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The use of an untrained index response in a conditioned suppression paradigm

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The need for extended training and motivational manipulations when using an instrumental index response in a conditioned suppression paradigm is often problematic. An index response requiring no prior training and no manipulation of motivational states is described. An experiment which exemplifies the use of this index response in a conditioned suppression paradigm is reported.

While a variety of techniques have been developed to obtain behavioral measures of conditioned fear (cf. McAllister & McAllister, 1971), probably the most frequently employed is the conditioned suppression paradigm. Generally this paradigm involves three distinct stages. First, animals are trained to emit a given response (the index response) until the rate of responding is relatively stable. Typically, positively reinforced responses such as barpresses (e.g., Estes & Skinner, 1941) or licks of a drinking tube (e.g., Leaf & Muller, 1965) are employed although responses maintained by negative reinforcement also have been used (e.g., Scobie, 1972). Once a stable response rate has been achieved, animals are subjected to varying numbers of CS-shock pairings intended to result in classical conditioning of fear to the CS. These CS-shock pairings may be presented either in the same apparatus used for instrumental training (e.g., Estes & Skinner, 1941) or in an entirely different apparatus (e.g., Ayres, 1966). Finally, as a measure of conditioned fear, the CS is presented while the animal is performing the previously acquired instrumental response. The degree to which the CS presentation results in suppression of responding is thought to reflect the amount of fear elicited by the CS. This suppression is often explained by the assumption that the CS elicits certain fear-related responses (e.g., crouching and freezing) which are incompatible with the ongoing instrumental response (cf. Miller, 1951).

Numerous problems accompany the use of this standard conditioned suppression paradigm. First, to obtain a valid comparison of conditioned fear among treatment groups, it is necessary that the groups be equated for rate of responding on the instrumental index response prior to the conditioning treatment. This equation of response rates can be difficult to attain in situations in which the groups consist of subjects with different characteristics, e.g., studies comparing strains or age groups. This problem is magnified when the subject characteristics involved are those which might be expected to affect instrumental learning rates (e.g., Pare, 1969).

A second problem relates to measuring retention of conditioned fear. Any retention test involving the conditioned suppression paradigm invariably measures not only retention of the classical CS-US pairings, but also retention of the index response. Even when groups have achieved equivalent response rates prior to fear conditioning, differential forgetting of the index response can result in inflated measures of retention of fear conditioning in one of the groups.

A third problem is that for learning of the index response to occur and for reliable responding to continue following fear conditioning, it is necessary for animals to be placed on a deprivation schedule or be confronted with aversive stimulation. As with the learning of the index response, groups differing with respect to certain subject characteristics may be difficult to equate in terms of motivational states (cf. Goodrick, 1972). Even with animals equated for motivational level, the degree to which a fear-eliciting CS results in suppression of instrumental responding often depends on the amount of deprivation, the intensity of the aversive stimulation, or the incentive properties of the reinforcements employed in the instrumental task (cf. McAllister & McAllister, 1971). Furthermore, certain motivational states may interact with treatments used to modify retention of conditioned fear, making it difficult to study the effects of these treatments. For example, whether or not direct electrical stimulation of the cortex produces amnesia for fear conditioning may well depend on an animal's motivational state at the time the stimulation is administered (e.g., Gold, Bueno, & McGaugh, 1973).

In an effort to circumvent some of these problems, we attempted to discover an index response that required little or no prior learning or motivational manipulation. And it was necessary that the response occur reliably in the absence of a fear-provoking CS and be suppressed when the CS was present. One response that meets these requirements is the reliable movement of most rodents

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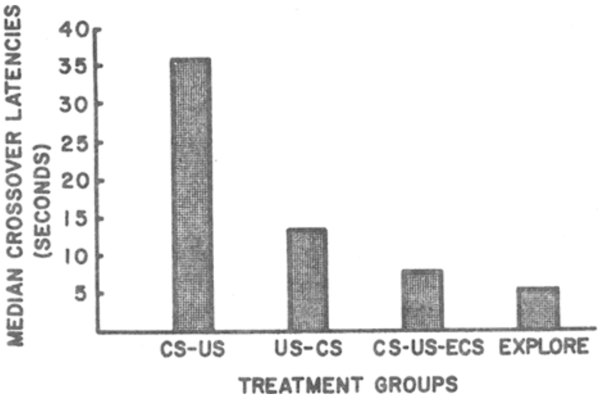

Figure 1. Median crossover latencies for the treatment groups on the test day trial.

from a lighted to a darkened field when allowed to choose environments. This paper reports an experiment exemplifying the use of this unlearned index response within a conditioned suppression paradigm.

METHOD

Subjects

The subjects were 48 naive male albino rats of the Sprague-Dawley strain, 70-80 days old at the beginning of the experiment.

Apparatus

The one-way avoidance box is described in detail by Gordon and Spear (1973). It consists of two equally sized compartments, one white (translucent) and the other black. Each compartment measures approximately 27.5 x 13 x 13 cm, and each contains a grid floor. The door spearating the two chambers can be lowered (opened) leaving a 3-cm hurdle between the chambers. The opening of the door initiates a .01-sec timer that can be terminated by the interruption of a photocell beam located 19 cm into the black compartment. A 7.5-W light bulb located 8 cm behind the white compartment can be operated such that the light flashes at a 2-Hz fequency (i.e., light is on for .25 sec followed by a .25-sec light offset).

The fear conditioning apparatus consists of a clear Plexiglas chamber with a grid floor through which shock can be administered. The chamber measures approximately 30 x 30 x 60 cm. Fastened to the top of the chamber is a white translucent box containing a 7.5-W light operated at a 2-Hz frequency. Located above the chamber is a pulley system carrying leads from an electroconvulsive shock (ECS) generator. The leads terminate in flattened alligator clips that can be fastened to a rat's ears and through which ECS can be administered. The pulley system allows an animal to move freely in the conditioning chamber with the ear clips attached.

Procedure

All rats received 3 days of adaptation to ear clips (5 min/day) outside the experimental room. On the 4th day, all animals were placed in the white chamber of the avoidance apparatus, and, after 3 sec, the flashing light was initiated. The door separating the two chambers was opened 10 sec after the light onset, and the time required for each animal to cross into the black chamber was recorded. Animals were randomly matched on the basis of crossover latencies; four approximately equivalent groups of 12 rats were formed.

On Day 5, all animals were placed in the conditioning chamber with ear clips attached. Animals in Group 1 (CS-US) received a 10-sec presentation of the flashing light, beginning 20 sec after introduction into the chamber. A 1.6-mA footshock of 3 sec duration was delivered 7 sec after the light onset. The CS and US were coterminous. Animals in Group 2 (US-CS) received the US 7 sec after being placed in the chamber and were presented the CS 10 sec after US offset. Group 3 animals (CS-US-ECS) received the same treatment as animals in Group,1 except that each received a 50-mA transpinnate ECS of a .5-sec duration immediately after the termination of the CS and US. Animals in Group 4 (explore) were placed in the conditioning chamber with ear clips attached for 30 sec with no treatment.

Twenty-four hours after the conditioning treatment, all animals were returned to the avoidance apparatus. The procedure for the test trial was the same as that used for the pretraining trial (Day 4). An important aspect of the testing procedure was that the door between the two chambers was not opened until 10 sec after light onset. In previous attempts to develop this paradigm, shorter intervals between CS onset and the door opening had been used (3-5 sec). The use of these shorter intervals sometimes resulted in animals "escaping" from the CS rather than "freezing." The use of the longer interval was necessary to produce reliable suppression by animals given prior fear conditioning.

RESULTS AND DISCUSSION

Crossover latencies on the pretraining trial ranged between 1.3 and 26.0 sec. Only 6 of 48 animals took longer than 10.0 sec to move into the dark compartment. These results suggest that the crossover response occurred reliably and without extreme variation on the part of the subjects employed in this experiment.

The median crossover latencies for the four treatment groups on the test trial are represented in Figure 1. The data in this figure suggest that substantial suppression of the crossover latencies occurred only in the CS-US group. Analyses of these data with Mann-Whitney U tests (Siegel, 1956) supported this suggestion. Animals in the CS-US group had significantly longer latencies than the US-CS animals ($U = 36$, $p < .05$), the CS-US-ECS animals ($U = 11$, $p < .002$) and the exploration animals ($U = 6$, $p < .002$). No other differences were significant.

These results are comparable to the results one would expect in a conditioned suppression paradigm employing a well-trained instrumental index response and highly motivated subjects. The crossover index response was suppressed (in comparison with a nontreated control group) by a CS that previously had been paired with shock in a forward conditioning sequence. Animals given a backward conditioning sequence did not significantly differ from controls, although a minor elevation of latencies was noted in this group. This slight elevation is not unusual, given certain similarities between the conditioning and test chambers (e.g., grid floors, same test room) that might have served as partial CSs for the mediation of fear. Finally, animals given ECS, and presumably rendered amnestic for the conditioning trial, also failed to show significant increases in crossover latencies.

The test latencies in the CS-US group were by no means maximal following the single conditioning trial. Eleven of 12 rats crossed into the black chamber in less than 100 sec, which suggests that greater suppression can be produced by increasing the number of conditioning trials or by changing certain conditioning parameters (e.g., shock intensity, duration). Preliminary work in our laboratory tends to support this suggestion of a graded suppression effect.

Our present paradigm appears to circumvent problems inherent in the more traditional conditional suppression paradigms to a great degree. Even though it remains necessary to equate groups for response rates prior to fear conditioning, most animals perform the crossover response in under 10 sec. Few index responses that require extended training exhibit this kind of minimal variability across subjects. More importantly, certain subject characteristics which often strongly influence rates of learning (e.g., age, sex) appear to influence the crossover response very little. For example, the mean crossover latencies of 20-day-old male (N = 23) and female (N = 20) albino rats were tested prior to fear conditioning. The latencies were 8.1 and 6.6 sec, respectively. This compares with the mean latency of 6.8 sec by subjects used in the present experiment (70-80-day-old males). Thus, the present index response can be used to equate response rates in groups that may learn alternative index responses at different rates.

The problem of confounding retention of fear with retention of the index response does not apply in the present paradigm since the crossover response requires no prior training. It also seems unlikely that any other long-term effects of the pretraining trial (e.g., habituation) might confound the retention of fear measure, since only minimal pre-exposure to the crossover apparatus is given (one short duration trial), and this exposure occurs 24 h prior to the fear conditioning trial.

Finally, the problems inherent in imposing deprivation or stress conditions on an animal during the training of an index response are also reduced. This does not mean that performance of the crossover response requires no motivational underpinning; animals appear motivated to cross from a light to a dark compartment. But, this motivation to explore or seek darkness may be inherent in all experiments using albino rats as subjects. The present paradigm simply alleviates the need to impose on animals special treatments that are known to interact importantly with fear conditioning and electroconvulsive shock treatments (e.g., Gold et al., 1973) and which may differentially influence groups differing in important subject characteristics.

Given the results of the present experiment and the advantages of the crossover paradigm, it appears that suppression of the crossover response may well be useful as an index of fear conditioning. Furthermore, the usefulness of the paradigm may be especially significant in situations in which prior training and motivational manipulations are problematic.

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