Escape-from-fear performance as affected by handling method and an additional CS-shock treatment

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Experiment 1 demonstrated that hurdle-jumping, escape-from-fear (EFF) performance was affected deleteriously when the handling of the rats was by the tail rather than the body and was facilitated by the administration, just prior to EFF training, of one additional CS-shock pairing along with the opportunity to jump the hurdle. Additional experiments elucidated the basis for the effect of these two variables. Experiment 2 indicated that tail handling degraded performance by decreasing reinforcement. In Experiment 3, the facilitatory effect of the extra CS-shock treatment was found to result from the occurrence of a response at the termination of the pairing and not from the CS-shock pairing per se.

In a typical escape-from-fear (EFF) task, classical fear-conditioning trials (CS-shock) are administered in one side of a two-compartment apparatus. Later, in the absence of shock, subjects are allowed to jump a hurdle to an adjacent safe compartment and, thereby, to escape the fear-eliciting CS and situational cues in the shock compartment. Theoretically, the learning of the instrumental hurdle-jumping response is motivated by these two sources of fear and is reinforced by the reduction of this fear following the response.

Recently, Crawford, Masterson, and Wilson (1977) have reported difficulty in consistently obtaining such instrumental learning based either on fear of the CS alone or on fear of both the CS and situational cues. In order to obtain learning, they found it necessary to administer not only the classical fear-conditioning trials but also an additional such trial, along with the opportunity to jump the hurdle, just prior to regular hurdle-jumping training. The necessity for the inclusion of such an additional step in the normal procedures is surprising because there is a relatively large literature reporting successful escape-from-fear learning without such a requirement. These studies have been conducted in a number of laboratories with different experimenters, with different strains and sex of rats, and with differences in a number of specific details of apparatus and procedure (e.g., Brown & Jacobs, 1949; Coover, Sutton, Welle, & Hart, 1978; Daly,

1968; Desiderato, Butler, & Meyer, 1966; Goldstein, 1960, 1976; Kalish, 1954; Kent, Wagner, & Gannon, 1960; King & Cairncross, 1974; D. E. McAllister, W. R. McAllister, Brooks, & Goldman, 1972; W. R. McAllister & D. E. McAllister, 1965; W. R. McAllister, D. E. McAllister, Weldin, & Cohen, 1974; Osborne, Mattingly, Redmon, & Osborne, 1975; Spear & Parsons, 1976).

According to Crawford (Note 1), the animals in the Crawford et al. (1977) experiments were handled by the tail during hurdle-jumping training. Potentially, such a procedure could degrade performance and, at least partially, account for their difficulty in obtaining learning of the hurdle-jumping task. Although the method of handling subjects was not reported in the successful studies from other laboratories cited above, the animals are always handled by the body in this laboratory. The relevance for EFF performance of the handling procedure as well as of the extra-pairing treatment was verified in Experiment 1. The bases for the effects of these two variables were identified in Experiments 2 and 3.

EXPERIMENT 1

Method

Apparatus. The apparatus used for the hurdle-jumping, EFF task consisted of a rectangular wooden box into which two separate compartments were inserted lengthwise. These compartments, constructed of Luminex glass, were each 248 mm long $\times$ 114 mm wide $\times$ 127 mm high. In the abutting ends of the compartments, a 70 mm high $\times$ 64 mm wide opening was centered 57 mm above the bottom to form a hurdle. A stainless steel assembly, fitted over the edges of the openings, contained tracks to hold a guillotine door that consisted of two pieces of Luminex glass placed in a metal frame. One compartment, used as the startbox, had a grid floor consisting of 21 stainless steel bars, 3 mm in diameter, placed 11 mm apart, whereas the other compartment, used as a safe box,

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had a Luminex glass floor, hinged so that its depression operated a switch. Color-Vu Gray 10 paper (laminated) was inserted behind the glass walls and floor of the safe box, whereas the walls of the startbox were backed with Color-Vu White paper. White and gray papers were placed appropriately between the glass pieces of the guillotine door so that the interior of the safe box was gray and that of the startbox, except for the floor, was white. Hinged to each compartment was a wooden box that served as a lid and contained light bulbs allowing for constant illumination of approximately 13 lx and for a light CS that increased the illumination to approximately 200 lx. The bottom of each lid was covered with hardware cloth, 70 mm above which was inserted a pane of ground glass. A wooden holding box, 200×200×190 mm, painted gray, and with Litter Green on the floor, was located next to the startbox and was used during hurdle-jumping training.

Classical fear-conditioning trials that preceded the EFF training were administered in the startbox of the apparatus described above and, so that subjects could be conditioned in pairs, also in the startbox of an identical apparatus located in the same room. The grid floors of the two startboxes were wired in parallel. The room illumination present throughout the experiment was approximately 5 lx.

Subjects. The subjects were 64 naive female hooded rats, 118–120 days of age, from the departmental colony. They were assigned at random to pairs, and then each pair was assigned at random to one of four groups ($n=16$) described below. Seven additional subjects were discarded, four because of apparatus failure and three for meeting a criterion which specified that a subject would be discarded for failure to make a hurdle-jumping response at least twice in the first 10 trials. The subjects were housed in pairs for the duration of the experiment, with food and water available ad lib except during the experimental treatments.

Design and Procedure. On Day 1, each pair of subjects received handling and exploration treatments in the experimental room. The sequence consisted of 5 min of handling (subjects alternately being petted and placed on a small table to explore), 10 min of exploration with one subject in each compartment of the hurdle-jumping apparatus with the guillotine door closed, 5 min of handling, and 10 min of exploration of the other compartment of the apparatus.

On Day 2, each pair of subjects received 35 fear-conditioning trials with an intertrial interval (ITI) of 2 min in either the startbox of the hurdle-jumping apparatus or in the other identical apparatus mentioned above. On each trial, the onset of the light CS was followed after 4 sec by a 2-sec inescapable shock of 125-V ac, delivered through 100 K Ω resistance. The CS and shock terminated simultaneously. The typical reaction of the subjects to the shock included vocalization and vigorous movement, and defecation occurred frequently.

On Day 3, 40 hurdle-jumping, EFF trials were administered in the absence of shock to each subject singly with a 30-sec ITI. On each trial, the subject was placed in the start compartment facing the guillotine door, which was raised after 10 sec simultaneously with the presentation of the CS and the activation of a Hunter Klockounter. If the subject jumped the hurdle, depression of the safe-box floor terminated the Klockounter and the CS, thus providing a measure, in units of .01 sec, of the latency of the response. After a jump, the guillotine door was closed immediately and the subject remained in the safe box for 10 sec before being removed to the holding box for the ITI. If no jump occurred within 60 sec, the subject was removed to the holding box for the ITI and a 60-sec latency was recorded.

The groups differed with respect to the manner in which the subjects were handled during the experiment. For two groups, the subjects were held by the body (B). That is, the experimenter grasped the subject by placing his hand over its back behind the forelegs. For the other two groups, during certain portions of the experiment, the subjects were handled by being grasped at the base of the tail (T). Specifically, during exploration and conditioning, these subjects were held by the tail when being placed into and

when being removed from the apparatus. During hurdle-jumping training, the subjects were removed from the holding box by the body, placed on the experimenter's lap, grasped by the tail, and placed in the startbox of the apparatus. When removed from the safe box (or from the startbox if no hurdle-jumping response occurred), the subjects were grasped by the tail and returned to the holding box. At all other times, the subjects in this condition were handled by the body. For both handling procedures, the subjects were held and transported in a gentle manner. At no time were gloves used when handling the subjects.

Within each handling condition, the subjects in one group received an extra CS-UCS pairing and an opportunity to jump the hurdle and escape the fear-eliciting CS and situational cues immediately prior to the initiation on Day 3 of the 40 EFF trials (Groups EP-B and EP-T), and the subjects in the other group did not (Groups NEP-B and NEP-T). On the EP (extra pairing) trial, the subject was placed with the appropriate handling procedure into the startbox facing the guillotine door and, 10 sec later, a conditioning trial, as described above, was administered, except that the CS remained on after shock termination. Immediately following the cessation of shock, the guillotine door was opened and the usual hurdle-jumping procedures were followed.

The treatments for each subject on each of the 3 days of the experiment were separated by approximately 24 h.

Results

Figure 1 shows the mean speed of hurdle jumping for each group as a function of blocks of five trials. Performance of the EP groups was superior to that of the NEP groups, irrespective of the handling procedure employed, and this superiority was apparent on the first trial block. Furthermore, it can be seen that, as training progressed, handling the subjects by the tail increasingly degraded performance relative to handling by the body under both the EP and NEP conditions. These observations were supported statistically. An ANOVA for repeated measures with the

Figure 1. Mean reciprocal of latency as a function of blocks of five trials for groups given an extra-pairing treatment (EP) or not (NEP) and handled by the body (B) or tail (T). Performance on Single Trials 1–5, and on the EP trial, is plotted in the inset. See text for explanation. (Experiment 1)

extra-pairing treatment (EP vs. NEP) and handling treatment (B vs. T) as the between-subjects factors and trial blocks as the within-subject factor yielded significant effects of extra pairing [$F(1,60)=11.74$, $p < .005$], trial blocks [$F(7,420)=126.28$, $p < .001$], and the interaction between trial blocks and handling [$F(7,420)=3.43$, $p < .005$]. None of the other factors reached acceptable levels of significance, although the effect of handling was marginal [$F(1,60)=3.24$, $p = .077$]. Because of the interaction, a factorial ANOVA was carried out on terminal performance (Trial Block 8). This analysis yielded a significant effect of handling [$F(1,60)=4.77$, $p < .05$] and of extra pairing [$F(1,60)=7.91$, $p < .01$]; the interaction was not significant ($F < 1$).

To determine the initial point at which the EP treatment became effective, the data from the single trials within the first trial block were evaluated. These data are plotted, along with the hurdle-jumping speeds on the EP trial, in the inset of Figure 1. A repeated-measures ANOVA over the five single trials yielded a significant effect of extra pairing [$F(1,60)=22.82$, $p < .001$] and of trials [$F(4,240)=13.33$, $p < .001$]. Neither the effect of handling nor any of the interactions was significant. Therefore, the facilitative effect of the EP treatment was evidenced on the very first trial.

Discussion

The facilitation of performance resulting from the extra-pairing treatment is consistent with the findings of Crawford et al. (1977). However, their failure to find learning in the absence of this treatment was not replicated, because learning occurred in the present experiment under both the EP and NEP conditions. Learning also occurred under both handling procedures but, as expected, tail handling clearly degraded performance.

The design of this experiment provided no basis for determining the reason for the effects obtained with either the extra-pairing or the tail-handling procedure. The following two experiments attempted to provide an explanation of these effects.

EXPERIMENT 2

This experiment was conducted in an attempt to isolate the aspect of tail handling that led to the detrimental effect on performance found in the first experiment.

Method

Apparatus. The apparatus was identical to that used in Experiment 1.

Subjects. The subjects were 64 naive female hooded rats, 113-120 days of age, from the departmental colony. They were assigned at random to pairs, and then each pair was assigned at random to one of the four groups ($n=16$) described below. Two additional subjects were eliminated for meeting the discard crite-

ria described in Experiment 1. The subjects were maintained as in the first experiment.

Design and Procedure. The procedures used for handling and exploration on Day 1 and fear conditioning on Day 2 were identical to those described in the first experiment, except that in the present experiment all subjects were handled by the body on these days.

The groups differed with respect to the handling procedure used during the hurdle-jumping trials on Day 3. Subjects in Group BB were handled by the body when being placed into the startbox and removed from the safe box to the holding box, whereas subjects in Group TT were handled by the tail. Thus, subjects in these groups were treated during hurdle jumping identically to those in Groups NEP-B and NEP-T of Experiment 1. Subjects in Group BT were placed into the startbox by the body and were removed from the safe box by the tail, whereas subjects in Group TB were placed into the startbox by the tail and removed from the safe box by the body. It should be clear that the first letter of the group designation indicates the handling procedure used in placing the subject into the startbox, and the second letter, the procedure used in removing the subject from the safe box. In all other respects, the hurdle-jumping procedures were the same as those used in Experiment 1, but the extra-pairing treatment was not administered to any of the groups.

Results

The mean speed of hurdle jumping in blocks of five trials for each of the groups is shown in Figure 2. As can be seen, performance increased over trial blocks for all groups. It is apparent that performance did not depend on the method of handling used in placing the subject into the apparatus. However, the handling procedure used in removing the subject from the apparatus affected performance markedly, with tail handling having a deleterious effect.

These observations were confirmed by a repeated-measures ANOVA with handling during placement into the apparatus and handling during removal from the apparatus as the between-subjects factors and

Figure 2. Mean reciprocal of latency as a function of blocks of five trials for groups handled by the body (B) or tail (T). The first letter indicates the handling treatment used for placement into the startbox, and the second, for removal from the safe box. (Experiment 2)

trial blocks as the within-subject factor. Handling during removal from the safe box [$F(1,60)=9.16$, $p < .005$], trial blocks [$F(7,420)=81.90$, $p < .001$], and the interaction between these two factors [$F(7,420)=6.67$, $p < .001$] were the only significant effects. Because of the interaction, terminal performance (Trial Block 8) was evaluated with a factorial ANOVA. Only handling during safe-box removal was significant [$F(1,60)=7.69$, $p < .01$].

Discussion

It seems clear that the degrading effect of tail handling observed in this experiment, as well as in Experiment 1, can be attributed to some negative aspect of the procedure that was manifested when it was used *following* the hurdle-jumping response. Thus, a reinforcement process is implicated. Probably, some aversiveness introduced by the tail-handling procedure decreased the reinforcement occurring with the escape from the fear-eliciting stimuli in the startbox. Despite this evidence that tail handling is aversive, the method of handling used in placing subjects into the startbox had no reliable effect on performance. Conceivably, because of the large amount of fear already present in the startbox, the additional aversiveness introduced would be proportionately small and, therefore, would not have a measurable effect. On the other hand, it is possible that the aversiveness resulting from tail handling would have waned by the time a trial started.

EXPERIMENT 3

The facilitatory effect resulting from the extra-pairing treatment in Experiment 1 could be due to either or both of two aspects of that treatment: the extra CS-shock pairing or the occurrence of the hurdle-jumping response immediately after shock termination. The present study was designed to resolve this issue.

Method

Apparatus. The apparatus was identical to that used in the previous experiments.

Subjects. The subjects were 60 naive female hooded rats, 118-121 days of age, from the departmental colony. They were assigned at random to pairs, and then each pair was assigned at random to one of three groups ($n=20$) described below. Two additional subjects were eliminated for meeting the discard criterion described in Experiment 1. The subjects were maintained as in the previous experiments.

Design and Procedure. The procedures used for handling and exploration on Day 1 and for fear conditioning on Day 2 were identical to those employed in Experiment 2. On Day 3, all groups received 40 EFF trials according to the procedures described in Experiment 1 for subjects handled by the body. The three groups differed with respect to the treatment received preceding these trials. Groups EP-R and NEP-NR were replications of Groups EP-B and NEP-B of Experiment 1. Thus, Group EP-R received an extra pairing of light and shock and an opportunity to escape to the safe box after shock termination, whereas Group NEP-NR

received neither the extra pairing nor the opportunity to respond. Group EP-NR received the extra pairing but was removed from the startbox to the holding box immediately following shock termination.

Results

Figure 3 presents the mean speed of hurdle jumping in blocks of five trials for the three groups. As may be seen, the performance of Group EP-R was superior to that of the other two groups throughout training, and the difference between the two EP groups tended to remain relatively constant. The performance of the EP-NR and NEP-NR groups was highly similar early in training, but the EP-NR group showed increasingly superior performance as training progressed. These data were evaluated statistically with a repeated-measures ANOVA with groups as the between-subjects factor and trial blocks as the within-subject factor. A significant effect of groups [$F(2,57)=5.09$, $p < .01$] and of trial blocks [$F(7,399)=207.59$, $p < .001$] was found. The interaction was not significant [$F(14,399)=1.31$, $p = .20$]. Newman-Keuls tests yielded a significant difference only between Groups EP-R and NEP-NR ($p < .01$).

Because, on the basis of Experiment 1, the EP treatment was expected to have an effect from the outset of training, the data of the single trials within the first trial block, plotted in the left portion of Figure 4, were analyzed with a repeated-measures ANOVA. Groups [$F(2,57)=13.33$, $p < .001$] and trials [$F(4,228)=21.75$, $p < .001$] were both significant, but the interaction was not ($F < 1$). Newman-Keuls tests revealed that Group EP-R was signifi-

Figure 3. Mean reciprocal of latency as a function of blocks of five trials for groups given a CS-shock pairing and allowed to jump the hurdle just prior to EFF training (EP-R), given a CS-shock pairing but not allowed to jump the hurdle (EP-NR), or given neither treatment (NEP-NR). (Experiment 3)

Figure 4. Mean reciprocal of latency as a function of Single Trials 1-5 and 36-40 for the three groups depicted in Figure 3. Performance on the EP trial for Group EP-R is also shown. (Experiment 3)

cantly superior to both of the other groups ($p < .01$), which did not differ. A similar analysis of the single-trial data comprising the final trial block, shown in the right portion of Figure 4, yielded a significant effect of groups [$F(2,57) = 4.88$, $p < .025$]. Neither trials nor the interaction was significant ($F_s < 1$). Newman-Keuls tests indicated a significant difference ($p < .01$) only between the EP-R and the NEP-NR groups. That is, Group EP-NR did not differ statistically from either of the other two groups, a result consistent with the tests based on the grand means.

Discussion

The data obtained on the initial trials of the present experiment indicate that the facilitatory effect of the EP treatment in Experiment 1 can be attributed to the occurrence of the hurdle-jumping response made following the CS-shock pairing and not to the extra pairing per se. This conclusion has two bases. First, of the two groups that received the extra CS-shock pairing, the performance of the group allowed to make the hurdle-jumping response (EP-R) was superior to the one not allowed to make the response (EP-NR). Second, the two groups not allowed to make the response (EP-NR and NEP-NR) did not differ in performance, even though one had received the extra CS-shock pairing. Theoretically, the initial superiority of the EP-R group may be attributed to the receipt of reinforcement from two sources following the response on the extra trial: (1) fear reduction occurring with the escape from the fear-eliciting stimuli (CS and situational cues) and (2) the progressive decrease in the presumably aversive persisting effects of shock occurring after shock termination.

It should be noted that the results pertaining to the effects of the EP treatment are contrary to a memorial interpretation. If the extra-pairing treatment

merely reinstated the memory of the pairings received on the previous day, it would be expected that the EP-NR group would be superior to the NEP-NR group, but it was not.

Because the results for the later stages of training are ambiguous, conclusions about the effect of the manipulated variables on terminal performance are, unfortunately, difficult to draw. The fact that the performance of the EP-R group was significantly superior to that of the EP-NR group early but not late in training may be attributed to one of two factors. First, the extra CS-shock pairing may have contributed to later performance of the EP-NR group even though it had no effect initially. There seems to be no ready explanation for such a possibility. On the other hand, the lack of a statistically significant difference between the groups in their terminal performance may have resulted from the operation of a ceiling effect in the EP-R group. The implication is that the superiority of this group shown initially would have continued to be reliable throughout training had no ceiling been present.

Because the facilitative effect of the EP-R treatment is apparently due to the occurrence of the reinforced response following the CS-shock pairing and not to the additional fear-conditioning trial itself, it can be assumed that the amount of conditioned fear present in the three groups at the outset of training was essentially the same. If so, it could be expected that, with training, the performance curves of the three groups would tend to converge. The indication that such convergence did not occur, but rather that different maximal levels of performance seem to have been reached, requires explanation. A likely interpretation arises from the fact that EFF training is a measure of resistance to extinction (W. R. McAllister & D. E. McAllister, 1971, p. 109). The faster escape speed shown initially by the EP-R group as a result of their extra-pairing treatment would reduce the amount of exposure to the fear-eliciting stimuli of the startbox, as compared to the other groups, and, hence, less extinction of fear would occur. This differential in the rate of extinction of fear should persist with trials and lead to the difference in levels of performance observed.

GENERAL DISCUSSION

Use of the tail-handling procedure, as opposed to body handling, was found to depress, but not to prevent, the learning of the EFF response. Therefore, tail handling can be considered to be a partial basis for the failure of Crawford et al. (1977) to obtain EFF learning under standard conditions. Presumably, some other aspect of their procedure was also non-optimal and, when combined with the degraded reinforcement from tail handling, was sufficient to prevent learning. One candidate for this role is the

procedure employed by Crawford et al. of delivering shock to the metal walls of their apparatus, including that containing the door, as well as to the grid floor. Thus, approaching the door to escape to the safe box would bring the subject closer to an object that had previously delivered shock, a circumstance that could debase performance. However, learning was obtained, even given these nonoptimal conditions, when an extra CS-shock treatment was administered. The results of Experiment 3 suggest that the crucial aspect of that treatment was the occurrence on this trial of the hurdle-jumping response. Presumably, the role of shock was to induce the response and, as described previously, to contribute to reinforcement so that it was sufficiently large to support learning.

In addition to yielding data of methodological importance, the results of the handling manipulation provide information concerning the nature of reinforcement in an aversively motivated instrumental learning task. Historically, reinforcement has been conceived of as resulting from some discrete event such as the termination of a conditioned stimulus. Yet there is a sizable body of data, to which the present results contribute, which indicates that all conditions which operate to affect the situation present after a response must be considered in identifying the source(s) of reinforcement. For example, whether the subject has explored the safe box prior to learning (W. R. McAllister & D. E. McAllister, 1967), the similarity of the safe and start compartments (Knapp, 1965; D. E. McAllister et al., 1972), the presence of extra-apparatus cues when a long time interval separates conditioning and test (W. R. McAllister & D. E. McAllister, 1965), and the presentation of a feedback stimulus following a response (e.g., Bower, Starr, & Lazarovitz, 1965) are among the events that have been found to affect the degree of reinforcement. The method of handling used after the response joins this list. It is probably not a coincidence that all of these events can be conceived of as affecting the amount of fear reduction.

In contrast to the above explanation, the learning of the hurdle-jumping response by the extra-pairing groups was attributed by Crawford et al. (1977, pp. 69-70) to the punishment on the single extra-pairing trial of the species-specific defense reaction (SSDR) of freezing. The resultant suppression of the freezing SSDR presumably allowed the flight SSDR to occur. This account raises a number of questions. If it is simply that the single extra CS-shock pairing punished and, thereby, suppressed freezing, then some explanation is needed for the failure of 35 such pairings, which were administered to all groups during fear conditioning, to serve the same function. It also seems gratuitous to argue that freezing was punished on the extra-pairing trial when the behavior of the subjects at the time shock was presented was

apparently not systematically observed. Furthermore, it is not made clear how the mere presence of a flight SSDR can account for the observed increases in speed of responding over trials (i.e., learning) in the extra-pairing groups. Complicating this problem is their report that some subjects not given the extra-pairing treatment jumped the hurdle on every trial but did not show learning. It would seem that such consistent hurdle jumping would imply the presence of a flight, rather than a freezing, SSDR. Therefore, it does not seem possible to predict whether or not learning will occur when there is evidence that a flight SSDR is present.

The above rejection of the SSDR account used by Crawford et al. to explain their findings is not meant to imply that freezing or crouching responses cannot under some circumstances play a role in determining whether or not active responses are learned. The occurrence of competing freezing responses in situations involving aversive stimuli has been an accepted fact for decades, and the potential influence that such responses may have on the learning of other, active responses has been discussed in detail by Brown and Jacobs (1949) and Miller (1948, 1951). However, in the standard EFF task, where freezing can occur but where the hurdle-jumping response is reinforced by fear reduction, the weight of the evidence is that punishing the freezing response by shock is not necessary to produce learning.

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