



## Making waves: Comparing Morris water task performance in rats and prairie voles

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### ABSTRACT

Spatial processing is a critical component for survival. This domain of information processing has been extensively studied in rats and mice. Limited work has examined the capacity of other rodent species, like the prairie vole (*Microtus ochrogaster*), to process spatial information. The Morris water task (MWT) is a classic spatial task that has been used to examine spatial cognition in rodents. This task involves an animal developing configural relationships between extra-maze cues and the location of a hidden platform to successfully escape from a pool of water. The current study compared performance in the MWT between rats and prairie voles. Rats were observed to outperform prairie voles in key aspects of the task including latency to find the platform, directness of swim paths to the platform, and degrees of heading error. These results may be attributed to potential interspecies differences in spatial cognition, stress reactivity, physiology, or motivation. This study provides the foundation for future work investigating the spatial cognition of prairie voles and the factors that contribute to water task performance in rodents.

### 1. Introduction

Animal models have become a vital tool for investigating psychological phenomena, with researchers often using rodents of the superfamily Muriodea. Specifically, many researchers have used rodent species of the subfamily Muridae, the largest and most diverse subfamily of Muriodea, with over 1300 recognized species [39], see [36,21]. Species of muriod rodents, such as rats (*Rattus norvegicus*) and mice (*Mus musculus*), have been the focus of models examining various cognitive processes including learning, memory, and spatial orientation. [10,2], see [41,28,30,52,22,15]. While much of the work examining rodent cognition has focused on rats and mice, far less work has been done using rodents from the subfamily Cricetidae. This subfamily of the Muriodea superfamily includes hamsters, voles, lemmings, and New World rats and mice [39]. The phylogeny of murids and cricetids, encompassing morphological differences in the hippocampal formation and genetic differences, suggests that these different species experienced divergent evolutionary pressures resulting in differential central nervous system development and cognition [20,43,21]. Therefore, a comparative analysis of rodents from these two subfamilies – more specifically rats and prairie voles – may prove useful for developing rodent models that can inform our understanding of mechanisms

underlying cognition in humans.

Performance in many standard laboratory behavioral tasks focused on cognition has not been sufficiently studied in prairie voles. Interestingly, prairie voles differ significantly from many murids in that their behavior and physiology is intimately tied to the surrounding social context. This species demonstrates features of social monogamy similar to humans, such as forming strong opposite-sex social bonds, displaying bi-parental care of offspring, and responding negatively to social environmental disruptions [7]; see [32]. Prairie voles have been used to examine social behavior, reproductive processes, mood disorders, and cardiovascular function, among other behavioral and neurobiological processes see [32] for review, [19,5,33,34]. These characteristics may offer a unique translational approach to future work investigating how aspects of an animal's social environment can influence cognition.

The Morris water task (MWT) has been used extensively to examine spatial cognition in rodents [37,50,38]; for review see [53]. For example, the progressive decrease in latency to find the hidden platform observed across days of place learning has been attributed to encoding the location of the platform relative to environmental cues. Changing task procedures provides additional insight to factors that may be influencing performance. For example, shifting the location of the hidden

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platform across days, associated with matching-to-place or spatial reversal, elicits significant within-day improvements in performance [55–57]. These observations have been attributed to using spatial working memory and updating the position of the goal between trials [50]. In contrast, attaching a proximal cue to the platform as it shifts position (cued testing) between each trial allows for the dissociation of sensorimotor and motivational factors that may vary between groups [37].

Given the value of the MWT for understanding spatial cognition, the current study seeks to examine spatial performance in rats and prairie voles. Use of the MWT to examine rodent spatial cognition has been primarily conducted with rats and mice (see [38]; [28,53]; for review). In comparison, little work has examined this type of information processing in voles [23,12–14], with only two papers directly examining spatial cognition of prairie voles using the MWT [46,45]. As of yet, no work has directly compared spatial cognition between rats and prairie voles. Therefore, the current study seeks to compare spatial processing in Long-Evans rats and prairie voles using a sequential analysis of MWT performance.

## 2. Materials and methods

### 2.1. Animals

Ten female Long-Evans rats and six female prairie voles, approximately 90 days old, obtained from Northern Illinois University served as subjects for the current study. Rats were pair housed in plastic cages in colony rooms with temperatures maintained at 21–23 °C and held on a 12 h light/dark cycle. Prairie voles were pair housed in polypropylene cages with temperatures maintained at 21–23 °C and held on a 14 h/10 h light/dark cycle. Ad libitum access to food (LabDiet 5P00/RMH3000, Animal Feeds & Needs, IL USA for rats; Purina Rabbit Chow, Purina, St. Louis, MO USA for prairie voles) and water was provided throughout the study. All procedures were conducted according to the National Institutes of Health's *Guide for the Care and Use of Laboratory Animals* and approved by NIU's Institutional Animal Care and Use Committee (IACUC).

Females were chosen for this initial experiment for several reasons. There is a longstanding paucity of research on females of all species in nearly all biomedical fields, with neuroscience being among the weakest (see discussion in [3,24]). Additionally, the use of females allows for comparisons with previous experiments conducted by our laboratories that have investigated behavioral variables in both prairie voles [17–19] and rats [31,26]. Finally, female prairie voles may be especially sensitive to the effects of environmental manipulations on behavioral outcomes (see for instance [9,18,34]), allowing for a foundation to conduct additional studies using tasks of spatial navigation in the prairie vole model.

### 2.2. Apparatus

The water task apparatus was a large circular pool (173 cm diameter × 60 cm height) filled with water (19–21 °C) which contained a hidden escape platform (15 cm diameter and 28 cm in height). To hide the platform, it was submerged 2 cm below the surface of the water for rats and 1 cm below the water for prairie voles. The water was made opaque by adding white non-toxic tempura paint (Sargent Art). The apparatus was located in a rectangular room (2.77 m × 4.42 m) containing many extra-maze visual cues. For example, a poster was placed on one wall while a metal cabinet was positioned adjacent to a sink on an opposite wall. The two remaining walls had wooden doors with unique duct-tape designs on them as additional cues. To record trials, a bullet camera, attached to a DVD recorder (Sony), was mounted to the ceiling directly above the pool.

### 2.3. Procedure

Consistent with other work employing the MWT ([37,50,38]; for review see [53]), all animals were trained under light conditions to swim to a hidden escape platform submerged below the water. Each trial consisted of an animal being transported from their holding cage (located just outside of the testing room) and placed in the water facing the pool wall at one of the four-cardinal (N,S,E,W) directions. After being released, each animal was allowed to swim freely until either it located the hidden escape platform or 60 s had elapsed. If the animal failed to locate the escape platform in the given time, it was guided to the platform by the experimenter. After locating or being led to the escape platform, all animals were left on the platform for an additional 30 s to encode their surroundings. After each trial was completed, the water in the pool was strained and stirred to displace potential odor cues [35].

#### 2.3.1. Place learning

Animals received seven days of place training with four trials per day. During place training, animals were trained to locate a stationary hidden platform located in the southwest quadrant of the pool.

#### 2.3.2. Probe trial

On the eighth day of testing, all animals experienced a single probe trial in which the hidden platform was removed from the pool. Similar to place training, animals were allowed to swim for 60 s; however, during the probe trial, the amount of time spent in each quadrant was analyzed and calculated as a Brown's score [6].

#### 2.3.3. Matching-to-place

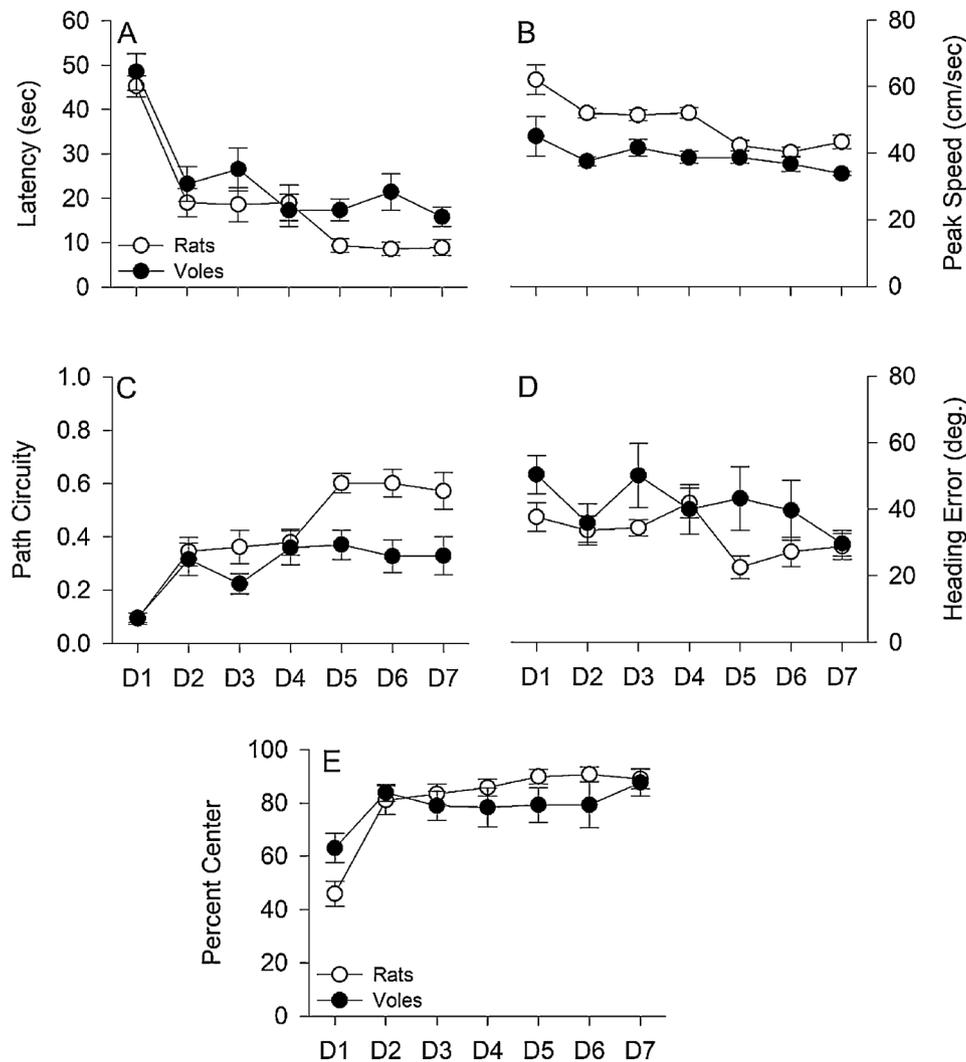
One day following the probe trial, animals experienced three days of matching-to-place (MTP) testing. Matching-to-place testing assess an animal's ability to learn a new place response each day [54]. During MTP, the location of the hidden escape platform changed every other trial. Each MTP day consisted of two trials: one in which the location of the platform was moved to a new quadrant (different from the previous day of testing) and an additional trial in which the platform remained in the same novel location. For example, if the platform was located in the southwest quadrant on the last day of place training, it would be moved to the northwest quadrant on the first trial of MTP testing. Following this trial, the platform remained in that location (northwest quadrant) and the latency to locate the platform was recorded. This pattern of paired trials continued for three days, such that the platform was moved from the southwest to northwest on the first day MTP testing, moved from northwest to southeast on the second, and from southeast to northeast on the final day of testing.

#### 2.3.4. Cued testing

Following the three days of matching-to-place, the water level was decreased such that the escape platform was now visible 1 cm above the water and the platform was covered with a dark colored sock. All animals then experienced three days of cued (visible platform) testing. Cued testing consisted of two daily trials in which the platform was moved to alternating corners of the swimming pool between each trial. For example, animals experienced the cued platform in the northeast quadrant for the first trial of cued and the platform was subsequently moved to the southeast quadrant. This pattern of trials consisted for an additional two days of testing.

### 2.4. Motion capture

Motion tracking software (NOLDUSEthovision 3.0, Leesburg, VA, USA) was used to quantify movement and performance characteristics of animals within the water task. Performance was analyzed for each trial across all days of testing.



**Fig. 1.** Movement characteristics are plotted for rats and prairie voles across the seven days of water task place learning. When compared to voles, rats located the hidden escape platform significantly faster (A), had significantly higher peak speeds (B), had significantly more direct swim paths (C), and had significantly lower heading errors (D). Lastly, a significant interaction was observed for the percentage of time spent swimming in the center of the pool, with rats initially swimming less time in the center on the first day of place learning (E).

2.4.1. General performance measures

Several measures were used to examine general water task performance characteristics. First, latency to reach the platform was recorded for each trial. As previously discussed, the maximum amount of time for a given trial was 60 s; after which the researcher guided the animal to the escape platform. Latency was averaged across the four trials for each day of place learning as well as across the two trials for each day of MTP and cued testing. In addition, the path circuity or the complexity of a swim path was measured by taking the outward shortest distance (the Euclidean distance between the release location and the escape platform location) and dividing it by the total distance traveled during that trial. Path circuity ranges from zero (most complex) to one (most direct). Path circuity was averaged across the four trials for each day of place learning as well as across the two trials for each day of MTP and cued testing. Next, the peak speed for each trial was recorded. The peak speed for a given trial is the point in which the highest velocity is achieved during a given swim path. Peak speeds were also averaged across the four trials for each day of place learning as well as across the two trials for each day of MTP and cued testing. Further, movement scaling, or the capacity of each rodent species to scale their peak movement speeds to the distance traveled was examined. Lastly, a center vs periphery analysis was conducted to examine the percentage of time spent swimming in the periphery of the pool as a measure of

thigmotaxis during place learning and matching-to-place. This measure was calculated by creating zones in the Noldus Ethovision tracking system that correspond with either the center of the pool or the periphery. These boundaries were determined using a two body-length distance from the wall of the pool for either rats (19 cm) or voles (14 cm). The resulting percentage of time in the center was then averaged across trials per day and examined between groups.

2.4.2. Spatial performance measures

Two measures were used to assess spatial performance in the task. First, heading error was assessed as a measure of direction estimation. Heading error was obtained by calculating the angle subtended by: (1) the point associated with the center of the escape platform, (2) the point associated with the center of the escape platform [2], the release point of a given swim path (N, S, E, W), and (3) the point in which the peak in speed occurred during the swim path [26]. In addition, the Brown's score [6] was used as an assessment of an animals' preference for the target quadrant of the pool (the quadrant that contained the hidden platform), during the probe day. The Brown's score reflects the percentage of time spent in the target quadrant ( $Q_{target}$ ) relative to the amount of time spent in the other three quadrants ( $Q_1, Q_2, \& Q_3$ ). It is calculated using the following formula:

$$\text{Brown's score} = [(Q_{\text{target}} - Q_1) + (Q_{\text{target}} - Q_2) + (Q_{\text{target}} - Q_3)] / 3$$

Brown's score values may range from -33 (never entering the target quadrant) to 100 (never leaving the target quadrant).

### 2.4.3. Statistical analysis

SPSS 23 (IBM, USA) was used to run repeated measures ANOVAs to evaluate main effects of group, day, and group by day interactions with alpha set at 0.05. Partial eta squared ( $\eta^2_p$ ) were reported for each main effect and interaction as a measure of effect size. The Greenhouse-Geisser correction was used in analyses where Mauchly's test indicated violations of the assumption of sphericity, and Tukey's Honest Significant Difference (HSD) post-hoc tests were used to evaluate significant main effects and interactions. T-tests were used to assess group differences in Brown's scores. Degrees of freedom were adjusted when Levene's test indicated violations of the assumption of homogeneity of variance.

## 3. Results

### 3.1. Place learning

#### 3.1.1. General performance characteristics

Several measures were used to quantify general performance characteristics associated with place learning or acquisition of the water task. First, latency was calculated for each trial across the seven days of place learning. A repeated-measures ANOVA was conducted for latency with group (rat vs. vole) as a between subjects measure and day (1, 2, 3, 4, 5, 6, and 7) as a within subjects measure. Lack of sphericity in latency across days resulted in the application of a Greenhouse-Geisser correction ( $\epsilon = 0.611$ ). The ANOVA revealed a significant main effect of group [ $F(1,14) = 6.268$ ,  $p = 0.025$ ,  $\eta^2_{\text{partial}} = 0.309$ ] and day [ $F(3.664, 51.295) = 30.834$ ,  $p < 0.001$ ,  $\eta^2_{\text{partial}} = 0.688$ ]; however, the group x day interaction [ $F(3.664, 51.295) = 1.150$ ,  $p = 0.342$ ,  $\eta^2_{\text{partial}} = 0.076$ ] was not significant. While both groups' latencies decreased across days of acquisition, rats took significantly less time to locate the hidden platform than voles (see Fig. 1A). In addition, a repeated-measures ANOVA was conducted for peak speeds. Lack of sphericity in peak speed across days resulted in the application of a Greenhouse-Geisser correction ( $\epsilon = 0.319$ ). The ANOVA revealed a significant main effect of group [ $F(1,14) = 22.103$ ,  $p < 0.001$ ,  $\eta^2_{\text{partial}} = 0.612$ ] and day [ $F(1.912, 26.773) = 11.305$ ,  $p < 0.001$ ,  $\eta^2_{\text{partial}} = 0.447$ ]; however, the group x day interaction [ $F(1.912, 26.773) = 2.674$ ,  $p = 0.089$ ,  $\eta^2_{\text{partial}} = 0.160$ ] was not significant. Both groups' peak speeds decreased across days of acquisition; however, rats swam significantly faster than voles (see Fig. 1B). Next, a repeated-measures ANOVA was conducted for path circuitry. Lack of sphericity in path circuitry across days resulted in the application of a Greenhouse-Geisser correction ( $\epsilon = 0.594$ ). The ANOVA revealed a significant main effect of group [ $F(1,14) = 13.510$ ,  $p = 0.002$ ,  $\eta^2_{\text{partial}} = 0.309$ ] and day [ $F(3.564, 49.898) = 12.652$ ,  $p < 0.001$ ,  $\eta^2_{\text{partial}} = 0.475$ ]; however, the group x day interaction [ $F(3.564, 49.898) = 2.388$ ,  $p = 0.070$ ,  $\eta^2_{\text{partial}} = 0.146$ ] was not significant. Swim path complexity was observed to decrease across days of acquisition; however, rats' swim paths were significantly more direct than voles (see Fig. 1C). Further, the repeated-measures ANOVA for heading error revealed a significant main effect of group [ $F(1,14) = 4.849$ ,  $p = 0.045$ ,  $\eta^2_{\text{partial}} = 0.257$ ] and day [ $F(4.390, 84) = 2.448$ ,  $p < 0.031$ ,  $\eta^2_{\text{partial}} = 0.149$ ]; however, the group x day interaction [ $F(4.390, 84) = 1.446$ ,  $p = 0.207$ ,  $\eta^2_{\text{partial}} = 0.094$ ] was not significant. Heading error was observed to decrease across days of acquisition; however, rats generated significantly less heading error when compared to voles (see Fig. 1D). Lastly, a repeated-measures ANOVA was conducted for percentage of time in center vs. surround. Lack of sphericity in across days resulted in the application of a Greenhouse-Geisser correction ( $\epsilon = 0.448$ ). The ANOVA revealed a significant main effect of day [ $F(2.691,$

$84) = 21.013$ ,  $p < 0.001$ ,  $\eta^2_{\text{partial}} = 0.600$ ] and a group x day interaction [ $F(2.691, 84) = 3.998$ ,  $p = 0.017$ ,  $\eta^2_{\text{partial}} = 0.222$ ]; however, the main effect of group [ $F(1,14) = 0.193$ ,  $p = 0.668$ ,  $\eta^2_{\text{partial}} = 0.014$ ] was not significant. Post hoc analysis revealed rats spent significantly less time in the center during the first day of place learning when compared to voles (HSD  $< 0.05$ ); however, this difference was not observed on subsequent days of testing (see Fig. 1E).

### 3.2. Probe day measures

#### 3.2.1. Brown's score

Following seven days of place learning, all animals experienced a probe day in which the hidden escape platform was removed as an assessment of location retention. Brown's score, and sequential analysis (change in heading, progression path circuitry, and movement scaling) were calculated from a 60 s probe trial. The independent samples t-test conducted for Brown's score revealed a significant group difference [ $T(13.527) = 2.237$ ,  $p = 0.043$ ,  $d = 1.059$ ]. Levene's test indicated unequal variances ( $F = 5.351$ ,  $p = 0.036$ ) so degrees of freedom were adjusted from 14 to 13.527. Brown's score analysis revealed that rats had significantly greater preference for the target quadrant when compared to voles (see Fig. 2A).

### 3.3. Matching-to-place measures

Following a probe day, all animals experienced three days of matching-to-place in which the location of the hidden escape platform was moved to novel quadrants of the pool. The repeated-measures ANOVA conducted for latency revealed a significant main effect of group [ $F(1,14) = 14.163$ ,  $p = 0.002$ ,  $\eta^2_{\text{partial}} = 0.503$ ], trial [ $F(1,14) = 5.660$ ,  $p = 0.032$ ,  $\eta^2_{\text{partial}} = 0.288$ ], and group x trial interaction [ $F(1,14) = 23.091$ ,  $p < 0.001$ ,  $\eta^2_{\text{partial}} = 0.623$ ]. Latency was shown to decrease across trials; however, this decrease was dependent on animal species. Post-hoc analysis revealed that rats' latency to reach the platform significantly decreased from trial one to trial two while voles' latency remained consistent (HSD  $p < 0.05$ ; see Fig. 3A). Additionally, the repeated-measures ANOVA conducted for peak speed revealed a significant main effect of group [ $F(1,14) = 44.046$ ,  $p < 0.001$ ,  $\eta^2_{\text{partial}} = 0.759$ ]; however, the main effect of trial [ $F(1,14) = 2.379$ ,  $p = 0.145$ ,  $\eta^2_{\text{partial}} = 0.145$ ] and the group x trial interaction [ $F(1,14) = 1.955$ ,  $p = 0.184$ ,  $\eta^2_{\text{partial}} = 0.123$ ] were not significant. Rats swam significantly faster than voles during matching-to-place testing (see Fig. 3B). Next, the repeated-measures ANOVA conducted for path circuitry revealed a significant main effect of group [ $F(1,14) = 15.151$ ,  $p = 0.002$ ,  $\eta^2_{\text{partial}} = 0.520$ ], trial [ $F(1,14) = 10.967$ ,  $p = 0.005$ ,  $\eta^2_{\text{partial}} = 0.439$ ], and group x trial interaction [ $F$

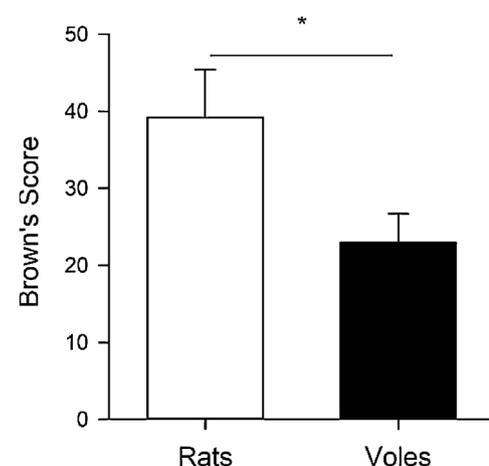
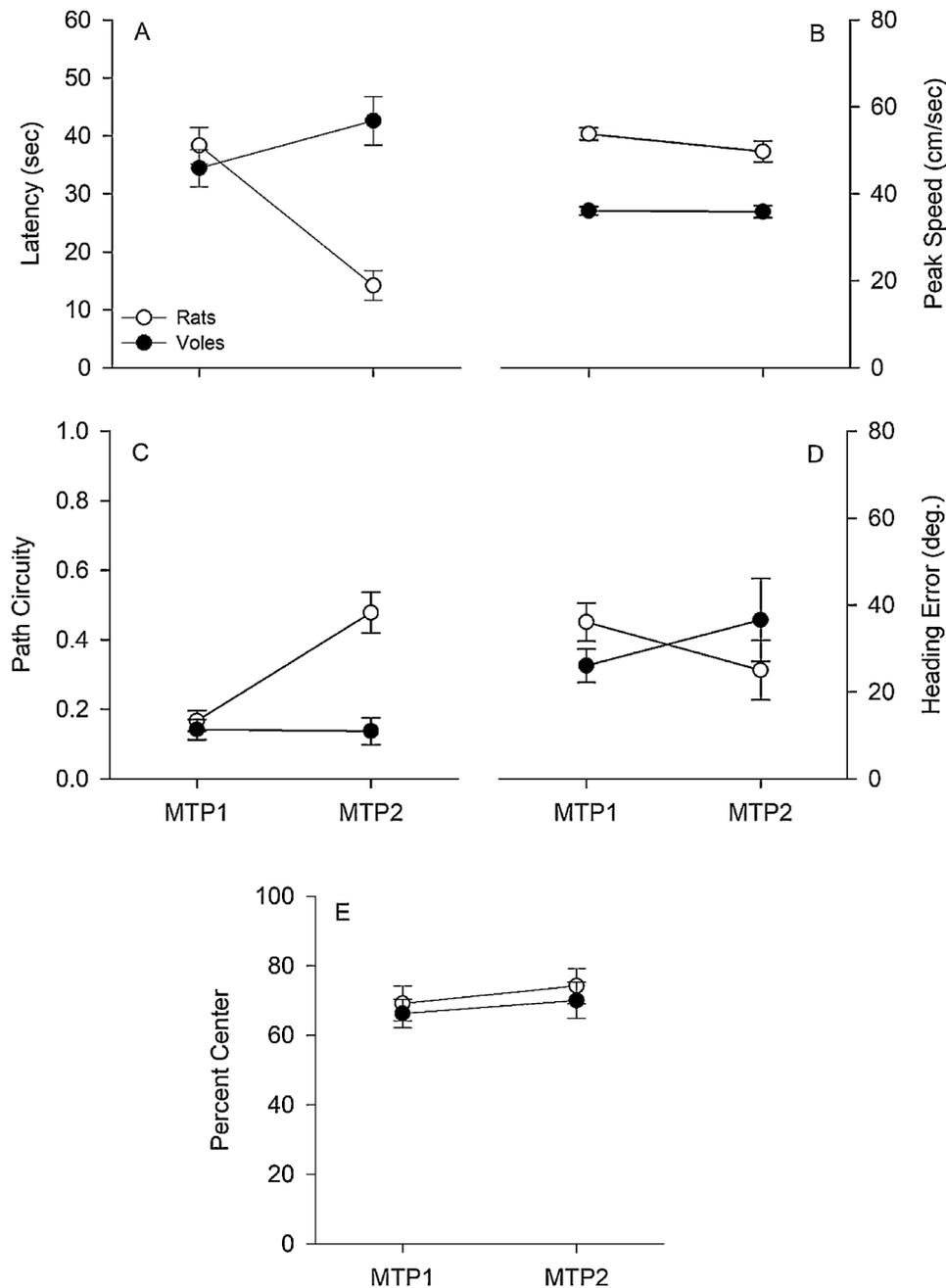


Fig. 2. Brown's score analysis revealed that rats spent significantly more time searching in the target quadrant when compared to prairie voles (A).



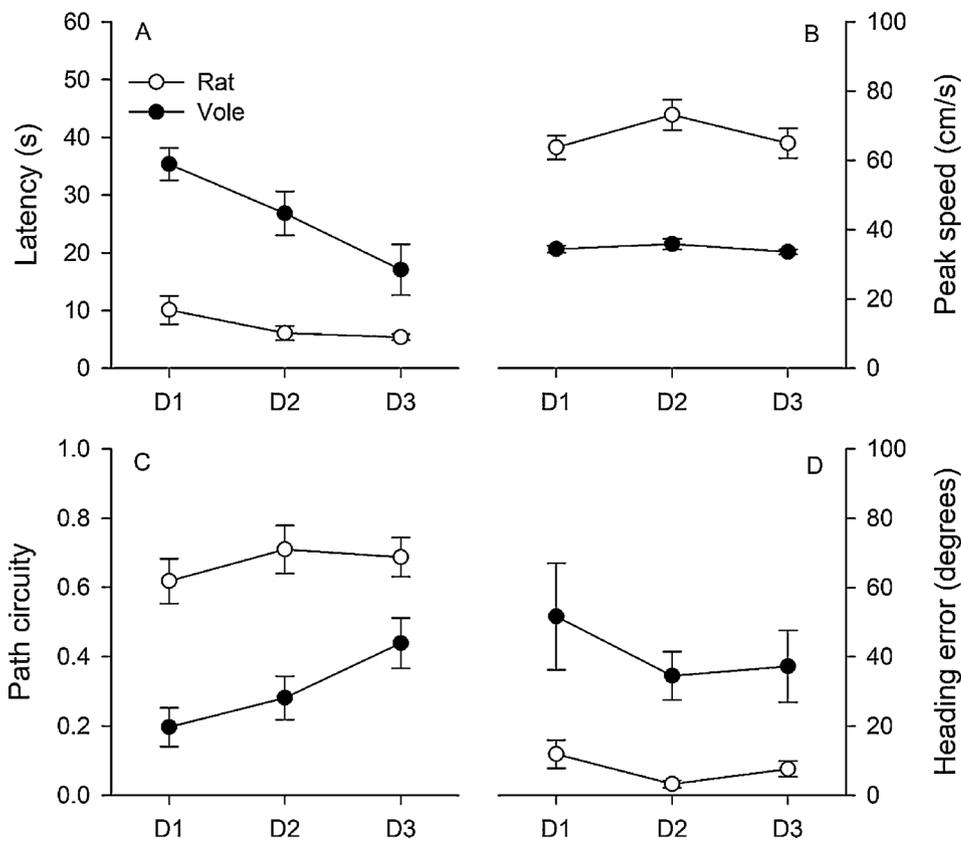
**Fig. 3.** Movement characteristics are plotted for both species across first and second trials of matching-to-place testing. When compared to voles, rats were significantly quicker to locate the hidden escape platform on the second matching-to-place trial (A), had significantly higher peak speeds (B), had significantly lower heading errors on the second matching-to-place trial (C), and had significantly more direct swim paths from MTP1 to MTP2 trials (D). Lastly, no significant group differences were observed for the percentage of time spent swimming in the center of the pool across both trials of matching-to-place (E).

(1,14) = 11.625,  $p = 0.004$ ,  $\eta^2_{\text{partial}} = 0.454$ ]. Path circuituity was shown to increase across trials; however, this increase was dependent on animal species. Post-hoc analysis revealed that rats' swim paths were significantly more direct from trial one to trial two while voles' circuitousness remained consistent across trials (HSD  $p < 0.05$ ; see Fig. 3C). Further, the repeated-measures ANOVA conducted for heading error failed to reveal a significant main effect of group [ $F(1,14) = 0.013$ ,  $p = 0.910$ ,  $\eta^2_{\text{partial}} = 0.001$ ], trial [ $F(1,14) = 0.002$ ,  $p = 0.969$ ,  $\eta^2_{\text{partial}} = 0.000$ ], or a group x trial interaction [ $F(1,14) = 2.572$ ,  $p = 0.131$ ,  $\eta^2_{\text{partial}} = 0.155$ ]. No differences were observed for heading error between species during matching-to-place (see Fig. 3D). Lastly, the repeated-measures ANOVA for percentage of time in center vs. surround failed to reveal a significant main effect of group [ $F(1,14) = 0.054$ ,  $p = 0.819$ ,  $\eta^2_{\text{partial}} = 0.004$ ] and trial [ $F$

(1,14) = 4.127,  $p = 0.062$ ,  $\eta^2_{\text{partial}} = 0.228$ ], or a group x day interaction [ $F(1,14) = 0.560$ ,  $p = 0.467$ ,  $\eta^2_{\text{partial}} = 0.038$ ]. All groups exhibited similar percentages of time spent swimming in the center of the pool across both trials of matching-to-place (see Fig. 3E).

### 3.4. Cued measures

Following three days of matching-to-place, all animals experienced three days of cued learning in which the level of the pool's water was decreased to one centimeter below the platform and the platform was covered with a dark colored sock. The repeated-measures ANOVA conducted for latency, with group (rat vs. vole) as a between subjects measure and day (D1, D2, and D3) as a within subjects measure, revealed a significant main effect of group [ $F(1,14) = 77.485$ ,



**Fig. 4.** Movement characteristics are plotted for rats and prairie voles across three days of cued testing. When compared to voles, rats were significantly quicker to locate the hidden escape platform on the second matching-to-place trial (A), had significantly higher peak speeds (B), had significantly lower heading errors on the second matching-to-place trial (C), and had significantly more direct swim paths from cued1 to cued2 trials (D).

$p < 0.001$ ,  $\eta^2_{\text{partial}} = 0.847$ ], day [ $F(2,28) = 11.517$ ,  $p < 0.001$ ,  $\eta^2_{\text{partial}} = 0.451$ ], and the group  $\times$  day interaction [ $F(2,28) = 4.122$ ,  $p = 0.027$ ,  $\eta^2_{\text{partial}} = 0.227$ ] was significant. Rats took significantly less time to locate the cued platform relative to voles. Although latency to find the platform decreased across days, voles exhibited a larger decrease in latency relative to rats across days (see Fig. 4A). In addition, the repeated-measures ANOVA conducted for peak speed revealed a significant main effect of group [ $F(1,14) = 49.771$ ,  $p < 0.001$ ,  $\eta^2_{\text{partial}} = 0.780$ ]; however, the main effect of day [ $F(2,28) = 3.269$ ,  $p = 0.053$ ,  $\eta^2_{\text{partial}} = 0.189$ ] and the group  $\times$  day interaction [ $F(2,28) = 1.502$ ,  $p = 0.240$ ,  $\eta^2_{\text{partial}} = 0.097$ ] were not significant. Rats exhibited significantly higher peaks in speed when compared to voles for cued days of testing (see Fig. 4B). Further, the repeated-measures ANOVA conducted for path circuitry revealed a significant main effect of group [ $F(1,14) = 29.879$ ,  $p < 0.001$ ,  $\eta^2_{\text{partial}} = 0.681$ ] and day [ $F(2,28) = 3.356$ ,  $p = 0.049$ ,  $\eta^2_{\text{partial}} = 0.193$ ]; however, the group  $\times$  day interaction [ $F(2,28) = 1.439$ ,  $p = 0.254$ ,  $\eta^2_{\text{partial}} = 0.093$ ] was not significant. Although swim paths became more direct across days, rat swim paths to the visible platform were significantly more direct relative to vole swim paths (see Fig. 4C). Finally, the repeated-measures ANOVA conducted for heading error revealed a significant main effect of group [ $F(1,14) = 52.252$ ,  $p < 0.001$ ,  $\eta^2_{\text{partial}} = 0.789$ ]; however, neither the main effect of day [ $F(2,28) = 1.783$ ,  $p = 0.187$ ,  $\eta^2_{\text{partial}} = 0.113$ ] nor the group  $\times$  day interaction [ $F(2,28) = 0.295$ ,  $p = 0.618$ ,  $\eta^2_{\text{partial}} = 0.021$ ] were found to be significant. Rats exhibited significantly lower heading error during cued testing (Fig. 4D).

#### 4. Discussion

The current study used the Morris water task (MWT) to investigate species differences in spatial learning. The performance of rats and prairie voles was consistent with a conserved capacity to use environmental cues to guide movement; however, several significant differences in performance were observed. First, during place training, rats

located the hidden platform faster, followed more direct swim paths, and accrued less heading error relative to prairie voles. Next, during the probe trial, rats exhibited a stronger preference for the quadrant associated with the position of the hidden platform relative to prairie voles. Further, during matching-to-place and cued testing, rats located the escape platform significantly quicker, followed more direct paths, and accrued less heading error relative to prairie voles. Multiple factors may contribute to the differences observed in performance. The following sections will consider this pattern of results and the mechanisms that may mediate the observed performance.

##### 4.1. Differences in spatial orientation

Rats were shown to perform significantly better in key spatial performance measures across each component of the MWT relative to prairie voles. One possible contributing factor to explain the differences observed is that rats and prairie voles may differ in their visual acuity. If prairie voles' visual acuity was not sufficient enough to discern among extra-maze cues in the testing room (i.e., posters, duct tape patterns, doors), this may have disrupted their ability to develop relationships between the cues and the hidden platform. Research on rodent visual acuity is limited [47,1,16]. One of the only studies to compare visual acuity across rodent species used a modified water task where rats and mice were trained to swim from a starting location down an alley that divided into two sides, each with their own computer monitor with a distinct display. To escape the water, rodents needed to choose the monitor displaying a vertical grating pattern as it contained a submerged escape platform [44]. Rats and mice were observed to learn the task quickly; however, successful side choice was observed to fall below criterion when the spatial frequency was increased beyond 0.5 cycles for mice and 1.0 cycles for rats. Currently, no work has examined the visual acuity of prairie voles. As such, it may be beneficial to utilize a similar acuity water task with prairie voles. The results of that work could influence the design of future methodology and facilitate

investigation of prairie vole spatial cognition. If prairie voles have worse visual acuity relative to rats, they may be limited to engaging navigational strategies that depend on visual cues that are proximal to the apparatus.

Rodents have access to a variety of navigational strategies with which to locate the hidden platform. During early acquisition, rodents tend to thigmotax around the edge of the swimming pool to locate the escape platform (see [53]). To escape, rodents need to learn that there is no escape around the perimeter of the pool and begin searching away from the wall. Excessive thigmotaxis may indicate that a rodent has yet to encode relationships among room cues and the escape platform location or that they are unable to shift to using other navigational strategies. During acquisition in the current study, prairie voles exhibited significantly more circuitous paths to the platform than rats. This pattern of behavior suggests that they may have engaged in more thigmotaxis than rats. Further, during the MTP portion of the task, rats' path circuitry was shown to become more direct from one trial to the next, suggesting that they learned the new location of the platform, whereas prairie voles' paths remained equally circuitous from one trial to the next. Despite differences in path circuitry during acquisition and MTP, when thigmotaxis was analyzed as a specific individual variable, via percent time spent swimming in the center of the pool when compared to swimming in the surround, no group differences were observed. Rather, a significant interaction was observed where rats exhibited a significantly lower percentage of swimming in the center on the first day of acquisition when compared to voles. This difference was no longer significant after the first day of testing. This result suggests that voles did not engage in excessive thigmotaxis, but differences in latency and path circuitry point to voles demonstrating inefficient use of spatial cues in the room. This could suggest that voles may have difficulty in utilizing an efficient navigational strategy to solve the task. Differences in the capacity to shift between navigational strategies may reflect varying survival needs of each species' natural habitats.

Field observations of wild prairie voles (*Microtus ochrogaster*) indicate that their habitats are restricted to very specific areas of prairie land, including tallgrass prairies, ungrazed pastures, fallow fields, weedy areas, or areas adjacent to soybean or alfalfa fields [48,27]. These areas often contain few, if any, salient landmarks or configurations in their environment. This may predispose prairie voles to utilizing other navigational information such as using celestial cues. Indeed, in one study, meadow voles (*Microtus pennsylvanicus*), which are often found in wet meadows, swampy pastures, and moist grasslands, were observed to acquire an outdoor water task adapted from the MWT that required them to make use of celestial cues [23]. To solve this outdoor variant of the task, meadow voles had to associate the location of the hidden platform with the position of the sun and its configuration with other celestial cues. While this previous study only examined meadow voles, it is plausible that prairie voles also rely on such cues to navigate. Specifically, the absence of celestial cues in the current study may have limited prairie voles' ability to navigate; thereby forcing them to use thigmotaxis, which may have negatively influenced performance.

In contrast to meadow or prairie voles, rats occupy a multitude of habitats ranging from garbage dumps and sewers to woodlands and farmland [40,42,59]. The dynamic ability of rats to thrive in various environments necessitates that they become flexible in their ability to navigate through them. This is consistent with previous work showing that rats are able to utilize various sources of spatial information and display flexibility in switching between navigational strategies to accommodate their current access to this information [29]. For example, when consistent extra-maze cues were present, and the goal location remained consistent, rats prioritized environmental cue-based strategies, like beacon homing or piloting to navigate. When cue access was restricted, accurate navigation to a refuge was thought to depend on using dead reckoning, a self-movement cue-based strategy. To further characterize the spatial cognition of each species as well as their capacity to shift navigational strategy, a follow up study could use

projections of celestial cues on the testing room walls as extra-maze cues for the task. These results may inform subsequent methodologies for investigating rat and prairie vole spatial capacities. Given that the MWT has shown to be hippocampal dependent, potential differences in the capacity to shift between navigational strategies may reflect differences in hippocampal morphology.

#### 4.2. Non-spatial factors influencing performance

Differences in swim latency could have been attributed to a range of other factors. First, differential stress reactivity may have influenced the pattern of results. For example, rat baseline levels of plasma corticosterone were observed at roughly 100 ng/ml and were observed to increase to 500 ng/ml following one trial in the MWT [4]. In contrast, prairie voles exhibit higher baseline levels of corticosterone (500 ng/ml) and the levels double subsequent to five minutes of swimming [51]. As of yet, no studies have directly compared stress reactivity or corticosterone level between rats and voles during training in the MWT. In addition, there are no comparative data for corticosterone after a first swim in rats or prairie voles. The current study establishes the foundation for future studies to investigate the role of stress reactivity in mediating species differences in performance.

Next, the size of the apparatus relative to the each species may have influenced the current pattern of results. In the current study, both species were tested in the same swim tank (173 cm diameter) with an escape platform (15 cm diameter). Previous work has observed differences in performance when using the same size swim tank to investigate spatial learning in mice and rats [55,58,49]. In addition, differences in performance have also been observed when the size of the apparatus was scaled relative the average mouse (100 cm diameter) or rat (180 cm diameter) body size [11]. Although the size of the escape platform (15 cm diameter) used in the current study was larger than typically used in these studies (10 cm diameter), it was sufficient to elicit species differences in performance. These observations demonstrate species differences in MWT performance are robust and maintained over a range of swim tank and escape platform sizes.

Although the apparatus size may play a limited role in mediating species differences in performance, the size of the rodent may have influenced performance. Specifically, rats are larger than voles and this size differential may have impacted the topographic or kinematic aspects of movement during swimming. This explanation, while possible, does not fit with the pattern of results observed in the current study. Specifically, the lack of a significant group effect for peak speeds during acquisition suggests that both species are capable of swimming at relatively equal speeds. These data suggest that physiological differences in body size or motor coordination should not have contributed to differences observed in latency to reach the platform.

Finally, prairie voles and rats may vary in their motivation to escape the water. Specifically, prairie voles may have been less motivated to escape when compared to rats. However, this explanation is not consistent with the results as latency was shown to decrease across days and peak speeds were not shown to significantly differ between species during acquisition, suggesting an equivalent motivation for each species to escape onto the platform. In addition, previous work has shown that exposure to water is aversive to rats and voles alike. In detail, when placed in a forced-swim task, rats will often display active coping behaviors (struggling, climbing, and swimming) in an attempt to remove themselves from the water (see [8]). Similar behaviors have been observed when testing naïve prairie voles [19,33,34]. In the current study, both rats and voles demonstrated active swimming behaviors in their search for the escape platform, providing further support for both species being equally motivated to escape from the averseness of the water.

## 5. Conclusions

Comparative analysis of spatial processing across species provides valuable insight for extending the use of rodent models in the understanding of spatial cognition. Many studies have examined differences in water task performance between rats and mice; however, no work has examined this comparison for rats and prairie voles. The prairie vole has been a useful rodent model for studying social stress, depression, emotion, and cardiovascular function [17,19,33,34], but few studies have examined the spatial cognition of these rodents. While both species were shown to have learned the task, as evidenced by decreased latencies to reach the platform across days of testing, rats were shown to outperform prairie voles in all (acquisition, probe, MTP, and cued) components of the task. These performance differences in acquisition were paralleled by differences in Brown's scores during a probe trial where rats spent significantly more time in the target quadrant when compared to voles. These results parallel other work showing that rats tend to outperform other rodent species, like mice, in water-based spatial tasks [58,11]. These differences may have been attributed to several mechanisms including differences in spatial cognition, responses to stress, physiology, or motivation. However, the lack of group differences in several control measures suggests that the species differences observed here may be due to differences in navigational strategy use or the flexibility to switch between strategies. This work provides the foundation for future comparative studies to investigate neural control of spatial navigation.

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