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Effect of Stress on Oral Fentanyl Consumption in Rats in an Operant Self-Administration Paradigm

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SHAHAM, Y., L. C. KLEIN, K. ALVARES AND N. E. GRUNBERG. *Effect of stress on oral fentanyl consumption in rats in an operant self-administration paradigm.* PHARMACOL BIOCHEM BEHAV 46(2) 315-322, 1993.—The effect of intermittent footshock stress (0.8 mA; 0.2 s on; 40 s off on the average; for 10 min/day) on oral fentanyl (50 or 75 μg/ml) self-administration (SA) in operant chambers was examined in male rats. In Experiment 1, after 1 month of initiation of the fentanyl SA by partial water deprivation, animals were tested for lever-pressing for fentanyl (75 μg/ml) under fixed-ratio-4 (FR-4) and progressive-ratio (PR) schedules of reinforcement for 30 min/day in operant chambers. Exposure to footshock stress increased fentanyl SA under the FR-4 and PR schedules compared with a nonstress condition. When water was substituted for the drug, the operant behavior persisted before extinction. In Experiment 2, different rats were tested for lever-pressing for fentanyl (50 μg/ml) under FR-6 and PR schedules. This experiment further assessed the role of taste in the stress-induced fentanyl SA and examined the effect of increasing the schedule requirements (i.e., FR-3, 6, and 12) on lever-pressing for fentanyl. Exposure to footshock stress increased lever-pressing for oral fentanyl SA under the FR schedules of reinforcement. When a quinine solution (30 μg/ml), matched for bitter taste with the fentanyl solution, was substituted for the drug solution, an extinction of the drug-reinforced behavior occurred, indicating that the stress-induced oral fentanyl SA is not related to stress-induced changes in taste sensitivity. In both experiments, no significant stress effects were observed for water consumption in home cage and lever-pressing on the nonoperative lever.

Fentanyl Operant chambers Opioid drugs Quinine Oral self-administration Stress Water deprivation

CLINICAL observations and reports suggest that exposure to stress is related to increased opioid consumption and increased relapse to opioid use (e.g., 15,21,27,31). However, methodological limitations of these epidemiological and clinical studies, such as retrospective assessment of stress and lack of appropriate control groups, prevent any firm conclusion regarding a causal link between stress and opioid abuse (12,22). Animal models of drug use can circumvent these methodological problems. In the animal literature, several studies have reported that aversive environmental events, such as social isolation (1,2,11) and food deprivation (see 3 for a review), are associated with increased opioid self-administration (SA). Unfortunately, these particular studies did not validate the stress response by biochemical (e.g., plasma catecholamine or corticosterone levels) or physiological (e.g., blood pressure) indices, whereas other studies reported that in rats, neither isolation (see 8,10,13) nor food-deprivation (see 4) affect biological measures of stress (e.g., plasma norepinephrine, plasma ACTH and corticosterone, blood pressure). Further, it may be that increased drug SA under conditions of food-deprivation are related to variables other than stress. For example, increased drug SA, under conditions of social or food deprivation, may illustrate a more general phenomenon, "reinforcement interaction," whereby decreased availability of one reinforcer increases responding maintained by another (1,3). Therefore, despite the potential value of animal models to investigate the relationship between stress and opioid abuse, few unequivocal reports in the literature indicate that stress causes increased opioid consumption.

Recently, using rats, we (25,26) examined the effect of 15 min/day of immobilization (IM) stress on oral opioid SA in home cages using an oral opioid SA procedure modified from

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[2] The opinions or assertions contained herein are the private ones of the authors and are not to be construed as official or reflecting the views of the Department of Defense or the Uniformed Services University of the Health Sciences.

Stolerman and Kumar (29). IM stress was chosen because it is associated with reliable changes in physiological measures of the stress response, including increased plasma corticosterone, ACTH, and catecholamine levels (15,16,19). The results of our studies indicated that IM stress increased oral opioid (fentanyl or morphine) SA compared with a nonstress control condition. To extend these findings, it is important to examine whether the effect of stress on opioid SA can generalize to other types of stressors that affect physiological measures of stress, and whether exposure to stress increases other types of behavioral responses to obtain the drug (i.e., operant lever-pressing for the drug) in addition to drinking opioid solutions in home cages.

A widely used stressor in the animal literature is electric footshock stress (15,16,24). Using this stressor for 15 min/day, Dib and colleagues reported that rats increased intracerebroventricular (6) and intrathecal (5) morphine SA. One limitation of these studies, however, was the fact that the increased morphine SA was only observed during exposure to the noxious electric footshock stressor, but not prior to or after the stressor administration. Therefore, the rats in these studies may have learned to increase their morphine SA to decrease the pain induced by the footshock. That is, the stress-induced morphine SA may not have been related to stress-induced changes in the reinforcement efficacy of opioids, but instead to the effectiveness of opioids to reduce the discomfort of electric footshock.

Taken together, it is not clear from the available literature whether other stressors in addition to IM increase opioid SA in animal models of drug use. Moreover, it is not clear whether the stress-induced opioid SA (25,26) can be generalized to other behavioral responses to obtain the drug (e.g., operant lever-pressing) in addition to drinking the drug solution in home cages.

The present two experiments examined whether IM stress-induced oral opioid SA reported previously in a home cage paradigm (25,26) also occurs for a different behavioral response to a different stressor. Specifically, we examined whether a mild electric footshock that precedes the drug SA period increases operant responding to obtain fentanyl (an opioid agonist) for oral SA in operant conditioning chambers. The present experiments were designed to minimize the possibility that opioid consumption was related to antinociceptive action by having the stress exposure precede the opioid SA period. It was hypothesized that the stressor would increase lever-pressing for fentanyl compared with a nonstress control condition.

EXPERIMENT 1

METHOD

Subjects

Eight male Wistar rats (14–16 weeks old [Charles River]) were the subjects. Animals were individually housed in polypropylene shoebox cages (35.6 cm × 15.2 cm × 20.3 cm) at a temperature of 23 °C, relative humidity of 50%, and light-dark cycles of 12 h each (light on 0700–1900). Food (Agway Prolab Animal Diet RMH 3500) was available continuously.

Drugs

Fentanyl-HCl (NIDA) in concentrations of 25–100 μg/ml dissolved in tap water was used. Naloxone-HCl (Dupont Pharmaceutical, Wilmington, DE), in a concentration of 0.4 mg/ml in 0.86% NaCl (saline) solution and a dosage of 1.0 mg/kg, was injected IP to precipitate the withdrawal syndrome.

Footshock Stress

Mild, constant-current, intermittent, inescapable, electric footshock was used. The inescapable shock was administered for 0.2 s every 40 s on the average under a variable interval schedule of shock administration with a range of 10–70 s. The shock intensity was 0.8 mA and it was delivered through a scrambler to the grid of the floor in the operant chamber for 10 min/day.

Apparatus

Four standard commercial, sound-attenuating, operant conditioning chambers (ENV-001, Med associate Inc., East Fairfield, VT), each equipped with two levers and two 65 ml liquid dispensers (ENV-201), were used. The two levers were located 7 cm above the grid floor. Pressing on the left lever (the operative lever) resulted in the activation of the left dispenser. This dispenser was initialized to administer 0.1 ml of solution. Lever presses on the right lever (the nonoperative lever) were recorded as a measure of nonspecific activity, but had no consequences. The operant chambers were connected to a cabinet and power supply (SG600/C) which, in turn, was connected via a 16 port interface (DIG-700) to a 386 VGA computer. MED-PC Medstate Notation (30), Turbo Pascal, Quattro-Pro, and SAS softwares were used to operate the operant conditioning chambers, and to record and analyze the data.

Procedure

During the Initiation Phase (days 1–29), animals were maintained under partial water deprivation (see below for details). Animals lever pressed for the fentanyl solution for 30 min/day in the operant chamber under a fixed-ratio-6 (FR-6) schedule of reinforcement (i.e., every sixth lever-pressing resulted in the delivery of 0.1 ml of the fentanyl solution). For the first 3 weeks of the Initiation Phase, the drug concentration was increased from 25 μg/ml to 100 μg/ml under a partial water deprivation condition in the home cage. Specifically, for the first 4 days, the drug concentration was 25 μg/ml and the animals were provided with 20 ml of water in their home cage. For the next 6 days the drug concentration was increased to 50 μg/ml and the animals were provided with 10–20 ml of water in their home cage. For the next 3 days the drug concentration was increased to 75 μg/ml and the animals were provided with 20 ml of water in their home cage. For the next 8 days the drug concentration was increased to 100 μg/ml and the amount of water in the home cage was increased from 10 ml/day to 35 ml/day. The drug concentration then was reduced to 75 μg/ml because this value resulted in the greatest dosage of fentanyl SA. For the last 8 days of the Initiation Phase (days 22–29 of this phase) the amount of water consumed in the home cage was increased by 5–10 ml/day (depending on the individual animal's response rate in the operant chamber) from an initial value of 30 ml until all animals drank less water than the amount available to them. Electric footshock was administered over a 10 min-period during the Initiation Phase so that the stress administration reliably predicted the drug SA period. During the sessions of the Initiation Phase, the houselight was on during exposure to electric footshock stress, the light above the operating lever was on during the drug SA period, and a white noise was turned on for 0.1 s

every time the animal met the schedule requirement. Withdrawal measures after naloxone (1 mg/kg) administration were assessed once during the Initiation Phase at the highest concentration (100 μg/ml).

During the Testing Phase, the FR schedule requirements were lowered to FR-4 because two animals responded at a low rate under an FR-6 schedule when water was gradually increased in home cages during the last week of the Initiation Phase. Animals were tested during the Testing Phase for lever-pressing for fentanyl under two different reinforcement schedules, an FR-4 or a progressive ratio (PR) schedule, while they were either exposed or not exposed to electric footshock prior to the SA period. During this phase, water was available continuously in the home cages. During the nonstress testing period, the animals were in the operant chambers for 10 min prior to the 30 min drug SA period while the shock and the houselight were turned off. In the PR sessions, the initial schedule of reinforcement was FR-1, and the schedule requirement was increased by one response at a time after each successful earning of a drug reinforcement (i.e., increasing the schedule requirement from FR-1 to FR-2, FR-3....FR-*n*). The Testing Phase included three cycles of fentanyl SA under conditions of exposure to stress, no stress, and another cycle of exposure to stress. During the stress conditions, the animals were exposed to shock stress for 10 min/day prior to the fentanyl SA period. Each of these cycles included 3–4 days of responding under an FR-4 schedule followed by 1 day of responding under the PR schedule. After this period, water was substituted for fentanyl to examine extinction of the drug-reinforced behavior, and the animals self-administered water under an FR-4 schedule for 4 days under conditions of stress, 4 days of no exposure to stress, and an additional 13 days of reexposure to stress.

At the end of the experiment, animals were exposed to electric footshock stressor under the conditions described (i.e., 0.8 mA; 10 min; 0.2 s on; 40 s off on the average) and were immediately decapitated without anaesthesia. Trunk blood was collected in heparinized tubes (50 μl of heparin/tube) and centrifuged for 20 min at 1500 × g at 4°C. Radioimmunoassay (RIA) for plasma corticosterone (ICN Biomedic) was performed. The RIA for corticosterone also included an additional five drug-free Wistar male rats (14–16 weeks old) that were not exposed to stress to examine the effectiveness of the stress manipulation. These animals were individually housed under the same conditions as the experimental animals with food and water continuously available.

Statistical Analyses

Repeated-measures analysis of variance (ANOVA) was used to examine the dependent measures of the number of responses and dosage levels under the FR-4 schedule and number of responses under the PR schedule. The data within each experimental condition (i.e., stress + drug, no stress + drug, stress + water, no stress + water) were averaged across days. Post-hoc analyses comparing individual time points utilized Fisher's Least Significant Difference Test. Significance level was based on $\alpha = 0.05$. Additional dependent measures included plasma corticosterone levels, 23-h water consumption in the home cage, rate of responding for the nonoperative lever, and latency until the first reinforcement after the termination of the stressor.

RESULTS AND DISCUSSION

One animal was excluded from the data analyses because it earned on the average less than one reinforcement per session during the first 20 days of the Testing Phase compared with at least three reinforcers per session earned by the other animals. All data from the other seven rats were included in the analyses with the exception that responses on the first day of the no-stress + drug and no-stress + water sessions were excluded because initial exposure to an apparatus in which shock was previously administered may elicit a stress reaction that is indistinguishable from exposure to the shock per se (7). In other words, based on Dunn's report (7), those transition days cannot be viewed as a no-stress condition. For the second stress + water SA condition that was longer than the other experimental conditions (13 days), the data were collapsed into four 3-day blocks to examine the rate of extinction of water lever-pressing during this period.

At the highest fentanyl concentration (100 μg/ml) during the Initiation Phase, naloxone (1 mg/kg) was injected at the end of one of the sessions. During this session, the rate of responses ranged from 156–300 responses/session, which corresponded to about 2.5–5 ml of the fentanyl solution (a dosage range of 0.6–1.2 mg/kg/session). Several withdrawal symptoms were observed during the 20 min of the withdrawal assessment: wet-dog shakes (0.8 ± 0.4, Mean ± SEM), teeth chattering (12.8 ± 3.8), ptosis (1.2 ± 1.0), excessive grooming (6.6 ± 1.8), and abnormal posture (3.7 ± 1.3). These results suggest that the animals developed physical dependence to fentanyl during the Initiation Phase.

Analysis of plasma corticosterone revealed that electric footshock caused a large increase in plasma corticosterone levels, indicating that the stress manipulation was effective. Significant differences were observed between the seven experimental animals exposed to electric footshock stress (180.3 ± 10.4 ng/ml) and the five control animals that were not exposed to stress (82.4 ± 27.8 ng/ml) [$F(1, 10) = 16.7$, $p < 0.05$].

Figure 1a presents average rate of responding on the operative lever during the Testing Phase for the FR-4 ratio schedule conducted during the drug sessions and water sessions. Exposure to shock stress increased rate of responding for fentanyl compared with a no-stress condition. In addition, over time, when water was substituted for fentanyl, there was an initial increase in rate of responding followed by an extinction of the drug-reinforced behavior. Repeated measures ANOVA revealed a significant time effect for the FR-4 schedule across the drug and water sessions [$F(10, 60) = 5.1$, $p < 0.05$]. Post-hoc condition differences were observed for the two stress + drug conditions versus the no-stress + drug condition; the first stress + water condition versus the no-stress + drug condition and the last stress + water condition; and the first stress + drug condition versus the last water condition (see Fig. 1a). Also, animals increased their rate of responding when water was initially introduced compared with the second stress + drug condition, but this difference did not reach statistical significance ($p = 0.11$).

Figure 1b presents dosage levels during the drug sessions. There was a significant time effect during the drug conditions [$F(2, 12) = 9.1$, $p < 0.05$]. Post-hoc condition differences were significant between the two stress + drug conditions versus the no-stress condition (see Fig. 1b). Exposure to stress also increased rate of responding under the PR schedule conducted on the last day of each of the drug conditions [$F(2, 12) = 5.2$, $p < 0.05$]. Post-hoc condition differences were significant between the two stress + drug conditions versus the no-stress condition (see Table 1).

Water SA during the no-stress condition was not significantly different from the water + stress conditions (see Fig.

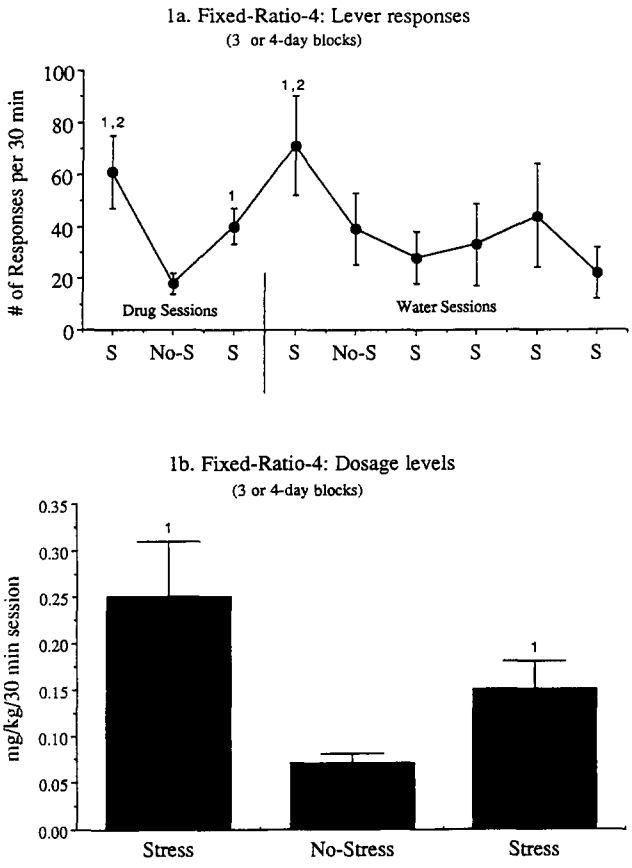

FIG. 1. Lever-pressing for fentanyl or water under an FR-4 schedule of reinforcement and dosage levels under conditions of exposure or no exposure to shock stress (±SEM). Abbreviations: S, Stress; 1, Significant differences from the no-stress drug condition, $p < 0.05$; 2, Significant differences from the last water condition, $p < 0.05$.

1a), indicating that the stress-induced fentanyl consumption was not a result of stress-induced thirst. Further, no significant experimental condition effects were observed for rate of on the nonoperative lever, indicating that footshock did not cause an increase in nonspecific activity (see Table 1). Also, shock stress did not significantly affect the latency for response for the drug and water consumption in home cages (see Table 1).

Taken together, the results of Experiment 1 indicate that electric footshock stress increases rate of responding for fentanyl compared with a no-stress condition. These results indicate that the stress-induced enhancement of opioid SA reported recently (25,26) generalizes to another type of stressor (electric footshock) and a different behavioral response to obtain the drug (operant lever-pressing). However, we are hesitant to reach broad conclusions about stress and opioid SA from this experiment alone for several reasons. First, the response rates observed during the sessions were relatively low (cf. 3). Second, the time duration of the extinction of the drug-reinforced behavior was rather long (cf. 3). Third, this experiment examined a single FR schedule of reinforcement and a single dosage level. Finally, fentanyl at the concentration used in this experiment (75 μg/ml) is a somewhat bitter solution. Therefore, the stress-induced changes in the drug-reinforced behavior might be partially related to stress-induced changes in sensitivity to taste. The bitter taste of the fentanyl solution may also have contributed to the relatively low response rate observed during the drug sessions.

Experiment 2 was designed to attempt to replicate the findings of Experiment 1 but in a way that would address the limitations of Experiment 1. Specifically, in Experiment 2, the animals self-administered fentanyl under a different schedule of reinforcement (FR-6) and the drug dosage was reduced to 50 μg/ml. Further, during the extinction phase, a somewhat bitter quinine solution (30 μg/ml) was used instead of water to examine whether stress-induced changes in the drug SA are associated with stress-induced changes in taste sensitivity or preference. In addition, after the extinction phase, the drug was reintroduced and the effect of stress on fentanyl SA was examined under three different schedules of reinforcement (FR-3, FR-6, and FR-12) to examine whether the animals increase their response rate as the schedule requirements are increased.

EXPERIMENT 2

METHOD

Subjects

Six naive male Wistar rats (14–16 weeks old [Charles River]) were the subjects. Animals were housed under the same conditions as described in Experiment 1.

Drugs

Fentanyl-HCl (NIDA) in a concentration of 50 μg/ml dissolved in tap water was used. Naloxone-HCl (Dupont Pharmaceutical), in a concentration of 0.4 mg/ml in 0.86% NaCl (saline) solution and a dosage of 1.5 mg/kg, was injected IP to precipitate the withdrawal syndrome. Quinine (Sigma, St. Louis, MO) in a concentration of 30 μg/ml was used.

Apparatus and Footshock Stress

The apparatus, shock parameters, and shock duration were identical to Experiment 1.

Procedure

After 48 h of water deprivation, animals were trained to lever press for water in the operant chamber under an FR-1 schedule. For the next 8 days, animals were given 10 ml/day of water in the home cage and lever pressed for water for 30 min/day in the operant chambers. During this period, the schedule requirements were increased from FR-1, FR-2, FR-4, FR-6, FR-8 to FR-12. Then, fentanyl (50 μg/ml) was substituted for water and the schedule requirement was reduced to FR-4. For the next 4 weeks (Initiation Phase), the amount of water available in the home cages was increased until all animals consumed less water than the amount available to them. Specifically, for the first week animals were provided with 10 ml/day of water in their home cage. During the second week the amount of water in the home cage was increased from 10 ml/day to 30 ml/day. During the third week the amount of water in the home cage was increased from 35 ml/day to 50 ml/day. During the last week of the Initiation Phase the amount of water in the home cage was increased by 5 ml/day until all animals drank less water than the amount available to them. For the first week of the Initiation Phase, the schedule

TABLE 1

AVERAGE LEVER-PRESSING DURING THE PROGRESSIVE RATIO DAYS, NONOPERATIVE LEVER RESPONSES, LATENCY FOR THE FIRST REINFORCEMENT, BODY WEIGHT, AND HOME CAGE DAILY WATER CONSUMPTION DURING THE FIRST PART OF THE TESTING PERIOD OF EXPERIMENT 1 (±SEM)

Measure/Phase	Stress + Fentanyl	No Stress + Fentanyl	Stress + Fentanyl	First Stress + Water period
Nonoperative lever responses (#/30 min)	3.5 ± 0.9	3.1 ± 1.2	3.8 ± 1.5	6.2 ± 1.8
Latency for 1st reinforcement (s)	128.9 ± 38.5	185.2 ± 95.7	208.89 ± 67.7	490.8 ± 187.8
Number of responses during progressive ratio days (#/30 min)	42.6 ± 10.9*	21.1 ± 7.1	36.7 ± 10.0*	Not done
Body weight (g)	453.4 ± 12.9	473.6 ± 14.0	489.7 ± 15.2	504.3 ± 16.1
Daily water consumption in the home cage (ml/day)	53.0 ± 2.7	57.7 ± 7.2	56.7 ± 6.1	59.1 ± 8.0

*Significant differences from the No-Stress period, $p < 0.05$.

requirement was increased from FR-4 to FR-12. During the second and third week of the Initiation Phase, however, the schedule requirement was decreased from FR-12 to FR-6 because one of the animals responded at a low rate at the higher schedule requirements (i.e., FR-8 or FR-12). As in Experiment 1, electric footshock was administered over a 10-min period prior to the drug SA session during the Initiation Phase, so that the stress administration reliably predicted the drug SA period. On the seventh day of the Initiation Phase, naloxone (1.5 mg/kg) was administered to precipitate the opioid withdrawal syndrome.

During the Testing Phase, animals were tested for lever-pressing for fentanyl under either an FR-6 or a PR schedule in the operant conditioning chamber while they were either exposed or not exposed to electric footshock prior to the SA period. During this period, water was available continuously in the home cages. During the no-stress testing period, the animals were in the operant chambers for 10 min prior to the 30 min of the drug SA period while the shock and the houselight were turned off. As in Experiment 1, the Testing Phase included three cycles of fentanyl SA under conditions of exposure to stress, no stress, and another cycle of exposure to stress. During the stress conditions, the animals were exposed to shock stress for 10 min/day prior to the fentanyl SA period. Each of these cycles included 3–4 days of responding under an FR-6 schedule, followed by 1 day of responding under the PR schedule. After this period, a quinine solution (30 μg/ml) was substituted for fentanyl and the animals self-administered the quinine solution for three additional cycles. The quinine concentration was chosen based on a pilot study in our laboratory that determined that approximately 50% fentanyl preference occurred in a two-bottle choice test in a home-cage setting when drug-naive animals were given a choice between 30 μg/ml of a quinine solution and 50 μg/ml of a fentanyl solution.

At the end of the quinine sessions, the animals were left in their home cages for 1 week and no measurements were taken. Consequently, fentanyl SA was reinstated by reintroducing the drug for one day under an FR-3 schedule, and the effect of shock stress on fentanyl SA was determined for three different schedule requirements (i.e., FR-3, 6, and 12). During this period, the schedule requirements were increased daily during the stress sessions and the no-stress sessions.

RESULTS AND DISCUSSION

Data analyses included all animals' data with the exception of the first day of the no-stress + drug and no-stress + quinine sessions. During the Initiation Phase, naloxone (1.5 mg/kg) was injected at the end of one of the sessions. During this session, the animals self-administered approximately 4.5–7 ml of the fentanyl solution (a dosage range of about 0.7–1.0 mg/kg/session). Several withdrawal symptoms were observed: wet-dog shakes (0.2 ± 0.4), teeth chattering (16.8 ± 11.9), ptosis (2.0 ± 2.9), excessive grooming (4.7 ± 2.2), and abnormal posture (6.2 ± 4.6). These results suggest that, as in Experiment 1, the animals developed physical dependence to fentanyl during the Initiation Phase.

Figures 2a presents average rate of responding on the operative lever during the Testing Phase for the FR-6 ratio schedule conducted during the drug sessions and quinine sessions. Exposure to shock stress increased rate of responding for fentanyl compared with the no-stress condition. In addition, over time, when quinine was substituted for fentanyl, there was an initial increase in rate of responding followed by an extinction of the drug-reinforced behavior. Repeated measures ANOVA revealed a significant time effect for the FR-6 schedule across the drug and quinine sessions [$F(5, 25) = 5.1, p < 0.05$]. Significant post-hoc differences were observed for the second stress + drug condition versus the no-stress + drug condition; the first stress + quinine condition versus the no-stress + drug condition and the last stress + quinine condition; and the two stress + drug conditions versus the last quinine condition (see Fig. 2a). Also, increases in rate of responding were observed between the first stress + drug condition versus the no-stress + drug condition ($p = 0.10$), and the stress + quinine condition versus the first and second stress + drug conditions, but these trends did not reach statistical significance ($p = 0.09$ and $p = 0.13$, respectively).

Figure 2b presents dosage levels during the drug sessions. A significant time effect was observed during the drug sessions

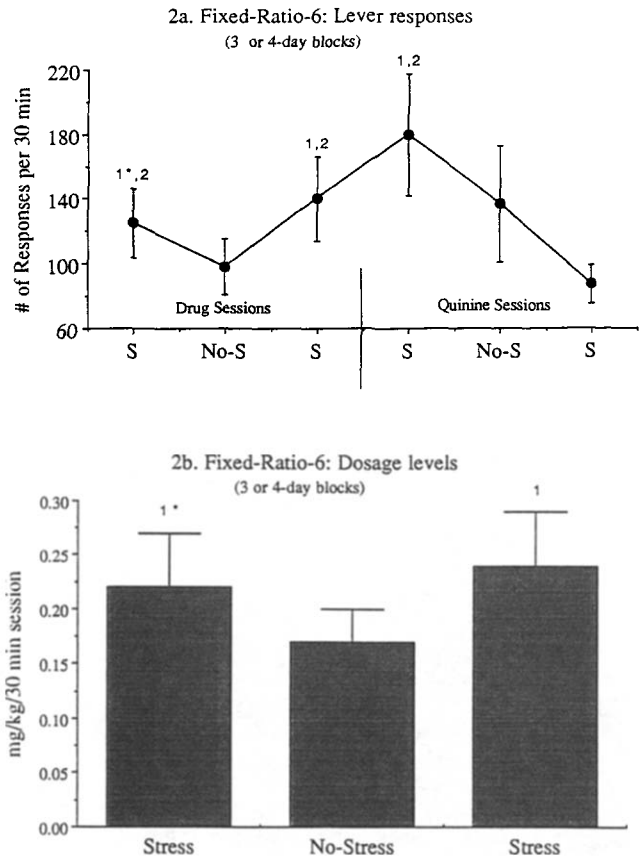

FIG. 2. Lever-pressing for fentanyl or quinine under an FR-6 schedule of reinforcement and dosage levels under conditions of exposure or no exposure to shock stress (±SEM). Abbreviations: S, Stress; 1, Significant differences from the no-stress drug condition, $p < 0.05$; 2, Significant differences from the last quinine condition, $p < 0.05$; 1*, Differences from the no-stress drug condition at $p = 0.1$ and $p = 0.06$ for lever responses and dosage levels, respectively.

[$F(2, 10) = 5.2$, $p < 0.05$]. Significant post-hoc differences were observed between the second stress + drug condition versus the no-stress condition (see Fig. 2b). Unlike Experiment 1, exposure to stress did not increase rate of responding for the PR schedule conducted on the last day of each of the drug sessions (see Table 2). No significant condition differences were observed for PR responding during the quinine conditions (first stress + quinine condition [151.2 ± 44.6], no-stress + quinine condition [188.5 ± 64.2], and last stress + quinine condition [126.8 ± 25.4]).

An important finding of this experiment was that stress did not alter quinine SA. That is, rate of quinine SA during the no-stress condition was not significantly different from the quinine + stress conditions (see Fig. 2a). Therefore, the stress-induced fentanyl consumption is not the result of stress-induced changes in sensitivity to taste. Further, no significant experimental condition effects were observed for rate of responding on the nonoperative lever indicating that, as in Experiment 1, stress did not increase nonspecific activity. Also, shock stress did not affect either water consumption in home cage or the latency for the response for the drug (see Table 2).

One week after the termination of the quinine sessions, the animals were tested for lever-pressing for fentanyl under FR-3, FR-6, and FR-12 schedules of reinforcement under conditions of stress and no stress. Figure 3 presents rates of responding and dosage levels for these schedules. Exposure to shock stress increased rate of responding for fentanyl under the three fixed-ratio schedules compared with the no-stress conditions. In addition, animals increased their rate of responding as the schedule requirements increased. Repeated-measures ANOVA for rate of responding and dosage levels revealed significant differences for stress condition [$F(1, 5) = 8.0$, $p < 0.05$, and $F(1, 5) = 11.4$, $p < 0.05$, for rate of responding and dosage levels, respectively]; and for fixed-ratio schedule requirements [$F(2, 10) = 28.1$, $p < 0.05$, and $F(2, 10) = 14.8$, $p < 0.05$, respectively]. In addition, a significant schedule requirement by stress condition interaction was observed for dosage levels [$F(2, 10) = 4.3$, $p < 0.05$], indicating that the effect of stress on dosage levels was larger under the lower FR schedules (FR-3 and FR-6) compared with the higher (FR-12) schedule. Significant post-hoc stress versus no-stress conditions differences for rate of responding and dosage levels were observed for the FR-3 and FR-6 schedules. A similar trend was observed for the FR-12 schedule (see Fig. 3), but the results did not reach statistically significant levels [$F(1, 5) = 2.3$, $p = 0.19$, and $F(1, 5) = 2.6$, $p = 0.17$, for response rate and dosage levels, respectively].

The results of Experiment 2, with the exception of the PR responding, replicated the findings in Experiment 1. These results indicate that stress-induced oral fentanyl SA occurs under several schedules of reinforcement and in response to a different drug concentration (50 μg/ml) as reported in Experiment 1. In addition, the response rates observed during the sessions were comparable to other published reports of oral opioid SA (see 3), indicating that the somewhat low response rate observed in Experiment 1 may have resulted from the higher concentration of the fentanyl solution (75 μg/ml). Further, the extinction of the drug-reinforced behavior when an equally bitter quinine solution was substituted for fentanyl indicates that changes in taste sensitivity are not likely to be involved in the effect of stress on oral fentanyl SA.

GENERAL DISCUSSION

The results of these experiments indicate that exposure to footshock stress increased fentanyl SA compared with a non-stress control condition, and that the increased lever-pressing for fentanyl after exposure to stress occurred under several fixed-ratio schedules and two fentanyl dosages. Also, over time, an extinction of the lever-pressing responding occurred when fentanyl was substituted with water or with a similarly bitter quinine solution, indicating that stress-induced fentanyl SA is not related to changes in thirst, taste sensitivity, or taste preference. In addition, there were no differences among the experimental conditions for rate of responding on the nonoperative lever, indicating that increased lever-pressing for fentanyl did not result from stress-induced activity. Therefore, it is unlikely that the stress-induced fentanyl SA observed in these experiments is related to nonspecific effects of stress on general activity levels, thirst, or sensitivity to taste.

The initial enhanced rate of responding upon substitution of the fentanyl with water or quinine followed by a subsequent extinction of lever-pressing for the vehicle is consistent with previous reports from the opioid SA animal literature (see 23,28). When a vehicle is substituted for opioid drugs, the typical behavioral response is an initial increase in rate of

TABLE 2

AVERAGE LEVER-PRESSING DURING THE PROGRESSIVE RATIO DAYS, NONOPERATIVE LEVER RESPONSES, LATENCY FOR THE FIRST REINFORCEMENT, BODY WEIGHT, AND HOME CAGE DAILY WATER CONSUMPTION DURING THE FIRST PART OF THE TESTING PERIOD OF EXPERIMENT 2 (±SEM)

Measure/Phase	Stress + Fentanyl	No Stress + Fentanyl	Stress + Fentanyl	First Stress + Quinine period
Nonoperative lever responses (#/30 min)	9.6 ± 1.1	10.0 ± 0.8	10.2 ± 7.5	12.4 ± 4.6
Latency for 1st reinforcement (s)	80.8 ± 15.1	35.6 ± 8.0	70.7 ± 27.1	61.7 ± 19.5
Number of responses during progressive ratio days (#/30 min)	210.0 ± 54.2	184.1 ± 49.6	183.7 ± 39.1	151.2 ± 44.6
Body weight (g)	457.8 ± 12.3	480.3 ± 13.7	490.7 ± 14.3	505.5 ± 16.5
Daily water consumption in the home cage (ml/day)	55.1 ± 9.3	57.0 ± 6.8	50.5 ± 8.1	55.1 ± 10.8

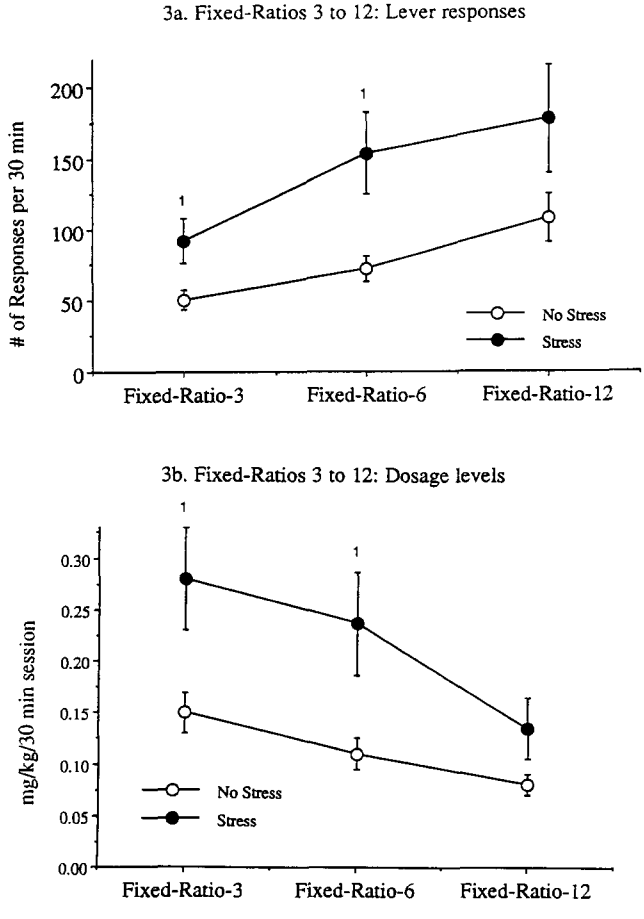

FIG. 3. Lever pressing for fentanyl under FR-3, FR-6 and FR-12 schedules of reinforcement and dosage levels under conditions of exposure or no exposure to shock stress (±SEM). 1, Significant differences from the no stress condition, $p < 0.05$.

responding followed by an extinction of this response. An unexpected finding in Experiment 1 was the long duration of water extinction observed under conditions of stress. Previous oral opioid SA studies usually have reported shorter extinction periods (3). Procedural differences exist between the present experiment and Carroll and Meisch's (3) studies (e.g., induction of the drug SA behavior, type of aversive environmental event, type of drug). But it may be that the long extinction duration in Experiment 1 was because the animals were exposed to repeated stress administration for a long period of time. In other words, exposure to stress may lead to a prolongation of the extinction phase of a given drug-seeking behavior. This prolongation of the extinction phase of opioid use may be related to the increased relapse to opioid use under conditions of stress reported in the human literature (e.g., 15,31). Alternatively, it may be that the long extinction period was related to the relatively low fixed-ratio (FR-4) used in Experiment 1. The shorter extinction period under an FR-6 schedule observed in Experiment 2, and previous reports indicating that a lack of extinction behavior is common at low FR schedules when the behavior is contingent upon the delivery of a strong reinforcer (see 9), lends support for the latter alternative.

The findings of these experiments also have broader relevance to the operant oral SA paradigm. The most common procedures used to establish orally delivered drugs as reinforcers are food-induced drinking (3,18) or schedule-induced drinking (17). In contrast, oral SA procedures in a home cage setting have mostly used water deprivation to induce drug SA (20,29). In one study, Leander and McMillan (17) induced etonitazene (a potent opioid agonist) lever-pressing in operant chambers by water deprivation. Using this induction technique, these investigators established that etonitazene can serve as a reinforcer (i.e., rates of drug SA administration exceeded vehicle SA after the termination of the induction procedure). Similarly, in the present experiments, a partial water deprivation induction procedure, at least under conditions of stress, induced fentanyl SA, and fentanyl functioned as a reinforcer after the termination of the induction phase. Specifically, fentanyl SA, under conditions of stress, met the two crucial criteria (i.e., drug presentation should show characteristic patterns of intermittently reinforced behavior, and

rates of drug-maintained behavior must exceed rates of vehicle maintained behavior) used to determine that a drug is serving as a reinforcer in the oral operant SA paradigm (18). In the present experiments, fentanyl SA was maintained under intermittent schedules of reinforcement; an increase in the schedule requirements resulted in an increase in the number of responses per session (see Fig. 3a); and an extinction of the drug-reinforced behavior was observed when a vehicle (water or quinine) was substituted for the drug. Taken together, these observations indicate that the water deprivation induction procedure used in the present experiments may prove useful in the examination of mechanisms underlying the stress-opioid interaction. This methodology may be further used to examine other environmental and biochemical factors that affect oral drug-reinforced behavior.

In conclusion, the results of the present experiments replicate and extend previous findings (25,26), and indicate that the effect of stress to increase opioid SA generalizes to a different stressor (mild electric footshock) and a different oral SA paradigm (operant lever-pressing for fentanyl). These findings indicate that a causal relationship exists between exposure to at least two stressors (immobilization and mild intermittent electric footshock) and increased oral opioid SA in two different behavioral paradigms (drug preference in home cages under limited fluid consumption schedule and operant oral SA paradigm). In addition, the partial water deprivation induction of oral opioid SA procedure described in the present experiments may be a useful technique to induce operant responding for drugs delivered via the oral route and to examine mechanisms underlying the stress-drug interaction.

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