Animal Learning & Behavior
1994, 22 (4), 379–383

Fear-potentiated startle using three conditioned stimulus modalities

WILLIAM A. FALLS and MICHAEL DAVIS
Yale University, New Haven, Connecticut
and Connecticut Mental Health Center, New Haven, Connecticut

The capacities of three different conditioned stimulus modalities (light, noise, and airflow produced by a fan) to produce fear-potentiated startle were evaluated. Previous experiments have shown that following either light–shock or noise–shock pairings, both the light and noise conditioned stimuli acquire the ability to potentiate the acoustically elicited startle response in rats (the so-called *fear-potentiated startle effect*). In Experiment 1, the ability of airflow produced by a fan to act as a conditioned stimulus was investigated. Rats were given either paired or unpaired fan–shock training followed by a test for fear-potentiated startle. The fan conditioned stimulus potentiated startle only in the group given explicit fan–shock pairings. In Experiment 2, we evaluated the discriminability of the three conditioned stimulus modalities. Rats were given light, noise, or fan–shock pairings and were subsequently tested for fear-potentiated startle with the trained conditioned stimulus as well as the two remaining novel conditioned stimuli. Only the trained conditioned stimulus potentiated startle. These results show that fear-potentiated startle can be produced with three discriminable conditioned stimulus modalities, allowing the future use of fear-potentiated startle in the investigation of higher order conditioning phenomena.

A conditioned stimulus (CS) that is repeatedly paired with a shock unconditioned stimulus (US) acquires the ability to facilitate the acoustically elicited startle reflex in rats (Brown, Kalish, & Farber, 1951; Davis & Astrachan, 1978). This fear-potentiated startle effect is one example of a class of procedures in which Pavlovian emotional conditioning is assessed via modulation of a separately initiated behavior (Rescorla & Solomon, 1967; Wagner & Brandon, 1989). A central state of fear is assumed to be the conditioned response in this paradigm (McAlister & McAlister, 1971). Conditioned fear is operationally defined by elevated startle amplitudes in the presence (vs. the absence) of a stimulus that was previously paired with shock.

Fear-potentiated startle has several advantages for the investigation of Pavlovian emotional conditioning: It does not require prior operant training, it can be produced after a single CS–US pairing (Davis, Schlesinger, & Sorenson, 1989), and it is retained over a very long time interval (Campeau, Liang, & Davis, 1990). Because fear-potentiated startle involves modulation of a rapid reflex that is elicited by a brief probe stimulus, the excitatory strength of the CS can be assessed at any time during the CS by manipulating the time at which the reflex is elicited (Davis et al., 1989). Fear-potentiated startle occurs following explicitly paired CS–US presentations, but not following explicitly unpaired or "random" CS–US presentations (Davis & Astrachan, 1978). Extinction of fear-potentiated startle has also been investigated (Falls, Miserendino, & Davis, 1992). On the other hand, higher order conditioning phenomena have not been demonstrated in this paradigm, because only two CS modalities (light and noise) have been used to produce fear-potentiated startle. Because of the limited parametric information on CS modality, in the present study we evaluated whether airflow produced by a fan can serve as a CS and, if so, whether rats can discriminate between the light, noise, and fan CSs.

Preparation of this article was supported by NIMH Grant MH-25642, MH-47840, Research Scientist Development Award MH-00004, a grant from the Air Force Office of Scientific Research, and the State of Connecticut. W. A. Falls is now at the Department of Psychology, Northern Illinois University, DeKalb, IL 60115 (e -mail: tj0waf@corn.cso.niu.edu). Correspondence and reprint requests should be addressed to M. Davis, Department of Psychiatry, Yale University, Abraham Ribicoff Research Facilities, Connecticut Mental Health Center, 34 Park Street, New Haven, CT 06508 (phone: 203-789-7448; fax: 203-562-7079).

EXPERIMENT 1

We exposed rats to either explicitly paired or unpaired fan–shock training and then tested for fear-potentiated startle. A no-training control group was included in which startle amplitude was measured in the presence and the absence of a neutral fan CS to assess whether the fan produced any unconditioned effects on the startle reflex.

Method

Subjects. A total of 44 male albino Sprague-Dawley rats (Charles River Co., Kingston, NY), weighing between 330 and 430 g were used. All the rats were housed in hanging wire cages (17 × 35 × 45 cm) in groups of 3–5. The rats were maintained on a 12:12-h light:dark cycle with lights on at 7:00 a.m. Food and water were continuously available.

Apparatus. Conditioning and fear-potentiated startle testing were conducted in five identical stabilimeter devices (see Cassella & Davis, 1986). Each stabilimeter consisted of an 8 × 15 × 15 cm Plexiglas and wire-mesh cage suspended between compression springs within a steel frame. The floor of each stabilimeter consisted of four 6.0-mm-diam stainless steel bars spaced 18 mm apart, through which shock could be administered. Cage movement resulted in displacement of an accelerometer with the resulting voltage being proportional to the velocity of displacement. The analog output of the accelerometer was amplified and digitized on a scale of 0–4096 units by a MacADIOS II board (GW Instruments, Somerville, MA) interfaced to a Macintosh II microcomputer. We defined startle amplitude as the peak accelerometer voltage that occurred during the first 200 msec after the startle stimulus onset.

Each stabilimeter was located within a 68.5 × 35.5 × 42 cm ventilated, plywood isolation box. This inner isolation box was located within an additional, outer, 76 × 47 × 51 cm, ventilated, plywood isolation box. This "double housing" of stabilimeter devices prevented ultrasonic communication among the rats. All five stabilimeter and double-housing isolation boxes were located in a ventilated, sound-attenuating chamber (2.5 × 2.5 × 2 m; Industrial Acoustics Co., Bronx, NY). A surveillance camera (Ikegami, Model ITC-40, Utsunomiya, Japan) was positioned behind each stabilimeter within the inner isolation box and connected to a TV monitor located outside of the Industrial Acoustics isolation chamber. A red light bulb (7.5 W), located 25 cm from the stabilimeter, illuminated the chamber at all times.

A white-noise generator (Grason-Stadler, Model 901B, West Concord, MA), connected to a 16.5-cm two-way speaker (Alpine, Model 6267AX, Alpine Electronics, Torrance, CA) and located 36.6 cm from the rear of each stabilimeter, provided background noise (0–20 kHz) at 55 dB. Ventilation fans attached to the side walls of the inner and outer plywood isolation boxes produced additional noise. The overall background noise level was 64 dB (SPL; A scale). The startle stimulus was a 95-dB (SPL; A scale), 50-msec burst of white noise provided through a high-frequency speaker (Radio Shack Super Tweeter, Tandy Inc., Fort Worth, TX) located 2 cm from the front of each stabilimeter. Brushless computer fans (Radio Shack, Model 273-243, Tandy Inc., Fort Worth, TX), located 12 cm above the floor of each startle cage, provided the fan CS used during conditioning. The fans were positioned so that the air flow was vertical and downward. The 12-V dc fans were operated by a power source that provided 11.5 V dc. Sound pressure measurements, which were made with a sensitive Brüel & Kjaer decibel meter (Model 2235, Brüel & Kjaer, Malborough, MA), revealed that the fan CS did not raise the overall noise level above the background. The unconditioned stimulus in each stabilimeter was a scrambled shock, generated by one of five Lehigh Valley constant-current shock generators (Model SGS-004, BRS/LVE Bettsville, MD) located outside of the industrial acoustics isolation chamber. Shock intensity was measured with a 1-kΩ resistor across a differential channel of an oscilloscope in series with a 100-kΩ resistor connected between adjacent floor bars within each stabilimeter. Current was defined as root-mean square voltage across the 1-kΩ resistor, where mA = 0.707 × 0.5 × peak-to-peak voltage. According to this method, shock intensity was 0.6 mA. The presentation and sequencing of all stimuli were controlled by a Macintosh II microcomputer (Apple Computer, Cupertino, CA).

Procedure. Baseline startle measurements were made prior to any training or testing. Each baseline session began with a 5-min period during which no stimuli were presented. Thereafter, we presented 10 startle-eliciting stimuli at each of 3 noise-burst intensities (viz., 90, 95, and 105 dB), for a total of 30 startle-eliciting stimuli. The three noise-burst intensities occurred in a balanced irregular order, with a 30-sec interval between successive startle stimuli. Baseline startle measurement served to familiarize the rats with handling, the apparatus, and the startle stimulus.

Training began 1 day after the baseline startle test and consisted of two consecutive daily sessions. The first training trial in each session occurred 5 min after we placed the rats in the stabilimeter devices. The rats in Group Paired (n = 17) received 10 fan–shock training trials consisting of a 3.7-sec fan operation that coterminated with a 500-msec, 0.6-mA scrambled footshock. The intertrial interval averaged 4 min (range, 3–5 min). The rats in Group Unpaired (n = 17) received the same number of fan and shock presentations, except that these events were explicitly unpaired with variable interstimulus intervals that ranged between 1.5 and 2.5 min. The rats in the no-training control group (n = 10) remained in the colony room.

Testing occurred in the stabilimeters 1 day after the last training session. The session began with a 5-min period during which no stimuli were presented. Thirty 95-dB noise bursts were then presented to habituate the rats to the startle-eliciting stimulus. All the rats subsequently received 15 presentations of the startle-eliciting noise burst alone (no-fan test trials) intermixed with 15 presentations of the startle-eliciting noise burst 3.2 sec after the onset of the 3.7-sec fan stimulus (fan test trials). The interval between successive noise bursts was 30 sec. No-fan test trials and fan test trials occurred in a pseudorandom sequence.

Results and Discussion

Figure 1 shows mean startle amplitudes during no-fan and fan test trials. The difference between responses on these two trial types also is shown. Startle amplitude during testing varied with prior training condition. Presentation of the fan stimulus in the no-training group produced a slight suppression of startle amplitude relative to no-fan trials. Somewhat greater suppression occurred to the fan CS following explicitly unpaired fan–shock training (Group Unpaired). Despite this sup-

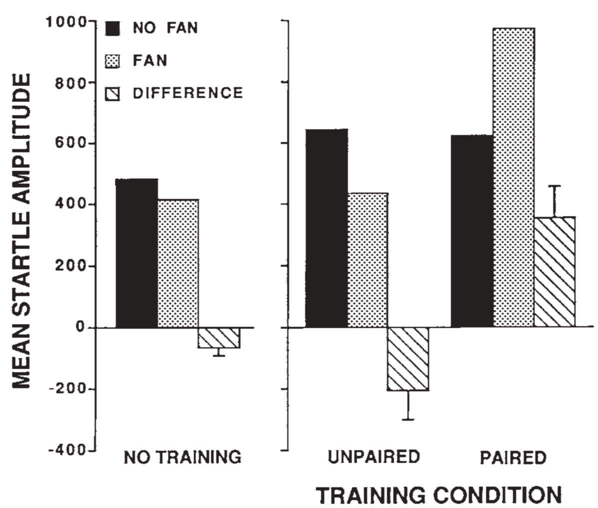

Figure 1. Mean startle amplitudes obtained on no-fan and fan test trials and their difference for groups that were given no-training, unpaired, or paired fan–shock trials. Error bars represent standard error of the mean.

pressive effect, the fan CS produced potentiated startle in Group Paired.

A two-variable analysis of variance (ANOVA; training group × trial type) conducted on mean startle amplitudes obtained from no-fan and fan test trials yielded a significant main effect of group [$F(2,41) = 3.24$, $p < .05$] and a significant group × trial type interaction [$F(2,41) = 11.44$, $p < .001$]. The main effect of trial type was not statistically significant ($F < 1$). The small suppressive effect of the fan in the no-training group was not statistically significant ($t < 1$). The fan significantly suppressed startle in the unpaired group [$t(16) = 2.44$, $p < .05$]; however, the difference in startle between the no-training and unpaired groups was not statistically significant [$t(25) = 1.16$, $p > .05$]. In contrast, the fan significantly potentiated startle following paired fan–shock training [$t(16) = 4.12$, $p < .001$]. These results indicate that a fan CS can produce fear-potentiated startle, and they extend previous work from our laboratory indicating that fear-potentiated startle occurs following explicitly paired, but not explicitly unpaired CS–US training (Campeau & Davis, 1992).

EXPERIMENT 2

Although fear-potentiated startle now can be produced using three different CS modalities, it is not known to what degree rats can discriminate among the three modalities. Campeau and Davis (1992) showed that rats can discriminate between two noise-frequency bands following differential conditioning in which one frequency is consistently followed by shock and the other is never followed by shock. However, it is possible that fear-potentiated startle is nonspecific in the absence of explicit discriminative conditioning and may occur to any salient change in background stimulation as the result of complete generalization. To assess this possibility, rats received fan, light, or noise–shock training, or no training. We tested all four groups for fear-potentiated startle using all three CS modalities so that the trained groups received the paired CS modality as well as the two novel CS modalities.

Method

Subjects. A total of 48 male albino Sprague-Dawley rats (Charles River Co., Kingston, NY), weighing between 330 and 430 g, were housed and maintained as in Experiment 1.

Apparatus. The apparatus was identical to that described in Experiment 1. In addition, an 8-W fluorescent bulb with a rise time of 100 msec was attached to the back of each stabilimeter and served as a light CS. The noise CS was a 70-dB (SPL, A-scale) white noise that was bandpass filtered with high and low passes set at 2000 Hz (Campeau & Davis, 1992). The noise CS was delivered through the same speaker that provided background noise.

Procedure. Baseline startle measurements were made prior to training or testing as described in Experiment 1.

Training occurred over 2 consecutive days and began 1 day after the baseline startle test. The rats in Groups Fan+, Light+, and Noise+ were given 10 CS–shock trials on each of the two training days. Each trial consisted of a 3.7-sec CS that coterminated with a 500-msec, 0.6-mA footshock. The intertrial interval averaged 4 min (range, 3–5 min). The CS was a fan ($n = 9$), a light ($n = 10$), or a 2-kHz noise ($n = 9$). The rats in a no-training group remained in the colony room.

One day later, all the rats received a test for fear-potentiated startle that included all three CS modalities. They were placed in the stabilimeter devices and, after a 5-min period during which no stimuli were administered, received 30 95-dB noise bursts so that

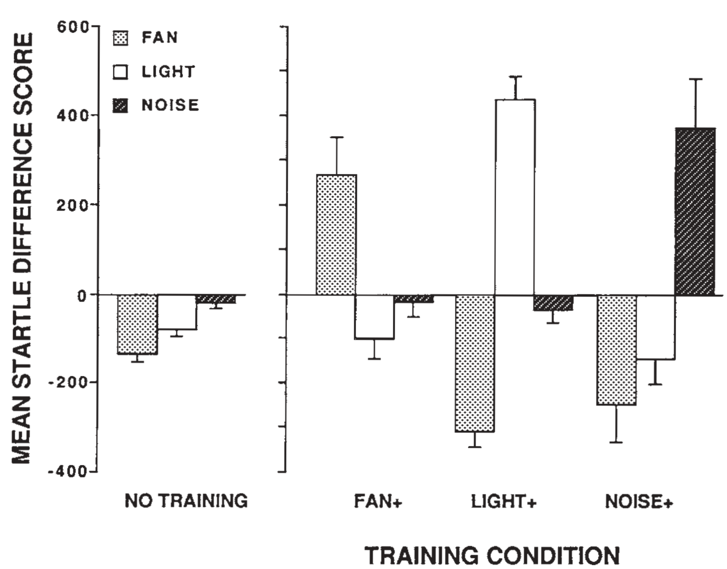

Figure 2. Mean startle difference scores obtained on fan, light, and noise CS test trials for groups that were given no training, fan–shock (Fan+), light–shock (Light+), or noise–shock (Noise+) training. Difference scores were calculated by subtracting the mean startle amplitude obtained on no-CS test trials from the amplitude obtained on CS test trials. Error bars represent the standard error of the mean.

they would be habituated to the startle-eliciting stimulus. Immediately thereafter, all the rats received 15 no-CS test trials intermixed with 15 each of fan, light, and noise CS test trials. On CS test trials, the startle-eliciting noise burst occurred 3.2 sec after the onset of a 3.7-sec CS. The interval between successive noise bursts was 30 sec.

Results and Discussion

Baseline startle amplitudes did not differ among the groups. Analysis of mean startle amplitudes obtained on no-CS test trials revealed no effect of group ($F < 1$). Therefore, mean startle difference scores were computed and analyzed separately. Difference scores were calculated for each rat by subtracting the mean startle amplitude obtained on no-CS test trials from the mean startle amplitude obtained on fan, light, and noise CS test trials. The resulting difference scores reflect the magnitude of fear-potentiated startle in the presence of each CS and are shown in Figure 2.

All three test stimuli resulted in sight suppression of startle during CS test trials relative to no-CS trials in the no-training group. The rats given CS–shock training prior to testing (Groups Fan+, Light+, and Noise+) showed reliable fear-potentiated startle only to their training CS. Novel CSs in these groups produced slight to substantial suppression of startle.

An ANOVA (group $\times$ test CS modality) yielded a significant group $\times$ test CS interaction [$F(6,88) = 35.36$, $p < .001$]. An analysis of the interaction indicated significant differences among the CS modalities in Groups Fan+ [$F(2,16) = 14.6$, $p < .001$], Light+ [$F(2,18) = 61.72$, $p < .001$], and Noise+ [$F(2,16) = 42.80$, $p < .001$]. We conducted *t* tests on the difference scores, and these indicated that significant potentiated startle occurred to the training CS in Groups Fan+ [$t(8) = 3.75$], Light+ [$t(9) = 6.44$], and Noise+ [$t(8) = 5.18$; ps $< .01$]. The suppression of startle occurring on CS test trials in Group No-Training was statistically different from startle on no-CS trials only for the fan CS [$t(19) = 2.76$, $p < .05$]. The fan CS also produced statistically reliable suppression in Groups Light+ [$t(9) = 4.54$, $p < .01$] and Noise+ [$t(8) = 3.45$, $p < .01$]. This suppression was greater in the trained groups (i.e., Groups Light+ and Noise+) than in the No-Training group [$t(28) = 4.33$, $p < .001$; $t(27) = 2.75$, $p < .05$, respectively, for Groups Light+ and Noise+]. Neither the light CS nor the noise CS produced reliable suppression of the startle reflex.

These results indicate that all three CS modalities can produce fear-potentiated startle following pairing with shock and, more importantly, demonstrate the specificity of fear-potentiated startle to the CS that was previously paired with shock.

GENERAL DISCUSSION

The fear-potentiated startle effect has proven useful for investigating behavioral, anatomical, and pharmacological bases of Pavlovian fear conditioning (Davis, 1992). Together with previous experiments (Campeau & Davis, 1992), the present study demonstrates that fear-potentiated startle can be produced by light, noise, or fan CSs following paired, but not unpaired, CS–shock training. Moreover, the rats showed fear-potentiated startle only to the CS modality that had been previously paired with shock.

Prior to the present experiment, the degree of stimulus control of fear-potentiated startle was not known. In the extreme case, following light–shock training, rats might have shown fear-potentiated startle to any salient stimulus or any significant change in background stimulation (e.g., complete generalization). Instead, the present results clearly show that fear-potentiated startle produced with these CSs is under explicit stimulus control. The lack of generalization among the three modalities, together with the fact that each modality produced comparable levels of potentiated startle, will permit the use of these three stimulus modalities interchangeably. This is critical in investigations of higher order conditioning, in which discriminability and stimulus salience are major concerns.

The three CS modalities tended to produce a modest suppression of the startle reflex in untrained rats, with the fan having the greatest (and only statistically reliable) suppressive effect. These unconditioned inhibitory effects were larger in rats that were given prior light–shock or noise–shock training (see Figure 2). Perhaps fear produced by the conditioning context or residual effects of the CS permits detection of the unconditioned effect of novel stimuli on baseline startle or enables the novel stimuli to inhibit the baseline startle reflex (e.g., by facilitating attention to the novel stimulus). Experiments are currently under way to evaluate these possibilities.

REFERENCES

Brown, J. S., Kalish, H. I., & Farber, I. E. (1951). Conditioned fear as revealed by magnitude of startle response to an auditory stimulus. *Journal of Experimental Psychology*, **41**, 317-328.

Campeau, S., & Davis, M. (1992). Fear potentiation of the acoustic startle reflex using noises of various spectral frequencies as conditioned stimuli. *Animal Learning & Behavior*, **20**, 177-186.

Campeau, S., Liang, K. C., & Davis, M. (1990). Long-term retention of fear-potentiated startle following a short training session. *Animal Learning & Behavior*, **18**, 462-468.

Cassella, J. V., & Davis, M. (1986). The design and calibration of a startle measurement system. *Physiology & Behavior*, **36**, 377-383.

Davis, M. (1992). The role of the amygdala in conditioned fear. In J. P. Aggleton (Ed.), *The amygdala: Neurobiological aspects of emotion, memory, and mental dysfunction* (pp. 255-305). New York: Wiley-Lis.

Davis, M., & Astrachan, D. I. (1978). Conditioned fear and startle magnitude: Effects of different footshock or backshock intensities used in training. *Journal of Experimental Psychology: Animal Behavior Processes*, **4**, 95-103.

Davis, M., Schlesinger, L. S., & Sorenson, C. A. (1989). Temporal specificity of fear-conditioning: Effects of different conditioned stimulus-unconditioned stimulus intervals on the fear-potentiated startle effect. *Journal of Experimental Psychology: Animal Behavior Processes*, **15**, 295-310.

Falls, W. A., Miserendino, M. J. D., & Davis, M. (1992). Extinction of fear-potentiated startle: Blockade by infusion of an excitatory amino acid antagonist into the amygdala. *Journal of Neuroscience*, **12**, 854-863.

McAlister, W. R., & McAlister, D. E. (1971). Behavioral measurements of conditioned fear. In F. R. Brush (Ed.), *Aversive conditioning and learning* (pp. 105-179). New York: Academic Press.

Rescorla, R. A., & Solomon, R. L. (1967). Two-process learning theory: Relationships between Pavlovian conditioning and instrumental learning. *Psychological Review*, **74**, 151-185.

Wagner, A. R., & Brandon, S. E. (1989). Evolution of a structured connectionist model of Pavlovian conditioning. In S. B. Klein & R. R. Mowrer (Eds.), *Contemporary learning theories: Pavlovian conditioning and the status of traditional learning theory* (pp. 149-190). Hillsdale, NJ: Erlbaum.

(Manuscript received October 5, 1993;
revision accepted for publication March 2, 1994.)