Spatial Memory in Hamsters (Mesocricetus auratus): Depleting/replenishing Environments and Pre-choice Behaviors in the Radial Arm Maze

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Rodents’ spatial memory is traditionally assessed in the radial arm-maze (RAM). An accurate response pattern in the RAM is described as the tendency to visit a new arm after each choice (i.e., win-shift strategy). When this response pattern is found, it is said that the animal remembers the places visited. In the present experiment, 12 hamsters were assessed in the RAM under 2 conditions: the depleting condition, in which feeders were not rebaited after each visit, and the replenishing condition, in which feeders were rebaited. We registered the number of new arms visited (hits), the time spent in the central area of the maze, and the behaviors emitted in the central area before each arm choice. Results showed that, regardless of condition, animals were significantly more likely to visit new arms. However, more pre-choice behaviors and a longer center time were observed in the depleting condition than in the replenishing one. It is discussed that hamsters have a win-shift strategy for hoarding behavior even when they do not need to remember the places visited, though they exhibited more pre-choice behaviors when searching for food in the depleting condition.

Keywords: radial arm-maze, spatial memory, pre-choice behaviors, hamsters

In the standard radial-arm maze (RAM) procedure, animals can obtain a piece of food at the end of each arm, and once the animal traverses the whole maze and obtains all pieces of food, the session is finished (Olton & Samuelson, 1976; for a review, see Foreman & Ermakova, 1998). Since only one piece of food is available in each arm, it is assumed that the optimal strategy would be to enter each arm only once. This strategy implies that subjects should discriminate the previously visited arms from the ones they have not yet visited. Extensive evidence shows that the subjects avoid revisiting locations significantly more than what would be expected by chance (Craig et al., 2012; Olton, Collison, & Werz, 1977; Olton & Samuelson, 1976). This performance is accounted for the assumption that subjects maintain each arm in working memory (Carrillo-Mora, Giordano, & Santamaría, 2009; Olton, 1978; Zhou & Crystal, 2011).

Nevertheless, experimental evidence has suggested that if animals used response algorithms or biases such as always visit the adjacent arm, then a discrimination process involving past items would be unnecessary (Brown & Cook, 1986; Yoerg & Kamil, 1982). If no algorithms are used, then picking-up visual, haptic, or smell information from arm entrances could play an important role in the spatial orientation required to choose among arms within the RAM (Brown, 1992; Suzuki, Augerinos, & Black, 1980; Zoladek & Roberts, 1978). If spatial orientation by context cues is determinant for performance in the RAM, then subjects should exhibit...
observational responses and explore different places before choosing to accept or reject an arm (Brown & Cook, 1986; Haga, 1998).

Since Tolman (1932, 1939) observed and theorized that organisms’ engagement in a behavioral pattern is characterized by “looking back and forth” at a choice point, the analysis of orientation and observation behaviors emitted before a final choice has generated wide interest (Bett et al., 2012; Bimonte & Denenberg, 2000; Browne & Dinsmoor, 1974; Hu, Xu, & González-Lima, 2005, 2006; Schmidt, Papale, Redish, & Markus, 2013). This pre-choice behavioral pattern is also known as vicarious-trial and error (VTE; Muenzinger, 1938), and it serves to orient and differentiate signals within a choice point (Tolman, 1938; for a recent review, see Redish, 2016).

Pre-choice behavior was analyzed by Brown and Cook (1986) as the dynamics of their performance in the RAM. The authors observed that rats increased the time spent at the central place of the RAM during the last few choices and engaged in investigatory behaviors at the entrance of the arms. Pre-choice behaviors such as door investigation, sniffing, examining the floor, pausing, or inquiring by moving the head in the center platform were called microchoices to distinguish them from the truthful arm choices called macrochoices (Brown, 1992).

Brown and Cook (1986) suggested that the dynamics of performance in the RAM within trials are related to a process of signal discrimination rather than to the guidance provided by a cognitive map to direct spatial orientation (Brown, 1992; Hu & Amsel, 1995). Hence, anticipatory discriminative responses (Blough, 1959) are most likely occurring well before the moment of choice, and key aspects of this process can be revealed by examining the distribution of observation responses (see Brooks & Wasserman, 2010).

Thus, the analysis of behavioral patterns before a final response is emitted has been insightful to better understand discrimination and decision-making processes in humans (Spivey, Grosjean, & Knoblich, 2005) as well as nonhuman animals (Blough, 1959; Brooks & Wasserman, 2010; Skinner, 1965).

Assuming that the function of pre-choice behaviors is to discriminate among places from within the RAM, then animals will surely perform such behaviors more frequently while under conditions that demand the recall of the places visited rather than under conditions in which memory is not required to complete the task. One way to test this hypothesis is to compare performance in a condition in which food is renewed after each visit (replenishing condition) to performance in a condition in which food is depleted after each visit (depleting condition). In the present experiment, we addressed whether animals modify their strategies to obtain food in the RAM between depleting and replenishing environments. Pre-choice behaviors, as well as arm-choice behavior, were analyzed in both conditions.

**Method**

**Subjects**

Twelve golden male hamsters (*Mesocricetus auratus*), inbred in the veterinary school of the University of Guadalajara, participated in the experiment. They were approximately 8-months-old upon arrival and had previous experience running an experiment on a straight-alley task. Each hamster lived in an individual home cage (21 × 22 × 24 cm) with free access to water and solid food (Purina © chow) in a temperature-controlled room set to a 12:12 h light/dark cycle with light beginning at 0700. Before starting the experiment, the experimenter weighed the amount of food intake for each hamster for 7 days. After the food-intake baseline was established, the experimenter supplied the average amount of food consumed at baseline at the end of each daily session. Because of
the development of a limp when walking, one hamster did not finish the experiment. The experiment followed the official Mexican norm NOM-062-ZOO-1999 “Technical Specification for Production, Use and Care of Laboratory Animals”.

Apparatus

An eight-arm radial maze (Model RAMRN; MED Associates, Inc., St. Albans, Vermont, USA) was used. The radial maze had an octagonal-shaped central platform from which eight arms arose, and the entrance of each arm was equipped with an automatic guillotine door. The size of the central platform of the maze was 27.5 cm in diameter, 17.1 cm width, and 27.5 cm high. Each arm had an interior size of 44.5 cm long, 9.09 cm wide, and 16.8 cm high. It was equipped with an automatic pellet dispenser (ENV-203-190IR) and a head entry detector (ENV-254) mounted at the food receptacle. The arms of the maze were completely covered with blackout-cloth, preventing subjects’ visual access to extra-maze cues.

Experimental events were controlled and recorded by MED-PC IV software, installed on an IBM-PC compatible computer. A video camera (Sony DCR-TRV 351) located about 30 cm above the top of the RAM recorded the hamsters’ behaviors at the central platform.

Procedure

Subjects were randomly assigned to one of two groups, differing in their order of exposure to two conditions in a counterbalanced order: depleting or replenishing. Thus, Phase 1 was the depleting condition for one group of hamsters and the replenishing condition for the other. In Phase 2, the conditions were reversed. In the depleting condition, only the first visit to each arm produced a food pellet (Bio-Serv®), and any subsequent visits did not. In contrast, the arms’ feeders were baited after each visit in the replenishing condition. Therefore, subsequent visits to an arm continued to deliver food until a total of eight pellets were obtained. Each condition lasted 18 daily sessions. Since subjects had experience traversing a runway for food, no previous training was necessary.

A session began by placing the hamster on the central platform. After a few seconds, all guillotine doors opened. A choice was counted once the hamster’s head entered the food receptacle at the end of an arm. Sessions ended when the subject obtained a total of 8 food pellets or after 15 min had elapsed, whichever occurred first. Then, the guillotine doors closed, and the subject was kindly removed from the RAM and placed in its home cage. The maze was wiped clean after each session with a 10% solution of alcohol to avoid any mark of each subject’s smell.

The videotapes of the last three depleting and replenishing sessions were selected for analysis of pre-choice behaviors. The analysis of the videos was done using CowLog software (Hänninen & Pastell, 2009). Two naïve and independent observers registered the following behaviors: pauses, when the hamster was walking on the central platform, stopped, and then continued walking in the same direction; pause-change direction, when the hamster was walking on the central platform, stopped, and then walked in a different direction; and, entrance exploration, when the animal touched the arm entrance edges with its nose.

Data Analysis

Hits were measured as the percentage of different arms chosen within the first eight choices. Data were obtained for each subject for the first five sessions and last five sessions of each condition and then averaged by group. Using Student’s one sample \( t \)-tests, hits were compared with the percentage obtained if choices were random.

The center time was measured as the time elapsed after exiting an arm until entering another arm. A linear mixed-effects model was run on the last five sessions of each condition, with subjects as a random effect and condition, successive choices, and group (order of treatment), and their interaction as fixed effects. Also, for the last five sessions of each condition, center time was averaged by each successive choice and group, and it was then fit to a linear function.

An analysis of variance (ANOVA) of two factors (Condition and Group) was performed on the mean of the cumulative pre-choice behaviors of the last three sessions of each condition.

Results

Despite the fact that the replenishing condition did not require rats to choose new arms to obtain food, the average percentage of new arms visited (hits) within the first eight choices was high. A Student’s \( t \)-test showed that, in all conditions, the hits were significantly above chance (\( p < 0.01 \); see Table 1). In Phase 1
(Table 1, upper row), hamsters of both the replenishing and depleting conditions began with about 70% hits in the first five sessions but finished with a slightly different performance in the last five sessions: 75.25% and 71.43%, respectively. In Phase 2 (Table 1, lower row), when conditions were switched, the hamsters exposed to the replenishing condition slightly increased to 74.29% and 73.81% (first and last five sessions, respectively); on the other hand, the hamsters exposed to the depleting condition had 75.43% and 73.71% of hits in the first and last five sessions, respectively. Neither conditions nor the order of exposure to conditions (Phases 1 or 2), or the level of experience with the task (first or last sessions) affected animal choices significantly.

Table 1
**Percentage of Hits by Condition During the First and Last 5 Sessions of Each Phase**

|                  | Replenishing |                  | Depleting |                  |
|------------------|--------------|------------------|-----------|------------------|
|                  | First sessions | Last sessions | First sessions | Last sessions |
|                  | $M \pm SEM$ | $df$ | $t$ | $M \pm SEM$ | $df$ | $t$ | $M \pm SEM$ | $df$ | $t$ |
| Phase 1          | 70.48±4.15 | 5  | 4.93* | 75.24±4.41 | 5  | 5.73* | 70.95±1.72 | 5  | 12.2* |
| Phase 2          | 74.29±3.76 | 5  | 6.46* | 73.81±5.08 | 5  | 4.69* | 75.43±3.68 | 4  | 6.91* |

*p<0.01

To evaluate whether the hamsters spent more time on the center platform for the choices in either the depleting or the replenishing condition, a linear function of the time each hamster spent at the central platform for successive choices was fitted (Figure 1). The left panel shows data from Phase 1, while the right panel shows data from Phase 2. In all cases, center time increased over the successive choices. A linear mixed effects model indicated that center time increased throughout the successive choices, $F(6, 54) = 6.33, p < 0.001$, and that effect was mediated by condition (Successive Choices × Condition interaction), $F(6, 54) = 2.60, p = 0.03$.

![Figure 1](image1.png)

**Figure 1.** Mean of center time as a function of successive choices in the RAM by condition (replenishing and depleting) in the last five sessions of each phase and the respective linear regression.

The cumulative sum of the three pre-choice behaviors registered while on the central platform (i.e., pauses, changes of direction, and entrance exploration) is shown in Figure 2 (left panel) as a function of successive choices. Open circles represent data from the depleting condition and filled circles correspond to
the replenishing condition; solid and dashed lines represent Phase 1 and Phase 2, respectively. This figure shows that, for the depleting condition, pre-choice behaviors increased more steeply than for the replenishing condition. However, higher values correspond to the subjects that were in the replenishing condition in Phase 1 and then switched to the depleting condition in Phase 2 (open circles dashed line); for those subjects, pre-choice behaviors increased up to four responses. An ANOVA showed significant effects of both condition, \( F(1, 9) = 11.11, p = 0.01 \), and an interaction, \( F(1, 9) = 5.25, p = 0.05 \), but no group effect was found, \( F(1, 9) = 0.22, p = 0.65 \).

The right panels of Figure 2 show the same data for each separate behavior: pauses (upper panel), changes of direction (middle panel), and entrance exploration (lower panel). The first two behaviors show a pattern similar to what was previously described for cumulative responses, but the behavior of entrance exploration only had a cumulative sum of 0.5 occurrences in all conditions.

![Figure 2](image)

**Figure 2.** Mean of cumulative responses as a function of successive choices in the RAM in the last three sessions of each phase and condition (replenishing and depleting). The right panel shows each behavior observed, and the left panel shows overall responses.

**Discussion**

In the present study, although the probability to choose new arms did not differ between replenishing and depleting conditions, all conditions were significantly above chance, including the replenishing condition, during which no spatial memory was required to obtain food. The values shown in Table 1 indicate that subjects’ performances were similar to what has been reported in other studies using hamsters without access to extramaze cues (Etienne, Sitbon, Dahn-Hurni, & Maurer, 1994) or using analogues of the RAM (Cabrera, 2009; Tonneau, Cabrera, & Corujo, 2012). The strong tendency to select new arms above chance has also been exhibited by rats in the absence of food rewards (Haga, 1995; Timberlake & White, 1990). Furthermore, in our experiment, this finding was observed in the first sessions of both conditions. This result suggests that what has been asserted about rats in the RAM is also true for hamsters: Almost no previous experience is required to search efficiently in the RAM (Timberlake, 2002). Not only for hamsters, but also for rats, niche-related mechanisms determine much of the win-shift strategy in maze behavior. Given that resources in a patch (i.e., food or water) are usually finite, then, as they become depleted, the probability to shift to a new patch increases (Stephens & Krebs, 1986). As far as hamsters’ food gathering, it has been observed that when a task demands low efforts for foraging, hamsters gather more food than they consume (Day & Bartness, 2001). This is also the case for the walking requirement to get food in the RAM (Jones, McGhee, & Wilkie, 1990).
Even though the hit probability was indistinguishable between depleting and replenishment conditions, as well as between early and late sessions, hamsters’ search for food clearly differed in the amount of pre-choice behaviors they exhibited. In the depleting condition, time spent on the center platform and the cumulating behaviors increased more steeply than in the replenishing condition, particularly when the depleting condition followed the replenishing one. The increase in time and in pre-choice behaviors was observed more consistently in later choices, when the discrimination of a nonvisited arm became more difficult. This result is in line with previous research that shows an increase in VTE behaviors at a choice point where it is more difficult to discriminate among places or stimuli (Bett et al., 2012; Bimonte & Denenberg, 2000; Redish, 2016; Tolman, 1939).

The main conclusion of this experiment is twofold. First, performance in choice procedures, such as in RAM, cannot be completely described by the probabilities and distributions of choices; therefore, it is necessary to consider the behavioral patterns before a choice is made. Second, pre-choice behaviors, such as pause and orientation towards potential travel routes (Schmidt et al., 2013), are highly sensitive to changes in the environmental requirements that demand memory processes to perform a task (Bett et al., 2012). Hence, notwithstanding the tendency to select new arms in both the depleting and replenishing conditions, these results indicate that hamsters foraging in the RAM seek potential cues that provide information about past places. So, it is possible that animals do not rely inherently upon their memory in a map-like representation but involve observing behaviors as in a discrimination process.

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