EXTINCTION, REINSTATEMENT AND SPONTANEOUS RECOVERY OF CONDITIONED SUPPRESSION IN RATS AND THE STABILITY OF CONDITIONED INHIBITOR PERFORMANCE

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Abstract. Acquisition of the suppressive properties of a conditioned stimulus (CS), terminating with shock, and of the attenuating properties of the conditioned inhibitor (CI), signaling that shock will not be given at the end of the CS, proceed at similar rates. The attenuation of suppression exerted by the CI and the decrement of the suppression due to extinction differed in both the time course and the distribution of bar-presses within the CS duration. After extinction the CS still retained some suppressing properties, which were limited to the last 20–30 s, whereas the CI attenuated the suppression throughout the duration of the CS. Extinction of the suppressive properties of the CS did not change the attenuating properties of the CI. The recovery of the suppressive properties of the CS after presentations of unsignaled reinstating shocks or after a long pause in experimental sessions was incomplete and short-lasting. Both procedures did not change the properties of the CS presented in compound with the CI.

INTRODUCTION

It has been shown (22) that the properties of a conditioned stimulus (CS), paired with the unavoidable shock, to suppress on-going bar-pressing for food may be completely removed when the CS is presented on
the background of a conditioned inhibitor (CI). In contrast to Hendry's experiments (6), we followed a Pavlovian procedure, since the conditioned defensive response was established first and only then did conditioned inhibitor training follow. Thus, conditioned inhibitor training consisted of presentation in predetermined order of two kinds of trials, both on a baseline of barpressing for food, reinforced according to a 2.5 min variable interval (VI) schedule. For one type of trial offset of the CS, used previously during conditioned suppression training, coincided with a brief unavoidable shock resulting in pronounced suppression of the on-going bar-pressing behavior. On other trials, the same CS was preceded and accompanied by a stimulus of another modality, and this compound was never paired with shock. After only brief training, the CS presented on the background of the CI lost nearly all of its suppressing properties, whereas the same CS presented alone exerted a stable suppressing effect on the on-going bar-pressing. The rates of acquisition of the suppressive properties of the CS and of the attenuating effects of the CI were similar, suggesting that two independent learning processes were involved acquiring of these two opposite modes of responding to the CS and to the CI.

However, it seems that the CI attenuating effect on the suppressing properties of the CS was related to the intensity of the CS. In full agreement with previous studies (11–14, 23, 27), we found (22) that the conditioned suppression was acquired earlier and suppression was more pronounced in a group trained with a more intense CS (70 dB white noise) than in a group in which the CS was less intense (50 dB white noise). At the beginning of inhibitory training, the attenuation of suppression was greater in a group trained with the less intense CS and less pronounced when the more intense CS was used. However in contrast to the first day, inhibitory training gradually became more effective in rats trained with the more intense, 70 dB CS. There was a tendency to reach the criterion of inhibitory training earlier in rats trained with the 70 dB CS than in rats trained with the 50 dB CS. Moreover, a statistically significant enhancement of barpressing was observed only during the CI + 70 dB CS presentations (22). This last result suggests that the efficiency of conditioned inhibitor training is related to the strength of the conditioned response on the basis of which the inhibitor has been established.

The aim of the present experiment was to test whether changes in the suppressive properties of the CS exert parallel effects on the efficiency of the conditioned inhibitor. The classical defensive response, strength of which was measured by suppression of the on-going barpressing for
food, was weakened using an extinction procedure, then reestablished using several experimental procedures, and the effects of the conditioned inhibitor were tested at the same time.

MATERIAL AND METHODS

The experiment was conducted on 15 experimentally naive, male hooded rats, approximately 3 months old at the beginning of the training. Training was conducted in four identical operant chambers, each having an electrifiable grid floor, a single bar on one of the walls and a food-tray under it. A pilot light centered on the top of the back chamber wall provided illumination in the vicinity of the bar equal to $205 \pm 5$ lx. Equipment for automatic programming and recording of the experiment was located in an adjoining room.

**Table I**

Overview of the experimental procedure

<table>
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<tr>
<th>Days of experiment</th>
<th>Stages of experiment</th>
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<td>1–14</td>
<td>Reduction of the body weight</td>
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<tr>
<td>15–19</td>
<td>Barpressing training</td>
<td>-</td>
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<td>Pretest-day</td>
<td>$4 \times CS$,</td>
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<td>21–26</td>
<td>CER acquisition</td>
<td>$4 \times CS + US$,</td>
</tr>
<tr>
<td>27–32</td>
<td>CI acquisition</td>
<td>$4 \times CS + US, 4 \times CI + CS.$</td>
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<td>33–39</td>
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<td>The 1st test session</td>
<td>$4 \times CS, 4 \times CI + CS$,</td>
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<td>Reinstatement procedure</td>
<td>$4 \times US$,</td>
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<td>42</td>
<td>The 2nd test session</td>
<td>$4 \times CS, 4 \times CI + CS$,</td>
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<td>43–48</td>
<td>CER and CI retraining</td>
<td>$4 \times CS + US, 4 \times CI + CS.$</td>
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<td>49–54</td>
<td>CER extinction (the 2nd series)</td>
<td>$4 \times CS$.</td>
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<td>55–84</td>
<td>Pause in experiment</td>
<td>-</td>
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<td>85–87</td>
<td>The 3rd–5th test sessions</td>
<td>$4 \times CS, 4 \times CI + CS.$</td>
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All the rats were subjected to the same experimental procedures in this extended experiment lasting nearly 3 months. Table I contains an outline of the consecutive stages of the experiment, which may be described as follows.

Prior to any training the rats were reduced to 75% of their ad lib. body weight and maintained at that weight on a 24 h feeding rhythm during all stages except for the pause in the experiment. The first day of barpressing training consisted of initial presentations of 40 “free” 45 mg food pellets on a 1-min VI schedule (magazine training), followed
immediately by a period with a continuous reinforcement schedule until 120 food pellets were delivered in a single session. After this, all consecutive sessions lasted 2 h, and the barpresses were reinforced according to 2.5-min VI food reinforcement schedule both during and between trials. The Pretest day (P-day) consisted of four presentations of a sporicadic CS, a 3 min period of 80 dB (re: 20 μN/m²) white noise. The CS was presented alone with onsets at 14.0, 49.0, 70.0, and 89.5 min after the beginning of the session. The next stage of the experiment consisted of six conditioned emotional response (CER) training sessions, when each 3-min presentation of the CS was terminated with 1 s of scrambled electric shock of 2-mA intensity. Onsets of the CS were at the same time as during P-day. During CI training the number of CS presentations was increased to eight with additional CS onsets at 24.5, 38.5, 59.5, and 101.5 min after the beginning of the 2-h session. Four presentations of the CS terminated with a 1 s 2-mA shock as in the previous stage (CS+ trials), whereas four others were preceded by offset of the house light one min before the CS onset and terminated without shock but together with the onset of the house light (CI + CS trials). The order of CS+ and CI + CS trials was changed each day in predetermined arrangements (see 11) in which the same kind of trials was never given more than twice in a row.

The procedure of CER extinction lasted 7 days and was very similar to that during the P-day: four 3-min periods of CS presentations, each without shock. The only difference was the times of CS presentations with the CS onsets at 14, 48, 71 and 79 min after beginning of the session.

Two kind of trials were given during the test sessions. In each test session there were four presentations of the CS not accompanied by shock and four regular CI + CS trials given always according to the arrangement: CS; CI + CS; CI + CS; CS; CI + CS; CS; CI + CS. Between the 1st and the 2nd sessions the reinstatement procedure was employed. It consisted of one session in which unsignaled shock of 1 s duration and 2-mA intensity was given four times in the last second of the 16th, 50th, 73rd, and 81st min after the beginning of the session.

After the 2nd test session the CER and CI were retrained and then the CER was extinguished for the second time. The CER and CI retraining lasted 6 days with the same procedure as during the conditioned inhibitor training. The second series of extinction sessions was exactly the same as the first one, except for duration which was six days. Then a 30 days pause in the experiment followed. Rats were in their home cages except for short periods necessary for daily weighing. During the first 20 days of the pause, subjects had free access to food all the time,
and during the last 10 days of the pause the 2 h feeding was renewed resulting in a gradual reaching of the 75% ad lib. body weight by the end of the pause. After the pause there were given three test sessions using exactly the same procedure as described above.

During all experimental sessions the numbers of bar-presses emitted in consecutive 30 s periods were collected and served as the main measure of behavior. The magnitude of changes in bar-pressing rate during the CS+ trials was measured by computing the “suppression ratio”, B/(A + B), where B represented the number of bar-presses emitted during the 3 min action of the CS and A was the number of responses during the 3-min period immediately before the CS onset. For CI + CS trials two ratios were computed, and in both cases A was the number of responses during the 3-min period immediately before the CI onset, i.e., before the offset of the house light. The effect of darkness, the CI, was measured by 3B1/(A + 3B1) ratio, where B1 represented number of bar-presses emitted during the one minute from house light offset to CS onset. The joint action of CI + CS was measured by the B/(A + B) ratio, similarly as on CS+ trials. Ratios were computed independently for each subject and trial. Additionally, daily ratios were calculated by summing the numbers of responses emitted during corresponding periods of time for all CS+ or CI + CS trials.

In some cases the suppression ratios calculated for CS+ trials were compared with so called “dummy” CI + CS suppression ratios. The “dummy” suppression ratios were estimated for periods in which no sporadic stimuli were presented but the CI + CS should be given according to the predetermined arrangements described above. Such comparisons provided additional control as to whether changes of the on-going barpressing during the presentation of the extinguished CS differ from fluctuations of the bar-pressing rate in the intertrial intervals. The method employing “dummy” suppression ratios was introduced in early CER studies by Kamin (13).

The measures used in CER studies are not always normally distributed. Thus nonparametric tests were always used parallelly to ANOVA tests. For the same reason medians were preferred over means for the illustration of the group data.

RESULTS

Pretest day. As seen from Fig. 1, introduction of the 70 dB CS exerted negligible effect on the on-going bar-pressing behavior, which was confirmed by the lack of any statistically significant changes between
the number of bar-presses before and during the action of the CS presented during this stage of the experiment.

*CER acquisition.* Inspection of Fig. 1 indicates that during the first day of CER training CS offset and shock presentations resulted in a decrease of the response rate. On each consecutive trial the bar-pressing rate was lower both during the CS+ action and during intertrial intervals. This effect was rather longlasting and as seen from Fig. 2 not only during the CER training but also in subsequent stages of the experiment, the frequency of bar-presses emitted during the A periods was lower than that during *P*-day. An analysis of variance of the number of bar-presses emitted by each subject during the four A periods of a given day (*P*-day and six CER training days) showed a significant day effect ($F_{6/84} = 8.05, P<0.001$) and further Duncan tests.
revealed differences between the P-day and all CER training days ($P_s < 0.01$ for all comparisons).

Comparisons of the numbers of bar-presses emitted in A and B periods of each trial independently showed that the CS evoked statistically significant suppressions of the on-going behavior on all trials of the 2nd through the 6th days of training inclusively. As seen from

![Fig. 2. Median numbers of bar-press responses per min emitted in pre-CS periods during all consecutive stages of the experiment.](image)

Fig. 2, in which daily suppression ratios are presented suppression in this experiment was not complete. Comparisons of daily scores for A and B periods showed that for each of the 2nd-6th days of CER training

![Fig. 3. Median daily suppression ratios during P-day, CER acquisition, CI acquisition and CER extinction (1st series). Filled circles denote the 80 dB white noise CS presented with the shock US, while open circles denote the same stimulus presented without the shock US, and triangles indicate the offset of the house light. The effects of 1 min CI action (triangles) were measured by the ratio, $3B_t/(A+3B_t)$, and the effects of 3 min CS (circles) and CI+CS (circles with triangle) actions were measured by the ratio, $B/(A+B)$. Crosses below the marks for daily ratios denote suppression significant at $P_s < 0.05$ or better, as estimated by the A-B comparisons (Wilcoxon tests).](image)
the decrease of bar-pressing rate during the CS presentations was significant \((P_s<0.01\) for each comparison, Wilcoxon matched-pairs two-tailed tests). An analysis of variance showed a significant day effect on daily suppression ratios \((F_{5/70} = 28.73, P<0.001\). Duncan tests confirmed differences between the first and all others days of CER training, between the 2nd and the 3rd day, and between the 2nd and the 5th day \((P<0.01\) for all comparisons). The pronounced suppressing effect of the CS during the last day of CER acquisition is readily seen from Fig. 4.

![Graph](image)

**Fig. 4.** Median numbers of bar-press responses emitted in consecutive 30 s periods before, during, and after each presentation of the CS+ and the CI+CS during the last day of CER acquisition and the first day of CI acquisition. Left panels show data for the last day of CER acquisition. Middle and right panels illustrate data for the first day of the CI acquisition. Numbers in circle denote consecutive trials within the session.

**CI acquisition.** As seen from Fig. 4 the first instance of the house light offset decreased the rate of the on-going bar-pressing behavior. This effect was significant as shown by the comparison of the number of bar-presses emitted before and during the 1 min action of the CI \((P<0.01,\) Wilcoxon test). As far as the next CI + CS trials are concerned, the unconditioned suppressing effect of the house light offset was much
weaker, not significant on the second and absent during the last trials of the 1st day of CI acquisition. The disruptive effect of the house light offset was restored during the first CI + CS trial of the 2nd day of CI acquisition (P<0.05, the same test) and then was never observed until the very end of the experiment. On the contrary, toward the end of the CI acquisition stage the house light offset tended to accelerate the barpressing rate, however, during this stage of the experiment this effect was not significant statistically.

The introduction of CI + CS trials resulted in the attenuation of the suppressing effect of the 80 dB white noise CS. These changes were minimal for the CS+ trials, when the CS together with shock US were presented and during the CI acquisition stage each CS+ trial denoted significant suppression of the barpresses (Ps<0.01 in all A-B comparisons, Wilcoxon test). When presented on the CI background the same CS also resulted in significant suppression on most of the trials. Non-significant differences between the number of bar-presses emitted before the CI + CS trial and during the action of the CI + CS compound were observed in two trials during the 3rd, in one trial during the 4th, in one trial during the 5th and in two trials during the 6th day of the CI acquisition. The smallest differences in suppressing properties of the CS presented alone and in compound with the CI and the largest trial-to-trial variability was observed during the first session of the CI acquisition stage of the experiment (Fig. 4). However, as seen in Fig. 3, during the 2nd–6th days of inhibitory training there were observed marked differences in the magnitude of the suppressing effect of the CS between the CS+ and CI + CS trials. Except on the first day of CI acquisition, daily suppression ratios computed for the CS+ trials and for the CI + CS compound differed significantly (Ps<0.01, Wilcoxon tests). Collectively, these data indicate rapid acquisition of the attenuating properties of the conditioned inhibitor.

A two-way analysis of variance of the daily suppression ratios showed significant effect from stimuli used (F223 = 57.35, P<0.001), but a lack of the progressive day effect (F370 = 2.17, P<0.05). A significant interaction of the two main effects (F10140 = 2.65, P<0.01) was clarified by Duncan tests showing that, except of the first day of CI acquisition, all daily suppression ratios for the CS+ trials were different from all suppression ratios for the CI and the CI + CS presentations (Ps<0.01).

It is reasonable to ask whether changes of the on-going behavior in the three stimuli situations (CS+, CI alone, and CI + CS compound) were related each other. To solve this problem the Kendall tau correlation coefficients between the daily suppression ratios for each pair of
stimulus situations were estimated for each day independently. A positive correlation coefficient would indicate that during a given session suppression ratios calculated for individual subjects maintained their ranks in the two stimulus situations. A negative correlation coefficient would indicate that these indices for individual subjects have opposite ranks within the group for the two stimulus situations compared. This analysis showed that there was a positive correlation in responding during the CS$^+$ and the CI + CS compound during the first ($P<0.05$) and the second ($P<0.01$) sessions of CI acquisition. All other correlation coefficients were nonsignificant. These comparisons indicate that only at the very beginning of training was the discrimination between the CS$^+$ and CI + CS weak or, more precisely, that during the 1st and the 2nd days of conditioned inhibitor training, the individual variability in responding to the CS$^+$ and CI + CS presentations was controlled by the same rule.

**CER extinction (1st series).** The course of the CER extinction in terms of daily suppression ratios is shown in Fig. 3. In spite of marked changes in values of the daily suppression ratios, the CS suppressed significantly the on-going bar-pressing behavior ($P<0.01$ for the 1st–6th extinction sessions, $P<0.05$ for the 7th extinction session, A-B comparisons, Wilcoxon tests). However, an analysis of variance showed significant day effect on daily suppression ratios ($F_{6/84} = 19.39$, $P<0.001$). Further Duncan tests revealed a gradual weakening of the suppressing properties of the CS presented without shock US. Daily suppression ratios for the 1st CER extinction day differed from those for the 3rd–7th days at $P<0.01$. The ratios for the 2nd CER extinction day differed from those for the 4th day at $P<0.05$ and from those for the 5th–7th days at $P<0.01$. The ratios for the 3rd day differed from those for the 5th day at $P<0.05$ and from those for the 6th and 7th days at $P<0.01$. The ratios for the 4th day differed from those for the 6th and the 7th days at $P<0.01$ each. The ratios for the 5th day differed from those for the 7th day at $P<0.01$. No other comparisons were significant.

Similar gradual weakening of the suppressing properties of the CS was observed when each consecutive CS presentations instead of daily suppression ratios were analyzed. As seen from Fig. 5 at the beginning of the CER extinction each presentation of the CS resulted in significant suppression of bar-pressing, whereas during the 6th and the 7th extinction days only in single trials the suppression was statistically significant.

The weakening of the suppressive effects of the CS was also shown by the comparisons of the daily suppression ratios estimated for the CS and for periods in which the CI + CS should be given using the “dummy” suppression ratios described in the Methods. Comparisons of suppression
ratios for the CS and the "dummy" CI + CS periods showed significant differences during all extinction days except for the 7th sessions ($P_s < 0.01$ for the 1st, 2nd, 3rd, 5th days, $P < 0.02$ for the 4th day, $P < 0.05$ for the 6th day; Wilcoxon tests).

The lack of the significant changes in barpressing rate during the last extinction day was additionally showed by an analysis employing the Kendall tau correlation coefficient similar to that used for the CI acquisition stage. Again in this case comparisons were done between daily suppression ratios estimated for the CS and "dummy" suppression ratios for time periods in which the CI as well the CI + CS should be given. Correlation coefficients between suppression ratios for the "dummy" CI and CI + CS periods have positive relationships reaching significance on the 1st, 3rd, 7th extinction days ($P_s < 0.01$) and at the 4th extinction day ($P < 0.05$). These significant correlation coefficients indicate that individual subjects maintained their ranks in different periods of the session as far as the rate of the baseline on-going barpressing behavior was concerned. During the 7th CER extinction day, and only during this day, suppression ratios for the CS presentations and for the "dummy" CI periods, as well as for the CS presentations and for the "dummy" CI + CS periods also showed such positive correlations ($P < 0.01$ in the first and $P < 0.05$ in the second case). All of these analyses clearly indicate that by the 7th CER extinction session the CS lost its suppressing properties nearly completely.

The 1st and the 2nd test sessions. The procedure used during the test sessions was a continuation of CER extinction procedure since the CS was presented without the shock US, however the CI + CS trials were reintroduced. Comparison of the daily suppression ratios for the CS

Fig. 5. Median suppression ratios for each presentation of the 80 dB white noise CS during seven consecutive sessions of the CER extinction (1st series) of the experiment. Denotations as in Fig. 3.
presentations during the last, 7th day of CER extinction, and during the 1st test day showed no differences (Wilcoxon test). Reintroduction of the CI + CS trials exerted some immediate effect on the rats' behavior. Comparisons of the number of responses emitted before and during the action of the CS during the 7th extinction session showed significant suppression (P<0.05, A-B comparison for daily scores, Wilcoxon test). A similar comparison for the 1st test day showed significance on the P<0.01 level, indicating that in spite of the lack of change in the magnitude of daily suppression ratios, the suppressing effect of the CS was more systematic during the 1st test day than during the last day of extinction. The analysis employing the Kendall tau correlation coefficient revealed that already during the 1st test day, the subjects were reacting to the CS and CI + CS situations differently. The significant positive correlation between ratios estimated for the CS and "dummy" CI + CS periods observed during the 7th extinction session was lost during the 1st test day. Similarly, there was no significant correlation between the CS and CI periods, whereas the correlation between the CI and the CI + CS periods remained significant at the P<0.01 level.

Much more interesting are the comparisons between the 1st and the 2nd test days, since they should reflect the effects of the unsignalled reinstating shock applications in the intervening session. Daily suppression ratios for each stimulus situation during the 1st and the 2nd test days are presented in Fig. 6. Comparisons of the daily suppression ratios for a given stimulus situation showed that only the ratios for the CI + CS compound were different due to the increase of the ratio value during the 2nd test day (P<0.02, Wilcoxon test). An analysis of variance based on daily suppression ratios showed the effect of stimulus situations (F_{2/28} = 9.22, P<0.001), but no effect of days. The interaction of the two main effects was significant (F_{2/28} = 8.33, P<0.01), and the Duncan tests revealed significant differences between suppression ratios for the

![Fig. 6. Median daily suppression ratios during the 1st and the 2nd test days. Denotations as in Fig. 3.](image-url)
CS and the two situations involving the conditioned inhibitor (P<0.01 in both cases). The two daily suppression ratios for the CS differed (P<0.05), as did the two daily suppression ratios for the CI + CS compound. There was no significant difference between two daily suppression ratios estimated for the CI periods.

Due to the decrease of the value of the suppression ratio for the CS and the increase of the value of the suppression ratio for the CI + CS compound, daily suppression ratios for these two stimulus situations differed during the 2nd test day (P<0.01, Wilcoxon test). During the 1st test day the difference between these stimulus situations was not observed.

All of these analyses indicate that unsignalled shocks resulted not only in the reinstatement of the suppressive effect of the CS, but also in some increase of the attenuating effect of the CI. In fact, during the 2nd test day the value of the daily suppression ratio calculated for the CI + CS compound exceeded the 0.5 level, indicating enhancement of on-going bar-pressing behavior. This enhancement was, however, not statistically significant (A-B comparison, Wilcoxon test).

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Fig. 7. Median numbers of bar-press responses emitted in consecutive 30 s periods before, during and after each presentation of the CS+ (left panels) and the CI+CS compound (right panels) during the first day of the CER and CI retraining. Denotations as in Fig. 1.
The correlation between suppression ratios for the CS and the CI, similarly as for the CS and the CI + CS compound, was very small and the tau correlations coefficients were close to zero. Conversely, the scores for the CI and the CI + CS were positively correlated ($P<0.01$). In this respect, the behavior of animals was similar to that observed at the beginning of the CER extinction stage of the experiment.

Fig. 8. Median daily suppression ratios during CER and CI retraining and CER extinction (2nd series). The "v" above the mark for daily CI ratio denotes acceleration of bar-presses significant at $P<0.05$, as estimated by the A-B comparisons (Wilcoxon test). Other denotations as in Fig. 3.

Finally, it should be mentioned that unsignalled reinstating shocks had no effect on the on-going bar-pressing responding (Fig. 2). The comparison of the daily A scores showed that response rate in the 1st and in the 2nd test sessions did not differ (Wilcoxon test).

**CER and CI retraining.** The behavior of rats during the first day of retraining is shown in Fig. 7 whereas the general course of retraining is illustrated in Fig. 8. Data presented in both Figures indicate rapid reacquisition of previously trained behavior, which was not completely lost during extinction and then was partially renewed due to the reinstatement procedure. Nevertheless, the first presentation of the CS$^+$, until the actual shock application, was still a continuation of the extinction procedure. The value of the suppression ratio for the first CS$^+$ presentation of this stage was higher than these for the first and the fourth CS presentations during the 2nd test day denoting weaker suppression of the on-going barpressing. Comparisons of these values revealed significant difference between the last CS presentation during the 2nd test day and the first CS$^+$ presentation during the CI reacquisition ($P<0.05$, Wilcoxon test). The first presentation of the CS$^+$ during the CER and CI reacquisition resulted in significant suppression of the on-
going behavior \( (P<0.01, \text{A-B comparison, Wilcoxon test}), \) which was, as seen in Fig. 6, restricted to the last 30 s of the CS. Already one CS-US pairing resulted in rapid decrease of the suppression ratio value, and comparison between the suppression ratios for the first and the second CS\(^+\) trials of the CER and CI reacquisition revealed significant difference \( (P<0.01, \text{Wilcoxon test}) \). In the subsequent CS\(^+\) presentations, the part of the CS-US interval with marked suppression of bar-pressing gradually extended toward the earlier portion of the CS action. In parallel the differences in numbers of responses emitted during the action of the CS\(^+\) and the CI + CS compound increased.

Comparisons of the numbers of bar-presses emitted in the A and B periods of each trial independently showed that each presentation of the CS\(^+\) evoked significant suppression \( (Ps<0.01, \text{Wilcoxon tests}) \). Only during one presentation (the 1st trial of the 6th day of reacquisition) did the CI + CS compound produce a significant decrease in bar-pressing rate \( (P<0.01, \text{the Wilcoxon test}) \). The CI presented alone produced a significant enhancement of barpressing in four cases \( (Ps<0.02 \text{ in two cases and } Ps<0.05 \text{ in another two}; \text{Wilcoxon tests}) \).

These data indicate much more efficient differentiation between the CS\(^+\) and the CI + CS trials during the CER and CI retraining than observed toward the end of the CI training stage of the experiment. This was further documented by a two-way analysis of variance of the daily suppression ratios for the 1st-6th days of CER and CI retraining. The effects of stimuli used \( (F_{2,28} = 72.81, P<0.001) \) and days \( (F_{5,70} = 2.48, P<0.05) \) were both significant, as was the interaction between the two main effects \( (F_{10,140} = 4.72, P<0.001) \). Duncan tests showed that daily suppression ratios for the CS\(^+\) during the 1st day of CER and CI retraining differed significantly from ratios for the CS\(^+\) during the 2nd-6th days \( (Ps<0.01 \text{ in each comparison}) \). Moreover, each daily suppression ratio for the CS\(^+\) differed on the same level from each daily ratio for the CI or the CI + CS presentations.

It is worth mentioning that all daily ratios computed for CI presentations exceeded the 0.5 value, and during the 2nd day of CI reacquisition the enhancement of barpressing by the CI was significant \( (P<0.02, \text{A-B comparison, Wilcoxon test}) \). Except for the 1st day of CI reacquisition, the daily ratios for the CI + CS compound also were higher than the 0.5 value. The Kendall tau correlation analysis showed that toward the end of the CER and CI retraining stage, there was a similarity in responding to the CI presentations and to the CI + CS compound. Correlation coefficients between daily ratios for the CI and the CI + CS situations were positive and reached significance in the 4th \( (P<0.05) \), 5th \( (P<0.02) \) and the 6th \( (P<0.05) \) days of the CER and CI retraining.
All correlations between other pairs of stimulus situations were non-significant.

**CER extinction (2nd series).** The course of the CER extinction in terms of daily suppression ratios is shown in Fig. 8. Comparisons with data presented in Fig. 3 indicated that extinction of the CER was more rapid in the 2nd series than in the 1st series. During the second extinction series the CS significantly suppressed the on-going behavior during the 1st–4th sessions ($P<0.01$ for the 1st–3rd and $P<0.05$ for the 4th day, A-B comparisons for daily scores, Wilcoxon tests). An analysis of variance showed a significant day effect ($F_{5,70} = 15.26, P<0.001$), and Duncan tests revealed differences between the suppression ratios for the 1st and all other days of extinction and also between the 2nd and the 3rd–6th days of extinction ($P<0.01$). Other comparisons failed to show significant differences.

More rapid extinction during the 2nd series was shown also by the comparison of the numbers of bar-presses emitted before and during each consecutive CS presentation. Only during the 1st and the 2nd CER extinction days did each presentation of the CS result in significant suppression of bar-presses ($P<0.01$ for each A-B comparison, Wilcoxon tests), whereas during the next sessions the CS exerted a significant effect on the on-going behavior only on some trials. During the 2nd extinction series, the CS resulted in significant suppression ($P<0.05$, at least) in 13 out of 24 trials, while during the 1st extinction series such an effect was observed in 21 out of 28 trials.

The rapid weakening of the suppressive properties of the CS was also shown by the comparisons of the daily suppression ratios estimated for the CS and for periods in which the CI + CS should be presented. Comparisons of suppression ratios for the CS and the “dummy” CI + CS periods showed significant differences only during the 1st, 2nd ($P<0.01$) and the 3rd ($P<0.02$) day of the 2nd series of extinction. The results of these comparisons showed perfect agreement with those obtained by the analysis of variance.

The Kendall tau correlation analysis showed significant positive correlation between the daily ratios for the CS and the CI + CS situation in the 1st extinction day ($P<0.05$), whereas correlations coefficient between other stimuli situations for all other days of extinction did not reach significance.

**The 3rd, 4th and 5th test sessions.** The first and the second presentations of the CS during the 3rd test session produced marked suppression of the on-going bar-pressing. Comparison of the number of bar-presses emitted before and during the first presentation of the CS after the pause in the experiment showed significant suppression ($P<0.01$)
as well as for the second CS presentation \((P<0.02\text{, Wilcoxon tests})\). This spontaneous recovery of the suppressive properties of the CS was, however, shortlasting, and similar comparisons for next CS presentations during the 3rd–5th test days did not revealed significant changes in the on-going behavior. Comparisons of the suppression ratios for the first CS presentation on the 3rd test day with the first and the fourth CS presentations on the last day of extinction also indicated the recovery of suppressing properties of the CS \((P<0.01\text{ and } P<0.05 \text{ respectively, Wilcoxon tests})\). The suppression ratio for the second CS presentation on the 3rd test day differed from the first CS presentation on the last day of extinction \((P<0.02\text{, Wilcoxon test})\). Comparison of the daily suppression ratios for the last extinction day and the 3rd test day also revealed recovery of the suppressing properties of the CS after the pause \((P<0.05\text{, Wilcoxon test})\).

As mentioned before, during the last extinction days there was no significant difference between daily ratios estimated for the CS and for the periods in which CI + CS should be presented. On the contrary, during the 3rd test day, daily ratios for the CS and for the CI + CS periods differed \((P<0.01\text{, Wilcoxon test})\), similar to the CER and CS retraining sessions before the extinction stage.

![Fig. 9. Median daily suppression ratios during the last day of the CER extinction (2nd series) and during the 3rd, 4th, and 5th test days. Denotations as in Figs. 3 and 8.](image)

As seen from Fig. 9 a spontaneous recovery of the CER was shortlasting and during the 4th and 5th test days the daily ratios for the CS indicated no change in the on-going bar-pressing. All daily ratios for the CI were above the 0.5 value and during the 4th test day this enhancing effect of the CI on bar-pressing rate was significant \((P<0.01\text{, A-B comparison, Wilcoxon test})\). The ratios for the CI + CS presentations fluctuated around the 0.5 value.
An analysis of variance based on daily scores for the CS, CI, and CI + CS stimulus situations employed during the 3rd–5th test days showed the effects of days ($F_{2/23} = 5.49, P<0.01$), stimuli ($F_{2/23} = 14.64, P<0.001$) and their interaction ($F_{4/56} = 9.2, P<0.001$). The suppression ratios for the CS presentations during the 3rd test day differed ($Ps<0.01$) from all others ratios computed for any stimulus situation and test day (Duncan tests). None of the Kendall tau correlation coefficients between any of the pairs of stimulus situations presented during the 3rd–5th test day reached significance.

**Baseline responding changes during the experiment.** As mentioned before, the beginning of CER training produced marked drop of the barpressing rate in intervals between stimuli presentations. To estimate whether the slow restoration of the on-going barpressing observed in Fig. 2 was significant, the numbers of barpresses emitted in four A periods preceding each CS presentation were summed, and these daily A scores for the last three days of the stages of CER acquisition, CI acquisition, CER extinction (1st series), CER and CI retraining, and CER extinction (2nd series) were compared. An analysis of variance did not show any effects of stage or day, however, the interaction of the two main effects was significant ($F_{8/112} = 3.08, P<0.01$). As shown by the further Duncan tests, the interaction was due to the highest rate of responding during the 5th day of the second series of extinction. Although the data presented in Fig. 2 suggest some recovery of the base-line responding, fluctuations of the daily scores were rather unsystematic.

**DISCUSSION**

Although the main index of the efficiency of conditioned inhibitor training was the comparison of performance during the conditioned stimulus and the inhibitory compound presentations, the present discussion of the results will start with the problem of differentiation between the CS and the CI. Acquisition of the two modes of behavior, suppression of on-going bar-pressing during the action of the 80 dB white noise CS paired with shock and regular bar-pressing rate during the house light offset CI, proceeded with the same rapidity. In fact significant suppression of on-going behavior was produced by the fifth presentation of the CS. Similarly, beginning with the sixth presentation of light offset, darkness ceased decreasing bar-pressing rate.

For proper evaluation of the rapidity of inhibitory training it should be noted that rats were not habituated to the house light offset so that this stimulus evoked a strong orienting response resulting in a marked arrest of bar-pressing during the first presentation. This orienting res-
Response was probably enhanced due to some cross-modality transfer from the noise CS, to which suppressing properties were firmly established in previous training. Moreover, each presentation of the CI was accompanied during the last three min of its action by the CS and during the first two days of CI acquisition each presentation of the CI + CS compound resulted in significant suppression of the on-going bar-pres- sing. In spite of these factors, no signs of second-order conditioning of suppression to the house light offset were noticed. In full agreement with our previous data (22), the present training results indicate perfect discrimination between the two stimuli of the 80 dB white noise and the house light offset.

Acquisition by the conditioned inhibitor of the property to inhibit suppressing effects of the CS required much longer training which was not completed during the six days of the CI acquisition stage. Although the amount of suppression produced by the CS+ and the CI + CS compound differed statistically as soon as the second day of CI acquisition, the suppression ratios for the two stimulus situations were positively correlated during the second, similar to the first day of CI acquisition. This positive correlation between the scores was lost on subsequent days of training, indicating the start of discrimination between the CS and CI + CS presentations. Nevertheless the CI + CS compound produced significant suppression in most trials until the end of the original CI training.

The weakening of the suppressive effects of the CS presented in compound with the CI was accompanied by a smaller but marked deterioration of the suppressive effects of the CS+. In light of data showing perfect discrimination between the CI alone and the CS, this weakening of the suppressive effects of the CS+ cannot be considered as generalization of the inhibitory properties from the CI (or CI + CS) to the CS alone. Moreover, the disruptive effect of introduction of the CI + CS trials on the suppressive properties of the CS+ was the strongest during the first day of CI training and then gradual restoration of the suppression to the CS+ was observed. Thus, other factors are responsible for these changes. Even in early papers on CER, it was noted that the increase of the proportion of time occupied by the CS resulted in weakening of the suppressing effects of the CS (4, 5, 21). During the CER acquisition stage of the present experiment, the CS+ occupied 10 per cent of the total duration of the sessions whereas during the CI acquisition stage 20 per cent of total time of the session was occupied by the CS presented either alone or in compound with the CI. These changes in proportion of time were less radical than those employed by previous researchers. Moreover, it was shown earlier (24)
that 15% of the total time occupied by white noise of the same intensity presented on the baseline of the same schedule of reinforcement as in the current experiment produced complete suppression of barpressing during the CS action. Nevertheless the change in the proportion of time occupied by the CS possibly exerted some influence that weakened the suppressive effects of the CS and furthered acquisition by the CI of the property to inhibit the suppressive effects of the CS. The introduction of the CI + CS trials into the experimental situation inevitably evoked an orienting response and changed the balance between the two motivations and the response patterns related to them. Independent of the mechanism responsible for rapid attenuation of the suppressive effects of the CS at the beginning of CI training, all these considerations emphasize the necessity for direct comparisons of the suppression ratios for the CS+ and the CI + CS compound as the main measure of the efficacy of the CI training. The scores for the two stimulus situations differed significantly beginning on the second day of CI training. In this respect the inhibitory training was very efficient.

Changes in suppressive properties of the CS observed during CI training were quite distinct to those observed during the CER extinction, when suppression of on-going behavior by the CS, without shock, decreased on each consecutive day. The extinction of the suppressive properties of the CS was, however, not complete. Both in the first and in the second extinction series, one CS presentation of the last session produced significant suppression. The figures presented in this paper showed that this residual suppressive effect of the extinguished CS was restricted to the short period of time just preceding the CS offset. Inspection of individual records revealed that in the course of extinction, the period of time with regular bar-pressing rate after the CS onset increased and, consequently, the period of time with a marked drop in bar-pressing rate became more and more limited to the end of the CS. On the contrary, the weakening of the suppressive properties of the CS+ observed after introduction of the CI + CS trials was abrupt and involved in a similar degree the whole period of the CS action.

A gradual weakening of the suppressive properties of the early phases of the CS and limitation of strong suppression to the final phases of the CS action was observed also during the prolonged CER training in which each CS presentation was accompanied by shock. These changes were considered as a result of acquisition of the inhibition of delay (24, 27). Some investigators have even observed enhanced bar-pressing in the earlier parts of CS action and suppressed bar-pressing restricted only to the end of the CS duration, just before the shock application, when a large part of the session has been occupied by consecutive CS presenta-
tions (7, 17). The present data indicate that chronic extinction of the CER, consisting of repeated presentations of the CS of normal duration scheduled at the same time as in regular training sessions but no longer accompanied by shock, markedly accelerated the development of inhibition of delay.

The involvement of the inhibition of delay mechanism in the process of CER extinction was additionally supported by changes in the distribution of bar-presses during CS action in the course of CER end CI retraining. Reintroduction of the CS-shock US pairings resulted in rapid restoration of the suppressing properties of the extinguished CS. Indeed, a single CS-shock US pairing resulted in a marked decrease in the value of the suppression ratio. As seen in figures presented in this paper, supported by inspection of individual records, the distribution of bar-presses within the CS period during the CER and CI retraining changed in just the opposite way to that observed during the CER extinction. Each consecutive CS presentation accompanied by the shock US resulted in the gradual extension of the final no-bar-pressing phase toward the earlier phases of the CS action.

On the basis of his experimental data, Konorski claimed that previously established associations are not lost in the course of extinction and even after counterconditioning the old associations still exist and may be brought to light (15, p. 316–340). Much experimental data has supported this view (1, 25), so that the term "extinction" is misleading (26). The present results showed that in the course of CER extinction the previously acquired CS-US association was not lost; even the memory of the moment of shock US presentation within the trial was retained, but the pattern of responding during the CS action was changed. In fact it was not extinction of the CER, but adaptive changes in performance that were observed in the course of CS presentations not accompanied by shock. These adaptive changes in performance were, as mentioned before, very similar to those observed when the duration of CS action or the number of CS presentations during the session increased and also when schedules with low rates of food reinforcement were used (2, 16).

The comparison between performance during the second test session following presentation of the four unsignaled reinstating shocks and the second session of the CER and CI retraining stage indicated that reinstatement of fear conditioned to the experimental context play very small role in recovery of the suppressing properties of the CS, relative to the restoration of the CS-US pairings. Also in other CER studies, presentations of unsignaled shocks resulted in shortlasting reinstatement of suppression which was much smaller than before extinction (1, 8–10, 18–20). In contrast, the value of the daily suppression ratio for the
second day of CER and CI retraining, after four presentations of the shock paired with the CS, was exactly the same as during the last session before extinction. Although several possibilities of the mechanisms responsible for the reinstatement of an "extinguished" suppression were discussed (1, 20) it has to be remembered that presentation of unsignaled shocks in the experimental context should weakened rather than strengthened the CS-US association. In the course of presentations of unsignaled shocks in the experimental context, new associations have to be formed, which may have similar disruptive effect on previously established CS-US associations as those acquired during backward conditioning (3). Thus, greater restoration of the suppressing properties of the CS should be expected as a result of spontaneous recovery of the "extinguished" CER after a long pause in experiments. Results of the present experiment were in accordance with these expectations.

One finding of the present experiment calls for special attention. Extinction of the CER exerted no effect on the mode of responding acquired previously by the conditioned inhibitor of the CER. On the contrary, the suppression ratios for the CI during the CI training, in the first and the second test sessions and consecutive sessions of the CER and CI retraining, form a smooth learning curve. Similar learning function that begins with a disruption and finishes with some enhancing effect on on-going bar-pressing form suppression ratios for CI + CS presentations. Clearly, properties of the conditioned inhibitor acquired in original training and signaling that shock will not be given during its action even when the CS is presented were fully preserved, independent of repeated presentations of the CS without shock during extinction and presentations of unsignaled reinstating shocks. Since both of those procedures have to exert opposite influences on the fear levels of rats, we may conclude tentatively that this drive has no effect on the conditioned inhibitor of the CER. Furthermore, it may be hypothetized that in the course of training, two opposite motivational states were conditioned to the CS terminated with shock, and to the CI never paired with shock, thus allowing the regular rate of bar-presses for food. The problem whether a conditioned inhibitor of the CER would be similarly resistant to changes in the alimentary motivation level will be discussed in next paper of this series.

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