Rapid Induction of Conditioned Opiate Withdrawal in the Rat

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Previous studies of conditioned opiate withdrawal in animals either have suffered from a lack of readily quantifiable data (e.g., measurement of diarrhea and vocalization in rodents) or were very long and costly (e.g., disruption of operant responding in monkeys). In this study, an attempt was made to produce a rapid and quantifiable measure of conditioned opiate withdrawal in the rat. Rats were trained to lever-press for food reinforcement on a fixed-ratio-15 schedule. All rats were then implanted with two subcutaneous 75-mg morphine pellets and allocated into three groups. The paired group received four naloxone injections (0.025 mg/kg SC) in the operant chambers paired with a distinctive tone and smell. The unpaired group was also exposed to the tone and smell in the chambers on four occasions, but received the naloxone injections in the home cage. The saline control animals were never exposed to naloxone or the tone and smell. On the test day, all rats were exposed to the tone and smell and injected with saline. The paired group showed a significant reduction in operant responding in response to the tone and smell when compared either with the other two groups, or to their own response rates on the previous day. In a second experiment, the paired and unpaired groups were again challenged with the tone and smell and a saline injection 1 month after removal of the morphine pellets. Again, the paired group showed a significant disruption of response. These results suggest that the conditioned stimulus acquired significant behavior-disruptive properties manifest even in the absence of opiate receptor occupancy. A rapidly acquired, quantifiable measure of conditioned withdrawal in the rat will allow systematic evaluation of the neurobiological basis for this phenomenon. ***[Neuropsychopharmacology 8:15–21, 1993]***

KEY WORDS: *Opiate; Morphine; Withdrawal; Dependence; Classical conditioning; Operant behavior*

Significant clinical evidence suggests that individuals experiencing drug withdrawal can become conditioned to environmental situations. Previously neutral stimuli can elicit many of the symptoms of opiate abstinence if paired with the withdrawal state, and this "conditioned withdrawal" may have motivational significance in drug dependence (O'Brien 1975; O'Brien et al. 1988).

Several animal models of conditioned withdrawal have been demonstrated in rats and monkeys. In early studies, rats injected with morphine for 6 weeks showed significant signs of physical withdrawal in a morphine-paired environment even up to 155 days after the last morphine injection (Wikler and Pescor 1967). In operant conditioning studies in monkeys, significant conditioned withdrawal was observed in morphine-dependent animals (2-month history of morphine injections): the monkeys showed an immediate suppression of food-maintained responding on a fixed-ratio-10 schedule in response to stimuli associated with injection of nalorphine (Goldberg and Schuster 1967). These conditioned stimuli acted as negative reinforcers since the monkeys lever-pressed to turn off the conditioned

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stimulus (Goldberg et al. 1971). The conditioned stimuli also appeared to have motivational significance in that the stimuli previously associated with opiate abstinence increased self-administration of morphine similar to that produced by the opiate antagonist (Goldberg et al. 1969).

Disruption of operant responding can also be observed in rats during precipitated opiate withdrawal. In particular, the rate of food-reinforced lever-pressing by morphine-dependent rats is reduced by naloxone administration (Gellert and Sparber 1977; Koob et al. 1989). The purpose of this study was to attempt to rapidly condition the withdrawal-induced disruption of food-reinforced operant responding in order to produce a quantifiable measure of conditioned morphine withdrawal in the rat. To accomplish this, rats were trained to respond for food on a fixed-ratio-15 (FR-15) schedule, implanted with morphine pellets to induce dependence, and then subjected to pairings of naloxone with a previously neutral smell and tone stimulus.

METHODS

Animals

Thirty-four male Wistar rats (Charles River) weighing 180 to 200 g at the start of the experiment were used. The rats were housed in groups of three in a room with a 12-hour light/12-hour dark cycle. Rats were given 10 to 15 g food per day in addition to that earned in the operant boxes. Water was freely available.

Operant Training

Rats were trained to lever-press in Coulborn operant chambers for 45-mg food pellets on a continuous reinforcement (FR-1) schedule. Subsequently, the fixed ratio (FR) schedule of reinforcement was gradually increased to 15. Rats then responded on an FR-15 schedule during 30-minute sessions 5 to 7 days per week until a stable rate of responding was achieved (± 10% of mean for 3 consecutive days).

Dependence Induction

Rats were anesthetized with halothane (1% v/v in air) and implanted subcutaneously with two 75-mg (0.13 mmol) morphine pellets wrapped in nylon mesh. After 3 days' recovery, training on the FR-15 schedule was resumed until the rate of responding returned to baseline.

Conditioning

Rats were randomly allocated to three groups. All groups were tested on the FR-15 schedule for 30 minutes daily for the following 9 days.

On 4 alternate days, Group 1 (the paired group; n = 13) received an injection of naloxone hydrochloride (0.025 mg/kg SC [70 nmol/kg] dissolved in saline) paired with a 7-kHz tone at 85 dB and a distinctive smell (anise extract) 10 minutes after the start of the session. On those days, the paired group received a saline injection in their home cage. On the other 5 alternate days, the paired group received saline 10 minutes after the start of the session and no tone or smell.

Group 2 (the unpaired group; n = 13) received a saline injection 10 minutes after the start of the session for 9 days and were exposed to the tone and smell on 4 of those alternate occasions. On those days the unpaired group also received an injection of naloxone (0.025 mg/kg SC) later in their home cage.

Group 3 (saline controls; n = 8) received saline injections both during the session and in their home cage and were never exposed to the tone or smell.

Test for Conditioned Withdrawal in Dependent Rats

After the 9 conditioning days, all rats were tested for conditioned withdrawal responses. The animals were tested on an FR-15 schedule for 30 minutes. Ten minutes after the start of the test, all animals received a saline injection and were exposed to the tone and smell.

Test for Conditioned Withdrawal in Postdependent Rats

The subcutaneous morphine pellets of the paired and unpaired groups were removed and the rats allowed 1 month's recovery. A baseline rate of responding was once again achieved, and animals were again tested for conditioned withdrawal. The paired and unpaired groups were tested for 30 minutes on an FR-15 schedule; 10 minutes after the start of the test, they received an injection of saline and were exposed to the tone and smell.

Statistical Analysis

Data collected in each 30-minute trial were the number of lever presses per 5-minute period. The pressing rate in the first 10 minutes of each trial was taken as the pretreatment baseline, and the data from the subsequent four 5-minute periods were calculated as percentages of baseline responding. Thus, the percentage changes in responding presented in the figures are derived from the data on baseline responding presented in Table 1. The pretreatment baseline response rates of the three groups before, during, and after conditioning were compared using two-way analysis of variance (ANOVA) with *treatment group* as the between-groups factor and *days* as the within-groups factor. Individual comparisons were performed at particular points using Neuman-Keuls post hoc tests. The effect of saline in-

Table 1. Pretreatment Baseline (First 10 minutes) Response Rates Before, During and After Conditioning

	Mean ± SEM Lever Presses/Min During First 10 Minutes						
Group	**Day 1**	**Day 2**	**Day 4**	**Day 6**	**Day 8**	**Test 1**	**Test 2**
Paired	71.1 ± 4.4	69.3 ± 7.8	71.3 ± 23.7	80.3 ± 6.5	76.7 ± 6.6	59.6 ± 8.4	62.7 ± 10.8
Unpaired	73.2 ± 3.9	64.8 ± 8.1	71.9 ± 7.5	76.1 ± 4.5	99.9 ± 6.0*	74.7 ± 7.8	82.0 ± 8.9
Saline Controls	92.4 ± 9.0	70.5 ± 8.7*	80.3 ± 11.1	74.3 ± 9.6*	84.9 ± 8.4	95.1 ± 8.3	

* Significantly different from day 1 ($p < 0.05$).

jection in the three groups of rats before conditioning was assessed by analyzing the data from day 1 of the conditioning procedure using two-way ANOVA with *treatment group* as the between-groups factor and *period* as the within-groups factor. To test for acquisition of the conditioned withdrawal response, data from the 4 conditioning days in which the paired group received naloxone paired with tone and smell were compared using two-way ANOVA with *days* and *period* as the repeated measures. To test for conditioned withdrawal in morphine-dependent and postdependent rats, data were analyzed using three-way ANOVA with *treatment group* as the between-subjects factor and *test day* and *period* as the within-subjects factors.

RESULTS

There were no significant differences between the three groups of rats with respect to baseline pretreatment response rates or response to saline injection. Naloxone (0.025 mg/kg SC) significantly reduced the response rate of the paired group (precipitated withdrawal) and there was an enhanced sensitivity to the effects of naloxone paired with tone and smell across the conditioning sessions (acquisition of the withdrawal response). When morphine-dependent rats were challenged with the tone and smell, the paired group showed a significant reduction in response rates compared with the other two groups and their own response rates on the previous day (conditioned withdrawal). In a second tone and smell challenge 1 month postdependence, the paired group again demonstrated significant disruption of response (conditioned withdrawal in postdependent rats). These results are presented in detail below.

Pretreatment Baseline Response Rates of the Paired, Unpaired, and Saline Control Groups Before, During, and After Conditioning

Table 1 shows the response rates during the first 10 minutes (i.e., pretreatment baseline) of the three groups of rats on days 1, 2, 4, 6, and 8 of training and on the 2 test days (the saline control group was not tested postdependence). Two-way ANOVA was performed with *treatment group* as the between-groups factor and *days* as the repeated measure. *Treatment group* had no significant effect (there was no difference between the pretreatment response rates of the three groups), but *days* had a significant effect [pretreatment response rates were variable during the study; $F(5,155) = 4.9$, $p < 0.001$]. There was also a significant *treatment group* × *days* interaction [the amount of variation in pretreatment response rates across the study was different for different groups on different days; $F(10,155) = 3.7$, $p < 0.001$]. Post hoc tests (Neuman-Keul's) comparing rats' pretreatment response rates on each day with their own response rates on day 1 showed (1) no significant differences in the paired group; (2) the pretreatment response rate on day 8 was significantly higher than on day 1 in the unpaired group ($p < 0.01$); and (3) the pretreatment response rates on days 2 and 6 were significantly lower than on day 1 in the saline control group ($p < 0.05$). Thus, it appears that the response rate across days was somewhat variable, but there was no clear pattern of changes coinciding with any particular treatment. Therefore, to correct for variation in baseline response rates, all proceeding data will be presented as a percentage of baseline.

Effect of Saline Injection in the Three Groups (Before Conditioning)

Table 2 shows the percentage of baseline responding by the three groups of rats on day 1 of the conditioning procedure. All rats received saline IP 10 minutes after the start of the test session but no tone or smell. Two-way ANOVA revealed a significant effect of *period* [response rates decreased across the 20-minute session; $F(3,93) = 12.9$, $p < 0.0001$] but no significant effect of *treatment group* (the three groups did not differ in their response to saline injection). The rats received this same treatment on days 3, 5, 7, and 9 of conditioning; data (not shown) were not significantly different from those shown in Table 1.

Naloxone Injection in Morphine-dependent Rats (Acquisition of Conditioned Withdrawal Response)

Figure 1 shows the percentage of baseline responding by the three groups of rats on days 2, 4, 6, and 8 of conditioning. Rats in Group 1 (the paired group) received an injection of naloxone (0.025 mg/kg SC) and were exposed to a tone and smell 10 minutes after the start of

Table 2. Effect of Saline Administration on Day 1 of Conditioning

Treatment Group	Responses/Min ± SEM (first 10 minutes)	Response Rate in Posttreatment 5-Minute Periods (% first 10 minutes)			
		1	2	3	4
Paired	71.1 ± 4.4	98.3 ± 5.9	92.7 ± 7.2	88.5 ± 7.1	80.2 ± 7.8
Unpaired	73.2 ± 3.9	101.3 ± 5.3	91.1 ± 8.4	73.0 ± 7.2	68.3 ± 10.4
Saline Controls	92.4 ± 9.0	99.4 ± 2.9	85.1 ± 2.2	76.9 ± 5.0	75.9 ± 8.1

Response rates in the first 10 minutes are presented as mean ± SEM lever presses/min. Posttreatment response rates are mean ± SEM response rates presented as percentages of the first 10 minutes.

the session. Group 2 (the unpaired group) received a saline injection 10 minutes after the start of the session and were exposed to the tone and smell (they received naloxone later in the home cage). The saline controls received saline and were not exposed to the tone or smell. Two-way ANOVA revealed a significant effect of *treatment group* [naloxone significantly reduced response rate; $F(2,31) = 129.3$, $p < 0.0001$] while the effect of *days* just missed significance [$F(3,93) = 2.6$, $p = 0.06$; Fig. 1]. One-way ANOVA of the data from the paired group alone showed a significant effect of *days* in this group [the effect of naloxone and tone and smell increased across conditioning days; $F(3,36) = 7.1$, $p < 0.001$]. Post hoc tests showed that the response rates of the paired group on days 4, 6, and 8 were significantly lower than on first exposure to the naloxone and tone and smell ($p < 0.01$; Fig. 1). Thus, these data demonstrate an enhanced sensitivity to the effects of naloxone across the conditioning sessions. This could reflect either an enhanced unconditioned response to naloxone or the contribution of a developing conditioned response to the original unconditioned response. Naloxone injection in morphine-dependent rats also produced diarrhea, vocalization, and weight loss, although this was not systematically quantified.

Test for Conditioned Withdrawal in Dependent Rats (After Completion of Conditioning)

Figure 2 shows the percentage of baseline responding by the three groups after injection of saline accompanied by the tone and smell. Three-way ANOVA revealed a significant effect of *treatment group* [conditioning had a significant effect on response rate; $F(2,31) = 21.2$, $p < 0.0001$], *test day* [presentation of the conditioned stimulus had a significant effect on response rates compared with saline injection alone; $F(1,31) = 8.8$, $p < 0.01$], and *period* [there were differences in the response rates across the 20-minute session; $F(3,93) = 14.2$, $p < 0.0001$]. The interactions between the three factors were also highly significant ($p < 0.0001$). Post hoc tests showed that the tone and smell significantly reduced the percentage response rate of the paired group compared with the unpaired and saline control groups and their own rate of responding on the previous day ($p < 0.05$). Some diarrhea and screeching were also observed, although this was not systematically quantified.

Test for Conditioned Withdrawal in Postdependent Rats

Figure 3 shows the response rates of the paired and unpaired groups during a second challenge for conditioned withdrawal 1 month after removal of the morphine pellets. Three-way ANOVA revealed a significant effect of *treatment group* [conditioning had a significant effect on response rate; $F(1,20) = 15.6$, $p < 0.001$], *test day* [presentation of the conditioned stimulus had a significant effect on response rate compared with sa-

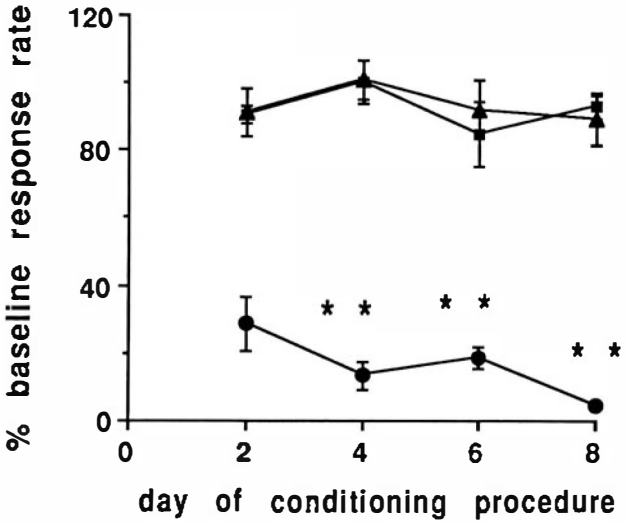

Figure 1. Mean ± SEM percent baseline response rate (total 20-minute posttreatment period) of the paired, unpaired, and saline control groups on days 2, 4, 6, and 8 of the conditioning procedure. Rats in the paired group received naloxone (0.025 mg/kg SC) paired with the tone and smell. The unpaired group received a saline injection and were exposed to the tone and smell. The saline controls received saline injection only. Asterisks indicate results significantly different from day 2 ($p < 0.01$). (●, paired group; ▲, unpaired group; ■, saline controls).

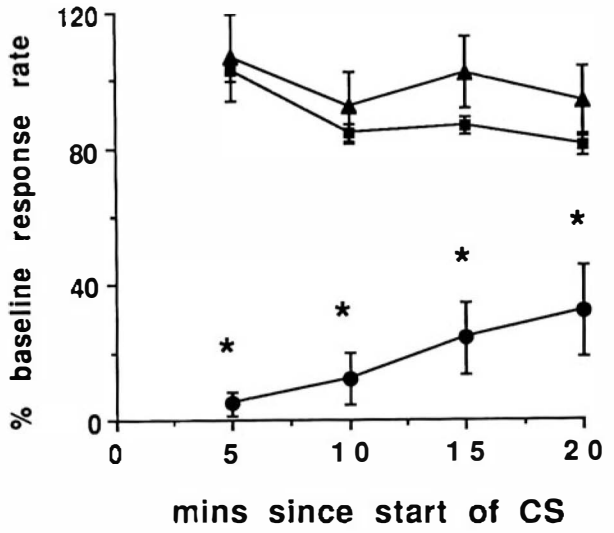

Figure 2. Test for conditioned withdrawal in morphine-dependent rats. Values are mean ± SEM percentages of baseline rate of lever pressing/minute. Mean ± SEM pretreatment baseline response rates on the test day were as follows: paired group = 60.0 ± 8.4; unpaired group = 74.7 ± 8.1; saline controls = 95.1 ± 8.7 lever presses/minute. Asterisks indicate results significantly different from the unpaired group and saline controls ($p < 0.05$). (●, paired group; ▲, unpaired group; ■, saline controls).

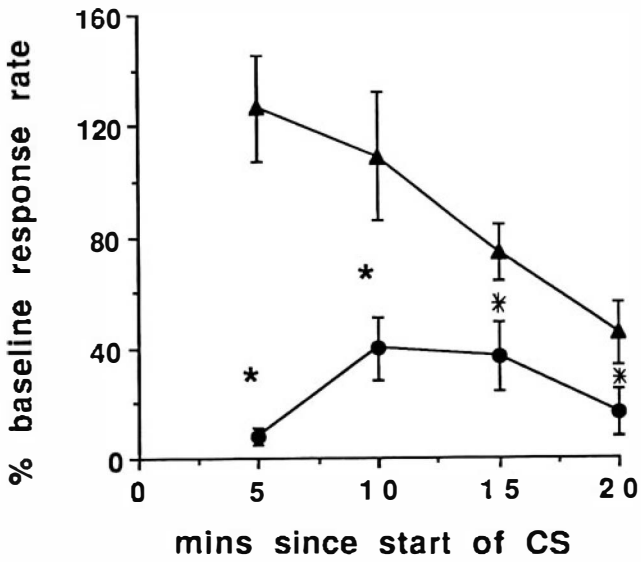

Figure 3. Test for conditioned withdrawal in postdependent rats. Values are mean ± SEM percentages of baseline rate of lever-pressing/minute. Mean ± SEM pretreatment baseline response rates on the test day were as follows: paired group = 66.0 ± 10.8; unpaired group = 81.9 ± 9.3 lever presses/minute. Asterisks indicate results significantly different from the unpaired group ($p < 0.05$). (●, paired group; ▲, unpaired group).

line injection alone; $F(1,20) = 4.1$, $p < 0.05$], and *period* [there were significant differences in the response rates across the 20-minute session; $F(3,60) = 17.1$, $p < 0.0001$]. The interactions between the three factors were also significant ($p < 0.05$). Post hoc comparisons showed that the percentage rate of responding of the paired group was significantly decreased by the tone and smell compared both with the previous day and the unpaired group ($p < 0.05$).

DISCUSSION

Induction of morphine dependence by subcutaneous implantation of morphine pellets is a simple procedure that does not appear to markedly disrupt food-reinforced lever-pressing in rats. However, there was some day-to-day variation in rates of responding and it is possible that this was, at least in part, due to the fact that the rats were morphine dependent. To control for this variation in the rate of lever-pressing, data from each animal were presented as percentages of their own pretreatment (first 10 minutes) response rate. On day 1 of the conditioning procedure, all rats received an injection of saline 10 minutes after the start of the session. Saline injection had no apparent effect on lever-pressing rates in morphine-dependent rats, although response rates declined gradually across each session as reported previously (Koob et al. 1989). This presumably reflects fatigue, satiation, or both, and is always seen in these experiments.

In agreement with previous observations (Gellert and Sparber 1977; Koob et al. 1989), naloxone (0.025 mg/kg SC) produced a disruption of food-reinforced lever-pressing in morphine-dependent rats. Other symptoms such as diarrhea, vocalization, and weight loss were also observed after naloxone administration to morphine-dependent rats. It could be argued that the disruption of food-reinforced responding could have been the result of an aversive action of naloxone itself, or morphine dependence. However, this dose of naloxone is totally without effect in rats that are not dependent on opiates (Gellert and Sparber 1977). Doses around 100 times higher (2 to 4 mg/kg SC) than that used in this study are required before any intrinsic effects of naloxone are observed in nondependent rats (Gellert and Sparber 1977). Thus, it is likely that the ability of this low dose of naloxone to disrupt food-reinforced responding and induce symptoms such as diarrhea in morphine-dependent rats was due to a precipitation of morphine withdrawal. In this study an attempt was made to condition rapidly this withdrawal response in order to produce a quantifiable operant measure of conditioned opiate withdrawal in the rat.

The paired group of morphine-dependent rats received four doses of naloxone (0.025 mg/kg SC) paired with a distinctive tone and smell. The unpaired group also received four naloxone injections and four ex-

periences of the tone and smell, but these were at different times and different locations. Finally, the saline controls never experienced the naloxone or the tone and smell. Acquisition of the conditioned response was demonstrated by the increasing effect that each successive administration of naloxone paired with the tone and smell had on lever-pressing rates. Finally, when morphine-dependent animals were challenged with the tone and smell in the absence of naloxone administration, there was a highly significant reduction in responding in the paired group compared with the other groups and the previous day's response rates. Thus, just like the physiological responses to opiate withdrawal (e.g., diarrhea, weight loss, etc.), the ability of naloxone to disrupt operant responding in morphine-dependent rats can be classically conditioned. Although this conditioning procedure was rapid (four pairings), comparison of the data on days 2 and 4 of the conditioning procedure shows that there was apparently significant acquisition after a single pairing. Further experiments are required to determine whether it would in fact be possible to demonstrate the conditioned withdrawal response after only a single pairing of the unconditioned and conditioned stimuli.

As mentioned earlier, the dose of naloxone used in these experiments has no intrinsic effects in nondependent rats. Thus, while a nondependent paired group (naloxone paired with tone and smell in the operant chambers) was not included, such a group would be expected to exhibit neither an unconditioned, nor a conditioned, response. Another group not included in this experiment was an unpaired morphine-dependent group that received naloxone (i.e., precipitated withdrawal) in the operant chamber and tone and smell in the home cage alternated with saline injections in the operant chamber; such a group would also not have been expected to have acquired the conditioned response to tone and smell, since this unconditioned stimulus had never been paired with withdrawal. It is possible that the presentation of naloxone in the operant chambers might serve to condition this context. The purpose of administering saline injections in the operant chambers on alternate days during conditioning was to avoid such a context conditioning. Context conditioning is unlikely to have occurred since there was no significant shift in the baseline responding of the paired group on days 1 to 8 of conditioning (Table 1).

In a further experiment, the paired and unpaired groups were again challenged with the tone and smell 1 month after removal of the subcutaneous morphine pellets. The paired group again displayed the conditioned disruption in lever-pressing. The response rates of the paired group were significantly lower than those of the unpaired group. The decrease in responding across the test session by the unpaired group presumably reflects fatigue, satiation, or both. Similar effects were seen with saline injections (Table 2). The enhanced effect (Fig. 3) probably reflects the increased weight and age of the rats after 6 weeks (i.e., they get lazy).

The plasma elimination half-life of subcutaneous morphine pellets has been estimated at 8.3 hours, and abstinence signs were observed 72 hours after their removal (Yoburn et al. 1985). It seems likely that the rats in the study were no longer dependent 1 month after removal of the morphine pellets. Thus, we have demonstrated that it is still possible to elicit a conditioned morphine withdrawal response even in postdependent rats. This is surprising, as one might predict that the unconditioned stimulus, naloxone injection alone, would be unable to produce a withdrawal response in postdependent rats. However, further experiments are required to determine whether that is the case.

In agreement with our findings, Goldberg (1976) showed that a stimulus paired with morphine withdrawal in rhesus monkeys disrupted food-reinforced responding in both dependent and postdependent animals. Monkeys injected with morphine or self-administering morphine for 1 to 2 months showed significant disruption of operant responding with presentation of a light or tone that had been previously paired with opiate antagonist administration (Goldberg and Schuster, 1967, 1969, 1970; Goldberg et al. 1969; Goldberg 1976). This ability of a conditioned stimulus to produce conditioned withdrawal-like responses persisted for 2 to 4 months postdependence (Goldberg 1976). There are also clinical reports that withdrawal symptoms associated with environmental situations can occur even in people who have abstained from opiates for very long periods of time (Wikler 1952; Teasdale 1973; O'Brien, 1975).

Therefore, the disruption of food-reinforced lever-pressing during precipitated morphine withdrawal can be rapidly conditioned (four pairings), and this conditioned withdrawal response persists even in postdependent rats. These data provide a quantifiable measure that should be of use in experiments to determine the neurochemical and neuroanatomical bases for conditioned opiate withdrawal.

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REFERENCES

Gellert VF, Sparber SB (1977): A comparison of the effects of naloxone upon body weight loss and suppression of

fixed-ratio operant behavior in morphine dependent rats. J Pharmacol Exp Ther 201:44–54

Goldberg SR (1976): Conditioned behavioral and physiological changes associated with injections of a narcotic antagonist in morphine-dependent monkeys. Pavlov J Biol Sci 11:203–221

Goldberg SR, Schuster CR (1967): Conditioned suppression by a stimulus associated with nalorphine in morphine dependent monkeys. J Exp Anal Behav 10:235–242

Goldberg SR, Schuster CR (1969): Nalorphine: Increased sensitivity of monkeys formerly dependent on morphine. Science 166:1548–1549

Goldberg SR, Schuster CR (1970): Conditioned nalorphine-induced abstinence changes: Persistence in post morphine-dependent monkeys. J Exp Anal Behav 14:33–46

Goldberg SR, Woods JH, Schuster CR (1969): Morphine: Conditioned increases in self-administration in monkeys. Science 166:1306–1307

Goldberg SR, Woods JH, Schuster CR (1971): Nalorphine-induced changes in morphine self-administration in rhesus monkeys. J Pharmacol Exp Ther 176:464–471

Koob GF, Wall TL, Bloom FE (1989): Nucleus accumbens as a substrate for the aversive stimulus effects of opiate withdrawal. Psychopharmacology 98:530–534

O'Brien C (1975): Experimental analysis of conditioning factors in human narcotic addiction. Pharmacol Rev 27: 533–543

O'Brien C, Childress A, McLellan A, Ehrman R, Ternes J (1988): Learning factors in substance abuse. In Ray BA (ed), NIDA Res Monogr 84:44–61

Teasdale J (1973): Conditioned abstinence in narcotic addicts. Int J Addict 8:273–292

Wikler A (1952): A psychodynamic study of a patient during experimental self-regulated readdiction to morphine. Psychiatr Q 26:270–293

Wikler A, Pescor FT (1967): Classical conditioning of a morphine abstinence phenomenon, reinforcement of opioid drinking behavior and "relapse" in morphine-addicted rats. Psychopharmacologia 10:255–284

Yoburn BC, Chen J, Huang T, Inturrisi CE (1985): Pharmacokinetics and pharmacodynamics of subcutaneous morphine pellets in the rat. J Pharmacol Exp Ther 235:282–286