Pharmacology Biochemistry and Behavior, Vol. 41, pp. 543-546, 1992
Printed in the U.S.A.

0091-3057/92 $5.00 + .00

Conditioned Withdrawal in Environments Associated With the Presence or Absence of Morphine

PAUL SCHNUR*[1] AND MARCY WAINWRIGHT†

**Department of Psychology, University of Southern Colorado, Pueblo, CO 81001-4901*
†Colorado College, Colorado Springs, CO 80903

Received 4 October 1991

SCHNUR, P. AND M. WAINWRIGHT. *Conditioned withdrawal in environments associated with the presence or absence of morphine.* PHARMACOL BIOCHEM BEHAV 41(3) 543-546, 1992. – An experiment designed to compare conditioned withdrawal in environments associated with the presence or absence of morphine was conducted in hamsters. For some animals, morphine administration was paired with distinctive environmental cues. For other animals, naloxone-precipitated withdrawal was paired with the distinctive environmental cues. For still other animals, naloxone-precipitated withdrawal and the distinctive environmental cues were unpaired. Following 12 days of training, animals were observed for signs of withdrawal (e.g., wet-dog shakes, etc.) in the distinctive environment following vehicle injections. Results indicated that more conditioned withdrawal responses occurred in the environment paired with the absence of morphine (naloxone-precipitated withdrawal) than in the environment paired with morphine administration.

Opiate withdrawal Conditioned withdrawal Naloxone-precipitated withdrawal
Morphine Naloxone Hamsters

BEHAVIORAL models of drug abuse propose that drug-seeking behavior is maintained, in part, by conditioned stimuli (CS's) associated with the positive reinforcing effects produced by drug ingestion, as well as by CS's associated with the negative reinforcing effects produced by the relief of withdrawal symptoms in the dependent animal (5,17,20). The focus of the present work is on the Pavlovian conditioning of opiate withdrawal responses in the hamster, *Mesocricetus auratus*. Recent work in our laboratory has demonstrated that opiate dependence develops readily in the hamster (10) and that both morphine tolerance (12) and dependence (11) can be brought under the control of Pavlovian CS's. The present experiment investigated the development of conditioned withdrawal responses to environmental stimuli associated with the presence vs. absence of opiates.

Previous research has demonstrated that environmental stimuli associated with the *absence* of opiates can function as Pavlovian conditioned stimuli to elicit conditioned withdrawal responses (CWR's) in opiate-dependent animals and humans (2,6,13,18,19). For example, Wikler and Pescor (19) demonstrated that formerly morphine-dependent rats exhibited withdrawal signs in a maze where they had experienced morphine abstinence. Similarly, in methadone-maintained human volunteers, a naloxone-precipitated withdrawal reaction was paired with the presentation of a tone/odor CS on 12 training trials. On test trials, the withdrawal reaction occurred following the presentation of the CS paired with a saline injection (6,8).

Conversely, other research has demonstrated that environmental stimuli associated with the *presence* of opiates can function as Pavlovian CS's to elicit CWR's in morphine-treated animals and humans (1,3,4,7,9,16). For example, Kelsey and colleagues demonstrated that opiate withdrawal in rats occurs in an environment associated with daily morphine injections (1,4). Similarly, opiate addicts experienced withdrawal symptoms during audiovisual presentations depicting stimuli formerly associated with injections of heroin (7).

Thus, the available evidence indicates that conditioned withdrawal responses can be elicited by stimuli associated either with the presence or absence of opiates. To date, however, there have been no studies comparing the magnitude of conditioned withdrawal elicited by environmental stimuli associated with the presence or absence of opiates. The present experiment was designed to make that comparison. For some animals, therefore, morphine administration was paired with placement in a distinctive environment that served as the CS. For other animals, naloxone-precipitated withdrawal was paired with placement in the CS environment. For still other

[1] Requests for reprints should be addressed to Paul Schnur, Department of Psychology, University of Southern Colorado, Pueblo, CO 81001-4901.

animals, naloxone-precipitated withdrawal and placement in the CS environment were unpaired.

METHOD

Subjects

Subjects were 16 golden Syrian hamsters (12 females, 4 males) with a mean weight of 130 g. They were housed individually in stainless steel hanging cages in a temperature-controlled vivarium under a 12 L: 12 D reversed lighting cycle (lights off at 0900). Animals had free access to water and Purina lab chow. All experiments were conducted during the dark phase of the hamster's circadian cycle and in accordance with NIH guidelines for the use and care of laboratory animals.

Apparatus and Materials

Hamsters were observed for symptoms of withdrawal in a transparent polycarbonate cage (45.7 × 24.1 × 20.3 cm). Morphine sulfate (15 mg/kg) and naloxone hydrochloride (10 mg/kg) were dissolved in saline and administered SC in the dorsal surface of the neck in 1-ml/kg volumes. Saline vehicle also was administered SC in 1-ml/kg volumes. Doses of morphine and naloxone refer to the salt.

Procedure

Animals were divided randomly into four groups ($n = 4$) for the training phase of the experiment, which lasted 12 days. On each day, Group M-HC/N-TC received a morphine injection (M) in the home cage (HC) and a naloxone injection (N) in the test cage (TC) 1 h later. These animals were removed from the home cage, injected with morphine, and immediately replaced in the home cage. One hour later, they were removed from the home cage, transported to an adjacent lab, and placed in the plastic test cage. After a 10-min period of observation, they were injected with naloxone and observed for an additional 30 min. For these animals, the plastic test cage was paired with naloxone-precipitated withdrawal. Group M-HC/S-TC was treated identically except that saline (S) was substituted for naloxone in the test cage injection. For this group, then, naloxone-precipitated withdrawal did not occur. The only withdrawal symptoms paired with the test cage would have been those elicited spontaneously 60–90 min after the home cage morphine injection. Group M-TC/N-HC received a morphine injection in the test cage and a naloxone injection in the home cage. These animals were removed from the home cage, transported to the adjacent lab, injected with morphine, and immediately placed in the plastic test cage. After a 10-min period of observation, these animals were injected with saline and then observed for an additional 30 min. Thirty minutes after being returned to the home cage, these animals were injected with naloxone. For these animals, the plastic test cage was paired with morphine and the home cage was paired with naloxone-precipitated withdrawal. The interval between the morphine and naloxone injections was identical in Group M-HC/N-TC and Group M-TC/N-HC; the groups differed only in where naloxone-precipitated withdrawal occurred. Group M-TC/S-HC was treated identically except that saline was substituted for naloxone in the home cage injection. For this group, then, morphine was paired with the test cage and withdrawal was not precipitated by naloxone. Following training, animals were left undisturbed in their home cages for 2 days. On the third day, animals were tested for conditioned withdrawal. On the test day, animals received injections according to the schedule used during training except that saline was substituted for morphine and naloxone.

Behavioral measures Animals were tested individually by a single observer who was not blind as to group assignment. Similar work in our laboratory with blind observers, however, has yielded interrater reliability estimates in excess of 0.90. Behavior was sampled continuously during the 40-min observation periods throughout the experiment. Signs of withdrawal including paw tremors, wet-dog shakes, abdominal writhing, teeth chattering, and yawning were counted. Paw tremors refer to vigorous shaking of the front or rear paws that is unrelated to grooming or scratching. Wet-dog shakes refer to torsional shakes involving the head and shoulders. Abdominal writhing was noted when the animal rotated its torso while pressing its abdomen to the floor, typically accompanied by arching of the back. Teeth chattering refers to tremors in the jaw muscles that produce visible movements of the mouth and muscles of the face, often accompanied by audible knocking of the teeth. Yawning needs no explanation. A composite withdrawal score was obtained by summing across response categories. A significance level of $p < 0.01$ was adopted throughout.

RESULTS AND DISCUSSION

Training

Data collected during the 30-min postinjection interval indicated that withdrawal symptoms (composite score) occurred in the test cage only in Group M-HC/N-TC, as expected, and that these symptoms increased across days of training. A 4 (groups) × 12 (days) mixed factorial analysis of variance (ANOVA) indicated that the effect of groups, $F(3,12) = 8.70$, the effect of days, $F(11,132) = 3.39$, and the interaction between groups and days, $F(33,132) = 3.40$, were significant. Posthoc comparisons using the Newman-Keuls test indicated that Group M-HC/N-TC exhibited more withdrawal signs than each of the other groups, which did not differ among themselves. These data confirm that naloxone was sufficient to precipitate withdrawal following a morphine injection, little or no spontaneous withdrawal occurred 60–90 min postmorphine, and, during training, no withdrawal occurred in the test cage either in Group M-TC/N-HC or M-TC/S-HC.

Previous research conducted in our laboratory has demonstrated the development of anticipatory CWR's in the 10 min prior to a daily naloxone injection in opiate-dependent hamsters (11). In the present experiment, analysis of CWR's in the first 10-min period of observation during training revealed that Group M-HC/N-TC gave more CWR's than any other group, but that the difference among groups was not significant. The failure to observe a significant number of anticipatory CWR's is likely due to the relatively low degree of dependence achieved in the present experiment, which relied on daily injections of a moderate dose of morphine (15 mg/kg). In the previous work, animals were made dependent by the subcutaneous implantation of two 75-mg pellets of morphine.

Test

Figure 1 shows the mean number of withdrawal responses (composite score) in each group during the 40-min test session.

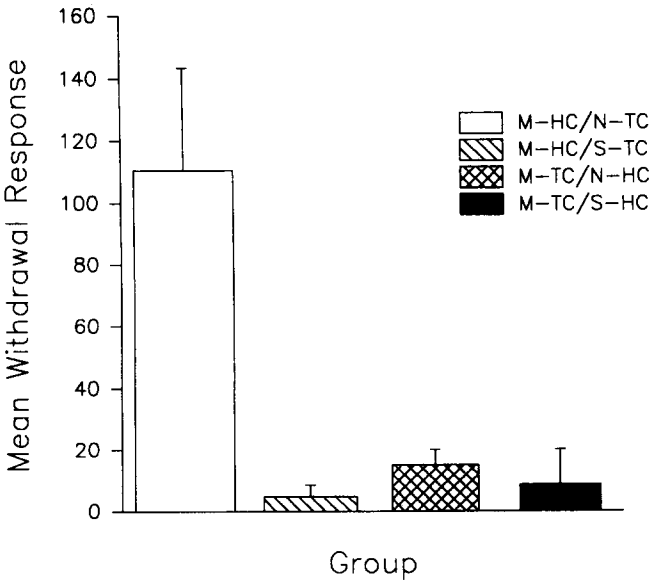

FIG. 1. Mean conditioned withdrawal responses on the test day for all groups.

It is evident that the number of CWR's in Group M-HC/N-TC was several times greater than that seen in the other groups. A one-way ANOVA indicated that the effect of groups was significant, $F(3,12) = 9.05$. Posthoc comparisons using Newman-Keuls test indicated that Group M-HC/N-TC differed from each of the other groups, which did not differ among themselves.

Thus, animals trained to associate the CS environment with naloxone-precipitated withdrawal exhibited significantly more CWR's during the saline test session than animals trained to associate the CS environment with morphine administration, whether or not naloxone-precipitated withdrawal occurred elsewhere (Groups M-TC/N-HC and M-TC/S-HC). Moreover, the low level of CWR's in Group M-HC/S-TC suggests that spontaneous withdrawal occurring 60-90 min after morphine makes little contribution to conditioned withdrawal in the CS environment.

Figure 1 also indicates that a small number of CWR's did occur during the test session in groups that received pairings of morphine with the CS environment. Indeed, the absolute number of CWR's in Groups M-TC/N-HC ($\bar{X} = 15$) and M-TC/S-HC ($\bar{X} = 8.5$) is similar to the number of CWR's reported in rats in experiments that paired morphine administration with a CS environment [e.g., (1,4)]. Since withdrawal was not elicited in the CS environment in these groups during training (see above), the presence of CWR's during the test session is consistent with the hypothesis that withdrawal responses develop as *compensatory* responses during morphine administration (14,15).

One might object to defining conditioned withdrawal in Groups M-TC/N-HC and M-TC/S-HC in the absence of control groups that were not opiate dependent. It is possible, for example, that control groups receiving only saline throughout training might have exhibited "CWR's" on the test day at a level comparable to that seen in Groups M-TC/N-HC and M-TC/S-HC. In previous work using similar procedures [e.g., (10,11)], however, the level of conditioned withdrawal in saline or placebo control groups was lower than that seen in these two groups. Nevertheless, comparisons across different experiments are hazardous and we do not mean to imply that CWR's have been demonstrated conclusively in Groups M-TC/N-HC and M-TC/S-HC. The purpose of this experiment was to compare conditioned withdrawal in environments associated with the presence or absence of morphine. The results conclusively demonstrate that CWR's develop more fully in CS environments paired with naloxone-precipitated withdrawal than in CS environments paired with the administration of morphine.

ACKNOWLEDGEMENT

This research was supported by a Scholarly Activities Grant from the University of Southern Colorado.

REFERENCES

1. Falls, W. A.; Kelsey, J. E. Procedures that produce context-specific tolerance to morphine in rats also produce context-specific withdrawal. Behav. Neurosci. 103:842-849; 1989.
2. Goldberg, S. R.; Schuster, C. R. Conditioned nalorphine-induced abstinence changes: Persistence in post morphine-dependent monkeys. J. Exp. Anal. Behav. 14:33-46; 1970.
3. Hinson, R. E.; Siegel, S. Nonpharmacological bases of drug tolerance and dependence. J. Psychosom. Res. 26:495-503; 1982.
4. Kelsey, J. E.; Aranow, J. S.; Matthews, R. T. Context-specific morphine withdrawal in rats: Duration and effects of clonidine. Behav. Neurosci. 104:704-710; 1990.
5. Lindesmith, A. Addiction and opiates, 2nd ed. Chicago: Aldine; 1968.
6. O'Brien, C. P.; Ehrman, R. N.; Ternes, J. W. Classical conditioning in human opioid dependence. In: Goldberg, S. R.; Stolerman, I. P., eds. Behavioral analysis of drug dependence. New York: Academic Press; 1986:329-356.
7. O'Brien, C. P.; Testa, T.; O'Brien, T. J.; Brady, J. P.; Wells, B. Conditioned narcotic withdrawal in humans. Science 195:1000-1002; 1977.
8. O'Brien, C. P.; Testa, T.; O'Brien, T. J.: Greenstein, R. Conditioning in human opiate addicts. Pavlov. J. Biol. Sci. 11:195-202; 1976.
9. Paletta, M. S.; Wagner, A. R. Development of context-specific tolerance to morphine: Support for a dual-process interpretation. Behav. Neurosci. 100:611-623; 1986.
10. Schnur, P. Acute morphine dependence in the hamster. Pharmacol. Biochem. Behav. 38:711-713; 1991.
11. Schnur, P. Conditioned morphine withdrawal in hamsters. Psychopharmacology (in press).
12. Schnur, P.; Martinez, R. A. Environmental control of morphine tolerance in the hamster. Anim. Learn. Behav. 17:322-327; 1989.
13. Sideroff, S. I.; Jarvik, M. E. Conditioned responses to a videotape showing heroin related stimuli. Int. J. Addict. 15:529-536; 1980.
14. Siegel, S. Morphine tolerance acquisition as an associative process. J. Exp. Psychol. [Anim. Behav.] 3:1-13; 1977.
15. Siegel, S. Classical conditioning, drug tolerance and dependence. In: Smart, R. G.; Glaser, F. B.; Israel, Y.; Kalant, H.; Popham, R. E.; Schmidt, R., eds. Research advances in alcohol and drug problems. New York: Plenum Press; 1983:207-246.
16. Siegel, S.; Hinson, R. E.; Krank, M. D.; McCully, J. Heroin "overdose" death: The contribution of drug associated environmental cues. Science 216:436-437; 1982.
17. Stewart, J.; de Wit, H.; Eikelboom, R. Role of unconditioned

and conditioned drug effects in the self administration of opiates and stimulants. Psychol. Rev. 91:251–268; 1984.
18. Teasdale, J. Conditioned abstinence in narcotic addicts. Int. J. Addict. 8:273–292; 1973.
19. Wikler, A.; Pescor, F. T. Classical conditioning of a morphine abstinence phenomenon, reinforcement of opioid drinking behavior and "relapse" in morphine addicted rats. Psychopharmacologia 10:255–284; 1967.
20. Wise, R. A.; Bozarth, M. A. A psychomotor stimulant theory of addiction. Psychol. Rev. 94:469–492; 1987.