

Immediate-Startle Stimulus Presentation Fails to Condition Freezing Responses to Contextual Cues

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Twenty-eight male Wistar rats were placed in a novel and distinctive environment. Eighteen of these received an intense startle-eliciting white noise stimulus. Animals that experienced a 60-s delay between placement and the startle stimulus demonstrated significant freezing in the context, both poststartle (Session 1) and on a later startle-free test (Session 2). Animals that received immediate startle, however, did not differ on either occasion from animals that did not experience the startle stimulus. The amplitude of the startle response was not affected by this manipulation, which indicates a dissociation between freezing and startle responses with immediate- versus delay-startle presentation. The findings are consistent with Fanselow's (1986) conditioned stimulus-based associative explanation of the immediate-shock freezing deficit.

Freezing, a crouchlike response of the rat (Blanchard & Blanchard, 1969) is a species-specific defense reaction (Bolles, 1970) that is both a conditioned and unconditioned response (Blanchard & Blanchard, 1969; Blanchard, Fukunaga, & Blanchard, 1976a; Fanselow & Sigmundi, 1986). It is conditionally elicited by cues that signal the onset of an aversive event and is most often studied as a response to cues associated with shock (Blanchard & Blanchard, 1969; Blanchard, Dielman, & Blanchard, 1968; Bouton & Bolles, 1980; Fanselow, 1980, 1982; Sigmundi, Bouton, & Bolles, 1980), but it has also been found to be elicited by cues associated with startle-eliciting auditory stimuli (Cranney, 1987).

Blanchard, Fukunaga, and Blanchard (1976b) found that rats placed in an inescapable environment and given footshock after a short delay show a reliable freezing response. Animals that are shocked immediately after placement, however, fail to freeze. This has been called the "immediate-shock freezing deficit" (Fanselow, 1986).

Blanchard et al. (1976b) explained this effect according to an environmental control hypothesis in which delay-shock animals are seen to have perceived the environment as inescapable and freeze rather than attempt to flee because flight is not supported in this environment. Animals in the immediate-shock condition, however, have not yet learned that the environment is inescapable, and hence flight, which is seen as the more dominant response, is selected. In contrast, Fanselow (1986) proposes that the basis of the immediate-shock freezing deficit is the lack of sufficient time in the immediate condition to (a) encode all the elements that make up the context and (b)

process these elements so that they form a unit that can be associated with the shock stimulus and so become a conditioned stimulus (CS). This explanation, therefore, assumes an associative deficit on the basis of inadequate processing of the CS (context) and is based on a prior assumption that freezing is a conditioned response (CR) to shock-associated cues but not an unconditioned response (UR) elicited by shock itself (Fanselow 1980, 1982).

There are significant reasons to expect that immediate-versus-delayed startle will have an effect on freezing responses similar to that caused by immediate-versus-delayed shock. Acoustic startle stimuli have been found to have similar properties to shock even at levels presumed to be too low to cause tissue damage. High-intensity startle stimuli (~105–120 dB) are able to condition freezing responses to contextual cues (Borszcz, Cranney, & Leaton, 1989; Cranney, 1987; Leaton & Cranney, 1990). Poststartle responding to intense startle stimuli, like postshock responding, is a biphasic response, that is, an abrupt startle response followed by prolonged freezing. Cues associated with the startle stimulus elicit freezing but not startle responding, a pattern very similar to that caused by shock. It appears, therefore, that startle stimuli may share many of the aversive conditioning properties of shock.

The present experiment was designed (a) to examine whether the freezing-deficit effect with immediate stimulation would generalize to another aversive event, the acoustic startle stimulus, and (b) to use the startle response to examine whether the UR is affected in the immediate condition. If the startle amplitude as well as poststartle freezing is attenuated in the immediate-startle condition, then the assumption that the freezing deficit is dependent on an associative deficit is questionable. In particular, if startle amplitude is also attenuated, then this would suggest that there is a problem with encoding the startle stimulus itself, which would indirectly lead to less conditioning. We predicted that if the freezing deficit is due to a direct disruption of conditioning, then an intense startle stimulus (the unconditioned stimulus, US) presented to an animal immediately after placement in a startle chamber (the CS) will elicit startle amplitudes (the UR) similar to those

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elicited by animals receiving delayed startle but that poststartle freezing (the CR) will be less in the immediate-startle group.

Method

Subjects

Twenty-eight experimentally naive male Wistar rats, which were obtained from the University of New South Wales (Kensington, New South Wales, Australia) Animal Breeding and Holding Unit, served as subjects for this experiment. There were 9 rats in each of the two experimental groups and 10 in the control group. Rats were matched as closely as possible by weight. When testing began, the mean weights ($\pm SD$) were 381.8 ± 15.63 g for the immediate-startle group, 381.5 ± 15.47 g for the delay-startle group, and 380.7 ± 18.01 g for the no-startle control group. Subjects were allowed free access to food and water and were maintained on a 12:12-hr light-dark cycle.

Apparatus and Stimuli

The apparatus was similar to that described by Cranney (1988). Briefly, rats were tested in one of two identical $20 \times 12 \times 12$ cm startle chambers that were constructed of Plexiglas and that had grid floors. Each cage was suspended from a Plexiglas sheet to which piezoelectric film had been laminated. The abrupt startle reflex movements made by the rat caused the Plexiglas to flex slightly, inducing a voltage in the piezoelectric film (see Leitner & Rosenberger, 1983). This voltage was digitized by a microcomputer system, and the peak positive voltage within 100 ms after startle stimulus onset was taken as the measure of startle response.

The startle-eliciting stimulus was a 117-dB (SPL, ~ 20 μ Pa), 50-ms white noise burst (0-ms rise-fall time). Continuous white noise (70 dB SPL, ~ 20 μ Pa) was used to mask any extraneous auditory stimuli, and all test stimuli were superimposed on this background. Stimulus and background intensity were measured with a Brüel and Kjaer sound-level meter (Type 2235), with a microphone placed in a standard, central position in each chamber.

Procedure

Animals were handled for 1 min each on 5 successive days, matched by weight on the last day of handling, and randomly allocated to one of the three experimental conditions. Before each rat was placed in the test chamber, the chamber was cleaned with a 0.5% acetic acid solution, and the underlying bedding was cleaned if necessary.

During Session 1, animals in the immediate-startle condition were placed in the test chamber by one experimenter who remained within the soundproof experimental room to record freezing. A startle stimulus was immediately triggered by a second experimenter (through closed-circuit video observation of the startle chamber) as soon as the door of the startle chamber was closed. Rats were left in the chambers for 120 s. During the first 60 s, the presence or absence of the poststartle freezing response was sampled at 1-s intervals. Freezing responses were defined as (a) the absence of movement of body or vibrissae, except for movement associated with respiration (Fanselow, 1980), (b) piloerection, and (c) a stance that did not include resting the abdomen on the base of the cage. Animals in the delay-startle condition received the startle stimulus 60 s after placement in the cage and were left in the cage for another 60 s, and the presence or absence of the freezing response was sampled at 1-s intervals. Subjects in the no-startle control condition were placed in the chambers for a total of 120 s, and the freezing response was time-sampled for the entire period.

After a 4-hr delay, during which the animals remained in their home cages, all subjects were returned to the test chambers for 60 s, and the freezing response was again time-sampled (Session 2).

Freezing scores for each subject were calculated by expressing the number of samples in which freezing was observed as a percentage of total number of samples in the observation period. Thus for each subject, a single score was calculated for Session 1, and a single score was calculated for Session 2. The only exception was for the no-startle control group, for which scores were calculated for each of the two 60-s observation periods of Session 1. All animals were videotaped during the observation periods to allow for a reliability check on freezing measures. An experienced rater, who was unaware of the condition of the subjects, scored the videotaped freezing responses of 6 randomly chosen rats during the period immediately after the offset of the startle stimulus. A reliability score of $r = .992$ was obtained for total percentage scores of the two raters.

All statistical analyses of freezing and startle responses were undertaken using a fully planned analysis with multivariate analysis of variance (MANOVA)-adjusted critical values to control for a family-wise error rate of $\alpha = .05$. Planned contrasts tested all possible group and repeat comparisons as well as Group \times Repeat interactions. Although MANOVA procedures are reasonably robust to violations of the normality and homogeneity assumptions (Stevens, 1986), confirmatory nonparametric Mann-Whitney U tests were conducted on all relevant between-groups comparisons on the freezing variate because there was little variation within two of the groups of this experiment. The upper-bound version of this test was used (Rohlf & Sokal, 1981; Sokal & Rohlf, 1969). Separate Friedman two-way analyses of variance (ANOVAs) were undertaken on the repeated measures for both the immediate- and delay-startle groups.

Results

In both the first and second 60-s periods of Session 1 for the no-startle control group, no freezing was observed. Therefore, both the immediate and delay startle groups were compared with a single control value of zero freezing. Figure 1 presents the mean percentage freezing by each group during Session 1 (poststartle) and Session 2 (context). Although some freezing was observed across the two sessions in the immediate-startle group, this did not differ significantly from zero, $F(1, 25) = 1.014$, $p > .05$. The delay-startle group, however, did show significantly more freezing than both the no-startle control, $F(1, 25) = 37.793$, $p < .05$, and immediate-startle, $F(1, 25) = 25.106$, $p < .05$, groups. No significant difference was found between the two test sessions, and no interactions reached significance. The significantly higher freezing obtained by the delay group in Session 1, therefore, was maintained in the subsequent context test. Nonparametric tests confirmed these analyses. A Friedman two-way ANOVA found no significant differences between Session 1 and Session 2 freezing scores for any group. Mann-Whitney U tests found significant differences on Session 1 and Session 2 scores between the immediate- and delay-startle groups, $Us(9, 9) = 70$ and 67 , respectively, $ps < .05$, and between the delay- and no-startle groups, $Us(9, 10) = 85$ and 90 , respectively, $ps < .05$, but no difference between immediate- and no-startle groups, $Us(9, 10) = 20$ and 30 , respectively, $ps < .05$.

There was no reliable difference in startle amplitude ($M \pm SD$) between the immediate- (104.7 ± 69) and delay-startle (136 ± 76.8) groups ($t = 0.911$, $p > .1$), despite the reliable difference in freezing.

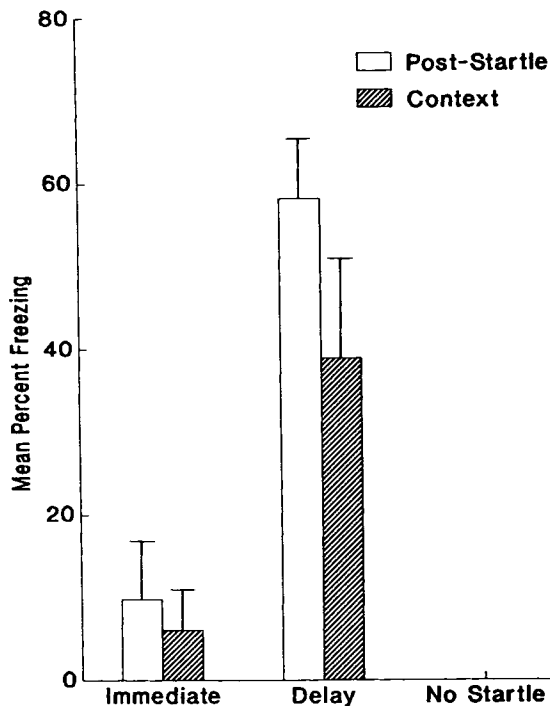


Figure 1. Mean percentage of freezing for the immediate-, delay-, and no-startle groups observed over 60 s, both immediately after startle (Session 1) and in the same context 4 hr later (Session 2).

Discussion

The pattern of findings with the freezing response replicates and extends the findings of Blanchard et al. (1976b) and Fanselow (1986) and further supports the suggestion that startle stimuli act in a manner similar to shock in supporting aversive conditioning (Cranney, 1987).

The results do not support the environmental control hypothesis of Blanchard et al. (1976b). If the fear elicited in the immediate-startle subjects was expressed as escape attempts instead of freezing, this study's repeated measures design should have enabled these subjects sufficient opportunity to learn that the context was inescapable in the poststartle period, so that when the contextual cues were presented again, freezing rather than escape should have followed. The absence of freezing in the same animals on both poststartle and context-alone tests, therefore, is not consistent with the response selection hypothesis.

The results are consistent with Fanselow's (1986) associative deficit account. Like delayed shock, delayed presentation of an intense startle stimulus in the experimental apparatus resulted in apparatus cues acquiring the ability to elicit freezing, as shown by freezing on a startle-free context test (Session 2). Startle delivered immediately after placement in the apparatus, however, not only resulted in significantly less freezing during the context test, but these animals did not differ significantly from animals that received no startle at all. This same pattern of freezing was also observed in the poststartle freezing test (Session 1), which suggests a strong relationship

between freezing in the post-US period and freezing to the CS (context) alone. Furthermore, the dissociation that was observed between freezing responses and startle amplitudes, with immediate startle attenuating the former but having no effect on the latter, is consistent with Fanselow's (1986) proposal that freezing is not elicited directly by the US itself but is conditioned by the US to contextual cues associated with it.

The results of this experiment have shown that the freezing deficit is generalizable to immediate-startle stimulus presentation and that this deficit is independent of any attenuation of startle amplitude. This pattern of dissociation between startle and freezing responses is consistent with the hypothesis that freezing is a CR and not a UR to aversive events. Caution must be used in interpreting this result, however, because the startle response is a relatively low-level response to the startle-eliciting stimulus (i.e., the startle reflex is mediated by the lower brainstem; Davis, Gendelman, Tischler, & Gendelman, 1982) and may not adequately reflect the integrity of higher processes associated with the US, such as (a) encoding a representation of the US or (b) inhibited or disrupted processing of the aversive nature of the US (e.g., analgesia that results from stress elicited by experimenter handling). Both of these proposals are consistent with an associative deficit explanation but suggest that US processing may be disrupted rather than CS processing.

Fanselow (1990) has recently shown that, in relation to animals with no preexposure, subjects given preexposure to the shock context before the conditioning session exhibit facilitated conditioning when very brief delays (about 9 s) are used between placement in the apparatus and shock delivery. Because rats given either (a) no preexposure and a brief delay during the conditioning session (Fanselow, 1990) or (b) preexposure but no delay (i.e., immediate shock) during the conditioning session (Fanselow, 1986) both fail to show enhanced freezing, it could be argued that the facilitation with preexposure and very brief delays is due to (a) the formation of a representation of the context during preexposure and (b) retrieval of that representation during the conditioning session. This "facilitation" effect, therefore, is consistent with Fanselow's (1986) CS-based associative deficit explanation of the immediate-shock freezing deficit because facilitation occurs when conditions favor the presence of an active representation of the context at the time of shock delivery. Thus, although an associative deficit based on disrupted US processing cannot be entirely dismissed, converging evidence suggests that a CS-based associative deficit is the most likely explanation for the deficit in freezing responses after immediate-shock or immediate-startle presentation.

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