000; Etienne and Jeffery, 2004; Wallace et al., 2008). Research has shown that damage to the hippocampus (Wallace et al., 2002; Wallace and Whishaw, 2003) or related structures (Martin et al., 2007; Winter et al., 2013) disrupts the direction and distance component of the homeward segment. Therefore, the current study examines the effects of damage, localized to structures within the medial frontal cortex, on the organization of exploratory behavior.

EXPERIMENTAL PROCEDURES

Animals

Twelve adult naïve female Long–Evans rats (University of Lethbridge vivarium), weighing 250–300 g, were housed in groups of two in Plexiglas cages. The colony room was maintained at 20–21 °C with a 12-h/12-h light/dark cycle. Rats were provided ad lib access to food and water in their home cages throughout the duration of the experiment.

Apparatus

The apparatus was a circular table (2.50 m in diameter) without walls mounted on ball bearings that permitted it to be rotated. The surface of the table was 0.64 m above the floor. The home base was a small, opaque Plexiglas box (0.20 m × 0.29 m × 0.22 m) with a circular hole (0.11 m) cut on one side. The home base was placed at the edge of the table such that the circular hole faced the center of the table. For each rat, the location of the home base remained stable across all exploration sessions; however, the table was rotated between rats and wiped down with ammonia cleaning solution after testing each rat.

A night vision camera was positioned perpendicular to the table in a light proof room; the room was completely dark when the lights were turned off. The camera was connected to a video recorder. The experimenter used night vision goggles to place the rats on the table under dark testing conditions.

Surgery

Rats were anesthetized with a mixture of isofluorane and oxygen during surgery. Medial frontal lesions ($n = 6$) involved making an approximately 2-mm wide trephine on both sides of the midline; thereby leaving a 2-mm wide piece of skull covering the sagittal sinus. The medial frontal cortex was removed by aspirating the overlying gray matter of the cortex. The control animals ($n = 6$) were also anesthetized and received an incision. Subsequent to the surgery, all rats were given two weeks to recover prior to testing.

Procedure

All testing was conducted during the light phase of rats' 12-h light/dark cycle. Each rat was individually transported from the colony to the testing room in an opaque Plexiglas cage covered with a cloth to limit rats' access to visual stimuli. During transportation from the colony room to the testing room, the cage was rotated a varying number of times and a different path was taken to the testing room each day. Upon entering the testing room the rat was placed in the home base and the researcher sat in a designated chair in the southeast corner of the testing room. All rats were given 50 min to explore the table. All rats were initially tested under light conditions for three days prior to being tested under dark conditions for three days. Previous work has shown that changes in the sequence of testing conditions do not appear to influence control rat performance (i.e., light first: Whishaw et al., 2001; Wallace and Whishaw, 2003; dark first: Wallace et al., 2006).

Analysis

Exploratory behavior. EthoVision (Noldus, Leesburg, VA, USA) was used to quantify two characteristics of exploratory behavior from the day the most exploratory trips were taken for light and dark conditions. For a majority of the rats, this occurred on their first days of light and dark exposure. First, the Brown's score (Brown and Whishaw, 2000) was used to assess establishment of a home base in the refuge. The Brown's score is calculated by finding the average difference in the percent time spent in the target quadrant (Q^t) relative to the other three quadrants (Q^1, Q^2, Q^3) as follows: $[(Q^t - Q^1) + (Q^t - Q^2) + (Q^t - Q^3)]/3$. Values may range from −33 (avoidance of the quadrant with the refuge) to 100 (not spending time outside of the quadrant with the refuge). Next, the total

distance traveled was used to assess the rats' activity level during the exploratory session. The total distance traveled was calculated from the *xy*-coordinates obtained from EthoVision by summing point-to-point distances.

The Peak Performance (Vicon, Denver, CO, USA) motion capture system was used to quantify the topographic and kinematic characteristics of movement during exploratory trips. An exploratory trip was defined as departure from the home base, locomotor activity that displaced the rat at least half the table diameter (1.25 m), and ended when the rat returned to the home base. This definition of an exploratory trip was intended to eliminate lingering behaviors (Drai et al., 2000) from the analysis. The first five exploratory trips, observed subsequent to home base establishment, were captured from the analog video recording and converted into digital computer files at a 30-Hz frame rate. Rat movements were tracked by manually clicking the position of the midpoint between the forelimbs every fifth frame. The resulting *xy*-coordinates were scaled to real world units and used to calculate moment-to-moment speeds and distance traveled. Previous work has demonstrated that exploratory trips are a series of non-circuitous progressions punctuated by stops (Wallace and Whishaw, 2003; Wallace et al., 2006). A progression was defined as a continuous bout of movement equal to or greater than 0.1 m/s and movement not exceeding 0.1 m/s was defined as a stop. Exploratory trips were divided into outward and homeward segments. The outward segment was defined as all of the progressions and stops until the final stop. The homeward segment was defined as the progression that occurred after the final stop. Topographic and kinematic characteristics of both segments were used to evaluate group differences in exploratory trip organization.

Several measures were used to characterize group differences in movement organization on the outward segment. First, distance traveled was calculated for each outward segment. Next, outward segment path circuity was calculated by dividing the Euclidian distance of the outward segment by the total distance of the outward segment. Path circuity values closer to 0.0 reflect highly complex paths; whereas values closer to 1.0 reflect more direct paths. Further, percent time spent on the periphery (approximately a body length or 0.18 m from the edge of the table) was calculated for outward segments. In addition, peak speed observed on the outward segment was recorded for each exploratory trip. Finally, additional measures of path complexity were included to analyze movement kinematics (see Blankenship et al., 2016). Briefly, velocities are decomposed into mutually orthogonal and scalar valued ''persistence" and ''turning" velocities (V^{ρ} and V^{t}, respectively) that are assumed to draw from normally distributed Weiner processes, and their associated moment-to-moment autocorrelations ρ^{ρ} and ρ^{t} give a measure of the tendency to respectively move in straight line segments or frequently turn. Comparisons can then be made among and within trajectories using the maximum likelihood ratio.

Several measures were used to characterize group differences in movement organization on the homeward segment. Distance traveled, path circuity, time spent on the periphery, and peak speed were calculated for homeward segments. The lack of complexity of homeward segments yields limited distinguishing information in the persistence and turning metrics. Two additional measures were included to assess the direction and distance estimation capacity on the homeward segment. First, heading error on the homeward segment was calculated as the angle subtended by: (1) the first point of the outward segment, (2) the first point of the homeward segment, and (3) the fastest point on the homeward segment. Finally, the peak error was calculated as the absolute difference between the proportion of the trip associated with the peak speed and 0.5. These measures characterize homeward segment kinematic and topographic organization.

Statistical analysis. SPSS V22.0 was used for all statistical analysis. Repeated measures mixed design ANOVAs with group (medial frontal vs. control) as a between-subjects factor and condition (light vs. dark) as a within-subjects factor were used to analyze all parameters of exploratory behavior. The average of each animal's five exploratory trips was calculated for all analyses. Partial eta squared and Cohen's d are provided as measures of effect size for each analysis as appropriate. The significance level was set at .05 for all analyses. All parametric analyses did not violate assumptions of normal distribution and homogeneity of variance. Repeated measures ANOVAs consisted of a between-subjects variable comprising two groups (MF vs. sham) and a within-subjects variable comprised of two levels (dark vs. light). Departures from homogeneity of variance (Levene's) and covariance (Box's M) were addressed by using the Greenhouse–Geisser correction.

Histology

At the conclusion of behavioral testing, rats were deeply anesthetized and perfused with phosphate-buffered saline followed by 4% paraformaldehyde. Brains were removed from the skull and stored in a 30% sucrose-formalin solution. Brains were frozen and cut at 40-μm coronal sections. Every fifth section was processed for Cresyl Violet, using a standard protocol. Cresyl Violet sections were viewed on a Leica DM4000 and lesion extent was recorded for each case. Sections were then digitized at 4000dpi on a flatbed scanner. Maximum and minimum lesion volumes were traced onto standardized sections (Paxinos and Watson, 2007) using Adobe Illustrator CS5 for PC (Adobe Systems, Mountain View, CA, USA).

RESULTS

Histology

In all lesion cases except M14, the lesion extended rostrocaudally from the anterior pole of the cortex to the level of the anterior commissure (ac; Fig. 1). In these cases, a bilateral lesion involving the medial frontal cortex was

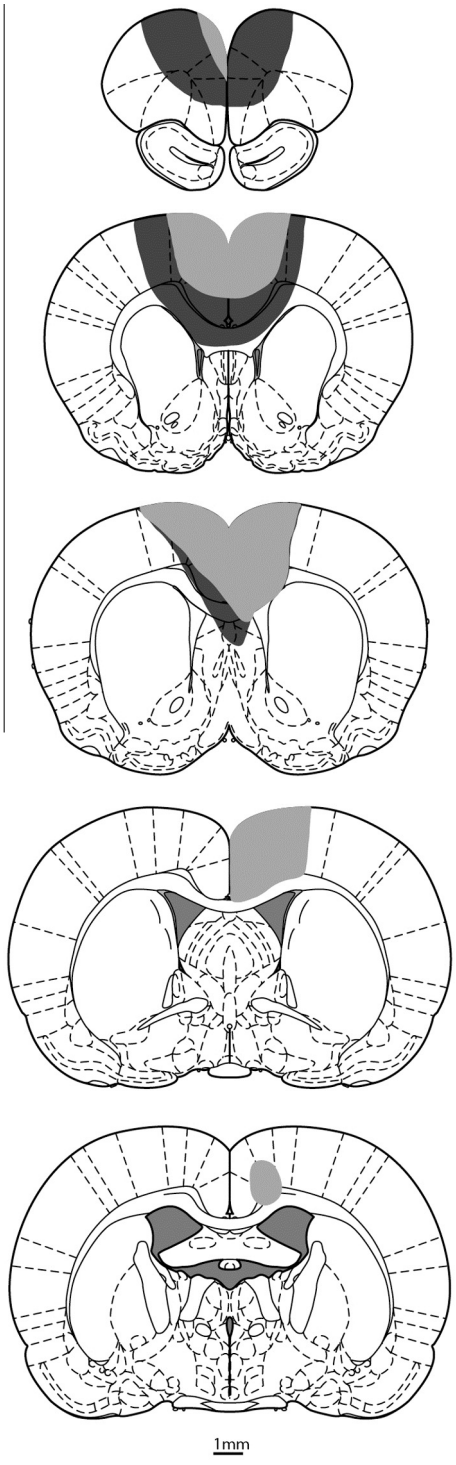

Fig. 1. Lesion localization and extent. Cases M14 (light gray) and M15 (dark gray) are depicted as example lesions (slides from top to bottom relative to Bregma: 5.16 mm, 2.16 mm, 1.08 mm, 0.00 mm, and −1.08 mm). Case M14 represents the minimal lesion in the rostral cortex but extends farther caudally than any other case. Case M15 is the largest lesion in the rostral brain but does not extend past bregma. Standardized plates adapted with permission from Paxinos and Watson, 2007.

visible beginning at or near the level of the forceps minor (fmi) of the corpus callosum (cc). Lesions at this level involved medial agranular cortex (AgM/M2), cingulate cortex (Cg1), prelimbic cortex (PrL) bilaterally. In all of these cases, the white matter of the fmi and the cingulum bundle (cg) were included in the lesion. The olfactory bulbs were not damaged in any case.

By the level of the genu of the corpus callosum (gcc), all cases demonstrated a visible lesion, including case M14. At this level, all lesions involved primary and secondary cingulate (Cg1 and Cg2) cortices, AGm, and a portion of the lateral agranular cortex (AGl). The cc was destroyed at the midline in all cases.

Between the gcc and the ac, lesions continued to affect Cg1, Cg2, AGm, and AGl bilaterally in all cases, again including the cc and the external capsule. In most cases, the dorsomedial aspect of the striatum was included in the lesion on at least one side. Also, most cases exhibited damage dorsal to the superior portion of the medial septum at the level of the ac. Only lesion case M14 exhibited a lesion caudal to the ac (Fig. 1). In this case, the lesion ended at the level of the fimbria.

Exploratory behavior

Both groups of rats explored the circular table and established a home base within the refuge. As previously mentioned, exploratory behavior was taken from the day the most exploratory trips were taken for light and dark conditions. For a majority of the rats, this occurred on their first days of light and dark exposure. When examined using non-parametric statistics, no significant difference for day of data was used ($U_{light} = -1.483$, $p = 0.394$; $U_{dark} = -0.383$, $p = 0.818$).

Brown's score. Tests of sphericity revealed an equality of variance violation which was addressed using the Greenhouse–Geisser correction. The mixed design repeated measures analysis of variance (ANOVA) conducted on the Brown's score, revealed a significant effect of condition [$F_{(1,10)} = 13.189$, $p < 0.005$, $\eta_p^2 = 0.569$]; however, the effect of group [$F_{(1,10)} = 0.622$, $p = 0.449$, $\eta_p^2 = 0$ .059] and Group × Condition interaction [$F_{(1,10)} = 0.017$, $p = 0.900$, $\eta_p^2 = 0.002$] was not significant. Rats spent significantly more time in the refuge under light conditions (M: 77.59, SE: 5.3) relative to dark (M: 44.06, SE: 5.3) conditions.

Travel distance. The mixed design repeated measures ANOVA conducted on the total distance traveled revealed a significant effect of condition [$F_{(1,10)} = 10.434$, $p < 0.009$, $\eta_p^2 = 0.511$]; however, the effect of group [$F_{(1,10)} = 0.008$, $p = 0.929$, $\eta_p^2 = 0.001$] and Group × Condition interaction [$F_{(1,10)} = 1.048$, $p = 0.330$, $\eta_p^2 = 0.095$] was not significant. Rats traveled significantly longer distances under dark conditions (M: 196.76 cm, SE: 15.90 cm) relative to light (M: 113.09 cm, SE: 16.73 cm) conditions. No group differences were observed in either measure of macro level organization of exploratory behavior. More exploratory behavior was observed under dark condition relative to light conditions.

Outward segment organization

Outward segment topographic profiles are provided for representative control and medial frontal rats under both conditions (see Fig. 2). In general, both groups exhibited similar performance on the outward segment of exploratory trips (see Fig. 3).

Travel distance. The mixed design repeated measures ANOVA conducted on outward segment distance failed to reveal a significant effect of condition [$F_{(1,10)} = 3.418$, $p = 0.094$, $\eta_p^2 = 0.255$], group [$F_{(1,10)} = 0.515$, $p = 0.498$, $\eta_p^2 = 0.049$], or Group × Condition interaction [$F_{(1,10)} = 1.932$, $p = 0.195$, $\eta_p^2 = 0.162$].

Circuity. The mixed design repeated measures ANOVA conducted on the outward segment path circuity failed to reveal a significant main effect of condition [$F_{(1,10)} = 3.616$, $p = 0.086$, $\eta_p^2 = 0.266$], group [$F_{(1,10)} = 0.039$, $p = 0.847$, $\eta_p^2 = 0.004$], and Group × Condition interaction [$F_{(1,10)} = 1.772$, $p = 0.213$, $\eta_p^2 = 0.150$].

Periphery time. Tests of sphericity revealed an equality of covariance violation which was addressed using the Greenhouse–Geisser correction. The mixed design repeated measures ANOVA conducted on the outward segment percent time spent on the periphery failed to reveal a significant effect of condition [$F_{(1,10)} = 0.021$, $p = 0.886$, $\eta_p^2 = 0.002$], group [$F_{(1,10)} = 1.189$, $p = 0.301$, $\eta_p^2 = 0.106$], and Group × Condition interaction [$F_{(1,10)} = 0.820$, $p = 0.387$, $\eta_p^2 = 0.076$].

Peak speed. The mixed design repeated measures ANOVA conducted on the outward segment peak speed failed to reveal a significant main effect of condition [$F_{(1,10)} = 0.267$, $p = 0.617$, $\eta_p^2 = 0.026$], group [$F_{(1,10)} = 3.331$, $p = 0.099$, $\eta_p^2 = 0.249$], and Group × Condition interaction [$F_{(1,10)} = 1.936$, $p = 0.194$, $\eta_p^2 = 0.162$]. These results are consistent with groups exhibiting similar behavioral characteristics on outward segments.

Topography and kinematics. Several differences were observed in outward segment organization of topographic and kinematic characteristics of movement. The mixed design repeated measures ANOVA conducted on persistence velocity autocorrelation (ρ^p) revealed a significant effect of condition [$F_{(1,10)} = 7.643$, $p < 0.02$, $\eta_p^2 = 0.433$]; however, neither the effect of group [$F_{(1,10)} = 0.001$, $p = 0.982$, $\eta_p^2 < 0.001$] nor the Group × Condition interaction [$F_{(1,10)} = 0.001$, $p = 0.980$, $\eta_p^2 < 0.001$] was significant. Outward segments were more persistent under dark conditions relative to light conditions (see panel E of Fig. 3). Tests of sphericity revealed an equality of variance violation which was addressed using the Greenhouse–Geisser correction. The measure of turning velocity autocorrelation (ρ^t) revealed a significant effect of group [$F_{(1,10)} = 17.371$, $p < 0.002$, $\eta_p^2 = 0.635$]; however, neither the effect of condition [$F_{(1,10)} = 0.217$, $p = 0.651$, $\eta_p^2 = 0.021$] nor the Group × Condition

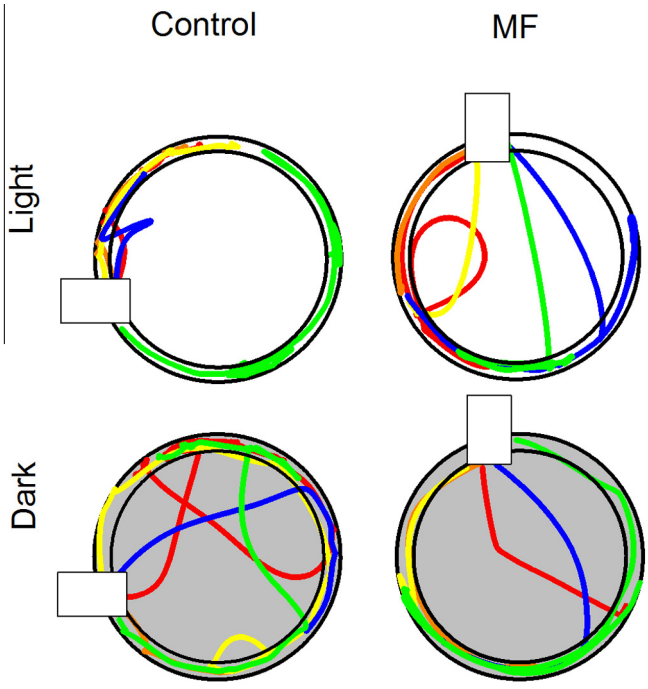

Fig. 2. Outward segment topographic profiles are provided for control (left panels) and medial frontal (right panels) groups under light (top panels) and dark (bottom panels) testing conditions. Each trial is labeled in a different color for ease of understanding (trial 1 is labeled in red, trial 2 in orange, trial 3 in yellow, trial 4 in blue, and trial 5 in green). The white rectangle represents the approximate orientation of the refuge. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

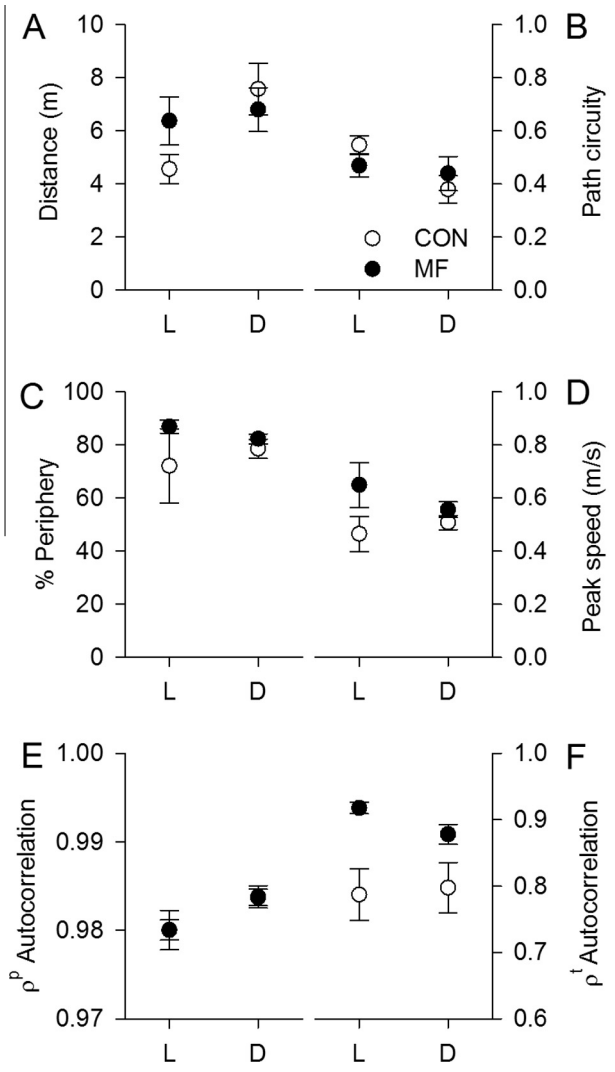

Fig. 3. Average outward segment distance traveled (panel A), path circuity (panel B), percent time on the periphery (panel C), and peak speed (panel D) is plotted for both groups under light and dark conditions. Each group's average outward segment V^p (panel E) and V^t (panel F) are plotted for light and dark conditions.

interaction [$F_{(1,10)} = 0.639$, $p = 0.443$, $\eta_p^2 = 0.060$] was significant. The medial frontal group exhibited higher turning velocity relative to the control group (see panel F of Fig. 3).

Homeward segment organization

Homeward segment topographic profiles are provided for representative control and medial frontal rats under both testing conditions (see Fig. 4). Group differences were observed on multiple characteristics of the homeward segment (see Fig. 5).

Distance. The mixed design repeated measures ANOVA conducted on homeward segment distance traveled revealed a significant effect of group [$F_{(1,10)} = 9.422$, $p < 0.012$, $\eta_p^2 = 0.485$]; however, neither the effect of condition [$F_{(1,10)} = 0.444$, $p = 0.520$, $\eta_p^2 = 0.043$] nor the Group × Condition interaction [$F_{(1,10)} = 0.099$, $p = 0.759$, $\eta_p^2 = 0.010$] was significant. The medial frontal group traveled a longer distance on the homeward segment than the control group.

Circuity. The mixed design repeated measures ANOVA conducted on homeward segment path circuity revealed a significant effect of group [$F_{(1,10)} = 18.194$, $p < 0.002$, $\eta_p^2 = 0.645$]; however, neither the effect of condition [$F_{(1,10)} = 1.168$, $p = 0.305$, $\eta_p^2 = 0.105$] nor Group × Condition interaction [$F_{(1,10)} = 0.458$, $p = 0.458$, $\eta_p^2 = 0.056$] was significant. Independent of testing condition, medial frontal rats followed more circuitous paths to the refuge relative to the control group.

Center vs periphery. The mixed design repeated measures ANOVA conducted on percent of the homeward segment located on the table periphery revealed a significant effect of group [$F_{(1,10)} = 18.194$, $p < 0.034$, $\eta_p^2 = 0.645$]; however, neither condition [$F_{(1,10)} = 0.195$, $p = 0.668$, $\eta_p^2 = 0.019$] nor Group × Condition interaction [$F_{(1,10)} = 0.733$, $p = 0.412$, $\eta_p^2 = 0.068$] was significant. Independent of testing condition, medial frontal rats exhibited homeward segments that had a higher percent of their paths restricted to the periphery relative to the control group.

Heading error. Tests of sphericity revealed an equality of variance violation which was addressed using the Greenhouse–Geisser correction. The mixed design repeated measures ANOVA conducted on homeward segment heading error revealed a significant effect of group [$F_{(1,10)} = 26.767$, $p < 0.001$, $\eta_p^2 = 0.728$] and condition [$F_{(1,10)} = 11.630$, $p < 0.007$, $\eta_p^2 = 0.538$]; however, the Group × Condition interaction [$F_{(1,10)} = 1.701$, $p = 0.221$, $\eta_p^2 = 0.145$] was not significant. The medial frontal group had a significantly larger homeward segment heading error relative to the control group. Both groups exhibited a significant increase in homeward segment heading error from light to dark conditions.

Peak speed. The mixed design repeated measures ANOVA conducted on homeward segment peak speed revealed a significant effect of condition [$F_{(1,10)} = 45.886$, $p < 0.001$, $\eta_p^2 = 0.821$] and Group × Condition interaction [$F_{(1,10)} = 8.048$, $p < 0.018$, $\eta_p^2 = 0.446$]; however, the effect of group [$F_{(1,10)} = 1.528$, $p = 0.245$, $\eta_p^2 = 0.133$] was not significant. Both groups' peak speeds were faster under light conditions relative to dark conditions. Post-hoc analysis revealed that the control group exhibited significantly faster peak speeds relative to the medial frontal group, but only under the light conditions (HSD $p < 0.05$).

Peak error. Tests of sphericity revealed an equality of variance violation which was addressed using the Greenhouse–Geisser correction. The mixed design ANOVA conducted on homeward segment peak error revealed a significant effect of group [$F_{(1,10)} = 13.379$,

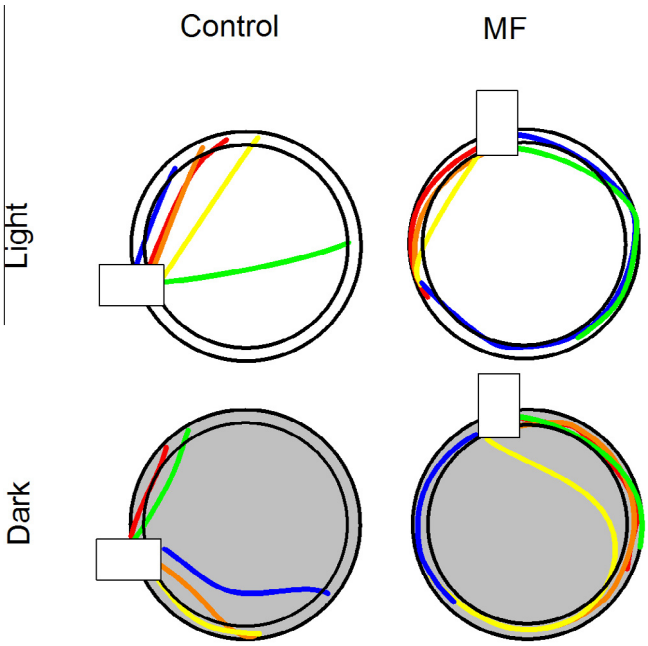

Fig. 4. Homeward segment topographic profiles are provided for control (left panels) and medial frontal (right panels) groups under light (top panels) and dark (bottom panels) testing conditions. Each exploratory homeward progression corresponds with specific outward progression and is labeled identically to Fig. 2 (trial 1 is labeled in red, trial 2 in orange, trial 3 in yellow, trial 4 in blue, and trial 5 in green). The white rectangle represents the approximate orientation of the refuge. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

$p < 0.004$, $\eta_p^2 = 0.572$] and Group × Condition interaction [$F_{(1,10)} = 5.067$, $p < 0.048$, $\eta_p^2 = 0.336$]; however, the effect of condition [$F_{(1,10)} = 2.622$, $p < 0.136$, $\eta_p^2 = 0.208$] was not significant. Post-hoc analysis revealed that the medial frontal group had a significantly larger peak error than the control group, these group differences were restricted to light conditions (HSD $p < 0.05$).

DISCUSSION

This study examined the effects of medial frontal lesions on exploratory behavior under light and dark conditions. Both groups established a home base in the refuge and organized their movements around that location. In general, group differences were restricted to the homeward segment and did not depend on the testing condition. For example, the medial frontal group exhibited homeward segments that were highly circuitous and followed the periphery of the table. Several exceptions were noted. First, medial frontal rats exhibited higher turning velocity autocorrelations (ρ^t) on outward segments under both testing conditions. Next, group differences in homeward segment peak speed and peak error were dependent on testing condition. Specifically, medial frontal rats exhibited slower peak speeds and larger peak errors on homeward segments when exploring under light conditions. This pattern of results demonstrates a role for the medial frontal cortex in organizing exploratory behavior. The following sections discuss whether the role of the medial frontal cortex in organizing exploratory behavior involves processing self-movement cues.

Role of the medial frontal cortex in exploration

Damage to the medial frontal cortex has been associated with multiple behavioral impairments that may have contributed to the disruptions in exploratory trip organization. First, previous work has demonstrated that damage to the medial frontal cortex impairs motor coordination (Kolb et al., 1983). For example, rats with medial frontal lesions will exhibit disruptions in food handling (i.e., frequently dropping the food item) and motor sequence learning (i.e., opening latches in a puzzle box). These impairments in motor coordination may have contributed to the group differences observed in outward segment turning velocity (V^t). Group differences in turning velocity (V^t) may have influenced self-movement cue processing. Previous work has demonstrated that outward segment movement coordination was associated with homeward segment direction estimation in dead reckoning tasks at the ambulatory (Wallace et al., 2006) and manipulatory (Wallace et al., 2010) scales. Therefore, the medial frontal cortex may be indirectly involved in self-movement cue processing by coordinating movement on the outward segment. Observing that the medial frontal lesions disrupted outward segment movement coordination and homeward segment direction estimation independent of testing condition is consistent with an indirect role for the medial frontal cortex in self-movement cue processing. However, it is possible that other functions of the medial frontal cortex contributed to the disruption in performance observed under both testing conditions.

In addition to motor coordination, the medial frontal cortex has been implicated in response inhibition (Kolb

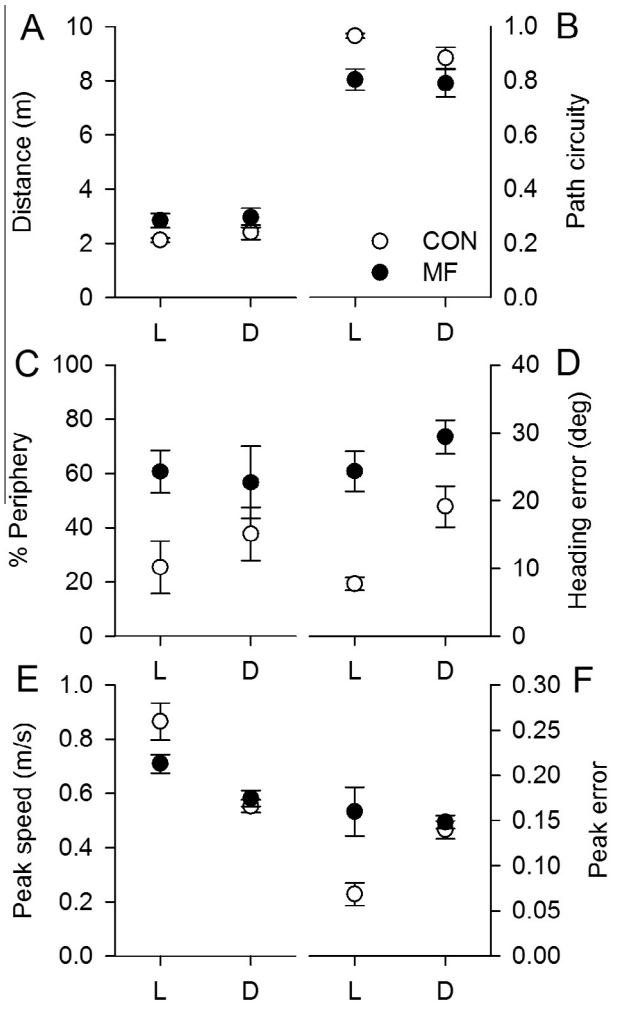

Fig. 5. Average homeward segment distance traveled (panel A), path circuity (panel B), percent time on the periphery (panel C), and heading error (panel D) are plotted for both groups under light and dark conditions. Each group's peak speed (panel E) and peak error (panel F) are plotted for light and dark conditions.

et al., 1974; Womack et al., 1993; Salazar et al., 2004). Specifically, medial frontal lesions attenuate performance in spatial reversal learning paradigms; rats with these lesions typically exhibit a perseverative pattern of responding. Impaired response inhibition may have contributed to the increased homeward segment path circuity observed under both testing conditions. Rats with medial frontal lesions may have perseverated in using a thigmotaxis strategy on the homeward segment rather than switching to using either self-movement or environmental cues to guide behavior on the homeward segment. This observation is in contrast to results obtained from studies investigating the role of the hippocampus in spatial orientation. Damage to the hippocampal formation impairs direction estimation when tested under dark conditions; however, direction estimation is spared under light conditions (Maaswinkel et al., 1999; Martin and Wallace, 2007; Winter et al., 2013; Köppen et al., 2013). Rats with medial frontal lesions did not successfully use environmental cues, available under light conditions, to compensate for deficits in self-movement cue processing. Impaired response inhibition associated with medial frontal lesions may have contributed to the current pattern of results; however, impaired spatial orientation cannot be excluded.

Previous work has provided evidence for a relationship between circulating levels of estradiol and use of specific spatial strategies. For example, female rats with higher levels of estradiol more quickly acquire place strategies; whereas lower levels of estradiol are associated with the acquisition of a response strategy (Korol et al., 2004). However, this hormone-mediated spatial strategy bias may be task-specific. Specifically, female rats have been observed to prioritize response strategies over place strategies to solve circular mazes (Daniel and Lee, 2004; Hawley et al., 2012). In the context of the current study, a hormone biased spatial strategy does not predict strong group differences. For example, if rats' estrous cycles are synchronized, then the performance should be same on the homeward segment. In addition, if rats' estrous cycles are not synchronized, then both groups should be variable in their performance on the homeward segment. The significant group differences (control rats followed non-circuitous homeward progressions directed toward the refuge and medial frontal rats followed circuitous homeward segments focused along the periphery of the table) observed in the current study are not consistent with the hormones biasing use of specific spatial strategies. Other work has shown phases of the estrous cycle do not influence female rats' use of self-movement cues to organize food hoarding behavior (Köppen et al., 2015). It is unlikely that phases of the estrous cycle influenced self-movement cue processing during exploratory behaviors; however, these data were not collected for the current study. As such, future work is needed to examine this possibility. Alternatively, it is possible that medial frontal lesions disrupted the estrous cycle (lack of evidence for this relationship in the literature is discussed in the next section) thereby differentially influencing spatial strategy use across groups. This being the case, the group differences observed in the current study are not likely consistent with a hormone biased spatial strategy; rather medial frontal damage may impair information processing and result in a perseveration in using a thigmotaxis strategy on the homeward segment.

Damage to structures within the medial frontal cortex has been shown to produce deficits in processing information from the egocentric reference frame (assessing orientation relative to the self). For example, bilateral or unilateral damage to the AGm (medial agranular cortex) has been shown to disrupt performance on right-left discrimination tasks (Kesner et al., 1989; Poucet, 1990; King and Corwin, 1992). Loss of information processing from the egocentric reference frame may have contributed to the disruptions in performance on the homeward segment. This multi-modal processing deficit is consistent with the disruptions in homeward segment organization observed independent of access to environmental cues.

It is also possible that motor coordination, response inhibition, and egocentric reference frame deficits may not be mutually exclusive. For example, an intact

egocentric reference frame is necessary for spared motor coordination or spared ability to shift from using tactile cues (thigmotaxis) to visual cues (beacon homing). All these factors reflect processing of sensory-motor information rather than influencing higher-level processing of environmental or self-movement cues.

In order to study the function of the medial frontal cortex, we carefully removed cortical tissue via the aspiration method. Care was taken to remove as much of the medial frontal cortex as possible while limiting damage to adjacent regions as much as possible. Even so, Cg1, Cg2, and some white matter structures (small sections of the cingulum bundle and the corpus callosum) were destroyed in all cases (see Fig. 1). Loss of white matter was limited in its rostrocaudal extent in these cases, and likely had little effect on the outcome of the study. Indeed, the present lesions are consistent with aspiration lesions found in previous studies of the function of the cortical areas included in our definition of the medial frontal cortex (Rosen et al., 1992; van Eden et al., 1998; Whishaw et al., 2001b).

Other contributing factors

In addition to sensory motor processing, the medial frontal cortex has been implicated in mediating other aspects of open field behavior. First, previous work has reported that medial frontal lesions have varying impact on open field locomotor activity. For example, medial frontal lesions in rats have been shown to increase locomotor behavior in an open field (Womack et al., 1993). In contrast, other work has shown that medial frontal lesions decreased travel distance in mice and rats during open field tasks (Silva et al., 1986; Mcallister et al., 2015). Further, medial frontal lesions have been found to not significantly influence open field behavior (Salazar et al., 2004). In the current study, groups did not significantly differ in total distance traveled under either exploratory session; however, rats with medial frontal lesions exhibited slower homeward segment peak speed under light conditions. The selective nature of this group difference is not consistent with a general change in locomotor function.

As discussed above, hormonal factors may also have influenced the results of the current study. While the estrous cycle has been associated with performance on spatial tasks like the water maze (Frye, 1995; Warren and Juraska, 1997), the nature of the relationship between estrous cycle and spatial task performance continues to be debated. Other work has failed to find effects of estrous cycle stage on water maze performance (Rubinow et al., 2004) or self-movement cue-based food hoarding task performance (Köppen et al., 2015). In addition, previous work has provided evidence that lesions of the medial frontal cortex spare estrous cycling. Following surgeries, female rats still exhibited consistent estrous cycles (Afonso et al., 2007; Baran et al., 2010). Therefore hormonal factors do not seem to be a likely influence for the group differences observed in the current study.

Differences in motivation to return the refuge may have contributed to the effect of medial frontal lesions on exploratory behavior. The medial frontal cortex has been implicated in the generation of the autonomic stress response. Specifically, damage to the medial frontal cortex has been shown to attenuate cardiac arousal elicited by stimuli previously associated with shock (Frysztak and Neafsey, 1994). In addition, rats with medial frontal lesions exhibit decreases in plasma corticosterone levels in response to either acute or repeated restraint stress (Sullivan and Gratton, 1999). During exploration, the refuge could be characterized as a safe location or a location that elicits relatively less autonomic arousal. As time or distance away from the refuge increases, autonomic arousal would be expected to increase and prompt a return to the refuge. Disruptions in performance observed on the homeward segment could be attributed to an attenuated autonomic stress response; however corticosterone data were not collected for the current study. As such, future work is needed to examine this possibility; however, given this explanation, rats with medial frontal lesions would be expected to have a lower preference for the quadrant with the refuge. In contrast, no group differences were observed in the time spent in the quadrant with the refuge (i.e., Brown's score) under either testing condition. Neither changes in general locomotor behavior nor varied motivation to return to the refuge provide a complete account of the effects of medial frontal lesion on the organization of exploratory behavior.

CONCLUSION

A growing literature has identified the neural systems that contribute to self-movement cue processing. The hippocampus is one structure that has been implicated in the processing of self-movement cues (Maaswinkel et al., 1999; Wallace and Whishaw, 2003). Once the hippocampus has integrated these self-movement cues, the resulting information may be transferred to motor planning regions in the cortex to organize behavior. Transfer of information could be supported by direct projections from the CA1 field of Ammon's horn to the medial frontal cortex (Swanson, 1981; Ferino et al., 1987). These anatomical characteristics may account for the effects of medial frontal cortex lesions on exploratory behavior.

The current study demonstrated that medial frontal cortex lesions disrupted the organization of exploratory behavior under light and dark conditions. The pattern of spared and impaired behavioral organization was consistent with a role of the medial frontal cortex in motor coordination, response inhibition, and egocentric spatial processing. These processes are essential for maintaining spatial orientation; however, they are peripheral to self-movement cue based estimation of direction and distance to the movement origination point. Therefore it may be that the medial frontal cortex provides movement processing resources to support dead reckoning in the hippocampus, entorhinal cortex, and subcortical structures.

Acknowledgment—We thank the anonymous reviewers for their thoughtful comments.

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(Accepted 24 August 2016)
(Available online 31 August 2016)