Short report

Returning to home cage serves as an effective reward for maze learning in rats

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ABSTRACT

This study examined the effectiveness in rats of 'returning to home cage' as a reward for learning a Lashley III maze. Rats could return to their home cage directly (Direct HC group) or they could be removed manually by an experimenter from the maze's goal box (Indirect HC group). In the third group, hungry rats received a food reward in the goal box (Food group). The Direct HC group reliably learned the maze and its performance was very similar to that of the Food group. In contrast, performance by the Indirect HC group was significantly poorer than the Direct HC group, as well as the Food group, possibly due to the negative impact of handling during removal from the goal box. These results suggest that a 'home cage reward' is as effective a procedure for rats in maze learning as previously reported in mice (Blizard et al., 2003, 2006).

1. Introduction

Presentation of food to deprived animals (e.g., Simmons, 1924) and escape from water (e.g., Morris, 1981) or bright light (Barnes, 1979), have proven to be effective reinforcers to motivate rats and mice to navigate mazes. Blizard et al. (2003) emphasized the importance of using various reinforcers for cognitive tasks because it has been shown that animals of different ages or strains may differ in sensitivity to food (e.g., Fuller and Cooper, 1967), pain (Mogil et al., 1999), or cold stress (Guerra et al., 1998). Differences in performance in a behavioral task among different ages or strains of rodents might represent not only age or strain related differences in the cognitive ability of interest, but also differences in sensitivity to the reinforcers used in the task. For example, if we compared young and aged animals in maze learning using only a food reward and observed better performance in young animals than in aged ones, differences in performance might represent not only differences in cognitive ability but also differences in sensitivity to the food reward between the groups. In contrast, if we consistently observed better performance in young animals than aged ones across various types of reinforcers used for maze learning, differences in performance between the groups could be persuasively attributed to differences in age. Thus, examination of performance in a cognitive task using various kinds of reinforcers is needed in order to appropriately compare cognitive abilities among different ages or strains of rodents. Blizard et al. (2003) found that two genetically heterogeneous groups of mice could learn a Lashley III maze using 'return to home cage' as a

reinforcer. That is, non-deprived mice learned the maze reliably without any explicit rewards such as food, if the mice could return to their home cage directly from the goal box of the maze. Blizard et al. (2006) also confirmed the effectiveness of the home cage reward when comparing mice of various age groups in HS and B6D2F1 mice. Bressler et al. (2010) confirmed the finding and provided valuable detailed illustrations of the procedure.

Almost a century ago, Simmons (1924) examined the home cage reward in rats' maze learning and found that it was not effective in rats. Blizard et al. (2003) speculated that, as the rats in Simmons (1924) were returned to their home cage by an experimenter, handling might interfere with the reward effect of returning to their home cage. However, since studies have not examined the effect of the home cage reward in rats' maze learning without the possible interference of manual handling by the experimenter, it is not clear whether the discrepancy between Simmons (1924) and Blizard et al. (2003, 2006) is due to the difference in the manner of returning subjects to their home cages or to species differences between rats and mice. Therefore, the purpose of our study is to examine learning of the Lashley III maze by rats if they are allowed to return to their home cage directly from the goal box of the maze. The possible interference of handling rats in the goal box and returning them to their home cage was also examined.

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2. Method

2.1. Subjects and apparatus

The subjects were 18 experimentally naïve, male Wistar rats, approximately 75 days old at the start of the experiment. These rats were acquired from Kiwa Laboratory Animals Co., Ltd. and they were maintained according to guidelines approved by Kanazawa University Animal Experimentation Regulations. Rats were kept on a controlled 12:12 light/dark cycle in which lights went on at 12:00 and off at 0:00. Experimental sessions were conducted during the dark phase between 9:00-11:00.

The experimental apparatus was a Lashley III maze with a wall height of 15 cm and runway width of 12 cm. The openings from one runway to the next were 12 cm wide and placed 40 cm from the end of each runway. The length of each runway was 144 cm and there were three runways connecting the start box (40 cm long and 12 cm wide) to the goal box (also 40 cm by 12 cm). The maze was constructed of plywood and painted flat gray. The start and goal boxes were separated from the runways by gray PVC guillotine doors that could be operated manually. The runways and start and goal boxes were covered with a wire-mesh top. The maze was elevated 18 cm above the floor of the experimental room with a closeable 12 cm by 12 cm floor hole at the end of the goal box. Each metal home cage (32 cm long, 23.5 cm wide, and 16 cm high) had a wire mesh cover, except for a closeable 12 cm by 12 cm hole in its top, which could be placed under the goal box, congruent with the maze floor opening. Depending on the experimental group, the hole in the floor of the goal box could be closed off by a gray PVC board, with either a food reward placed in a cup (9 cm in diameter) in the goal box or no food and manual removal to home cage, or rats could freely enter their home cage through the opened hole in the floor of the goal box. The illumination level was approximately 2 lx on the floor of the maze, and the rats' performance was monitored and recorded from above by a video camera (SONY DCR-TRV33) with an infrared light mode.

2.2. Procedure

The 18 rats were randomly assigned to one of three groups: food reward (Food group, $n = 6$), direct home cage reward (Direct HC group, $n = 6$), or indirect home cage reward (Indirect HC group, $n = 6$). During the first 10 days, all rats were handled for 1 min a day and the rats in the Food group were reduced to 90% of their pre-deprivation body weight and maintained at this body weight throughout the remaining experiment by adjusting the amount of food given in the home cage. The Direct HC and the Indirect HC groups did not receive any food deprivation throughout the experiment except during the short daily experimental session. Water was always available except during the daily experimental session for all groups. From Day 11 to 14, habituation to the goal box was conducted. Rats in the Direct HC group were placed directly in the goal box with the hole in the floor leading to the home cage opened. The hole in the goal box was closed by a PVC board after the rat entered his home cage. Then the experimenter removed the home cage from under the goal box and returned it to its original place on the breeding shelf. Rats in the Indirect HC group were kept in the goal box for durations that matched the individual response latencies of rats in the Direct HC group (2–22 s) and then carefully moved by the experimenter manually from the goal box to their home cage, placed on the floor next to the goal box, after which the home cage was returned to its original place on the breeding shelf. For the Food group, the floor hole in the goal box was closed and a food cup with five 45 mg food pellets was set at the end of the goal box. Rats were moved to their home cage placed on the floor next to the goal box after they consumed the food reward, and then the home cage was returned to its place on the breeding shelf. One habituation trial was given per day.

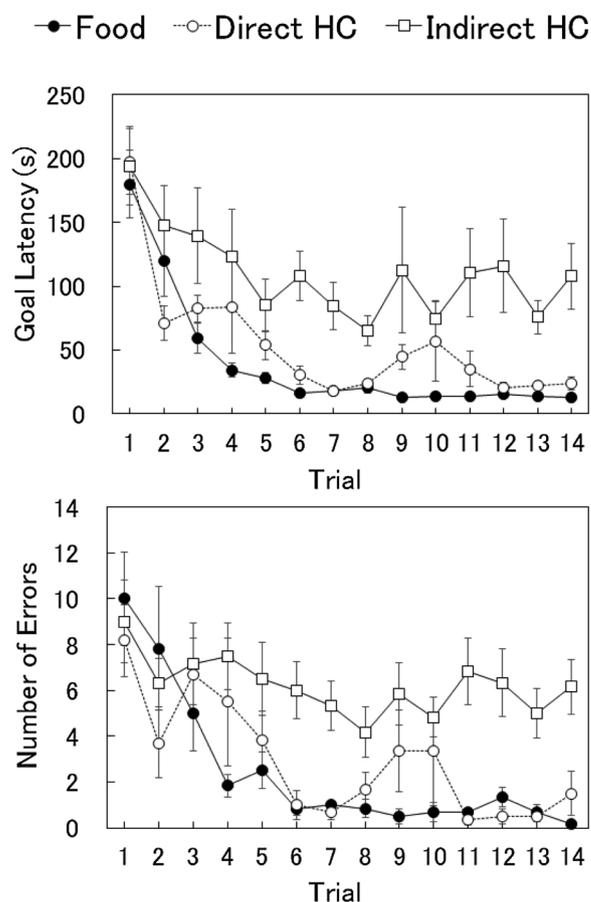Acquisition training began on Day 15 and lasted for 14 days. The

Fig. 1. Mean goal latency (top panel) and the number of errors (bottom panel) of Food, Direct HC, and Indirect HC groups during acquisition training. Error bars represent *SEM*.

food cup and water bottle were removed from the home cage immediately before a trial and then the rat was placed in the start box. The rat was kept in the start box for 60 s, after which the start door was opened. During this 60 s interval, the home cage of the subject was set on the floor below (Direct HC group) or next to (Food and Indirect HC groups) the goal box. The goal box door was closed when the rat's four legs entered the goal box. One hour after being returned to the breeding shelf, the rats in all groups were transferred to clean cages with fresh bedding materials, food cups, and water bottles. The appropriate amount of food to maintain 90% of pre-deprivation body weight was given in the food cups for Food group. One trial was conducted per day.

3. Results

During the four habituation trials, the Food group came to eat the food reward immediately and the Direct HC group learned to smoothly enter their home cage from the goal box within 3 s. The mean latency to reach the goal box for the three groups during acquisition training is shown in the top panel of Fig. 1. On the first trial, there was no significant difference among the groups. Although all groups demonstrated an increasingly shorter latency, it was more marked in the Food and Direct HC groups than in the Indirect HC group. Planned comparison using multiple *t*-tests with nominal significance levels regulated by Ryan's method showed that there was no significant difference among the groups on Trial 1 ($ps > .05$). However, goal latency was significantly shorter overall in the Food and Direct HC groups than in the Indirect HC group ($ps < .05$) and the difference between the Food and Direct HC groups was not significant ($p > .05$) by Trial 14. A two-factor ANOVA of Group and Trial showed a significant main effect of

Group ($F(2, 15) = 26.187$, $p = .001$, $\eta_p^2 = .777$) and Trial ($F(13, 195) = 13.308$, $p = .001$, $\eta_p^2 = .470$). However, interaction between Group and Trial was not significant ($F(26, 195) = .889$, $p = .624$, $\eta_p^2 = .106$). Post hoc Tukey HSD test for the significant main effect of Group showed that goal latency was significantly shorter in the Food and Direct HC groups than in the Indirect HC group ($ps < .05$), and that the difference between the Food and Direct HC groups was not significant ($p > .05$).

The number of errors in the three groups during acquisition training is shown in the bottom panel of Fig. 1. Errors were scored when all four paws of a rat either entered into a runway but went in the wrong direction, passed by the opening to the next runway, or went back to a previous runway. Although there was no significant difference among the groups on the first trial, the Food group and the Direct HC group showed a greater reduction in errors during acquisition than the Indirect HC group. Planned comparison using multiple t-tests with nominal significance levels regulated by Ryan's method showed that there was no significant difference among the groups on Trial 1 ($ps > .05$), but the total number of errors was significantly less in the Food and Direct HC groups than in the Indirect HC group ($ps < .05$), and the difference between the Food and Direct HC groups was not significant ($p > .05$) on Trial 14. A two-factor ANOVA of Group and Trial showed a significant main effect of Group ($F(2, 15) = 18.040$, $p = .001$, $\eta_p^2 = .706$) and Trial ($F(13, 195) = 8.215$ $p = .001$, $\eta_p^2 = .354$). Interaction between Group and Trial was not significant ($F(26, 195) = 1.470$, $p = .075$, $\eta_p^2 = .164$). Post hoc Tukey HSD test for the significant main effect of Group showed that the number of errors was reliably less in the Food and Direct HC groups than in the Indirect HC group ($ps < .05$) and that the difference between the Food and Direct HC groups was not significant ($p > .05$).

When compared to the Food group, performance by the Direct HC group appeared to be somewhat unstable since it deteriorated to some extent on Trials 9 and 10 after it had reached asymptote on Trials 7 and 8, but the differences between the groups were not statistically significant and the Direct HC group showed a clear learning curve in both goal latency and number of errors. Moreover, although interaction between Group and Trial was not significant for both goal latency and the number of errors, the results of the planned comparison and the post hoc analysis for the significant main effect of Group suggest that rats learned the maze not only for Food reward but also for a Direct HC reward and that Indirect HC was not as effective for maze learning. Therefore, the results suggest that a Direct HC reward could serve as an effective reinforcer for Lashley III maze learning in rats.

4. Discussion

The results of this study show that rats can learn the Lashley III maze for a Direct HC reward as well as a Food reward. This suggests that the Direct HC reward is effective for both rats and mice (Blizard et al., 2003, 2006). The present study also showed that an Indirect HC reward was not as effective as a Direct HC reward, providing a rationale for the differences between Blizard et al. (2003, 2006) and Simmons (1924) regarding the effectiveness of a Home Cage reward. Thus, manual removal of animals from the goal box and placement in the home cage by the experimenter in Simmons (1924) may have interfered with the effectiveness of the home cage reward. Our current study results suggest that a Direct HC reward can serve as a useful alternative reinforcer for maze learning in rats. As Blizard et al. (2003, 2006) noted, responsiveness to various reinforcers might be different among different ages or strains of rodents. Therefore, to compare cognitive abilities among different ages or strains appropriately, it is necessary to use various kinds of reinforcers for cognitive tasks. In this regard, the findings of the present study would contribute by adding the HC reward as a possible reinforcer in rat maze learning.

Nevertheless, several issues still need to be clarified. First, Blizard et al. (2003) showed that HS mice learned the maze as effectively when,

on their own, they returned, not to their own home cage, but to one that was identical to their own cage but washed and with clean shavings. We did not examine whether the reward effect of returning to the home cage would be limited strictly to the rat's particular *home* cage. Specifically, would the HC reward differ in effectiveness as a reinforcer if rats returned on their own to a cage that was other than their particular cage? Or is the HC reward actually the absence of being handled by an experimenter? The aspects of a cage, i.e., familiarity, size, shape, bedding material, etc., which characterize the effectiveness of a cage as a reward, should be scrutinized. We might predict the effectiveness of a cage as a reinforcer for maze learning by examining the relative time spent staying in the cage versus the maze (Premack, 1959). Premack's principle of reinforcement predicts that the more time rats spontaneously spend in a cage connected to the goal, rather than in the maze, the more the cage would serve as an effective reinforcer for maze learning.

Also, we did not precisely match the delay in returning rats from the goal box to their home cage between the Direct and Indirect HC groups during acquisition. It is well known that delay in reinforcement reduces the effectiveness of that reinforcement (e.g., Dickinson et al., 1992). According to the experimenter's informal observation, there was no obvious difference in the delay between Direct and Indirect HC groups because the rats in these groups frequently turned back toward the closed goal door after they entered the goal box. However, we should compare the effectiveness of direct and indirect HC rewards with precisely matched delays from the goal box to their home cage. Additionally, although the experimenter in the present study was very experienced in handling rats, the effectiveness of the Indirect HC reward might be affected by the experimenter's proficiency in handling rats.

Finally, Blizard et al. (2003, 2006) replicated the effectiveness of the HC reward for differences in age, sex, and strains of mice. The present study confirmed the effectiveness of a direct HC reward only for adult male Wistar rats. Clearly, the generalizability of the effectiveness of the HC reward for different ages, sexes, and strains of rats must be examined.

Acknowledgment

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