LEARNING AND MOTIVATION **28,** 404–423 (1997)
ARTICLE NO. LM970979

Forgetting of the CS Duration in Rats: The Role of Retention Interval and Training Level

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Three experiments were conducted on long-term memory for conditioned stimulus (CS) duration using the conditioned suppression paradigm with rats. After training in each experiment, suppression systematically increased during successive fifths of a 150-s CS that ended in shock, indicating a temporal discrimination. Experiment 1 tested the effect of a retention interval (3, 7, 15, or 20 days). The other two experiments tested the effect of two different levels of training (45 or 90 trials) on the retrieval of temporal information after retention intervals of 3 or 20 days (Experiment 2), and 7 or 15 days (Experiment 3). The temporal discrimination was well retained 3 days after training but was progressively forgotten as the retention interval increased. Forgetting of time was inferred from the flattening of the temporal discrimination gradient, which was complete after the 20-day retention interval. This flattening was accompanied by an increase in conditioned suppression that was greater at the beginning of the CS. These results suggest that rats forgot the CS duration but remembered the CS–US excitatory relationship. Training level had a slight effect on the rate of forgetting and reacquisition but did not affect the way it happened. © 1997 Academic Press

Studies of long-term retention of a temporal discrimination have not always produced clear-cut results. In some of them, temporal information was hardly forgotten with the passage of time. For instance, Crouse and Cohen (1987), using a fixed interval (FI) reinforcement schedule, found that the typical increase in response rate across the fixed interval was still kept after a 27-

The first author is now at The University of Pennsylvania. This research was supported by a grant from the Basque Government's Programa de Formación de Investigadores (Ref. BFI90.129) and by Grant PB88-0105 from the D.G.I.C.Y.T. The authors thank Geoffrey Hall, Robert Rescorla, and Sadahiko Nakajima for valuable comments, and Mark Bouton for invaluable assistance in the preparation of a previous version of this manuscript.

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day retention interval between training and testing. Similarly, using a differential low-rate (DRL) reinforcement schedule, Hamm, Dixon, and Knisely (1984) did not obtain a loss of temporal discrimination 21 days after training. However, the lack of forgetting could be due to some idiosyncracy of these experiments, such as a repeated within-subjects testing procedure that could have strengthened the temporal memory (Crouse & Cohen, 1987), or a retention interval that was too short (Hamm *et al.,* 1984). Indeed, forgetting of temporal discrimination has been found using a FI reinforcement schedule when the test was conducted between 10 and 25 days after training (Campbell, Krauter & Wallace, 1980; Gleitman & Bernheim, 1963; Gleitman, Steinman, & Bernheim, 1965), and after a retention interval of 90 days with a DRL reinforcement schedule (Lejeune, 1989).

When forgetting of a temporal discrimination has occurred, it has not been accompanied by a loss in the response itself. On the contrary, forgetting was usually reflected as an increase in the response but at inappropriate times. It seems that rats remembered the response, but forgot the time when the response was most likely reinforced. This result has not been so clearly obtained in the scarce studies conducted within a classical conditioning paradigm, such as conditioned suppression (Hammond & Maser, 1970; Rosas & Alonso, 1996). These studies used a procedure akin to inhibition of delay (Pavlov, 1927), where a long-duration conditioned stimulus (CS) is followed by an unconditioned stimulus (US). After training in this procedure temporal discrimination is displayed as an increasing suppression over time within the CS, with its maximum toward the end of it, just before the US presentation (e.g., Davis, McIntire, & Cohen, 1969; Hendry & Van-Toller, 1965; Holmes, Jackson, & Byrum, 1971; Libby & Church, 1975; Millenson & Hendry, 1967; Schachtman, Channell, & Hall, 1987; Zielinsky, 1966). Hammond and Maser (1970) found forgetting of the temporal discrimination after a 25-day retention interval though overall suppression did not change between the end of training and testing. However, as Rosas and Alonso (1996) pointed out, a more careful review shows that the loss of temporal discrimination in Hammond and Maser's study was actually caused by a decrease in suppression to the end of the CS, so by a loss of conditional emotional response (CER) to the CS. This result has been recently challenged by Rosas and Alonso (1996). Using a similar procedure, they found that forgetting of inhibition-of-delay was displayed after a 20-day retention interval as an *increase* in the suppression to the beginning of the CS. That is, forgetting of the temporal discrimination yielded a strong CR to the whole CS, a result that is in agreement with the ones reported previously with operant conditioning (e.g., Gleitman & Bernheim, 1963).

As Rosas and Alonso (1996) noted, the loss of excitation found by Hammond and Maser (1970) might have been caused because they presented weak shocks in the absence of the CS that could have led to weaker excitatory conditioning to the CS, and therefore to easier loss of it with the passage of time. On the other hand, the retrievability of both temporal discrimination and excitatory conditioning might have been enhanced in Rosas and Alonso' experiment because a within-subject design was used—the same rats were tested with retention intervals of 3, 7, and 20 days, in that order. The conditioning sessions involved in each test could have strengthened the memory for the next test, preventing both the observation of time forgetting after retention intervals shorter than 20 days and forgetting of excitation at the 20-day retention interval. Moreover, although reliable temporal discrimination was reached after about 30 conditioning trials, Rosas and Alonso continued training to a total of 90 conditioning trials. It is well known that overtraining has an enhancement effect on the information learned (e.g., Hoffeld, 1962) and may protect it against memory deficits (e.g., Perez-Ruiz & Prado-Alcala, 1989; Sullivan & Willson, 1993).

Therefore, although the excitatory CR in conditioned suppression seems very difficult to be forgotten using standard (i.e., short) CS–US training (e.g., Gleitman & Holmes, 1967; Hendersen, 1985), there is yet room to claim that a previous training of inhibition-of-delay might increase its sensitivity to forgetting. This could have been prevented in Rosas and Alonso' experiment because of their within-subject design and overtraining. In the present experiments, we used between-subjects designs to test training level and retention interval effects upon forgetting of temporal discrimination.

EXPERIMENT 1

In Experiment 1, we tested the effects of four different retention intervals (3, 7, 15, and 20 days) on a temporal discrimination of a 150-s CS acquired in the conditioned suppression paradigm. We expected a greater impairment with longer retention intervals that would be shown as a gradual flattening of the temporal discrimination gradient.

Method

Subjects

Thirty-two naive male Wistar rats provided by Letica Instruments were used. They ranged in ad libitum weight from 328 to 442 g just before the experiment began. They were housed individually in acrylic plastic cages located in an acclimatized room, with constant temperature (23°C) and humidity (50%) that was maintained on a 12-h light/dark cycle with light on at 2:30 AM. The experiment was conducted during the dark portion of the cycle in a room adjacent to the home room. Water was available throughout the experiment. Access to dry food was limited: first, the rats spent 2 days without food, and then they received a food ration daily until they reached 80% of ad libitum weight. This weight level was maintained to the end of the experiment. However, a correction procedure was used to adjust for the natural growth of the rat. All rats were weighed before deprivation started and the proportional deviation from the mean weight was then calculated for each rat. Using the natural growth curve provided by the breeders, the 80% target weight was calculated every week by multiplying 80% of the mean expected weight by the proportional deviation corresponding to each rat.

Apparatus

Eight operant boxes (31 × 25.5 × 33 cm), made by Coulbourn Instruments, were used. The front panel, ceiling, and rear wall were made of aluminum, whereas the side walls and door were made of clear Plexiglas. A food cup (magazine) was 2 cm from the floor in the center of the front wall. An external pellet dispenser delivered 45-mg food pellets (provided by Letica Instruments) into the food cup through a plastic tube. To the right of the magazine was a lever, 6 cm above the floor. Ambient illumination was provided by a 2-lux white incandescent bulb placed 20.5 cm above the food cup. The CS was provided by a speaker located to the right of the bulb, 25.5 cm above the floor. The floor of the box was composed of stainless-steel rods 6 mm in diameter and spaced 1.5 cm apart center-to-center. The floor could be electrified by an AC shock generator. Each box was housed in a sound-attenuating cubicle equipped with a fan that supplied a background noise of 40 dB. All experimental contingencies and response recordings were controlled by a Fujitsu AT microcomputer.

Procedure

Unless noted otherwise all sessions lasted 60 min and were conducted daily.

Pretraining. Rats initially received magazine training sessions. Each session had a maximum duration of 30 min. In each, food pellets were delivered on a variable-time (VT) 60-s schedule while lever press responses were continuously reinforced. Each rat finished magazine training when it made 100 lever press responses.

Training of the lever press response (baseline). Rats received eight further sessions training the lever press response. The lever press response was reinforced with one food pellet on a variable interval (VI) 30-s schedule in the first session. In the remaining sessions, reinforcement was delivered according to a VI 60-s schedule.

Aversive classical conditioning. Thirty on-baseline classical conditioning sessions were then conducted. A rest day and a lever press baseline recovery session (equal to the sessions used to train the lever press response) were inserted after every five sessions. Rats received three conditioning trials per session. On each trial, a 4.5-kHz tone CS (85 dB) was presented five times per second (5 Hz) for 150 s, followed immediately by an electric shock of 0.5 mA and 0.5-s duration. The intertrial interval (ITI) was variable around a mean of 780 s. The first trial appeared at a mean of 600 s after the beginning of the session. At the end of this conditioning, rats were assigned to four

TABLE 1
Mean Conditioned Suppression Ratios Plotted Across Fifths of the CS Duration over the Six Blocks of Five Training Sessions in Experiment 1

Block	1st fifth	2nd fifth	3rd fifth	4th fifth	5th fifth
1	.11	.11	.11	.12	.12
2	.16	.09	.07	.07	.07
3	.29	.20	.15	.12	.12
4	.39	.29	.18	.13	.11
5	.45	.35	.24	.17	.14
6	.48	.38	.25	.20	.17

groups of eight subjects that were matched on the temporal discrimination level in the last trial of training and on the mean rate of lever pressing in the last conditioning session.

Test. Rats then received a test session after a retention interval elapsed. That retention interval was 3 days (Group 3), 7 days (Group 7), 15 days (Group 15), or 20 days (Group 20). During the retention interval, rats rested in their home cages, except for one baseline recovery session (VI 60-s training) given the day before the test session. Food deprivation was maintained through the retention interval. The test session was the same as the conditioning session except that the CS was not followed by the electrical shock. Only data of the first test trial were considered in the analyses.

Data analysis. Lever press responses were recorded and suppression ratios to the CS were calculated. Five ratios were obtained in each trial, each corresponding to successive 30-s fifths of the CS duration. Ratios were computed by the $A/A + B$ formula proposed by Annau and Kamin (1961) with a slight modification. A was the number of lever press responses during a period equal to a fifth of the CS duration, and B was the number of lever press responses during the same duration period in absence of the CS (calculated from the mean response rate during the session when the CS was not on). Suppression ratios were submitted to analyses of variance (ANOVA), and planned comparisons were made using the methods discussed by Howell (1987, pp. 431–443). The rejection criterion was $p < .05$.

Results and Discussion

Table 1 shows the mean conditioned suppression ratios plotted across fifths of the CS duration over the six blocks of five conditioning sessions. Suppression to the whole CS was evident in Block 1, but suppression to the first and second CS fifths decreased in the following blocks, while it remained high and relatively constant in later fifths. A 5 (Fifth) × 6 (Block) ANOVA found a significant main effect of Fifth, $F(4,124) = 217.3$, and Block, $F(5,155) = 89.48$, and also a significant Fifth by Block interaction, $F(20,620) = 61.45$.

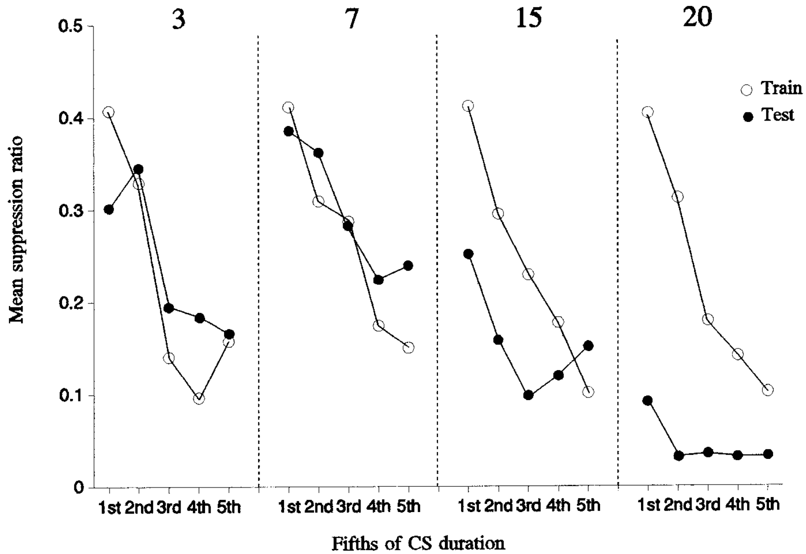

FIG. 1. Mean conditioned suppression ratios plotted across fifths of the CS duration during the last trial of conditioning and the first trial of the test for Groups 3, 7, 15, and 20 (from left to right) in Experiment 1.

Subsequent analyses revealed that the CER was significantly different across fifths of the CS duration in every block except in Block 1, Fs(4,340) $\geqslant$ 24.9. Thus, rats discriminated between the beginning and the end of the CS by 30 conditioning trials. They also revealed that the block simple effect was significant in every fifth, Fs(5,525) $\geqslant$ 11.78, reflecting a general decrease in the suppression level as training progressed. However, rats finally displayed a higher CER toward the end of the CS, from which the acquisition of a reliable temporal discrimination was inferred.

Figure 1 shows the mean conditioned suppression ratios plotted across fifths of the CS duration during the last conditioning trial and the first test trial for Groups 3, 7, 15, and 20 (from left to right). This figure suggests the temporal gradient only changed over the longest retention intervals (15 and 20 days). A 4 (Group) $\times$ 5 (Fifth) $\times$ 2 (Trial) ANOVA revealed a significant main effect of Fifth, $F(4,112) = 31$, and Trial, $F(1,28) = 4.95$. Group by Trial, $F(3,28) = 3.82$, and Fifth by Trial, $F(4,112) = 5.8$, interactions were significant. The rest of factors or interactions were not significant, largest $F(3,28) = 1.4$.

Subsequent analyses run to explore the Fifth by Trial interaction found that the simple effect of Trial was reliable in the first and second fifths, Fs(1,88) $\geqslant$ 5.6, showing a general increase in the suppression to the beginning of the CS during the test. Most important, subsequent analyses conducted to explore the Group by Trial interaction found that the simple effect of Group was significant during the test, $F(3,40) = 3.6$. This effect was found as a higher suppression in group 20 than in groups 3 and 7, Fs$(1,40) \geqslant 5.5$. Thus, between-group comparisons suggested an increase in suppression after the longest retention interval with respect to the shorter ones. According to these results, the Trial effect (training vs test) only was significant in groups 15 and 20, Fs$(1,88) \geqslant 7.4$. So, within-group comparisons suggest an increase in the suppression to the CS 15 and 20 days after training.

Taken together, these results suggest an absence of forgetting of the CS duration 3 and 7 days after training. Forgetting of temporal discrimination appears after 15- and 20-day retention intervals as a flattening of the temporal discrimination gradient. This flattening was caused by an increase in suppression that was stronger at the beginning of the CS. This pattern suggests that forgetting of temporal discrimination leaves the memory of the CS–US excitatory association intact. However, forgetting of the temporal discrimination does not seem enough to explain fully the increase in suppression to the end of the CS found at the 20-day retention interval. We will go back to this issue in the General Discussion. The results of this experiment appear to be entirely consistent with the results reported by Rosas and Alonso (1996) even though on that occasion a repeated-measures design was used. Therefore, the results of this experiment show first that even avoiding the source of memory enhancement provided by the within-subjects design used by Rosas and Alonso (1996), the loss of excitatory CR to the end of the CS found by Hammond and Maser (1970) did not appear here. Second, there was not any evidence of forgetting in the 3- and 7-day tests.

Finally, the mean rates of lever press responding in absence of the CS during the last session of aversive classical conditioning were 38, 38, 40, and 38 responses per minute (rpm) for Groups 3, 7, 15, and 20, respectively. The mean rates during the test session were 30, 24, 27, and 21 rpm for Groups 3, 7, 15, and 20, respectively. None of these differences among groups were significant, Fs$(3,28) \leqslant 1.7$, suggesting that the retention interval did not affect baseline operant responding.

EXPERIMENT 2

The lack of both forgetting of the excitatory CR at the 20-day test and forgetting of the CS temporal discrimination at the 3- and 7-day tests could have been due to an overtraining effect. In Experiment 1, 90 conditioning trials were used but, as Table 1 shows, the CS–US excitatory association was readily acquired, and temporal discrimination was already reliable after only 30 training trials. The additional training might have strengthened the memory, as indeed has been found elsewhere (e.g., Schendel & Hagman, 1982; Sullivan & Wilson, 1993), allowing good retrieval of the CS duration memory after 3- and 7-day retention intervals, and preventing the forgetting of the CS excitatory response after the 20-day retention interval.

The next two experiments were done with the aim of assessing the effect of the amount of training on the retention of the temporal discrimination. Two different levels of training were used (45 and 90 trials). Thus, half of the subjects received the same level of training used in Experiment 1 (90 trials), and half received just one-half of it. If overtraining enhances retention, a reduction in the level of training should produce faster forgetting than that found in previous experiments; forgetting of the CS duration might be found at the short retention interval, while the long one might lead to forgetting of the CS–US excitatory association. In Experiment 2, the effect of these two training levels on the retrieval of the temporal discrimination was tested 3 and 20 days after training.

Method

Subjects and Apparatus

Thirty-two naive male Wistar rats were used. They were provided by Letica Instruments and ranged in ad libitum weight from 173 to 207 g before the experiment began. The animals' upkeep, as well as the apparatus, was the same as described in Experiment 1.

Procedure

Rats received pretraining, training, conditioning, and testing as described in Experiment 1, except for the following. Ten sessions of lever press response training, instead of 8, were run. A 2×2 factorial design was used with aversive conditioning training level (45 vs 90 trials) as one factor and retention interval (3 vs 20 days) as the other. After 15 conditioning sessions (45 conditioning trials), half of the rats were assigned to Group 45 and the other half were assigned to Group 90, matched on the suppression level to the CS fifths at the 45th trial of training. Conditioning finished there for Group 45, but it continued for Group 90 until it received twice the training (a total of 90 trials and 30 conditioning sessions). Once conditioning was finished in each case, a retention interval began. The retention interval was 3 days for half of the subjects in each previous condition (Groups 45/3 and 90/3) and 20 days for the other half (Groups 45/20 and 90/20).

After the retention interval, all rats received a test. In this experiment, testing consisted of daily reacquisition sessions of aversive classical conditioning following the procedure used before. Testing was finished for each group when suppression during the first and last fifth of the CS differed significantly on nine consecutive trials.

Results and Discussion

Figure 2 shows the mean conditioned suppression ratios plotted across fifths of the CS duration during the last trial of training and the first trial of testing for groups 45/3 and 45/20 (left panel) and for groups 90/3 and 90/20

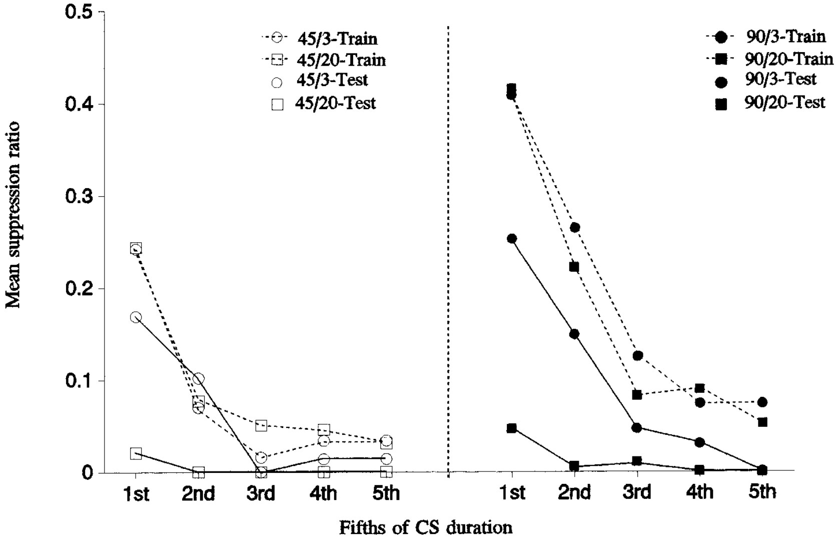

FIG. 2. Mean conditioned suppression ratios plotted across fifths of the CS duration during the last trial of training and the first test trial for groups 45/3 and 45/20 (left) and for groups 90/3 and 90/20 (right) in Experiment 2.

(right panel). The data from the last training trial reflect an improvement in the temporal discrimination in the more trained groups. The first test trial data showed that while the 3-day retention interval had a small effect on the temporal discrimination, the 20-day retention interval clearly impaired it. More important, this effect did not differ essentially as a function of the training level. A 2 (Retention Interval) × 2 (Training Level) × 5 (Fifth) × 2 (Trial) ANOVA found main effects of Training Level, $F(1,28) = 4.8$, Fifth, $F(4,112) = 45.4$, and Trial, $F(1,28) = 17.9$. The Retention Interval by Fifth Interaction, the Training Level by Fifth interaction and the Fifth by Trial interaction were significant, $Fs(4,112) \geqslant 2.5$. Finally, the Retention Interval by Fifth by Trial interaction was also significant, $F(2,112) = 3.25$. None of the other factors or interactions were significant, largest $F(1,28) = 2.98$.

Analyses run to explore the Retention Interval by Fifth by Trial interaction found a significant simple effect of Trial in the first CS fifth independently of the Retention Interval, $Fs(1,78) \geqslant 8.4$, showing a general increase in suppression to the first part of the CS during the test. Also in the first fifth, the simple effect of Retention Interval was significant only during the test, $F(1,78) = 19.4$; the increase in suppression to the beginning of the CS was greater after 20 than after 3 days. The simple effect of Trial at the 20-day test was significant in every Fifth, $Fs(1,112) \geqslant 4.2$, except the last one. Therefore, 20 days after training an increase in suppression to the whole CS except for its final part was found. Finally, the simple effect of Fifth was significant only at the 3-day test, $F(4,112) = 14.5$, showing that temporal discrimination, well conserved 3 days after training, was lost after the 20-day retention interval.

With respect to the main purpose of this experiment, analyses run to explore the Training Level by Fifth interaction found that the simple effect of Training Level was statistically reliable in the first two fifths of the CS, $Fs(1,70) \geqslant 7.8$. Thus, additional training yielded a decrease in suppression to the beginning of the CS that could be interpreted as better temporal discrimination. Most important, as the Training Level by Fifth interaction was independent from either trial or retention interval, the effect of the larger training might be better seen as improvement on learning, rather than as improvement on the memory of the temporal discrimination.

In summary, a general effect of training level was found leading to a better temporal discrimination with longer training. The effect of larger training was evident at both the end of training and during the test. With respect to the effect of the retention interval, this was first shown as an increase in the suppression to the CS onset. While this suggests that some forgetting of the CS duration happened independently of the retention interval, additional analyses show that temporal discrimination is well kept 3 days after training, disappearing 20 days later. This was a consequence of an increase in suppression to the first part of the CS at the 20-day test either with respect to the 3-day test or to the end of training. This increase in suppression led to a flattening in the temporal discrimination gradient similar to that found in Experiment 1.

Baseline responding did not complicate the interpretation of the results. The mean lever press rates during session 15 of aversive conditioning were 22 and 26 rpm for groups 45 and 90, respectively, $F(1,30) < 1$. In the last conditioning session before testing (session 15 for groups 45/3 and 45/20 and session 30 for groups 90/3 and 90/20), the mean rates were 20, 24, 20, and 26 rpm, respectively. Differences among groups were not statistically significant, $F(3,28) < 1$. Finally, during the first session of testing, the mean rates were 19, 19, 22, and 20 rpm for groups 45/3, 45/20, 90/3, and 90/20, respectively. These rates also did not differ, $F(3,28) < 1$.

Reacquisition data. The reacquisition score (number of trials until a reliable difference between the first and last fifths was found in nine consecutive trials—taken as an index of temporal discrimination) for groups 45/3 and 90/3 was 9 (the minimum). Groups 45/20 and 90/20 required 25 and 16 trials, respectively. These data suggest a strong retention interval effect upon temporal discrimination independently of the training level. They also suggest that the training level affected the retrieval of the temporal information only when the test was conducted 20 days after training. Figure 3 shows the mean conditioned suppression ratios plotted across fifths of the CS duration during the first three 3-trial blocks of reacquisition for groups 45/3, 45/20, 90/3, and 90/20. Groups tested at the 3-day retention interval showed better temporal

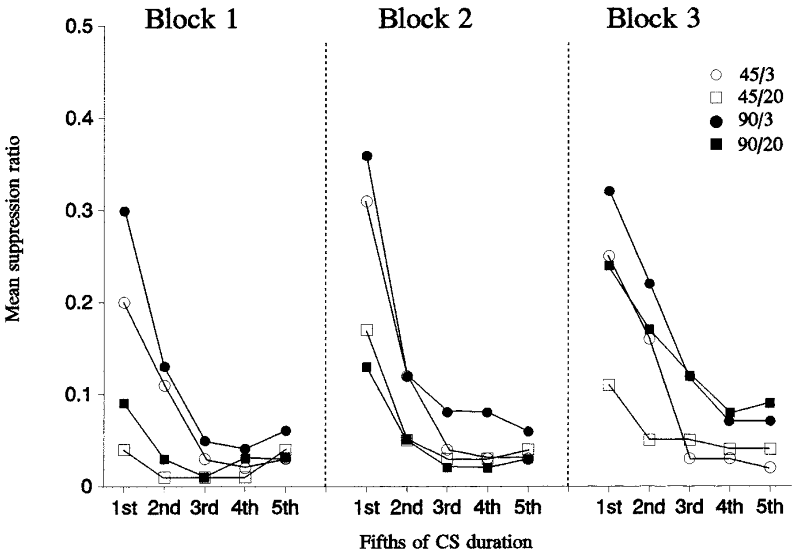

FIG. 3. Mean conditioned suppression ratios plotted across fifths of the CS duration during the first three 3-trial blocks of reacquisition for groups 45/3, 45/20, 90/3, and 90/20 in Experiment 2.

discrimination than groups tested after the 20-day retention interval in the two first 3-trial blocks. The effect of training level was found as a greater suppression to the CS onset in the less trained groups in block 1 independently of the retention interval, and to the whole CS in block 3 in the 20-day test. A 2 (retention interval) × 2 (training level) × 5 (fifth) × 3 (block) ANOVA conducted during the reacquisition phase found significant main effects of Fifth, $F(4,112) = 63.7$, and Block, $F(2,56) = 6.1$. The Retention Interval by Fifth, $F(4,112) = 13.4$, and Fifth by Block, $F(8,224) = 6.13$, interactions were significant. Most important, however, the three-way Training Level by Fifth by Block interaction was significant, $F(8,224) = 2$. This reflects first, that the Training Level effect was evident during the reacquisition and, second, that this effect was independent of the retention interval. None of the other factors or interactions were significant, largest $F(1,28) = 3.9$.

Planned comparisons exploring the training level by fifth by block interaction found a simple effect of Training Level in the first fifth in block 1, and in the first and second fifths in block 3, $Fs(1,120) \geqslant 8$. The simple effect of Block was significant in the first fifth independently of the Training Level, $Fs(2,120) \leqslant 5.3$. However, in the second fifth this effect was only significant in the subjects that received 90 trials of training, $F(2,120) = 13.6$. Conditioned suppression to the second CS fifth decreased during reacquisition only in the most trained subjects. Thus, the analyses conducted during this phase show that the decrease in the suppression level to the beginning of the CS was reestablished slightly faster in the groups that had received more training. This suggests that the temporal memory was more accessible after longer training independently of the retention interval.

In summary, there was practically no forgetting of the temporal discrimination after the 3-day retention interval, while it was complete at the 20-day test. As previously found, an increase in suppression to the first part of the CS gave rise to a clear-cut flattening of the temporal discrimination gradient 20 days after training. This effect was found independent on the training level. However, reacquisition data suggest that temporal memory might have been more accessible for rats that had received additional training, even though the temporal discrimination seemed to be equally forgotten with the two training levels. This better accessibility of the temporal memory probably was a consequence of the better learning shown after longer training, more than just an effect upon the retrievability of the temporal discrimination. Finally, it is worth noting that even reducing the level of training to one-half, there is no trend of loss of the excitatory response to the CS at the 20-day test (cf. Hammond & Maser, 1970).

EXPERIMENT 3

Experiment 2 showed that 3 days after training there was almost no forgetting, and that 20 days later temporal discrimination seems to be completely forgotten. Thus, it is possible that floor and ceiling effects could have made it more difficult to observe a stronger effect of the level of training with these particularly extreme retention intervals. In Experiment 3, we therefore assessed the effect of two intermediate retention intervals: 7 and 15 days.

Method

Subjects and Apparatus

Thirty-two naive male Wistar rats were used, provided by Letica Instruments. They ranged in ad libitum weight from 210 to 250 g before the experiment. Maintenance conditions and apparatus were the same as those described in Experiment 1.

Procedure

Rats received pretraining, training, conditioning, and testing under the conditions described in Experiment 2. However, in this case testing started 7 days after the end of training for half of the animals (Groups 45/7 and 90/7), and 15 days after training for the other half (Groups 45/15 and 90/15).

Results and Discussion

Figure 4 shows the mean suppression ratios plotted across fifths of the CS during the last conditioning trial and the first test trial for groups 45/7 and 45/15 (left panel) and groups 90/7 and 90/15 (right panel). The data from the

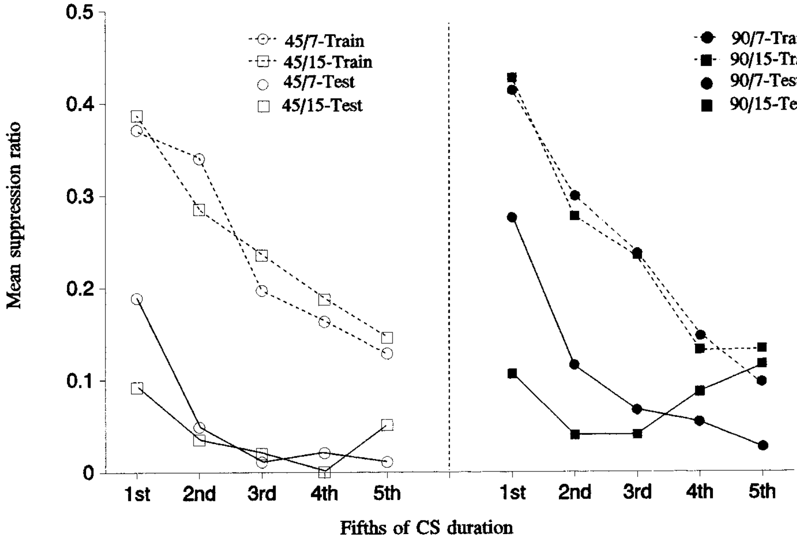

FIG. 4. Mean conditioned suppression ratios plotted across fifths of the CS duration during the last trial of training and the first test trial for groups 45/7 and 45/15 (left) and for groups 90/7 and 90/15 (right) in Experiment 3.

last training trial did not reveal a clear difference in performance as a function of the level of training. Temporal discrimination was attenuated 7 days after training, and practically lost at the 15-day test. This decrement seemed more accentuated for rats that received only 45 trials of training. In addition, a clear increase of the suppression level with the passage of time can be observed in every case. A 2 (Retention Interval) × 2 (Training Level) × 5(Fifth) × 2(Trial) ANOVA found significant main effects of Fifth, $F(4,112) = 36.9$, and Trial $F(1,28) = 30.1$. The Retention Interval by Fifth and Fifth by Trial interactions were also significant, $Fs(4,112) \geqslant 2.7$. It also found a significant Retention Interval by Fifth by Trial interaction, $F(4,112) = 2.5$. The rest of factors or interactions were not significant, $Fs < 1$. This was especially relevant for the main aim of our experiment, since none of the analyses involving the training level were significant.

Analyses run to explore the retention interval by fifth by trial interaction found that the simple effect of Retention Interval was significant during the test in the first CS fifth, $F(1,61) = 6.2$. Thus, suppression to the beginning of the CS was greater at the 15-day test than at the 7-day test. The simple effect of Fifth was significant only at the 7-day test, $F(4,112) = 12.2$, showing that temporal discrimination was lost at the 15-day retention interval. Finally, the simple effect of Trial was significant in every fifth at either the 7-day or the 15-day test, $F(1,112) \geqslant 7.1$, except for the last fifth at the 15-day test, $F(1,112) = 5.4$. Thus, a general increase in suppression was found during

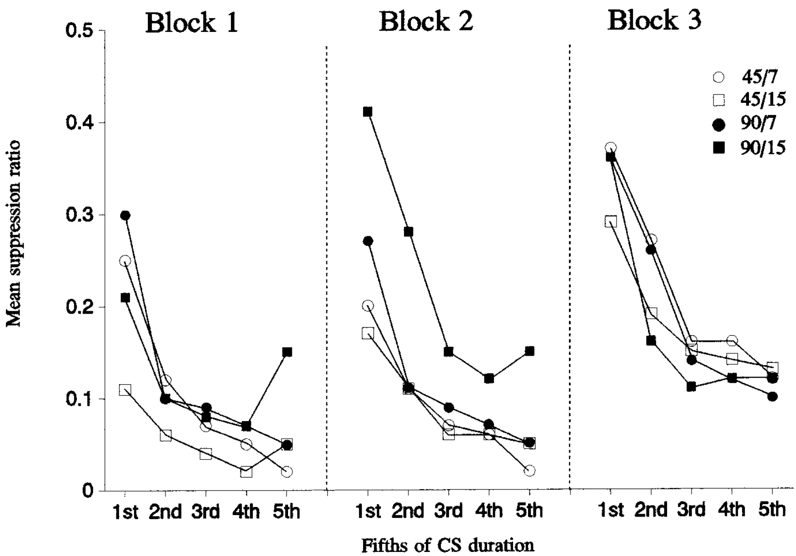

FIG. 5. Mean conditioned suppression ratios plotted across fifths of the CS duration during the first three 3-trial blocks of reacquisition for groups 45/7, 45/15, 90/7, and 90/15 in Experiment 3.

the test after both the 7-day and the 15-day retention interval. However, rats were still able to discriminate the CS duration at the 7-day test. These results confirm and extend those found in Experiment 1, showing that there was little forgetting of the temporal discrimination after a 7-day retention interval and that forgetting of the CS duration was almost complete after 15 days. Once again, the training level effect did not affect the subjects performance during the first test trial.

Finally the mean lever press rates were 24 and 28 rpm for groups 45 and 90, respectively, at session 15 of aversive conditioning, when they were first assigned. The difference was not significant, $F(1,30) < 1$. On the last session of aversive conditioning, the mean rates were 23, 25, 31, and 34 rpm for groups 45/7, 45/15 (session 15), and 90/7, 90/15 (session 30), respectively. Here again differences among groups were not significant, $F(3,28) < 1$. The mean response rate during the first session of the test was 17, 15, 32, and 24 rpm for groups 45/7, 45/15, 90/7, and 90/15, respectively, $F(3,28) < 1$. Thus, the differences found during the test cannot be attributed to changes in the operant level of baseline responding.

Reacquisition data. The reacquisition score for group 45/7 was 13, while it was just 9 (the minimum) for group 90/7. In the same way, groups 45/15 and 90/15 required 19 and 10 trials, respectively. These data suggest that the reestablishment of the temporal discrimination was slower after the short training. Figure 5 shows the mean conditioned suppression ratios plotted across fifths of the CS duration during the first three 3-trial blocks of reacquisition for groups 45/7, 45/15, 90/7, and 90/15. Groups receiving long training showed better performance than the others during the first two 3-trial blocks. This was specially true in groups tested 15 days after training. A 2 (Retention Interval) × 2 (Training Level) × 5 (Fifth) × 3 (Block) ANOVA conducted during the reacquisition phase found significant main effects of Fifth, $F(4,112) = 71$, and Block, $F(2,56) = 17$. The Retention Interval by Fifth, $F(4,112) = 2.6$, Retention Interval by Block, $F(2,56) = 5.1$, and Fifth by Block, $F(8,224) = 2.1$, interactions were significant. Most important for the main purpose of this experiment, the Training Level by Fifth, $F(4,112) = 2.5$, and Training Level by Block, $F(2,56) = 4.9$, interactions were significant. The Retention Interval by Fifth by Block interaction was also significant, $F(8,224) = 4.2$. The rest of the factors or interactions were not, largest $F(1,28) = 1.9$.

Planned comparisons run to explore the Training level by Block interaction found that the simple effect of Training Level was significant in block 2,$F(1,48) = 5.4$. Finally, analyses run to explore the Training Level by Fifth interaction found that the simple effect of Training Level was significant in the first Fifth, $F(1,48) = 7$. Thus, the Training Level effect was mostly detected at the very beginning of the CS duration and, as it was found in Experiment 2, reflected easier reacquisition of the temporal discrimination after longer training. Indeed, in this experiment the Training Level effect was only found during the reacquisition phase, and not at the end of conditioning; so, although smaller than in Experiment 2, it seems to be reflecting a memory enhancement more than a learning improvement.

Taken together, the results obtained in this experiment suggest a greater forgetting of temporal discrimination 15 days after training than 7 days after it. Actually, there seems to be no effect of the 7-day retention interval upon temporal discrimination, confirming the results obtained in Experiment 1. Unlike in Experiment 1, the 7-day retention interval led to a general increase in the suppression to the CS. It is not clear what the reason for this difference could be. Group 90/7 in this experiment, and group 7 in Experiment 1 received identical treatment, and thus, there was no reason to expect differences between them. However, this experiment shows that a general increase in conditioned suppression over time might be a feature of this procedure, and thus deserves an explanation. We will go back to it in the general discussion.

With respect to the effect of training level, it was quite small and detected only in the reacquisition phase. Temporal memory seems to be slightly more accessible after longer training. However, training level did not affect the way forgetting happened. Independently of the amount of training, forgetting was always found as a general increase in the suppression that was more intense to the beginning of the CS, accompanied by a flattening of the temporal gradient.

GENERAL DISCUSSION

In all three experiments, rats were able to discriminate the duration of a 150-s CS followed by an electrical shock. Rats displayed initially a strong

CER of fear to the whole CS. As training progressed, conditioned suppression gradually decreased, this decrease being greater at the beginning of the CS. At the end of training, conditioned suppression to the beginning of the CS was negligible, increasing gradually toward the end of it. Temporal discrimination was inferred from this pattern (see Rosas & Alonso, 1996). Unlike in our previous findings, some of these experiments found that the developing of temporal discrimination was accompanied by a decrease in conditioned suppression to the end of the CS (see also, Rosas & Alonso, 1997). This decrease is similar to the one known to occur in conditioned suppression after prolonged training, and it is usually attributed to habituation of fear (e.g., Annau & Kamin, 1961; Hendry & Van-Toller, 1965; Millenson & Dent, 1971).

Most important, tests run following different retention intervals found a gradual increase in conditioned suppression that was greater at the beginning of the CS. This led to flattening of the temporal discrimination gradient, suggesting that rats forgot the CS duration. Although forgetting depended clearly on the retention interval, there was relatively little evidence that it was affected by the amount of previous training. Thus, slight or no forgetting was observed 3 and 7 days after training and forgetting of the temporal discrimination was clear 15 days later, and maximum at the 20-day test. These results did not change when the number of conditioning trials (45 vs 90) was varied in different groups. However, reacquisition of temporal discrimination was slightly faster after larger original training.

These results replicate and extend those reported by Rosas and Alonso (1996). Forgetting of temporal discrimination was not accompanied by the loss of conditioned suppression to the CS found by Hammond and Maser (1970). In fact, conditioned suppression increased over time. Thus, CS excitatory properties did not seem to be forgotten here, in a situation where its retrievability has not been so enhanced by overtraining on the temporal discrimination, and/or the reacquisition sessions of a repeated-measures design. This pattern of results seems quite similar to that found in the literature using FI (e.g., Gleitman & Bernheim 1963) or DRL reinforcement schedules (Lejeune, 1989) in rats. It is also consistent with forgetting found in discrimination of other stimulus dimensions. For instance, in the case of the wavelength discriminations (e.g., Thomas, 1981; Thomas & Lopez, 1962), subjects show greater generalization (responding more to a wavelength that has never been reinforced) as retention interval increases, which suggests that they forgot which specific wavelength was initially reinforced (see also, Hall, 1991; Riccio, Ackil, & Burck-Vernon, 1992; Riccio, Rabinowitz & Alxerod, 1994; Riccio, Richardson, & Ebner, 1984). Similarly, rats here seem to have forgotten what part of the CS duration is immediately followed by the US, showing a similar level of response to different temporal parts of the CS. In short, rats forgot the time, but not the CS–US excitatory relationship.

The general increase on conditioned suppression found in these experiments with the retention interval, however, deserves further consideration. It might be claimed that the results obtained here were caused by an increase in the suppression to the CS that is not specific to the beginning of if. For instance, these results may reflect simple incubation of fear over time (e.g., Pinel & Cooper, 1966). This might have yielded an increase in general suppression that would be better detected at the beginning of the CS because suppression was lower there. Following this argument, it could be claimed that rats might be keeping the memory of the CS duration, but that a ceiling effect in suppression would impede its detection. An explanation in terms of incubation of fear cannot be completely ruled out based in the results of our experiments. However, there are several reasons that suggest simple incubation would not be enough to explain the results from these experiments.

First, although some studies have found incubation of a weak aversion over time in other conditioning situations (e.g., Batsell & Best, 1994; Pinel & Cooper, 1966; but see, Rosas & Bouton, 1996, Experiment 3), studies conducted with the conditioned suppression technique show no changes in mild suppression over time (e.g., Gleitman & Holmes, 1967; Hendersen, 1985). Thus, according to the literature simple incubation of conditioned suppression seems unlikely (see Spear & Riccio, 1994, for a review). At any rate, incubation does not suffice to explain the results reported here. Incubation could account for the general increase in conditioned suppression but it cannot account in a simple way for the beneficial effect of overtraining upon reacquisition of the temporal discrimination. However, this effect is readily explained by assuming that temporal discrimination was forgotten, and that this forgetting was weaker when the memory of the temporal discrimination was strengthened through overtraining.

These results could be alternatively explained if the instrumental response (lever press) would become more easily disrupted by the retention interval. According to this account, the animals could remember the CS duration after the retention interval, but a greater disruptability of the lever press response would impede detection of such temporal discrimination. Again, the disruptability of the lever press response would be more easily detected at the beginning of the CS, as this was the part where the rat was responding more. However, this account seems unlikely. Baseline lever press responding in the absence of the CS did not change over the retention interval. Furthermore, the test took place one day after a baseline training session of the instrumental response that would have attenuated any weakening of the lever press responding over the retention interval.

In order to explain the fear increase over time found here within a learning theory framework it should be noted that rats learned first the CS–US excitatory association, and that the conditioned fear response from which this association is inferred was gradually hidden by the developing of a temporal discrimination (Rosas & Alonso, 1996)—or by an inhibitory association between the beginning of the CS and the US (Pavlov, 1927)—that counteracted the excitatory association developed earlier. It seems likely that this process, combined with the fear habituation process mentioned earlier, yielded the general decrease in conditioned suppression that often accompany the developing of the temporal discrimination gradient (e.g., Rosas & Alonso, 1997). Temporal discrimination (e.g., Gleitman & Bernheim, 1963; Lejeune, 1989; Rosas & Alonso, 1996) and habituation (e.g., Carew, Pinsker, & Kandel, 1972; Hall & Schachtman, 1987; Leaton, 1974) both have been shown to be attenuated by retention intervals. Thus, it seems reasonable to think that the increase of conditioned suppression or fear over time was due to forgetting of the temporal discrimination combined with spontaneous recovery of habituated fear.

Most interesting, these arguments place the explanation of the phenomenon within the scope of modern theory of interference and forgetting (e.g., Bouton, 1993, 1994). This theory would explain the faster forgetting of temporal discrimination because it was learned in the second place and/or because it was caused by the developing of an inhibitory association between the CS onset and the US (Pavlov, 1927) that counteracted the CS–US excitatory association learned earlier. From this point of view, the memory for both second-learned information and inhibition is considered more easily affected by the retention interval than the memory for information learned in the first place and/or information about the CS–US excitatory association (e.g., Bouton, 1993). A similar argument can be used to explain why habituation of fear (acquired second) is forgotten while the memory about the excitatory properties of the CS is not.

Finally, the effect of training level, although weak, pointed in the same direction as that obtained in other situations (e.g., Schendel & Hagman, 1982; Sullivan & Wilson, 1993), suggesting a better memory after overtraining. Indeed, the training level only affected either the strength of the discrimination or the rate of reacquisition but did not affect the way forgetting occurred.

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Received February 26, 1997
Revised April 22, 1997