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A Deficit in One-Trial Context Fear Conditioning Is Not Due to Opioid Analgesia

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BEVINS, R. A. AND J. J. B. AYRES. *A deficit in one-trial context fear conditioning is not due to opioid analgesia.* PHARMACOL BIOCHEM BEHAV 49(1) 183–186, 1994. – Rats given a foot shock immediately after placement in a box subsequently freeze (immobility) much less in that box than rats given the same shock 2 min after placement. A possible explanation of this result is that these two procedures might induce different levels of opioid analgesia at the time of shock. Opioids might be present immediately after handling, transporting, and exposure to a new situation, but absent 2 min later. Two experiments examined this possibility by giving the opioid antagonist naloxone before conditioning (Experiment 1) or before conditioning and testing (Experiment 2). There was no effect of naloxone relative to saline controls. The results do not support the analgesia hypothesis. Experiment 2 precludes a state-dependent learning account of the results.

Context fear conditioning Opioid analgesia Naloxone Immediate-shock deficit One trial Freezing

RATS given a single foot shock immediately after placement in a context subsequently show much less conditioned immobility (freezing) in that context than rats that receive the same shock 2 min after placement in the context (1,2,5,6). Despite the fact that the same shock was given in the same context, recent work has concluded that the weak freezing seen with the immediate shock does reflect a learning (conditioning) deficit (1,5,6). This lack of context conditioning with the immediate-shock procedure has been termed the immediate-shock deficit.

In order for context fear conditioning to occur, it is thought that stimulus (contextual) input from the hippocampus must cojointly occur with shock input at the lateral, basolateral, and/or central amygdala nuclei (4,13,14,17). One explanation of the immediate-shock deficit has attributed the lack of context conditioning with the immediate shock to the hippocampus not having enough time to process the context and subsequently activate the amygdala before the shock input arrives (9).

Another possible explanation for the immediate-shock deficit is that the immediate shock is not as effective as the delay shock due to the presence of endogenous opioids (i.e., analgesia). It has been shown that the combined handling and transporting of rats to a novel context can lead to opioid release and, thus, produces an opioid analgesia (11,18). The effect of these opioids would be to decrease the pain produced by the shock (10). More opioids may be present at the time of shock for the immediate than for the delay-shock condition, thus decreasing the perceived intensity of the immediate shock. Presumably the 2 min of being undisturbed before shock in the delay condition would allow opioid levels to drop.

EXPERIMENT 1

Experiment 1 tested the opioid analgesia account of the immediate-shock deficit by giving a general opioid antagonist, naloxone, to the rats before conditioning. If the conditioning deficit is due to analgesia, naloxone should block the analgesic effect, making the immediate shock more effective (10).

Method

Animals. The subjects were 42 male Holtzman-derived albino rats (394 to 659 g) from our breeding colony at the University of Massachusetts Amherst. The rats were individually housed in hanging stainless steel cages and had 24-h access to food and water. Six of the rats were 150 days old; the rest were 85 to 90 days old. The colony was on a 16 L : 8 D cycle. All experiments were run during the light phase. On each of the 5 days before the start of the study, each rat was handled for about 1 min. Rats were randomly assigned to groups with

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the restriction that each of the six treatment groups contained one of the 150-day-old rats and that there be seven rats per group.

Apparatus. The inside dimensions of the box used were 19.4 × 20.3 × 22.2 cm (h × w × l). The floor was made of 20 stainless steel rods, 2 mm in diameter, spaced 1.2 cm apart. The back wall and ceiling were Plexiglas with black cardboard mounted on the outside. The end walls were painted glossy black. The front wall was clear Plexiglas. A 100 W 120 V frosted white bulb mounted about 30 cm in front of the clear Plexiglas wall about 27 cm above the grid floor provided general lighting. A 68 dB masking noise was provided by a room air conditioner. Noise level was measured with a General Radio model 1565-B sound meter set on the Cs scale with the microphone placed in the center of the box. A high voltage, high resistance shock source provided a 2-s 1-mA foot shock scrambled through a relay sequencing scrambler (12). Before each rat, the box was cleaned with a 5% vinegar (5% acidity) and 95% tap water. Rats were video taped using a Panasonic video camera (Model AG-180).

Procedure. The design was a 2 × 3 factorial in which the drug given before conditioning (saline vs. naloxone) was crossed with the conditioning procedure (immediate, delay, or no-shock). Naloxone hydrochloride (Sigma), 4 mg/kg in an isotonic saline vehicle (0.9% NaCl), was given intraperitoneally (IP) on day 1 to the rats in the naloxone groups [Del(N), Imm(N), and No(N)]. The same volume of saline alone was given IP to the rats in the saline groups [Del(S), Imm(S), and No(S)]. After the injection, the rats were given either the immediate-, delay-, or no-shock procedure. Each rat in the immediate-shock procedure received a 2-s 1-mA foot shock immediately after placement in the box and lid closure. Rats in the delay-shock procedure received the same shock 2 min after lid closure. For the immediate and the delay groups, the time between injection and shock was 5 min. We chose this interval so that the present work would be comparable to other research examining decreases in shock potency due to opioid analgesia [see (8,15)]. The no-shock groups were treated like the immediate groups but without the shock. On day 2, each rat was placed in the box and filmed for 5 min.

Behavioral observations. Freezing and not freezing for each rat was scored from the video tapes. Freezing was defined as the absence of movement except that of the rat's sides needed for breathing. Not freezing was defined as anything else (5). Observations were paced by a relay click that was present on day 2 filming. This click cycled on for 0.2 s then off for 1.8 s. Given the 5-min observation period, the 2-s sampling interval yielded 150 observations per rat.

Statistical analyses. Because the assumption of a normally distributed population required by standard parametric statistics was violated (e.g., mostly 0 scores in the no-shock group), nonparametric statistics were used. The effect of naloxone on the shock groups was assessed using a Wilson's (χ^2) 2 × 2 nonparametric analysis of variance (21). The factors were drug (saline vs. naloxone) and condition (immediate vs. delay shock). A two-tailed rejection region of 0.05 was used for all tests.

Results and Discussion

The bars in Fig. 1 show the median percentage of freezing for each group. The dots show the data for each rat. Looking first at the saline groups (empty bars), one can see that the rats in group Del(S) froze more than the rats in groups Imm(S) or No(S). Thus, previous work demonstrating the immediate-shock deficit (1,2,5,6) was replicated here with the saline groups. The results for the naloxone groups (striped bars) reveal a similar pattern. The 2 × 2 nonparametric analysis of variance revealed a main effect of condition, $\chi^2(1) = 7.04$, denoting more freezing in the delay-shock condition than in the immediate condition. There was no main effect of drug or condition × drug interaction (χ^2s < 1).

The similarity in the pattern of results for the saline and naloxone groups suggests that the immediate-shock deficit is not due to the greater presence of endogenous opioids in the immediate-shock group. If it were, then naloxone should have enhanced freezing in group Imm(N) relative to group Imm(S). This did not occur. Also, given that group Del(N) and Del(S) did not differ, it does not appear that opioid analgesia was decreasing the perceived potency of the delay shock either. This result is consistent with other reports that naloxone does not enhance context conditioning produced by a single delayed shock [e.g., (8)].

A state-dependent learning account (16) could explain the failure to find an effect of naloxone. Naloxone was given only on the conditioning day. Perhaps the physiological state induced by naloxone was a crucial aspect of what was conditioned. If so, then the failure to reestablish this state on the testing day (i.e., give naloxone) could explain why enhanced freezing was not observed in either the immediate or the delay condition. Experiment 2 of the present report was designed to directly assess this state-dependency account.

Some one-trial context fear conditioning work has found evidence for weak conditioning with an immediate shock (2), whereas other work has failed to find evidence for conditioning (5,6,9). Thus, it is of interest to compare freezing in the immediate-shock conditions with that in the no-shock conditions. A Wilson's 2 × 2 nonparametric analysis of variance proved inappropriate, however, because all scores were at or above the median resulting in a χ^2 of 0. The test was, therefore, insensitive to differences that existed between groups. Given that there was no effect of naloxone, the saline and naloxone groups within each condition (immediate and no-shock) were combined and a Mann–Whitney *U*-test was used. That test revealed that rats in the immediate groups froze more than those in the no-shock groups, $U(14, 14) = 36$. This result adds to the evidence that the immediate shock can condition some context fear.

EXPERIMENT 2

As mentioned earlier, the failure during testing to reinstate the physiological conditions that were present at the time of conditioning could explain the lack of a naloxone effect in Experiment 1. Experiment 2 tested this state-dependent learning account by giving naloxone or saline injections on both the conditioning and testing day to rats in either the immediate or the delay condition.

Method

Animals. The subjects were 36 male albino rats like those from Experiment 1. They weighed from 367 to 564 g and were 85 to 92 days old. Rats were randomly assigned to one of four groups such that there were nine rats per group.

Apparatus. The apparatus was unchanged except for the following modification. The click pacing stimulus was replaced with a 28 V white indicator lamp. The lamp was mounted on a black metal stand and placed in front of the box. The lamp cycled on for 0.1 s and off for 1.9 s during all sessions of the experiment.

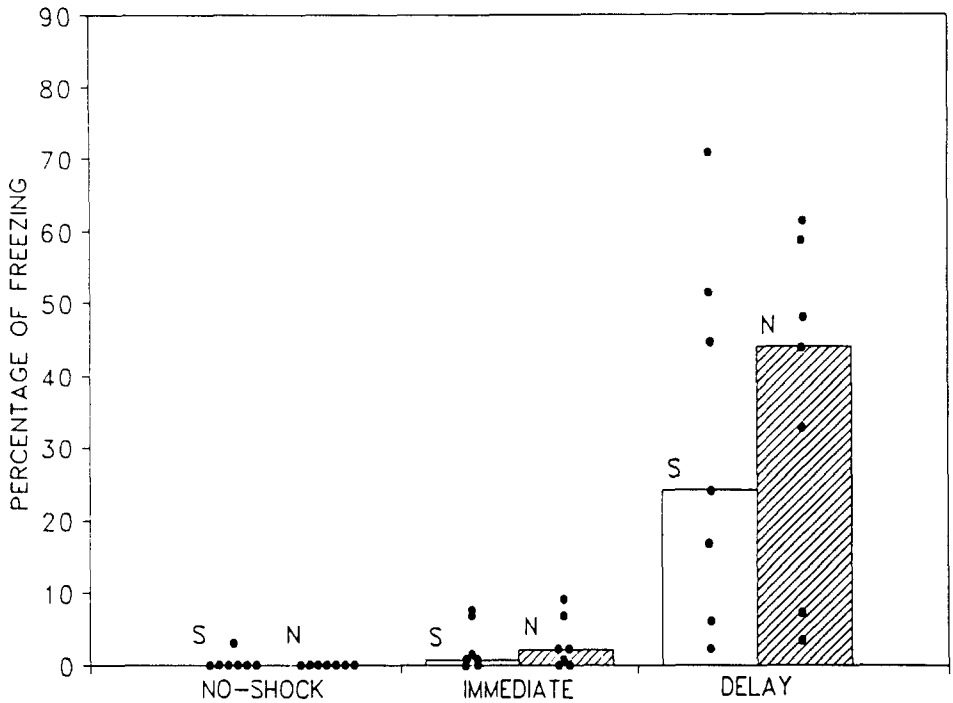

FIG. 1. Median percentage of freezing for the saline (S) groups (empty bars) and the naloxone (N) groups (striped bars) in Experiment 1. The dots show the data for each rat.

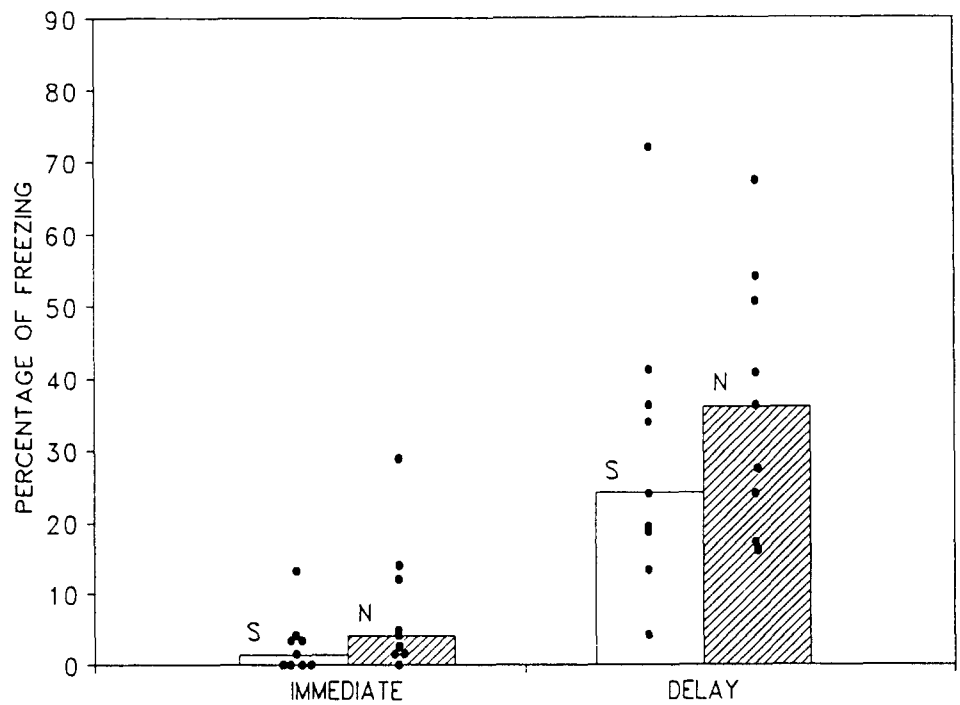

FIG. 2. Median percentage of freezing for the saline (S) groups (empty bars) and the naloxone (N) groups (striped bars) in Experiment 2. The dots show the data for each rat.

Procedure. The procedural details were those of Experiment 1 except for the following: a) only the immediate and delay conditions were used. b) Injections were given on both the conditioning day and the test day. Time between injection and shock on the test day was identical to that on the conditioning day (i.e., 5 min). Thus, the design was a 2 × 2 factorial in which the drug given before conditioning and testing (saline vs. naloxone) was crossed with procedure (immediate vs. delay shock).

Results and Discussion

The bars in Fig. 2 show the median percentage of freezing for each group, and the dots show the data for each rat. As in Experiment 1, the saline and naloxone groups showed the immediate-shock deficit (i.e., more freezing in the delay than in the immediate group). However, naloxone given on the conditioning day and test day did not affect freezing. These impressions were confirmed by a main effect of condition, $\chi^2(1) = 21.78$, but no main effect of drug or condition × drug interaction, χ^2s(1) < 1.78. Also note that a majority of the rats in the immediate groups froze to some extent. Although the no-shock control group was not run here, this result, like those of Experiment 1, argues that some context conditioning occurred with the immediate shock (2).

The fact that naloxone did not enhance freezing in the immediate condition when naloxone was given on both the conditioning day and test day argues against a state-dependency interpretation of the results in Experiment 1. Thus, the results of Experiments 1 and 2 combined argue that the weak conditioning with the immediate shock is not due to the greater presence of opioid analgesia in the immediate-shock group than in the delay-shock group.

Although an opioid-mediated analgesia does not seem to be responsible for the immediate-shock deficit, the present work does not eliminate the possibility of a nonopioid-mediated analgesia (20). For several reasons, however, a nonopioid mechanism seems unlikely. First, the type of analgesic response (opioid vs. nonopioid) found using similar preparations appears to be dependent on the intensity of the event (or stressor) producing the analgesia. Nonopioid analgesia is produced by more severe stressors (3,7). For instance, analgesia produced by a 3-min 2-mA foot shock is opioid mediated, but analgesia evoked by a 3.5-mA shock of the same duration appears nonopioid mediated (19). It is hard to imagine that the stressor received by the rats in the present work (handling, transport, and novel situation) is as severe as receiving a 3-min shock along with being handled, transported, and placed in a novel situation. Another reason why a nonopioid analgesia account of the immediate-shock deficit seems unlikely is that previous work examining handling- and transport-induced analgesia under similar conditions as the present work found that analgesia to be blocked by an opioid antagonist (11). Finally, recent work has found that under certain conditions an immediate shock can be as effective as a delay shock at conditioning context fear (1). For example, rats exposed to box A for 2 min, then lifted quickly out of that context and given a foot shock immediately upon placement in a novel context (box B) freeze in box A at levels comparable to rats that received a delay shock in box A.

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