Appetitive context conditioning proactively, but transiently, interferes with expression of counterconditioned context fear

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Four experiments used rats to study appetitive–aversive transfer. Rats trained to eat a palatable food in a distinctive context and shocked in that context ate and did not freeze when tested 1 d later but froze and did not eat when tested 14 d later. These results were associatively mediated (Experiments 1 and 2), observed when rats were or were not food deprived (Experiments 1 and 2), and were not due to latent inhibition (Experiment 3). In contrast, rats trained to eat in the context and shocked there 13 d later froze and did not eat when tested 1 d after the shocked exposure. However, rats that received an additional eating session in the context 1 d before the shocked exposure ate and did not freeze when tested 1 d after the shocked exposure (Experiment 4). The results show that appetitive conditioning transiently interferes with aversive conditioning. They are discussed in terms of a weak context–shock association becoming stronger with the lapse of time (so-called fear incubation) or of the interference by the context–food association becoming weaker with the lapse of time.

Pavlovian conditioning imbues neutral stimuli with emotional and/or motivational significance. A relatively innocuous stimulus paired with an aversive shock unconditioned stimulus (US) becomes a conditioned stimulus (CS), eliciting protective reflexes and defensive responses. That CS also suppresses appetitive activity (e.g., Estes and Skinner 1941), punishes contingent instrumental responses (e.g., Azrin 1960; for review, see Azrin and Holz 1966), and increases avoidance responses (e.g., Rescorla and LoLordo 1965; for review, see Rescorla and Solomon 1967). In contrast, a CS paired with an attractive food US elicits consummatory reflexes and approach responses, suppresses defensive responses (e.g., Grossen et al. 1969; Bull 1970; Overmier and Bull 1970; Overmier et al. 1971), reinforces contingent instrumental responses (for review, see Wike 1966; Hendry 1969; Fantino 1977), and can increase responding in Pavlovian-Instrumental tasks (e.g., Estes 1948; for review, see Holmes et al. 2010). Such results have led to opponent-process theories of motivation (e.g., Konorski 1967; Bindra 1974; Gray 1975; Dickinson and Dearing 1979; Toates 1986). These theories differ in several respects but share the view that aversive and appetitive CSs excite contrasting central motivational states or systems and that excitation of one depresses activity in the other (for review, see Dickinson and Pearce 1977).

An implication of these theories is that the development of defensive responses across pairings of an appetitive CS and an aversive US will be impaired, as will approach responses across pairings of an aversive CS and an appetitive US. This impairment will occur because the motivational information originally encoded by the CS is the opposite of that appropriate to the new US. Consistent with this implication, the development of approach responses is impaired across pairings of an aversive CS and an appetitive US (e.g., Peck and Bouton 1990), as is the development of withdrawal responses across counterconditioning of an appetitive CS by an aversive US (e.g., Konorski and Szejkowska 1956; Nasser and McNally 2012, 2013). However, the source of this impairment is unclear: It could be due to an associative failure whereby the CS

of one motivational class fails to enter into the subsequent association with the US of the opposite class; alternatively, the association between the CS and the new US could be formed but fail to elicit the responses appropriate to that US.

Preexposure to a CS also impairs the development of responding. A CS repeatedly presented in the absence of any other scheduled events is slower to elicit responses across its pairings with a US than is a novel CS. However, there is evidence that the responding which is impaired shortly after conditioning recovers with the lapse of time since conditioning. Killcross et al. (1998a,b) shocked rats in either an extensively preexposed or a novel context and tested rats from each of these groups either 1 d or 14 d later. The preexposed rats showed few fear (freezing) responses whereas the control animals showed substantial levels of freezing when tested after a 1-d delay, demonstrating a latent inhibitory effect of context preexposure. Both preexposed and control rats showed similar and substantial levels of freezing when tested after a 14-d delay, demonstrating that the latent inhibitory effect of context preexposure was lost across the delay between conditioning and testing (see also Westbrook et al. 2000; Leung et al. 2007; but see De la Casa and Lubow 2002; Wheeler et al. 2004 for the opposite effect). A similar loss of latent inhibition across a delay has been reported in rats made sick by an injection of lithium chloride after ingestion of a preexposed flavor (Aguado et al. 1994).

The present experiments shocked rats in an appetitively conditioned context and tested them in that context 1 d or 14 d later. The aim was to determine whether an appetitively conditioned context, like a preexposed context, transiently impairs the expression of subsequent context-conditioned fear (freezing) responses. In each of four experiments, rats were exposed to two contexts, designated as A and B. They were provided with a palatable

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(38% sugar) breakfast cereal, Froot Loops (FLs), in one of these contexts, e.g., A, but not in the other, B. They were then shocked in A and tested there with FLs either 1 d or 14 d after context–shock pairings. We found that rats shocked in the context where they had previously eaten FLs ate and did not freeze when tested 1 d later but froze and did not eat when tested 14 d later. The subsequent experiments examined some of the factors that regulate this proactive interference effect and its loss with the lapse of time.

Experiment 1

Experiment 1 examined whether a history of eating FLs in a context interferes with subsequent fear conditioning of that context. The design is shown in Figure 1A. All rats were exposed to FLs in one context, A, but not in a second context, B, and were then allocated to four groups. The physical identity of the two contexts was counterbalanced in each group. Two groups were shocked in A (Groups A1 and A14); the other two groups were shocked in B (Groups B1 and B14). FLs were not present when the context was shocked. All groups were tested in Context A with FLs present. Groups A1 and B1 were tested 1 d after the shocked exposure to the context, while Groups A14 and B14 were tested 14 d after this exposure. The number of FLs consumed across training was measured. The behavior of each rat across the shocked exposure and on test was recorded to DVD. A time sampling procedure was used in which each rat was observed every 2 sec and scored by two observers as freezing or not freezing on the shocked exposure and as freezing, eating, or neither freezing nor eating on test. Both observers were unaware of the subject's group allocation and one was unaware of the design of the experiment.

Rats shocked in A should show fear when tested there. If this fear is a consequence of rats having been shocked per se, those shocked in B should also show fear when tested in A; if the fear expected in A is associatively mediated, rats shocked in B should not show fear when tested in A. Moreover, if the history of eating FLs in A interfered with subsequent associative formation, rats shocked in A would eat rather than freeze when tested at either the 1-d or 14-d retention interval. In contrast, if the history of eating in A proactively interfered with retrieval and/or expression of the context–shock association, rats shocked in A would eat and not freeze at the 1-d interval but freeze and not eat at the 14-d retention interval.

Results

The numbers of FLs eaten each day increased across the initial phase of training. On the final day of training (Day 6), the mean (+SEM) number eaten by each group was 12.1 (+1.3) in Group A1, 11.3 (+2.3) in Group B1, 9.4 (+1.3) in Group A14, and 10.8 (+1.5) in Group B14. Context fear conditioning (Day 7) proceeded without incident. In the final minute of this session, the mean (+SEM) level of freezing in each group was: 43.8% (+12.4%) in Group A1, 57.9% (+10.2%) in Group B1, 54.2% (+9.1%) in Group A14, and 54.6% (+7.4%) in Group B14. The groups did not statistically differ in the number of FLs consumed across training or in the levels of freezing on the final minute of the shocked exposure, largest $F < 1$.

Figure 1B shows the mean percentage of time rats in each of the groups spent eating (left) and freezing (right) during the 5 min test session. Among rats tested 1 d after context–shock pairings, the performance of rats that had been shocked in Context A (Group A1) was indistinguishable from that of rats that had been shocked in Context B (Group B1): Neither group showed evidence of fear (they ate and did not freeze); in contrast, among rats tested 14 d after context–shock pairings, those that had been shocked in Context A (Group A14) showed more fear (they spent less time eating and more time freezing) than rats that had been shocked in Context B (Group B14).

These impressions were confirmed statistically. Eating: On average, rats tested 14 d after context–shock pairings spent less time eating than those tested 1 d later, $F_{(1,26)} = 16.18$, $P < 0.05$, partial $\eta^2 = 0.38$, 95% CI = 0.72, 2.22. The overall effect of where rats were shocked, A versus B, was not significant, $F_{(1,26)} = 2.96$, but there was a significant context × time interaction, $F_{(1,26)} = 6.0$, $P < 0.05$, partial $\eta^2 = 0.19$, 95% CI = 0.14, 1.65. On the test conducted 1 d after the shocked exposure to the context, there was no statistically significant difference between Groups A1 and B1, $F < 1$, but on the test conducted 14 d after the shocked exposure, Group A14 spent significantly less time eating than rats in Group B14, $F_{(1,26)} = 9.32$, $P < 0.05$, $d = 2.04$, 95% CI = 0.50, 2.55. Freezing: On average, rats tested 14 d after the shocked exposure to the context froze more than those tested 1 d later, $F_{(1,26)} = 6.19$, $P < 0.05$, partial $\eta^2 = 0.19$, 95% CI = 0.16, 1.66; and, on average, rats that had been shocked in Context A froze more than those that had been shocked in Context B, $F_{(1,26)} = 7.57$, $P < 0.05$, partial $\eta^2 = 0.23$, 95% CI = 0.25, 1.76. There was no statistically significant interaction between these factors, $F_{(1,26)} = 2.57$. Post
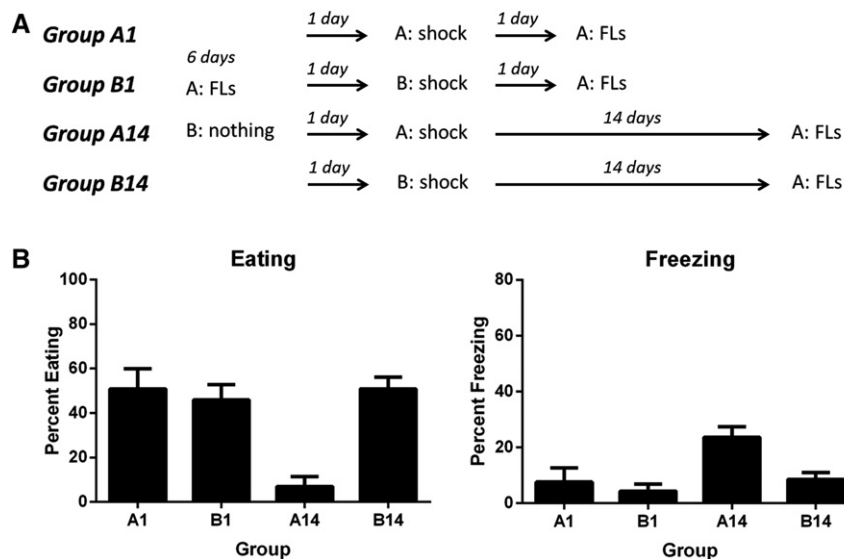

Figure 1. (A) Schematic showing the design of Experiment 1. All rats were trained with Fruit Loops in Context A but not B. They differed with respect to where they were shocked (Groups A1 and A14 were shocked in A; Groups B1 and B14 were shocked in B) and when they were tested in relation to context fear conditioning (Groups A1 and B1 were tested 24 h later; Groups A14 and B14 were tested 14 d later). Contexts A and B were chambers that differed in size, shape, scent, and location within the laboratory. FLs denote the presence of Kellogg's Fruit Loops. (B) Mean (+SEM) time spent eating (left) and freezing (right) by each of the groups during the 5-min test session in Experiment 1. Two rats did not eat FLs across the 6 d of training in phase 1 and were thus excluded from the remainder of the experiment. The final group sizes were: $n = 8$ for Group A1, $n = 7$ for Group B1, $n = 8$ for Group A14, and $n = 7$ for Group B14.

hoc tests confirmed that there was no statistically significant difference between Groups A1 and B1, $F < 1$, on the 1-d test, but revealed that Group A14 froze significantly more than rats in Group B14, $F_{(1,26)} = 8.97$, $P < 0.05$, $d = 1.68$, 95% CI = 0.47, 2.52, on the 14-d test.

Summary

Rats shocked in the food-associated context, A, ate and did not freeze when tested there 1 d later but froze and did not eat when tested there 14 d later. The performance observed at the 14-d retention interval was due to the association between A and shock, rather than shock per se, as rats shocked in B ate and did not freeze when tested in A at either the 1-d or 14-d retention intervals. The contrasting performances between the 1-d and 14-d tests suggests that rats in Groups A1 and A14 had formed the context–shock association but did not express this association in their behavior at the 1-d retention interval while doing so at the 14-d interval.

Experiment 2

In the previous experiment, rats had continuous access to chow in their home cages across training and testing with the FLs. It is possible, therefore, that rats trained and tested while hungry would fail to show evidence for context-conditioned fear at the long retention interval; that is, hungry rats trained with FLs in A and tested there 14 d later might eat rather freeze. Accordingly, Experiment 2 examined whether a motivational state of hunger influenced the retardation and recovery of context-conditioned fear with the lapse of time. The design was the same as that used in Experiment 1 and is shown in Figure 2A. The procedure differed from the previous experiment in two respects. First, rats were provided with 2-h access to chow each day (shortly after training) rather than with continuous access across the course of the experiment. Second, the number of FLs provided in each session of initial context–FL training was matched to the number eaten in Experiment 1 when the rats had unrestricted access to chow.
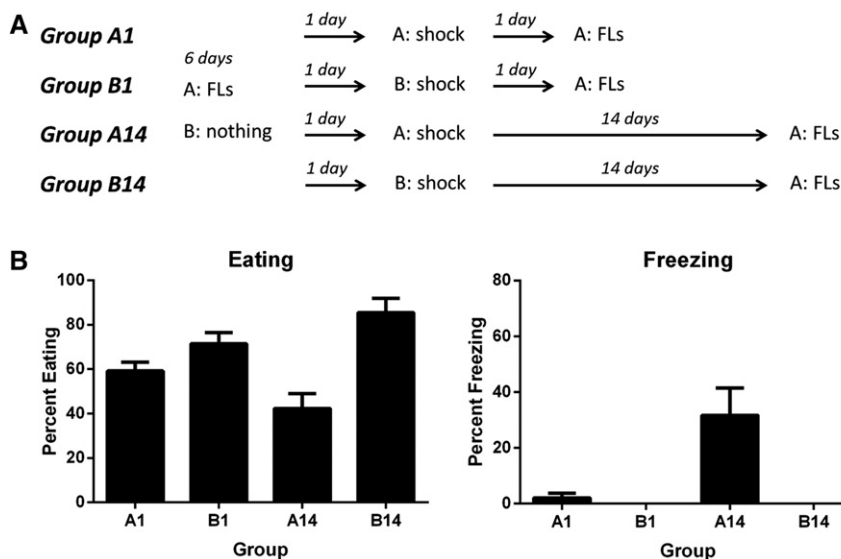
Results

All rats ate all the FLs provided on each of the 6 d of context–FL training. Context fear conditioning (Day 7) proceeded without incident. In the final minute of this session, the mean (+SEM) level of freezing in each group was 32.1% (+8.9%) in Group A1, 29.2% (+6.0%) in Group B1, 30.8% (+8.0%) in Group A14, and 28.8% (+9.1%) in Group B14. The differences between the groups in freezing were not statistically significant, largest $F < 1$.

Figure 2B shows the mean percentage of time rats in each of the groups spent eating (left) and freezing (right) during the 5 min test session. Relative to the previous experiment where rats were trained and tested sated, rats trained and tested hungry in Experiment 2 tended to spend more time eating and less time freezing. However, across the groups, the pattern of results was identical to that observed in Experiment 1. Among rats tested 1 d after context–shock pairings (Groups A1 and B1), neither group showed evidence of fear (they ate and did not freeze); in contrast, among rats tested 14 d after context–shock pairings (Groups A14 and B14), those that had been shocked in Context A (Group A14) showed more fear (they spent less time eating and more time freezing) than rats that had been shocked in Context B (Group B14). Statistical analysis confirmed this description of the results. Eating: On average, rats shocked in Context A spent less time eating than rats shocked in Context B, $F_{(1,27)} = 22.90$, $P < 0.05$, partial $\eta^2 = 0.46$, 95% CI = 0.98, 2.46. The main effect of context was moderated by a context × time interaction, $F_{(1,27)} = 7.11$, $P < 0.05$, partial $\eta^2 = 0.21$, 95% CI = 0.22, 1.70. Specifically, relative to rats shocked in B, rats shocked in A spent less time eating when tested 14 d, $F_{(1,13)} = 22.57$, $P < 0.05$, $d = 2.32$, but not 1 d, $F < 1$, later. The main effect of time was not significant, $F < 1$. Freezing: On average, rats shocked in Context A froze more than rats shocked in Context B, $F_{(1,27)} = 10.82$, $P < 0.05$, partial $\eta^2 = 0.29$, 95% CI = 0.45, 1.92; and rats tested at the 14-d retention interval froze more than rats tested at the 1-d retention interval, $F_{(1,27)} = 8.32$, $P < 0.05$, partial $\eta^2 = 0.24$, 95% CI = 0.30, 1.78. The context × time interaction was also significant, $F_{(1,27)} = 8.32$, $P < 0.05$, partial $\eta^2 = 0.24$, 95% CI = 0.30, 1.78, showing that rats in Group A14 froze more than rats in Group B14, $F_{(1,14)} = 10.53$, $P < 0.05$, $d = 1.56$, although Groups A1 and B1 did not differ, $F < 1$.

Summary

This experiment has shown that rats maintained on restricted access to food, like rats maintained on unrestricted access to food, ate the FLs and did not freeze at the 1-d retention interval but froze and did not eat at the 14-d interval. The increase in fear expression with the lapse of time between context–shock pairings and test is thus independent of whether or not rats were hungry across training and test.

Experiment 3

The results of Experiments 1 and 2 could be due to the latent inhibitory effect of context preexposure rather than to the association between the context and food. As noted previously, rats shocked in an extensively preexposed context, like rats in the previous experiments,

Figure 2. (*A*) Schematic showing the design of Experiment 2. This experiment differed from the previous one only in that rats had restricted access to food across the course of training and testing, and thus, were maintained hungry. Contexts A and B were chambers that differed in size, shape, scent, and location within the laboratory. FLs denote the presence of Kellogg's Froot Loops. (*B*) Mean (+SEM) time spent eating (*left*) and freezing (*right*) by each of the groups during the 5 min test session in Experiment 2. The final group sizes: $n = 8$ for Group A1, $n = 8$ for Group B1, $n = 8$ for Group A14, and $n = 7$ for Group B14.

exhibited little freezing when tested 1 d after the shocked exposure but substantial levels of freezing when tested 14 d after that exposure (Killcross et al. 1998a,b). The present experiment examined the role of context preexposure in regulating the test performances observed in the previous experiments. The design is shown in Figure 3A. Four groups of rats were exposed to FLs in B but not in A. Two groups were then shocked in A (Groups A1 and A14) and the other two groups were shocked in B (Groups B1 and B14). FLs were absent on the shocked exposure. All groups were tested in A, either 1 d (Groups A1 and B1) or 14 d (Groups A14 and B14) later. If preexposure to A per se mediated the results observed in the previous experiments, rats preexposed, shocked and tested in A will eat and not freeze when tested at the 1-d interval but freeze and not eat at the 14-d retention interval. In contrast, if the history of eating food in A was critical for the results of the previous experiments, rats preexposed, shocked, and tested in A will freeze and not eat at both retention intervals.

Results

The number of FLs eaten on each session increased across training. In the final training session (Day 6), the mean (+SEM) number eaten by each group was: 15.3 (+1.1) in Group A1, 16.5 (+0.8) in Group B1, 15.0 (+2.0) in Group A14, and 14.3 (+1.9) in Group B14. These differences were not statistically significant, $F < 1$. Context fear conditioning (on Day 7) proceeded without incident. In the final minute of this session, the mean (+SEM) level of freezing in each group was 57.1% (+8.7%) in Group A1, 45.7% (+11.0%) in Group B1, 59.0% (+9.8%) in Group A14, and 48.1% (+7.4%) in Group B14. The differences between the groups in freezing were not statistically significant, largest $F < 1$.

Figure 3B shows the mean percentage of time rats in each of the groups spent eating (left) and freezing (right) during the 5-min test. It is clear that rats that had been shocked in Context A (Groups A1 and A14) showed more fear (they spent less time eating and more time freezing) than those that had been shocked in Context B (Groups B1 and B14). This was equally true among rats tested either 1 d or 14 d after context–shock pairings. Eating: On average, rats shocked in Context A spent less time eating than rats shocked in Context B, $F_{(1,24)} = 4.72$, $P < 0.05$, partial $\eta^2 = 0.16$, 95% CI = 0.04, 1.60. The main effect of time, $F_{(1,24)} = 1.40$, and the time × context interaction, $F < 1$, were not significant. Freezing: On average, rats shocked in Context A froze more than rats shocked in Context B, $F_{(1,24)} = 12.39$, $P < 0.05$, partial $\eta^2 = 0.34$, 95% CI = 0.55, 2.11. The main effect of time, $F_{(1,24)} = 4.10$, and the time × context interaction, $F < 1$, were not significant.

Summary

Rats shocked in A froze more and ate less than rats shocked in B when both were tested in A at 1-d or 14-d retention intervals. In contrast to the results obtained in the previous experiments, the test performances of rats shocked in A were not affected by the retention interval: Those tested 1 d after the shocked exposure to A spent as much time freezing and as little time eating as rats tested 14 d after this exposure. These results among rats preexposed, shocked and tested in A show that, with the parameters used, preexposure per se is not sufficient to regulate when the context–shock was expressed. They imply that the regulation of this association observed in the previous experiments was due to a history of eating in the shocked context.

Experiment 4

The design used in Experiments 1 and 2 involved training rats with FLs in Context A, shocking them in that context 1 d later, and testing the rats either 1 d or 14 d after the shocked exposure. In these experiments, therefore, the focus of interest was the effect of the retention interval between context conditioning and testing. The present experiment examined the effect of the retention interval between training with FLs and context conditioning. There were two aims. The first was to examine whether a 14-d retention interval between training with FLs and the shocked exposure resulted in freezing rather than eating when rats were tested 1 d after the shocked exposure. The second aim was to examine whether these effects of a long retention interval between the context–food training and the shocked exposure to the context were reversed by a context–food reminder session before the shocked exposure.

The design is shown in Figure 4A. There were four groups of rats. Two groups of rats (A1 and A14) were exposed to FLs in A but not in B across six consecutive days. Both groups were shocked in A and tested there 1 d later. The groups differed in the interval between the final session of context–food training and context fear conditioning. This interval was 1 d for Group A1 and 13 d for Group A14. Based on the results of the previous experiments, rats that received a 1-d retention interval between the final
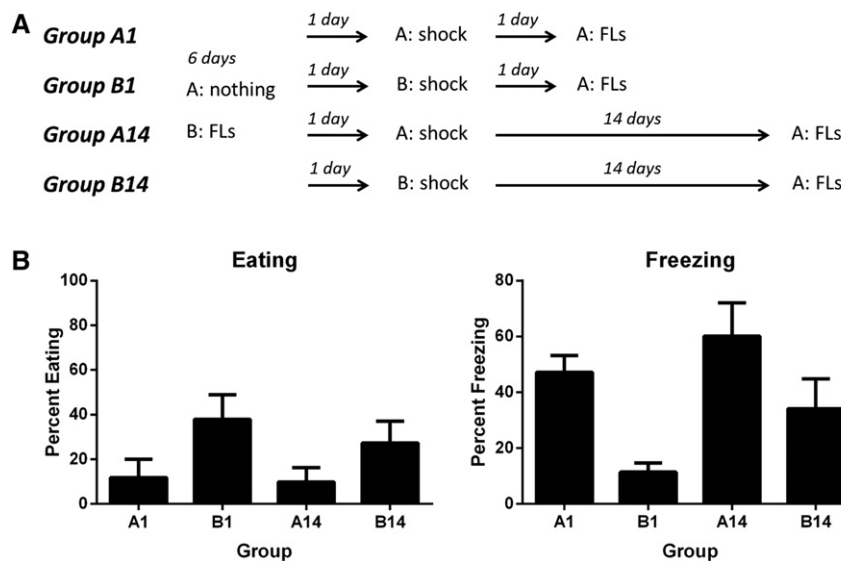

Figure 3. (A) Schematic showing the design of Experiment 3. Rats had unrestricted access to food across the course of training and testing. All rats were trained with Fruit Loops in Context B but not A. They differed with respect to where they were shocked (Groups A1 and A14 were shocked in A; Groups B1 and B14 were shocked in B) and when they were tested in relation to context fear conditioning (Groups A1 and B1 were tested 24 h later; Groups A14 and B14 were tested 14 d later). Contexts A and B were chambers that differed in size, shape, scent, and location within the laboratory. FLs denote the presence of Kellogg's Froot Loops. (B) Mean (+SEM) time spent eating (left) and freezing (right) by each of the groups during the 5-min test session in Experiment 3. A squad of four rats had to be excluded from the experiment as they were incorrectly shocked in Context B during the initial phase of training, and thus ceased eating FLs: The final group sizes were $n = 7$ in Group A1, $n = 7$ in Group B1, $n = 7$ in Group A14, and $n = 7$ in Group B14.
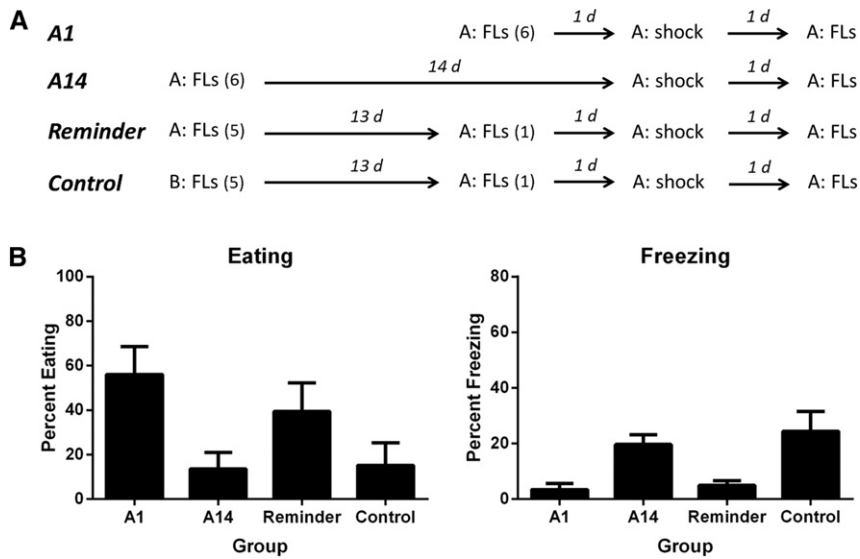
Figure 4. (*A*) Schematic showing the design of Experiment 4. Groups A1 and A14 were trained and tested as described in Experiment 1. Group Reminder was exposed to five sessions of Fruit Loops in A but not B, and after a 13-d retention interval, to an additional day of this training. Group Control was exposed to five sessions of FLs in B but not A, and after a 13-d retention interval, to one session of FLs in A but not B. All groups were shocked in A 24 h after the final training session, and after a further 24 h, were tested for expression of context-conditioned fear in A. Contexts A and B were chambers that differed in size, shape, scent, and location within the laboratory. FLs denote the presence of Kellogg's Froot Loops. (*B*) Mean (+SEM) time spent eating (*left*) and freezing (*right*) by each of the groups during the 5-min test session in Experiment 4. One rat was excluded from the experiment as it did not eat the FLs across its initial training. The final group sizes were: $n = 7$ in Group A1, $n = 7$ in Group A14, $n = 8$ in Group Reminder, and $n = 8$ in Group Control.

context–FL exposure and the shocked exposure will eat and not freeze when tested 1 d after the shocked exposure. The first question of interest was whether rats that received the 13-d retention interval between the final context–FL exposure and the shocked exposure will freeze and not eat when tested 1 d after the shocked exposure.

The second question of interest was whether any such effect (freezing rather than eating) was reversed by an additional exposure to FLs after the long retention interval but before the shocked exposure. The third group (Reminder) examined this question. Rats in this group were trained with FLs in A but not in B across five, rather than the standard six consecutive days. Thirteen days after the final context–FL exposure, Group Reminder received their sixth exposure to FLs in A and no food in B. One day later, this group was shocked in A and tested there 1 d after the shocked exposure. A fourth group (Control) received exposures to FLs in Context B but not in Context A across five consecutive days. Following a 13-d interval, Group Control received an additional exposure to both contexts but FLs were now present in A but not in B. One day later, this group was shocked in A and tested there 1 d after the shocked exposure. Groups Reminder and control are identical except that the former received the initial 5 d of training with FLs in A but not in B whereas Group Control received the initial 5 d of training with FLs in B but not in A. Group Control was included to assess whether the single session of FLs in A before the shocked exposure was sufficient to result in eating rather than freezing in Group Reminder or whether the initial five sessions of eating in A were necessary for the additional session in A to produce such results in Group Reminder.

Results

The numbers of FLs eaten per session increased across the initial phase of training. The mean (+SEM) number eaten by Groups

A1 and A14 on the sixth and final training session in Context A was 18.5 (+1.0), and 16.0 (+2.0), respectively. The mean number eaten by Groups Reminder on their reminder exposure to FLs in Context A was 16.1 (+1.9) and was 16.5 (+1.3) by Group Control, indicating that the presentation of FLs in Context A after the history of FLs presentation in Context B did not elicit any neophobia in Group Control. The statistical analysis confirmed that there were no significant differences among the groups in the number of FLs eaten on the final training exposure, $F < 1$. Context-fear conditioning proceeded without incident. In the final minute of this session, the mean (+SEM) level of freezing in each group was: 24.2% (+10.6%) in Group A1, 40.0% (+6.2%) in Group A14, 37.1% (+3.9%) in Group Reminder, and 35.0% (+7.8%) in Group Control. None of the differences between the groups were statistically significant, largest $F_{(1,26)} < 2.4$.

Figure 4B shows the mean percentage of time rats in each of the groups spent eating (left) and freezing (right) during the 5-min test session. As in previous experiments, rats exposed to six consecutive days of food in A and shocked the next day (Group A1) ate and did not freeze when tested 1 d later. Interpolation of a 13-d interval between eating in A and the shocked exposure resulted in freezing rather than eating when tested 1 d later (Group A14). However, when the long retention interval ended in an additional eating session in A, rats shocked in A ate and did not freeze when tested 1 d later (Group Reminder). Moreover, this additional eating session acted as a reminder rather than itself being sufficient to produce the effect on test as rats who had eaten food in B and, 13 d later exposed to FLs in A, froze and did not eat when tested 1 d after the shocked exposure (Group Control).

The statistical analysis confirmed these impressions. Eating: Rats that received five exposures to FLs in B but not in A and then a single exposure to FLs in A but not in B (Group Control) spent less time eating than groups that had been exposed to six exposures to FLs in Context A, $F_{(1,26)} = 5.58$, $P < 0.05$, $d = 0.96$, 95% CI = 0.13, 1.91. Rats remotely exposed to 6 d of A–FL training, Group A14, spent less time eating than those that had been recently exposed to at least one session of A–FL training (Groups A1 and Reminder), $F_{(1,26)} = 5.0$, $P < 0.05$, $d = 1.04$, 95% CI = 0.09, 2.06. There was no statistically significant difference in eating between the latter groups, $F_{(1,26)} = 1.1$. Freezing: Rats exposed to a single day of A–FL training (Group Control) spent more time freezing than groups that had been exposed to 6 d of A–FL training, $F_{(1,26)} = 15.46$, $P < 0.05$, $d = 1.66$, 95% CI = 0.81, 2.59. Rats remotely exposed to 6 d of A–FL training, Group A14, spent more time freezing than those that had been recently exposed to at least one session of A–FL training (Groups A1 and Reminder), $F_{(1,26)} = 7.31$, $P < 0.05$, $d = 2.34$, 95% CI = 0.31, 2.28. There was no statistically significant difference in freezing between the latter groups, $F < 1$.

Summary

This experiment again demonstrated that prior context–FL training proactively interferes with expression of context conditioned

fear. It additionally demonstrated three novel findings. The first is that prior context–FL training is necessary but not sufficient to generate the interference effect: Proactive interference was significantly reduced when context–FL training terminated 13 d as opposed to 1 d before context–shock pairings. The second novel finding is that the capacity of remote context–FL training to proactively interfere with expression of context-conditioned fear was restored when a single reminder session was conducted 1 d before context–shock pairings. Finally, a single session of context–FL training was not sufficient to produce proactive interference.

Discussion

The present experiments have shown that pairing an appetitively conditioned context with an aversive shock US impairs the ability of that context to elicit fear responses when rats are tested 1 d but not 14 d after that shocked exposure. The fear (freezing and reduced eating) observed among the rats shocked in A when tested at the longer retention interval was due to the association between A and shock rather than shock per se (Experiment 1). Moreover, these contrasting effects of the retention interval on eating and freezing were obtained when the rats had unrestricted or restricted access to chow in their home cages, showing that the effects were independent of the levels of food deprivation (Experiment 2).

The absence of fear at the 1-d retention interval among rats shocked in A was contingent on multiple exposures to food in A. Rats given a single session of food in A before the shocked exposure to A froze and did not eat when tested in A at both the 1-d and 14-d retention intervals. However, multiple sessions are not sufficient; the additional requirement is that these sessions are located relatively close to the context–shock exposure. Rats trained to eat in A and subjected to the shocked exposure 13 d later froze and did not eat when tested there 1 d after that shocked exposure. Finally, this effect of the long interval between the initial training with food in A and the shocked exposure was reversed when the rats received an additional exposure to food in A shortly before the shocked exposure. These rats ate, rather than froze, when tested in A 1 d after the shocked exposure (Experiment 4). The single eating session reinstated the effects of the remote eating sessions when the rats were tested 1 d after the shocked exposure.

The contrasting effects of the short and longer retention intervals on the expression of context-conditioned fear are similar to those obtained when a preexposed context is paired with a foot shock US or a preexposed flavor is paired with the malaise induced by lithium chloride (LiCl). Preexposed subjects show low levels of conditioned responding when tested shortly after the shocked exposure (e.g., Killcross et al. 1998a,b) or the flavor-LiCL pairing (e.g., Kraemer and Roberts 1984; Kraemer et al. 1988; Bakner et al. 1991; Aguado et al. 1994), but substantial levels when tested sometime after that conditioning. Experiment 3 examined whether the effects of context–food training on the expression of the subsequent context–shock association were due to context preexposure rather than a history of eating in that context. However, that experiment failed to detect any evidence for a latent inhibitory effect of context alone exposure; rats trained to eat FLs in B but not A, shocked in A and tested in A froze and did not eat at either the 1-d or the 14-d retention intervals. Thus, a history of context preexposure was not sufficient to generate the proactive interference effect: It was only observed among rats that had been trained with FLs in A before A–shock pairings (Experiments 1, 2, and 4). It is worth noting that the present procedure differed in several ways from those used to show that preexposure transiently interferes with the expression of conditioned fear or conditioned aversions. One of these ways was that the pres-

ent procedure effectively trained a discrimination in which food was present in B but not in A across the initial training, whereas previous procedures just preexposed to a context or a flavor. The discrimination training used here may have resulted in A becoming a signal for the absence of food which could have maintained attention to A or imbued A with aversive properties that interacted with those conditioned by the shock to promote fear and suppression of food at both the 1- and 14-d retention intervals.

One explanation for the increase in expression of context-conditioned fear across the retention interval among rats shocked in the appetitively conditioned context is so-called fear incubation (e.g., McMichael 1966; Zammit-Montebello et al. 1969; Pinel and Mucha 1973; Houston et al. 1999; Balogh et al. 2002; Pickens et al. 2009a,b, 2010, 2013). This incubation could be due to better consolidation, and hence, better retrieval of the CS-shock association with time, or to recovery from a hypothesized inhibitory effect of recent conditioning (Pickens et al. 2009a,b, 2010, 2013; see also Bouton et al. 2008). Regardless of the underlying mechanism, the hypothesis is that conditioned fear grows across time. For example, Lubow and colleagues (De la Casa and Lubow 2005; De la Casa et al. 2005; Lubow and De la Casa 2005a,b) have argued that a preexposed context is impaired in its ability to associate with shock as is a preexposed flavor impaired in its ability to associate with malaise. However, the weak aversive association in each of these cases grows with the lapse of time, resulting in substantial levels of freezing or flavor avoidance at the longer retention interval. According to this hypothesis, the appetitively conditioned context was likewise impaired in its ability to associate with shock, resulting in little or no fear, at the 1-d retention interval, but substantial levels of fear at the 14-d interval due to the growth in the strength of the context–shock association. It should be noted that tests of the incubation hypothesis which have controlled for the influence of variables such as the recency of shock exposure failed to find any evidence that conditioned fear grows across time (Leung and Westbrook 2008).

Rather than a weak context–shock association becoming stronger with the lapse of time, the present results could also be explained by a weakening of the context–food association across time. According to this explanation, the context–food association was strong and interfered with retrieval and/or expression of the context–shock association at the 1-d interval but was weaker and failed to interfere with the context–shock association at the longer interval. A weakening of the context–food association over time explains why interpolation of a long retention interval between eating in the target context and shocked exposure to that context resulted in freezing and not eating when rats were tested 1 d after the shocked exposure to the context. Moreover, the fact that rats exposed to a reminder session before the context–shock pairing ate and did not freeze when tested 1 d after the shocked exposure implies that any such waning in the strength of the context–food association with time was restored by the single context–food exposure.

The absence of fear among rats shocked in the appetitively conditioned context and tested 1 d later are consistent with the retardation in the development of fear responses across pairings of an appetitive CS with an aversive US reported in previous experiments (e.g., Nasser and McNally 2012). They differ from previous experiments in showing that the fear is present among rats shocked in that context and tested 14 d later. It remains to be determined whether the absence of fear across the initial pairings of the appetitive CS and the aversive US reported previously would be replaced by fear if a long retention interval was interpolated between those initial pairings and the test of the CS. The role played by the number of occasions on which the appetitive CS is paired with the aversive US may also bear upon the contrast between the present results and those reported in a previous examination

of appetitive–aversive transfer. Specifically, Bouton and Peck (1992) exposed rats to pairings of a CS and an appetitive US and then paired the CS with an aversive US (or vice versa). Finally, they tested rats either shortly (1 d) or sometime (28 d) after the counterconditioning of the appetitive CS with the aversive US (or the aversive US with the appetitive US). They confirmed that rats were impaired in developing fear responses (freezing) across pairings of the appetitive CS and the aversive US (as were rats impaired in developing appetitive responses [head jerking] across pairings of the aversive CS and the appetitive US). However, the tests conducted after counterconditioned responses were at asymptote revealed that in each protocol, the CS elicited contrasting responses depending on the interval of time between counterconditioning and test: The appetitive CS paired with shock elicited fear responses at the short retention interval but appetitive responses at the longer retention interval; the aversive CS paired with the appetitive US elicited appetitive responses at the short interval but fear responses at the longer interval.

In these experiments, the influence of counterconditioning on performance waned with the lapse of time, being replaced with control over performance by the original conditioning. In contrast, the present experiments have shown that the influence of the original conditioning (the context–food association) wanes with the lapse of time, being replaced by the control over performance by the counterconditioning (the context–shock association). There are several differences between the protocols used in these experiments (e.g., discrete CS versus context; test of CS alone versus eating or freezing in the context) that may explain the contrasting results. However, perhaps the most interesting difference is in the amount of counterconditioning. In the experiments reported by Bouton and Peck (1992), as noted, subjects were counterconditioned until the impairments produced by the original conditioning had been removed. In the present experiments, subjects received a single context-fear conditioning episode, to allow the detection of the proactive interference effect. Therefore, the amount of counterconditioning may determine its interactions with the original conditioning. With a small amount of counterconditioning, there is a transient proactive interference effect; the original conditioning controls performance at a short retention interval whereas counterconditioning controls performance at a longer retention interval. With extensive counterconditioning, there is a transient retroactive interference effect: The counterconditioning controls performance at a short retention interval, whereas the original conditioning controls performance at a longer retention interval.

Materials and Methods

Subjects

Subjects were 126 experimentally naïve adult male Wistar rats (*Rattus norvegicus*) weighing between 350 and 400 g at the beginning of the experiment. They were obtained from a commercial supplier (Animal Research Centre, Perth, Australia) and housed in groups of eight in opaque plastic boxes (22-cm height × 67-cm length × 40-cm width). The boxes were kept in an air-conditioned colony room maintained on a 12:12 light–dark cycle (lights on at 7:00 a.m.). In Experiments 1, 3, and 4, food and water were continuously available in the home cage during all phases of the experiment. In Experiment 2, access to food was progressively reduced from 4 h to 2 h per day prior to the start of the experiment. During the experiment, rats continued to receive 2 h access to food per day at the termination of the final experimental session. This was in addition to any FLs consumed during the experimental sessions and was sufficient to maintain rats at 90% of their free-feeding weights. Each rat was handled for 2–3 min each day for 4 d prior to the start of the experiment. Two days prior to the start of each experiment, rats were familiarized with Kellogg's

Fruit Loops in their home cage. All experimental procedures occurred between 9 a.m. and 6 p.m. The procedures were consistent with the ethical guidelines established by the American Psychological Association and were approved by the Animal Care and Ethics Committee of the University of New South Wales.

Apparatus

Each experiment was conducted in two sets of four chambers. Each chamber of one set measured 33 cm (height) × 31 cm (length) × 26 cm (width). The chambers were located in separate compartments of a wooden cabinet, the floor, walls, and ceiling of which were painted black. The sidewalls and ceiling of the chambers themselves were made of aluminum and the back and front walls were made of clear plastic. The floor consisted of stainless steel rods, 5 mm in diameter, spaced 10 mm apart, (center to center). A flat steel tray was placed ∼5 mm below the stainless steel rods and was used to ensure that, when present, the Fruit Loops were easily accessible to the rat in the chamber.

The second set of chambers was located in a separate room. Each chamber of the second set measured 19.5 cm (height) × 23.5 cm (length) × 20.5 cm (width), and each was placed in separate compartments of a wooden cabinet which was identical to that described above. The front and rear walls of these chambers, as well as the hinged lid, were made of clear plastic, and the side walls were made of aluminum. The floor of each chamber consisted of stainless steel rods, 2 mm in diameter, spaced 10 mm apart, (center to center). A flat steel tray was placed ∼5 mm below the stainless steel rods and was used to ensure that, when present, the Fruit Loops were easily accessible to the rat in the chamber.

A constant-current shock generator, capable of delivering unscrambled AC 50 Hz to the floor of each chamber, was used for the presentation of a 0.8 mA, 0.5-sec footshock US. Illumination of each chamber was provided by an infrared light source (940 ± 25 nm). A camera mounted on the back wall of each shell was used to record the behavior of each rat. Each camera was connected to a monitor and a DVD recorder located in another room of the laboratory. This room contained the computer that controlled stimulus presentations via appropriate software (LabView, National Instruments).

Experiment 1

Procedure

Phase 1—training

On Days 1–6, rats received two 20-min training sessions per day, one in Context A and the other in Context B. The physical identity of the contexts designated A and B were fully counterbalanced in this and subsequent experiments. Half the rats were exposed to Context A in the morning and Context B in the afternoon, while remaining rats were exposed to these contexts in the reverse order. The interval between context exposures on the same day was a minimum of 2 h. In Context A, all rats were provided with 20 Froot Loops (FLs) in the center of the chamber, and the number of FLs eaten in the 20-min session was recorded. Fruit Loops were chosen because they are highly palatable, standardized in their size, and easily retrieved between the grids on the floor. In Context B, the animals were exposed to the context alone. At the end of training on Day 6, rats were assigned to one of 4 groups. The group designators A1, A14, B1, and B14 were used to denote the context where shock occurred (A or B) and time of test (1 or 14 d after shock). These groups were matched for their consumption of FLs across the initial 6 d of training.

Phase 2—fear conditioning

On Day 7, rats received a single session of context fear conditioning. Rats were placed in either Context A (Groups A1 and A14) or B (Groups B1 and B14) and shocked twice: The first shock occurred

after 3 min of context exposure and the second occurred 1 min later, yielding a session of length 5 min. No FLs were present in the chambers during this session.

Phase 3—test

On Day 8, two groups of rats—Groups A1 and B1—were returned to Context A for a 5-min test session. During this test, rats were presented with 20 Fruit Loops and the number of Fruit Loops eaten, the latency to begin eating, as well as the time rats spent eating and freezing were recorded. On Day 22, the remaining two groups—Group A14 and B14—were tested in the same way.

Scoring and statistics

The last day of training (Day 6), the single session of fear conditioning (Day 7), and the subsequent test sessions (Days 8 and 22) were recorded to DVD. The behavior of each rat was then scored by two naïve observers. Specifically, each rat was observed every 2 sec and scored for the presence of either freezing (defined as the absence of all movement except those required for respiration; Fanselow 1980) or eating. In general, rats tended to pick up a single FL and eat it for 20–30 sec until the entire FL had been consumed. Thus, at any given moment, a rat may have been freezing, eating, or doing neither. A rat could not, by definition, have been eating and freezing during the same observation. Eating and freezing results were analyzed using separate analyses of variance (ANOVAs) with the criterion for rejection of the null hypothesis set at $\alpha = 0.05$. In general, these were modest negative correlations between these two measures, which served as complementary indexes of the same learning, i.e., context-conditioned fear. Confidence intervals (95% standardized) are reported for each significant comparison, partial eta squared (η^2) as a measure of effect size for significant results in ANOVA, and Cohen's d as a measure of effect size for significant contrasts.

Experiment 2

Procedure

Four groups of rats were trained and tested identically to their counterparts in Experiment 1 in all but three respects. First, rats were maintained hungry across the course of the experiment. Second, the number of Fruit Loops to which rats were exposed across each session of Phase 1 training was equal to the average number of FLs consumed in the same session by rats in Experiment 1. This restriction on FLs access was imposed to equate FLs consumption between rats in Experiments 1 and 2, thereby facilitating their comparison. Third, all rats were tested for expression of context-conditioned fear at the same time but differed with respect to the recency of context–shock pairings in Phase 2; that is, rats in Groups A1 and B1 were subjected to consecutive days of appetitive and then aversive context conditioning which terminated 24 h prior to the test session; in contrast, rats in Groups A14 and B14 were subjected to consecutive days of appetitive and then aversive context conditioning which terminated 14 d prior to the test session.

Experiment 3

Procedure

Four groups of rats were trained and tested identically to their counterparts in Experiment 1 in all but one respect: During the initial phase of training, rats were trained to eat FLs in Context B, not in A, and exposed to A, not B, alone. Thereafter, Groups A1 and A14 were shocked in Context A, while Groups B1 and

B14 were shocked in Context B. Finally, Groups A1 and B1 were tested in A 1 d after context–shock pairings, while Groups A14 and B14 were tested 14 d after context–shock pairings.

Experiment 4

Procedure

There were four groups of rats. Group A1 and Group A14 were exposed to FLs in Context A but not B on each of six consecutive training days. These groups differed with respect to the interval between the final session of this training and subsequent A–shock pairings: For Group A1, this interval was 24 h; for Group A14, it was 14 d. Both groups were finally tested in Context A 24 h after A–shock pairings. The start of training was delayed for rats in Group A1 such that the two groups were tested at the same time. For the other two groups in this experiment, Group Reminder and Group Control, the initial phase of training in these groups was not conducted across consecutive days. Group Reminder was exposed to Fruit Loops in Context A but not B over five consecutive days; then after a 13-d retention interval, they received an additional day of the same training: exposure to Fruit Loops in A and B alone. In contrast, Group Control was exposed to FLs in B but not A over five consecutive days; then after the same 13-d retention interval, they received an additional day of the opposite training: exposure to FLs in A and B alone. Both groups were exposed to A–shock pairings 24 h later; and tested in A after a further 24 h. Training of these groups commenced at the same time as training of Group A14, such that all groups in this experiment were tested at the same time. In all other respects, the details for training, counterconditioning, and test were identical to those previously described for Experiment 1.

Data analysis

The data for percentage time eating and percentage time freezing were analyzed using planned orthogonal contrasts in ANOVA (Hays 1963). The Type I error rate was controlled at $\alpha = 0.05$ using the Bonferroni correction for multiple comparisons. We anticipated that a proactive interference effect would again be evident in Group A1, such that these rats would not show fear when finally tested in A. The question of interest was whether interpolating a retention interval between phase one training and A–shock pairings would attenuate this interference effect. We anticipated that just a single day of FLs in A would not be sufficient to generate the proactive interference effect, and thus, that Group Control, would show more fear at test relative to Group A1 for which A–FL exposures occurred immediately before A–shock pairings. The question of interest was whether the effectiveness of a single A–FL training session in generating proactive interference can be improved among animals with a prior history of A–FLs training. If this is the case, Group Reminder should show less fear at test than Group Control; indeed, if the restoration of proactive interference is complete, Group Reminder should show as little fear at test as Group A1.

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