Retention of conditioned autonomic and behavioral responses in preweanling rats: Forgetting and reinstatement

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Prewanling rats, 16 days of age, responded to an olfactory conditioned stimulus (CS) paired with a shock unconditioned stimulus (US) with increases in heart rate and behavioral activation. In two experiments this finding was replicated and, in addition, it was found that the form of these conditioned responses (CRs) changed after a retention interval. When tested 24 h after CS-US pairings, the subjects displayed a decrease in heart rate accompanied by CS-elicited freezing. Giving two unsignaled shocks prior to the delayed test effectively reinstated the tachycardia and behavioral arousal CRs. The results are discussed in terms of contextual influences on the form of the CR and how changes in the magnitude of context fear may alter responding to an olfactory CS.

Recently, Hunt, Hess, and Campbell (1997) reported an ontogenetic change in the form of conditioned responding to an olfactory conditioned stimulus (CS) paired with a footshock unconditioned stimulus (US). Both heart rate (HR) and somatomotor responses to this CS differed as a function of the age of training. During CS-US pairings, preweanling rats, 16 days of age, responded to the CS with cardioacceleration accompanied by a slight behavioral arousal. In contrast, adults given identical training responded to the CS with cardiodeceleration and freezing. It was hypothesized that the conditioning of a tachycardic defensive response in the preweanling subjects, as opposed to the bradycardic, perhaps attentional, response in the adults might be related to greater salience of the olfactory stimulus for the preweanling subjects. Given preweanlings' relative inexperience with stimuli from other modalities, such as audition and vision, olfactory stimuli may be of special importance to the young animal, which would make olfactory cues particularly salient events for learning (see Wilson & Sullivan, 1994).

An alternative explanation for the divergent conditioned responses (CRs) observed by Hunt et al. (1997) focuses on age differences in the contribution of context conditioning. The preweanlings in that study exhibited more freezing in the context in which CS-US pairings occurred than did the adults. The CR pattern elicited by the olfactory CS in the preweanlings could have resulted from a summation of fear conditioned to the CS with fear conditioned to the context (Bouton & Bolles, 1985; Miller & Schachtman, 1985; see also Rescorla, Durlach,

& Grau, 1985). In adult rats the context and the CS are assumed to compete for association with the US (see, e.g., Marlin, 1981; Odling-Smee, 1975; Rescorla & Wagner, 1972), so that learning about one occurs at the expense of learning about the other, but in the young rat, learning about contextual stimuli has been reported to enhance, or potentiate, responding to a CS and vice versa (see, e.g., McKinzie & Spear, 1995). Accordingly, when the CS was presented against an existing baseline level of fear to the context, responding to the CS appeared to be intensified (i.e., increased heart rate and behavioral activation) with respect to that observed in the adults (i.e., decreased heart rate and freezing). By inference, then, the CRs to the olfactory CS would have consisted of cardiac decelerations and freezing in the pups as well, without the contribution of contextual fear.

One prediction that arises from the hypothesis regarding the additive effects of context conditioning to CS responding in preweanling subjects is that the form of the CR to the olfactory CS should change with manipulations of the degree of contextual fear. Specifically, if context fear is attenuated, the pattern of CRs should change from tachycardia/arousal to bradycardia/freezing. Rudy and Morledge (1994) have reported that context fear is forgotten very rapidly in preweanling animals, due perhaps to the functional immaturity of a hippocampal long-term memory system. In effect, the capacity for short-term contextual fear conditioning is available to the young rat (Rudy & Morledge, 1994), and perhaps disproportionately so (Lariviere, Chen, & Spear, 1990; McKinzie & Spear, 1995), but the propensity for *long-term* storage of this information is not readily available until after weaning (Rudy, 1993; Rudy & Morledge, 1994). In other words, context fear can be acquired by the preweanling rat, but this information cannot be transferred to a long-term memory store. If the conditioned tachycardia and increased activity of the preweanlings reported by Hunt

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et al. (1997) and others (Hunt, Hess, & Campbell, 1994; Sananes, Gaddy, & Campbell, 1988) did result from the summation of CS fear and contextual fear, as hypothesized, the form of the CR should change after an interval sufficient to allow forgetting of contextual information. In the present study, preweanling rats given pairings of an olfactory CS with footshock were predicted to show (1) tachycardia and flight/escape (arousal) behaviors during CS-US pairings, (2) a high degree of contextual fear during training as inferred by freezing during the intertrial intervals (ITIs), and (3) a change in CS-elicited response topography to include bradycardia and freezing after a 24-h retention interval, when forgetting of contextual fear should be complete (Rudy & Morledge, 1994).

EXPERIMENT 1

The purpose of the first experiment was to test the prediction that there would be a change in the form of both the cardiac and behavioral CRs to an olfactory CS paired with footshock in preweanling rats after a 24-h retention interval.

Method

Subjects

The subjects in this experiment were 20 experimentally naive male and female 16-day-old rat pups. Animals were born and reared in the vivarium in the psychology department at Princeton University. Pregnant females were checked daily for births. Two days after birth (day of birth = Postnatal Day 0), litters were culled to 8 pups each. Animals were given ad lib access to Purina Rat Chow and water and were maintained in a temperature-controlled vivarium with a 12:12-h light:dark illumination cycle. Light onset occurred at 0800 h and all training and testing occurred between 0900 and 1700 h. All procedures were approved by the Princeton University Animal Care and Use Committee.

Apparatus

The apparatus used for training and testing consisted of a cylindrical Plexiglas tube, 25 cm in length and 19 cm in diameter. The tube was mounted horizontally in a custom-built sound-attenuating chamber with interior dimensions of 66.0 × 37.0 × 81.5 cm. A 4-watt white bulb was mounted on an inside wall of the sound-attenuating chamber to provide constant low-level illumination. The bottom of the Plexiglas tube was cut out and fitted with 3-mm diameter stainless steel bars, spaced 7 mm apart (center-to-center). The bars were connected to a constant-current shock generator. The US was a 1-sec 0.6-mA electric shock delivered through the grid floor. The CS was a 30-sec presentation of amyl acetate (0.5 ml amyl acetate dissolved in 40 ml water), delivered using an olfactometer system that has been described in detail elsewhere (Hunt et al., 1997; Sananes et al., 1988). An air stream remained on at all times and was used to maintain temperature in the chamber at 30°C. The olfactory stimulus was evacuated from the chamber by negative pressure generated by an exhaust fan.

Heart rate was recorded through two transcutaneous electrodes shaped like safety pins. The electrodes were acutely implanted prior to training and testing. One electrode was implanted at the nape of the neck and the other near the base of the tail. Implantation required approximately 10 sec per subject. The electrodes and attached wire leads (32-ga, Model 2840/7, Alpha Wire Co., Elizabeth, NJ) did not appear to impede animals' movements or cause any discomfort. Cardiac potentials obtained through the electrodes were amplified by a Grass preamplifier (Model P15). The R-spike activated a Schmitt

trigger (Model S21-06, Coulbourn Instruments). A microcomputer stored each interbeat interval (IBI) measured to the nearest millisecond. The computer controlled all stimulus presentations and data collection. The cardiac signal was continuously displayed on a Hitachi oscilloscope (Model V-212). The training and testing sessions were videotaped using a Panasonic low-light camera (Model WV-1854) that was mounted against a clear window in the sound-attenuating shell and connected to a Panasonic VCR (Model AG-6720).

Procedure

On the day of training, animals were removed from the home cage and heart rate recording electrodes were implanted. Subjects were then placed into the conditioning chamber for a 15-min period of acclimation (Richardson, Siegel, & Campbell, 1988). Subjects were randomly divided into one of two groups, designated as paired or unpaired (10 per group), with an equal number of males and females in each group. No more than 2 subjects from a litter served in each treatment condition. Following acclimation, subjects assigned to the paired group were given 15 pairings of a 30-sec olfactory CS terminating with the onset of footshock. The ITIs ranged from 75 to 285 sec. Subjects assigned to the unpaired group were treated similarly except that the USs were presented during the ITIs. A random timing program generated one US during each ITI, with the stipulation that a US could not occur either 30 sec before or 30 sec after a CS. Following the conditioning session, subjects were earmarked for later identification and returned to their home cages.

Approximately 24 h after training, subjects were again removed from the home cage, heart rate recording electrodes were implanted, and animals were allowed 15 min to adapt to the test chamber (the same one as that used for conditioning). Subjects were then given four nonreinforced 30-sec olfactory CS trials with ITIs ranging from 60 to 120 sec. No shocks were delivered during the test phase.

On each CS trial during both training and testing, HR was recorded for 5 sec prior to and for 30 sec during the CS. The IBIs were converted to a beats-per-minute (BPM) measure. The mean HR recorded during the last second of the prestimulus interval was subtracted from the mean HR recorded during each second of the CS period, yielding difference scores. Positive difference scores indicated HR acceleration (tachycardia) whereas negative difference scores indicated HR deceleration (bradycardia).

The conditioning and testing sessions were also videotaped for later scoring of CS-elicited behaviors. For 10 sec prior to each CS and during the 30 sec of the CS, subjects were briefly observed at 2-sec intervals. At each observation period, ongoing behavior was noted. The behaviors of interest were as follows: head lifting, raising of the head in a gradual motion, usually occurring repeatedly and accompanied by sniffing; sniffing, the rhythmic movements of the nostrils; turning, a full-body circling movement that changed the orientation of the subject; forward locomotion, movement in a forward manner that changed the subject's location in the chamber; rearing, lifting of the forepaws off the grid floor, sometimes in stationary contact with the sides of the chamber, in the absence of grooming; wall climbing, both forepaws on the sides of the chamber and engaged in climbing motions; digging, attempts to burrow between the grids; freezing, the absence of all movements except those required for respiration.

Data Analyses

For training, the BPM changes in HR on each of the 30-sec CSs were averaged over blocks of three trials, yielding a total of five trial blocks. The data for Trial Blocks 1, 3, and 5 were used in the analyses. The individual behaviors described above were collapsed into three categories for analysis: activity, orienting, and freezing. The orienting measure comprised the behaviors of rearing, head lifting, and sniffing (Hayne, Richardson, & Campbell, 1991). Activity was composed of forward locomotion, turning, digging, and

wall climbing. Finally, freezing was a separate category. With some exception (e.g., freezing), behaviors were not mutually exclusive, allowing for the scoring of more than one behavior during a single observation interval. The measures used were percent change scores, calculated by subtracting the percent of intervals scored with one of the behaviors during the 10-sec pre-CS period from the percent of intervals scored with that behavior during the 30-sec CS. A positive change indicated more of that behavior during the CS than during the pre-CS period. The behavioral data were likewise averaged over blocks of three trials for analysis during training. For test, the cardiac and behavioral data were analyzed separately for each trial.

Both the cardiac and the behavioral data were analyzed using mixed-design analyses of variance (ANOVAs). In all cases where a repeated measure was included, the Geisser–Greenhouse correction procedure was employed (Keppel, 1982). Post hoc comparisons, where appropriate, were made using Neuman–Keuls tests, with the rejection level set at .05.

Results

Acquisition

Cardiac CRs. The BPM changes in HR during Trial Blocks 1, 3, and 5 for the paired and unpaired groups are presented in the top panels of Figure 1. As can be seen, the paired subjects began to display an increase in HR to the CS by the third trial block. Initially, presentation of the olfactory CS elicited bradycardia that did not differ between the two groups ($F < 1$; data not shown; see Hayne

et al., 1991; Hunt et al., 1994). The cardiac response of the unpaired group habituated to near-zero levels within the first trial block and remained near baseline throughout training. The 2 (conditioning treatment) $\times$ 3 (trial block) $\times$ 30 (seconds) mixed-design ANOVA yielded a significant main effect of trial block [$F(2,36) = 6.47, p < .01$]. The conditioning treatment $\times$ trial block and the trial block $\times$ seconds interactions were also significant [smallest $F(58,1044) = 2.87, p < .05$].

Baseline HRs. The 2 (conditioning treatment) $\times$ 3 (trial block) mixed-design ANOVA on baseline HRs yielded nonsignificant effects, although there was a trend for baseline HRs of the paired group to decrease across trial blocks and for those of the unpaired group to remain stable. Mean ($\pm$ SEM) baseline HRs on Trial Blocks 1, 3, and 5 were as follows: for the paired group, 497.6 (10.5), 478.1 (10.4), and 472.2 (6.4) BPM; for the unpaired group, 480.9 (8.8), 490.4 (9.1), and 483.0 (12.9) BPM.

Behavioral CRs. The behavioral responses of the paired and unpaired groups during Trial Blocks 1, 3, and 5 are shown in the top panels of Figure 2. Both the paired and unpaired subjects showed a CS-elicited increase in general activity and orienting during the first trial block (see also Hayne et al., 1991). The percent change in these behaviors decreased over trial blocks in the unpaired

Figure 1. Mean beat-per-minute (BPM) changes in heart rate (HR) during the 30-sec olfactory conditioned stimulus in Trial Blocks 1, 3, and 5 of training for paired and unpaired subjects of Experiments 1 (top panels) and 2 (bottom panels).

group. For the paired group, however, the amount of CS-elicited activity increased across training, whereas orienting behaviors declined. Neither group showed a substantial change in freezing. The 2 (conditioning treatment) $\times$ 3 (trial block) $\times$ 3 (behavior) mixed-design ANOVA yielded a significant main effect of behavior [$F(2,36) = 20.92, p < .001$] and a significant conditioning treatment $\times$ behavior interaction [$F(2,36) = 13.42, p < .001$].

Pre-CS behaviors. Similar analysis of the behaviors observed prior to CS onset on each of the three trial blocks yielded a reliable main effect of behavior [$F(2,36) = 9.11, p < .01$] and a conditioning treatment $\times$ behavior interaction [$F(2,36) = 13.45, p < .001$]. Post hoc comparisons indicated that the paired, but not the unpaired, group exhibited substantial freezing prior to CS onset on Trial Blocks 3 and 5. Unpaired subjects tended to be more active during this time and, interestingly, did not show the level of pre-CS freezing that the paired group did. This pre-CS freezing is assumed to indicate contextual fear (Fanselow, 1980, 1982; Rudy, 1993; Rudy & Morledge, 1994). Neither group showed any substantial orienting prior to onset of the CS. Mean ($\pm$ SEM) pre-CS activity and freezing of both groups are shown in the top panels of Table 1.

Testing

Cardiac CRs. The changes in HR recorded during the test trials given 24 h after training are presented in Figure 3A. The paired subjects responded to the CS with bradycardia, whereas the HR of the unpaired group remained near baseline, with a slight increase in rate during the beginning of the CS. The 2 (conditioning treatment) $\times$ 4 (trials) $\times$ 30 (seconds) mixed-design ANOVA yielded no significant effect of trials. The main effects of conditioning treatment [$F(1,18) = 4.47, p < .05$] and seconds [$F(29,522) = 5.93, p < .001$] and the conditioning treatment $\times$ seconds interactions [$F(29,522) = 2.77, p < .05$] were all significant.

Baseline HRs. The 2 (conditioning treatment) $\times$ 4 (trials) ANOVA conducted on the baseline HR data yielded nonsignificant main effects and interaction. Mean ($\pm$ SEM) baseline HR for paired and unpaired groups, collapsed across the four trials, were 482.0 (13.0) and 493.0 (16.9) BPM, respectively. These baseline HRs were similar to those recorded during training.

Behavioral CRs. Figure 3B shows the behavioral responses of the paired and unpaired groups averaged across the four test trials. The unpaired group exhibited significantly more orienting behavior than did the paired group, whereas the paired group showed substantially more freezing than did the unpaired group. The 2 (conditioning treatment) $\times$ 3 (behavior) mixed-design ANOVA yielded a significant main effect of behavior [$F(2,36) = 4.35, p < .05$] and a conditioning treatment $\times$ behavior interaction [$F(2,36) = 16.58, p < .001$].

Figure 2. Changes in percent of intervals scored as activity, orienting, and freezing by paired and unpaired subjects of Experiments 1 (top panels) and 2 (bottom panels) in Trial Blocks 1, 3, and 5 (see text for definitions of behaviors).

Table 1
Mean $\pm$ SEM Percentage of the Pre-Conditioned-Stimulus Period Spent Active and Freezing in Experiments 1 and 2 During Trial Blocks 1, 3, and 5

Experiment	Block											
	1				3				5			
	Active		Freezing		Active		Freezing		Active		Freezing	
	M	SEM	M	SEM	M	SEM	M	SEM	M	SEM	M	SEM
1												
Paired	13.3	3.5	17.3	8.1	18.0	7.9	42.0	11.0	19.3	6.4	35.0	10.5
Unpaired	26.0	2.2	16.7	6.0	35.3	9.4	6.7	3.6	36.0	7.6	2.0	2.1
2												
Paired	16.7	3.3	22.7	5.6	6.7	2.6	45.7	7.3	17.7	3.9	38.3	7.5
Unpaired	17.3	3.7	17.3	5.8	36.3	4.9	8.7	2.9	32.0	4.5	3.0	1.9

Pre-CS behaviors. There was little evidence of pre-CS freezing in either group. Both paired and unpaired subjects were generally active prior to the CS trials. These pre-CS behaviors were quite different from those observed during the training session, at least for the paired group. There was little pre-CS orienting behavior in either group. The analysis of pre-CS behaviors yielded a significant main effect of behavior [$F(2,36) = 6.04, p < .01$]. Mean ($\pm$ SEM) percent pre-CS activity and freezing behaviors are shown in the top portion of Table 2.

Discussion

The results of this experiment indicate that both the cardiac and behavioral CRs to the olfactory CS changed over a 24-h retention interval in preweanling rats. During acquisition, 16-day-old rats displayed tachycardia to the CS after only a few CS-US pairings. These data replicate several other reports from this laboratory (Hunt et al., 1994, 1997; Sananes et al., 1988). Accompanying this cardioacceleration were indications of behavioral arousal. At CS onset, paired subjects were observed to become quite active, whereas unpaired subjects showed much less CS-elicited activity. Qualitatively, the activity of the paired

group might be characterized as flight/ escape responses. Upon CS onset, paired subjects ran rapidly to the opposite end of the chamber from the odor source and engaged in rapid turning, running, wall climbing, and digging at the grid floor. These behaviors were not observed in the unpaired subjects. After an interval of 24 h, responding to the CS changed dramatically in the paired group. During the test, the subjects exhibited bradycardia accompanied by an increase in freezing. The tachycardia and arousal behaviors observed during training, therefore, were not persistent but were seen only during CS-US pairings, and perhaps also for a short time thereafter (cf. Hunt et al., 1994).

This change in response form was anticipated on the basis of the assumption that during training, the CRs of the young subjects reflected a summation of fear to the CS and fear to the context. The results of Experiment 1 suggested that context fear may indeed have contributed to the topography of the CR, since paired subjects displayed a higher degree of pre-CS freezing during training than did the unpaired group. Also, the tendency for baseline HRs to decrease across the training session in paired, but not unpaired, subjects might indicate context-

Figure 3. Mean beat-per-minute (BPM) changes in heart rate (HR) (left panel) and changes in percent of intervals scored as activity, orienting, and freezing (right panel) of paired and unpaired subjects averaged across four nonreinforced test trials in Experiment 1.

Table 2
Mean $\pm$ SEM Percentage of the Pre-CS Period Spent Active and Freezing During the Test Phase of Experiments 1 and 2

Experiment	Active		Freezing	
	<i>M</i>	<i>SEM</i>	<i>M</i>	<i>SEM</i>
1				
Paired	18.8	3.7	12.0	5.5
Unpaired	16.0	3.9	0	0
2				
Paired-no shock	34.0	13.4	0	0
Unpaired-no shock	32.0	11.8	0	0
Paired-shock	16.0	5.3	30.0	14.1
Unpaired-shock	28.0	11.8	34.0	15.1

tual conditioning as well. After an interval of 24 h, preweanlings no longer exhibited overt context fear (Table 2), and there was a corresponding change in CS-elicited response form over this interval.

That context fear appears to have been forgotten over the 24-h retention interval is in agreement with data reported by Rudy and Morledge (1994; see also Rudy, 1993). These researchers showed that although context freezing was evident in preweanling rats immediately following a context-shock pairing, the response was severely attenuated when testing occurred after an interval as short as 1 h. Although in agreement with Rudy, an equally likely hypothesis for the data presented in this experiment is that context fear was extinguished prior to the delayed test. In these experiments, subjects were given 15 min of exposure to the context before the CS test trials that might have resulted in the extinction of context fear. It is typically reported, however, that contexts require prolonged exposure for significant extinction to obtain (e.g., Bouton & Bolles, 1979). Because context freezing was not recorded immediately upon reexposure to the context, these two alternatives cannot be adequately contrasted. Nevertheless, regardless of the underlying mechanism—forgetting or extinction—contextual stimuli prior to the CS test trials did not support freezing behavior and therefore, according to the present analysis, could not summate with the CS to promote the tachycardia and flight/escape responses.

EXPERIMENT 2

The purpose of Experiment 2 was to further study the change in conditioned responding reported in Experiment 1 by examining the effects of reconditioning context fear prior to the 24-h test. To accomplish this, unsignaled footshocks were given in the test context shortly before nonreinforced CS trials. If the differences in responding observed between training and test reflect the loss of context fear (through forgetting or extinction), then unsignaled USs might effectively reinstate the training responses via the reestablishment of fear to the contextual cues (Fanselow, 1980, 1982; Rudy, 1993; Rudy & Morledge, 1994).

Method

Subjects

The subjects in this experiment were 40 experimentally naive male and female 16-day-old rats from the same source and reared under the same conditions as those in Experiment 1.

Apparatus

The apparatus was the same as that in Experiment 1.

Procedure

Subjects were randomly assigned to one of four groups, designated according to training contingency (paired or unpaired) and the presence or absence of unsignaled shocks given in the context prior to the test trials (shock or no shock). Two groups of paired (P) and two groups of unpaired (UP) subjects (10 per group) were trained exactly as described in Experiment 1. Subjects were given fifteen 30-sec olfactory CS presentations either paired or explicitly unpaired with a 1-sec 0.6-mA footshock. HR and behavioral responses were recorded during training, as in Experiment 1. Following conditioning, all subjects were earmarked for identification and returned to their home cages.

Following a 24-h retention interval, subjects were given four nonreinforced olfactory CS test trials, as described previously. Subjects were removed from the home cage, HR electrodes were implanted, and a 15-min adaptation period began. Following this, subjects were given one of two treatments. For subjects in the shock conditions (P-shock, UP-shock), two footshocks of the same intensity and duration as those used for training were presented, separated by a 60-sec interval. Two minutes after the second shock, nonreinforced test trials were given. The no-shock groups (P-no shock, UP-no shock) remained in the context for an additional 3 min to equate context exposure with the shock groups prior to nonreinforced CS trials. During testing, HR was recorded for 5 sec prior to the CS and during the 30-sec CS period. The test session was also videotaped for later analysis of CS-elicited behaviors.

Results

Acquisition

Cardiac CRs. Cardiac CRs were similar to those reported in Experiment 1 and are shown in the bottom panels of Figure 1. By the third block of trials, both paired groups were exhibiting tachycardia to the olfactory CS, whereas the unpaired groups were showing a slight initial acceleration followed by a return of HR to baseline later during the CS period. The 2 (conditioning treatment) $\times$ 3 (trial block) $\times$ 30 (seconds) mixed-design ANOVA yielded significant main effects of trial block and seconds [smallest $F(29,1102) = 7.26, p < .001$]. The conditioning treatment $\times$ seconds and trial block $\times$ seconds interactions were also significant [smallest $F(29,1102) = 2.62, p < .05$].

Baseline HRs. Analyses of baseline HRs during training yielded significant main effects of conditioning treatment and trial block [smallest $F(2,76) = 7.03, p < .01$] as well as a significant conditioning treatment $\times$ trial block interaction [$F(2,76) = 4.63, p < .05$]. Across the training session, baseline HRs of paired subjects declined, whereas those of unpaired subjects remained stable. On Trial Blocks 1, 3, and 5, the mean baseline HRs of the paired groups were 482.1, 450.0, and 456.1 BPM; those of the unpaired groups were 489.9, 487.4, and 485.3 BPM.

Behavioral CRs. The behavioral CRs were again similar to those reported in Experiment 1 and are shown in the bottom panels of Figure 2. Paired subjects exhibited a conditioned increase in general activity at CS onset by Trial Block 3, accompanied by a reduction in freezing. Unpaired subjects showed orienting behaviors and increased general activity initially, but with more CS exposure orienting and activity habituated. The 2 (conditioning treatment) $\times$ 3 (trial block) $\times$ 3 (behavior) mixed-design ANOVA yielded significant main effects of behavior [$F(2,76) = 58.77, p < .001$] and trial block [$F(2,76) = 2.99, p = .05$]. Also, the conditioning treatment $\times$ behavior [$F(2,76) = 26.19, p < .001$] and conditioning treatment $\times$ trial block $\times$ behavior [$F(4,152) = 2.62, p = .05$] interactions were significant.

Pre-CS behaviors. The analysis of pre-CS behaviors yielded a significant main effect of behavior [$F(2,76) = 12.91, p < .001$]. In addition, the conditioning treatment $\times$ behavior, trial block $\times$ behavior, and conditioning treatment $\times$ trial block $\times$ behavior interactions were significant [smallest $F(4,152) = 3.14, p < .05$]. Post hoc comparisons indicated that the paired and unpaired groups did not differ in pre-CS behaviors on Trial Block 1, although they did on Trial Blocks 3 and 5. By Trial Block 3, the paired group was showing substantial pre-CS freezing, whereas the unpaired group was active. These results replicate those of Experiment 1 and are shown in the bottom of Table 1.

Testing

Cardiac CRs. The cardiac response to the CS on the first test trial differed according to conditioning treatment and the presence/absence of the pretest shocks. As can be

seen in Figure 4, the shocks administered prior to nonreinforced CS presentations served to reinstate the conditioned tachycardia. Those subjects in the paired group given no shocks showed CS-elicited bradycardia as reported in Experiment 1. The 2 (conditioning treatment) $\times$ 2 (shock) $\times$ 4 (trial) $\times$ 30 (seconds) mixed-design ANOVA yielded a marginally significant shock $\times$ trial interaction [$F(3,99) = 2.38, p = .07$]. Therefore, the responses on the first test trial only were used for further analyses. A subsequent ANOVA yielded a significant conditioning treatment $\times$ shock interaction [$F(1,36) = 7.03, p < .05$].

Baseline HRs. The 2 (conditioning treatment) $\times$ 2 (shock) analysis of baseline HRs recorded prior to the first test trial yielded nonsignificant effects. The subjects given the prior shocks tended to have slightly higher baselines than those not given the pretest shocks, although this difference was unreliable. Mean ($\pm$ SEM) baseline HRs for shock and no-shock groups, respectively, were 514.5 (9.7) and 483.5 (12.7) BPM.

Behavioral CRs. Similar to the pattern obtained with the HR response, pretest shock administration (P-shock) resulted in the conditioned behavioral response changing from primarily freezing to the pattern obtained at the end of training—behavioral activation and a reduction in freezing. These data are shown in Figure 5. In the absence of pretest shocks, the predominant CR to the CS in the P-no-shock group was freezing. For both unpaired groups (UP-shock and UP-no shock), there was a reappearance of general activity and orienting behaviors similar to that reported in Experiment 1. The 2 (conditioning treatment) $\times$ 2 (shock) $\times$ 3 (behavior) mixed-design ANOVA conducted on the data from Test Trial 1 yielded a significant main effect of behavior [$F(2,72) = 7.62, p < .01$]. The

Figure 4. Mean beat-per-minute (BPM) changes in heart rate (HR) on the first test trial of Experiment 2. Subjects were given either paired or explicitly unpaired presentations of the olfactory conditioned stimulus with footshock and tested after 24 h. Prior to test, some subjects were given two unsignaled shocks (shock) while the others (no shock) remained in the context without shock.

conditioning treatment $\times$ behavior, shock $\times$ behavior, and conditioning treatment $\times$ shock $\times$ behavior interactions were also significant [smallest $F(2,72) = 5.55, p < .01$].

Pre-CS behaviors. Similar analyses were conducted on the pre-CS behavior of the four groups. This analysis yielded significant conditioning treatment $\times$ shock [$F(1,36) = 3.26, p < .05$] and shock $\times$ behavior [$F(2,72) = 6.24, p < .01$] interactions. Prior to the first test trial, both shock groups (P-shock and UP-shock) exhibited freezing whereas the no-shock groups (P-no shock and UP-no shock) were active. The percent of the pre-CS period spent active and freezing for all four groups is shown in the bottom portion of Table 2.

Discussion

This experiment has shown not only that the pattern of conditioned responding to an olfactory CS changes after a 24-h retention interval, but also that both the cardiac and somatomotor CRs can be reinstated. Following two unsignaled USs, paired subjects responded to the CS with tachycardia and behavioral activation similar to that seen during the training session. Following the unsignaled shocks, the contextual stimuli once again effectively elicited freezing behavior.

Context appears to have contributed to the form of the CRs, perhaps by generating a tonic level of fear. This was inferred on the basis of the substantial pre-CS freezing in the subjects given CS-US pairings. Interestingly, the unpaired groups in these experiments showed little indication of contextual conditioning. There have been reports of similar findings in preweanling rats (e.g., McKinzie & Spear, 1995). Under some conditions, preweanling rats appear to learn more about the context if explicit CS-US pairings are given in the context than if

the CS and US are explicitly unpaired. Spear and colleagues (Lariviere, Chen, & Spear, 1990; McKinzie & Spear, 1995) have suggested that the context and the explicit CS become integrated in a manner unlike that observed in adults. In adults, the context and the CS are assumed to compete for association with the US (Marlin, 1981; Odling-Smee, 1975), whereas in preweanlings the context and the CS appear to become configured. In effect, conditioning to the context occurs more readily when CS-US pairings are presented than when the US is unsignaled (e.g., unpaired procedure) because the occurrence of the "CS + context" compound results in the potentiation of learning about the context by the presence of the CS.

The unsignaled shocks given before the test trials in this experiment were sufficient for conditioning contextual fear in both shock groups (P-shock and UP-shock; see Table 2). This finding is in contrast to that discussed above. Presenting the US explicitly unpaired with the CS failed to establish observable context fear, whereas prior to the test trials in Experiment 2, unsignaled USs were effective in this regard. The difference may reside in the parameters of shock delivery. In Experiment 2, the two pretest shocks were separated by a relatively short interval (60 sec). The two shocks presented in this manner may have constituted a more intense US than that used during training, when the intervals between shocks were considerably longer. Had a single shock instead been given prior to test in Experiment 2, significant context fear probably would not have been observed. Notably, Rudy's (Rudy, 1993; Rudy & Morledge, 1994) US for single-trial contextual conditioning in the young rat (2-sec, 1 mA) was more intense and of longer duration than that used in the present experiments.

Figure 5. Change in percent of intervals scored as activity, orienting, or freezing on the first test trial of Experiment 2. Subjects were tested 24 h after either paired or unpaired presentations of the 30-sec olfactory conditioned stimulus with shock. Just prior to test some subjects (shock) were given two unsignaled shocks in the testing context while others (no shock) remained in the context without shock.

GENERAL DISCUSSION

To summarize, the results of these two experiments indicate that the forms of both the cardiac and somatomotor CRs of preweanling rats to an olfactory CS change when measured 24 h after conditioning. During CS-US pairings, preweanling rats exhibited a rapidly acquired cardioacceleratory CR that was accompanied by behavioral flight/escape. Unpaired subjects showed habituation of the initial orienting responses to the CS and responded with little change in either HR or behavior after the first few trials. Following a 24-h retention interval, the CS elicited a conditioned decrease in HR accompanied by substantial freezing. Finally, the forms of both the cardiac and behavioral CRs seen during training could be reinstated at the delayed test by presenting two unpaired shocks prior to the CS trials.

One possible role for the unpaired shocks in reinstating both the cardiac and somatomotor CRs evident during training is through the conditioning of context fear. As discussed in the introduction, it was hypothesized that the responses of the preweanlings seen during training represented the summation of fear to the CS and fear to the context (Bouton & Bolles, 1979, 1985; Miller & Schachtman, 1985; Rescorla et al., 1985). The pre-CS freezing behavior of paired subjects during CS-US pairings corroborates this interpretation. By the time of the 24-h retention test, context fear had dissipated (Table 2), either via forgetting or explicit extinction. It was assumed that this CS fear in the absence of background context fear would be manifest as freezing and bradycardia. This also was confirmed. By giving two shocks prior to test, fear to the context was reestablished and could subsequently summate with fear to the CS, resulting in the reinstatement of tachycardia and behavioral activation to the CS.

The idea that context fear can modulate CS-elicited responses can be likened in some ways to the reinstatement effect studied by Bouton and Bolles (1979) and Rescorla and Heth (1975). In these studies, responding to a previously extinguished CS was resumed (i.e., reinstated) following exposure to the US. The finding of the present experiments is surprisingly similar to the postextinction reinstatement phenomenon, although the CS in the present experiments was not explicitly manipulated (i.e., extinguished) prior to test. Bouton and Bolles (1979) have additionally shown that the reinstating USs must be given in the testing context in order to be fully effective for restoring the CR. Such context specificity of reinstated responding is assumed to be true with respect to the present findings as well.

The effects of shock exposure on CS responding at test in Experiment 2 were thought to result from the reconditioning of contextual fear. However, there are alternative explanations for these results. Giving shocks in the context may have been effective for reactivating a partially forgotten memory to a more accessible state, quite apart from any new learning about the context. Unpaired USs have frequently been used as effective

methods for reactivating forgotten memories in both young and adult rats (e.g., MacArdy & Riccio, 1995; Miller, Jagielo, & Spear, 1990; Riccio & Spear, 1991; Spear & Parsons, 1976). By this account, the shocks given prior to test may not have explicitly conditioned fear to context, but rather functioned in facilitating the retrieval of the memory for the training episode as a whole. It is theoretically possible to differentiate these two alternatives with a rather simple experiment. If unpaired shock exposure prior to test served to condition fear to the context that contributed in an additive fashion to the fear responses expressed toward the CS, then giving these pretest shocks in a different context should not reinstate the training pattern of responding (see Bouton & Bolles, 1979). This is because the testing context would remain associatively neutral. Alternatively, if shocks served some other function not related to explicit contextual fear (i.e., memory reactivation), then shocks presented in a different context likely would be effective for reinstating the training CRs. This experiment would serve to define the specific role of the shocks in reinstating the training response pattern.

Throughout this paper, it has been assumed that the observed tachycardia and arousal behaviors reflect a more intense fear state than do bradycardia and freezing. This assumption is based on several ecologically oriented theories of animal defensive behavior (see, e.g., Hollis, 1982; Ratner, 1967). For example, Fanselow and colleagues (Fanselow, 1991; Fanselow, DeCola, De Oca, & Landeira-Fernandez, 1995; Fanselow & Lester, 1988) view defensive behavior along a continuum representing the perceived level of threat, and note that behavioral and physiological changes can be used to define at least three rather discrete stages: preencounter, postencounter, and circa-strike. The latter two are particularly relevant to the present analysis. According to Fanselow (1991), when a potentially threatening stimulus is detected, the animal enters into a postencounter defensive mode that is characterized by a variety of behavioral and physiological changes including freezing, analgesia, and specific autonomic adjustments. It follows from the present data that the autonomic changes accompanying postencounter defense include bradycardia. However, when threat becomes more imminent, such as when a predator is about to make physical contact, the response strategy changes to circa-strike, evident by flight and escape behaviors. Increases in sympathetic activity manifest through tachycardia would especially facilitate these behaviors. It is important to emphasize that the determinants of the topography of the defensive response are related to the degree of perceived threat (Fanselow, 1991). A nociceptive event like electric shock unconditionally activates the circa-strike mode of defense, whereas a stimulus associated with shock becomes capable of activating postencounter defense (Fanselow, 1989).

The behavioral and autonomic CRs observed in Experiments 1 and 2 fit well into this conceptual framework. During CS-US pairings, the situational (contextual) stimuli elicit fear that induces a postencounter

defensive mode (freezing). Upon detection of the olfactory CS, the level of perceived threat increases, thereby causing a shift to the circa-strike mode (flight/escape). When the pups are tested for responding to the CS after an interval of 24 h, they no longer begin this sequence in postencounter defense, as evident by the lack of observable freezing to the environmental cues. Onset of the olfactory CS elicits fear, but the degree of this fear is not sufficient in itself to activate circa-strike. Instead, this CS elicits postencounter behavioral and autonomic responses (Fanselow, 1989). The baseline degree of perceived threat provided by the environmental cues can once again be increased sufficiently to themselves evoke postencounter behaviors by giving unsignaled shocks in that context. This model fares well in explaining the form of defensive responses observed during training, the change in CR topography at the 24-h retention test, and also the reinstating effects of pretest shocks.

The role of contextual information in learning and performance has been a topic of considerable theoretical interest (see Balsam & Tomie, 1985). Complex associations between context and discrete CSs have been found with phenomena such as overshadowing, blocking, summation, and occasion setting, to mention a few. Such interactions between events become even more complicated in view of the unique principles governing learning in the young organism (Spear, 1979). Preweanlings appear to have a specialized disposition for learning about context (Lariviere et al., 1990; McKinzie & Spear, 1995), and the present data suggest a prominent role for context in modulating observable responding to a CS. Further research extending the putative role of context fear on the expression of target-relevant behaviors during development is clearly required for a comprehensive theory of the ontogeny of learning and performance.

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