



## **Persistent Memory Retention of Reward Events and Proactive Interference in Reward Series Learning By Mice**

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This study examined acquisition of a single alternating series of reward quantities in mice. Four male ICR mice, trained in a straight runway, showed differential response to items in a 3-0-3-0-3-0-3 series, constructed from a varying number of 0.045 g food pellets under inter-trial intervals (ITI) of 30 s (Experiment 1) or 20 min (Experiment 2), by running more slowly to nonrewards than rewards. Although mice showed reliable item anticipation under 20 min ITIs, nonreward anticipation became poorer in later serial positions than in earlier positions. It is possible that gradual deterioration of nonreward anticipation in a series is caused by proactive interference from previous item memories, since the nonreward anticipation was improved when the target item was divided by a long 120 min interval from prior items that were a potential source of proactive interference (Experiment 3). In Experiment 4, mice learned to respond differentially to the second item of 5-0 and 0-5 series with an ITI of 180 min. These results suggest that mice can discriminate reward magnitudes by forming item-associations between adjacent items and retain information of a previous item for a long interval, and that proactive interference occurs among item memories in a series.

Given that events in natural or experimental settings often have sequential order, animals may predict coming events from prior events (Capaldi, 1994) and choose possible behavioral options by considering the order of events (Phelps & Roberts, 1991; Wathen & Roberts, 1994) or temporal delays from a time point to multiple serial events simultaneously (Brunner, 1999; Brunner & Gibbon, 1995). The ability to learn the order of events has been studied in the form of serial learning. Serial learning in primates and birds has been examined mainly by the simultaneous chain task method, where all of the items are presented simultaneously and animals are trained to respond to these items in a fixed order (for a review, see Terrace, 2005). However, serial learning in rodents has been typically studied in the form of reward-serial learning, where items of a series are constituted from varying amounts of 0.045 g food pellets (e.g., Hulse & Dorsky, 1977) or a varying quality of food (e.g., Capaldi & Miller, 1988b). These food items are placed in the goal box of a straight runway in a fixed order on consecutive trials. Learning is indexed by slower running to smaller rewards, especially to nonrewards, and faster running to larger rewards.

It has been demonstrated that rats learn a series with a simple structure (e.g., 14-7-3-1-0) faster than one with a more complex structure (e.g., 14-1-3-7-0; Hulse & Dorsky, 1977), transfer learning between series with the same formal structure (Hulse & Dorsky, 1979; Taniuchi, 1995), extrapolate a novel item based on series structure (Fountain & Hulse, 1981), and facilitate learning by phrasing complex series into simple sub-

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patterns (Fountain, Henne, & Hulse, 1984). These results were interpreted as evidence that rats encode and represent the abstract structure of the series. This rule learning view has been strongly challenged by a memory-discrimination learning theory that explains serial learning by formation of item-association and stimulus generalization among signals of the associations (Capaldi, 1994; Capaldi & Molina, 1979; Capaldi, Nawrocki, Miller, & Verry, 1986; Capaldi, Verry, & Davidson, 1980; Capaldi, Verry, Nawrocki, & Miller, 1984; Haggbloom & Brooks, 1985).

Whether rats can encode abstract rule structure is not clear (Capaldi, 1994). In contrast, formation of item-association between adjacent items and stimulus generalization among signals in item-association is clearly demonstrated for rats (Capaldi et al., 1980). Moreover, it has been shown that rats can form an association between remote items (Capaldi et al., 1983; Capaldi & Miller, 1988b; Capaldi & Verry, 1981) and utilize the whole of a series representation as a discriminative stimulus for a succeeding series (Capaldi, 1992; Capaldi, Miller, Alptekin, & Barry, 1990; Haggbloom, Birmingham, & Scranton, 1992).

Although reward serial learning in rats has been studied extensively, generality of the findings across species has not been examined, even in other rodents. In serial pattern learning, which requires animals to track signals presented in different spatial locations, temporal phrasing of complex patterns into simple sub-patterns facilitates learning in rats (Fountain & Rowan, 1995; Stempowski, Carman, & Fountain, 1999), whereas it impairs performance of mice (Fountain, Krauchunas, & Rowan, 1999). The inconsistency of the effects of temporal phrasing on rats versus mice motivated us to investigate whether the findings in rat reward serial learning generalize to other rodent species. This study examined the mouse's basic capacity to learn reward series through acquisition of single-alternation series and concurrent learning of simple two-item series.

## Experiment 1

The goal of Experiment 1 was to examine the basic cognitive ability of mice to learn reward series through acquisition of a single alternation series. It has been well documented that rats could learn to anticipate a reward event on a current trial of single alternation series, utilizing the preceding reward event as a discriminative cue (Tyler, Woltz, & Bitterman, 1953). The single alternation series is one of the simplest reward series, where only two types of reward events, reward (R) or nonreward (N), are presented alternately. The ability to associate memories of a preceding reward event with a following event is critical for learning in a single alternation series. The single alternation series can be mastered by forming R'-N (apostrophe meaning "memory" of a reward event) and N'-R associations between items. In Experiment 1, mice received a 3-0-3-0-3-0-3 series, where each R-N and N-R transition occurred three times in a series presentation. The inter-trial interval (ITI) was 30 s, and mice were required to retain item information only for this short period. If mice could master the series, anticipation of reward magnitude should be displayed by running faster to reward items and slower to nonreward items.

Differential response to reward and nonreward items might also be induced for other reasons, such as a slight, transient reduction in hunger level by consumption of a food reward, possibly resulting in slower running to the following nonreward. Another possible source of differential response might be a reinforcement-omission effect, referring to an invigoration of response following a nonreward but not following a reward (e.g., Amsel, 1958; Stout, Boughner, & Papini, 2003). To check for these possibilities, mice were tested with a quasi-random series 3-3-0-0-3-0-3, after acquisition training with the single alternation series was completed. If either a transient reduction of hunger level following a reward or the reinforcement-omission effect were the main cause of differential responses to reward and nonreward trials, it would be expected that mice would run more slowly to a nonreward following a reward than on a trial after a nonreward in this quasi-random series. However, item-association learning predicts a deterioration of differential response to reward items and nonreward items in the quasi-random series because inconsistent outcomes were signaled by identical prior items, that is, R'-R and R'-N, as well as N'-R and N'-N.

## Method

**Subjects.** The subjects were four male ICR mice approximately 150 days old. These mice had experience as intruders in a resident-intruder test for an experiment on aggressiveness in mice, but they had not participated in any discrimination learning experiments.

**Apparatus.** The apparatus was an enclosed straight runway, 132.0 cm long, 10.0 cm wide, and 16.0 cm high, covered by wire mesh on hinged frames. The start box and the goal box were 15.0 cm and 20.0 cm long, respectively, and could be closed off by guillotine doors. The inside of the runway was painted flat black. A digital timer started when the start door was raised and stopped when the infrared ray, located 10.0 cm into the goal box, was interrupted by a mouse. The goal door was lowered when the infrared ray was interrupted, confining the mouse in the goal box. At the end of the goal box, 0.045 g food pellets could be placed in a food cup, 1.5 cm in diameter and 0.5 cm in depth.

**Pretraining.** During the first fourteen days, mice were handled for 1 min per day and their ad-lib body weight was reduced to 85% by food deprivation. These body weights were maintained throughout the remainder of the experiment. On Days 8-12, each mouse was given 10 min of exploration of the runway with all the doors open and permitted to eat six food pellets scattered on the runway floor. On Days 13-14, mice were confined to the goal box with the door lowered and allowed to eat three food pellets from the food cup.

**Experimental training.** Acquisition training began on Day 15 and continued for 80 days. Mice were brought into the experiment room in their home cage. A 3-0-3-0-3-0-3 series was presented once per day under ITIs of 30 s. A trial began with placement of the mouse in the start box with the start door raised 5 s later. On rewarded trials, the mouse was removed to the home cage after the reward was consumed. On nonrewarded trials, the mouse was confined to the goal box with no reward for 20 s and then removed to the home cage. If a mouse failed to complete a trial in 60 sec, it was picked up by the experimenter and placed in the goal box to receive the reward scheduled for that trial with a time score of 60 s assigned for that trial. A single mouse completed all trials of the series before the next mouse was run and the order of runs was varied and counterbalanced in blocks of four days. After completion of acquisition training with the 3-0-3-0-3-0-3 single alternation series, the series was shifted to a 3-3-0-0-3-0-3 quasi-random series for four days.

## Results and Discussion

Figure 1 shows running speeds on each trial of 3-0-3-0-3-0-3 series grouped in blocks of four days. With training, anticipation of reward magnitudes developed, with faster trials to a reward and slower trials to a nonreward. The data from the single alternation series were subjected to a Trials \* Blocks \* Subjects analysis of variance. The analysis revealed significant main effects of Trials ( $F(6, 18) = 12.86, p < 0.01$ ) and Blocks ( $F(19, 57) = 15.88, p < 0.01$ ), and the Trials \* Blocks interaction ( $F(114, 342) = 4.00, p < 0.01$ ). Simple effects of Trials were significant at Blocks 8-20 ( $F_s(6, 360) = 2.99, 5.22, 8.61, 2.49, 6.20, 8.20, 8.15, 9.12, 15.73, 11.43, 10.50, 19.20, \text{ and } 19.47, p_s < 0.05$ ) for Blocks 8-20. Paired comparisons by t-test with a nominal significance level regulated by Ryan's method were performed over the data of the last block, Block 20. The analysis revealed that mice ran faster to all the reward items than to all the nonreward items ( $p_s < 0.05$ ), and that difference in running speeds was not significant among reward items or among nonreward items ( $p_s > 0.05$ ).

When the series was shifted to a 3-3-0-0-3-0-3 quasi-random series, slower running to nonrewards was completely eliminated. A Trials \* Blocks \* Subjects analysis of variance on the quasi-random series data revealed significant main effects of Trials ( $F(6, 18) = 12.34, p < 0.01$ ) and paired comparisons by t-test with a nominal significance level regulated by Ryan's method showed that Trial 1 and Trial 2 were significantly

slower than all the other trials ( $ps < 0.05$ ) and that the difference between Trial 1 and Trial 2 was not significant.

Reliable differential response between reward and nonreward in the single alternation series was developed as a result of training, and it was completely eliminated by the series shift to a quasi-random series. In particular for the 3-3-0-0-3-0-3 quasi-random series, running speeds did not differ between the third or sixth nonreward following a reward and fourth nonreward following a nonreward. If the transient reduction of hunger level by consumption of a reward or an invigoration of response by the reinforcement-omission effect (e.g., Amsel, 1958; Stout et al., 2003) were the main cause of slower running to a nonreward, mice would be expected to run more slowly to a nonreward after a reward than to a nonreward following a nonreward. Thus, the results of the quasi-random test do not support an explanation in terms of differential hunger levels or the reinforcement-omission effect. On the contrary, item-association learning appropriately predicts such deterioration of differential response in the quasi-random series because it offers a difficult discrimination task, where different outcomes are signaled by identical prior items in the series (R'-R and R'-N or N'-R and N'-N). Therefore the results of Experiment 1 support the view that mice, as well as rats, can utilize memory of a reward event on a prior trial as a discriminative cue for a following reward event, and that they have the basic ability to learn a reward series. Although mice showed reliable nonreward anticipation on Block 8, it took about thirty presentations of the series, that is, about 90 R-N transitions, to develop a reliable anticipation of reward magnitude. While we cannot compare the present mouse performance with previous rat studies because of differences in some experimental parameters, such as reward magnitude or ITI, the performance of mice appears somewhat poorer than that of rats, which showed a clear response patterning to the single alternation series after about 50 R-N transitions (Flaherty & Davenport, 1972; Tyler et al., 1953).

## Experiment 2

The results in Experiment 1 showed that mice could acquire a single alternation series and retain item information for 30 s. Several studies have shown that rats manifested anticipation of reward magnitude under considerably longer ITIs, ranging from minutes to 24 h (Capaldi & Lynch, 1966; Capaldi, Nawrocki, Miller, & Verry, 1985). Experiment 2 examined whether mice could anticipate reward events in a single alternation series with a longer ITI of 20 min versus the 30 s ITI of Experiment 1. If mice could retain item information during a 20 min ITI, the differential response to reward and nonreward items of the single alternation series must occur as in Experiment 1.

### Method

**Subjects.** Mice that participated in Experiment 1 were used as subjects. The animals were allowed free access to food and water for 64 days after Experiment 1 was finished and then received pretraining for Experiment 2.

**Apparatus.** The apparatus was the same as that employed in Experiment 1.

**Pretraining.** During the first 12 days, mice were reduced to 85% of ad-lib body weight by food deprivation and were maintained at that body weight throughout the remainder of the experiment. On Days 1-7, mice were handled for 1 min per day. On Days 8-12, each animal was confined to the goal box with the door lowered and allowed to eat three food pellets from the food cup.

**Experimental training.** Training began on Day 13 and continued for 40 days. A 3-0-3-0-3-0-3 series was presented once per day under an ITI of 20 min. During a subject's ITI, trials for other subjects were

conducted. The order of the running of mice was consistent each day but it varied and was counterbalanced in blocks of four days. All other aspects of the training procedure were as in Experiment 1.

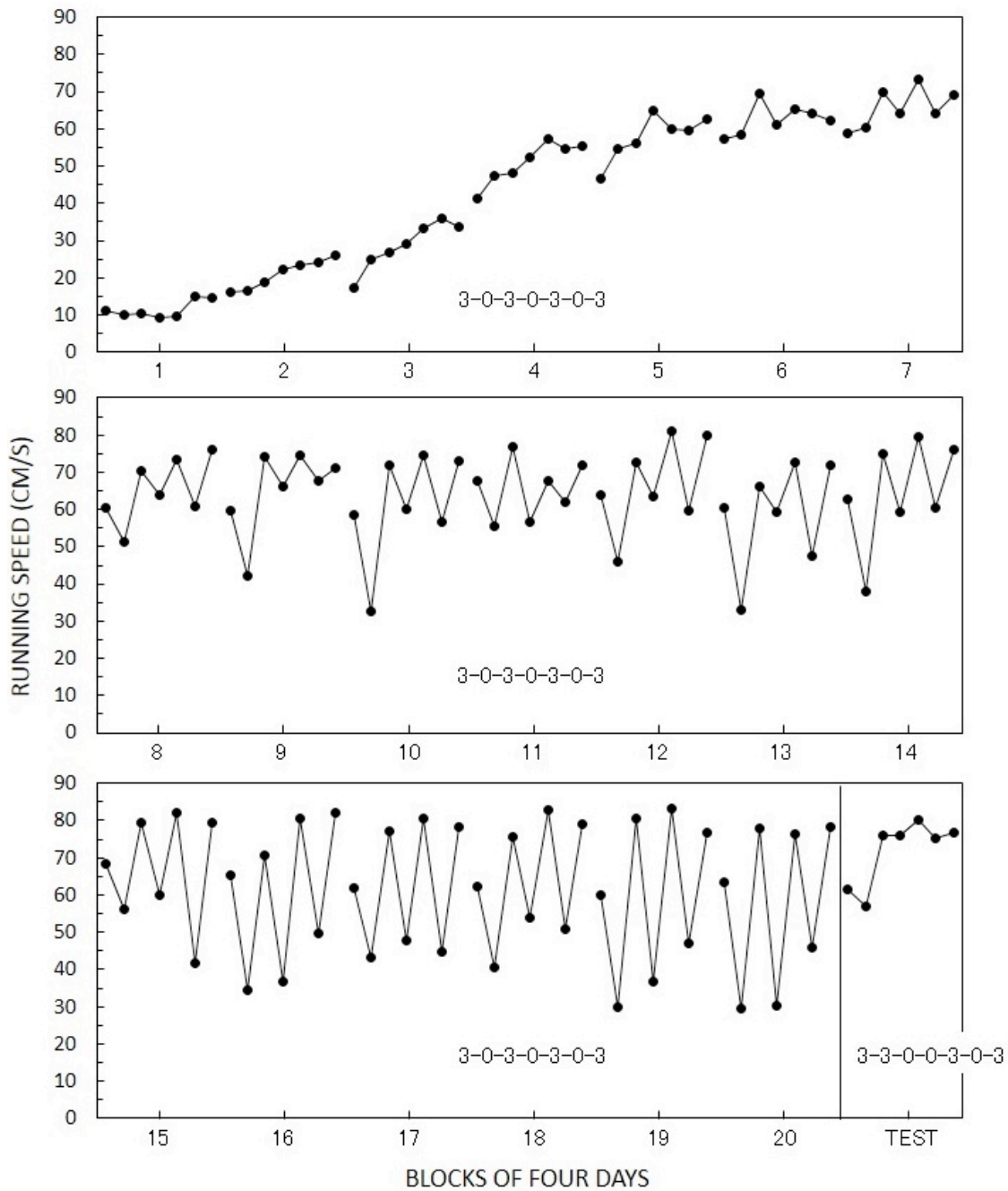


Figure 1. Running speeds on trials in a 3-0-3-0-3-0-3 series and a 3-3-0-0-3-0-3 series in blocks of four days in Experiment 1. Trials were separated by a 30 s ITI.



immediately preceding trial should be present. Given that the order of running was varied irregularly day by day, the possible odor cue might be valid for three-fourths of the sessions for all subjects, but not valid for one-fourth of the sessions. Therefore, if an odor cue from other subjects were a dominant discriminative cue for differential running, the mice's performance should be better when they were run on the second, third, or fourth turn than on the first turn in a session. Order \* Trials \* Subjects analysis of variance on the last two blocks revealed a significant main effect of Trials ( $F(6, 18) = 25.40, p < 0.01$ ). But the main effect of Order ( $F(1, 3) = 0.13$ ) and the interaction of Order \* Trials ( $F(6, 18) = 0.74$ ) were not significant. Absence of significant effects related to running order in a session suggests that the odor cue emitted by other subjects was not a dominant discriminative cue for reward anticipation in Experiment 2.

This discrepancy between the present study and Ludvingson and Sytsma (1967) on the effect of the odor cue from preceding subjects might be explained by the availability of adjacent item associations. Learning a single alternation series could be regarded as a discrimination task that requires animals to discriminate a memory cue of R' (R'-N) from N' (N'-R), where memory of reward item (R') signals a following nonreward item and memory of a nonreward (N') signals a following reward item. In contrast, because the identical memory cues of R' (R'-R and R'-N) and N' (N'-R and N'-N) are followed by both reward and nonreward in a double alternation series, for example, R-R-N-N-R-R-N-N or N-N-R-R-N-N-R-R, a single item memory cue is not an effective discriminative stimulus for a following reward event. Indeed, if the odor cue from a preceding subject became ineffective by destroying a stable relationship between reward events for that preceding subject and the subject that followed, rats could not learn to anticipate reward events in a double alternation series at all (Ludvingson & Sytsma, 1967). The item memory cue might overshadow the odor cue emitted by preceding subjects when it is a good discriminative stimulus for a following reward event. Of course, the inconsistent results with regard to the effect of the odor cue might result from species differences between mice in the present study and rats in Ludvingson and Sytsma (1967). Examining discrimination learning of the odor cue from other subjects in mice would be a worthwhile topic of research. Present results indicate that mice, like rats, can retain information of reward items for a relatively long interval. However, some deterioration of nonreward anticipation was observed on later serial positions. One possible cause of the gradual deterioration of nonreward anticipation is proactive interference. As mentioned above, learning a single alternation series could be regarded as a discrimination task of a memory cue of R' (R'-N) and N' (N'-R). If memory of a preceding nonrewarded trial (N'), which signals a reward item, is persistent in working memory during a nonreward trial following a rewarded trial, then it will interfere with memory of the reward (R') that signals a nonreward on the current trial. In a spatial working memory task using a radial maze, it was reported that rats could not reset memory of events in preceding trials and showed gradual deterioration of performance caused by proactive interference on subsequent trials (e.g., Cohen, Reid, & Chew, 1994; Roberts & Dale, 1981). The same may hold true for mice in reward serial learning. That is, if mice cannot reset their memory of prior items in a single alternation series, interference among item memories must be greater on later trials than on earlier trials.

### Experiment 3

In Experiment 2, nonreward anticipation gradually deteriorated as serial position advanced. One possible explanation for this phenomenon is proactive interference produced by confusion among preceding item memories. Roberts and Dale (1981) explained the magnitude of proactive interference in rats' radial maze performance in terms of temporal discriminability. That is, in subsequent trials, rats have to discriminate event memories of preceding trials from a current trial in terms of a temporal feature determined by the time elapsed from the occurrence of those events. Thus, the temporal discriminability view predicts superior performance given a longer interval between interfering events and target events, which ensures a higher temporal discriminability of those events. Cohen et al. (1994) confirmed this prediction by showing the release of proactive interference in a radial maze task by lengthening the inter-trial interval.

Experiment 3 examined the effects of temporal discriminability among item-memories on a single alternation series performance. Procedures were similar to those of Experiment 2, where a 3-0-3-0-3-0-3-0 series was presented under ITI of 20 min, but in Experiment 3, a 60 min (Phase 1) or a 120 min (Phase 2) interval was inserted between the sixth and the seventh trial (3-0-3-0-3-0/3-0, where the slash represents the lengthened interval). The target behavior in this experiment was the anticipation of the eighth nonreward, signaled mainly by memory of the seventh reward (R'[7]-N[8]). If progressive deterioration of nonreward anticipation was caused by proactive interference among memories of reward events, insertion of the longer interval would be expected to increase discriminability between R'[7] and the preceding memory of nonreward that signaled reward, for example N'[6]-R, and improve the anticipation of N[8].

The other possible cause of progressive deterioration of nonreward anticipation is the influence of differences in hunger level. Because the mice were fed after daily training and first nonreward and last nonreward were separated by more than 120 min under the condition of a 20 min ITI, the level of hunger might have been lowest on the initial trials and highest at the end of the series. A higher hunger level may hinder mice from inhibiting their response to an unbaited goal. Thus, different hunger levels could be a cause of the progressive deterioration of nonreward anticipation. To control for this possible hunger effect, subjects received control trials in which the long interval was inserted between the second and third items (3-0/3-0-3-0-3-0). Therefore, hunger strength should be same on the target eighth nonreward trial of the experimental 3-0-3-0-3-0/3-0 trial and the control 3-0/3-0-3-0-3-0 trial because the elapsed time from the preceding day's feeding to the target eighth item was scheduled to be the same between these conditions. If anticipation of the eighth nonreward item were better on the experimental trials than on the controls, it would suggest that proactive interference among item memories is for a factor in the progressive deterioration of nonreward anticipation.

## Method

**Subjects.** Mice from Experiments 1 and 2 were used. The mice were continuously maintained at 85% of ad-lib body weight from the start of Experiment 2 onwards.

**Apparatus.** The apparatus was the same as that employed in Experiments 1 and 2.

**Experimental training.** Phase 1 of Experiment 3 began the day after Experiment 2 finished and lasted for 20 days. A 3-0-3-0-3-0-3-0 series was presented under an ITI of 20 min once per day. A long ITI of 60 min was inserted between sixth and seventh items (3-0-3-0-3-0/3-0) of the experimental trials, whereas a long ITI was inserted between second and third items (3-0/3-0-3-0-3-0) of the control trials. Half of the subjects received the experimental trial on odd days and the control trial on even days. For the other half of the subjects, assignments were reversed. All of other aspects of the training procedure were the same as in Experiment 2.

Phase 2 of Experiment 3 began the day after Phase 1 finished and lasted for 20 days. In Phase 2, the long ITI was extended to 120 min. All other aspects of the training procedure were the same as in Phase 1.

## Results and Discussion

The top panel of Figure 3 shows running speeds for each trial of the experimental condition (3-0-3-0-3-0/3-0) and the control condition (3-0/3-0-3-0-3-0) in Phase 1. Planned comparisons using the Dunn-Sidak method showed no reliable differences between the experimental and control conditions in all serial positions ( $DS = -0.68, -0.92, 1.75, 1.10, -0.96, 0.77, -0.46, \text{ and } 1.96, ps > 0.05$  for Runs 1-8, respectively). The data shown in the top panel of Figure 3 were subjected to a Trials \* Conditions \* Subjects analysis of variance. The

analysis revealed a significant main effect of Trials ( $F(7, 21) = 19.24, p < 0.01$ ). Paired comparisons by t-test with a nominal significance level regulated by Ryan's method revealed that running speeds were slower on all nonreward trials than on all reward trial ( $ps < 0.05$ ). In addition, mice ran more slowly to a nonreward on Trial 2 than on Trial 6 and Trial 8 ( $ps < 0.05$ ). These results show reliable nonreward anticipation and its progressive deterioration, but they do not suggest a recovery from the progressive deterioration of nonreward anticipation by the insertion of the 60 min interval.

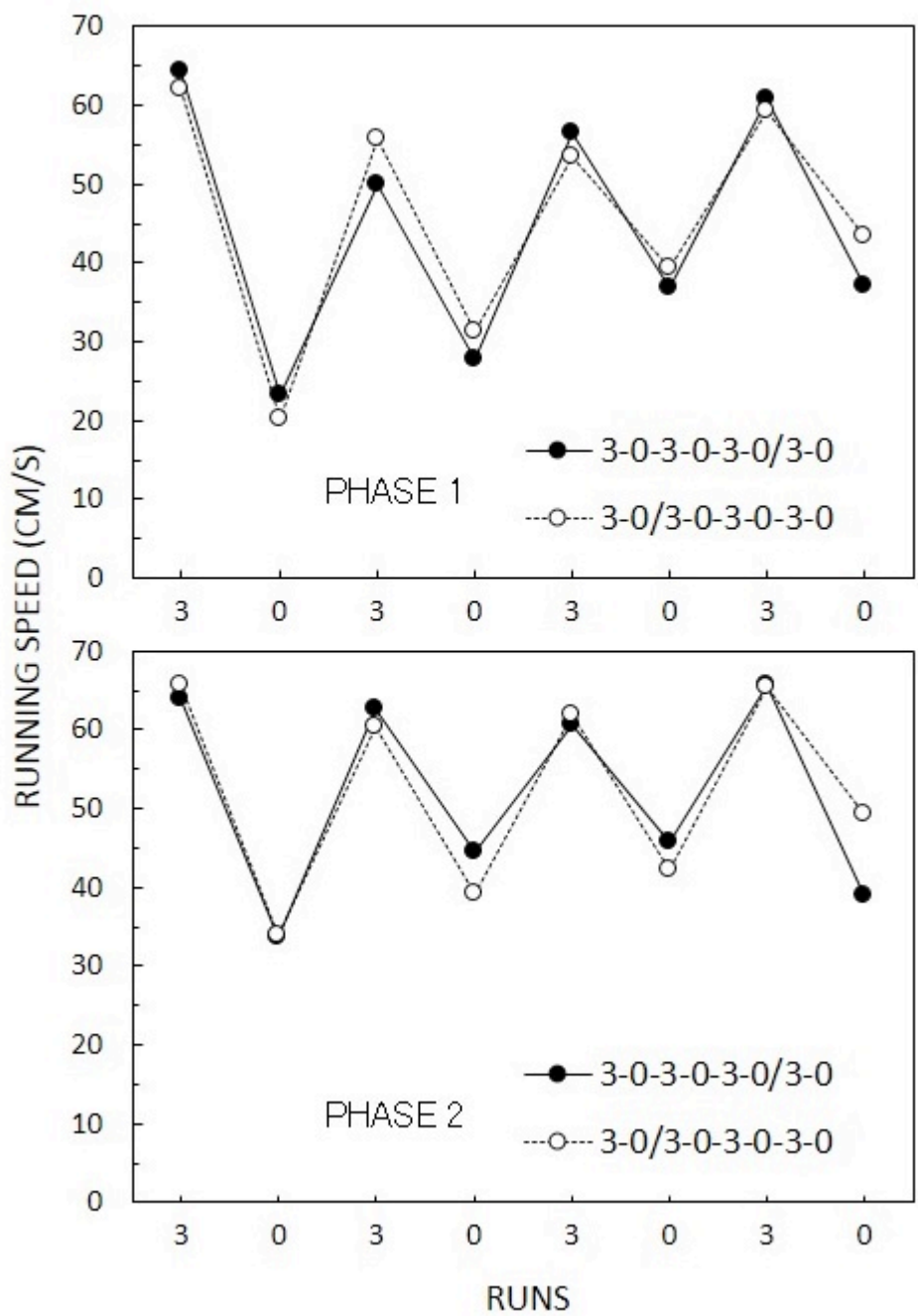


Figure 3. Running speeds on trials in a 3-0-3-0-3-0-3-0 series in Experiment 3. A 60 min (Phase 1: top panel) or 120 min (Phase 2: bottom panel) ITI was inserted between the fifth and sixth trials (3-0-3-0-3-0/3-0) or the second and third trials (3-0/3-0-3-0-3-0). Other ITIs, represented by hyphens, lasted 20 min.

Roberts and Dale (1981) predicted a lessening of proactive interference by increased temporal discriminability among events to be memorized. They did not find any decrease in the proactive interference effect in a radial maze task by extending the inter-trial interval from 1 min to 4 min. However, later studies found a complete release from proactive interference in a spatial memory task with a 120 min ITI (e.g., Cohen et al., 1994). Thus, it is probable that insertion of a 60 min interval was not adequate to provide sufficient temporal discriminability for recovery from proactive interference among memories of reward events in Phase 1. To test this possibility, the long ITI was extended to 120 min in Phase 2.

The bottom panel of Figure 3 shows running speeds in Phase 2. Planned comparisons using the Dunn-Sidak method showed that mice ran more slowly on Trial 8 of the experimental condition than on that of control condition and that running speeds did not differ significantly on all other trials ( $DS = 3.10, p < 0.05$  for Run 8;  $DS = 0.54, 0.10, -0.70, -1.55, 0.34, -1.01, \text{ and } -0.09, p > 0.05$  for Runs 1-7, respectively). A Trials \* Conditions \* Subjects analysis of variance revealed a significant main effect of Trials ( $F(7, 21) = 29.08, p < 0.01$ ) and interaction of Trials \* Conditions ( $F(7, 21) = 2.53, p < 0.05$ ). Simple effects of Conditions on each trial were significant for Trial 8 ( $F(1, 24) = 9.63, p < 0.01$ ) but not for the other trials ( $ps > 0.13$ ).

Results revealed by planned comparisons and ANOVA demonstrate a reliable decrease in proactive interference by the insertion of a 120 min ITI between the signal of a target nonreward and prior items. Since appetitive motivational strength for food reward should not have differed between the experimental and control conditions, the slower running for the eighth target nonreward in the experimental condition versus the control condition may provide evidence of a decrease in proactive interference among preceding item memories. The influence of the reinforcement-omission effect (e.g., Amsel, 1958; Stout et al., 2003) does not seem to be an adequate explanation for the results of Experiment 3. First, we know that the reinforcement-omission effect occurs within a relatively short temporal interval. For example, Stout et al. (2003) reported that lengthening the interval between the sudden omission of reinforcement and the next opportunity of response from 2 s to 20 s eliminated the reinforcement-omission effect completely. Although the experimental situation and variables in the present study differ from Stout et al. (2003), it is unlikely that lengthening ITI from 20 min to 120 min decreases the possible reinforcement-omission effect substantially and affects performance on subsequent trials. Second, no apparent decrease of response invigoration on the third or seventh reward trial following the 120 min interval was observed. If insertion of the longer 120 min ITI were to decrease frustration and result in response invigoration, running speed should have decreased even on reward trials following the longer ITI. Therefore, the influence of the reinforcement-omission effect seems to be excluded as a possible explanation of slower running on the eighth nonreward trial following the longer ITI.

One may also ask why a gradual deterioration of anticipation of the reward event was observed only on the nonreward trials since proactive interference should have produced greater confusion of reward and nonreward trials, even on the later reward trials. This problem might be related to the experimental paradigm of the present study. Learning a single alternation series of reward and nonreward could be regarded as one variation of a Go/No-Go task. In the Go/No-Go discrimination paradigm, poor performance is generally characterized by an indifferent active response to No-Go negative stimuli as well as Go positive ones, whereas good performance is usually shown by the development of response suppression only on No-Go trials (e.g., Izumi, 2001). The same is true for rats' serial learning, where poor reward anticipation is generally shown by indifferent fast running to larger rewards as well as smaller or nonrewards (e.g., Capaldi et al., 1986; Hulse & Dorsky, 1977, 1979). Gradual deterioration of nonreward anticipation might not be detectable in reward trials in Experiment 3 because both good anticipation of a reward and poor discrimination of a reward event, caused by proactive interference, could lead to fast running on reward trials in a single alternation series. It would be worth reexamining proactive interference in single alternation learning using another discrimination paradigm, for example, spatial alternation learning in a T-maze, because gradual deterioration of performance on both item trials, left and right goals, could be detected correctly in such a simultaneous discrimination.

A decrease in proactive interference by increased temporal discriminability among memories has been supported by many different types of animal memory studies, such as spatial memory tasks on the radial maze (Cohen et al., 1994) or nonspatial matching to sample tasks (D'Amato, 1973). The findings in Experiment 3 confirm the further generality of effects of temporal discriminability on proactive interference in serial learning.

Another factor said to affect the strength of proactive interference is item discriminability based on item materials. In a rat spatial memory task, a change in the floor texture of arms of the radial maze decreased proactive interference (Cohen et al., 1994). A rhesus monkey study in which animals were required to memorize photographs revealed release from proactive interference by changing the categories of objects in photographs (Jitsumori, Wright, & Cook, 1989; Jitsumori, Wright, & Shyan, 1989). Examining the effects of change in item quality on proactive inference in mice reward serial learning may clarify these issues.

### Experiment 4

Experiments 2 and 3 demonstrated that mice could retain item information for at least 20 min. In addition, mice run reliably faster to a reward that follows a 60 min or 120 min interval than to a nonreward. Although this faster running to a reward following to a long interval might reflect the persistent retention of a prior nonreward item by mice, it also could be interpreted as a nondifferential response caused by forgetting prior item information. It has been shown that rats can still respond differentially to reward and nonreward of a single alternation series under an ITI of 24 h (Capaldi & Lynch, 1966; Jobe, Mellgren, Feinberg, Littlejohn, & Rigby, 1977). Mice also show persistent retention of spatial information assessed by spontaneous alternation behavior in a T-maze (e.g., Jaffard, Dubois, & Galey, 1981), but there has been no previous research examining the temporal persistence of memory retention of reward events in mice. Experiment 4 investigated the ability of mice to retain reward item information over a longer interval than in Experiments 2 and 3 by examining the concurrent acquisition of a 5-0 and 0-5 series presented with a 180 min ITI. If mice could retain information about the first items for 180 min, then differentiated responses to the second items of a 5-0 and 0-5 series would be expected.

Several studies have shown that rats can utilize different levels of food deprivation as an interoceptive discriminative cue in a conditional place discrimination task (Bloomberg & Webb, 1949; Jenkins & Hanratty, 1949) or in an aversive classical conditioning situation (Davidson, Flynn, & Jarrard, 1992). Therefore, potential differential motivational levels caused by consumption of the first items of a 5-0 and 0-5 series could possibly mediate discriminative responding to second items in these series. In order to eliminate this possibility, five food pellets were delivered to the waiting cage 1 min after the completion of the first trial of a 0-5 series in Phase 2 of Experiment 4. This procedure ensured an equalization of the potential motivational level on the second trial of both series. If a differential response to second items were observed under this condition, it would strongly suggest that memories of first items of both series served as discriminative stimuli for the second items.

### Method

**Subjects.** Mice from Experiments 1-3 were used as subjects. The body weights of the subjects were continuously maintained at the same level as Experiment 3.

**Apparatus.** The apparatus was the same as that employed in the previous experiments.

**Experimental training.** Phase 1 began on the day after Experiment 3 was finished and lasted for 40 days. Subjects received either of a 5-0 and 0-5 series once per day under an ITI of 180 min. Half of the subjects received the 5-0 and 0-5 series in random order per two-day blocks and the other half in reverse order of presentation. All other aspects of the training procedure were same as in Experiment 1.

Phase 2 began the day after Phase 1 was finished and lasted for 40 days. In Phase 2, mice received five 45 mg food pellets in the waiting cage about 1 min after the mice received the first nonreward of the 0-5 series and were placed in the waiting cage. All other aspects of the training procedure were the same as in Phase 1.

### Results and Discussion

The left panel of Figure 4 shows running speeds on each trial of the 5-0 and 0-5 series under an ITI of 180 min in Phase 1. With training, a differential response developed on Trial 2. The data shown in the left panel of Figure 4 were subjected to a Series \* Blocks \* Trials \* Subjects analysis of variance. The analysis revealed a significant main effect of Series ( $F(1, 3) = 36.84, p < 0.01$ ) and Trials ( $F(1, 3) = 73.72, p < 0.01$ ), and Series \* Trials interaction ( $F(1, 3) = 11.17, p < 0.05$ ). Simple main effects showed that running speeds of the two series were significantly differentiated on Trial 2 ( $p < 0.01$ ) but not on Trial 1.

The right panel of Figure 4 shows running speeds in Phase 2. Although a differential response to the second item disappeared in the first block, it recovered soon with training. A Series \* Blocks \* Trials \* Subjects analysis of variance revealed a significant main effect of Series ( $F(1, 3) = 22.25, p < 0.02$ ) and Blocks ( $F(3, 9) = 11.55, p < 0.01$ ), and Series \* Trials interaction ( $F(1, 3) = 64.85, p < 0.01$ ). Simple main effects showed that running speeds of the two series were significantly differentiated on Trial 2 ( $p < 0.01$ ) but not on Trial 1.

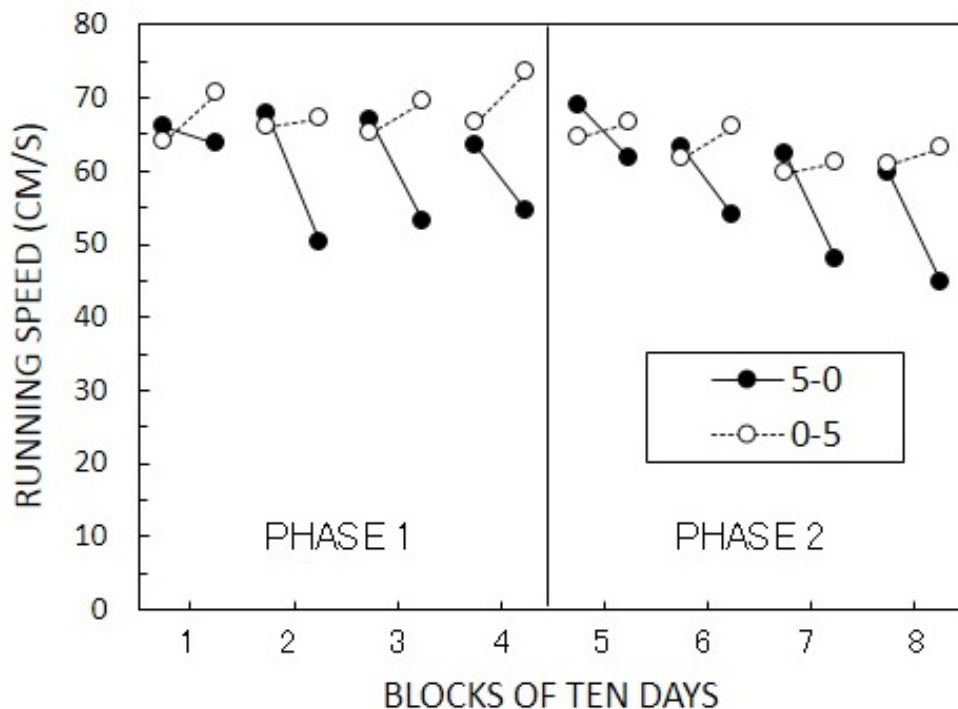


Figure 4. Running speeds on trials in a 5-0 and 0-5 series in blocks of ten days in Experiment 4. Runs were separated by a 180 min ITI. In Phase 2, five food pellets were delivered in a waiting cage 60 s after the first 0 pellet trial of the 0-5 series was presented.

On the first trial of Phase 1, mice only received food rewards in the 5-0 series, not in the 0-5 series, whereas in Phase 2, they received food rewards on the first trial of both the 5-0 series (in the goal box) and the 0-5 series (in the waiting cage). Mice showed reliable anticipation of reward magnitude under an ITI of 180 min in Phase 1, and this performance was maintained under conditions where motivational differences would not have served as discriminative stimuli in Phase 2. These findings indicate that mice can retain item information of a reward series, as well as spatial information (Jaffard et al., 1981), for long intervals.

Anticipation of the second item temporarily deteriorated when feeding control in the waiting cage was introduced in Phase 2. However, if a motivational level were utilized as discriminative stimuli during Phase 1, rapid recover of performance in Phase 2 would not be expected. Therefore, the deterioration of item anticipation at the beginning of Phase 2 might reflect some retrospective interference from the reward event that occurred in waiting cage to memory of the first item of a 0-5 series. Although it is shown that feeding during the retention period did not interfere in rat spatial memory tasks, where spatial item information and intervening reward stimulus were dissimilar (Maki, Brokofsky, & Berg, 1979), in the present study, the item event to be retained and the intervening event were quite similar. This similarity of the item event and the intervening event might produce a retrospective interference effect.

### **General Discussion**

In rat reward serial learning, rewards delivered in a fixed order for instrumental responses have been probed as to function as sources signaling following reward events, as well as reinforcers for instrumental responses. In the present study, mice could also utilize reward events as discriminative stimuli to predict following reward events, running faster to rewards and slower to nonrewards. Moreover, such differential responses, manifested under ITIs of 20 min or 180 min, demonstrated persistent retention of item information in mice. These results are similar to those of rat studies and confirm some generality of basic cognitive capacity for reward serial learning among rodents.

Although many rat studies did not report running speed on each serial position of single alteration series, Ishida (1981) reported no gradual deterioration in anticipation of nonreward in rats. In a single alteration series of reward events, there are only two types of reward events, that is R and N. Thus, strength of proactive interference is mainly decided by the persistence of a preceding item memory and the ability to discriminate those item memories in terms of temporal information. Since it has been documented, as previously mentioned, that rats could retain item information for 24 h (Capaldi & Lynch, 1966; Jobe et al., 1977), the absence of evidence for the proactive interference effect may represent the rats' prominent ability to discriminate item memories based on the elapsed time information from the occurrence of the events. This view may be consistent with the results of Experiment 1, which showed slower learning of a single alternation series in mice compared with previous rat studies (Flaherty & Davenport, 1972; Tyler et al., 1953).

Given that there are no guarantees about the equality of experimental variables for previous rat studies and the present mouse experiments, some inconsistency might be produced by differences in effects of some experimental variables among the experiments with these two species, rather than in the cognitive capacities of rats and mice. The type of experimental paradigm might also affect single alternation series learning. As mentioned above, acquisition of a single alternation series of reward and nonreward events could be regarded as a form of the Go/No-Go task. It has been pointed out that the Go/No-Go task not only measures discrimination learning ability but also impulsivity that affects response suppression on No-Go trials (e.g., Winstanley, 2011). Therefore, species differences between rats and mice in single alternation learning in the present study may reflect not only differences in serial learning ability but also in impulsivity between these species.

In addition, the small number of subjects and their continuous participation across the four experiments might make it difficult to interpret some of the statistical results in the present study. For example, a tendency toward increasing running speeds as the series progressed was shown for reward trials in Experiment 1 and nonreward trials in Experiment 2, but this tendency was significant only for Experiment 2. Due to the small number of subjects, it may not be possible to detect some phenomena statistically in the present study. Also, as this gradually increasing tendency of running speeds was obvious on reward trials in Experiment 1, but not in Experiment 2, it is difficult to determine whether such differences can be attributed to differences in ITIs between the experiments or if the extended training of subjects from Experiment 1 to Experiment 2 produced a ceiling effect on running performance. Examination of the generality of proactive interference effects in mice across a wide range of variables and experimental paradigms with a larger number of subjects is necessary to identify possible causes for the inconsistent results between rats and mice.

Although acquisition of a single alteration series suggests that mice can utilize item information when there are only two variations in this information, which can be clearly distinguished from each other, rats have proved that they are able to learn a reward series constructed from varied food quantities, to form item associations between remote items as well as adjacent items (Capaldi & Miller, 1988b), and to utilize an integrated series chunk as a discriminative stimulus for a later series (Capaldi, 1992; Capaldi et al., 1990). Moreover, rats can choose the larger food item among four different spatial locations by anticipating it based on concurrent learning of four different reward series (Phelps & Roberts, 1991; Wathen & Roberts, 1994). As a next step for research, more complex or higher order serial learning should be examined in mice.

In addition, several cognitive processes which mediate rats' serial learning have been hypothesized. Memory-discrimination learning theory (Capaldi & Molina, 1979) assumes that rats form item-associations, and that stimulus generalization among signals of the associations and reward-signal strength of each signal, determines the strength of instrumental response to items in a series. When similar items signal dissimilar items in a series, anticipation of those items becomes difficult (Capaldi et al., 1980). SPAM (Sequential Pairwise Associative Memory), an associative model of serial learning developed by Wallace and Fountain (2002), assumes composition of item memory as a determinant of item retrieval (Fountain, 2008; Wallace & Fountain, 2002). In addition to item association, rats seem to be able to learn the serial position of items or the number of trials, in circumstances where a simple item cue could not be an effective discriminative cue for a subsequent item (Capaldi & Miller, 1988a; Taniuchi, 2000). Moreover, rats may extract the abstract relationship between adjacent items and learn the formal structure of a series. Rats can learn to anticipate a nonreward of a 14-7-3-1-0 monotonic series more easily than a 14-1-3-7-0 non-monotonic series (Hulse & Dorsky, 1977), and they learn a long series more smoothly when it is "phrased" into subpatterns with a simple formal structure (Fountain, Henne, & Hulse, 1984). Therefore, rats' serial learning suggests that it is mediated by different learning processes that require encoding of item information at various levels. However, these various phenomena and possible learning processes in reward serial learning have not been examined in other rodents.

In serial pattern learning, which requires animals to track signals presented in different spatial locations, temporal phrasing of complex patterns into simple sub-patterns facilitates learning in rats (Fountain & Rowan, 1995; Stempowski et al., 1999), whereas it impairs performance of mice (Fountain et al., 1999). This inconsistency in the effects of temporal phrasing on rats versus mice strongly suggests the necessity of examining whether we can generalize the findings in rat reward serial learning to other rodent species.

Fountain et al. (1999) suggested two possible cognitive differences between rats and mice based on different effects of phrasing in spatial serial learning. First, that rats and mice differ qualitatively in using a phrasing cue. This possibility might be related to cognitive ability in chunking multiple events. Second, rats and mice share similar cognitive abilities, but mice have less working memory capacity such that they cannot process serial events and phrasing cues concurrently. These ideas might guide our future examination of species differences between rats and mice in serial learning. That is, we may be able to compare these species

by a chunking paradigm in a runway situation (Capaldi, 1992; Capaldi et al., 1990; Fountain et al., 1984), a multiple series learning paradigm (e.g., Phelps & Roberts, 1991; Wathen & Roberts, 1994), or in terms of simple working memory capacity (e.g., Cole & Chappell-Stephenson, 2003). Species differences between rats and mice have also been reported in spatial memory (Frick, Stillner, & Berger-Sweeney, 2000; Stranahan, 2011) but not in object recognition memory (Frick et al., 2000). Considering the discrepancy in results between rats and mice in phrasing effects and some memory tasks, it is obvious that the generality of a wide range of findings in rat reward serial learning and assumed learning processes should be examined in mice to identify species' differences in cognitive processes.

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