Conditioned suppression in the gerbil*

PETER FREY, SANDRA ENG, and WILLIAM GAVIN†
Northwestern University, Evanston, Illinois 60201

The present report describes apparatus and procedures for investigating conditioned suppression of a running response in the gerbil. Data are presented which demonstrate that the conditioned suppression observed in this situation is not an artifact of alpha responses, pseudoconditioning, or sensitization. An analysis of differential conditioning indicated that cue similarity and US intensity are negatively related to cue-specific differential responding.

The conditioned suppression procedure developed by Estes and Skinner (1941) has proven to be a useful paradigm for investigating Pavlovian fear conditioning; barpress responses have been studied with the rat (e.g., Kamin, 1965) and keypecks with the pigeon (e.g., Hoffman, 1969). This procedure typically involves the pairing of a visual or auditory CS with an aversive footshock while an animal is working for food reinforcement on a VI schedule. The degree of suppression of performance in the appetitive task produced by CS presentation is used as a measure of the level of fear conditioning. The present investigations demonstrate the successful generalization of this procedure to a new response system, the running response, and to another species, the gerbil (*Meriones unguiculatus*).

Because of the gerbil's inquisitiveness, tameness, and relatively inexpensive maintenance costs, many investigators have recently attempted to develop laboratory procedures for studying learning in this animal. Reynierse, Scavio, and Spanier (1970) investigated the gerbil's running performance in a 6-ft straight alley for food and/or water reward under several different deprivation conditions. Although some improvement in running performance occurred under the appropriate reward conditions, asymptotic running speed occurred at relatively low speeds (approximately 7 in./sec). The gerbil seems to perform fairly well in shock-motivated tasks such as two-way shuttle avoidance (Powell & Peck, 1969), Sidman avoidance (Powell & Peck, 1969), and passive avoidance (Lippman, Galosy, & Thompson, 1970; Walters & Abel, 1971), even though rate of acquisition in these tasks may be slower than that observed for the rat. These performance deficits are probably not indicative of a lack of learning ability since Blass and Rollin (1969) have demonstrated, with a miniaturized Wisconsin General Test Apparatus, that the gerbil's performance on object-discrimination learning sets is only slightly below that observed for the cat.

The present experiments examined the effect of pairing an auditory CS with footshock on the gerbil's performance in an automated runway. The initial experiment compared a standard conditioning procedure with two control procedures. The second experiment examined differential conditioning in the suppression situation.

EXPERIMENT 1

Method

Twelve male and 12 female gerbils, 12 to 24 months of age, were used as Ss. They were caged individually, and Purina Rat Chow was available for a 4-h feeding period after each daily experimental session.

A narrow automated runway, 25 in. long x 2½ in. wide x 10 in. high, was constructed of Plexiglas and varnished plywood. The floor of the runway consisted of two identical pieces of aluminum angle (1 x 1 x 25 in.) that could be used for presentation of a footshock US. Cave-like cylindrical openings (1¾ in. in diam and 2-1/8 in. in depth) were positioned at both ends of the runway centered in the end wall and located 1 in. above the level of the runway floor. A light beam and photocell system was located in each opening to record movement of the S's head into the end wells. A food pellet magazine (Scientific Prototype Corporation Model D 700) dispensed 45-mg Noyes pellets into one of the end walls. A diagram of this apparatus is presented in Fig. 1.

During food magazine training, the animals, placed on a food-deprivation schedule 1 week before (food available 4 h each day—and the animals learned to eat Noyes pellets on an open table), were given 16 daily sessions of 20 min duration in which food pellets could be received in the runway. Delivery of the pellets was contingent upon breaking the photobeam at the end opposite the foodwell, followed in sequence by breaking the photobeam at the foodwell end. The two photobeams were designated as the "setup" and the "foodwell" photobeams. Only one pellet could be set up at a time. Electromechanical counters recorded the number of times the two photobeams were each broken during the session and the number of pellets delivered.

In Phase 2, 12 animals were assigned to a fear conditioning treatment (P) and 6 were assigned to each of two control treatments consisting of either CS alone (C) trials or CS and US explicitly unpaired (EU) trials. All Ss received one trial per session for 22 sessions of 20 min duration. The CS was presented between the 3rd and 15th minute of each session, the time each day being ordered quasi-randomly. The 3/sec clicker CS was presented over a small speaker

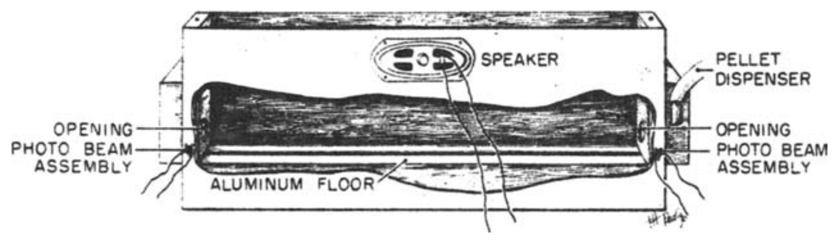

Fig. 1. A schematic diagram of the automated runway apparatus for investigating conditioned suppression in the gerbil.

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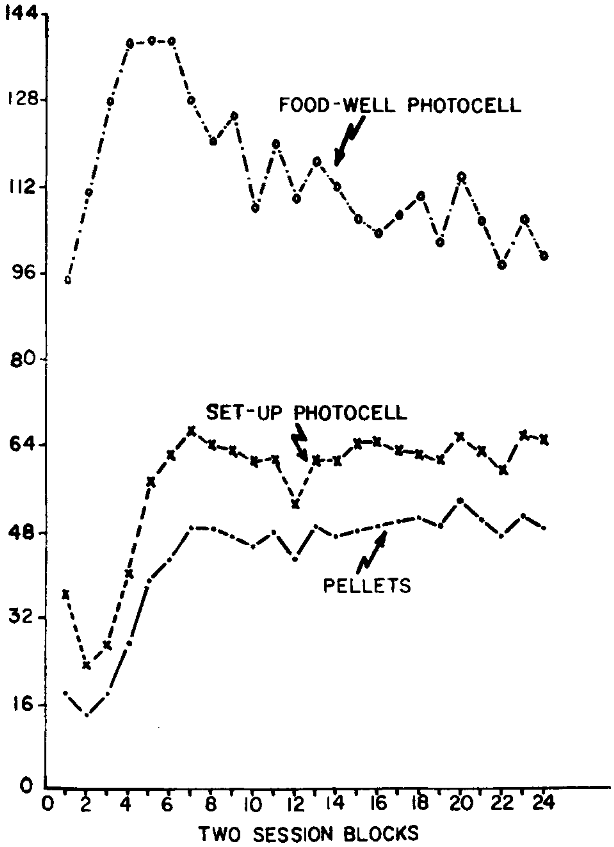

Fig. 2. Runway activity as indexed by the number of photobeam breaks per session and by the number of pellets delivered each session.

mounted on the wall of the runway. CS duration in every case was 75 sec at an intensity level of 70 dB (re: .0002 dynes/cm²). The conditioning treatment (P) in which the CS was paired with shock involved a CS-US interval of 75 sec. In the explicitly unpaired treatment (EU), the shock US was presented first each session, followed by the 75-sec CS after a constant delay of 90 sec. The US was a 1-sec 4-mA constant current ac shock delivered across the two sides of the aluminum floor. Interruption of either photobeam was recorded separately during CS presentation. The dependent measure for the fear response was the degree of suppression of the food-getting response, as monitored by the frequency of photobeam crossing. A suppression index was defined as $\alpha/(\alpha + \beta)$, where α is the number of photobeam breaks/minute during the CS and β is the number of breaks/minute during non-CS periods (see Church, 1969, pp. 114-118). In the present experiments, the activity during all non-CS times was averaged to provide an index of baseline activity.

In Phase 3, transfer effects were assessed by reassigning animals to one of the other treatments. Half the animals with their initial experience with the P treatment were switched to the A treatment, and the other half were switched to the EU treatment. Animals starting with either the A or the EU treatment were switched to the P treatment. The transfer from P to either A or EU assessed the differential extinction effects of CS alone vs CS/US explicitly unpaired. The transfer from A or EU to P assessed the differential effects of pretreatment with CS alone or CS/US explicitly unpaired on acqusition of the fear response under the conventional Pavlovian paradigm.

Results

The gerbils acquired a stable instrumental running response for food in 12 or fewer sessions, as indicated in Fig. 2. The initial decrease in pellets received (sign test: 22+, 2—, $p < .001$) was accompanied by a decrease in activity at the setup photocell end of the runway. This was probably because the gerbils quickly learned that the food pellets were always delivered at the foodwell end of the runway. The 12 sessions required to establish stable running can probably be attributed to the fact that the animals had to run away from the foodwell in order to set up the next pellet. Figure 2 indicates that the gerbils initially learned to spend most of their time at the foodwell end of the runway and only gradually did this pattern change to one in which both ends of the runway were visited at a

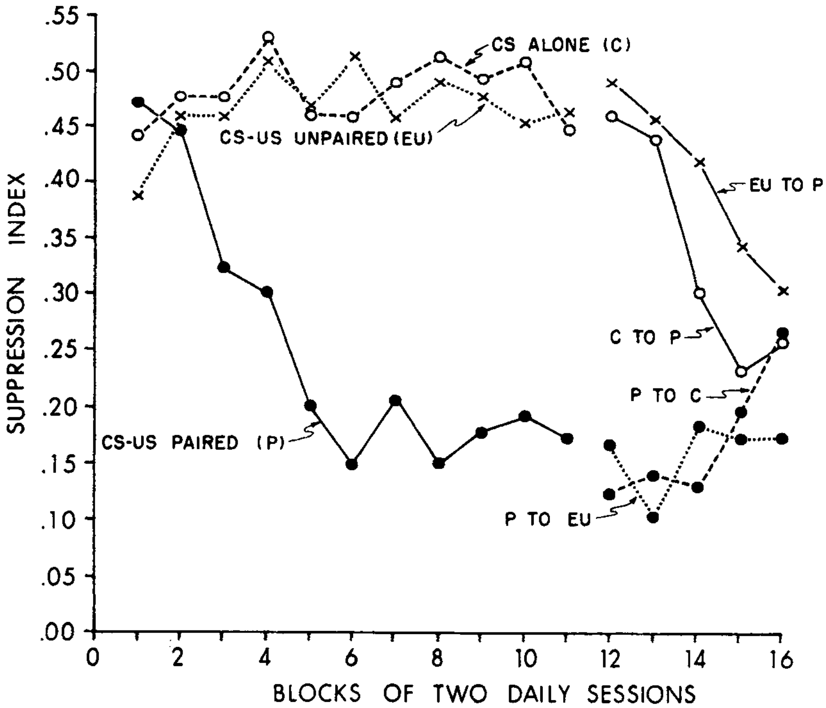

Fig. 3. Conditioned suppression of runway activity under a conditioning and two control procedures and after transfer among these procedures.

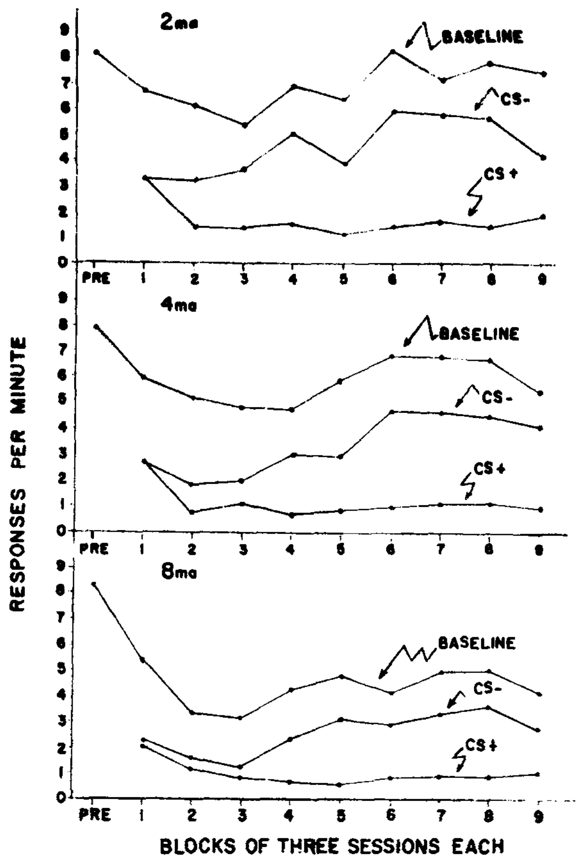

Fig. 4. Differential conditioned suppression as a function of US intensity. The lines in each graph represent activity during non-CS periods, activity during CS—, and activity during CS+.

high frequency. Although the number of pellets received leveled off after the 16th session at about 50 per 20-min session, the frequency of crossing the photobeam at the foodwell end continued to decline gradually throughout the 48 sessions.

The effects of the fear conditioning treatment and the two control procedures is indicated in Fig. 3. In the paired treatment (P), in which the footshock always followed the CS by 75 sec, reliable suppression to the CS was observed after approximately 10 CS-US pairings. The animals receiving only CS presentations (C) or receiving the CS 90 sec after presentation of the 1-sec US (EU) showed no evidence of suppression of the running response during the CS. The difference in suppression level on Sessions 17-22 between Group P (.17) and Groups C (.49) and EU (.47) is highly reliable ($F = 107.30$, $df = 2,21$, $p < .001$). There were also reliable differences in the baseline activity of these groups during non-CS periods in these same sessions. Group C, with 10.3 photobeam breaks/minute, showed a higher level of general activity than either of the groups receiving footshock. The baseline for Group P was 8.5 photobeam breaks/minute and for Group EU was 7.4 photobeam breaks/minute. These differences were reliable ($F = 3.84$; $df = 2,21$; $p < .05$).

In the third phase of the experiment, each of the groups was switched to one of the other training procedures. The groups that had previously experienced the C and EU procedures were switched to the P procedure. The animals that had learned to suppress under the P procedure were switched either to the C procedure or to the EU procedure. Figure 2 indicates that the switch from C to P and from EU to P resulted in the development of suppression to the CS. The rate of learning was more rapid, however, for the C-to-P transfer than for the EU-to-P transfer (performance on Blocks 14-16: C-to-P = .27, EU-to-P = .36; $t = 2.31$, $df = 10$, $p < .05$).

The animals that were transferred from P to C and from P to EU did not show a rapid loss of suppression to the CS. On Block 16, however, the P-to-C animals were showing reliably less suppression than the P-to-EU Ss (P-to-C = .27, P-to-EU = .18; $t = 2.47$, $df = 10$, $p < .05$). These data indicate that suppression is more readily conditioned after pretreatment with C as opposed to EU and that suppression is "extinguished" more rapidly using C as opposed to EU.

EXPERIMENT 2

The first experiment provided reasonable evidence that the gerbil apparatus and procedure were effective for studying conditioned suppression. Additional research assessed the relative contribution of CS and non-CS cues in eliciting suppression by contrasting a differential conditioning procedure with two control procedures. The apparatus and magazine training procedure were essentially the same as in the previous experiment. The differential conditioning procedure involved a 1,000-Hz tone as CS+ and a 800-Hz tone as CS—, with each tone presented once during each 20-min session. The CS-US interval was 75 sec and the CSs were presented for 75 sec at 15 dB above a background white noise level of 60 dB. The footshock US was a constant 4-mA ac shock of 1 sec duration. The gerbils acquired the running response for food at essentially the same rate as the animals had in Experiment 1, and rapid and reliable conditioned suppression was observed when the tones and footshock were introduced. Unfortunately, there was no evidence of differential suppression to CS+ and CS—. The second formal experiment was designed to investigate several variables that might have been responsible for the absence of differential responding in the pilot investigation.

Method

The Ss were 27 male and 27 female gerbils, 12 to 24 months of age. The animals were maintained on a feeding schedule with Purina Lab Chow available 4 h a day following each day's experimental sessions. The apparatus and magazine training were essentially the same as in the previous two experiments.

The design of this experiment was a 3 by 3 factorial with three levels of footshock intensity (2, 4, or 8 mA) and three levels of cue similarity (CS+ was always a 1,500-Hz tone and CS— was either a 1,200-Hz tone, a 400-Hz tone, or a 3/sec click. All groups received differential conditioning training. In contrast to the previous experiment, the CS-US interval was 20 sec and both CS+ and CS— were each presented four times per session. CS duration was 20 sec and US duration was 1 sec. The two CSs were presented in alternation, with CS+ occurring first each session. The intertrial interval was a fixed 135 sec, with 20-min training sessions. Fear conditioning was started after all animals displayed stable performance of the running response for food.

Results

Analysis of variance on differential responding and on baseline activity indicated reliable effects of both main variables, but there were no interactive effects. For this reason, the data are presented separately for each main variable. Figure 4 demonstrates the effects produced by manipulation of footshock intensity. The frequency of photobeam breaks during CS+, CS—, and non-CS periods (baseline) are presented as a function of training sessions separately for each shock intensity. The top line in each graph is baseline performance, the bottom line is performance during CS+, and the middle line is performance to CS—. Differential conditioning was observed at each shock intensity and was slightly better at the low-intensity shock level (photobeam breaks/minute to CS— minus photobeam breaks/min to CS+ over Sessions 5-9; linear trend, $F = 4.77$, $df = 1{,}45$, $p < .05$). Footshock intensity produced an even more dramatic effect on food-getting activity during non-CS periods. The baseline on Sessions 5-9 was suppressed as a function of US intensity (linear trend, $F = 13.86$, $df = 1{,}45$, $p < .001$). It is probable that the inferior differential performance at high shock levels is a direct result of the general decrease in food-getting activity in the high shock intensity condition. With high-intensity footshock, the gerbil shows strong suppression during non-CS periods.

The effect of manipulating the similarity of CS+ and CS— is shown in Fig. 5. The top line in each graph is baseline performance, the bottom line is performance during CS+, and the middle line is performance to CS—. Cue similarity had a reliable effect on differential responding (photobeam breaks/minute CS— minus CS+ on Sessions 5-9; $F = 6.40$, $df = 2.45$, $p < .005$) but not upon the baseline response level (photobeam breaks/minute during non-CS period on Sessions 5-9; $F = 2.16$, $df = 2{,}45$).

These data indicate that differential conditioning can be reliably demonstrated using the present suppression procedure. Manipulation of cue similarity exerted a strong influence on differential responding. When CS+ and CS— were similar (1,500 Hz vs 1,200 Hz), the gerbils responded differentially, but some suppression was observed to both cues. When CS+ and CS— were reasonably dissimilar, suppression was observed to CS+, but performance to CS— was similar to baseline performance. The US intensity manipulation primarily affected the gerbil's baseline activity. At 8 mA, there was a marked suppression of food-getting activity during non-CS periods. At 2 mA, there was very little suppression during non-CS periods.

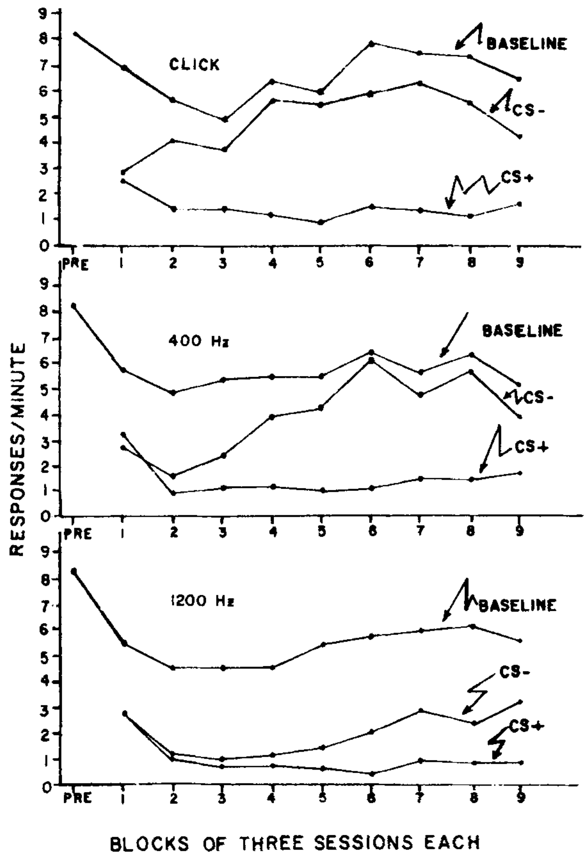

Fig. 5. Differential conditioned suppression as a function of cue similarity. CS+ was always a 1,500-Hz tone and CS— was either a 1,200-Hz tone, a 400-Hz tone, or a 3/sec click.

GENERAL DISCUSSION

The present experiments clearly demonstrate that the gerbil is an excellent S for investigating fear conditioning. The initial experiment indicated that CS-related suppression could be conditioned after only a few pairings of an auditory CS with footshock. Control groups indicated that this conditioned suppression was not attributable to alpha responses, to pseudoconditioning, or to sensitization. In contrast to pilot research, the second experiment demonstrated reliable differential conditioning in this situation. There were a number of procedural differences that might account for these dissimilar outcomes. In the pilot work, CS+ and CS— were each presented only once per session. In the formal experiment, each was presented four times per session. Within-session contrast of the two cues may be important in developing differential responding to the cues. A 75-sec CS-US interval was employed in the pilot study, while a 20-sec interval was used in Experiment 2. This shorter interval may have been helpful in associating the onset of each of the auditory cues with its appropriate consequence. Also, the tones employed as CS+ (1,000 Hz) and CS— (800 Hz) in the pilot research were probably too similar. The data from Experiment 2 indicate that cue similarity was an important determinant of the degree of differentiation.

The successful demonstration of CS-specific fear conditioning in these experiments provides evidence that the gerbil runway procedure is a useful alternative to the more typical rat barpress or pigeon keypeck suppression techniques. The present procedure has several advantages which may be important to some investigators. Because of the gerbil's relatively high baseline activity level, conditioned suppression in this species is usually a matter of degree rather than the all-or-none suppression behavior commonly observed in the rat. This is usually reflected by smaller within-group variances. Another point is that the present apparatus is no more expensive to construct than the apparatus normally used with the rat or pigeon. The gerbil has a major advantage in terms of initial purchase cost and maintenance costs. These considerations, in conjunction with the present behavioral data, provide an excellent rationale for using the gerbil runway procedure for investigating Pavlovian fear conditioning.

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