

Preweanling Rats Solve the Morris Water Task via Directional Navigation

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Adult rats show a preference for directional navigation over place navigation in the Morris water task. Here, the authors investigated whether preweanling rats with a newly developed ability to perform the water task also solve the task via directional navigation. After 24-day-old rats were trained to find a hidden platform in a fixed spatial location, a no-platform probe trial was conducted with the pool either in the same position as that used during training (*no shift group*) or shifted to a new position in the room (*shift group*). The authors found that rats in the shift group did not search for the platform at its absolute spatial location but rather navigated in the same direction that the platform was located during training and searched at the correct distance from the pool wall, resulting in a search at a location that was never trained. This pattern of results suggests that young rats learn to solve the water task by navigating in a particular direction rather than navigating to a precise place—a finding that may have implications for understanding hippocampal development.

Keywords: spatial learning, spatial navigation, cognitive mapping, hippocampus, ontogeny

Studies on the development of spatial navigation in the rat have generally agreed that the ability to learn to navigate to a hidden platform in the Morris water task (Morris, 1981, 1984) emerges toward the end of the 3rd week of life (Akers & Hamilton, 2007; Brown & Whishaw, 2000; Carman & Mactutus, 2001; Kraemer & Randall, 1995; Rudy & Paylor, 1988; Rudy, Stadler-Morris, & Albert, 1987; Tonkiss, Shultz, & Galler, 1993). In these studies, it has typically been assumed that preweanling rats learn to solve the water task by navigating to a precise place in the environment (i.e., the hidden platform) based on its spatial relationship to a constellation of distal visual cues. There are, however, other means by which rats can reach a goal location—such as navigating in a particular direction in the environment (Blodgett, McCutchan, & Mathews, 1949; Hamilton, Akers, Weisend, & Sutherland, 2007; Skinner et al., 2003)—that may appear behaviorally identical to place navigation unless the two types of navigation are disambiguated with certain experimental procedures, such as moving the test apparatus within the environment. Given the standard methodologies used within the existing developmental water task literature, therefore, it is unclear whether preweanling rats solve the task using true place navigation or a different strategy, such as directional navigation.

To dissociate place and directional navigation among adult rats in the water task, Hamilton et al. (2007) trained rats to navigate to a hidden platform in a fixed location and then conducted a no-platform probe trial with the pool either in the same position as that used during training (*no shift condition*) or shifted to a new position (*shift condition*). In the shift condition, the absolute spatial location of the platform remained within the perimeter of the pool

but was located in the opposite quadrant; thus, to reach the absolute location, rats had to navigate in a direction opposite to what was trained. The results clearly showed that instead of navigating to the absolute location, rats in the shift condition navigated to and persisted in swimming at the relative location of the platform. That is, shift rats navigated in the same direction in the room that the platform was located during training and searched at the correct distance from the pool wall, resulting in a swim path centered around a location that was never trained. This pattern of results suggests that adult rats learn to solve the water task by using a combination of direction and distance information from the distal cues and the pool wall, respectively, which is inconsistent with the hypothesis that rats learn the precise spatial location of the platform. In the present study, we used similar methodology as Hamilton et al. to determine whether the newly developed ability of preweanling rats to learn to locate a hidden platform in the Morris water task is—like adult rats—reflective of learning to navigate in a particular direction in the environment as opposed to true place navigation.

Method

Subjects

All procedures were in accordance with the Institutional Animal Care and Use Committee at the University of New Mexico. Two pregnant dams (Harlan, Indianapolis, IN) arrived 5 days prior to giving birth. The day of birth was designated as Postnatal Day 0. Pups were housed with their dam in plastic cages (51 × 25 × 22 cm) with a 12-hr light–dark cycle (lights on at 8:00 a.m.); food and water were provided ad libitum. When rat pups reached 24 days of age, 16 pups underwent water task testing, with 8 pups in each group (shift or no shift). Groups contained equal numbers of rats from each litter, with numbers of males and females from each litter matched as much as possible (shift: $n = 4$ males, $n = 4$ females; no shift: $n = 5$ males, $n = 3$ females). Weaning occurred at the end of testing.

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Apparatus

A circular white pool (1.5 m diameter, 48 cm high) was set on a wooden frame (48 cm high) that rested on appliance rollers, allowing the pool to be repositioned when filled with water. The pool was filled to a depth of 27 cm with $\sim 24^\circ\text{C}$ water, which was made opaque by the addition of a small amount of nontoxic powdered white paint. The platform (16 \times 16 cm), located ~ 1 cm below the water surface, was made of white plastic and covered in a wire grid to assist rats in climbing onto it. Figure 1 shows the layout of the testing room; the most prominent extramaze visual cues included office furniture (north and east walls), a shower curtain hung in front of a closet (northwest corner), a wooden wiring channel at the junction of the wall and ceiling (south and west walls), a tarp (southeast corner) and chalkboard (southwest corner) hung on the walls, and a door that remained closed during testing (south wall). Behavior was videotaped by an overhead camera attached to a digital camcorder. Videos were transferred to a Linux workstation for tracking and analysis.

Procedure

All training and probe trials were conducted within a single day. Training consisted of six sessions of four trials. Rats were trained with the pool in either Position 1 or Position 2 (see Figure 1), with equal numbers of rats assigned to each position. Regardless of pool

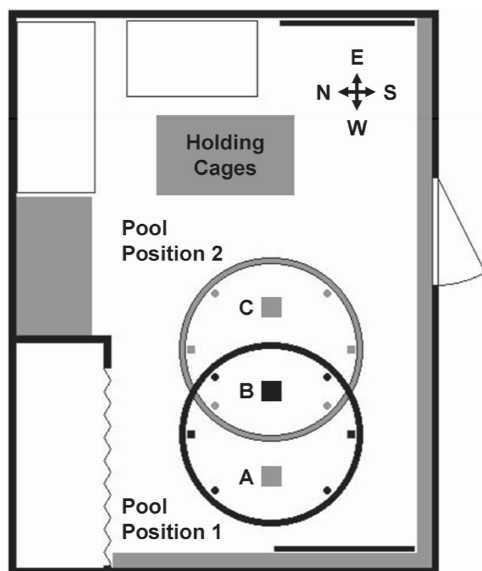


Figure 1. Room layout showing the two possible pool positions. During training, the pool was either in Position 1 or Position 2, and the platform was located at the same absolute location (the black rectangle labeled B). After training, a no-platform probe trial was administered during which the pool either remained in the same position as that used during training (no shift group) or was shifted to the other position (shift group). The gray rectangles (Locations A and C) mark comparison locations (relative/opposite) used for probe trial analyses. Pool Positions 1 and 2 were separated by a distance equal to the radius of the pool. The small circles around the pool perimeter (SW, SE, NW, and NE) mark release points used during training, and the small squares (N or S) mark release points used during the probe trial.

position, the hidden platform was at Location B (i.e., the same absolute location in the room). Rats were released facing the pool wall from one of four points equally spaced around the pool perimeter (northeast, southeast, northwest, southwest). Each release point was used once within a session; the sequence of release points was randomized within and across sessions for each rat. To prevent fatigue and hypothermia in the young rats, trials lasted a maximum of 45 s; if a rat did not find the platform within this time limit, it was retrieved by the experimenter and placed on the platform. Rats remained on the platform for approximately 5 s before being removed by the experimenter; this duration was selected to further limit exposure to the cool water and to reinforce navigation to the platform by quickly removing the rat from the water. The intertrial interval was 15 s, during which rats were placed in a holding cage. Immediately after each session, rats were towel-dried in front of a heater and returned to their individual cages, which contained an ample amount of bedding and were located in close proximity to a heater. Before each session (inter-session interval was ~ 10 min), we measured the rats' core body temperature using a rectal thermometer (Physitemp Instruments, Clifton, NJ). Rats were not run in the next session of trials until core body temperature was within 1°C of baseline body temperature (37°C). The dependent measure during training was latency to find the platform, which was averaged within sessions. Immediately after training, a 30-s probe trial was conducted during which the platform was removed from the pool. For the no shift group, the pool remained in the same position (Position 1 or Position 2) as that used during training. For the shift group, the pool was shifted a distance equal to the radius of the pool to the other position (see Figure 1). Both no shift and shift groups contained equal numbers of rats that received training with the pool in Position 1 or Position 2. Rats were released from one of two release points (north or south) that were not used during training; release points were randomly selected with the constraint that each release point was used equally often for each group.

Four dependent measures were taken during the probe trial for each of two critical locations that were the same size as the platform. One critical location was the absolute location of the platform in the room during training (Location B), and the other critical location was in the diametrically opposite quadrant of the pool (Location A or C). For the shift group, the opposite location corresponds to the relative location of the platform in the pool, which could be reached by swimming in the same direction (i.e., eastward or westward) that the platform was located during training. For the no shift group, the opposite location serves as a comparison location that has the same spatial relationship to the absolute location as does the relative location for the shift group (i.e., in the opposite quadrant). Thus, the critical locations of interest for the shift group were the absolute location of the platform in the room and the relative location in the pool that the platform occupied during training, whereas the critical locations of interest for the no shift group were the absolute location of the platform in the room and the opposite location in the pool. The number of times each critical location was crossed and the average distance from each location were measured; the latter measure was adapted from the goal proximity measure described by Gallagher, Burwell, and Burchinal (1993). Latency to enter and time spent in circular regions (66 cm in diameter) centered around each critical location were also measured.

We analyzed training data using repeated measures analyses of variance (ANOVAs) with Group, Sex, and Litter as between-subjects factors, and Session as a within-subject factor. We analyzed probe trial data using repeated measures ANOVAs with Group, Sex, and Litter as between-subjects factors, and Location (absolute vs. relative/opposite) as a within-subject factor. Given the presence of significant Group \times Location interaction effects, additional ANOVAs were performed to determine (a) whether, within the shift and no shift conditions, rats exhibited a preference for the absolute versus relative/opposite platform location and (b) whether shift and no shift rats differed in their preference for the absolute and relative/opposite platform locations.

Results

Analyses of training data revealed a significant Session effect, $F(5, 40) = 51.15$, $p < .001$, resulting from a decrease in latency to reach the platform across sessions. No other main effects or interactions were significant ($ps > .072$). No shift and shift groups displayed similar latencies during the last session, finding the platform in 4.22 ± 0.64 s and 4.81 ± 0.62 s ($M \pm SEM$), respectively.

Representative probe trial swim paths for no shift and shift groups are shown in Figure 2A; group means for probe trial dependent measures are shown in Figures 2B–2E. Analyses of probe trial data revealed significant Group \times Location interactions for all four dependent measures: latency to enter the circular regions surrounding the critical locations, $F(1, 8) = 14.74$, $p = .005$; average distance from the critical locations, $F(1, 8) = 5.74$, $p = .043$; number of critical location crossings, $F(1, 8) = 6.19$, $p = .037$; and time spent in the circular regions surrounding the critical locations, $F(1, 8) = 7.76$, $p = .024$. No other main effects or interactions were significant ($ps > .110$). Because no effects involving Sex or Litter were significant, these factors were dropped from subsequent analyses.

Of the 8 rats in the no shift group, 6 rats entered the absolute region first. No shift rats entered the absolute region faster, $F(1, 7) = 8.58$, $p = .022$, and spent more time in the absolute region, $F(1, 7) = 6.38$, $p = .040$, than the opposite region. No shift rats also navigated closer to and crossed the absolute location more frequently than the opposite location, but these comparisons did not reach significance (average distance: $p = .075$; crossings: $p = .125$). Of the 8 rats in the shift group, 7 rats entered the relative region first. Compared with the absolute region, shift rats entered the relative region faster, $F(1, 7) = 10.79$, $p = .013$; navigated closer to the relative location, $F(1, 7) = 8.33$, $p = .023$; crossed the relative location more frequently, $F(1, 7) = 6.67$, $p = .036$; and spent more time in the relative region, $F(1, 7) = 7.23$, $p = .031$.

No shift rats entered the absolute region faster than shift rats, $F(1, 14) = 13.14$, $p = .003$; navigated closer to the absolute location, $F(1, 14) = 14.62$, $p = .002$; crossed the absolute location more frequently, $F(1, 14) = 4.90$, $p = .044$; and spent more time in the absolute region, $F(1, 14) = 7.63$, $p = .015$. In contrast, shift rats entered the relative region faster than no shift rats entered the opposite region, $F(1, 14) = 12.94$, $p = .003$; navigated closer to the relative location, $F(1, 14) = 7.22$, $p = .018$; and spent more time in the relative region, $F(1, 14) = 10.13$, $p = .007$. Shift rats also crossed the relative location more frequently than no shift rats crossed the opposite location, but this comparison did not reach

significance ($p = .053$). Comparisons between the absolute location for no shift rats and the relative location for shift rats failed to detect significant differences for any of the dependent measures ($ps > .512$). Likewise, comparisons between the opposite location for no shift rats and the absolute location for shift rats failed to detect any significant differences ($ps > .517$).

Discussion

We investigated whether 24-day-old rats learn to solve the Morris water task by navigating to the absolute spatial location of the hidden platform or, rather, by navigating in a particular direction in the environment. We found that when the pool was shifted to a new position during a posttraining probe trial, rats did not navigate to the absolute spatial location of the platform; instead, they navigated in the same direction that the platform was located during training and searched at the correct distance from the pool wall, resulting in a search at a location in the room that was never trained. In fact, rats in the shift group treated this relative location in much the same way as rats in the no shift group treated the absolute location in terms of both initial trajectory and spatial distribution of the swim path, while treating the absolute location as an arbitrary, untrained location in the pool. This pattern of results is consistent with the hypothesis that preweanling rats use a combination of direction information from the distal cues and distance information from the pool wall to locate a hidden platform in the water task; it is inconsistent with the hypothesis that preweanling rats learn to navigate to the precise location of the platform. These findings suggest that the developing ability of young rats to solve the Morris water task more accurately reflects learning to use distal cues to guide navigation in a particular direction than learning to use those cues to guide navigation to a particular place. This means that previous water task studies among preweanling rats (Akers & Hamilton, 2007; Brown & Whishaw, 2000; Carman & Mactutus, 2001; Kraemer & Randall, 1995; Rudy & Paylor, 1988; Rudy et al., 1987; Schenk, 1985; Tonkiss et al., 1993) most likely offer a characterization of the emergence of directional navigation instead of place navigation.

Our present finding of directional responding in the water task among preweanling rats is nearly identical to that observed among adult rats (Hamilton et al., 2007), suggesting that rats solve the water task using directional navigation regardless of their developmental stage. Furthermore, Hamilton et al. (2007) found that rats perform directional responses after minimal training (eight trials) as well as after more extended training (36 trials) in the water task, suggesting that the navigational strategy used to solve a spatial task does not change with the amount of training, as has been suggested by previous studies (Chang & Gold, 2003; Packard & McGaugh, 1996). Thus, the present and previous (Hamilton et al., 2007) studies taken together suggest that directional responding is the principal type of navigation in the water task regardless of rats' age or amount of experience with the task. These studies, however, do not imply that rats cannot utilize place navigation to solve spatial tasks, although it has been shown that spatial tasks requiring place navigation are often more difficult to learn than tasks that can be solved via directional navigation (Blodgett et al., 1949; Skinner et al., 2003). Thus, the existing evidence suggests that directional responding—not place responding—may be the predominant manner by which rats navigate in a spatial environment.

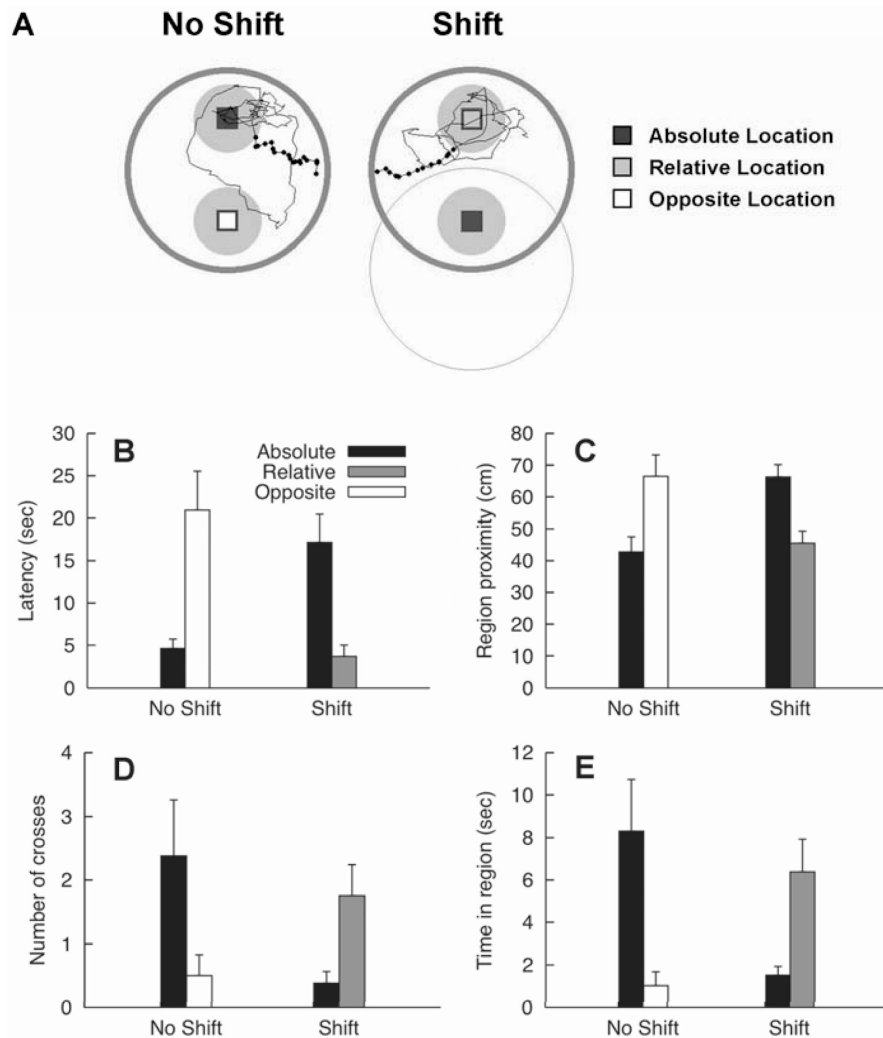


Figure 2. (A) Representative probe trial swim paths for no shift and shift groups. Selected paths were from rats with median latencies to enter the preferred region during the probe trial (absolute for the no shift group, and relative for the shift group). The large thin circle shown for the shift group indicates the pool position used during training. The large thick circles indicate the pool position during the probe trial. The smaller gray circles within the pool mark the circular regions around the critical locations (absolute vs. relative/opposite) that were used for analyses. The initial trajectory, defined as the path taken from the release point until one of the two circular regions was entered, is shown in filled black circles. The remainder of the path is shown as a thin black line. (B) Latency to enter the circular region around the two critical locations of interest. (C) Average distance from the two critical locations. (D) Number of times each critical location was crossed. (E) Time spent in each of the two circular regions. All data panels (B–E) show $M_s \pm SEMs$.

It is important to note that the directional navigation observed in the present study is neither the same as simple response-based navigation (i.e., turning left or right in a consistent manner) nor the same as learning to enact a particular sequence of movements, as several different release points were used throughout training, and novel release points were used during the probe trial. Furthermore, the directional navigation observed here is not equivalent to cued navigation, which consists of navigating toward a single proximal cue that marks the goal location. Although the swim paths observed among rats in the shift group were consistent with the use of an intramaze cue—the pool wall itself—to provide information

regarding where to search for the platform (i.e., how far from the wall to search), information regarding the direction in which to navigate must have been provided by extramaze cues, as the pool wall alone offered no disambiguating information regarding direction. In other words, whereas cued navigation involves the use of a single cue to guide navigation to a goal location, directional navigation involves using a combination of direction and distance information from the distal cues and the wall of the test apparatus, respectively. Further evidence that directional and cued navigation do not reflect the same process comes from previous studies (Akers & Hamilton, 2007; Brown & Whishaw, 2000; Rudy &

Paylor, 1988; Rudy et al., 1987) reporting that the ability to navigate in the water task using a proximal cue emerges at least a couple days earlier in development than the ability to navigate using distal cues (see discussion in Akers & Hamilton, 2007).

Both place and directional learning in a dry land T-maze have been found to be impaired by hippocampal lesions (Stringer, Martin, & Skinner, 2005), indicating that the two types of navigation have a common neuroanatomical basis. The existence of separate populations of place cells (O'Keefe, 1979) and head direction cells (Taube, Goodridge, Golob, Dudchenko, & Stackman, 1996) in the hippocampal formation, however, suggests that place and directional navigation rely, at least to some degree, on distinct neurophysiological mechanisms. Whether the neural mechanisms underlying place and directional navigation exhibit different developmental trajectories, however, remains to be determined, as thus far the developmental studies of spatial navigational abilities in preweanling rats have presumably assessed only directional navigation. Because adult rats generally learn directional tasks more easily than place tasks (Blodgett et al., 1949; Skinner et al., 2003), it is possible that directional responding may be a simpler form of navigation than place responding, and thus the ability to navigate in a particular direction may developmentally precede the ability to navigate to a specific place. Provided that a variant of the Morris water task can be derived that requires true place navigation, investigation of the developmental trajectories of directional versus place navigation may offer additional insight into whether the neural mechanisms underlying each form of navigation can be dissociated.

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