Changes in Urination/Defecation, Auditory Startle Response, and Startle-Induced Ultrasonic Vocalizations in Rats Undergoing Morphine Withdrawal: Similarities and Differences between Acute and Chronic Dependence

MIKHAIL KALINICHEV and STEPHEN G. HOLTZMAN
Department of Pharmacology, Emory University School of Medicine, Atlanta, Georgia
Received September 10, 2002; accepted October 25, 2002

ABSTRACT
In drug-free subjects, a single dose of morphine followed by an opioid antagonist a few hours later results in signs of a withdrawal syndrome, suggesting a state of physical dependence. Increased urination/defecation, altered startle, and ultrasonic vocalizations (USV) are some signs of the withdrawal syndrome in rats chronically dependent on morphine. We investigated whether naltrexone stimulates urination/defecation and alters startle and USV in male rats that were pretreated with only a single dose of morphine and compared these indices to the ones of chronic dependence. Separate groups of rats were pretreated with either a single dose (10 mg/kg) or with a continuous s.c. infusion of morphine via an osmotic pump. Naltrexone (0.01–1.0 mg/kg) was administered 2 to 6 h after the single dose of morphine and on days 7 to 11 of the infusion. Subjects chronically exposed to morphine also were tested during spontaneous withdrawal 3 to 24 h after pump removal. Naltrexone increased urination/defecation in subjects pretreated with morphine either chronically or acutely; it increased startle and USV in acutely dependent rats but decreased them in chronically dependent rats. In the latter group, changes in the four variables during spontaneous withdrawal were qualitatively similar to those during precipitated withdrawal but smaller in magnitude. Differences in withdrawal signs between acute and chronic dependence suggest that the neural substrates that mediate those particular components of the withdrawal syndrome are affected differently in the two states of dependence.

The state of opioid dependence and its underlying neural substrates are typically revealed by the withdrawal syndrome that develops as the result of an abrupt termination of agonist delivery or administration of an opioid antagonist. In the rat, the withdrawal syndrome from chronic opioid dependence is characterized by a variety of somatic signs as well as by changes in several behavioral measures reflective of “motivation”. Somatic signs of either spontaneous or antagonist-precipitated withdrawal from chronic morphine treatment in rats include loss of body weight, defecation/diarrhea, “wet dog shakes”, jumping, abdominal contractions, and abnormal postures (Martin et al., 1963; Blasig et al., 1973). Changes in behavioral measures include suppression of operand responding (Gellert and Sparber, 1977), suppression of locomotor activity (Brady and Holtzman, 1981), conditioned place aversion (Mucha, 1987), and increased thresholds for intracranial self-stimulation (Schaefler and Michael, 1986). These behavioral signs have been considered as operational measures of the affective/motivational aspects of the opiate withdrawal syndrome (Koob et al., 1989) and appear to have neural substrates that are distinct from those of somatic signs (Bozarth and Wise, 1984; Koob et al., 1992).

Opioid antagonists can precipitate withdrawal-like signs and symptoms in humans and animals pretreated with only a single dose of morphine, presumably revealing a state of acute physical dependence (Eisenberg, 1982; Young, 1986; Bickel et al., 1987; Schulteis et al., 1997). For example, naltrexone causes a number of somatic signs of withdrawal in rats pretreated with a single dose of morphine 4 h earlier; although compared with withdrawal from chronic morphine, there are fewer signs and a lower withdrawal score (Easterling and Holtzman, 1999).

The assessment of withdrawal processes based on operant behaviors has contributed significantly to the understanding of the state of opioid dependence and its underlying neural substrates.
of interoceptive and motivational aspects of acute opioid dependence. That rats discriminating the combination of acute morphine and naltrexone generalized dose dependently and completely to naltrexone administered during chronic morphine treatment provided evidence that withdrawal from acute and chronic dependence are associated with similar interoceptive states (Easterling and Holtzman, 1999). Other studies confirmed that a single dose of morphine increased sensitivity to opioid antagonists in altering behaviors maintained by food or brain stimulation in a fashion similar to that of chronic morphine (Adams and Holtzman, 1990b; Easterling and Holtzman, 1997).

Despite these findings, little research has been done on acute opioid dependence and withdrawal using unconditioned behaviors, especially in procedures that address the question of whether affective changes accompanying acute withdrawal are similar to those of chronic withdrawal. The first objective of this study was to determine whether auditory startle responses and startle-induced ultrasonic vocalizations could be used as behavioral measures of withdrawal from a single dose of morphine. Changes in these behaviors provide objective and reliable measures of withdrawal from chronic morphine (Vivian and Miczek, 1991; Mansbach et al., 1992). Auditory startle amplitudes and startle-induced ultrasonic vocalizations were evaluated in groups of rats undergoing withdrawal from acute or chronic morphine to compare the magnitude of these changes across different states of dependence. The second objective was to determine whether somatic signs, increased urination, and defecation could provide objective quantitative measures of withdrawal from acute dependence as they do withdrawal from chronic dependence (Ho et al., 1979; Pinelli and Trivulzio, 1997). To determine an optimal pretreatment interval for these somatic and behavioral indices of withdrawal, morphine pretreatment times were varied between 2 and 6 h. In a recent drug discrimination study in which rats were trained to discriminate acutely administered morphine followed by naltrexone from saline followed by naltrexone, the maximal discriminative effects occurred at 3 to 4 h (Easterling and Holtzman, 1999). In contrast, the maximal potentiation of naltrexone-induced drinking suppression by a single dose of morphine occurred at a 2-h interval (White and Holtzman, 2001). Based on these findings, we hypothesized that the optimal pretreatment time would vary across somatic or behavioral signs.

**Materials and Methods**

**Animals.** The subjects were adult male Sprague-Dawley rats (Charles River Breeding Laboratories, Raleigh, NC) weighing between 220 and 300 g at the start of the study. Animals were housed two to three per cage and maintained in the Emory University Division of Animal Resources Care Facility (Atlanta, Georgia). Food and water were always available. The colony room was maintained on a 12-h light/dark cycle, with lights on at 7:00 AM. This study was performed in accordance with the 1996 Guide for the Care and Use of Laboratory Animals (National Academy of Sciences, Washington, DC), and the research protocol was approved by the Institutional Animal Care and Use Committee of Emory University.

**Induction of Chronic Dependence.** After a rat was anesthetized with halothane, a 1.5-cm incision was made between the scapulae. A small cavity was made using a blunt-ended hemostat and two Alzet osmotic pumps [model 2ML2 (14 day); Alza, Palo Alto, CA] were inserted. One group of rats (n = 24) received osmotic pumps that delivered a total of approximately 20 to 22 mg/kg morphine per day. Drug concentrations were based on the infusion rate of the pump and on the weight of the animal. This treatment reliably produces tolerance to and physical dependence upon morphine in the rat (Adams and Holtzman, 1990b; Easterling and Holtzman, 1997). Another group of rats (n = 17) received pumps filled with saline. Half of the animals in each group were used in the experiments on urination and defecation, and the other half were used in the experiments on acoustic startle and startle-induced ultrasonic vocalization.

To precipitate withdrawal, we injected naltrexone (0.01, 0.1, and 1.0 mg/kg) on days 7, 9, and 11 according to a Latin square design (animals tested for startle also received a saline injection on day 5). Immediately after the injection animals were placed into animal holders for a 20-min session (see below). We induced spontaneous withdrawal by removing the pumps on day 13 while the rats were anesthetized with halothane. The appropriate 20-min sessions were repeated in the same animals 3, 6, and 24 h after pump removal to determine the effects of spontaneous withdrawal from morphine.

**Induction of Acute Dependence.** An injection of either morphine (10 mg/kg; n = 42) or saline (n = 20) was given to otherwise drug-free rats. Two to six hours later all animals were given a single injection of saline and were immediately placed into animal holders for a 20-min session. The whole procedure was repeated at 2- to 3-day intervals, except naltrexone (0.01, 0.1 and 1.0 mg/kg) was administered instead of saline, according to a Latin square design.

**Measurement of Urination and Defecation.** All measurements were taken inside the sound-attenuating cubicles (MED Associates, St. Albans, VT), with continuous 55-db background white noise maintained constantly. Rats were placed into animal holders (16 × 8 × 8 cm) fixed on the top of the platform (25 × 11 cm) that was used to measure the acoustic startle response; however, no startle stimuli were presented. All four sides of the animal holders were made of stainless steel bars placed 4 cm apart. Strips of Fisherbrand Absorbent Underpads (Fisher Scientific, Pittsburgh, PA), weighed previously, were placed under each holder to collect urine and fecal boli. At the end of the 20-min session, fecal boli were counted and removed from the pad; each pad was then weighed again, and the difference in the weight of the pad before and after the session was used to estimate the amount of urine deposited on it.

**Measurement of Auditory Startle Response.** Rats were put into animal holders that were placed inside sound-attenuating cubicles, as described above. Startle was measured via recording the force produced by a rat on a single point load cell (the startle-transducing platform). Startle-inducing auditory stimuli (white noise) were delivered through a speaker placed approximately at the level of the rat’s ears. The startle response was recorded during the 500 ms following the onset of the auditory stimulus. Startle peak was defined as the first peak of the downward force that was produced by the rat on the platform, with a minimum latency of 20 ms, a minimum peak value of 50 (range 50–2047), and a minimum peak time of 30 ms. All events during the 20-min session were controlled and recorded by software from Med Associates (Startle Reflex, version 3).

Before the start of the experiment, all auditory stimuli were calibrated (precision of 0.5 db) in the four cubicles, with the aid of the Digital Sound Level Meter (model S4022; Sper Scientific, Scottsdale, AZ). Each startle platform was calibrated by using a constant force produced by a weight displaced around a 45° angle by a rotating-step motor. The four startle platforms varied by less than 2% in the startle calibration procedure. During the 20-min session, each animal was first habituated to the animal holder for 3 min. After that, a series of 18 (three blocks of six trials) discrete (30 ms) auditory pulses (0-ms rise/fall time) of the same intensity (105 db; intertrial interval of 30–45 s) was delivered. The responses of each rat were averaged over the 18 trials.

**Measurement of Ultrasonic Vocalizations.** Ultrasonic vocalizations (20–28 kHz) were recorded and transformed into an audible signal (0.2–10 kHz) with the aid of Mini-3 Bat Detectors (Ultra...
Sound Advice, London, UK). After passing through an audio filter (Noldus Information Technology, Inc., Sterling, VA), the signal was sent to a computer, where it was digitized and analyzed with the aid of the software UltraVox (Noldus Information Technology, Inc.). Ultrasonic distress vocalizations were recorded for the entire duration of each session for every rat in the study.

**Drugs.** Morphine sulfate (Penicillin Co., St. Louis, MO) and naltrexone hydrochloride (Sigma-Aldrich, St. Louis, MO) were prepared in 0.9% saline. Both drugs were injected s.c. in a volume of 1.0 ml/kg. In addition, morphine and saline were administered by continuous s.c. infusion via osmotic pumps, as described above. All doses are expressed as the free base.

**Data Analysis.** Bartlett’s test for the homogeneity of variance was applied to the interval scale data. Analysis of variance (ANOVA) was performed on the interval scale data suitable for parametric analysis to evaluate the main effect, followed by Student-Neuman-Keuls post hoc test when appropriate. All proportion data (percentage of animals emitting ultrasonic vocalizations) were analyzed with the χ-square test. The α level chosen was p ≤ 0.05.

**Results**

**Uration**

**Chronic Dependence.** Following pump removal, animals undergoing spontaneous withdrawal from chronic morphine did not differ from saline-exposed controls in the amount of urine deposited, which was uniformly low at each of the three time points (Fig. 1a). In animals chronically dependent on morphine, naltrexone challenge produced significant, dose-dependent (F2,40 = 11.02; p = 0.001) increases in urination (Fig. 1b). There was a significant main effect of the treatment group (F1,40 = 11.02; p < 0.01) and a significant treatment group × dose interaction (F2,40 = 5.96; p < 0.01). The follow-up comparisons revealed that the increase in urination occurred in response to the two highest doses of naltrexone (0.1 and 1.0 mg/kg; Fig. 1a).

**Acute Dependence.** Naltrexone (0.01–1.0 mg/kg) had no effect on urination in animals pretreated with saline. In contrast, naltrexone-stimulated urination in rats pretreated with a single dose of morphine, depending upon the pretreatment interval (Fig. 1c). An overall ANOVA revealed a significant main effect of pretreatment group (F3,56 = 28.78; p < 0.0001) and of dose (F2,56 = 6.21; p < 0.01); a pretreatment group × dose interaction approached, but did not reach statistical significance (F8,56 = 2.08; p = 0.07). All three doses of naltrexone stimulated urination after 2-h pretreatment with morphine, and no dose did after 6-h pretreatment (Fig. 1c). In 4-h pretreated animals, 0.01 and 1.0 mg/kg of naltrexone increased urination significantly (Fig. 1c). The peak effect of naltrexone, which was reached after as little as 0.01 mg/kg in 2-h pretreated rats, was almost twice the peak effect observed in rats chronically dependent upon morphine.

**Defecation**

**Chronic Dependence.** Following pump removal, the number of fecal boli was significantly (F1,40 = 23.21; p = 0.0001) and consistently higher in animals that had received morphine chronically than it was in animals that had saline in their pump (Fig. 2a). There was also a significant main effect of time (F2,40 = 3.92; p < 0.05), with the peak effect at 3 h after the pumps had been removed. There was no significant treatment group × time interaction (F1,40 = 2, 12; p > 0.05). In animals chronically dependent on morphine, naltrexone produced robust dose-dependent (F2,40 = 127.02; p < 0.0001) increases in defecation (Fig. 2b). There was a significant main effect of treatment group (F1,40 = 353.23; p < 0.0001; Fig. 2b) and a significant treatment group × dose interaction (F2,40 = 139.96; p < 0.0001). Although all doses of naltrexone stimulated defecation in morphine-exposed rats, the two higher doses (0.1 and 1.0 mg/kg) of naltrexone produced a particularly dramatic effect (Fig. 2b).

**Acute Dependence.** Naltrexone (0.01–1.0 mg/kg) had no effect on defecation in rats pretreated with saline but dose-dependently increased the number of fecal boli in the three groups pretreated with morphine (Fig. 2c). An overall ANOVA revealed significant main effects of pretreatment group (F3,56 = 8.54; p < 0.001) and dose (F2,56 = 23.75; p < 0.001) as well as a significant pretreatment group × dose interaction (F6,56 = 3.92; p = 0.01). The highest dose of naltrexone (1.0 mg/kg) stimulated defecation in all morphine-pretreated rats, the intermediate dose (0.1 mg/kg) increased defecation only in 6-h pretreated animals, and the lowest dose (0.01 mg/kg) had no effect in any group (Fig. 2c).

**Auditory Startle Response**

**Chronic Dependence.** Auditory startle amplitudes were significantly (F1,36 = 8.96; p < 0.01) lower in animals under-

![Fig. 1](https://example.com/fig1.png)

**Fig. 1.** Increases in the amount of urine (grams) excreted by rats undergoing spontaneous withdrawal from chronic morphine (a) or naltrexone-precipitated withdrawal from chronic (b) or acute (c) morphine. Chronic dependence was induced by continuous infusion of morphine (20–22 mg/kg/day) via osmotic pumps. Acute dependence was induced by pretreatment with a single dose of morphine (10 mg/kg) administered 2, 4, or 6 h before naltrexone. Values are means ± S.E.M. (n = 8–12/group). The S.E.M. does not appear if it was less than the radius of the point. *, p < 0.05; **, p < 0.01; ***, p < 0.001 compared with group treated with saline.
going spontaneous withdrawal from chronic morphine than they were in animals previously exposed to saline (Fig. 3a). There was also a significant main effect of time ($F_{2,36} = 3.96$; $p < 0.05$) and a significant treatment group × time interaction ($F_{2,36} = 4.55$; $p < 0.05$). Compared with saline-exposed controls, animals undergoing spontaneous withdrawal from chronic morphine had lower startle amplitudes 3- and 24-h following removal of the pump (Fig. 3a). Startle amplitude was not affected by any dose of naltrexone (0.01–1.0 mg/kg) in rats that had pumps containing only saline nor was it affected by the chronic morphine treatment, as evidenced by the similar outcomes of the saline challenge to the two groups (Fig. 3b, points above Sal). It was, however, reduced more than 50% by 0.1 and 1.0 mg/kg of naltrexone in animals that were physically dependent upon morphine (Fig. 3b). ANOVA confirmed a significant main effect of treatment group ($F_{1,36} = 16.69; p < 0.001$) and of dose ($F_{3,36} = 7.19; p < 0.01$) and revealed a significant treatment group × dose interaction ($F_{2,36} = 9.57; p < 0.001$).

**Acute Dependence.** The effect of naltrexone on startle amplitude in animals pretreated with a single dose of morphine was the opposite of that in animals chronically dependent upon morphine. Naltrexone (0.1 and 1.0 mg/kg) increased startle amplitudes in rats that had been injected with 10 mg/kg of morphine either 2 or 6 h earlier, and the lowest dose, 0.01 mg/kg, increased startle amplitude in the 2-h pretreatment group. ANOVA revealed a significant main effect of the pretreatment group ($F_{2,54} = 4.73; p < 0.05$) and of dose ($F_{2,54} = 3.86; p < 0.05$) as well as a significant pretreatment group × dose interaction ($F_{4,54} = 4.01; p < 0.01$). Naltrexone did not affect startle amplitudes in rats pretreated with saline (Fig. 3c), consistent with the outcome in rats that had received infusions of saline via osmotic pumps (Fig. 3b), nor did morphine alone at either pretreatment interval (Fig. 3c, points above Sal).

**Startle-Induced Ultrasonic Vocalizations**

**Chronic Dependence.** Animals undergoing spontaneous withdrawal from morphine were less likely to vocalize 3 h after pump removal than were the corresponding control animals ($\chi^2(1, n = 20) = 10.90; p = 0.001$; Fig. 4a). There was no difference between the two groups in the percentage of animals vocalizing at later time points (6- and 24-h; Fig. 4a) or in the duration of vocalization at any of the three time points (Fig. 4d). Naltrexone dose dependently decreased startle-induced ultrasonic vocalizations in morphine-dependent animals (Fig. 4, b and e). Compared with saline-exposed controls, morphine-dependent animals were less likely to vocalize in response to 0.1 ($\chi^2(1, n = 20) = 10.90; p = 0.001$), and 1.0 mg/kg of naltrexone ($\chi^2(1, n = 20) = 16.15; p < 0.0001$; Fig. 4b). Furthermore, all doses of naltrexone decreased the duration of vocalization (Fig. 4e); there was a significant main effect of treatment group ($F_{3,36} = 42.57; p < 0.0001$) and dose ($F_{2,36} = 5.83; p < 0.01$), but no significant treatment group × dose interaction. In fact, the highest dose of naltrexone totally suppressed vocalizing. Naltrexone had no effect...
on either measure of startle-induced vocalizing in rats that were receiving a continuous infusion of saline via osmotic pump.

**Acute Dependence.** Rats pretreated with 10 mg/kg of morphine 2 h earlier and then given an injection of saline were less likely to vocalize ($\chi^2 (2, n = 29) = 8.86; p = 0.01$) than were rats treated with saline instead of morphine (Fig. 4c). In addition, the duration of vocalizing by this group was significantly shorter ($F_{2,27} = 4.83; p = 0.01$) than that of saline-pretreated animals, as well as of animals pretreated with morphine 6 h earlier (Fig. 4f). The two higher doses of naltrexone tended to increase vocalizing in rats pretreated with 10 mg/kg of morphine regardless of the pretreatment interval. Compared with saline-pretreated controls, rats pretreated with morphine 6 h earlier were more likely to vocalize after 1.0 mg/kg of naltrexone ($\chi^2 (2, n = 29) = 5.64; p = 0.05$), and those pretreated 2 h earlier were more likely to vocalize after 0.1 mg/kg naltrexone ($\chi^2 (2, n = 29) = 6.00 p = 0.01$). For duration of vocalization, there was a significant main effect of group ($F_{2,54} = 5.03; p = 0.01$) and a significant group × dose interaction ($F_{2,54} = 9.01; p < 0.0001$). The intermediate dose of naltrexone (0.1 mg/kg) increased duration of vocalization in both of the morphine-pretreated groups relative to the group pretreated with saline, whereas 1.0 mg/kg of naltrexone had this effect only on 6-h pretreated animals. Naltrexone did not affect either measure of vocalization in rats that were pretreated with saline instead of morphine (Fig. 4, c and f).

**Discussion**

Emergence of the withdrawal syndrome in chronically morphine-dependent rats was characterized by urination and defecation as well as by significant changes in auditory startle responses and startle-induced USV, as others have shown (Ho et al., 1979; Vivian and Miczek, 1991; Mansbach et al., 1992; Pinelli and Trivulzio, 1997). These somatic and behavioral indices of withdrawal from chronic dependence also were reliably present in animals treated with an opioid antagonist a few hours after pretreatment with only a single dose of morphine, presumably reflecting the state of acute physical dependence. Like withdrawal from chronic morphine treatment, withdrawal from acute morphine treatment stimulated urination and defecation in morphine pretreated animals. Withdrawal from chronic morphine administration, however, affected auditory startle and USV in the opposite manner from withdrawal from acute dependence. Naltrexone had no affect on any of the dependent measures in the absence of morphine. This is the first study to compare these components of the morphine withdrawal syndrome during spontaneous withdrawal from chronic dependence and antagonist-precipitated withdrawal from chronic and acute dependence.

Naltrexone produced robust increases in the number of fecal boli and the amount of urine excreted by morphine-dependent animals. Potentiation of defecation was particularly dramatic in rats chronically dependent upon morphine; the number of fecal boli increased by more than 80-fold. The somatic signs associated with spontaneous withdrawal from chronic morphine, as expected, were qualitatively similar to those of naltrexone-precipitated withdrawal, albeit milder. Signs of spontaneous withdrawal from chronic morphine administration usually are less intense than are the withdrawal signs precipitated by an opioid antagonist (Wei and Way, 1975; Jasinski, 1977).

This is the first study that provides a quantitative evaluation of urination and defecation in rats undergoing with-
withdrawal from acute morphine. In accord with the withdrawal from chronic dependence, naltrexone precipitated dose- and time-dependent increases in urination and defecation in rats pretreated with only a single dose of morphine. Thus, as in chronic opioid dependence, increases in urination or defecation can be used as valid somatic signs of acute physical dependence in the rat. The magnitudes of these increases varied from sign to sign. For example, in chronically morphine-dependent animals, increases in number of fecal boli were particularly dramatic in response to the two higher doses (0.1 and 1.0 mg/kg) of naltrexone. In animals pretreated with only a single dose of morphine, the same doses of naltrexone produced more modest increases in number of fecal. Unlike defecation, potentiation of urination was more robust in animals undergoing withdrawal from acute rather than chronic dependence. Thus, defecation and urination, like other signs of antagonist-precipitated withdrawal from morphine in rats (Blasig et al., 1973), vary quantitatively as a function of degree of physical dependence and dose of antagonist.

In rats chronically-dependent on morphine, naltrexone precipitated dose-dependent decreases in startle amplitude, the two higher doses decreasing amplitude by 50 to 60%. These data are consistent with those from a study in which naloxone dose dependently decreased auditory startle amplitude in chronically morphine-dependent rats (Mansbach et al., 1992). The magnitude of startle decrease and effectiveness of the opioid antagonists in the two studies were similar, even though different antagonists (naltrexone versus naloxone) and different regimens of chronic morphine exposure (osmotic pumps versus pellets) were used.

In accord with precipitated-withdrawal data, animals undergoing spontaneous withdrawal from chronic morphine exhibited a time-dependent decrease in acoustic startle responses. Compared with saline-exposed controls, morphine-dependent rats exhibited significant (30%) decreases in startle amplitudes as early as 3 h after pump removal. Startle amplitudes continued to decrease with time; by 24 h after pump removal, they were 50% lower than those of saline controls. In contrast to morphine withdrawal, withdrawal from chronic exposure to psychostimulants, benzodiazepines, or alcohol is associated primarily with increases in startle amplitudes (Rassnick et al., 1992; Miczek and Vivian, 1993; Barros and Miczek, 1996).

Naltrexone dose and time dependently increased startle amplitudes in rats pretreated with a single dose of morphine, in contrast to our expectations and the results of withdrawal from chronic morphine treatment. Two-hour pretreatment with 10 mg/kg morphine resulted in the largest effect, with all doses of naltrexone producing similar potentiation of the startle response. These increases in startle amplitude were not merely a consequence of naltrexone reversing a depressant effect of morphine on this response because morphine alone did not affect startle amplitude at either pretreatment interval. Others also have found that startle amplitude is unaffected by morphine (Davis, 1979; Mansbach et al., 1992).

Similar to some other aversive stimuli, such as foot- or tail-shock (Tonoue et al., 1986; van der Poel et al., 1989), social defeat (Tornatzky and Miczek, 1995), or cues that predict those stimuli (Burgdorf et al., 2001), startle-inducing auditory stimuli induce rats to emit USV in the 20- to 28-kHz frequency range (Kaltwasser, 1990). There appear to be significant strain differences in the capacity of auditory stimuli to induce USV. In response to 105-db acoustic stimuli, up to 90% of drug-naive Sprague-Dawley male rats emitted USV; male rats of other strains (56% Long-Evans; 40% Wistar) were less likely to vocalize (Kaltwasser, 1990). Naltrexone dose dependently decreased the occurrence and the total duration of vocalization in rats chronically dependent on morphine. In response to the highest dose of naltrexone (1.0 mg/kg), 80% of saline-exposed animals vocalized, whereas vocalization was completely suppressed in the morphine-dependent group. In accord with precipitated-withdrawal data, as early as 3 h after pump removal animals undergoing spontaneous withdrawal from chronic morphine were less likely to vocalize than were saline-exposed controls. In contrast to our findings, rats that were made chronically dependent upon morphine by pellet implantation had an increased total duration of USV during spontaneous withdrawal (Vivian and Miczek, 1991). In that study, however, vocalizations were not emitted in response to startle-inducing auditory stimuli, and rats vocalized only when tested in dyads; potentiation of vocalization did not occur in animals without prior social (agonistic or sexual) experience (Vivian and Miczek, 1991). Furthermore, that study was done with Long-Evans rats, which vocalize less in response to startle-inducing auditory stimuli than do Sprague-Dawley rats (vida supra).

Naltrexone precipitated time- and dose-dependent increases in ultrasonic vocalization in rats treated with a single dose of morphine. Therefore, like changes in the auditory startle response, changes in vocalization during withdrawal from acute morphine dependence were in the opposite direction of changes during withdrawal from chronic dependence. Animals pretreated with a single dose of morphine 2 h (but not 6 h) before testing were less likely to vocalize and vocalized for a shorter time than did saline-pretreated controls. In accord with these findings, morphine dose dependently attenuated USV induced by electric foot shocks (Tonoue et al., 1986) or by social defeat (Vivian and Miczek, 1993).

As we hypothesized, the maximal effect of naltrexone in animals injected with only a single dose of morphine occurred at different pretreatment intervals across different somatic and behavioral signs. The maximal potentiation of urination and acoustic startle by naltrexone was observed with 2-h morphine pretreatment. With longer pretreatment intervals (4–6 h), potentiation of these signs was milder (urination) or absent (acoustic startle). In accord with these findings, potentiation of naltrexone-induced drinking suppression by a single dose of morphine also occurred at a 2-h pretreatment interval (White and Holtzman, 2001). Other somatic and behavioral signs, such as increases in defecation and USV, appear to require longer (e.g., 6 h) pretreatment times.

There is large body of evidence suggesting that the state of acute opioid withdrawal is qualitatively similar to the state of chronic opioid withdrawal (see Introduction). There is some evidence, however, that mechanisms underlying chronic and acute opioid dependence are not identical and, in some cases, can be opposite to each other. For example, morphine given acutely inhibits adenylyl cyclase, leading to a reduction of cAMP in the cell, whereas chronically administered morphine up-regulates this second messenger system (Nestler and Aghajanian, 1997). The opposite changes in auditory startle response and USV during withdrawal from acute and chronic morphine complement these findings.
Thus, it appears that the phenotypic expression and the underlying mechanisms of withdrawal from acute dependence and chronic dependence can be similar or different, depending upon the neural substrates that mediate a particular component of the syndrome. Nonetheless, our results demonstrate that changes in urination, defecation, auditory startle response amplitude, and startle-induced USV are objectively quantifiable components of the withdrawal syndrome from acute morphine dependence as well as of the syndrome of withdrawal from chronic morphine dependence.

Acknowledgments
We thank Dr. Michael Davis for advice on measuring the acoustic startle response.

References


Davis M (1979) Morphine and naloxone: effects on conditioned fear as measured with the potentiated startle paradigm. Eur J Pharmacol 54:341–347.


Jasinski DR (1977) Assessment of the abuse potential of morphine-like drugs (meth-