

## Startle Responding and Context Conditioning Naloxone® Pretreatment and Stimulus Intensity

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**Abstract**—The possibility that acoustic startle stimuli could support a conditional response (freezing) to contextual stimuli was investigated. Rats were exposed to three acoustic startle stimuli on the first day, and one on the second day. On day 1, 20 rats received naloxone pretreatment and another 20 received saline (placebo) pretreatment. Half of each group received a high-intensity acoustic stimulus, the other half a low-intensity acoustic stimulus. Both the higher stimulus intensity and the naloxone pretreatment led to greater freezing behavior during the 3-minute test period before the single startle stimulus on day 2. These findings support the notion that increased actual or perceived intensity of the acoustic startle stimulus increases conditioning to contextual stimuli as indexed by freezing behavior.

AN ELECTRIC FOOTSHOCK elicits an immediate burst of activity in rats, followed 2 to 10 seconds later by a period of immobility called freezing (Anisman and Waller 1973, Myer 1971). This freezing response is one of the rat's species-specific defense reactions (Bolles 1970), and represents a conditional reaction to apparatus stimuli that have become associated with shock (Blanchard and Blanchard 1969, Bolles and Collier 1976, Fanselow 1980).

Fanselow (1981, 1984, Fanselow and Bolles 1979) reported that naloxone, an opiate antagonist, enhances both the activity burst and freezing components of the postshock reaction. On the basis of these and other findings, Fanselow (1979, 1984) argued that naloxone enhances the perceived intensity of the shock by antagonizing endogenous opioid analgesic systems. He also argued that these effects are specific to nociceptive stimuli, as naloxone decreased the activity burst elicited by a nonnociceptive acoustic startling stimulus, and had no effect on the freezing that followed the nonnociceptive stimulus (Fanselow 1984). This finding corroborates previous reports in the startle literature of no effect of opioid manipulations on the startle response (*e.g.*, Davis 1979, Warren and Ison 1982), and confirms the generally held notion that the acoustic startle stimulus is not painful (Myer 1971), and so cannot support conditional responding.

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Data that appear to contradict this notion have recently been reported in two separate areas of inquiry. First, Kelly and Leitner (1984), in investigating opioid and nonopioid stress analgesia, reported increased startle responsiveness after the administration of naloxone. Second, Borszcz and colleagues (Borszcz, Cranney, and Leaton 1984, 1985) reported findings that suggested that increased startle responsiveness and freezing behavior result from an association formed between the initially aversive startle stimulus and the contextual stimuli of the startle apparatus. These findings suggest that the acoustic startle stimulus has nociceptive properties and can support a conditional response, particularly freezing to contextual stimuli.

Given the numerous methodologic differences among these previous studies, it is not surprising that different conclusions were drawn. For example, the intensity of the auditory stimulus varied across the studies; Warren and Ison (1982) used a 110-dB white noise stimulus, whereas Kelly and Leitner (1984) used a 120-dB white noise stimulus. The lower intensity used by Warren and Ison (1982) may be related to the lack of influence of opioid manipulations. That is, the more intense stimulus, as employed by Kelly and Leitner (1984), may have nociceptive properties, and the presence of such properties may be a necessary condition for opioid manipulations to exert an influence. Accordingly, the current study varied the intensity of the acoustic startle stimulus, substituting the acoustic for the electrical stimulus in the experimental paradigm of Fanselow (1984, Fanselow and Tighe 1984). The procedure had two phases that were carried out 24 hours apart.

On day 1, the training day, the rat was simply exposed to a few stimuli in the experimental apparatus in order to establish a freezing baseline. On day 2, the test day, the rat was returned to the experimental apparatus and given a single stimulus 3 minutes later. The percentage of time spent freezing during the 3-minute prestimulus period is an index of the amount of freezing conditioned to apparatus stimuli during the training day.

The opioid manipulation employed in the current study was pretreatment on day 1 with the opioid antagonist naloxone. If the acoustic startle stimulus has nociceptive properties, then naloxone will increase the perceived intensity and aversiveness of the stimulus (Fanselow 1984), and thus increase conditioning to contextual stimuli, as indexed by freezing behavior during the prestimulus period on day 2.

### Methods

#### Subjects

Forty male albino Sprague-Dawley rats were used. None had undergone experiments before. The rats were 100 days old at the beginning of the experiment. They were individually housed, maintained on a 14:10 hour light/dark cycle, and allowed free access to food and water. All experimental procedures were carried out during the lighted portion of the cycle. Rats were handled for at least 20 seconds twice a day for 5 days before the experiment began.

#### Apparatus and Stimuli

The apparatus has been described previously (Leaton 1976). Briefly, animals were tested in a 20 × 12 × 12-cm startle chamber enclosed within a dimly illuminated, sound-attenuating box, with an observation window in the front wall. Vertical displacement of the chamber moved an attached magnet within a fixed coil and induced a voltage that was amplified and integrated (Model 7P10, Grass). The integrated output was adjusted to reset to baseline each second. Stimulus presentations were locked to the reset. The integrated output was digitized and strobed to a Digital microcomputer, where the raw integrated values were transformed so that the range corresponded to the digital conversion limits (0-255 A-D [analogue to digital] units). Startle response amplitude was measured during the 200-msec period after the startle stimulus onset. Before each rat was placed in the experimental apparatus, the chamber was cleaned with a solution of ammonium hydroxide and tap water.

The startle-eliciting stimulus was a 100-msec burst of white noise with a 0-msec rise/fall time,

and with an intensity of either 100 or 130 dB SPL (re. 20 micro Pa). It was delivered through a 9-cm piezo-electric tweeter (S-864A, Herald Electronics), centered 12 cm from the long wall of the startle chamber. Continuous white noise (70 dB SPL re. 20 micro Pa) masked extraneous auditory stimuli. All test stimuli were superimposed on this background of white noise. Stimulus and background intensity were measured with a General Radio sound level meter (Type 1551-C, 20-KHz setting), and a General Radio impact noise analyzer (Type 1556-B), with the microphone placed in a standard position within the chamber.

#### Procedure

Rats were first matched by weight, and then randomly assigned to one of four cells formed by a 2 × 2 factorial design: intensity (high, low) × pretreatment (saline, naloxone). On day 1, the naloxone groups were injected (intraperitoneally) with naloxone hydrochloride dissolved in isotonic saline, at a concentration of 4 mg/ml/kg. The saline groups were injected with an equivalent volume of the saline vehicle. One minute after injection, each rat was placed in the test chamber, and 4 minutes after injection received three acoustic startle stimuli spaced 54 seconds apart. The rat was removed 1 minute after the third stimulus. On the second day, each rat was placed in the chamber, and 3 minutes later received a single acoustic startle stimulus. The intensity of the stimulus was the same as that given on day 1. The session ended 5 minutes after the startle stimulus.

For both sessions, while the rat was in the chamber, its behavior was observed according to a time-sampling procedure: every 2 seconds the rat's behavior was scored as either freezing or activity. Freezing was defined as the absence of all visible movement of the body and vibrissae except for movement necessitated by respiration (Fanselow 1984). Interrater agreement on a random sample of animals was 93%, a level comparable to that reported by Fanselow (1984). The observer was always ignorant of the drug administered to the rat. The percentage of samples scored as freezing during the pre- and post-stimulus periods on each day was calculated.

### Results

#### Freezing

During the prestimulus period on day 1, only one rat, in the low-intensity, saline group, showed any freezing behavior (4.0%). Figure 1 shows the mean per cent freezing scores for the poststimulus period on day 1, and for both the pre- and

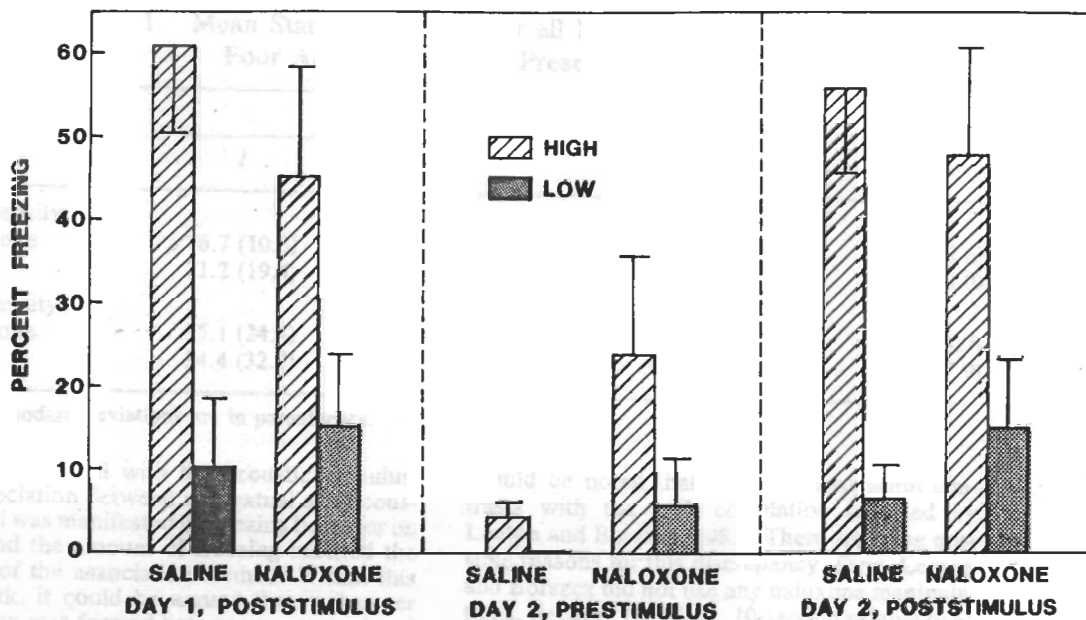


FIG. 1. Mean percentages of freezing for the 1-minute poststimulus period on day 1, and for the 3-minute prestimulus period and 5-minute poststimulus period on day 2, as a function of stimulus intensity (high, low) and drug pretreatment (saline, naloxone). The low-intensity saline group did not freeze during the prestimulus period on day 2. Vertical bars are SE.

poststimulus periods on day 2. During the poststimulus period on day 1, the high-intensity groups displayed more freezing behavior than did the low-intensity groups (Mann-Whitney  $U$  test = 51,  $P < .05$ ); there were no other differences. During the prestimulus period on day 2, the high-intensity groups displayed more freezing behavior than did the low-intensity groups ( $U = 23$ ,  $P < .05$ ), and the naloxone groups displayed more freezing behavior than did the saline groups ( $U = 92$ ,  $P < .05$ ); there were no other differences. During the poststimulus period on day 2, the high-intensity groups displayed more freezing behavior than did the low-intensity groups ( $U = 49$ ,  $P < .05$ ); there were no other differences.

**Startle Response**

Table 1 shows the mean startle amplitude for all four groups across the four startle stimulus presentations. The high-intensity groups responded significantly more than did the low-intensity groups,  $F(1,36) = 35.2$ ,  $P < .05$ ; no other differences were significant. That is, there were no main or interaction effects for drug pretreatment.

**Discussion**

Results indicate that freezing behavior during the critical prestimulus test period on day 2 was facilitated by stimulus intensity and by the naloxone pretreatment. These findings support the notion that increased intensity of the acoustic startle stimulus increases its nociceptive properties and thus increases conditioning to contextual stimuli during day 1 as indexed by freezing behavior. That naloxone pretreatment also increased freezing behaviour supports Fanselow's (1979, 1984) argument that naloxone increases the perceived intensity and aversiveness of stimuli.

In this experiment, the effects of the higher physical intensity and of the naloxone pretreatment were both evident during the 3-minute prestimulus test period on day 2. Since the animals had not yet received further acoustic stimulus presentations or drug treatment, the differential freezing behavior during the test period can be attributed to the different treatments during the training phase on day 1. In subsequent research, it was found that naloxone pretreatment without startle stimuli on day 1 does not lead to freezing during the prestimulus period on day 2. During the training phase, the contextual stimuli

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TABLE 1. Mean Startle Amplitude for all Four Groups Across the Four Acoustic Stimulus Presentations

Group	Day 1			Day 2
	1	2	3	1
High intensity				
Naloxone	76.7 (10.5)	71.8 (15.8)	62.7 (18.6)	80.4 (20.6)
Saline	71.2 (19.4)	78.6 (22.5)	81.4 (16.2)	75.5 (20.0)
Low intensity				
Naloxone	35.1 (24.4)	34.2 (23.9)	27.8 (25.5)	34.8 (17.7)
Saline	34.4 (32.7)	42.5 (28.5)	34.6 (33.2)	29.7 (27.9)

Note: Standard deviations are in parentheses.

became associated with the acoustic stimulus. This association between contextual and acoustic stimuli was manifested in freezing behavior on day 2, and the amount of freezing indexed the strength of the association formed. Within this framework, it could be argued that a stronger association was formed between contextual and startle stimuli when the acoustic prestimulus was of a greater actual or perceived intensity. The lack of a naloxone pretreatment effect in the day 1 and 2 poststimulus periods can be explained in terms of the stronger short-term effects of the immediately preceding startle stimulus presentation. These short-term effects are reflected in the greater overall freezing levels during the poststimulus periods.

There was substantial variability in freezing behavior among animals in each group, as reflected by the large standard errors (see Figure 1). A similar phenomenon is evident with weak levels of electrical shock (M. S. Fanselow, personal communication, June 1984), and it is possible that the phenomenon reflects individual differences in auditory and pain sensitivity. Future research could investigate this possibility by independently assessing auditory and pain thresholds.

The amplitude of the startle response was facilitated by the increased stimulus intensity, corroborating previous research (*e.g.*, Hoffman and Ison 1980). The startle response was not facilitated by naloxone pretreatment. Given the previous contradictory reports of decreased activity (Fanselow 1984) and increased activity (Kelly and Leitner 1984), it is possible that naloxone does not have a systematic effect on startle response. That naloxone clearly affects freezing, but not startle, suggests that at some level there is a divergence in the processes underlying the two responses. This notion is supported by the relatively low correlation,  $r = 0.30$ ,  $P = 0.06$ , between the prestimulus day 2 freezing scores and their associated startle response amplitudes. It

should be noted that this low correlation contrasts with the high correlation reported by Leaton and Borszcz (1985). There are three possible reasons for this discrepancy. First, Leaton and Borszcz did not use any naloxone manipulations. Second, the short, 10-second sample used by Leaton and Borszcz (1985) should bear a closer relationship to subsequent startle responding than the long, 3-minute sample employed here, since there would be more varied activity in the longer sample. Third, the level of freezing in the current study was relatively low, so the range for correlation was not as great as in the Leaton and Borszcz (1984) study.

#### Conclusion

Overall, the results of this study suggest that acoustic startle stimuli can support conditional responding, but that this process depends on the aversive properties of the acoustic startle stimulus, which are determined to a large degree by the actual and perceived intensity of the stimulus. Future research needs to further explore the nature and boundaries of this phenomenon. Apart from varying the parameters of stimulus presentation, investigation of the opioid system underlying the naloxone effect and how it relates to the same phenomenon with shock (Fanselow 1984) and to stress-induced analgesia effects (Kelly and Leitner 1984, Leitner and Kelly 1984) would appear to be a particularly interesting area of study.

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