

## SHORT COMMUNICATION

### **Unexpected Post-CS Events During Extinction and the Slow Reacquisition Effect**

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Rats were used to examine the effects of a surprising post-CS event on reacquisition of an extinguished conditioned taste aversion. One flavor CS was paired with LiCl and then followed by many CS-alone extinction trials. Following these extinction trials, subjects received the CS paired again with LiCl to assess the extent of reacquisition. For some subjects, the final extinction exposure was immediately followed by a surprising second flavor CS. The surprising event did not influence the degree of reacquisition. Additional results found that the second flavor did influence habituation of neophobia to a flavor showing that the event does influence consumption in some circumstances. These results are discussed with respect to the role of attention on extinction and reacquisition of a conditioned taste aversion.

Recent studies have examined treatments influencing extinction of conditioned responding. Such treatments include demonstrations of the context-specificity of extinction (e.g., Bouton & King, 1983; Chelonis et al., 1999; Lovibond, Preston, & Mackintosh, 1984) and the blocking potential of an extinguished conditioned stimulus (e.g., Schachtman et al., 1992). Another extinction phenomenon is the slow reacquisition effect. If a conditioned stimulus (CS) is given extensive CS-alone extinction presentations following CS-US pairings and then the CS is paired again with the US, reconditioning is slow relative to a group conditioned with a novel CS (e.g., Bouton, 1986; Danguir & Nicolaidis, 1977; Hart, Bourne, & Schachtman, 1995). There have been a number of recent notions regarding the associative processes underlying extinction (e.g., Bouton, 1993; Miller, Kaspro, & Schachtman, 1986; Robbins, 1990), including the slow reacquisition effect; but no definitive process has emerged (see Falls, 1998; Richardson et al., 1999).

Robbins (1990) proposed that extinction occurs because the CS-alone exposures result in a loss of attention. Moreover, several conditioning models claim that extinction results in low attention/associability of the CS (e.g., Lubow, Weiner, & Schnur, 1981; Pearce & Hall, 1980). A loss of attention readily explains the slow reconditioning that can occur following extensive extinction. A low level of attention to a CS as a result of extinction treatment should produce poor subsequent conditioning (Bouton & Nelson, 1998). Indeed, Kaye and Pearce (1984) have shown that, assuming that an orienting response to a light is a direct measure of associability/attention, attention to a light declined over the course of extinction after an initial increase when the US was first omitted.

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Hall and Pearce (1982) found that if a CS was first given CS-alone exposures (i.e., latent inhibition training) and then a surprising event occurred following a presentation of the CS, subsequent conditioning of the CS was enhanced because the associability of the CS was increased by the surprising event. Surprising post-trial events have also been shown to enhance conditioning of an added CS in a blocking procedure (e.g., Dickinson, Hall, & Mackintosh, 1976). The present experiments used a surprising event to examine its influence on the slow reacquisition of an extensively extinguished CS.

In this conditioned taste aversion experiment, a novel flavor was administered as a surprising event immediately after the final CS extinction trial in an attempt to restore the flavor's associability and produce rapid reacquisition. Many previously published studies have shown that a sensitivity to the effects of flavors as post-CS events (e.g., Kasproff & Schachtman, 1993; Kaye, Gambini, & Mackintosh, 1988; Robertson & Garrud, 1983).

## Method

### *Subjects and Apparatus*

Twenty-four male Sprague-Dawley rats (mean body weight = 199 g) purchased from a commercial breeder (Sasco, Indiana, U.S.A.) were used. Twelve additional male Sprague-Dawley-derived rats (mean body weight = 429 g) were used as subjects in a follow-up experiment described below. Rats were individually housed in hanging, wire mesh cages with ad libitum access to lab chow. Subjects were gradually water deprived prior to the start of the experiment, culminating in 15-min water access each day. Water access occurred in the home cage after each day's experimental manipulations. The room housing the animals was on a 16:8 h light:dark cycle; and treatments occurred during the middle of the light portion of the cycle. All treatments occurred in the home cage. A clip on the front of each cage could hold a plastic drinking tube (a modified, inverted 50-ml syringe) with a metal lick tube attached. LiCl was administered using a 25-ga, 1.59-cm hypodermic needle.

### *Procedure*

On Day 1, half the animals received 10-min access to a drinking tube containing a 0.1% (w/v) saccharin solution (Sac, Sigma, Missouri, U.S.A.) while the other half of the animals received 10 min access to a 1% (w/v) decaffeinated coffee (Sanka) solution (Coff). Immediately after exposure to the solution, each animal received an i. p. injection of 0.3 M LiCl (Sigma) at 1.33% body weight. Intake of all flavored solutions was recorded by weighing the drinking tubes before and after treatment. If an animal did not drink 1 ml or more of the target solution during the initial exposure to the solution, the subject was eliminated from the experiment due to insufficient CS exposure. Day 2 was a recovery day in which the animals only received their daily exposure to water. Extinction occurred on Days 3-11. For the first eight days of extinction (Days 3-10), each subject received a 10 min exposure to the same solution that it had received on Day 1 but without LiCl.

Following the extinction exposure on Day 10, four of the subjects that had been conditioned and extinguished with Sac and four of the subjects that had been conditioned and extinguished with Coff were randomly assigned to each of Groups Reac, Reac-Vin, and Control ( $n = 8$ ), except for the counterbalancing for body weight and consumption of solutions on Days 1, 3, and 10. On the final extinction day, Day 11, the subjects in Groups Reac, Reac-Vin, and Control received a 10 min exposure to the same solution that they had received on Days 3-10; however, all of the rats in Group Reac-Vin and four of the rats in Group Control (two that had been receiving Sac and two that had been receiving Coff) also received a 10 min exposure to a second flavor (a 3% vinegar solution, Vin, Heinz, U.S.A.) in a drinking tube for 10 min immediately after exposure to Sac or Coff. The Vin exposure was expected to have little impact for those rats in Group Control. Group Reac and the remaining four rats in Group Control did not receive a second exposure to a drinking tube on Day 11.

That is, Group Reac and four rats in Group Control received a ninth extinction trial exactly like the previous eight trials.

Days 12 and 14 served as reacquisition trials for Groups Reac and Reac-Vin. On these days, these subjects received access to the same solution (Coff or Sac) that they had received during earlier phases of training for 10 min followed by an injection of 0.15 M LiCl at 1% body weight. Days 13 and 15 were recovery days. The animals in Group Control that had received initial conditioning and extinction with Sac received conditioning trials with Coff on Days 12 and 14 (and 2 had received Vin after their Sac on Day 11 and two had not). The rats in Group Control that had received initial conditioning and extinction with Coff received conditioning trials with Sac on Days 12 and 14 (and 2 had received Vin after their Sac on Day 11 and two had not). Hence, Group Control received conditioning and extinction with one flavor prior to subsequent conditioning with a different flavor on Days 12 and 14 in order to assess the degree of slow reacquisition seen in Groups Reac and Reac-Vin. Testing occurred on Days 16-19 when all subjects were given 10 min exposures to Sac.

A follow-up experiment sought evidence that a posttarget flavor influences processing of a target flavor by examining whether this second flavor could produce an attenuation of neophobia to a target flavor. Animals were first acclimated to receiving water from a syringe. Subjects were then divided into two groups ( $n = 6$ ), counterbalanced for body weight: Group Control and Group Vinegar.

On Day 1, all subjects received a 2 ml exposure to a 3% coffee solution (Coff) via oral infusion with a syringe. The infusion procedure ensured that both groups drank comparable amounts of Coff. The 3% coffee concentration was used because previous unpublished work in this laboratory demonstrated neophobia with this flavor. Other flavors, such as saccharin, can yield neophobia, but, based on evidence from our laboratory, are less sensitive to the effects of posttarget flavors on neophobia (see also Green & Parker, 1975; Kasprow and Schachtman, 1993; Kaye, Gambini, & Mackintosh, 1988; Robertson & Garrud, 1983).

Immediately after oral infusion of Coff, Group Control was given 5 min access to water in the drinking tubes while Group Vinegar was given drinking tubes containing Vin. The test for neophobia to Coff occurred on Days 2 and 3 during which time all rats were given a drinking tube containing 3% Coff for 10 min. Hence, the only way in which the two critical groups differed in the experiment above was whether Vin followed the target flavor on Day 11 prior to (and following reconditioning) a 10-min test on the target. The only way that the current groups differed was whether Vin followed the target flavor on Day 1 prior to a 10-min test on the target.

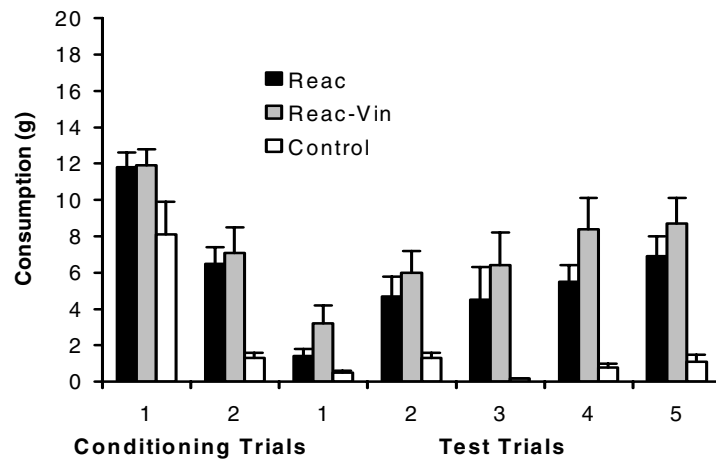
## Results

All data were analyzed using an analysis of variance (ANOVA). The taste aversion results are discussed first. Three rats were eliminated from the experiment (one from each group) for failing to drink sufficient solution on Day 1 of the experiment (all consumed less than 0.2 g). With one exception, there were no significant differences between the Coff and Sac as a flavor when it was used as a factor in the analyses and so the scores were collapsed over this counterbalancing variable. The exception was that Coff produced neophobia for rats in Group Control that were shifted to coffee on Day 12. Analysis on this day produced a group by flavor interaction,  $F(2, 15) = 8.37, p < 0.005$ , which was due solely to the low consumption of coffee by the three rats in Group Control (a mean of 3.7 g) whereas the means for all other conditions (including Sac consumed by the four rats in Group Control) were quite high (means of more than 10.0 g). Even these three rats showed extremely strong conditioning on Day 14 (a mean of 1.0 g) stemming from Day 12 treatment. Moreover, there was no significant difference between the Reac-Vin and Reac groups on this day with respect to an effect of Flavor or interaction,  $F_s < 1.24, p_s > 0.25$ ). Also the subgroups of Group Control (those receiving Vin and those that did not) were compared and no differences occurred for these subconditions, all  $F_s < 1.61, p_s > 0.20$ ; hence, these rats were combined for Group Control.

On Day 1, all three conditions consumed comparable amounts of solution ( $F < 1$ ). An ANOVA conducted on the data from the Day 1 conditioning trial and the first extinction trial revealed a main effect of Day,  $F(2, 18) = 51.03$ ,  $p < 0.0001$ , indicating that conditioning had occurred, but there was no effect of Group or interaction,  $F_s < 1$ . An ANOVA conducted on the nine days of extinction obtained no effect of Group or an interaction,  $F_s < 1$ , but all groups showed the expected increase in consumption across the extinction trials,  $F(8, 144) = 42.68$ ,  $p < 0.001$ .

There were no differences among the groups on the initial eight extinction trials or on the ninth extinction trial,  $F_s < 1$ . The rats in Group Reac-Vin and the four rats in Group Control that received Vin consumed similar amounts of vinegar,  $F(1, 9) = 1.94$ ,  $p > 0.15$ . The rats in Group Reac-Vin consumed 1.3 ( $\pm 0.4$ ) g of Vin on Day 11 while the four rats in Group Control that received Vin consumed 0.6 ( $\pm 0.2$ ) g.

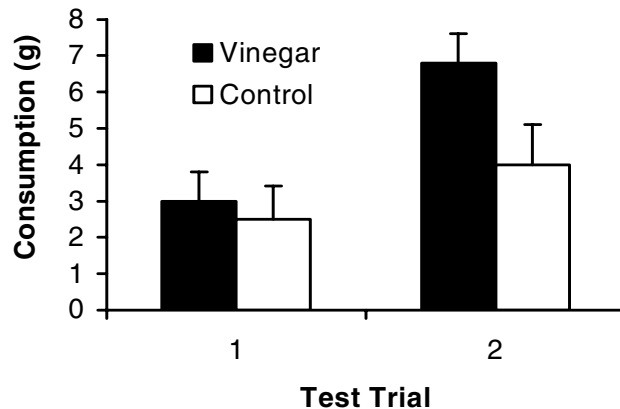
The data represented in the left side of Figure 1 (Conditioning Trials 1 and 2) show consumption on the conditioning trials on Days 12 and 14. A 3 x 2 (Group x Day) ANOVA conducted on the Day 12 and Day 14 data obtained a main effect of Group,  $F(2, 18) = 8.00$ ,  $p < 0.005$ , an effect of Day,  $F(1, 18) = 60.40$ ,  $p < 0.0001$ , and no interaction,  $F < 1$ . There was a tendency for the three groups to drink different amounts of flavor on the first reacquisition trial,  $F(2, 18) = 2.92$ ,  $p = 0.08$ , but this was due to the reacquisition groups consuming more than Group Control, possibly related to the apparent neophobia effect produced by coffee consumption, as mentioned above,  $F_s > 4.25$ ,  $p < 0.054$ ; the reacquisition groups did not differ from each other,  $F < 1$ . An analysis conducted on the Day 14 data obtained a main effect of Group,  $F(2, 18) = 10.34$ ,  $p < 0.001$ . Again, Groups Reac and Reac-Vin did not differ from each other on Day 14,  $F < 1$ . However, both Groups Reac and Group Reac-Vin drank more than the rats in Group Control,  $F_s(1, 18) > 13.52$ ,  $p_s < 0.002$ , revealing a slow reacquisition effect for the latter two groups.



**Figure 1.** Mean intake ( $\pm$  SEMs) during the conditioning trials on Days 12 and 14 and the test trials in Experiment 1.

The data in the right side of Figure 1 indicate group differences during consumption of Sac on the five test trials (Days 16-20). An ANOVA conducted on these scores obtained a main effect of Group,  $F(2, 18) = 9.65, p < 0.0015$ . An ANOVA indicated that Groups Reac and Reac-Vin consumed more Sac on the test trials than Group Control,  $F_s(1, 18) > 8.30, p_s < 0.01$ . No difference in Sac consumption was found between Groups Reac and Reac-Vin as revealed by no main effect of Group,  $F(1, 12) = 1.38, p > 0.25$ , and no Group x Day interaction,  $F < 1$ . Thus, slow reacquisition occurred for Groups Reac and Reac-Vin, but no effect of the posttarget flavor Vin was observed in Group Reac-Vin. In fact, with respect to the numerical difference between the means, consumption of the test flavor was greater for Group Reac-Vin, revealing, if anything, the distractor flavor on the last day of extinction produced weaker learning about the target CS. There was no indication from this experiment that a presumably surprising flavor on the final extinction trial produced more rapid reacquisition for a CS. An additional experiment, nearly identical to that described above, using Sac and 3% vinegar produced very similar results. Moreover, other events used as posttrial surprising events, such as a swim experience, yielded no effects on reacquisition.

The data from the follow-up neophobia experiment are shown in Figure 2. The subjects in Group Vinegar consumed 2.8 ( $\pm 1.0$ ) g of Vin on Day 1. Group Vinegar consumed 3.0 ( $\pm 0.8$ ) g and 6.8 ( $\pm 0.8$ ) g of Coff on the two test trials respectively, while Group Control consumed 2.5 ( $\pm 0.9$ ) g and 4.0 ( $\pm 1.1$ ) g of Coff on these two test trials respectively. An ANOVA conducted on these test data revealed that Group Vinegar drank more Coff than did Group Control,  $F(1, 10) = 6.27, p < 0.05$ , due primarily to the difference on Day 2.



**Figure 2.** Mean intake ( $\pm$  SEMs) during the test trials in Experiment 2.

Thus, presenting Coff followed by Vin as a posttarget stimulus facilitated the habituation of neophobia by potentially promoting greater attention to the CS and, thus, causing greater habituation. The posttarget flavor, Vin, influenced processing of the target flavor. The difference in consumption was not due to differences in motivation (i.e., thirst), as all rats had sufficient opportunity to replete themselves with water in the home cage after the previous day's experimental treatment. It is possible that some stimulus generalization may have occurred between Coff and Vin, such that Group Vinegar received effectively "more" of the

target solution than Group Control. Stimulus generalization is difficult to rule out completely when an attenuation of neophobia is promoted by a posttarget flavor. Nonetheless, data from this laboratory suggests that generalization between the coffee and vinegar concentration is minimal; a number of experiments were conducted with these flavors showing that subjects clearly differentiate these flavors (e.g., Gustavson et al., 1992; Schachtman et al., 1992). Of course, this experiment did not assess the flavor-specificity of the effect of vinegar on neophobia to coffee (i.e., perhaps a surprising second flavor enhances consumption of any flavor on a subsequent day) although such a general effect of surprise on consumption seems unlikely.

### **Discussion**

There are a number of views regarding the processes underlying extinction including degradation of the US representation (e.g., Rescorla, 1979; Rescorla & Heth, 1975; see also Richardson et al., 1999, for a discussion), interference and retrieval processes (Bouton, 1993; Kraemer & Spear, 1992; Miller et al., 1986), and the acquisition of inhibitory associations (e.g., Calton, Mitchell, & Schachtman, 1996; Delamater, 1996). Robbins (1990) claimed that a loss of attention or associability underlies extinction. To the extent that this latter view predicts that nontarget surprising events presented on the final extinction trial should enhance attention to the target CS and facilitate reacquisition, the present results do not support the attention/associability view of extinction. No effect of a posttarget surprising event was observed in the present experiments.

However, it is possible that low associability for an extinguished CS is not modified by posttrial events in the same manner that the loss of associability of a preexposed stimulus (i.e., latent inhibition) is impacted (Hall & Pearce, 1982). Of course, surprising posttrial events have also been shown to have a disruptive effect on conditioning (Wagner, Rudy, & Whitlow, 1973). Nonetheless, although some authors have claimed that latent inhibition exposures and extinction exposures may involve similar processes (Kraemer & Spear, 1992; Miller et al., 1986), the present results suggest that some treatments may differentially influence latent inhibition and extinction.

If surprising posttrial events during extinction are capable of influencing performance to a CS (i.e., the rate of extinction, see Taylor & Boakes, 2002), but not reconditioning of the extinguished CS, then one might question whether a loss of associability is involved in extinction. The question remains as to why posttrial events have been found to influence performance to a flavor (Kasproff & Schachtman, 1993; Taylor & Boakes, 2002). Extinction may result in the conditioning of a motivational state (which, say, opposes the motivational state resulting from the reinforcer used during the CS-US pairings), thereby reducing the CR, and posttrial events may influence this state (hence, influencing performance, see Delamater, 1996). Evidence supporting the notion that extinction results in the formation of inhibitory stimulus-response associations has been reported (e.g., Delamater, 1996), and posttrial events may influence performance based on such associations but without influencing subsequent reacquisition. Thus, posttrial nontarget events can impact performance to target CSs. However, given the present proce-

sure such events do not appear to appreciably restore associability as assessed by reconditioning.

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