LEARNING AND MOTIVATION **28,** 311–322 (1997)
ARTICLE NO. LM960966

Effects of a Retention Interval on the US-Preexposure Phenomenon in Flavor Aversion Learning

Luis Aguado and Isabel De Brugada

Universidad Complutense de Madrid, Madrid, Spain

and

Geoffrey Hall

University of York, York, United Kingdom

Two experiments, using rats as subjects, examined the effects of a retention interval on the retardation of flavor aversion learning produced by prior exposure to the unconditioned stimulus (US). Experiment 1 showed that the US-preexposure effect was attenuated when a 15-day retention interval was interposed between preexposure and conditioning, but that the same interval was without effect when it occurred between conditioning and testing. Experiment 2 confirmed these findings and also demonstrated that these retention intervals did not influence the conditioned aversion shown by control subjects not given US preexposure. These results are consistent with the proposal that the US preexposure has its effect by interfering with the formation of the target association; they provide no support for the suggestion that the effect depends on interference at the test stage.

Pavlovian learning is retarded by prior exposure to either of the stimuli that will later be associated during conditioning. Prior exposure to the conditioned stimulus (CS) results in latent inhibition; prior exposure to the unconditioned stimulus (US) produces the retardation known as the US preexposure effect. Both phenomena are readily observed in flavor aversion learning, the conditioning procedure used in the experiments reported here (see Lubow, 1989; Randich & LoLordo, 1979, for reviews). Both phenomena have been interpreted as showing that preexposure interferes with the formation of the CS–US

This work was supported, in part, by a grant under the Acciones Integradas programme of the British Council and the Spanish Ministerio de Educación y Ciencia. We thank C. Bonardi and M. Symonds for helpful discussion.

Address reprint requests and correspondence to L. Aguado, Facultad de Psicología, Campus de Somosaguas, 28023 Madrid, Spain.

0023-9690/97 $25.00

association. For latent inhibition it is suggested that the preexposed CS suffers a loss of associability (e.g., Mackintosh, 1975; Pearce & Hall, 1980) and is thus less able to form an association with the US. A preexposed US might suffer a loss of effectiveness through a process of habituation (but see Best, 1983); alternatively the preexposure procedure could allow the formation of an association between the context and the US which might then act to block the formation of the CS–US association when CS–US pairings are given in the same context (e.g., Batson & Best, 1979).

For the case of latent inhibition, however, an alternative interpretation has been offered. It is suggested that the preexposure and conditioning phases of the latent inhibition procedure generate independent memories so that, although the CS–US association may be well formed during conditioning, the memory of the preexposure experience interferes with retrieval at the time of testing (e.g., Bouton, 1991; Kasprow, Catterson, Schachtman, & Miller, 1984). Evidence to support this interpretation comes from studies in which a retention interval has been imposed between the phases of a latent inhibition experiment. Thus Aguado, Symonds, and Hall (1994; see also Kraemer & Roberts, 1984), in experiments using the flavor-aversion paradigm, found that a retention interval reduced the size of the latent inhibition effect, both when this interval occurred between preexposure and conditioning and when it occurred between conditioning and the test. They interpreted these results as implying that the memory of the initial experience of nonreinforced exposure to the CS became less retrievable with time, so that after a long preexposure to test interval, it was no longer able to interfere with retrieval of information acquired during the conditioning episode.

The experiments reported here use the same rationale to investigate the possibility that interference at the retrieval stage might also play a role in the US preexposure effect: that preexposure to the US might have its effects not because it prevents the acquisition of the CS–US association but because it interferes with the expression of this association on test. This proposal is not inconsistent with the idea that the US-preexposure effect is a consequence of the formation of a context-US association during preexposure; it differs from standard accounts which emphasize the role of blocking by contextual cues (see Randich & LoLordo, 1979) only in supposing that these cues exert their effect during test rather than during conditioning (cf. Balaz, Gutsin, Cacheiro, & Miller, 1982). The two accounts differ, however, in the predictions they make about the likely effects of imposing a retention interval in this training procedure.

Both accounts accept that such an interval might result in a loss of strength by the context-US association formed during preexposure, as contextual cues (or cues like them) will be experienced in the absence of the US. Both can predict, therefore, that an interval imposed between preexposure and conditioning should reduce the size of the US-preexposure effect. There is already some experimental evidence to support this prediction (e.g. Cannon,

TABLE 1
Experimental Designs

Experiment 1

Group	Pre	RI 1	Cond	RI 2	Test
Pre S-S	3 Li	2 days	Sac → Li	2 days	Sac
Pre S-L	3 Li	2 days	Sac → Li	15 days	Sac
Pre L-S	3 Li	15 days	Sac → Li	2 days	Sac
Control	3 sal	2 days	Sac → Li	2 days	Sac

Experiment 2

Group	Pre	Cond 1	RI	Test 1	Cond 2	Test 2
Pre S	3 Li	Sac → Li	2 days	4 Sac	3 Sac → Li	Sac
Pre L	3 Li	Sac → Li	15 days	4 Sac	3 Sac → Li	Sac
Con S	3 sal	Sac → Li	2 days	4 Sac	3 Sac → Li	Sac
Con L	3 sal	Sac → Li	15 days	4 Sac	3 Sac → Li	Sac

Note. RI, retention interval; Pre, preexposure; Cond, conditioning; Sac, saccharin; Li, injection of lithium chloride; sal, saline injection.

Berman, Baker, & Atkinson, 1975). Where the accounts differ is in their predictions about the effects of imposing an interval between conditioning and the test. If US preexposure has its effects because it blocks acquisition of the CS–US association, then such a retention interval will be without effect on the size of the US-preexposure effect ultimately obtained. But if the effect depends on interference at the time of the test, then extinction of the context-US association in the interval between conditioning and the test will be effective in attenuating it. There is little experimental work that bears directly on this prediction, but some support comes from a study by Miller, Jagielo, and Spear (1993) who found evidence for just such an attenuation of the US-preexposure effect. Unfortunately their experiment involved some unusual procedural details (in particular, they gave training with a compound CS but tested just one of the elements of the compound) which give rise to possible doubts about the generality of their result. Accordingly the experiment to be described next investigates the effects of retention interval on the US-preexposure effect in a paradigm more typical of those that have been used routinely to demonstrate the effect.

EXPERIMENT 1

The treatment given to the four groups of rats used in this experiment is summarized in the upper part of Table 1. All subjects received flavor aversion conditioning with saccharin as the CS and an injection of lithium chloride (LiCl) as the US. Control subjects received no preexposure to the US and

were expected to show a substantial aversion to saccharin on the test, given 2 days after conditioning. Subjects in Group Pre S-S were treated just like the controls except for receiving a series of three preliminary US presentations, the last of these 2 days before conditioning. (Pre indicates preexposure to the US; S-S that the intervals between preexposure and conditioning and between conditioning and the test were both short.) These subjects should show the US-preexposure effect (i.e., show less of an aversion than the Control group in the final test). The remaining two groups received the same training as Group Pre S-S, but with a lengthened retention interval. For Group Pre L-S, the interval between preexposure and conditioning (RI 1 in Table 1) was increased to 15 days. We expected that this would attenuate the US-preexposure effect and that this group would show more of an aversion on test than that shown by Group Pre S-S. The fourth group (Pre S-L) experienced the 15-day interval between conditioning and the test (RI 15 in the table). The question of central interest was whether this group would show any reduction in the size of the US-preexposure effect.

Method

Subjects. The subjects were 32 male Wistar rats, assigned at random to one of the four equal-sized training groups. They had a mean weight of 325 g at the start of the experiment. They were housed in individual home cages, with food freely available, and under a 12-h light/12-h dark illumination cycle, with the lights coming on at 8:00 am. All experimental treatments were given in the home cages and in the morning, during the light period of the illumination cycle. Fluids were presented in 50-ml plastic tubes equipped with a metal drinking spout.

Procedure. Before the start of training, a schedule of water deprivation was imposed. After a day with no access to water, there were 3 days on which animals were given access to water in the drinking tubes for two periods of 30 min, one in the morning and one in the evening. This schedule was maintained throughout the experiment, except for treatment days, on which a saccharin solution rather than water was presented in the morning session, and for the first three days of the long retention intervals for Groups Pre S-L and Pre L-S when *ad libitum* access to water was allowed.

The next three days constituted the preexposure phase (see Table 1). On each day all subjects were given an intraperitoneal injection immediately after the morning drinking period. For subjects in Group Control, this was of isotonic saline (at 10 ml/kg body weight); all other groups received .3 M LiCl, 10 ml/kg. Then followed RI 1, lasting 15 days for Group Pre L-S, but only 2 days for the other three groups. On the conditioning day, all animals were given 10 ml of a .1% saccharin solution for 30 min, after which they received an injection of LiCl. The effects of this conditioning trial were assessed in a final test session on which all subjects were given free access

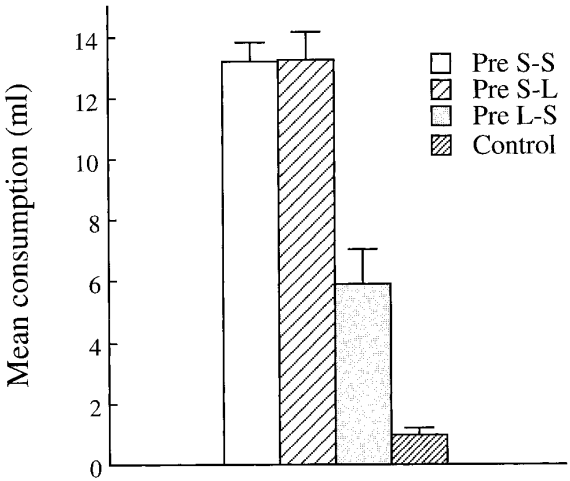

FIG. 1. Experiment 1: Group mean (+ SE) amounts of saccharin consumed on test after flavor-aversion conditioning. Groups labeled Pre had received prior exposure to the US. S-S means that there were short intervals both between preexposure and conditioning and between conditioning and the test. S-L means that the first of these intervals was short and the second long. L-S means that the first of these intervals was long and the second short.

to the saccharin solution for 30 min. The test occurred after an interval (RI 2) of 15 days for Group Pre S-L, and of 2 days for the other groups.

Results and Discussion

Figure 1 presents group mean amounts of saccharin consumed on the test day. The US preexposure effect is evident in the comparison between Group Control and Group Pre S-S. Control subjects showed a strong aversion whereas those in Group Pre S-S did not. The size of this effect appears to be diminished by inserting a retention interval between preexposure and conditioning, in that Group Pre L-S showed a substantially stronger aversion than Group Pre S-S; but a retention interval between conditioning and test is without effect; Groups Pre S-S and Pre S-L did not differ. These impressions were confirmed by statistical analysis. An analysis of variance performed on the data summarized in Fig. 1 yielded a significant effect of group, $F(3,38) = 56.65$. (The rejection level adopted for this and all subsequent analyses was $p < .05$.) Comparisons between pairs of means using Duncan's test showed that each of the preexposed groups differed from the control group; that Group Pre L-S differed from the other two preexposed groups, Pre S-S and Pre S-L, and that the two latter groups did not differ from one another.

Thus this experiment has successfully replicated previous results showing attenuation of the US-preexposure effect with a long preexposure-to-conditioning retention interval (e.g., Cannon *et al.*, 1975; Cappel & LeBlanc, 1975).

We did not, however, replicate the attenuation found by Miller *et al.* (1993) when a long retention interval was interposed between conditioning and the test day. This pattern of results is consistent with the traditional explanation of the US-preexposure effect as being the result of an acquisition deficit and gives no support to an interpretation in terms of interference at retrieval. Before pursuing the theoretical implications of this conclusion, we report a further experiment intended to confirm the reliability of the present results.

EXPERIMENT 2

The conclusion that the effect of US preexposure is independent of the conditioning-to-test interval depends on the absence of any difference in Experiment 1 between Groups Pre S-S and Pre S-L. A single null result is unlikely to be convincing and accordingly, in the present experiment, we attempted to confirm this finding. Two groups (labelled Pre S and Pre L according to the length of the conditioning-to-test interval; all subjects in this experiment experienced a short preexposure to conditioning interval) received training identical to that given to the critical groups in Experiment 1. (See the lower part of Table 1 for a summary of the experimental design.) The procedure differed only in that the test was extended over four trials in the hope of increasing its sensitivity.

In addition, we included further control conditions that would allow us to assess a possible explanation for the null result of Experiment 1. The proposal that a long conditioning-to-test interval might attenuate the US-preexposure effect depends on assuming not only that what has been learned during preexposure is lost to some extent over this interval but also on assuming that the CS−US association formed during conditioning will remain intact. Nonetheless, it remains possible that the CS−US association itself loses strength over the interval. If so, the reduced size of the aversion consequent on this loss of strength would tend to disguise any effect produced on test by a loss of the US-preexposure effect. In order to evaluate this suggestion we included two further groups, Con S and Con L (see Table 1). These matched Groups Pre S and Pre L in their conditioning-to-test intervals but received no US preexposure. Any loss in strength of the CS−US association over the retention interval would thus be evident in a reduced aversion in Group Con L.

Finally, we included a further phase of training designed to confirm the importance of the preexposure-to-conditioning interval in the US-preexposure effect. After completion of the test (Test 1 in Table 1), all subjects received further conditioning trials until the aversion extinguished over the course of the nonreinforced Test-1 trials was reestablished. They then received a further test trial (Test 2). For Group Pre S, this second phase of conditioning occurred a (relatively) short time after the phase of US preexposure and we might again expect to see a retardation of conditioning. For Group Pre L, on the other hand, the interval between the preexposure phase and the second phase of conditioning is lengthened by the 15-day retention interval that preceded

Test 1. If the interval between preexposure and conditioning is critical, these subjects can now be expected to show a loss of the US-preexposure effect.

Method

32 male Wistar rats with a mean weight of 270 g at the start of the experiment were used as subjects. They were maintained in the same conditions as the subjects in Experiment 1.

During the preexposure phase, subjects in Groups Pre S and Pre L received three injections of LiCl; subjects in the control groups received three saline injections at this stage. After an interval of 2 days, all underwent conditioning which consisted, as in the previous experiment, of a presentation of saccharin followed by an injection of LiCl. The aversion established by this trial was assessed in Test 1 which comprised four daily 30-min presentations of saccharin. This test took place 2 days after conditioning for Groups Pre S and Con S, and 15 days after conditioning for Groups Pre L and Con L. The second conditioning phase consisted of three saccharin–LiCl pairings. A recovery day on which no treatment was given followed each trial. Finally, in Test 2, all subjects were presented with saccharin for 30 min. Any procedural details not specified here were the same as those described for Experiment 1.

Results and Discussion

Figure 2 presents group means for saccharin consumption over the four trials of Test 1. (Data were lost for two subjects, one in Group Con L and one in Group Pre S, reducing the group size to seven in each of these cases.) It shows that the groups differed on the first test trial but that these differences soon disappeared as the aversion extinguished over the course of repeated testing. An analysis of variance conducted on these data with group and trial as the factors revealed a significant effect of trials, $F(3,78) = 69.44$, but not of group ($F < 1$). The Group × Trial interaction was significant, $F(9,78) = 3.61$. An analysis of simple main effects showed that the difference among the groups was significant only on trial 1, $F(3,72) = 4.71$ (for trial 2, $F = 2.29$; for trial 3, $F < 1$; for trial 4, $F = 2.36$). The data for trial 1 were therefore subjected to a further analysis, the factors being preexposure condition (Pre vs. Con) and length of retention interval (S vs. L). The effect of the preexposure condition was significant, $F(1,26) = 7.49$; the effect of the retention interval was not significant ($F < 1$), nor was the interaction, $F(1,26) = 1.74$. It may be noted that the absolute amounts of fluid consumed by groups Pre L and Pre S on the first of these test trials was somewhat less than that consumed by the comparable groups (Pre S-S and Pre S-L) of Experiment 1. We assume that this difference is a simple consequence of the fact that the rats used in this experiment were somewhat smaller than those used in Experiment 1.

This pattern of results thus confirms the finding of Experiment 1, that US preexposure results in a reduced degree of aversion on test and does so even

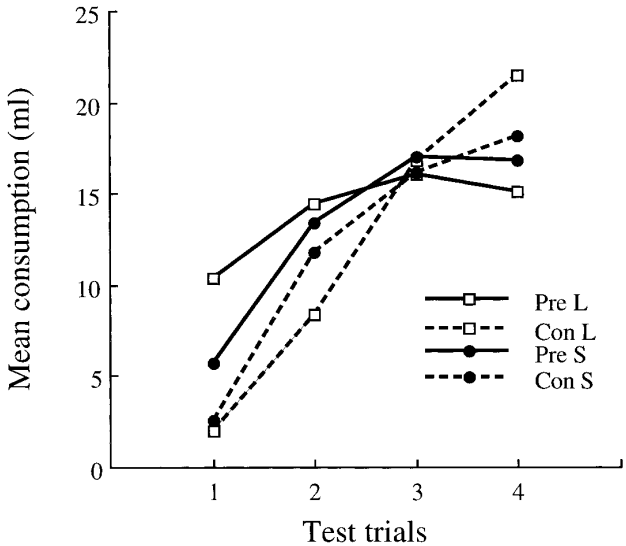

FIG. 2. Experiment 2: Group mean consumption of saccharin over the four test trials of Test 1. Pre groups had received preexposure to the US prior to flavor-aversion conditioning; Con groups received no preexposure. L means a long interval between conditioning and this test; S means a short interval.

when there is a long retention interval between conditioning and the test. In fact the appearance of the figure suggests (although the effect was not statistically reliable) that the size of the US-preexposure effect was bigger in Group Pre L than in Group Pre S. There was no sign that a long retention interval reduced the aversion shown by the control groups. Groups Con S and Con L did not differ reliably on any of the test trials. The test performance of these two control groups is also relevant to another issue. It is possible, both in this experiment and in Experiment 1, that the more prolonged exposure to the deprivation schedule experienced by the L groups might act to inflate the amount consumed by these groups on the test trials. If so, then in this experiment, Group Con L would be expected to consume more on test than Group Con S. The lack of a difference between these groups argues against the suggestion that L and S groups generally were in substantially different motivational states at the time of the test.

Group mean scores for Test 2, given after the aversion to saccharin had been reestablished by the second phase of conditioning, are shown in Fig. 3. All groups show a substantial aversion, apart from Group Pre S. An analysis of variance carried out on the data summarized in the figure showed there to be a significant difference among the groups, $F(3,26) = 10.29$. Comparison of individual means using Duncan's test showed that Group Pre S differed significantly from each of the other groups, which did not differ among

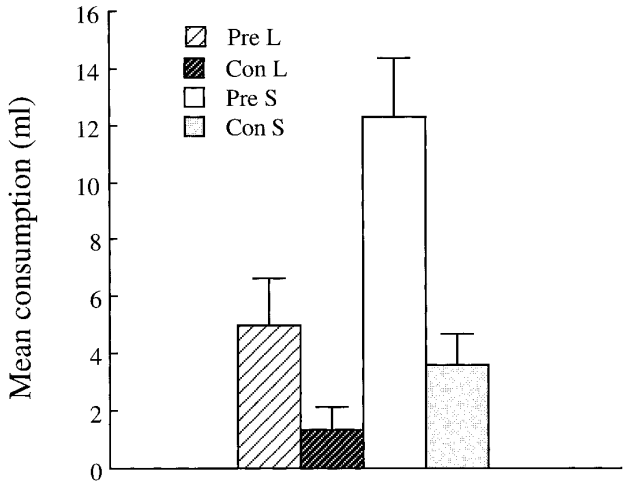

FIG. 3. Experiment 2: Group mean (+ SE) amounts of saccharin consumed on Test 2 given after the completion of Test 1 (see Figure 2) and reconditioning of the aversion.

themselves. Thus this test confirms that subjects that had experienced the shorter interval from preexposure to conditioning (Group Pre S) showed poor reacquisition of the aversion. Group Pre L, on the other hand, reacquired the aversion readily. In these subjects, although the long retention interval did not reduce the effects of preexposure on an already established aversion (Test 1), it did eliminate the effect on reacquisition of the aversion (Test 2). Taken together these results demonstrate that (contrary to what we have found for the case of latent inhibition; Aguado *et al.*, 1994), the critical retention interval in the case of the US-preexposure effect is not that between preexposure and test, but that between preexposure and conditioning.

GENERAL DISCUSSION

These experiments confirm that the aversion evoked by a flavor CS after CS–US pairing will be reduced in magnitude when the subjects have been given initial exposure to the US. This effect is attenuated when there is a long interval between preexposure and conditioning (Experiment 1; Experiment 2, Test 2). It is not influenced by a long retention interval interposed between conditioning and the test (Experiments 1 and 2); the aversion shown by nonpreexposed control subjects is similarly not influenced by such an interval (Experiment 2).

These results stand in marked contrast to those that have been reported for the effects of preexposure to the CS in this same training paradigm (e.g., Aguado *et al.*, 1994; Kraemer & Roberts, 1994). In this case, the critical interval has been shown to be that between preexposure and the test, a result

that has been interpreted as suggesting that the latent inhibition effect derives, at least in part, from interference during the test phase between what was learned during preexposure and what was learned during conditioning. The present results, on the other hand, accord with the proposal that US preexposure has its effects at acquisition rather than at retrieval; according to this analysis, a long interval following acquisition would be without effect whereas (assuming the effects of preexposure tend to diminish with time), a long interval between preexposure and conditioning would be expected to reduce the size of the effect.

The present results also contrast with those reported by Miller *et al.* (1993) who found, for rats given US preexposure, that a retention interval inserted between conditioning and testing brought about the recovery of a conditioned aversion. It is difficult to know which of the several differences that distinguish our procedure from that used by Miller *et al.* might be responsible for the discrepancy in outcome. Perhaps the most striking is that Miller *et al.* gave conditioning trials with a flavor/odor compound as the CS but gave the test with just the flavor. How this might affect the outcome, however, is a matter for speculation. One possibility is that this stimulus change induced a neophobic response which, summating with such aversion as was acquired during conditioning, resulted in reduced consumption of the flavor. Why this postulated neophobia should be sensitive to the length of the retention interval that preceded the test remains to be explained; in general, habituation of neophobia to flavors tends to be unaffected by a retention interval of the duration used here (e.g., Siegel, 1974; Domjan, 1977).

Another feature of Miller *et al.*'s (1993) experiment that deserves consideration is that the animals were transferred from their home cages to a different and distinctive environment for the preexposure, conditioning, and test treatments. In our experiments the home cages were used throughout. This procedural difference raises the possibility that the role played by context-US associations was different in the two sets of studies. One obvious implication is that a retention interval spent in a home cage that had also served as the preexposure environment would be likely to allow extinction of any context-US association formed during preexposure. Our experiments, therefore, could be construed as revealing the effects of context extinction given before or after conditioning, whereas that by Miller *et al.* might be sensitive to other changes occurring during the retention interval. However this may be, it remains true that the theoretical account offered by Miller *et al.*, which attributes the US-preexposure effect to context-blocking acting at the time of the test, would expect an effect of the conditioning-to-test interval to have been evident in our results.

A second possible consequence of using a novel cage as the experimental context is that the formation of a context-US association would be expected to proceed more readily than if the home cage were used. A novel environment would be highly associable; the very familiar home cage would have suffered

latent inhibition and would thus be less likely to form an association with the US. (See Miller *et al.*, 1993, Experiment 4.) To adopt this view leads to the suggestion that context-blocking at retrieval might still be the explanation for the results reported by Miller *et al.* (1993), but that our US preexposure effect depends on some other mechanism, and one that has its effect at the conditioning stage. The only obvious candidate here is the notion that US preexposure allows habituation to occur so that the injection of LiCl is less effective as a reinforcer on the conditioning trial. A retention interval after conditioning would thus be without effect, but one before conditioning, if it permitted spontaneous recovery of the habituated response to the US, would lead to an attenuation of the effects of preexposure.

In conclusion, the results reported here do not allow a choice between the alternative mechanisms (context blocking and US habituation) that have been proposed for the US-preexposure effect; nor can we fully resolve the discrepancy between these results and those of Miller *et al.* (1993). What we can say, however, is that in our training situation (which is one that has been used routinely in demonstrations of the US-preexposure effect), a retention interval between preexposure and conditioning attenuates the size of the effect, whereas an interval between conditioning and test does not. The mechanism responsible is thus one that acts on the acquisition of the CS–US association rather than at the time of the test.

REFERENCES

Aguado, L., Symonds, M., & Hall, G. (1994). Interval between preexposure and test determines the magnitude of latent inhibition: Implications for an interference account. *Animal Learning and Behavior,* **22,** 188–194.

Balaz, M. A., Gutsin, P., Cacheiro, H., & Miller, R. R. (1982). Blocking as retrieval failure: Reactivation of associations to a blocked stimulus. *Quarterly Journal of Experimental Psychology,* **34B,** 99–113.

Batson, J. D., & Best, P. J. (1979). Drug-preexposure effects in flavor-aversion learning: Associative interference by conditioned environmental stimuli. *Journal of Experimental Psychology: Animal Behavior Processes,* **3,** 273–283.

Bouton, M. E. (1991). Context and retrieval in extinction and in other examples of interference in simple associative learning. In L. W. Dachowski & C. F. Flaherty (Eds.), *Current topics in animal learning: Brain, emotion and cognition,* (pp. 25–53). Hillsdale, NJ: Lawrence Erlbaum Associates.

Cannon, D. S., Berman, R. F., Baker, T. B., & Atkinson, C. A. (1975). Effect of preconditioning unconditioned stimulus experience on learned taste aversion. *Journal of Experimental Psychology: Animal Behavior Processes,* **1,** 270–284.

Cappell, H., & LeBlanc, A. E. (1975). Conditioned aversion by amphetamine: Rates of acquisition and loss of attenuating effects of prior exposure. *Psychopharmacology,* **43,** 157–162.

Domjan, M. (1977). Attenuation and enhancement of neophobia for edible substances. In L. M. Barker, M. R. Best, & M. Domjan (Eds.), *Learning mechanisms in food selection,* (pp. 151–179). Waco, TX: Baylor University Press.

Kasprow, W. J., Catterson, D., Schachtman, T. R., & Miller, R. R. (1984). Attenuation of latent inhibition by postacquisition reminder. *Quarterly Journal of Experimental Psychology,* **36B,** 53–63.

Kraemer, P. J., & Roberts, W. A. (1984). The influence of flavor preexposure and test interval on conditioned taste aversions in the rat. *Learning & Motivation,* **15,** 259–278.

Lubow, R. E. (1989). *Latent inhibition and conditioned attention theory*. Cambridge: Cambridge University Press.

Mackintosh, N. J. (1975). A theory of attention: Variations in the associability of stimuli with reinforcement. *Psychological Review,* **82,** 276–298.

Miller, J. S., Jagielo, J. A., & Spear, N. E. (1993). The influence of retention interval on the US preexposure effect: Changes in contextual blocking over time. *Learning and Motivation,* **24,** 376–394.

Pearce, J. M., & Hall, G. (1980). A model for Pavlovian learning: Variations in the effectiveness of conditioned but not of unconditioned stimuli. *Psychological Review,* **87,** 532–552.

Randich, A., & LoLordo, V. M. (1979). Associative and nonassociative theories of the UCS preexposure phenomenon: Implications for Pavlovian conditioning. *Psychological Bulletin,* **86,** 523–548.

Siegel, S. (1974). Flavor preexposure and ''learned safety''. *Journal of Comparative and Physiological Psychology,* **87,** 1073–1082.

Received July 29, 1996
Revised November 21, 1996