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Ontogeny of Contextual Fear Conditioning in Rats: Implications for Consolidation, Infantile Amnesia, and Hippocampal System Function

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The authors present developmental evidence that contextual fear conditioning is supported by a short-term memory system that supports conditioning immediately after a shock and by a long-term memory system that supports contextual conditioning 24 hr after training. This is based on the finding that after 1 conditioning trial, rats 18 to 32 days old show the same amount of conditioned freezing when tested immediately after conditioning but 18-day-old rats show much less conditioned freezing than the older rats when the retention interval is 24 hr. The data also suggest that the long-term memory representation of context that mediates conditioned fear is not available until several hours after the conditioning trial. Implications of these findings for memory consolidation processes, infantile amnesia, and hippocampal formation development are discussed.

Two views of how the experimental context in which the events of a conditioning experience occur can be identified. One view (e.g., Rescorla & Wagner, 1972) is that the context is just the sum of its elements (the individual auditory, visual, olfactory, and tactile cues that the rat experiences) and that these cues have the same associative properties as the phasic events that constitute the conditioned stimulus. The alternative view is that the elements of the context are conjoined into a unitary representation, a spatial map (Nadel & Willner, 1980; Nadel, Willner, & Kurz, 1985), a configuration (Rudy, 1993), or dynamic stereotype (Fanselow, 1990; Pavlov, 1962). The first view implies that conditioning to context and to the conditioned stimulus (CS) are mediated by the same neural systems, but the second view suggests that they depend on different systems.

Studies of conditioned fear, as assessed by the rat's tendency to crouch or freeze (Blanchard & Blanchard, 1969), recently have provided evidence for the second view. In these studies, rats were exposed to pairings of an auditory cue and an electrical-shock unconditioned stimulus (US) and subsequently were tested for their fear to both the context and to the auditory cue. Normal adult rats showed a conditioned fear response to both the context in which the shock occurred and to the auditory cue. Both Kim and Fanselow (1992) and Phillips and LeDoux (1992), however, report that damage to the hippocampal formation impairs contextual fear conditioning but has no effect on fear conditioning to the auditory cue. Rudy (1993) has reported a similar dissociation during development. Rats 18 and 23 days old display equivalent fear conditioning to an auditory cue paired with shock, but 18-day-old rats showed significantly less contextual fear conditioning than 23-day-old rats.

The purpose of the experiments we report was to further analyze ontogenetic differences in contextual and auditory fear conditioning by studying the retention of these two forms of fear conditioning as a function of age. One reason for studying the retention of contextual fear conditioning is that in the Rudy (1993) study the rats were only tested for conditioned fear 24 hr after the conditioning experience. Young rats, however, are known to show more rapid forgetting than older rats (Campbell & Spear, 1972), so it is possible that 18-day-old rats displayed less contextual fear than 23-day-old rats because the younger rats could not retain the context fear experience for 24 hr. This hypothesis turned out to be wrong, but testing it yielded several unexpected results that suggest novel hypotheses about the memory processes mediating contextual conditioning.

General Method

Subjects

The subjects were male and female Long-Evans–derived hooded rat pups born to rats obtained from Charles River Breeders and bred at the University of Colorado. Subjects were between 18 and 32 days old at the start of training. They were maintained on ad-lib food and a 12:12-hr light–dark cycle. Checks for birth were made each morning between 8 and 10 a.m. Litters were culled to 9 pups (5 males and 4 females) on Day 3 following birth. Pups were weaned when 21 days old.

Apparatus

Training took place in two identical Igloo ice chests (54 cm × 30 cm × 27 cm) with white interiors. A speaker and a 24-V DC lightbulb were mounted on the ceiling of the chest. A clear plastic window (30 cm × 18 cm) was cut in the door of the chest so that the subjects could be observed. The four sides of the (26 cm × 21 cm × 10 cm) conditioning chamber that were placed inside each chest were made of clear plastic with a window screen top. The 2-s, 1-mA shock US was delivered through a removable floor composed of stainless steel rods 1.5 mm in diameter and spaced 1.2 cm center to center. Each rod was wired to a shock generator and scrambler (BRS, Lehigh Valley,

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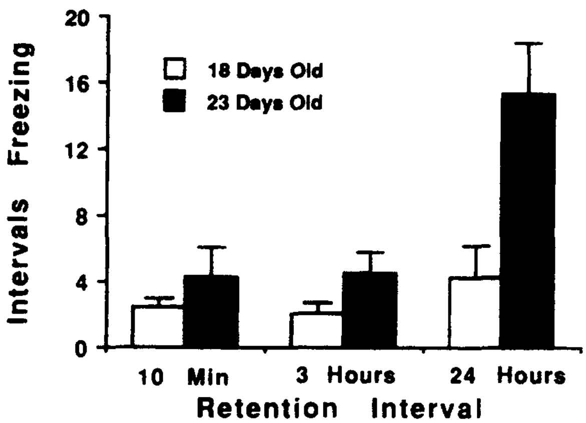

Figure 1. Mean number of intervals the freezing response was observed during the preconditioned stimulus (CS) and CS periods of auditory CS test of Experiment 1 as a function of retention interval. Error bars represent standard error.

Laurel, MD). The conditioning chamber rested on the steel rods. The rods and floor of each chamber were cleaned with water before each rat was trained or tested. An auditory stimulus, an 85-dB, 6-cps train of clicks generated by a Commodore brand computer, served as the CS. Ventilation fans provided background noise (68 dB). In all experiments, rats were given a single conditioning trial. In some experiments, the US was preceded by a 30-s presentation of the auditory CS; in others only the US was presented. In all experiments, the rats were placed into the conditioning chamber for 2 min prior to US onset and removed 30 s after US termination.

Contextual conditioning was assessed by placing the rat in the original conditioning context for 5 min. During the test period, a trained observer with no knowledge of the purpose of the experiment scored the rat's tendency to freeze. Observation was carried out using a time sampling procedure. Every 10 s, each rat was judged as either freezing or active during the test. This judgment was made at the instant the sample was taken. *Freezing* was defined as the absence of visible movement, except for respiration. Scoring began approximately 20 s after the rat was placed in the chamber.

Conditioned freezing to the auditory cue was assessed approximately 30 min after the context test. During this test, the original context was altered to reduce the contribution the training context might make to conditioned freezing. The rod floor was removed, and the chamber rested on a clear plastic floor. A clear plastic panel (34 cm × 10 cm) was inserted into the chamber connecting two diagonal corners. The diagonal panel reduced the size of the test chamber and altered its geometry from a rectangle to a triangle. During the auditory cue test, the rats were scored for 6 min. During the first 3 min, the auditory CS was absent, but during the second 3 min, it was present.

Experiment 1

As noted, it is possible that the 18-day-old rats showed less contextual fear conditioning than 23-day-old rats in the Rudy (1993) study because they were unable to retain the experience when tested 24 hr later. So, 18- and 23-day-old rats were given a single conditioning trial in which an auditory CS was paired with the US, and the rats were tested 10 min, 3 hr, or 24 hr later.

Subjects

Four litters of 18-day-old pups and five litters of 23-day-old pups provided subjects. Three rats from each litter were assigned to either the 10-min, 3-hr, or 24-hr retention condition, with the constraint that at least 1 male and 1 female from each litter were assigned to condition. On the recommendation of Abby and Hoffman (1973), the litter, rather than the rat, was the unit of analysis. The median score for the 3 rats assigned to a treatment was used to represent the litter's contribution.

Results and Discussion

Figure 1 shows the results of the context test. The 18-day-old rats showed little evidence of freezing at any retention interval. The 23-day-old rats also showed little freezing during the 10-min and 3-hr retention tests, but their freezing increased substantially during the 24-hr test. An analysis of variance (ANOVA) revealed a significant effect of age, $F(1, 21) = 14.3$, $p < .001$, and retention, $F(2, 21) = 4.4$, $p < .03$, and a significant Age × Retention interaction, $F(2, 21) = 5.06, p < .02$. The simple effects analysis indicated that the two age groups only differed on the 24-hr test, $F(1, 21) = 10.3, p < .001$, and that retention interval was significant only for 23-day-old rats, $F(2, 21) = 22.8, p < .001$.

Figure 2 shows the results of the auditory cue test. Freezing during the pre-CS period was uniformly low for all groups but increased significantly when the auditory CS was presented. The older rats showed somewhat more freezing during the CS than did the younger rats, but retention interval did not influence performance at either age. An ANOVA on this data revealed a significant effect of test period (pre-CS vs. CS), $F(1, 21) = 114.2, p < .001$, and age, $F(1, 21) = 6.8, p < .02$, and a significant Age × Test period interaction, $F(1, 21) = 4.3, p < .05$. There was no effect of retention interval, $F(2, 21) = 1.4$, and this variable did not interact with age or test period ($Fs < 1$).

The context test results at the 24-hr interval replicated Rudy's (1993) finding that 18-day-old rats show less contextual conditioning than 23-day-old rats. This difference cannot be

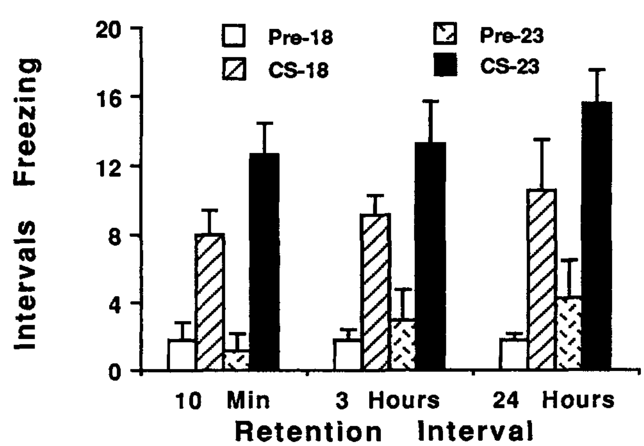

Figure 2. Mean number of intervals the freezing response was observed during the context of Experiment 1 as a function of retention interval. Pre-18 = preconditioned stimulus, 18-day-old rats; CS-18 = conditioned stimulus, 18-day-old rats; Pre-23 = preconditioned stimulus, 23-day-old rats; CS-23 = conditioned stimulus, 23-day-old rats. Error bars represent standard error.

attributed to more rapid forgetting by 18-day-old rats that occurs between 10 min and 24 hr after training, however, because neither 18- nor 23-day-old rats showed much freezing at the 10-min and 3-hr retention tests. Instead, it was due to an increase in freezing by 23-day-old rats that occurred between 3 hr and 24 hr.

As previously reported (Rudy, 1993), both the 18- and 23-day-old rats showed more freezing when the auditory cue was present than when it was absent, indicating that subjects at both ages were conditioned to the auditory cue. The older rats, however, responded more to the clicker than did the younger rats in this experiment, whereas the 2 age groups did not differ in the previous report.

Experiment 2

The context test of Experiment 1 revealed an unexpected result: The 23-day-old rats showed very little freezing behavior at the 10-min retention interval, but, as the retention interval increased from 10 min to 24 hrs, their freezing behavior increased dramatically. Typically, it is expected that there would either be no change in responding across these retention intervals or there would be a decrease in freezing as the retention interval increased. Experiment 2 served two purposes. It asked if this effect would also be displayed by older rats, and if it depended on the presence of the auditory cue during conditioning.

Subjects

In this experiment, 5 litters of 32-day-old rats were conditioned and tested 10 min, 3 hr, or 24 hr after training. Three rats from each litter were assigned to each group. The auditory cue was not presented during conditioning or testing.

Results and Discussion

Figure 3 shows that the 32-day-old rats' freezing response during the context test increased significantly as the retention interval increased, $F(2, 12) = 8.7, p < .005$. Newman-Keuls tests indicated that there was significantly more freezing during the 24-hr test than there was during the 10-min ($p < .01$) or 3-hr test ($p < .05$). So, 32-day-old rats showed a retention function similar to that shown by 23-day-old rats in Experiment 1. Contextual freezing increased dramatically between 10 min and 24 hr after training. This function evidently does not depend on the presence of the auditory CS at the time of the shock because the auditory stimulus was not presented in this experiment.

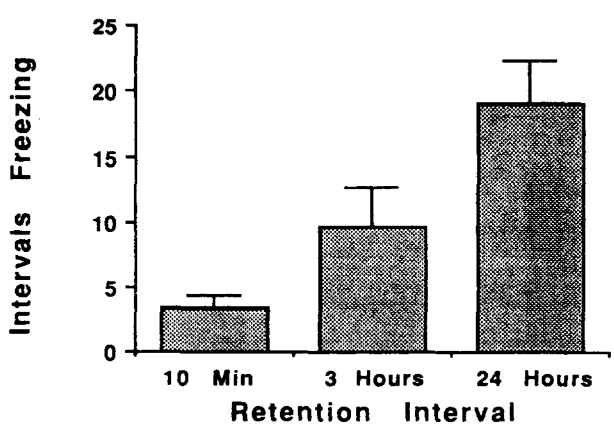

Figure 3. Mean number of intervals the freezing response was observed during Experiment 2 as a function of retention interval. The rats were 32 days old. Error bars represent standard error.

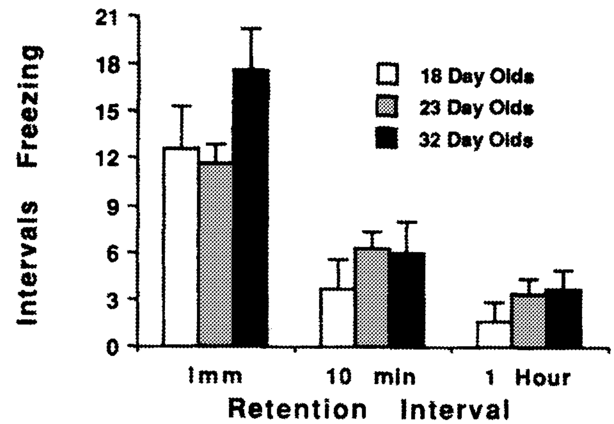

Figure 4. Mean number of intervals the freezing response was observed during Experiment 3 as a function of retention interval. Error bars represent standard error. Imm = immediate.

Experiment 3

In both Experiments 1 and 2, little evidence of contextual fear conditioning was present when subjects were tested either 10 min or 3 hr after conditioning. Fanselow (1980), however, has reported significant freezing by adult rats tested immediately after the conditioning trial. To more completely characterize the function relating age to retention of contextual fear conditioning, 18-, 23-, and 32-day-old rats were given a single shock and tested immediately, 10 min, or 1 hr after training.

Subjects and Procedure

Five litters at each respective age contributed 3 subjects to each group. Testing for rats in the immediate condition began 30 s after the US terminated, and the rats were not removed from the conditioning chamber until the test was completed.

Results and Discussion

The results for this experiment are shown in Figure 4. Rats at each age displayed the same general pattern of responding. Substantial freezing was observed when testing occurred immediately after the US terminated, but it decreased significantly as the interval increased to 10 min and 1 hr. An ANOVA revealed a significant effect of retention interval, $F(2, 36) = 42.3, p < .001$. A Newman-Keuls test indicated that freezing was significantly higher at the immediate test than at the 10-min or 1-hr test ($p < .01$). Although there was a significant main effect of age in this experiment, $F(2, 36) = 3.2$,

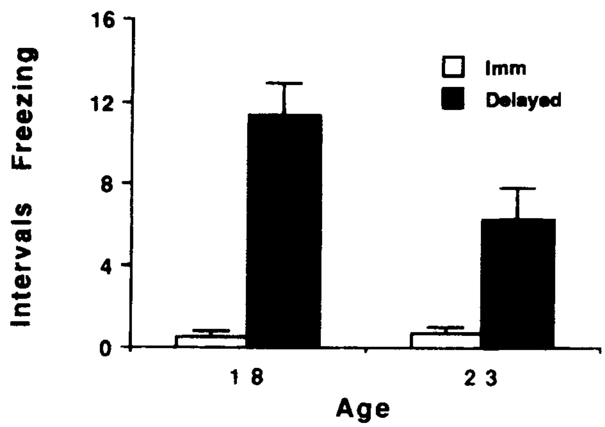

Figure 5. Mean number of intervals the freezing response was observed during Experiment 4 as a function of age. Error bars represent standard error. Imm = immediate.

$p < .05$, the Newman-Keuls post hoc test detected no differences among the age groups. The Age × Retention Interval interaction did not approach statistical significance, $F(4, 36) = 1.1$.

These results indicate that significant freezing can be observed when rat pups are tested immediately after the conditioning trial. The results also provide no evidence that the 18-day-old rats forget more rapidly than the older rats.

Experiment 4

When tested immediately after the conditioning episode, rats at each age showed appreciable freezing, but the amount of freezing decreased significantly as the retention interval increased to 10 min and remained low at the 1-hr and 3-hr intervals. When the interval was increased to 24 hr, however, the freezing response of the 23- and 32-day-old rats increased significantly, whereas the 18-day-old pups' freezing did not. These results suggest that contextual conditioning is mediated by two memory systems. One system supports freezing immediately after the learning episode but loses information quickly. The other supports freezing 24 hr later but requires some "incubation" period before information becomes accessible. The first system, the one supporting freezing immediately after shock, is available to 18- to 32-day-old rats, but 18-day-old rats do not appear to have access to the second system that supports contextual fear on the 24-hr retention test.

There is, however, an alternative interpretation of the developing rats' immediate freezing response. It may be that freezing displayed immediately after the conditioning trial is not a memory-based response but is an unconditioned response to shock and does not depend on the pups associating the context with shock. The purpose of Experiment 4 was to evaluate the possibility that freezing measured immediately after the shock is an unconditioned response. We used the strategy that Kim, Fanselow, DeCola, and Landeira-Fernandez (1992) used to address the same question in adult rats. They compared the immediate freezing response of adult rats trained under two conditions. In one case, the rats were shocked immediately after being placed into the conditioning chamber, and, in the other case, the rats were not shocked until they had been in the chamber for 3 min. If freezing observed in the immediate postshock period is just an unconditioned response to shock, then both groups should display equivalent freezing. If, however, freezing is a conditioned response, then it should depend on the rats' experiencing the context prior to shock. Kim et al. (1992) reported that the rats who experienced shock after 3 min in the chamber showed more freezing than rats shocked immediately. So, they concluded that freezing observed immediately after shock depends on associative processes (see also Blanchard, Fukunaga, & Blanchard, 1976; Fanselow, 1986).

To ensure that the freezing that developing rats show immediately after the shock was not an unconditioned response, we assessed the freezing response of 18- and 23-day-old rats. Half the rats at each age were shocked immediately after being placed in the conditioning chamber, and half were exposed to the chamber for 2 min before being shocked.

Subjects and Procedure

The subjects were taken from 6 litters of 18-day-old pups and 6 litters of 23-day-old pups. Three pups from each litter were assigned to the immediate shock condition, and 3 pups were assigned to the delayed shock condition. Rats in the immediate condition were placed into the conditioning chamber and shocked as soon as the door was closed. Rats in the delayed condition were not shocked until 2 min after being placed into the chamber. Scoring of freezing began immediately after the termination of the shock.

Results and Discussion

As shown in Figure 5, regardless of age, the subjects in the delayed condition displayed significantly more freezing than the subjects in the immediate condition. However, in this experiment the 18-day-old pups displayed more freezing than the 23-day-old pups. Consistent with this description, there was a significant effect of training condition, $F(1, 20) = 61.4$, $p < .001$, and a significant effect of age, $F(1, 20) = 5.4, p < .03$. The Age × Training condition also was statistically significant, $F(1, 20) = 6.1, p < .03$.

These results replicate Kim et al.'s (1992) findings with adult rats and rule out the possibility that postshock freezing observed in Experiment 3 was simply an unconditioned response to shock. If freezing was just an unconditioned response to shock, then both groups should have displayed equivalent freezing. The results support the hypothesis that freezing by rats placed in the context for 2 min before the shock occurred is sustained by a short-term memory process. So, together with the results of the other experiments, these data support the conclusion that contextual fear is mediated by two memory systems.

It should be noted that, using a different approach, Kim et al. (1992) also reached the same conclusion. Specifically, they reported that the administration of the competitive *N*-methyl-D-aspartate (NMDA) antagonist APV (DL-2-2-amino-5-phosphonovalerate) prevented contextual fear conditioning when adult rats were tested 24 hr after training but did not block contextual conditioning when the rats were tested immediately after training. They concluded, "There are two temporally distinct associative processes, a short-term NMDA-independent conditional fear and a long-term NMDA-dependent conditional fear" (Kim et al., 1992, p. 591).

Experiment 5

The previous experiments revealed an unexpected result: A negligible amount of contextual freezing was observed when the rats were tested 10 min to 3 hr after training, but freezing increased significantly at the 24-hr retention interval. The purpose of the final experiment was to provide some understanding of this result. Fanselow (1990) has suggested that in order to condition to context, a rat has to construct a configural representation of the context. He used Pavlov's (1962) term, *dynamic stereotype,* to represent the concept, but the idea is that the various elemental features of the context (its tactile, olfactory, visual, and auditory components) are conjoined into a unitary representation that becomes associated with the shock. Without this representation, the rat does not condition to the context. Fanselow's (1990) hypothesis derived from his work on the immediate-shock effect that was described earlier. Rats that receive a single shock immediately after being placed in the conditioning chamber show significantly less freezing than rats that are allowed 2 min in the chamber before the shock is presented.

Fanselow suggested that rats in the immediate shock group failed to condition because they did not have time to construct a representation of the context before the shock occurred. He supported this interpretation by showing that rats preexposed to the context 24 hr prior to training conditioned to the context even when shock occurred immediately (Fanselow, 1990). Presumably, the preexposure treatment permitted the rats to construct the configural representation of the context needed for conditioning when shock occurred immediately after the rat was placed in the chamber.

Building on Fanselow's hypothesis, one interpretation of why contextual fear increased substantially between the 10-min and 24-hr tests is that the configural representation of context requires time to construct. Experiment 4 was designed to test this hypothesis. Half the rats were preexposed to the conditioning context for 2 min. They received no shock during this period. They were returned to the conditioning chambers either 10 min or 24 hr later and given a single conditioning trial. The remaining rats were not exposed to the context. They only received the single conditioning trial. All rats were returned to the training context for testing 10 min after they received the shock.

In the preceding experiments, rats tested 10 min following the conditioning episode showed little contextual freezing, whereas rats tested 24 hr later showed much more freezing. If this difference is due to the time it takes to construct a usable representation of context, then rats who are preexposed to the context 24 hr prior to conditioning should show contextual freezing when tested 10 min after the conditioning trial, because they have had time to construct a representation of the context prior to the conditioning trial. Rats conditioned only 10 min after context preexposure, however, should not have time to construct the representation and so should not benefit from preexposure.

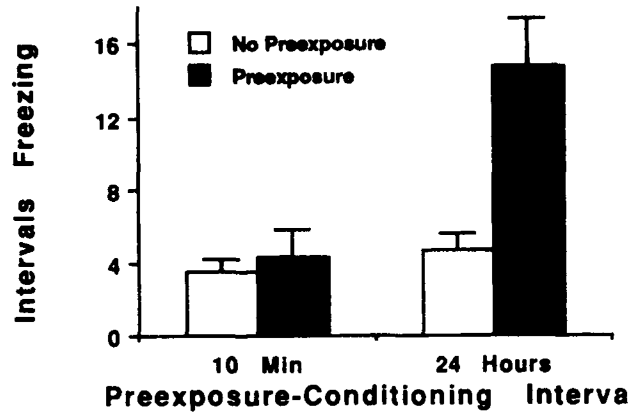

Figure 6. Mean number of intervals the freezing response was observed during the context test of Experiment 4 as a function of the interval separating context preexposure and the context conditioning trial. Error bars represent standard error.

Subjects and Design

The subjects were 23-day-old rats and were drawn from 10 litters. There were four basic training conditions. Two groups of rats were preexposed to the context for 2 min and 2 control groups of rats were not exposed to context prior to conditioning. One set of preexposed rats received their conditioning trial 10 min after being preexposed, and the corresponding control rats were conditioned on that day. These 2 sets of rats were 23 days old at the time of testing. Another set of preexposed rats were conditioned 24 hr after context exposure and the corresponding control rats were conditioned on that day. These 2 sets of rats were 24 days old on the day of testing.

There were two replications of this basic design. In the first replication, the auditory CS was paired with the shock on the conditioning trial. Five litters each contributed 2 subjects to each of these 4 groups. In the second replication, the auditory CS was not presented during conditioning. Five litters each contributed 2 subjects to each of these 4 groups.

Results and Discussion

The auditory cue had no influence on the outcome of the experiment. The mean number of intervals of freezing for rats in the condition with the auditory cue present were 3.2, 11.3, 2, and 2.8 s, respectively, for the preexposed rats in the 10-min and 24-hr retention interval and the appropriate nonpreexposed control rats. The mean number of intervals of freezing for the rats in the no-auditory-cue-present condition were 4.6, 18.2, 3.5, and 6.5 s. The data for the two replications are combined and presented in Figure 6. Rats preexposed to the context for 2 min displayed significantly more freezing than did control rats but only when the interval separating context preexposure and conditioning was 24 hr. When the preexposure-conditioning interval was only 10 min, preexposure had no influence on test performance. An ANOVA found a significant effect of preexposure, $F(1, 36) = 12.6, p < .001$, and retention interval, $F(1, 36) = 13.9, p < .01$, and a significant

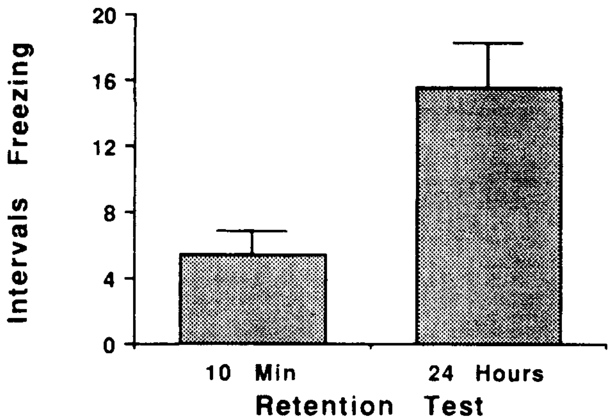

Figure 7. Mean number of intervals the freezing response was observed for control rats in Experiment 4 during their first test 10 min after the conditioning trial and on their second test 24 hr after the conditioning trial. Error bars represent standard error.

Preexposure × Retention Interval interaction, $F(1, 36) = 9.1$, $p < .005$. The simple effects analysis showed that preexposure was only significant when the rats were preexposed 24 hr prior to conditioning, $F(1, 36) = 22.7$, $p < .001$, and that rats preexposed 24 hr prior to conditioning displayed more freezing than those preexposed 10 min prior to conditioning, $F(1, 36) = 21.6, p < .001$.

In one replication, the auditory CS was not present. Rats in the 2 no-preexposure control groups of this replication were tested twice: 10 min following training and 24 hr later. The results of these two tests are shown in Figure 7. Notably, the results showed more freezing 24 hr after training than at the 10-min test, $F(1, 18) = 23.5, p < .001$.

In the preceding experiments, rats that were tested 10 min after the conditioning trial showed little freezing. Preexposure to the context 24 hr prior to conditioning, however, significantly altered this outcome. Rats in this condition showed significantly more freezing during the 10-min retention test than rats not preexposed and more freezing than rats preexposed to the context only 10 min prior to the conditioning trial. These findings provide strong support for the idea that it requires a significant period of time for the rat to construct a representation of the context that can then be used to access the shock experience. Perhaps it is for this reason that rats in the previous experiments displayed more freezing when the retention interval was 24 hr than when it was 10 min.

It is worth noting that the two tests with control subjects provided a within-subject replication of the 10-min versus 24-hr effect observed in Experiments 1 and 2. The same rats showed significantly more freezing during the 24-hr test than during the 10-min test. This fact is interesting because it provides additional support for the view that a representation of context is not available at the 10-min test. From one perspective, the test at the 10-min interval can be considered an extinction trial. Consequently, it might have been expected that these rats would show little or no freezing on the second test 24 hr later. Yet, their level of freezing was as high as that shown by 23-day-old rats in the other experiments that were only tested once. So, it appears that a functional representation of the context is not available 10 min after conditioning.

General Discussion

Previous studies have found that contextual and auditory cue fear conditioning can be dissociated by damage to the hippocampal formation (Kim & Fanselow, 1992; Phillips & LeDoux, 1992) and by age (Rudy, 1993), suggesting that these two types of fear conditioning depend on different neural substrates. In Experiment 1, both 18- and 23-day-old rats showed equivalent retention for conditioned fear to the auditory cue when tested 10 min, 3 hr, or 24 hr after training. In contrast, over these retention intervals, contextual fear conditioning increased significantly for the 23- to 32-day-old rats and remained low for the 18-day-old rats. These results provide additional evidence that contextual and auditory fear conditioning are mediated by different processes.

The complete retention pattern suggests that contextual fear conditioning per se depends on two memory systems: (a) a short-term memory system that supports contextual conditioning immediately after training but loses information quickly, and (b) a long-term memory system that supports contextual fear hours after the training occurs. These two systems appear to dissociate during ontogeny: The 18-day-old rats only showed evidence for the short-term memory system, but 23- to 32-day-old rats showed evidence for both systems. As noted, Kim et al. (1992) found that APV selectively blocks contextual freezing when rats are tested 24 hr after conditioning, but it does not block contextual freezing displayed immediately after conditioning. On the basis of this finding, they also concluded that contextual fear conditioning is supported by distinct short-term and long-term associative fear processes. Our developmental results thus support Kim et al.'s (1992) prior conclusion.

A unique finding from these experiments is that there is a period of time, between 10 min and 24 hr after the conditioning trial, during which the older rats display little contextual fear conditioning. In Experiments 1 and 2, this effect was seen as a between-subjects effect: Rats tested at the 10-min and 3-hr retention intervals showed little contextual freezing compared with other rats that were tested at the 24-hr interval. In Experiment 4, this was seen as a within-subjects effect: The same rats showed more contextual freezing during the 24-hr retention test than they did during the 10-min retention test. It is worth noting that Fanselow (1980) may have observed a similar outcome when he compared adult rats tested 30 s and 24 hr after training. His rats showed somewhat more contextual fear when tested 24 hr after training. Fanselow, however, did not test his rats at intermediate retention intervals.

It should be mentioned that the nonmonotonic retention function we obtained with the 23- and 27-day-old rats is reminiscent of results obtained some years ago in studies of passive avoidance with goldfish and mice (Riege & Cherkin, 1971, 1972; Zerbolio, 1969). In these studies, passive avoidance was assessed at various intervals following a single encounter with aversive stimulus. For example, Zerbolio (1969), who studied mice, found that about 70% of the mice avoided the shock location when the retention interval was 15 min or 120 min, but only 31% of the mice avoided it when the retention interval was 30 min. Thus, the retention function Zerbolio obtained over the 15-min (73%), 30-min (31%), and 120-min (70%) retention interval is similar in shape to what we observed in our 23- and 27-day-old rats, although the time course per se was quite different. Whether the same basic processes are acting in both situations, however, is impossible to determine without additional experimental work.

We suggested that the period between 10 min and 24 hr after training could reflect the time it takes for the rat to construct a representation of the context that can be used to retrieve the shock experience. In support of this idea, we found that preexposure to the conditioning context 24 hr prior to training significantly increased the amount of freezing observed when the rats were tested 10 min after the conditioning trial. Presumably rats that were preexposed to the context were able to construct a representation of the context prior to training that could be used to retrieve the shock experience 10 min later. If this analysis is correct, then an important difference between 18-day-old rats and older rats rests in the processes that enable the rat to construct or retain a representation of the context. Determining the neurobiological basis of this ontogenetic difference could provide important clues into the neural substrates of long-term memory.

Since Ribot's (1882) classic work, it has been recognized that the age of a memory can influence its susceptibility to loss that results from brain insult. Older memories tend to be more resistant to loss than newer memories. This empirical generalization implies a time-dependent consolidation process that stabilizes the memory (Rozin, 1976; Squire, Cohen, & Nadel, 1984). Kim and Fanselow (1992) recently reported evidence that the hippocampal system is involved in consolidation of memory for contextual fear conditioning. Rats conditioned to context and subsequently experiencing damage to the hippocampal system displayed a temporally graded, retrograde amnesia. Specifically, damage to the hippocampal system 1 day after a conditioning session eliminated contextual conditioning, but damage 7 or 14 days after conditioning had less effect, and damage produced 28 days after conditioning had no effect on test performance. So, as the time between conditioning and testing increases, the memory representation of contextual fear becomes stable and more resistant to the effects of damage to the hippocampal formation. We may have discovered an unsuspected property of the hippocampal-dependent consolidation process: During the initial stage of the process, the representation of context is not accessible and cannot be used to retrieve the memory of the shock experience.

Young rats show more rapid forgetting than do older rats (Campbell & Spear, 1972; Spear, 1979). This phenomenon, *infantile amnesia,* can be seen in the present results if performance at only the immediate and 24-hr retention intervals is studied. Notably, 18-, 23-, and 32-day-old rats showed a similar level of freezing on the immediate retention test, but 18-day-old rats showed much less freezing than did older rats on the 24-hr retention test (rapid forgetting). If we have correctly interpreted the complete pattern of results as reflecting a contribution of both short-term and long-term memory systems, then infantile amnesia in this instance occurs because the 18-day-old rats have not yet fully developed the neural substrates for the second memory system that constructs a stable long-term configural representation of context.

Evidence of long-term memory for contextual fear conditioning emerges between 18 and 23 days after birth. The hippocampal system is critically involved in the memory for contextual fear conditioning (Kim & Fanselow, 1992; Phillips & Ledoux, 1992), and the rat's ability to perform other tasks (e.g., place learning and delayed conditioned alternation) that depend on the integrity of the hippocampal system (Aggleton, Hunt, & Rawlins, 1986; Green & Stanton, 1989; Morris, Garrud, Rawlins, & O'Keefe, 1982; Rudy, Stadler-Morris, & Alberts, 1987; Sutherland, Whishaw, & Kolb, 1982) also emerges during this period of development. So, it is possible that maturation differences in the hippocampal system are responsible both for the age-related differences in long-term memory for contextual conditioning that we observed and for the disappearance of infantile amnesia. This hypothesis is reasonable because it is known that the hippocampus undergoes a protracted postnatal maturation (e.g., Altman & Bayer, 1975; Cotman, Taylor, & Lynch, 1973; Coyle & Yamamura, 1976; Crain, Cotman, Taylor, & Lynch, 1973; Wilson, 1984).

As noted, Kim et al. (1992) reported that the selective NMDA antagonist APV blocked the acquisition of conditioned contextual fear that is observed 24 hr after conditioning, but it does not block the conditioned freezing that is observed when the test is performed immediately after the conditioning trial. This finding implies that NMDA receptor function is critical for establishing long-term contextual fear conditioning. Thus, it is possible that immature NMDA receptor function is responsible for the impaired long-term contextual conditioning shown by 18-day-old rats. Unfortunately, the existing literature offers no support for this hypothesis, because NMDA receptor function appears well established before rats are 18 days old (Boje & Slotnick, 1992; McDonald, Johnston, & Young, 1990; Tremblay, Roisin, Represa, Charriaut-Marlangue, & Ben Ari, 1988).

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