Vulnerability of long-term memory to temporal delays in amphibians

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ABSTRACT

Two experiments with toads were designed to test the memory-decay hypothesis that extinction (i.e., nonreinforced) performance is a function of time since the last reinforcement. In Experiment 1, toads (*Rhinella arenarum*, formerly *Bufo arenarum*) received 15 daily acquisition trials each reinforced with access to water during 300 s in a runway and were then randomly assigned to one of 6 retention intervals (RIs): 1, 4, 8, 16, 32, and 64 days. Extinction started after the RI and lasted 8 additional daily trials. Overall extinction performance was a logarithmic function of the RI. Although 4 extinction trials produced similar performance than 4 days of RI (consistent with memory decay), 8 extinction trials produced lower performance than 8 days of RI (consistent with a decremental effect of nonreinforcement). In Experiment 2, two groups of toads received 15 daily acquisition trials each reinforced with access to water for either 30 or 600 s, thus producing two reward magnitudes. After an 8-day RI, extinction performance was weaker after training with the small, than with the large reward magnitude. These results suggest that, at least in early extinction, the instrumental performance of toads is strongly influenced by the time since the last reinforcement.

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1. Introduction

Mammalian long-term memory consolidated during a period of acquisition training and assessed during a period of extinction training is stable across time. For example, spaced-trial (e.g., 1 trial every 3 days) runway acquisition training under either continuous or 50% partial reinforcement (i.e., an unsignaled, pseudorandom sequence of reinforced and nonreinforced trials) yields faster extinction after continuous reinforcement (CR) than after partial reinforcement (PR; Rashotte and Surridge, 1969). This so-called partial reinforcement extinction effect (PREE) emerges in rats even when original PR training takes place in a different situation, under different deprivation conditions, and based on a different incentive (Ross, 1964). Amsel (1992) suggested the term "dispositional memory" to describe the striking stability of the associative knowledge acquired under these spaced-trial conditions that seems to survive extensive retention intervals and significant changes in internal and external conditions. Would such dispositional memory effects be exhibited by animals that show a reversed PREE, that is, faster extinction after PR, rather than after CR?

Several nonmammalian vertebrates have been shown to exhibit reversed PREEs under spaced-training conditions-typically, one trial per day (see Papini, 2003, 2006), including toads (*Rhinella arenarum*, formerly *Bufo arenarum*), the species used in the present experiments. Toads trained under analogous conditions with water as the incentive and goal approach in a runway as the instrumental behavior typically exhibit slower acquisition rate and faster extinction rate under 50% PR training than under CR training (e.g., Muzio et al., 1992, 1994). A similar effect occurs when nonreinforcement is replaced by exposure to a hypertonic solution that leads to water dehydration (Muzio et al., 2011), an outcome that toads find aversive and can be easily trained to avoid (Daneri et al., 2007; Puddington et al., 2012). These results suggest that nonreinforced (and also aversive) trials play a key decremental role in instrumental learning. Consistent with this hypothesis, instrumental performance is usually significantly lower a day after a nonreinforced trial compared to its level a day after a reinforced trial-called a reward-following effect (Muzio et al., 1992). Whereas this interpretation is parsimonious and consistent with some influential contemporary models of learning (e.g., Rescorla and Wagner, 1972), recent evidence suggests that the amphibian reversed PREE may be based on an entirely different mechanism.

Muzio et al. (2006) trained three groups of toads under the usual runway conditions, with two groups receiving CR and PR, matched on total number of acquisition trials. A third group was designed to receive training only on the same days when PR animals were scheduled to receive a reinforced trial, but to remain in their cages in days when PR animals received a nonreinforced trial.

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Thus, for this third group, there were half the number of training trials, they were all reinforced (i.e., a CR condition), but the number and temporal distribution of reinforced trials matched that of PR animals. Surprisingly, the extinction rate of this group matched that of PR animals, being significantly faster than that of the regular CR condition. These results suggested that the reversed PREE in toads is more related to reward distribution than to partial reinforcement per se. Muzio et al. (2006) suggested a memory decay interpretation according to which the memory of a past reward loses strength in time. This interpretation is also consistent with the reward-following effect, but it interprets it as arising out of memory decay, rather than active strength reduction after experiencing a nonreinforced trial.

The notion of memory decay has been extensively used in animal memory experiments to account to changes in time for recently acquired information. Typically these changes are measured after intervals ranging from seconds to minutes (e.g., Bolhuis et al., 1986; Roberts and Grant, 1976). Retention intervals in the order of days have been used in developmental research (e.g., McKinzie et al., 1998) and as a posttraining manipulation in animal learning experiments (e.g., Leung et al., 2007). Although events during the retention interval are critical determinants of the memory effect observed, the present research was designed to test a simple view of memory decay. It was hypothesized here that, in amphibians, memory strength for a reinforcement event spontaneously decays in time, ignoring for the moment the potential effects of events occurring during the retention interval on the level of performance observed during extinction testing. It was further assumed that memory strength holds a direct relationship with the level of extinction performance.

The current experiments were designed to test the memory-decay interpretation of the reversed PREE in toads using widely-spaced training (one trial per day). In Experiment 1, the retention interval between the last acquisition and first extinction trials was varied extensively (from 1 to 64 days in six groups). Of interest was to determine the shape of the decay function between the two critical trials. If memory decay reflects residual activity in a critical neural network left by a specific experience, then one would predict that the effects should be stronger early in the retention interval and eventually achieve an asymptotic value. Thus, if the memory decay hypothesis applies to the reversed PREE previously observed, extinction latencies should be described by a monotonic decreasing function of the retention interval. In Experiment 2, groups received acquisition training with either a large or a small incentive (600 vs. 30 s of hydration per trial in the goal box) and then shifted to extinction after an 8-day retention interval. A simple assumption was made for this experiment, namely, that the initial strength of the incentive memory would be a direct function of reward magnitude. On this basis, the memory decay hypothesis predicts lower extinction performance after acquisition with a small incentive than with a large incentive, despite an equal number of acquisition trials and retention interval.

2. Experiment 1

2.1. Method

2.1.1. Subjects

The subjects were 40 experimentally naive, adult, male toads (*R. arenarum*, formerly *B. arenarum*) collected from ponds around Buenos Aires, Argentina. This species is not listed as threatened (IUCN, 2010). Animals were maintained according to the guidelines outlined by the NIH Guide for Care and Use of Laboratory Animals. Upon arrival in the lab, all animals were placed in group cages with running tap water during at least the following two weeks.

The vivarium was kept at a temperature between 21 °C and 23 °C, and under a 16:8 h light:dark cycle (lights from 03:00 to 19:00 h). Reflexes were tested to detect possible neurological disorders. Subjects were treated with antibiotics and antiparasitic medication mixed with the meal. The antibiotic medication feeding regimen lasted 7 days; during this period, every toad received a daily feeding ration of insectivore bird ground and a live mealworm larva. A dose of antiparasitic medication was also administrated on the first day. This feeding regime ended at least one week before the start of training. The standard weight (weight of the hydrated animal after the urinary bladder has been emptied) of each animal was recorded 2 days before the start of the experiment (Ruibal, 1962). Standard weights varied between 62 and 199 g, and did not differ significantly across groups, $F(5, 34) = 2.15$, $p > 0.05$. Toads were dehydrated at 80% of their standard weight at the beginning of each pretraining and training trial. During the experiment, animals were kept in small, dry, individual plastic cages.

2.1.2. Apparatus

We used a runway built with black Plexiglas, divided into a start compartment and a goal compartment, both 20 cm long, separated by a 60 cm long alley. The runway was 12 cm wide and 20 cm high, and it was covered with translucent Plexiglas lids that allowed constant observation of the animals through a mirror. The goal compartment contained a green Plexiglas container (13 cm long, 10 cm wide, and 3 cm high) filled with deionized water. At the beginning and end of the alley there were guillotine doors that controlled the passage between compartments. In each section, diffuse illumination was provided by three light bulbs (15 W) placed on the cover of each compartment. Animals were observed through a mirror that concealed the presence of the experimenter. Temperature was maintained at 21–23 °C and relative humidity at 40–50% in the testing room. A constant white noise (20–30,000 Hz) was played during training session.

2.1.3. Procedure

Animals received two pretraining trials, one per day, in which they were placed in the starting box for 30 s, then both doors were opened, and then the toads were able to move about the runway for 10 min. In the first pretraining trial, drops of deionized water were scattered in the alley section of the runway. In the second pretraining trial, drops of water were placed only in the middle section of the alley and next to the second guillotine door, in front of the goal box. The container located in the goal box was always filled with deionized water.

Training involved one trial per day, 7 days per week. Each toad received 15 acquisition trials, followed by 8 extinction trials. In each trial, toads were placed in the start compartment for 30 s and then the guillotine doors were raised. Two dependent variables were registered: (1) Running latency (in seconds): Time from the moment the animal had its four legs in the alley and out of the start box, to the moment it entered the goal box with its four legs. This variable was recorded by the manual operation of a digital timer (1 s units) and transformed to the $\log_{10}$ to improve normality and allow for parametric statistics. (2) Weight variation (g/100 g): The weight of every subject (in grams) was registered before and after each trial to estimate water consumption. The difference between these two weights was divided by the standard weight and multiplied by 100 to provide a relative measure of water uptake corrected for individual differences in body weight. Acquisition trials ended in the goal compartment with 300 s of access to deionized water. Extinction trials also ended with 300 s in the goal compartment, but without access to deionized water.

Trials were run between 12:00 and 19:00 h. After each trial, animals were transferred to dry cages where they remained until the next day. At least 30 min after each daily trial, animals that had lost
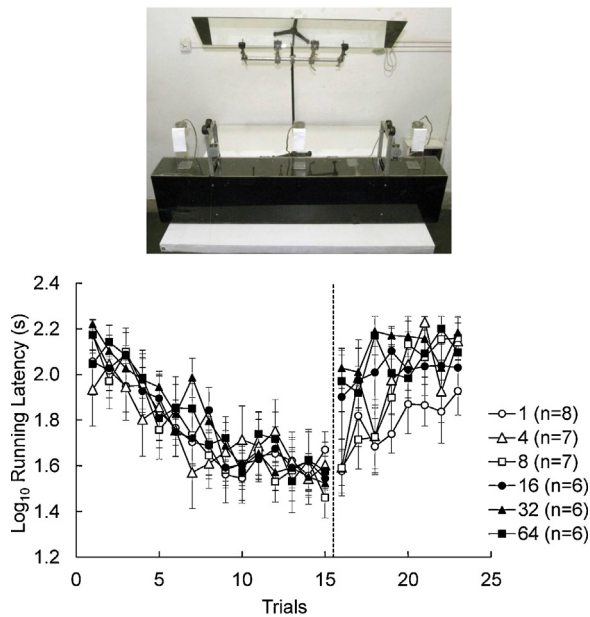
Fig. 1. Runway with observational mirror above lids (top). Running latency means (±SEMs) of groups of toads receiving acquisition training during 15 daily trials with access to water during 300 s followed by 8 extinction trials after retention intervals of 1, 4, 8, 16, 32, and 64 days (bottom). Results from Experiment 1.

Fig. 2. Mean (±SEMs) performance of groups of toads during four extinction trials after six different retention intervals (top) or during the entire extinction phase (average of 8 extinction trials; bottom). The logarithmic function was obtained using Excel. Results from Experiment 2.

weight during the trial were supplemented with deionized water, whereas those that had gained weight were dehydrated. This procedure ensured that animals would be at 79–81% of their standard weight at the start of each trial. A trial started when the animal was placed in the start box; after 30 s, the guillotine door was raised and the animal was allowed a maximum of 180 s to leave the start box or a maximum of 180 s to enter the goal box if already in the alley. A maximum latency of 180 s was assigned (1) when the animal failed to leave the start compartment after 180 s, or (2) when the animal left the start compartment before the maximum 180 s, but failed to enter the goal compartment within the following 180 s. In incomplete trials, toads were gently guided to the goal box where they received 300 s of access to the deionized water (guided trial). The mean (±SEM) percentage of guided trials in acquisition was 13.6% (±0.01) and in extinction was 47.1% (±0.05).

After acquisition ended, animals were randomly assigned to one of 6 retention interval conditions: 1, 4, 8, 16, 32, and 64 days. The retention interval spanned between the last day of acquisition and the first day of extinction. During the retention interval, animals remained in their cages, were fed 2 live Tenebrio larvae every week, and were hydrated daily to maintain them at 80% of their standard weight.

Extinction started the day after the retention interval ended, depending on the group. The six conditions were as follows: Groups 1 ($n=8$), 4 ($n=7$), 8 ($n=7$), 16 ($n=6$), 32 ($n=6$), and 64 ($n=6$). Water was present in the goal box, but not accessible during extinction trials. In all statistical tests, the alpha value set to less than 0.05.

2.2. Results

Fig. 1 shows the running latency for each group during acquisition and extinction trials. A Retention Interval (1, 4, 8, 16, 32, 64 days) × Trial (1–15) analysis of acquisition data indicated only significant acquisition effect, $F(14, 476) = 32.52$, $p < 0.001$. As expected given that no treatments were administered yet, there were nonsignificant effects for retention interval and the interaction in acquisition trials, $F_s < 1$. Therefore, there was no detectable
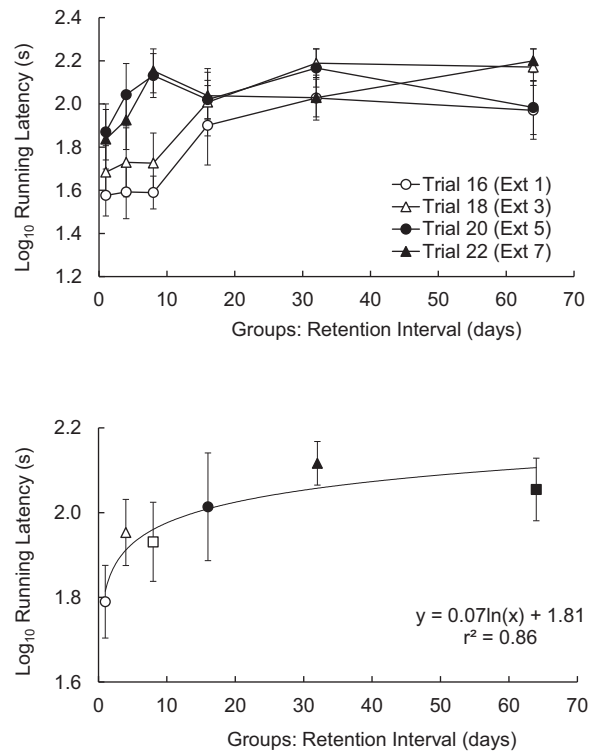
assignment bias before the retention interval was introduced and all groups showed similar acquisition rates.

Fig. 1 also shows the extinction performance of the six groups. Additionally, Fig. 2, top panel, displays the performance in four selected extinction trials, but now represents extinction as a function of the retention interval. It is clear that a retention interval of 16 or more days resulted in virtually no retention of the levels achieved toward the end of the acquisition period. In contrast, retention intervals between 1 and 8 days resulted in measurable change in performance across sessions. A Retention Interval × Trial (16–23) analysis was conducted to examine extinction performance. In this case, there was a significant retention interval by trial interaction, $F(35, 238) = 1.73$, $p < 0.01$. The extinction effects was also significant, $F(7, 238) = 9.32$, $p < 0.001$, but the group effect was not significant, $F(5, 34) = 1.76$, $p > 0.14$. LSD pair wise comparisons with the error term derived from this main analysis indicated that the significant interaction was derived from group differences in the first and third extinction trials, $F_s(5, 34) > 3.16$, $p_s < 0.02$.

To test whether memory decay time alone was responsible for the level of performance in extinction, we compared the performance of pairs of groups after the same number of days had elapsed since the last acquisition trial. Thus, Groups 1 and 4 did not differ on trials 4 and 1, respectively, $F(1, 13) = 1.02$, $p > 0.33$, suggesting that four extinction trials (Group 1) and four days without training since acquisition (Group 4) produce comparable performance. However, latencies for Groups 1 on Trial 8 were significantly longer than for Group 8 on Trial 1, $F(1, 13) = 6.41$, $p < 0.03$. This last result suggests that extinction trials do have a measurable effect on behavior, that is, that it is not just temporal decay that affects extinction performance.

Fig. 2, bottom panel, shows that the overall average extinction performance of these groups was described by a logarithmic function (i.e., a type of monotonic decreasing function) with a high

coefficient of determination, $r^2 = 0.86$. This function thus indicates that when 8 nonreinforced trials are taken into account, the majority of the change in extinction performance occurred at the lower end of the retention interval scale.

As is usual in these experiments (e.g., Muzio et al., 1992), toads increased their weight across acquisition trials. Standard weights (g/100 g) varied between an overall mean of 0.64 ($\pm$0.06) on Trial 1, to 1.46 ($\pm$0.13) on Trial 15. However, there were no obvious differences across groups. An analysis showed only a significant change across acquisition trials, $F(14, 476) = 16.79$, $p < 0.001$. The effects of groups and the group by trial interaction were not significant, $F_s < 1.48$, $p_s > 0.22$.

3. Experiment 2

The memory-decay hypothesis assumes that the initial strength of the memory for the last reward is a positive function of the magnitude of the reward. This is consistent with previous results showing that running latencies in extinction were shorter after acquisition with large rewards rather than small rewards (Muzio et al., 1992). Here, an 8-day retention interval was interpolated between the end of acquisition training and the start of extinction training to challenge the memory of the last incentive event. This retention interval was shown to produce an initial extinction performance similar to that of the typical 1-day interval when a relatively large incentive was used in training (300 s of access to deionized water in the goal box). However, this retention interval also led to measurable extinction of behavior over the course of 8 trials (e.g., see Fig. 2, top panel). To maximize the potential for observing incentive magnitude effects, the large magnitude was increased to 600 s of access to water, whereas the small magnitude was decreased to 30 s of access to water. Magnitudes ranging between 20 and 1280 s of access to deionized water were successfully used previously (Muzio et al., 1992).

3.1. Method

3.1.1. Subjects and apparatus

The subjects were 12 experimentally naive, adult, male toads. They were obtained from the same source and maintained as described in Experiment 1. The training apparatus was the same described in Experiment 1.

3.1.2. Procedure

Toads were randomly assigned to one of two groups, differing in terms of the time allowed in the goal box. Toads in Group 30 ($n = 6$) received 30 s of access to deionized water during each of 15 acquisition trials, whereas toads in Group 600 ($n = 6$) received 600 s of access to deionized water per trial. After the 15 acquisition trials, toads remained in their cages for 8 days (retention interval). During the retention interval, toads were kept at the same level of water deprivation implemented during acquisition. Animals then received 8 final extinction trials. Training conditions were as described in Experiment 1. In this experiment, the mean ($\pm$SEM) percentage of guided trials in acquisition was 12.8% ($\pm$0.02) and in extinction was 66.7% ($\pm$0.04).

3.2. Results

Fig. 3 shows the running latencies for acquisition and extinction in both groups. A Magnitude (600 s, 30 s) × Trial (1–15) analysis indicated a significant interaction effect, $F(14, 140) = 2.82$, $p < 0.002$. There was also a significant acquisition effect, $F(14, 140) = 5.32$, $p < 0.001$, but although borderline, the magnitude effect was not significant, $F(1, 10) = 4.76$, $p < 0.055$. Pair wise LSD comparisons with
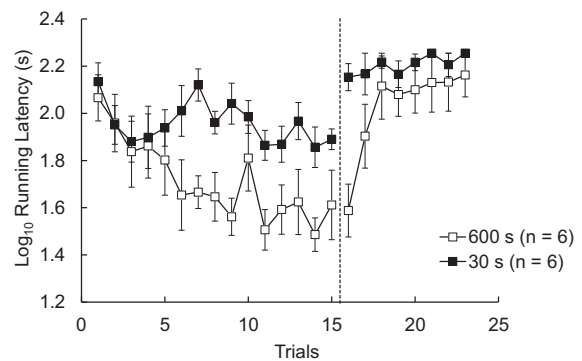

Fig. 3. Mean ($\pm$SEMs) performance of groups of toads during acquisition and extinction, after an 8-day retention interval. Acquisition trials ended with access to water for either 600 s or 30 s, thus producing different magnitudes of water uptake. Results from Experiment 2.

the error term from the main analysis indicated that latencies in Group 600 were significantly below Group 30 on Trials 7, 8, 9, 11, and 14, $F_s(1, 10) > 7.63$, $p_s < 0.03$. Group 30 did not show much change across trials. A separate analysis of this group that included all the acquisition trials fell short of significance, $F(14, 70) = 1.74$, $p = 0.066$, but an independent analysis of Trials 1 vs. 15 (first vs. last acquisition trial) indicated a significant decline in latencies, $F(1, 5) = 7.08$, $p < 0.05$.

Fig. 3 also shows that whereas an 8-day retention interval resulted in virtually no decrement in performance in Group 600, it almost completely erased the modest gains in latency exhibited by Group 30 during acquisition. A Magnitude × Trial (16–23) analysis revealed a significant interaction, $F(7, 70) = 3.28$, $p < 0.006$. There was also a significant extinction effect, $F(7, 70) = 5.90$, $p < 0.001$, but the group difference was not significant, $F(1, 10) = 3.81$, $p < 0.08$. Pair wise LSD comparisons with the error term derived from the main analysis indicated that the groups differed in the first extinction trial, $F(1, 10) = 20.27$, $p < 0.002$, but not in any of the subsequent trials, $F_s < 2.66$, $p_s > 0.13$.

The two groups were also significantly different in weight variation during the 15 acquisition trials. For Group 30, standard weights (g/100 g) varied between −0.02 ($\pm$0.05) on Trial 1 and −0.03 ($\pm$0.03) on Trial 15 (i.e., neither hydrating nor dehydrating substantially during the trial), whereas for Group 600, standard weights varied between 1.67 ($\pm$0.20) on Trial 1 and 2.59 ($\pm$0.34) on Trial 15 (i.e., hydrating substantially during the trial). The analysis of these data yielded significant interaction, group, and acquisition effects, $F_s > 3.41$, $p_s < 0.001$, indicating that the incentive magnitude operation successfully produced differential amounts of water uptake.

4. General discussion

Experiment 1 showed that running latencies in early extinction tend to increase as a function of the time since the last acquisition (i.e., reinforced) trial. Retention intervals between 1 and 8 days do not lead to significant changes during the initial extinction trials, but after 16 days there was a clear tendency for latencies to increase, and after 32 days the increase was significant. Such trend dissipated as extinction progressed. The overall extinction performance was fit reasonably well by a logarithmic function, suggesting that, with some amount of exposure to extinction, most of the change in behavior occurs with lower values of retention intervals. In addition, Experiment 2 shows that after a moderate retention interval of 8 days, toads that received acquisition training with a large reward exhibited better retention than toads receiving acquisition training with a small reward.

These results are consistent with a parsimonious account of instrumental performance based on the notion of memory decay (e.g., Bolhuis et al., 1986; Roberts and Grant, 1976). This memory decay hypothesis was suggested by data showing that the reversed PREE found in previous research with toads (Muzio et al., 1992, 2006) was related more to the distribution of rewarded trials than to the actual schedule of partial reinforcement. Groups that had received either continuous or partial reinforcement training during acquisition, but with the same temporal distribution of rewarded trials, exhibited virtually identical extinction rates. This result implied that nonreinforcement plays virtually no role in instrumental learning in toads. Experiment 1 confirmed that the extinction performance of continuously reinforced groups of toads resembled that of partially reinforced toads as the retention interval between the last acquisition and first extinction trials was increased from 1 to 64 days. However, as shown in Experiment 1, eight trials of extinction in Group 1 cause more decrement than eight days of retention interval in Group 8, which implies that, in addition to memory decay, nonreinforcement also contributes to response decrement in extinction.

The memory decay hypothesis is consistent with assuming that the strength of the incentive memory should be a function of reward magnitude. If that were the case, then extinction performance following a retention interval should be better after acquisition with a large reward than after acquisition with a small reward. Experiment 2 confirmed this prediction using a retention interval of 8 days and reward magnitudes greater and smaller than those used in the previous experiment.

These results combined seem to suggest that the instrumental behavior of toads is reduced during extinction due to memory decay, in addition to the well-established role of nonreinforcement. To the extent that the role of memory decay in extinction is confirmed in further experiments, it may shed light on the apparent evolutionary divergence in mechanisms of instrumental extinction between amphibians and mammals (Papini, 2003, 2006). Thus, just as extinction is hastened by long retention intervals, it may be possible to retard the rate of response decrement if the memory of the incentive were to remain active. Similar retardation was reported by Rescorla and Skucy (1969), when extinction of lever pressing in rats occurred in the context of unsignaled food delivery, although different mechanisms were suggested for the effect. This intriguing notion, which does not seem to apply to the relatively stable long-term memories formed during instrumental training by adult mammals (see Amsel, 1992), suggests that extinction could be retarded if concurrent reinforcement with the same incentive is administered even in a discriminably different situation. For example, reinforced training in two runways (contexts X+ and Y+) followed by extinction in only one of them (X−, Y+) should lead to little or no decrement in performance in either one of them. This would be because Y+ trials would keep the incentive representation active, preventing or reducing memory decay.

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