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# Anxiogenic-Like Effects of Spontaneous and Naloxone-Precipitated Opiate Withdrawal in the Elevated Plus-Maze

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SCHULTEIS, G., M. YACKEY, V. RISBROUGH AND G. F. KOOB. *Anxiogenic-like effects of spontaneous and naloxone-precipitated opiate withdrawal in the elevated plus-maze.* PHARMACOL BIOCHEM BEHAV **60**(3) 727–731, 1998.—Withdrawal from opiates and other drugs of abuse in human addicts is associated with a state of anxiety that may be of motivational relevance for the maintenance of drug addiction. Previous attempts with rats to model the anxiogenic-like effects of opiate withdrawal using the elevated plus-maze have met with mixed success. The current study sought to determine whether spontaneous and naloxone-precipitated opiate withdrawal could be observed reliably in rats made dependent on morphine through implantation of two morphine pellets (75 mg morphine base each). Seventy-two hours after implantation of either morphine or placebo pellets, rats were tested in the elevated plus-maze. In Experiment 1, pellets were removed 8 or 12 h prior to test; results indicated an anxiogenic-like effect (reduction in time spent in the open arms) of opiate withdrawal at 8 but not 12 h postpellet removal. In Experiment 2, pellets were not removed, but withdrawal was precipitated with naloxone (0.003–0.03 mg/kg SC). Naloxone dose dependently precipitated a reduction in exploration of the open arms of the plus-maze. The results suggest that both spontaneous and precipitated withdrawal from continuous morphine administration via pellet implantation result in demonstrable anxiogenic-like effects in the plus-maze. © 1998 Elsevier Science Inc.

Opiates Morphine Opiate withdrawal Opiate dependence Anxiety Elevated plus-maze

OPIATE withdrawal in humans is associated with a number of well-characterized somatic withdrawal signs, and a number of subjective or affective signs, including dysphoric mood, restlessness and irritability, and anxiety (1,18,19,25). Recent work has established a number of sensitive, reliable behavioral models of these subjective signs of opiate withdrawal, including elevations in intracranial self-stimulation thresholds (24,27) and conditioned place aversions (16,20,27,29).

These models have been valuable in the delineation of the neuroanatomical and neurochemical substrates that contribute to the dysphoria and aversive stimulus effects of opiate withdrawal, respectively.

Although a number of well-validated animal models of anxiety have been developed and employed in the assessment of withdrawal-induced anxiety for a number of drugs of abuse (4,7–12,15), attempts to develop animal models of the anxiety associated with opiate withdrawal in humans have yielded mixed results. For example, previous reports have indicated that spontaneous withdrawal from chronic intermittent morphine injections in rats was associated with an increase in anxiogenic-like behavior 24–48 h postmorphine as measured in the defensive probe-burying paradigm (15) or the elevated plus-maze (4). However, more recently it was reported that rats undergoing spontaneous withdrawal from continuous morphine delivered via osmotic minipumps showed no anxiogenic-like profile in either the elevated plus-maze or the social interaction test (14) when tested 30 h postmorphine.

Taken together, these seemingly discrepant results might suggest that the ability to detect anxiogenic-like effects of opiate withdrawal in animal models is task specific (14). Alternatively, it is possible that the time course for the expression of anxiogenic-effects of opiate withdrawal may differ as a func-

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tion of the opiate dependence induction regimen. The current study sought to evaluate this possibility by examining the effects of a single morphine withdrawal episode, either spontaneous or naloxone-precipitated, on behavior in the elevated plus-maze model of anxiety using a continuous morphine delivery system (subcutaneous morphine pellets). Spontaneous withdrawal in the present study was assessed at 8–12-h time points postmorphine. These time points differed from those employed by Grasing and colleagues [30 h postmorphine; see (14)], but were similar to those employed by our laboratory in studies of anxiogenic-like effects of ethanol withdrawal (23).

## GENERAL METHOD

All work was approved by the Institutional Animal Care and Use Committee of The Scripps Research Institute, and was conducted in strict adherence to the guidelines set forth in the Guide for the Care and Use of Laboratory Animals.

### *Animals*

Subjects were 137 naive male Wistar rats (Charles River Laboratory, Kensington, NY) weighing 260–280 g upon arrival. Animals were group housed (two to three per cage) in acrylic cages in a temperature- and humidity-controlled environment, given ad lib access to food and water, and maintained on a 12 L:12 D cycle, with lights on at 2200 h. Four to 7 days after arrival in the laboratory, all rats were handled for 10 min. Rats were handled briefly (3–5 min) two additional times prior to morphine or placebo pellet implantation (2 weeks after arrival), and again 24 h prior to plus-maze testing. All behavioral testing took place between 1700–2100 h during the dark (active) phase of the rats' daily cycle.

### *Drugs*

Naloxone hydrochloride (Sigma Biochemicals, St. Louis, MO) was dissolved in physiological saline prior to subcutaneous (SC) administration. All injections were made in a volume of 0.1 ml/100 g body weight. Morphine pellets (75 mg morphine base per pellet) and placebo pellets were provided by the National Institute on Drug Abuse (Rockville, MD). Two pellets (either morphine or placebo) were wrapped in nylon mesh (pore size approximately 0.2–0.5 mm after stretching over the pellets) to facilitate subsequent removal (Experiment 1) and implanted SC under halothane anesthesia.

### *Elevated Plus-Maze Paradigm*

As described previously (21–23), the plus-maze apparatus consisted of four arms elevated 50 cm above the floor, with each arm (10 cm wide, 50 cm long) positioned at 90° relative to the adjacent arms. Two of the arms were enclosed with 40-cm high walls, and the other two arms were open. Testing was conducted in a quiet room in which the ventilation system provided approximately 65 dB background noise. The testing room was illuminated only by a dim light centered along one wall of the room such that the open arms were illuminated from the side and not down the length of the arm. A light meter was used to adjust the lighting for all test sessions: <1 lux in the enclosed arms, 350 lx with the light meter pointing towards the light on the open arm, 22 lux with the light meter pointing down the length of the open arm.

To begin a test session, rats were placed in the center of the maze facing towards the enclosed arm that pointed in the direction of the light source. Photobeam sensors located at the entry points to each arm were used to monitor the position of the rat in the maze. Two behavioral measures were recorded by computer for each rat: 1) duration of time spent in open arms and closed arms, and 2) activity as measured by the total number of beam breaks at the entry points to closed arms. This latter measure has been reported as the most reliable measure of general activity in the plus-maze (8). Between each trial, the maze was wiped clean with a damp sponge and dried with paper towels. An adjacent antechamber served as a holding room where animals were kept before drug administration, during the interval between injection and testing, and following maze testing.

### *Data Analysis*

All data were analyzed using analysis of variance (ANOVA) followed by individual mean comparisons using the Newman–Keuls test for multiple planned comparisons. For plus-maze data, the amount of time spent in the open arms was expressed as a fraction of the total time spent exploring both the open and closed arms for each rat. The total number of beam breaks at the entry points to closed arms served as a measure of overall activity for each rat.

## EXPERIMENT 1: SPONTANEOUS MORPHINE WITHDRAWAL

### *Procedure*

To determine the feasibility of testing rats in the plus-maze shortly after anesthesia and surgery (to remove pellets), a preliminary experiment was conducted in which rats ($n = 12$) were implanted either with placebo or morphine pellets, and 72 h later the rats were anesthetized, a sham surgical procedure was conducted, but the pellets were not removed. Eight hours later the rats were given a vehicle injection 5 min prior to placement onto the elevated plus-maze apparatus. Results of this "sham" withdrawal experiment indicated that chronic morphine did not significantly affect plus-maze performance relative to the placebo group, $F(1, 10) < 1.0$, $p > 0.85$; data not shown. Furthermore, exploration of the open arms 8 h after brief halothane anesthesia and surgery (24.9 ± 8.7% time in open arms) was sufficient to permit detection of anxiogenic effects.

Accordingly, in a subsequent experiment rats ($n = 70$) were handled as described in the General Method section prior to plus-maze testing. Seventy-two hours prior to testing, the rats received SC implants of two morphine (75 mg base each; $n = 34$) or two placebo pellets ($n = 36$) wrapped in nylon mesh under light halothane anesthesia. Eight or 12 h prior to plus-maze testing the rats were anesthetized a second time, and the pellets were removed. To control for possible fluctuations in plus-maze behavior across the daily activity cycle, time of plus-maze testing was held constant in this experiment, and the time of pellet removal was varied for the 8 and 12 h withdrawal groups. The rats were placed in the holding room at least 1 h prior to the start of testing.

### *Results*

The results of this experiment are summarized in Fig. 1. Two-factor analysis of variance revealed only a significant main effect of pellet condition on percent time spent exploring the open arms of the plus-maze, $F(1, 66) = 4.04$, $p < 0.05$. Subsequent comparisons of morphine and placebo pellet conditions for each time of test revealed a significant effect at the 8-h time point ($p < 0.05$) but not the 12-h time point, suggesting a transient reduction in time spent exploring the open arms in rats withdrawing from morphine compared to pla-

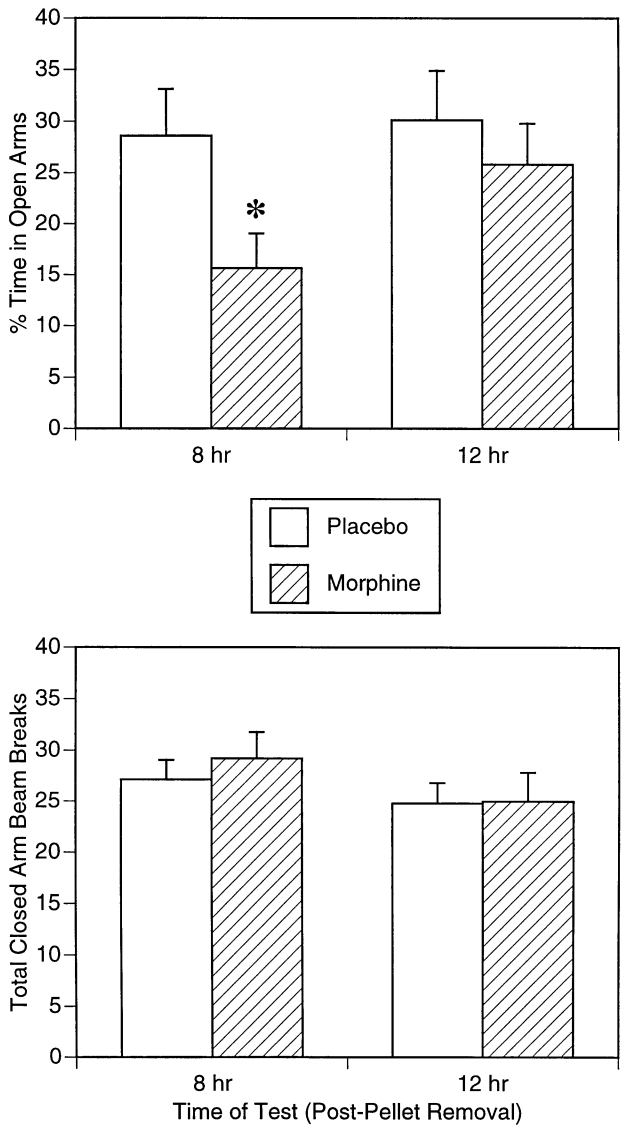


FIG. 1. Removal of morphine pellets (hatched bars) 8 h but not 12 h prior to test in the elevated plus-maze results in a significant reduction in time spent exploring the open arms of the maze relative to removal of placebo pellets (open bars, top panel). No significant effects of pellet condition were observed on activity in the closed arms of the plus-maze (total closed arm beam breaks, bottom panel). Data represent Mean ± SEM, and significance is denoted by $*p < 0.05$ vs. placebo control, Newman–Keuls post hoc test. $n$ = 14–21/group.

cebo–pellet-implanted control rats. There was no significant main effect of pellet, time of withdrawal, or pellet × time interaction for activity in the closed arms ($F$s $< 1.87$, $p$s $> 0.17$).

## EXPERIMENT 2: NALOXONE-PRECIPITATED OPIATE WITHDRAWAL

### *Procedure*

Rats ($n$ = 67) were handled as described in the General Method section prior to plus-maze testing, with each rat receiving a SC injection of saline during the last handling session to acclimate the rats to the injection procedure. Seventy-two hours prior to plus-maze testing, the rats received SC implants of two morphine (75 mg base each) or two placebo pellets wrapped in nylon mesh under halothane anesthesia. The rats were placed in the holding room at least 1 h prior to the start of testing. Morphine pellet-implanted rats ($n$ = 52) were given a SC injection of vehicle or naloxone (0.003, 0.01, 0.03 mg/kg) and 5 min later were tested in the elevated plus-maze. Placebo pellet-implanted rats ($n$ = 15) received a vehicle injection prior to testing. A preliminary study (28) revealed that doses of naloxone as high as 1.0 mg/kg were without effect on exploration of open arms in placebo-pellet implanted rats.

### RESULTS

The results for naloxone-precipitated withdrawal are summarized in Fig. 2. One-way ANOVA comparing placebo and morphine pellet-implanted rats receiving vehicle prior to test revealed no significant effect of chronic morphine on percent time spent in exploration of the open arms, $F(1, 28) < 1.0$, or in total beam breaks at the entrance to closed arms, $F(1, 28) = 1.47$, $p > 0.23$.

A one-factor ANOVA comparing morphine pellet-implanted rats treated with different doses of naloxone revealed a significant treatment effect on time spent in the open arms of the maze (Fig. 2, top panel), $F(3, 48) = 8.90$, $p < 0.0001$. Subsequent individual comparisons of each naloxone-treated group to the vehicle-treated control group revealed that doses of 0.01 mg/kg and 0.03 mg/kg (but not 0.003 mg/kg) produced a significant reduction in time spent exploring the open arms of the maze. In contrast, one-factor ANOVA on the activity measure (total beam breaks at entrance to closed arms) revealed no significant effect of naloxone treatment (Fig. 2, bottom panel), $F(3, 48) = 1.77$, $p > 0.16$.

### GENERAL DISCUSSION

The results of the current study indicate that both spontaneous and naloxone-precipitated withdrawal from continuously delivered morphine produce anxiogenic-like effects in the elevated plus-maze. These data support earlier reports of the anxiogenic-like nature of spontaneous opiate withdrawal in rodents using the defensive probe-burying and elevated plus-maze paradigms (4,15). Moreover, the results are consistent with an earlier report of naloxone-precipitated withdrawal producing an anxiogenic-like profile in the elevated plus-maze [unpublished results cited by Higgins and Sellers (17)]. The minimum dose of naloxone that produced reliable decreases in open-arm exploration in Experiment 2 (0.01 mg/kg SC) is identical to the minimum dose reported previously to produce other affective symptoms of withdrawal such as conditioned place aversions and elevations in brain stimulation reward thresholds (27,28). Although naloxone at this dose produces some somatic signs of withdrawal such as penile grooming and teeth chattering, most prominent somatic signs of withdrawal such as ptosis, diarrhea, weight loss, wet dog shakes, and jumping are elicited only at higher doses [0.03–1.0 mg/kg, see (27,28)].

Although the spontaneous withdrawal data of Experiment 1 differ somewhat from the results reported by Grasing and colleagues (14), who also employed the elevated plus-maze paradigm, these investigators reported the effects of spontaneous morphine withdrawal at 30 h (or later) after cessation of continuous morphine delivery via an osmotic minipump. The present data provide evidence that the effects of a single episode of spontaneous withdrawal from continuous mor-

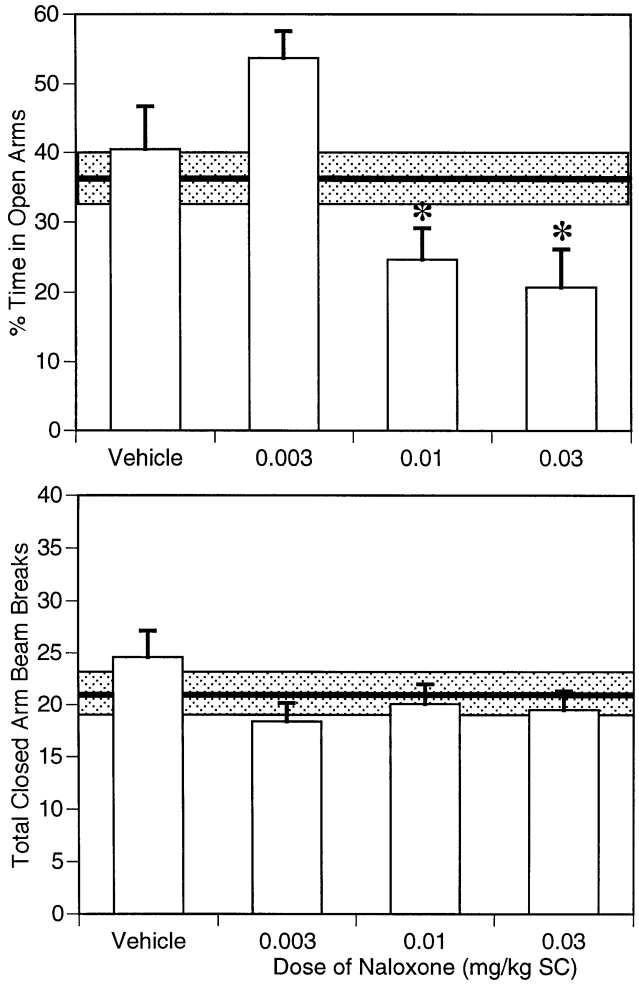


FIG. 2. Naloxone in morphine-dependent rats dose-dependently suppresses time spent exploring the open arms of the elevated plus-maze in comparison to vehicle treatment of morphine-dependent rats (top panel). Doses of 0.01 and 0.03 mg/kg produced significant decreases in time spent on the open arms. A slight increase in open arm time observed at the 0.003 mg/kg dose of naloxone relative to vehicle controls was not statistically reliable. No significant reductions in activity (total closed arm beam breaks, bottom panel) were produced by any doses of naloxone tested. Data represent mean ± SEM, and significance is denoted by $*p < 0.05$ vs. morphine pellet-implanted, vehicle-treated control, Newman–Keuls post hoc test. For comparison purposes, data from placebo pellet-implanted control rats are represented by the solid horizontal line (mean) and shaded horizontal bar (SEM). $n = 12$–14/group.

phine administration may be transient in nature, with a significant reduction in open-arm exploration seen at 8 h but not 12 h postmorphine pellet removal. Together with the results of Grasing and colleagues, the results would appear to indicate a transient profile of anxiogenic-like effects following spontaneous withdrawal from continuous morphine delivery by SC pellets.

These results potentially also could be interpreted as a residual effect of chronic morphine, and not a withdrawal effect per se, but several lines of evidence argue against this interpretation. First, a preliminary experiment indicated that sham pellet removal 8 h prior to plus-maze testing resulted in similar exploratory activity in the plus-maze for groups implanted with morphine and placebo pellets. Second, Yoburn and colleagues have reported that removal of two morphine pellets after 72 h implantation, conditions identical to those employed in Experiment 1 of the current study, resulted in a rapid biexponential clearance of morphine from the plasma, with an initial half-life of 0.74 h, and a terminal half-life of 8.3 h (30). Thus, rats tested at 8 h postpellet removal in the current study were definitely in a state of opiate abstinence, with the majority of morphine having already been cleared. Finally, in Experiment 2 it was found that rats implanted with morphine pellets and treated with vehicle prior to testing in the plus-maze showed no anxiogenic-like effects in comparison to placebo pellet-implanted rats. Taken together, these findings indicate that the most likely explanation for the time-dependent effect of spontaneous morphine withdrawal seen in Experiment 1 is a transient effect of withdrawal on this behavioral measure under the experimental conditions employed herein, and not a confound of residual morphine effect.

Earlier studies had indicated that anxiogenic-like effects of morphine withdrawal could be detected from 24–72 h post-morphine using either the elevated plus-maze paradigm or the defensive probe-burying paradigm as models of anxiety (4,15). However, in these studies morphine was administered as a series of once-daily or twice-daily injections, resulting in peaks and troughs in plasma (and brain) morphine concentrations. By contrast, the morphine pellet regimen employed herein and the osmotic minipump procedure employed by Grasing and colleagues (14) were designed to result in steady concentrations of morphine in plasma (13,30). It is possible, therefore, that the anxiogenic-like effects of opiate withdrawal may vary as a function of the morphine dependence induction regimen. In other words, the prolonged time course of the anxiogenic-like effects of opiate withdrawal seen with intermittent injection regimens may require repeated mini-withdrawal episodes produced by intermittent administration of morphine, a systematic sensitization or "kindling" phenomenon similar to that seen in the proconvulsant effects of alcohol withdrawal [e.g., (3)].

In combination with results of other studies, the present results indicate that anxiogenic-like effects appear to be a common affective component of withdrawal from multiple classes of abused drugs. For example, withdrawal from cocaine (2,15), alcohol (11,12,23), benzodiazepines (7,10), nicotine (4), and morphine [(4,15); current study], all result in measurable anxiogenic-like effects in the elevated plus-maze and/or defensive probe-burying paradigms, despite distinct pharmacodynamic mechanisms of action among these abused drugs. Furthermore, Lal, Emmett-Oglesby, and colleagues have reported that withdrawal from most of these drugs generalizes to the anxiogenic-like stimulus properties of pentylenetetrazol in a drug discrimination paradigm (5,6). In addition to anxiogenic-like effects, it also has been shown that alterations in brain reward circuitry, measured as elevations in brain stimulation reward thresholds, are a common element of withdrawal from multiple classes of abused drugs [see (25,26)].

In contrast, somatic withdrawal syndromes appear to vary dramatically between these different drugs. Opiate withdrawal in humans has been characterized as an intense flu-like state with somatic signs such as muscle aches, abdominal cramps, diarrhea, rhinorrhea, lacrimation, nausea and vomiting, fever, autonomic hyperactivity, and sweating, among others (1,18 ,19,25). Although withdrawal from chronic sedative/hypnotics (e.g., alcohol, benzodiazepines) also results in autonomic hyperactivity, other symptoms such as tremors, hallucinations, and seizures distinguish the sedative/hypnotic withdrawal syn-

drome from opiate withdrawal (1,7,18,26). Withdrawal from psychostimulants produces relatively minor physiological and somatic disturbances, most notably fatigue and suppressed heart rate (1,18). Thus, drugs that differ widely with respect to the somatic disturbances that characterize their abstinence syndromes nonetheless appear to share common symptomatology in an affective or emotional domain, including dysphoria and anxiety. Using animal models such as those employed herein and elsewhere to identify the mechanisms that contribute to neuroadaptations in the emotional and reward circuitry of the brain that are expressed as affective symptoms of drug withdrawal may provide a key for understanding drug addiction.

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