The interval between the CS and the UCS as a determiner of generalization performance

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Rats were trained in a conditioned taste aversion paradigm in order to determine whether a trace interval between the conditioned stimulus (CS) and the unconditioned stimulus (UCS) would result in the forgetting of stimulus attributes. Accordingly, subjects were conditioned with milk (CS), given either an immediate or a delayed injection of lithium chloride (UCS), and tested 48 h later with either milk or chocolate milk, a generalized flavor. The rats conditioned immediately following the presentation of the CS avoided milk more than chocolate milk, indicating discrimination between the two flavors. Those conditioned after a trace interval avoided both flavors equally, suggesting a loss of stimulus attributes of the original CS. Delay rats, however, still exhibited substantial learning when compared with controls not experiencing the UCS. These results allege a role for the forgetting of stimulus attributes during a trace interval in addition to following a complete learning episode.

Since the seminal studies showing stimulus generalization in Little Albert (Watson & Raynor, 1920), a variety of studies have shown that organisms will respond to stimuli other than those with which they were trained (e.g., Guttman & Kalish, 1956; Jenkins & Harrison, 1960; Pavlov, 1927). Further, investigations have documented the effect of time on the phenomenon of stimulus generalization: As the temporal interval between training and testing increases, so does the likelihood of responding to a greater range of stimuli (McAllister & McAllister, 1963; Perkins & Weyant, 1958; D. R. Thomas & Lopez, 1962). That is, subjects demonstrate poorer retention of the characteristics of the target stimulus than of the response that is appropriate; stimulus discrimination is forgotten before the response (for reviews, see Riccio, Ackil, & Burch-Vernon, 1992; Riccio, Rabinowitz, & Axelrod, 1994). Thus, this type of forgetting results not in a loss of responding, but in the performance of the conditioned reaction to a greater variety of stimuli. The reliability of this phenomenon has been exhibited with a wide range of stimuli, including wavelength (D. R. Thomas & Lopez, 1962), contextual cues (Zhou & Riccio, 1996), drawings (Bahrick, Clark, & Bahrick, 1967), pharmacological agents (MacArdy & Riccio, 1991), auditory stimuli such as tones (D. A. Thomas & Riccio, 1979), and flavors (Richardson, Williams, & Riccio, 1984).

If these findings represent loss of memory for the details of a stimulus representation, temporal intervals might produce similar effects if introduced at other stages in conditioning. For example, manipulating the interval between the conditioned stimulus (CS) and the uncondi-

tioned stimulus (UCS) in classical conditioning might alter the accuracy of the CS representation at the time the association is formed. Put slightly differently, delaying the presentation of the UCS may have effects upon the representation of the CS similar to those of postponing a test following completion of training. It is well known that a long trace interval reduces the strength of an association, but does that interval between the CS and the UCS also result in loss of memory for the specific attributes of the target? If so, a test following the conditioning episode might reveal the anomalous outcome of a weaker conditioned response (CR) to the CS but a relatively stronger CR to a generalized cue in the trace group than in the immediate condition.

In the present experiment, the temporal interval between presentation of the CS and UCS in a classical conditioning paradigm was manipulated to examine this possibility. A conditioned taste aversion (CTA) model was used, whereby rats are exposed to a novel liquid flavor followed by a toxic agent. Because robust conditioned taste aversion can be demonstrated with delays of poison administration for several hours following presentation of the taste (Garcia, Ervin, & Koelling, 1966; Revusky & Garcia, 1970), this task seems especially suited for use in the investigation of the nature of the trace representation.

In order to determine whether delaying UCS presentation affects the CS representation, rats drank a certain flavor (the CS) and had either immediate or delayed exposure to illness (the UCS). Half of those in the immediate group were tested with the original flavor, and half with a different one. They should show discrimination between the flavors, avoiding the original CS more than the foil. Half of the delay group rats also were tested with each flavor. If forgetting of stimulus attributes occurs between CS and UCS presentation, these rats may avoid the original and foil flavors equally.

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METHOD

Subjects

Sixty-one male albino rats (Hilltop Farms) were used in the experiment. Animals were housed singly in hanging wire mesh cages in the colony room, fed ad lib, and maintained on a 14:10-h light:dark cycle. Subjects were approximately 150 days old at the time of training and weighed between 360 and 510 g.

Procedure

In order to reduce the potential for context overshadowing during a delay (Marlin, 1981), procedures took place in the animals' highly familiar (latently inhibited) home cages. Partial water deprivation, whereby subjects had access to 15 min of water per day, was begun 3 days prior to training. On Day 4, animals were randomly divided into seven groups, and drank, instead of water, either a milk mixture or a chocolate milk mixture (the CS). All animals had a 15-min exposure to 40 ml of the CS. Either immediately or 2 h after presentation of the CS, they were injected with lithium chloride (LiCl). One hour following this injection, water bottles were replaced for 2 h to allow subjects to replenish their body fluids. Testing was conducted 48 h later and consisted of a 15-min exposure to 40 ml of the test flavor. Neophobic reactions at training yielding consumption scores of fewer than 5 ml were excluded from the study and resulted in unequal sample sizes among groups.

The groups were characterized by the flavors presented to them at training and test, as well as the timing of the US presentation (Table 1). They were assigned as follows: Group Milk-Immediate-Milk (M-I-M; $n = 7$) drank milk (50% water + 50% Carnation Brand Vitamin D Evaporated Milk) on training day, received an LiCl injection *directly* after training consumption, and was tested with milk. (This corresponds to a standard CTA paradigm). In contrast, Group Milk-Delay-Milk (M-D-M; $n = 9$) drank milk at training, was injected with the US 2 h after consumption, and was tested with milk. Group Milk-Immediate-Chocolate (M-I-C; $n = 8$) drank milk at training, was injected immediately with LiCl, and had chocolate milk (50% water + 50% Carnation + 10% Hershey's Chocolate Syrup) at test. Milk-Delay-Chocolate (M-D-C; $n = 9$) was identical to Group M-I-C except that this group's injection was delayed 2 h following the CS. It was expected that those animals receiving immediate UCS presentations would show less generalization at testing; their representations of the CS would be more precise than those of animals experiencing the UCS following a lag.

Several control groups were included to control for possible performance artifacts. Group Milk-no UCS-Milk (M-noUCS-M; $n = 10$) drank milk at both training and test, but did not experience the UCS. This group was included in order to determine how much milk rats would normally consume at its second presentation. Group Chocolate-no UCS-Chocolate (C-noUCS-C; $n = 10$) was included for the same reason as well as to determine whether there were any initial intake differences between milk and chocolate milk because it is important to ascertain that rats were not particularly neophobic to either flavor. Group Chocolate-Immediate-Chocolate (C-I-C; $n = 8$) was trained to avoid chocolate milk; the inclusion of this group allows the strength of the conditioned aversions to milk and to chocolate milk to be directly compared. If the aversions learned to chocolate and regular milk are equiv-

alent, consumption differences between the two flavors at test cannot be due to their differential palatability.

The dependent variable was the amount (milliliters) consumed during the 15-min test, which occurred 48 h following CS consumption. We emphasize that for every group, the test occurred at the identical interval following CS presentation. Only the interval *between* the CS and UCS presentations differed. Those animals that consume more liquid are considered to evidence a weaker aversion to the flavor; low consumption scores indicate good acquisition of the avoidance response.

RESULTS

As can be seen in Figure 1, the intake of flavors was less suppressed with a 2-h delay between the CS and UCS than with the immediate pairing. Further, although differential consumption between the target and generalized flavors occurred in the immediate condition, intakes appeared comparable in the delayed groups.

These impressions were confirmed by a two-way analysis of variance (ANOVA) (interval $\times$ test flavor) that yielded a significant main effect of delay [$F(1,29) = 15.131$; $p < .001$] among the four groups of major interest, namely those rats tested with either milk or chocolate that experienced an immediate UCS (M-I-M and M-I-C) or a delayed UCS presentation (M-D-M and M-D-C). This supports the finding that postponed UCS exposure attenuates subsequent avoidance performance. Although no main effect of flavor was identified [$F(1,29) = 1.558$; n.s.], there was an interaction between delay condition (immediate or delay) and flavor (chocolate or milk) at test [$F(1,29) = 3.959$; $p = .056$]. The critical comparison between M-I-M and M-I-C was made using a t test with the usual rejection criterion of .05; each subsequent comparison was tested at an alpha level (α) of half that of the previous test to maintain an acceptable Type I error rate.

Those rats that experienced a delayed injection of LiCl appeared to have a less potent aversion than animals in the immediate injection groups; M-I-M rats drank less, and thus showed a stronger aversion, than M-D-M rats at test ($t = 4.251$; $p < .0001$; $\alpha = 0.025$). M-I-C animals, however, did not consume less chocolate milk than M-D-C rats ($t = 1.383$; $p = 0.19$, n.s.), suggesting that unlike the target CS, the generalized flavor was about equally aversive with short or long delay intervals.

Examination of the differential generalization with delay showed that varying the interval between the CS and the UCS affected later intake, since Group M-I-C drank more ($t = 2.409$; $p < .03$, $\alpha = .05$) than group M-I-M, demonstrating that the immediate UCS presentation allowed them to later discriminate between milk and chocolate milk. This outcome is consistent with the view that subjects in the immediate condition formed a relatively specific association to the CS. However, those groups given LiCl after a 2-h delay (M-D-M and M-D-C) showed equivalent aversions to the two flavors in terms of milliliters consumed, indicating a lack of discrimination between the flavors. This lack of discrimination is similar to that which occurs when *testing* is delayed in a CTA paradigm (Richardson et al., 1984).

Table 1
Experimental Procedures and Design

Group	CS	Delay	Test
M-I-M	Milk	< 5 min	LiCl
M-I-C	Milk	< 5 min	Chocolate
M-D-M	Milk	2 h	Milk
M-D-C	Milk	2 h	Chocolate
C-I-C	Chocolate	< 5 min	Chocolate
M-noUCS-M	Milk	no conditioning	Milk
C-noUCS-C	Chocolate	no conditioning	Chocolate

Note—M-I-M, milk-immediate-milk; M-I-C, milk-immediate-chocolate; M-D-M, milk-delay-milk; M-D-C, milk-delay-chocolate; C-I-C, chocolate-immediate-chocolate; M-noUCS-M, milk-noUCS-milk; C-noUCS-C, chocolate-noUCS-chocolate.

Figure 1. Mean consumption of test substance plotted by group. Subjects in Group M-I-M drank less than their chocolate counterparts (M-I-C). Groups M-D-M and M-D-C did not differ, however, indicating a generalization between these two flavors if the UCS is administered at a delay following the CS. Groups M-noUCS-M and C-noUCS-C did not avoid the test flavor since they did not develop aversions. Group C-I-C developed an aversion equivalent to that of M-I-M, demonstrating similar conditioning to both flavors. M-I-M, milk-immediate-milk; M-I-C, milk-immediate-chocolate; M-D-M, milk-delay-milk; M-D-C, milk-delay-chocolate; M-noUCS-M, milk-no UCS-milk; C-noUCS-C, chocolate-noUCS-chocolate; C-I-C, chocolate-immediate-chocolate.

In order to reduce the Type I error rate, control groups were compared with the critical experimental groups in the following manner: The means of Groups M-noUCS-M and C-noUCS-C were compared, using a *t* test, with the highest consumption mean of the experimental groups (Group M-D-M; $M = 7$ ml), which was the score numerically closest to them. The comparisons were made with an alpha level of 0.0125, and the differences were significant in both cases (M-noUCS-M vs. M-D-M, $t = 7.119$; CC-NoLiCl vs. M-D-M; $t = 7.581$). These control groups consumed more than 15 ml (16.3 ml, C-noUCS-C; 18.0 ml, M-noUCS-M) of test substance, clearly showing by contrasts that all the trained groups did develop aversions. The results for Group C-I-C, which showed no difference in aversion strength from that of animals trained with milk in the M-I-M group ($t = 1.129$, n.s.), are included in Figure 1. Further, Group C-I-C was not different from Group M-I-C, indicating that the pairing of these flavors with LiCl resulted in equivalent aversions.

DISCUSSION

It appears that the processes acting on representations over time following conditioning also act during acquisition if a trace interval intervenes between the CS and the UCS. These effects appear to operate on what has been termed "associative memory" (Revusky, 1971), affecting the retrievability of the attributes of a "CS" before it becomes a CS. The

forgetting of specific CS characteristics was demonstrated here using taste aversion conditioning, in which rats not only learned to avoid a novel flavor paired with illness, but also became less able to distinguish that taste from another, similar flavor in a subsequent test when presentation of the UCS was delayed during the conditioning episode.

Why does the timing of the presentation of the unconditioned stimulus, influence the fidelity of the CS representation that enters into the association? It seems likely that the representation of the CS attributes begins to fade or degrade, and, when the UCS is presented at a delay, the CS representation with which it becomes associated is not as clear as when it was initially presented. Presumably, whatever the state of the percept at the time of the aversive input, it is stored as such. If the percept is intact, as with the brief delay, it will be connected with the aversive outcome in that form, and, if it has suffered degradation, it will be captured in that state. Although the etiology of the degradation of the trace is undefined, it is possible that interference plays a role since the animals could have sampled the food on their cages during the 2-h trace interval (Bevins, personal communication, May 1997).

One criticism that has been leveled against data documenting weaker taste aversions with delays between the CS and the UCS, such as those reported here, involves the possible role of neophobia (DeCola & Fanselow, 1995; Mitchell, Scott, & Mitchell, 1977). According to this interpretation, the delay group's reluctance to consume the CS at testing is not a result of conditioning, but of sensitization induced by LiCl administration. Rather than learned avoidance to a particular flavor, the rats would be expressing neophobia to any novel flavor following sickness. Both DeCola and Fanselow and Mitchell et al. reported data that they interpreted as supporting the sensitization/neophobia theory of behavior following delay "conditioning." However, this explanation and these data do not seem applicable to our results for several reasons. First, in both earlier reports, rats were tested with a two-bottle preference test, pitting the conditioned flavor against water, whereas the rats in the present experiment were tested with one bottle containing the test substance. The former paradigm has been shown to be more sensitive to detecting absolute aversions, whereas the latter is superior for detecting differences in aversion strength (Batsell & Best, 1993), which is why we chose this method. In addition, comparison of data collected with one-bottle tests to that derived from two-bottle testing is tenuous. Moreover, the DeCola and Fanselow and Mitchell et al. studies began testing 24 h following illness and continued testing in extinction; it was in the later testing days that differences among their groups arose. In contrast, we gave our rats a single test 48 h following CS presentation and compared consumption differences from this single test. Further, delaying the test until 48 h following conditioning ensures that it is well outside of the time window in which effects of illness-induced neophobia is observed (Domjan, 1977). So, although under some conditions, LiCl may sensitize animals to novel flavors, we believe this is not the case here.

In order to examine directly the likelihood of observing aversion-like avoidance without a contingency in the present paradigm, an ad hoc experiment was conducted. Fifteen female albino rats, weighing between 220 and 340 g on conditioning day, were trained as follows. After 3 days of partial water deprivation, as described previously, rats were randomly divided into three groups: Group Delay, Group Unpaired Milk, and Group Unpaired Chocolate. On the 4th day, all rats were allowed access to 40 ml of milk (as reported above) for 15 min. Two hours following milk presentation, delay rats only were injected with LiCl (2 mg/kg; 0.75 M). This group was identical to the M-D-M group in the main experiment. Groups Unpaired Milk and Unpaired Chocolate were injected identically 24 h later to create two explicitly unpaired conditions. All rats were tested 48 h following the injection, mimicking conditions of the main experiment. Delay rats were tested with 15 min access to 40 ml of milk, as were unpaired milk rats. Those in the unpaired chocolate group were tested instead with the chocolate mixture detailed in the main experiment. It was assumed that if the data we reported were due to sensitization from LiCl exposure, the delay rats should consume comparable amounts of test substance as the unpaired groups. Alternatively, if a delay between the CS and the UCS resulted in an aversion, albeit a weaker one, delay rats should drink substantially less than unpaired ones.

The data clearly demonstrate that delay rats developed an aversion, whereas unpaired milk and chocolate rats did not [$F(2,12) = 7.004$; $p < .01$; consumption means: delay = 8.6 ml; unpaired milk = 15.6; unpaired

chocolate = 13.8 ml]. Planned comparisons (t tests) indicated that delay rats were significantly different from both unpaired milk ($p < .01$) and unpaired chocolate ($p < .05$), whereas the unpaired groups did not differ from each other. Thus, the aversions of our previous delay groups cannot easily be attributed to neophobic responses following illness.

It is clear that processes correlated with time affect the representation of a CS prior to its coupling with a UCS. The representation seems to be changed in two respects. First, as the trace literature has consistently revealed, conditioning is poorer with longer intervals between the CS and the UCS, presumably reflecting a weaker CS representation. Second, and the novel contribution of this study, is that the representation seems to become less precise, so that at later testing a discriminably different flavor is avoided much like the target CS. We assume that with the long trace interval, the taste representation that enters into the association is something like a general "milky" flavor rather than a specific "plain milk" one.

Temporal intervals, then, lead to the forgetting of stimulus attributes whether they occur after a learning experience or during one, as demonstrated in the present experiment. This result has both theoretical implications, since lack of discrimination due to an extended CS–UCS interval has not previously been documented, and practical implications, since generalization of responding may increase not only during retention intervals but also during trace intervals embedded in the conditioning process as well.

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