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Second-order conditioning of the conditioned emotional response: Some methodological considerations

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Employing rats in a CER procedure, the present study sought to determine the extent to which the second-order conditioning effects reported by Rizley and Rescorla (1972) represented first- rather than second-order conditioning. Subjects receiving first-order pairings of flashing light (CS_1) and shock followed by second-order pairings of noise (CS_2) and CS_1 displayed greater suppression to CS_2 than did control subjects receiving second-order pairings in the absence of first-order conditioning. This was true whether or not control subjects had experienced unsignaled shock or habituation to CS_1 prior to CS_2-CS_1 pairings. Simple stimulus pairings did produce some suppression to CS_2, however. The procedure developed by Rizley and Rescorla (1972) appears to be a reliable means for producing and studying second-order aversive conditioning.

As Rescorla and his colleagues have argued (Holland & Rescorla, 1975; Rescorla, 1973; Rizley & Rescorla, 1972), an adequate demonstration of second-order conditioning (SOC) must show the presumed SOC effect to be dependent on both first- and second-order stimulus pairings (see Rizley & Rescorla, 1972). To date, only three reports of SOC have included the control procedures necessary to accomplish this end (Holland & Rescorla, 1975, Experiment 1; Rashotte, Griffin, & Sisk, 1977, Experiment 1; Rizley & Rescorla, 1972, Experiment 1). Of these, the Rizley and Rescorla study is unique in employing an aversive conditioning procedure, the conditioned emotional response (CER). The present study sought to replicate the SOC demonstration of Rizley and Rescorla, and to explore the possibility that first-order conditioning substantially contributed to the effects they observed.

The first-order conditioned stimulus (CS_1) employed by Rizley and Rescorla (1972, Experiment 1) was a 10-sec flashing light, occurring in an otherwise darkened environment. It seems plausible that, for the rat, this event could function as a fear-eliciting unconditioned stimulus (US) in its own right, independent of any first-order conditioning it might receive. If so, when subsequently paired with the second-order CS (CS_2), CS_1 might appear to support "second-order" conditioning when, in fact, first-order effects were being observed. To account for this possibility, Rizley and Rescorla gave control subjects unpaired first-order presentations of CS_1 and the US (shock), followed by the same CS_2-CS_1 pairings experienced by SOC animals. While significant responding to CS_2 was observed with this control (indicating that CS_1 could, indeed, support first-order conditioning), SOC subjects displayed still greater responding.

Though seeming to rule out a first-order conditioning account of their SOC effects, however, the first-order control utilized by Rizley and Rescorla may have introduced problems of its own. First, subjects receiving this treatment might be expected to display disinhibition effects following first-order unsignaled shock experience (Brimer, 1970). That is, if operant responding were sufficiently depressed following exposure to unsignaled shock, presentations of the novel CS_2 might act to increase response rates momentarily. This would tend to counteract any suppression brought about by CS_2-CS_1 pairings, while those pairings exerted their full effect among "SOC" subjects (cf. Brimer & Kamin, 1963). Second, there is substantial evidence that repeated unsignaled presentations of a US can retard subsequent conditioning (Baker, 1976; Cannon, Berman, Baker, & Atkinson, 1975; Engberg, Hanson, Welker, & Thomas, 1972; Kamin, 1961; Mis & Moore, 1973; Seligman, 1968). Thus, if CS_2-CS_1 pairings, by themselves, were able to establish "second-order" suppression to CS_2, such suppression might not appear among control animals that receive those pairings following unsignaled shock. Finally, since subjects receiving unpaired presentation of CS_1 and shock are, in effect, being habituated to the flashing light, the possibility is raised that the *pairings* of CS_1 and shock administered to SOC subjects serve not to establish first-order suppression to light, but to maintain the *unconditioned*

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effects of that stimulus. There is evidence, for example, that habituation to a given stimulus can be prevented if, on each presentation, that stimulus is followed by another stimulus (Lubow, Schnur, & Rifkin, 1976; Wagner, 1976).

To examine these possibilities and to replicate the SOC demonstration of Rizley and Rescorla (1972, Experiment 1), the present study employed five groups of rat subjects in a CER paradigm. Two groups, PP and UP, received, respectively, paired or unpaired presentations of light and shock, followed by pairings of white noise (CS_2) and light. A third group, U'P, received the same treatment as UP, except that all first-order shocks were signaled by an otherwise irrelevant warning stimulus. This group controls for the possible effects of unsignaled shock on second-order CS pairings. A fourth group, NP, received no first-order stimulus presentations, but did receive second-order pairings of noise and light. This group provides a direct assessment of the first-order conditioning capabilities of the flashing-light CS. Finally, Group PU received first-order light-shock pairings, followed by unpaired presentations of noise and light. This group is designed to show the dependence of SOC effects on second-order stimulus pairings, and has been employed by numerous investigators of SOC using the CER paradigm (Kamil, 1968; Kamin & Szakmary, 1977; Rizley & Rescorla, 1972).

METHOD

Subjects

The subjects were 80 experimentally naive male albino rats (mean weight = 332 g), obtained from Holtzman Co., Madison, Wisconsin. Prior to the experiment, the rats were randomly assigned to groups, placed on a restricted food diet, and reduced to 75% of ad-lib weight.

Apparatus

The apparatus consisted of eight standard operant conditioning units, individually housed in sound- and light-resistant chests. The side walls and ceiling of each chamber were constructed of clear acrylic plastic, with a sheet of frosted translucent plastic covering each ceiling. The front and rear walls were made of aluminum sheeting, with the front wall containing a bar, a food cup, and a loudspeaker. The floor of each chamber consisted of 18 stainless steel grid rods connected to a Grason-Stadler shock generator (E1064 GS). There was a 7.5-W light mounted above the ceiling of each chamber. The normal condition of the chambers was complete darkness.

The CS was either a 10-sec intermittent illumination (1/sec) of the overhead light or the delivery of a 10-sec 1,800-Hz tone or 30-sec 80-dB pulsating white noise. The US was a .5-sec shock with a nominal intensity of 1.0 mA. Programming and recording equipment was located in a room adjacent to the experimental room.

Procedure

Following initial magazine and barpress training, each subject received five daily 2-h sessions of barpressing on a 2.5-min variable-interval (VI) schedule of food reinforcement (Noyes pellets, 45 mg). During the last 2 days of this VI training, all animals received a total of four pretest presentations each of the 30-sec white noise, the 10-sec flashing light, and the 10-sec tone. The 2-h barpressing sessions continued throughout the experiment.

Phase I, first-order conditioning. On each of the 4 days of Phase I, subjects in Groups PP (n = 24), UP (n = 16), U'P (n = 16), and PU (n = 8) received four presentations of the 10-sec flashing light (CS_1), four presentations of the 1.0-mA shock, and four presentations of the 10-sec tone, spread throughout the 2-h session. The only thing which distinguished the various groups was the timing of the shock. For Groups PP and PU, shock onset coincided with offset of the light, thus providing first-order conditioning of that stimulus. For Group UP, shocks were explicitly unpaired with both light and tone. For Group U'P, shock onset coincided with offset of the tone. Finally, subjects in Group NP (n = 16) received only the 2-h VI sessions during Phase I, with no stimuli delivered. Following Phase I, all subjects received a single 2-h barpressing "recovery" session, with no stimuli being delivered.

Phase II, second-order conditioning. On each of the 2 days of Phase II, subjects in Groups PP, UP, U'P, and NP received four pairings of the 30-sec white noise and the 10-sec flashing light. Light onset coincided with offset of the noise. Animals in Group PU received the same number of daily presentations of noise and light, explicitly unpaired. No shock was delivered during this phase of the experiment.

Measures. Responding to stimuli was assessed in terms of suppression ratios. The ratio is of the form B/(A+B), with B representing the rate of barpressing during the CS, and A the rate of barpressing in the 1-min interval immediately preceding the CS. A ratio of .50 represents no suppression to the CS, while a ratio of .00 indicates complete suppression. Except as noted, all statistical analyses were based on two-tailed tests of significance.[1]

RESULTS

Pretesting and Phase I

The flashing light CS, as expected, elicited substantial unconditioned suppression during pretesting. The combined mean suppression ratio (n = 80) for the four pretest trials was .14 for light, compared to .40 and .41 for tone and noise, respectively.

Within Groups PP and PU, the first-order conditioning treatment established slight, but measurable conditioning to light. The mean Phase I ratio for these subjects was .08, a significant increase in suppression relative to the pretest (Wilcoxon $T = 108$, $N = 27$, $p < .03$, one-tailed). When unpaired with shock in Groups UP and U'P, the light lost much of its unconditioned suppressive capacity. The mean Phase I ratio for these two groups was .33, a significant decrease in suppression relative to the pretest ($T = 1.5$, $N = 32$, $p < .001$). Thus, Groups UP and U'P had apparently undergone substantial habituation to light prior to Phase II, when noise and light were paired for these animals.

Phase II

There were no differences among the groups in operant responding following the barpressing recovery day. The results of Phase II, second-order conditioning, appear in Figure 1, which plots mean suppression ratios to noise (CS_2) for each of the eight Phase II trials. Considering first Day 1 (Trials 2-4), Group PP appears to display SOC relative to Group PU, the second-order control group. The latter group shows some acceleration of barpressing to noise, a characteristic unconditioned effect of that stimulus (cf. Kamin & Szakmary, 1977). Group PP, however, does not seem to differ from Groups UP, U'P, and NP, all

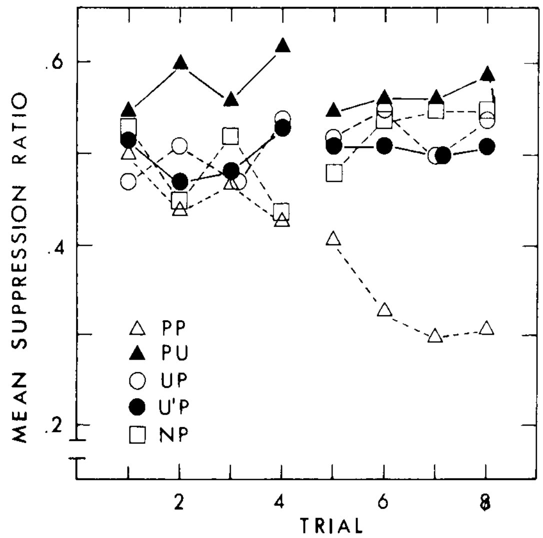

Figure 1. Mean suppression ratios to noise (CS_2) for the eight trials of Phase II.

of which—like Group PP—received noise-light pairings during Phase II.

To further assess these Day 1 effects, each subject was assigned its mean noise ratio across Trials 2-4. A Kruskal-Wallis test revealed significant overall differences among groups [$H(4) = 252.60$, $p < .001$], and subsequent Mann-Whitney U tests determined Group PU to be less suppressed than any of Groups PP, UP, and NP (all $ps < .05$). The difference between Groups PU and U'P was marginally significant ($p = .06$).

In contrast to Day 1, Day 2 of Phase II produced clear evidence of SOC effects. As can be seen in the figure, all groups but PP displayed accelerated responding to noise, while Group PP developed further suppression to that stimulus. When subjects were assigned their mean noise ratios across Trials 2-8 of Phase II, significant differences appeared among the groups [Kruskal-Wallis $H(4) = 262.64$, $p < .001$], with Group PP showing greater suppression than any of the controls (all Mann-Whitney $ps < .01$). The control groups did not differ among themselves over this period [Kruskal-Wallis $H(3) = 4.46$].

DISCUSSION

The present data confirm the work of Rizley and Rescorla (1972, Experiment 1) and make clear that the SOC effects they observed were not an artifact of the first-order control procedure they employed. That procedure, it will be recalled, exposed control subjects to unpaired presentations of CS_1 (flashing light) and shock, followed by pairings of CS_2 and CS_1. It seemed that, if CS_2-CS_1 pairings alone were able to support conditioning among "SOC" subjects, such conditioning might not appear among control animals as a consequence of their prior exposure to CS_1 and/or unsignaled shock. The present data indicate that, while CS_2-CS_1 pairings do produce some first-order conditioning, and thus contribute to the observation of SOC, this effect is quite small and is not measurably altered by prior experience with CS_1 and shock. Thus, relative to Group PU, the only group not receiving Phase II stimulus pairings, the four groups (including SOC subjects) which experienced noise-light pairings were alike in displaying some suppression to noise over the early trials of Phase II. However, at no point during Phase II was the behavior of Group UP, the first-order control of Rizley and Rescorla, different from that of an equivalent group (U'P) that did not experience unsignaled shock in Phase I. There was no evidence that the unsignaled shocks experienced by Group UP resulted in either disinhibition effects to CS_2 or interference with the small amount of suppression produced by the CS_2-CS_1 pairings. Likewise, at no point in Phase II did Group UP differ from Group NP, which experienced neither CS_1 nor shock in Phase I. This was true despite the fact that UP subjects, but not NP animals. had undergone substantial habituation of their unconditioned suppression to CS_1 during Phase I. Thus, the small amount of suppression brought about by mere noise-light pairings evidently did not depend on the level of unconditioned suppression to light during Phase II.

While the suppression displayed by SOC animals on Day 1 of Phase II may be most parsimoniously ascribed to first-order conditioning, the behavior of those animals on Day 2 very definitely represented second-order conditioning. Over Trials 5-8 of Phase II, SOC subjects showed increasing suppression to CS_2, while the control groups displayed no suppression to that stimulus. The Day 2 performance of SOC animals thus depended on the prior first-order conditioning accorded to CS_1.

Finally, it might be noted that the level of suppression to CS_2 among SOC subjects was markedly less than that observed by Rizley and Rescorla (1972). Despite the attempt to closely replicate the Rizley-Rescorla study, the present experiment apparently differed from that work in ways (e.g., shock source, subject deprivation) that affected the level of conditioning observed. Nevertheless, the present SOC effect very closely resembles that obtained in other, less well-controlled investigations of SOC employing the CER procedure (Davenport, 1966; Kamil, 1968, 1969; Kamin & Szakmary, 1977).

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NOTE

1. The various experimental groups were run in sets of eight subjects in the following combinations: PP, UP, U'P; UP, U'P; PP, OP; PP, OP, PU.

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