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Some Parameters of the Second-Order Conditioning of Fear in Rats

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Abstract
The effects of CS_1 duration, partial reinforcement, and trace conditioning on second-order conditioned suppression were investigated, employing 64 rats as *S*s. In Experiment 1, the clearest second-order conditioning was obtained when CS_1 duration was the same during both the first- and the second-order conditioning phases of the experiment. In Experiment 2, somewhat better second-order conditioning was obtained when first-order conditioning had been under conditions of partial reinforcement, delay CS-US pairing. In neither experiment were CS_1 suppression and CS_2 suppression during second-order testing well correlated. This aspect of the results raised the question of the influence of nonclassical conditioning factors on the conditioned suppression measure of emotional conditioning.

The purpose of the present research was to investigate the effects of several parameters on second-order conditioning in rats. Recently, Davenport (1966) and Kamil (1968) have demonstrated that second-order conditioning can be obtained readily, using the conditioned suppression technique. In these experiments, a multistage paradigm has been employed. Following the establishment of a steady state of operant responding on a partial reinforcement schedule, two stimuli are separately preexposed with no programmed consequences. In the next stage, first-order conditioning (FOC), one of these stimuli, CS_1, is paired with the US, electric shock. After conditioned suppression (a reduction in response rate during the CS) is established, the second stimulus, CS_2, is again preexposed. Finally, during second-order conditioning (SOC), CS_2 is paired with CS_1 (no US presented) and second-order suppression to CS_2 measured. The present research represents an attempt to initiate parametric investigation into second-order conditioned suppression.

Experiment 1

In this experiment, CS_1 duration during FOC and CS_1 duration during SOC were manipulated in 2 × 2 design. Assuming that the degree of second-order suppression obtained should be directly related to the magnitude of the conditioned emotional response elicited by CS_1 on SOC trials, two predictions were made. First, less second-order suppression should be expected when CS_1 duration is changed from FOC to SOC, a procedure Davenport (1966) has employed, because generalization decrement should decrease suppression to CS_1 during SOC. Second, maximal second-order suppression may be expected when short CS_1 durations are employed, both because of possible inhibition of delay conditioning during FOC and because faster extinction of CS_1 suppression may be found with longer CS_1 durations.

Method

Subjects. The *S*s were 32 experimentally naive male Holtzman rats 125-135 days old at the start of the experiment. They were maintained at 80% of their free-feeding weight for 2 wk. prior to, and throughout the course of, the experiment.

Apparatus. The apparatus consisted of four identical Gerbrands Model C test chambers enclosed in ventilated sound-attenuating boxes. A Lehigh Valley Electronics lever, two green 1-in. cue lights, and a Gerbrands recessed food cup were mounted on the response panel of each chamber. A small clear house light was mounted on the top of each chamber. A 3-in. speaker and a Gerbrands pellet feeder which dispensed 45-mg. Noyes rat pellets were mounted behind each response panel. The four chambers were controlled by standard electromechanical equipment located in an adjacent room.

Procedure. The Ss were run 6-7 days per week with a daily session duration of 26.8 min. Except as noted below, one trial was given each session. Trials were presented with random starting times during the middle 16.8 min. of a session. When no trial was in progress, 78 db. of white noise and noise from the exhaust fan provided masking, and the house light and cue lights were illuminated by 10 v. ac.

All *S*s first received bar-press training consisting of 50 "free" pellets on a VI 1-min. schedule followed by 40 continuously reinforced responses. This was followed by six sessions of a partial reinforcement schedule during which reinforcement was made available at random intervals, averaging 1 min., and remained available for 15 sec. or until the first bar press, whichever occurred first. The limited hold contingency was added to the VI 1-min. schedule in order to minimize adventitious reinforcement of pauses in responding, especially since such pauses are often the result of the fear-conditioning process. The limited hold contingency considerably reduced the probability of food reinforcement of the first posttrial response following conditioned suppression.

In Preexposure 1, 24 trials were given: 12 on which CS_1 was presented without programmed consequence (with the duration to be used in FOC) and 12 similar CS_2 trials. The CS_1 consisted of the elimination of the white-noise background and the presentation of a 450-cps tone of 74 db. (when measured with the exhaust fan on), with no illumination change. The CS_2 consisted of a 30-sec. increase in voltage to the cue lights and house light to 30 v. ac during Preexposure 1 and to 20 v. ac during Preexposure 2 and SOC. (The reduction to 20 v. was made because of difficulty in adapting novelty effects to the 30-v. value.) The CS_1 and CS_2 trials were randomly intermixed.

Next, 25 FOC trials were given. The CS_1 was paired with the US, which was a .5-sec. scrambled electric shock delivered by a Grason-Stadler shock generator, set at 2.5 ma., to the grid floors of the four chambers wired in parallel. A delay-conditioning procedure in which onset of the US coincided with offset of CS_1 was employed. Six sessions in which no trial was presented were interspersed among the 25 FOC sessions.

On the day following FOC Trial 25, Preexposure 2 (CS_2 only) was begun and continued for 19 trials. The first 10 trials were given at 2 trials per session.

Following completion of Preexposure 2, 19 SOC trials were then presented. On these trials, CS_2 onset was simultaneous with CS_1 offset.

The 32 *S*s of Experiment 1 were randomly assigned to four equal *n* groups designated 100-100, 100-10, 10-100, and 10-10. These numbers designate CS_1 duration in seconds during FOC and SOC. Thus, Group 100-10 received a CS_1 duration of 100 sec. during FOC and of 10 sec. during SOC. Three *S*s were discarded because of illness, two from Group 10-100 and one from Group 10-10.

The dependent variables recorded during the experiment were measures of suppression of bar pressing, using Kamin-type (1965) ratios of the form $R_I/(R_I + R_P)$, in which R_P is the rate of responding during a 100-sec. pretrial base-line interval, and R_I is the response rate during the interval under consideration. Note that the use of response rate in this equation compensates for different interval durations.

Results and Discussion

The only significant effect on CS_2 trials during Preexposure 1 was a reduction in suppression across trials ($F = 11.99$, $df = 1/25$, $p < .01$). The Greenhouse and Geisser (1959) correction for heterogeneity of covariance was employed in all repeated-measures analyses. The overall mean suppression ratio on Trial 12 was .39.

Two significant effects were found in the analysis of CS_1 effects during Preexposure 1: (a) a trials effect ($F = 8.53$, df - 1/25, $p < .01$) due to a decrease in CS_1 suppression with preexposure and (b) an effect of CS_1 duration ($F = 9.52$, $df = 1/25$, $p < .01$) due to more suppression by the 10-sec. groups on the first few trials. Newman-Keuls tests showed that there were no significant differences among the groups on any of the last five CS_1 preexposure trials. The overall mean ratio on these trials was .50.

During FOC, a significant increase in suppression across trials was obtained ($F = 26.43$, $df = 1/25$, $p < .001$), representing the acquisition of conditioned suppression. The CS_1 duration also had clear effects during FOC. The 100-sec. groups conditioned at a reliably slower rate than the 10-sec. groups ($F = 12.25$, $df = 1/25$, $p < .01$). A Mann-Whitney *U* test indicated that this difference was statistically reliable ($p < .02$, two-tailed) on the last FOC trial. On this trial, the mean CS_1 suppression ratio was .03 for the 10-sec. groups and .08 for the 100-sec. groups. This group difference replicates, in small part, the extensive work of Kamin (1965) which indicates that more conditioned suppression is obtained with short CS-US intervals.

The only significant effect in the analysis of Preexposure 2 was an overall decrease in CS_2 suppression over trials ($F = 9.55$, $df = 1/25$, $p < .01$), with the groups reaching an asymptote of .51.

The results obtained during SOC are shown in Figure 1. Analysis of the CS_2 suppression data revealed a significant trials effect ($F = 5.11$, $df = 1/25$, $p < .05$), representing the acquisition of second-order conditioned suppression. A significant interaction between CS_1 duration in FOC and CS_1 duration in SOC was also obtained ($F = 5.62$, $df = 1/25$, $p < .05$). As can be seen in the lower panel of Figure 1, Groups 10-10 and 100-100 displayed more sustained and complete second-order suppression to CS_2 than Groups 10-100 and 100-10. A further analysis of these data was performed. A criterion score, the number of SOC trials on which the CS_2 suppression ratio was equal to or below .30, was calculated for each *S*. The results of this procedure yielded a mean of 6.6 for Group 10-10, 3.0 for Group 10-100, 2.0 for Group 100-10, and 5.5 for Group 100-100. (Similar results were also obtained with criteria of .20 and .40.) Analysis of variance again revealed a significant interaction of CS_1 duration in FOC by CS_1 duration in SOC ($F = 12.50$, $df = 1/25$, $p < .01$).

Second-order conditioned suppression was clearly obtained. As predicted by the generalization decrement hypothesis, the groups which received consistent CS_1 durations during FOC and SOC displayed the most second-order conditioned suppression. However, contrary to the inhibition of delay hypothesis, there was no main effect of CS_1 duration on second-order suppression.

Analysis of variance of CS_1 suppression during SOC revealed several significant effects. A significant main effect of the length of CS_1 in SOC was obtained ($F = 7.52$, $df = 1/25$, $p < .05$), which reflects the more rapid extinction of first-order suppression by the 100-sec. groups. A significant interaction between CS_1 duration in FOC and SOC was also obtained ($F = 8.72$, $df = 1/25$, $p < .01$). A subsequent Newman-Keuls test indicated that over all SOC trials, Group 10-10 suppressed significantly more during CS_1 than did Group 10-100 ($p < .05$). None of the other possible group differences attained significance. A significant overall decrease in CS_1 suppression across SOC trials was also obtained ($F = 24.04$, $df = 1/25$, $p < .001$).

Lastly, the three-way interaction between trials and CS_1 duration during FOC and SOC approached statistical significance ($F = 3.89$, $df = 1/25$, $p < .075$). Because of the known conservative nature of the Greenhouse and Geisser (1959) correction and the intrinsic importance of this effect, further analyses with subsequent tests were carried out. These indicated that three distinct rates of extinction were shown: (a) At Trial 1, none of the groups was significantly different (Kruskal-Wallis, $p > .30$); (6) at Trial 10, Group 10-100 was suppressing significantly less than any of the other groups (Newman-Keuls, $p < .01$); and (c) at Trial 19, Group 10-10 was suppressing significantly more than any other group (Newman-Keuls, $p < .01$), These results seem to have been produced by the combination of two main effects. In general, the groups receiving consistent CS_1 durations and the groups receiving the 10-sec. CS_1 duration during SOC showed the slowest extinction of first-order conditioned suppression.

The finding during FOC of a significant effect of CS_1 duration does not seem to complicate the interpretation of the SOC findings; this effect was not present on SOC Trial 1. Additionally, the results of neither CS_2 nor CS_1 suppression during SOC paralleled the FOC effect.

Experiment 2

In this experiment, which was run concurrently with Experiment 1, partial reinforcement during FOC and trace conditioning during FOC were manipulated in a 2 × 2 design. Two experiments (Brimer & Dockrill, 1966; Willis & Lundin, 1966) have found that extinction of first-order conditioned suppression was retarded by partial reinforcement during conditioning. Therefore, it was hypothesized that partial reinforcement during FOC would retard extinction of the suppression elicited by CS_1 during SOC, producing more persistent second-order conditioned suppression. The inclusion of the trace variable was based upon the hypothesis that trace FOC, especially in combination with partial reinforcement, would increase uncertainty of US occurrence and thus make US omission during SOC less discriminable, retarding extinction of suppression to CS_1 during SOC, leading to more sustained second-order suppression.

Method

Except for the changes necessitated by its different independent variables, Experiment 2 was run exactly as Experiment 1. The 32 *S*s were randomly divided into four equal *n* groups, designated 100% delay (100D), 100% trace (100T), 50% delay (50D), and 50% trace (50T), which received different treatments during FOC. After the completion of Preexposure 1, the *S*s of Group 100D were given 33 delay-conditioning trials (CS_1 was of 30-sec. duration throughout the experiment for all *S*s). The *Ss* of Group 100T received 33 CS_1-US pairings during which the trace interval between CS_1 offset and US onset was progressively increased. The interval was 0 sec. for the first 3 trials, 10 sec. for the next 3 trials, 20 sec. for the next 12 trials, and 30 sec. for the last 15 trials. During the trace interval, white noise resumed and the "off-trial" stimulus complex resumed. Group 50D received 49 FOC trials consisting of 33 CS_1–US pairings and 16 CS_1-only trials. Trials 5, 9, 12, 14, 16, 26, 29, 31, 32, 36, 37, 39, 42, 43, 44, and 47 were the CS_1-only trials. The percentage of paired trials slowly decreased until it was 50% over the last 18 trials. Group 50T also received 49 FOC trials in the same pattern as group 50D. For this group the trace interval was introduced gradually, as for group 100T.

In order to keep the 50% and 100% groups together in time, on days when the 50% groups received CS_1-only trials, the 100% groups were not run. Nine sessions with no trial presentation were interspersed among the FOC sessions.

On the day following the last FOC trial, Preexposure 2 was begun and continued for 17 trials (the first 10 trials were given at 2 trials per session). After Preexposure 2, 25 SOC trials were run, CS_2 followed by CS_1 in a delay-conditioning paradigm. The CS_1 onset coincided with CS_2 offset for all groups.

Results

The only significant term in the analysis of CS_2 trials during Preexposure 1 was a decrease in suppression across trials ($F = 12.04$, $df = 1/28$, $p < .01$). The overall mean on Trials 11–12 was .34. In the analysis of CS_1 trials during Preexposure 1, a significant Trace × Partial Reinforcement interaction was obtained ($F = 5.77$, $df = 1/28$, $p < .05$), Group 100T showing somewhat more suppression than the other groups. Subsequent Newman-Keuls tests indicated that there were no significant differences among the groups on any of the last five CS_1 trials during Preexposure 1. A significant decrease in suppression across trials was also obtained ($F = 8.07$, $df = 1/28$, $p < .01$).

Because of the differing number of trials given the 50% and 100% groups during FOC, several separate analyses were performed, one within each percentage condition, and one over the first 20 trials of all groups. In all cases the only significant effect was trials ($p < .01$, in all cases), representing acquisition of first-order conditioned suppression. Nonparametric tests indicated there were no reliable differences among any of the four groups in CS_1 suppression on the last FOC trial and that, in suppression during the trace interval, there was similarly no difference between groups 100T and 50T.

The only significant effect in Preexposure 2 was a decrease in suppression across trials ($F = 10.98$, $df = 1/28$, $p < .01$). The overall mean CS_2 ratio on Preexposure 2, Trial 17, was .51.

The results obtained during SOC are shown in Figure 2. The only significant effect in an analysis of variance of the CS_2 suppression data was a main effect of trials ($F = 5.89$, $df = 1/28$, $p < .05$), representing acquisition of second-order suppression. A criterion-score analysis of these data, similar to that performed in Experiment 1, was carried out. This resulted in means of 4.0 for Group 100D, 5.0 for Group 100T, 10.5 for Group 50D, and 3.6 for Group 50T. (Similar results were obtained with criteria of .20 and .40.) Analysis of variance revealed a reliable partial Reinforcement × Trace interaction ($F = 7.08$, $df = 1/28$, $p < .05$), clearly due to the high score of Group 50D.

Analysis of the SOC CS_1 suppression data revealed two significant effects. The trace groups displayed significantly less CS_1 suppression during SOC than the delay groups ($F = 12.02$, $df = 1/28$, $p < .01$). There was also a significant decrease in CS_1 suppression across SOC trials ($F = 34.18$, $df = 1/28$, $p < .001$). An analysis of variance of suppression during the trace interval following CS_1 offset (Figure 2, top panel) by Groups 50T and 100T yielded only a reliable trials effect ($F = 18.28$, $df = < .001$).

Discussion

The SOC results of Experiment 2 offer only very tentative support for the partial reinforcement hypothesis proposed. By the criterion-score analysis, more second-order suppression was obtained following a 50% FOC contingency within delay-conditioning procedures. However, this conclusion was not supported by the general analysis of the data. This result seems even more questionable when the CS_1 suppression data of SOC are examined. No effects of partial reinforcement on CS_1 or trace suppression paralleling the possible CS_2 effect were seen.

This noncorrespondence of CS_1 and CS_2 suppression during SOC, which also appeared in Experiment 1 (e.g., Groups 10-10 and 100-100 were the same in CS_2 performance but differed in CS_1 performance), is puzzling since the classical conditioning model of second-order conditioning maintains that second-order suppression is the result of pairing CS_2 with the suppression response elicited by CS_1. Clearly, this model predicts that CS_1 and CS_2 suppression performance should be well correlated.

The extent to which this noncorrespondence may be due to differential sensitivity of the measures involved cannot be judged from the present data; however, they raise the question of the extent to which the conditioned suppression dependent variable is a function of factors other than the assumed internal, classically conditioned emotional response to CS_1. This same question is also raised in a recent study by Wagner, Siegel, and Fein (1967) which appeared during the data collection of the present experiments. These investigators obtained the partial reinforcement-extinction effect with a bar-in procedure, but found that when the conditioning trials were conducted with the bar blocked off no such effect appeared. Wagner et al. (1967) pointed out that these data strongly suggest that the partial reinforcement-extinction effect in conditioned suppression may be due to nonclassical conditioning factors associated with the ongoing rewarded operant behavior against which suppression is measured.

Many such factors can be hypothesized, especially when it is considered that adventitious factors may operate not only through the law of effect, as Wagner et al. (1967) suggest, but also through counterconditioning. The receipt of food probably elicits an internal response antagonistic to the CR elicited by CS_1. However, in the absence of either converging operations on these factors, or of more direct measurement of the internal state of the *S* during conditioned suppression experiments, speculation seems somewhat useless. It is quite possible that these potential effects exert a large influence on conditioned suppression only during extinction or analogous SOC procedures when response rate, and therefore probability of reinforcement during CS_1, begins to rise. Thus these results, along with those of Wagner et al. (1967), suggest that the conditioned suppression paradigm can involve instrumental as well as classical conditioning factors.

Experiment 2 did offer a partial test of the issues raised by Wagner et al. (1967) to the extent that the SOC procedure involves extinction of first-order conditioned suppression. Two of the possible adventitious factors mentioned by Wagner et al. were minimized in Experiment 2. The limited hold base-line schedule reduced the probability of food reinforcement for pauses in, or suppression of, responding. Secondly, the successful gradual introduction of the 50% US contingency prevented the occurrence of group differences in the acquisition of first-order conditioned suppression. Such differences can result in group differences in, for example, amount of reward during the CS. In Experiment 2, with these factors controlled, as in the Wagner et al. bar-out groups (a procedure which reduces instrumental factors during acquisition of suppression by removing the manipulandum), no partial-reinforcement extinction effect was found.

Lastly, the delay-trace factor of Experiment 2 did not produce the expected results. There was no apparent effect on second-order conditioned suppression, and the clearly higher rate of dissipation of CS_1 suppression during SOC by the trace groups was in a direction opposite to that predicted. However, it does agree, to an extent, with the findings of Kamin (1965) in that Kamin showed conditioned suppression to be poorer with a trace procedure.

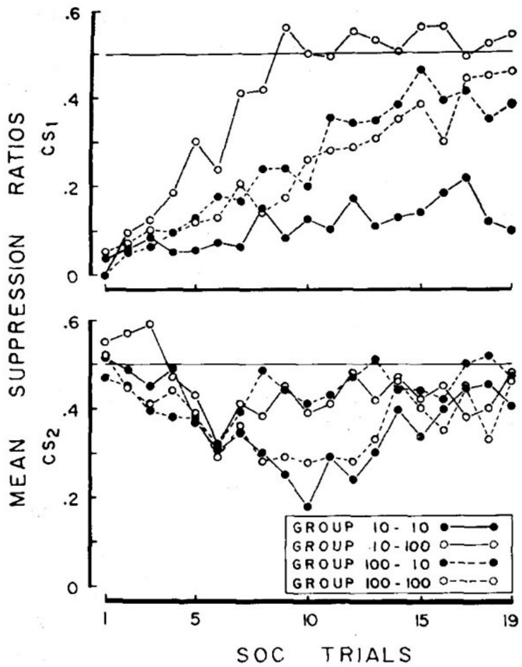

Figure 1. Mean CS_1 and CS_2 suppression performance of the groups of Experiment 1 during SOC.

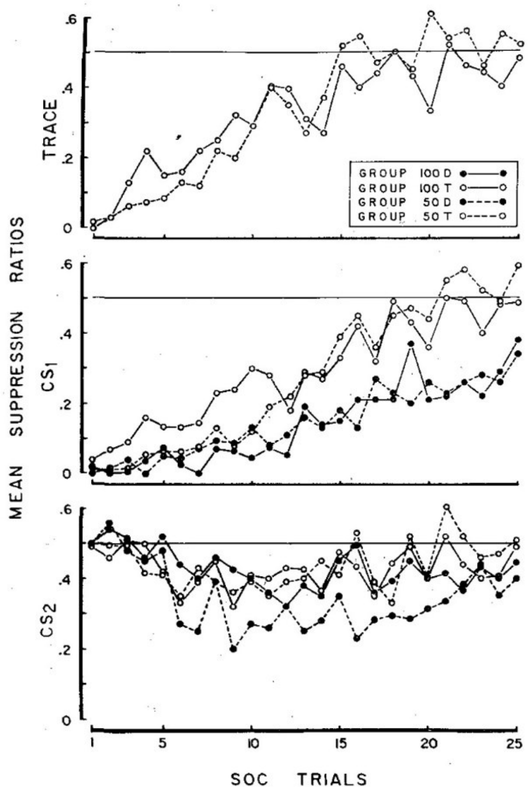

Figure 2. Mean trace, CS_1, and CS_2 suppression performance of the groups of Experiment 2 during SOC.

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