

# Brain reward deficits accompany naloxone-precipitated withdrawal from acute opioid dependence

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## Abstract

Single injections with morphine can induce a state of acute opioid dependence in humans and animals, typically measured as precipitated withdrawal when an antagonist such as naloxone is administered 4–24 h after morphine. Repeated treatment with morphine results in a progressive shift in potency of naloxone to produce such acute withdrawal signs. The current study examined alterations in brain reward thresholds after acute and repeated treatment with morphine (5.6 mg/kg) using a discrete-trial current-intensity brain-stimulation reward procedure. Rats with stimulation electrodes aimed at the medial forebrain bundle at the level of the lateral hypothalamus were tested in twice daily sessions separated by 4 h. Separate groups of rats received treatment with morphine immediately after the first daily test session, and one of several doses of naloxone (0.10, 0.33, 1.0 mg/kg) 4 h later and immediately before the second session; these morphine and naloxone treatments were repeated for four consecutive days (Morphine–Repeat NAL). Additional groups examined the independent contribution of repeated morphine or repeated naloxone. One control group (Morphine–Vehicle) received morphine on all four treatment days, but vehicle before the second test session. A second group (Morphine–Single NAL) also received morphine on all four treatment days, but received 1.0 mg/kg only once after the final morphine pretreatment. A final control group received no morphine at all but received the 1.0-mg/kg dose of naloxone four times (Vehicle–Repeat NAL) before the second daily test session. Repeated naloxone alone (Vehicle–Repeat NAL) produced no changes in brain reward thresholds. Repeated morphine alone (Morphine–Vehicle) failed to alter reward thresholds measured 4 h postmorphine, but produced a slight increase in thresholds in the test sessions that occurred before morphine treatment on Days 3 and 4 (and hence 23.5 h after the previous day's morphine injection). This suggested the development of a modest spontaneous withdrawal-induced reward deficit measurable at 23.5 but not 4 h postmorphine. Naloxone dose-dependently increased brain reward thresholds 4 h after a single morphine pretreatment, with a further shift to the left in the naloxone dose-effect function resulting from repeated morphine and naloxone administration (Morphine–Repeat NAL). However, when the highest dose of naloxone was tested only after the final morphine pretreatment (Morphine–Single NAL), its potency was no different than when administered after the first morphine pretreatment. The results indicate that neuroadaptation within brain reward circuitry results in significant reward deficits after a single morphine pretreatment, and this deficit increases rapidly with repeated morphine and naloxone-induced withdrawal experience.

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## 1. Introduction

Acute opioid dependence can be defined as “a state in which abstinence can be demonstrated or precipitated after

either a single dose or a short-term infusion of [an opioid]” (Bickel et al., 1988; Martin and Eades, 1964). This phenomenon has been reliably measured in a large number of studies with both human subjects and a variety of animal species (Adams and Holtzman, 1990; Azar et al., 2003; Azorlosa et al., 1994; Heishman et al., 1989a,b; Jacob and Michaud, 1974; Jones, 1980; Martin and Eades, 1964; Parker and Joshi, 1998; Schulteis et al., 1997, 1999, 2003,

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2004; Wiley and Downs, 1979; Young, 1986). Importantly, acute opioid dependence can be observed in humans (Jones, 1980; Azorlosa et al., 1994) and animals (Azar et al., 2003; Parker and Joshi, 1998; Schulteis et al., 1997, 1999, 2003, 2004) with no previous history of opioid exposure, suggesting that even a single exposure to opioids can induce a mild dependence-like state measurable in the form of opioid withdrawal signs upon antagonist administration. It is also well established that repeated treatments with morphine at daily or weekly intervals can increase the severity of withdrawal-like signs elicited upon antagonist administration (Adams and Holtzman, 1990; Azorlosa et al., 1994; Schulteis et al., 1997, 1999, 2003, 2004).

Many neuroadaptational models of drug addiction favor the view that the progressive development of a state of drug dependence reflects “homeostatic neuronal adaptations and synaptic plasticity in specific brain regions, changes that ultimately contribute to the addictive phenotype” (Shaw-Lutchman et al., 2002; see also Di Chiara et al., 1999; Koob and Le Moal, 1997, 2001). Within an affective or hedonic domain, the positive affective (rewarding) effects of a drug may come to be offset by opposing negative affective responses, including feelings of anxiety, restlessness, and depression/dysphoria. It has been argued that such affective components of the withdrawal syndrome may be of greater motivational relevance than somatic signs in maintaining drug-seeking behavior and compulsive drug use (Haertzen and Hooks, 1969; Henningfield et al., 1987; Jasinski et al., 1985; Koob and Le Moal, 2001). Accordingly, animal models of these affective signs of withdrawal are essential to the study of the neural mechanisms underlying opioid dependence and addiction (Koob and Le Moal, 1997, 2001; Schulteis et al., 1994).

A number of well-characterized behavioral measures of the negative affective consequences of withdrawal from chronic opioid treatment have been developed using rodents as subjects, and include antagonist-precipitated suppression of operant responding for food, conditioned place aversion (CPA), elevation of brain stimulation reward thresholds, and anxiogenic-like effects measured in the elevated plus maze or fear-potentiated startle paradigms (Fendt and Mucha, 2001; Gellert and Sparber, 1977; Higgins and Sellers, 1994; Koob et al., 1989; Schaefer and Michael, 1986; Schulteis et al., 1994, 1998; Stinus et al., 1990). Two of these models, suppression of operant responding and CPA, serve as general measures of the aversive stimulus effects of opioid withdrawal, and to date these have been the most extensively applied models to the study of acute opioid dependence (Adams and Holtzman, 1990; Azar et al., 2003; Parker and Joshi, 1998; Schulteis et al., 1997, 1999, 2003, 2004; Young, 1986). The aversive state(s) that results in suppression of operant responding and CPA during opioid withdrawal could reflect the elicitation of one or more affective components of withdrawal such as anxiety or dysphoria. In rats exposed chronically to morphine, these aversive states are produced by low doses of opioid

antagonists that fail to elicit profound somatic signs of withdrawal (e.g., escape jumps, wet dog shakes, abdominal constrictions, diarrhea, body weight loss, profuse salivation; Higgins and Sellers, 1994; Schulteis et al., 1994). In contrast, naloxone elevates brain reward thresholds and produces anxiogenic-like effects at doses comparable to those that produce suppression of operant responding and CPA (Fendt and Mucha, 2001; Higgins and Sellers, 1994; Schulteis et al., 1994, 1998).

If the aversive states measured via suppression of operant responding and CPA reflect underlying brain reward deficits and/or anxiogenic-like states, then these affective components of withdrawal also should be measurable under conditions of acute morphine dependence that result in suppression of responding and CPA. Earlier work by Easterling and Holtzman (1997) and Easterling et al. (2000) provided evidence for modest increases in brain reward thresholds when naltrexone was used to precipitate withdrawal from acute morphine pretreatment. The investigators used both an autotitration procedure (Easterling and Holtzman, 1997) and a progressive ratio schedule (Easterling et al., 2000), and observed with both procedures that 10–20% changes in brain reward thresholds were simultaneously accompanied by profound (60–70%) suppression of responding for the brain stimulation. However, even with such dramatic reductions in response rate, responding for brain stimulation reward on the progressive ratio schedule remained at levels as high as 0.75 responses per second in many test conditions (Easterling et al., 2000). Accordingly, Easterling and colleagues reasoned that perhaps the “remaining stimulation was rewarding enough to buffer against a precipitous decline in [reward threshold].”

The current study sought to further establish whether brain reward deficits reliably accompany withdrawal from acute exposure to morphine and whether the magnitude of said deficits was indeed limited (10–20%) or could approach the magnitude of deficit observed in chronic dependence (>60%) under certain conditions. To accomplish this goal, a discrete-trial current-intensity threshold procedure was used with low response requirements and a limited number of reinforcement opportunities (see Kornetsky and Esposito, 1979; Markou and Koob, 1993; Schulteis et al., 1994).

## 2. Materials and methods

### 2.1. Animals

Male Wistar rats ( $N=55$ , Harlan Labs, Indianapolis, IN) weighing 300–400 g at the time of testing were used. All rats were pair-housed in a temperature- and humidity-controlled room with a 12-h light/12-h dark cycle (lights on at 6:00 a.m.). Rats had ad-libitum access to food and water at all times. All training and testing took place from 9:00

a.m. to 5:00 p.m. daily, Monday through Friday. All experimental procedures were approved by the Subcommittee on Animal Studies of the VA San Diego Healthcare System, an AAALAC-accredited facility, and are in strict accordance with the *Guide for the Care and Use of Laboratory Animals* (revised 1996).

## 2.2. Drugs

Morphine sulfate was purchased from King Pharmaceuticals (Bristol, TN), and naloxone HCl was purchased from Sigma (St. Louis, MO). Both drugs were prepared for injection in sterile physiological saline, and all injections were made subcutaneously in a volume of 0.1 ml/100 g body weight. Doses of both drugs are expressed as the salt.

## 2.3. Brain stimulation reward procedure

The surgery, procedure, and apparatus have been described in detail elsewhere (Markou and Koob, 1993; Schulteis et al., 1994). For surgery, rats were anesthetized with halothane and a stainless-steel bipolar electrode (Plastic Product, Roanoke, VA) was implanted in the lateral hypothalamus unilaterally (AP-0.5 mm from bregma, L 1.7 mm, 8.3 mm ventral from dura, incisor bar 5.0 mm above interaural line). To counterbalance any possible brain asymmetries, half the rats received implants on the right side of the brain, the other half on the left side.

Using a discrete-trial current–intensity threshold procedure (Kornetsky and Esposito, 1979), stimulation was delivered by constant current stimulators using 60-Hz sinusoidal waves, with a train duration set at 250 ms. To start each trial, a rat received a noncontingent electrical stimulus. A correct response was recorded if a rat rotated a wheel manipulandum at least 1/4 turn within 7.5 s of the noncontingent electrical stimulus; each correct response produced a contingent stimulus identical in all parameters to the noncontingent stimulus. After each correct response, there was an intertrial interval (ITI) averaging 10 s (7.5–12.5 s). If no response occurred within 7.5 s of the noncontingent stimulus, the ITI followed and that trial ended. Any

responding during the ITI resulted in a 10-s delay before the start of the next trial.

Stimulus intensities varied according to the method of limits and were presented in alternating ascending and descending series (two of each) with a step size of 5  $\mu$ A; a given stimulus intensity was presented three times within each series. Threshold was defined for each series as the midpoint between the current intensity level at which at least two correct responses occurred and that level at which fewer than two correct responses occurred; the mean of the four series thresholds served as the estimated threshold for a given session. The duration of each session was approximately 30–40 min, and rats received two sessions per day, separated by 4 h (end of first session to start of second).

## 2.4. Acute dependence and withdrawal testing regimen

After the establishment of stable baseline thresholds ( $\pm 15\%$  on five consecutive days), rats were habituated on three consecutive days to subcutaneous vehicle injections, with one injection occurring immediately after the first session on a given day, and the second injection occurring 5 min before the second session. The thresholds on the final two days of testing were averaged and served as the baseline value against which all subsequent thresholds were compared. Twenty-four hr after the final vehicle baseline session, all rats were again injected with vehicle after the first session, but separate groups were then injected with vehicle or one of several doses of naloxone (0.1, 0.3, 1.0 mg/kg) 5 min before the second session to establish a baseline response to naloxone before onset of any repeated morphine or naloxone treatment (see Table 1 for details).

Beginning 4 days after this initial naloxone injection, all groups of rats except one were injected daily with morphine (5.6 mg/kg) for four consecutive days (see Table 1). The morphine injection occurred immediately after the first session on a given day. Four groups of rats received these repeated morphine treatments, with each group receiving a single dose of vehicle (Morphine–Vehicle) or naloxone (0.10, 0.30, 1.0 mg/kg; Morphine–Repeat NAL) 4 h after

Table 1  
Details of experimental design and treatment conditions

Treatment group	Naloxone baseline day <sup>a</sup>		Repeated treatment days 1–3 <sup>a</sup>		Repeated treatment day 4 <sup>a</sup>	
	Postsession 1	Pre-session 2	Postsession 1	Pre-session 2	Postsession 1	Pre-session 2
Morphine–Vehicle ( $n=9$ )	Vehicle	Vehicle	Morphine	Vehicle	Morphine	Vehicle
Morphine–Repeat NAL						
NAL 0.10 <sup>b</sup> ( $n=9$ )	Vehicle	NAL 0.10	Morphine	NAL 0.10	Morphine	NAL 0.10
NAL 0.33 ( $n=8$ )	Vehicle	NAL 0.33	Morphine	NAL 0.33	Morphine	NAL 0.33
NAL 1.0 ( $n=11$ )	Vehicle	NAL 1.0	Morphine	NAL 1.0	Morphine	NAL 1.0
Morphine–Single NAL ( $n=12$ )	Vehicle	NAL 1.0	Morphine	Vehicle	Morphine	NAL 1.0
Vehicle–Repeat NAL ( $n=6$ )	Vehicle	NAL 1.0	Vehicle	NAL 1.0	Vehicle	NAL 1.0

<sup>a</sup> Daily Session 1 was followed by an injection of vehicle or morphine (5.6 mg/kg sc), whereas Daily Session 2 was preceded by an injection of vehicle or naloxone (NAL) at the dose indicated.

<sup>b</sup> Dose of naloxone in mg/kg sc.

each morphine pretreatment. The second daily test session followed 5 min after naloxone administration. The dose of morphine and interval between morphine and naloxone were chosen based on earlier work indicating reliable signs of acute opioid dependence with these parameters (Adams and Holtzman, 1990; Azar et al., 2003; Schulteis et al., 1997, 1999, 2003, 2004; Young, 1986).

To determine the individual contributions of repeated naloxone vs. repeated morphine to any observed increases in brain reward thresholds across days of treatment, two

additional control groups were tested. One group of rats received repeated vehicle instead of repeated morphine, and the highest dose of naloxone before the second daily test session (Vehicle–Repeat NAL; see Table 1). A final group received repeated morphine, but naloxone (1.0 mg/kg) only after the fourth and final morphine treatment (Morphine–Single NAL; see Table 1). This was critical given in our previous work, which demonstrated that under certain conditions repeated naloxone experience in the presence of morphine resulted in the potentiation of withdrawal

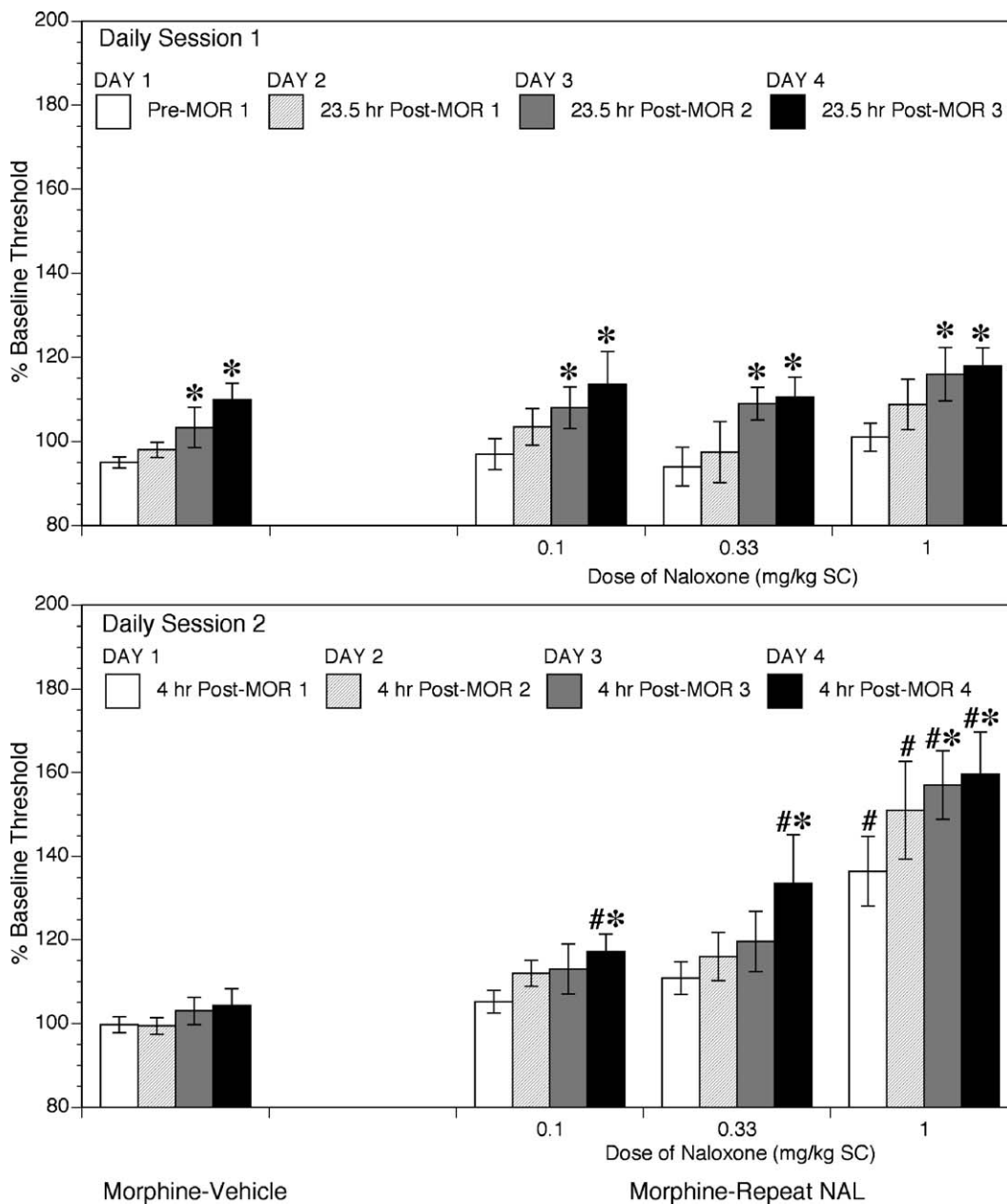


Fig. 1. Effects of acute and repeated morphine and naloxone on brain reward thresholds. Note that data collected in Daily Session 1 (upper panel) reflect reward thresholds measured 23.5 h after the preceding day's morphine dose, whereas data collected in Daily Session 2 (lower panel) reflect reward thresholds measured 4 h after that day's morphine injection and 5 min after that day's naloxone injection. All groups of rats were treated on four consecutive days with morphine (5.6 mg/kg), but differed with respect to the treatment administered 4 h later (vehicle [Morphine–Vehicle] or naloxone 0.10, 0.33, 1.0 mg/kg [Morphine–Repeat NAL groups]). Data represent mean ( $\pm$ S.E.M.) percent of baseline threshold. \* $P < .05$  vs. threshold measured in same treatment group on Day 1; # $P < .05$  vs. Morphine–Vehicle group on the same treatment day.

severity through a process of context-specific conditioning (Schulteis et al., 1999, 2003, 2004).

### 2.5. Statistical analysis

Data on all experimental treatment days were expressed as percent of threshold in the corresponding operant session (first or second) on the baseline days. Subsequently, the converted percent baseline response rate data were entered into appropriate single-factor (within or between subjects) ANOVAs, or two-factor mixed-design ANOVAs, as dictated by the treatment groups and conditions entered into the analysis. Follow-up comparisons consisted of interaction contrasts or simple main effects followed by individual means comparisons, as dictated by the outcome of the overall ANOVA.

## 3. Results

When tested before any morphine treatment (Naloxone Baseline Day), naloxone by itself did not significantly alter brain reward thresholds at any dose tested relative to vehicle treatment [ $F(3,33)=1.51$ ,  $P>.20$ ; data not shown]. Furthermore, repeated administration of the highest (1.0 mg/kg) dose of naloxone by itself (Vehicle–Repeat NAL group) did not significantly alter brain reward thresholds over time [ $F(3,15)=1.97$ ,  $P>.15$ ; see Fig. 2].

As shown in the lower panel of Fig. 1, there was no apparent change in brain reward thresholds measured 4 h after a single morphine pretreatment in the absence of any naloxone (Morphine–Vehicle), and repeated morphine pretreatment for a total of 4 days did not reveal any significant change in reward thresholds measured 4 h after each morphine injection [ $F(3,24)=2.59$ ,  $P>.05$ ]. However,

in this same Morphine–Vehicle group, repeated morphine pretreatment did result in a modest increase in threshold in the first of the two daily sessions [ $F(3,24)=5.40$ ,  $P<.006$ ; see upper panel, Fig. 1]; these sessions occurred immediately before the morphine injection on a given treatment day, but consequently occurred 23.5 h after the previous day's morphine injection. Follow-up comparisons indicated that this effect first reached significance on Morphine Day 3 (23.5 h after the second morphine treatment). Further inspection of Fig. 1 (upper panel) reveals that this threshold increase measured 23.5 h postmorphine was observed in all treatment groups, and the magnitude of the increase was not influenced significantly by treatment with any dose of naloxone. This was confirmed by a significant main effect of treatment day [ $F(3,99)=16.18$ ,  $P<.0001$ ] but no significant effect of naloxone dose [ $F(3,33)=1.80$ ,  $P>.15$ ] or Naloxone Dose  $\times$  Treatment Day interaction [ $F(9,99)=0.80$ ,  $P>.60$ ] in a two-factor mixed-design ANOVA. Follow-up comparisons revealed significant threshold increases on Morphine Days 3 and 4 (23.5 h after the second and third morphine treatments, respectively) in all groups, regardless of naloxone dose condition. This effect may therefore reflect modest brain reward threshold increases associated with the emergence over repeated morphine treatments of a spontaneous withdrawal state (rather than naloxone precipitated) that is detectable at 23.5 h but not 4 h postmorphine.

Whereas repeated morphine alone (Morphine–Vehicle) did not alter brain reward thresholds measured 4 h postmorphine, naloxone given 4 h postmorphine resulted in a significant dose-dependent increase in brain reward thresholds (Morphine–Repeat NAL, Fig. 1, lower panel). This was significant even after a single morphine pretreatment at the highest dose of naloxone tested (1.0 mg/kg). Moreover, naloxone potency to elicit brain reward threshold increases was further enhanced upon repeated morphine and

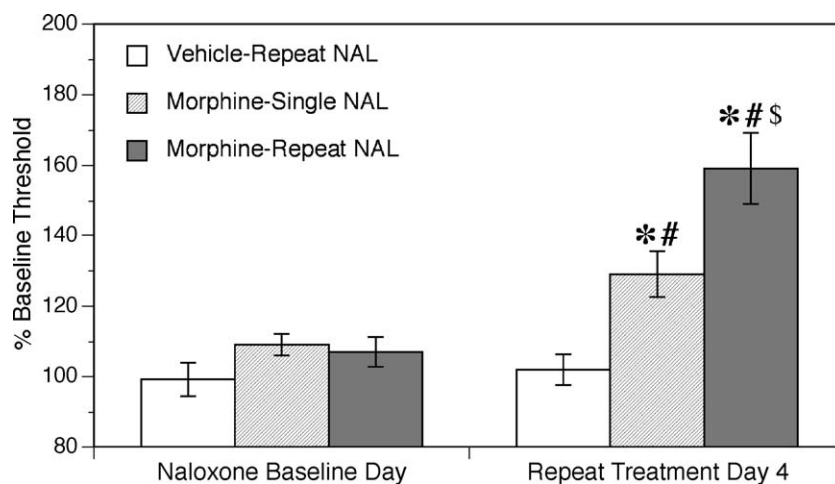


Fig. 2. Effects of 1.0 mg/kg naloxone on brain reward thresholds measured before any repeated treatment (Naloxone Baseline Day) or on Repeat Treatment Day 4, as a function of treatment condition (Vehicle–Repeat NAL, Morphine–Single NAL, Morphine–Repeat NAL). Data reflect mean ( $\pm$ S.E.M.) percent of baseline threshold measured 5 min after naloxone injection on the indicated treatment day. Note that data for Morphine–Repeat NAL are the same as those shown in Fig. 1 for the 1.0-mg/kg dose of naloxone. \* $P<.05$  vs. threshold measured in same treatment group on naloxone baseline day; # $P<.05$  vs. Vehicle–Repeat NAL group on Repeat Treatment Day 4; \$ $P<.05$  vs. Morphine–Single NAL group on Repeat Treatment Day 4.

naloxone administration. A two-factor mixed-design ANOVA with naloxone dose as the between-subjects factor and treatment day as the within-subjects factor revealed a significant main effect of naloxone dose [ $F(3,33)=15.02$ ,  $P<.0001$ ], as well as a significant main effect of treatment day [ $F(3,99)=6.47$ ,  $P<.0005$ ]. Follow-up comparisons indicated that on Days 1, 2, and 3, only the highest dose of naloxone tested (1.0 mg/kg) significantly increased reward thresholds relative to the Morphine–Vehicle group; moreover, the effect on Days 3 and 4 produced by this dose of naloxone was significantly greater than the effect on Day 1. In addition, by Day 4 doses of 0.33 and 0.10 mg/kg also significantly increased reward thresholds.

As shown in Fig. 2, the increase in reward threshold produced by 1.0 mg/kg of naloxone when administered repeatedly after morphine (Morphine–Repeat NAL) appears attributable to the interaction of morphine and naloxone, rather than a specific effect of one of the two treatments alone. An overall two-factor mixed-design ANOVA revealed a significant main effect of Morphine–Naloxone treatment (Vehicle–Repeat NAL, Morphine–Repeat NAL, Morphine–Single NAL), a significant main effect of treatment day (Naloxone Baseline Day vs. Repeated Treatment Day 4), and a significant Treatment $\times$ Day interaction (all  $F$ s  $>8.04$ ,  $P$ s  $<.002$ ). Follow-up comparisons revealed that none of the treatment groups differed from each other when naloxone was administered before onset of morphine treatment (Naloxone Baseline Day). On the final treatment day, rats receiving naloxone only after the final morphine pretreatment (Morphine–Single NAL) showed a significant increase in reward thresholds relative to the Vehicle–Repeat NAL condition. However, the threshold increase was still significantly greater when naloxone followed morphine pretreatment on all days (Morphine–Repeat NAL vs. Morphine–Single NAL).

#### 4. Discussion

Based on the current study and earlier work (Easterling and Holtzman, 1997; Easterling et al., 2000), it is clear that acute opioid dependence produced by a single treatment with a moderate dose of morphine is accompanied by brain reward deficits. For example, Easterling and Holtzman (1997) and Easterling et al. (2000) reported modest (10–20%) brain reward threshold increases produced by the opioid antagonist naltrexone after a single morphine pretreatment using autotitration and progressive ratio procedures. The current study supports and extends this earlier work using yet a third brain reward threshold procedure (discrete trial current–intensity; Kornetsky and Esposito, 1979; Markou and Koob, 1993). Herein we report roughly 35% increases in brain reward thresholds when a 1.0-mg/kg dose of naloxone was administered 4 h after a single morphine pretreatment (5.6 mg/kg sc). This reflected a specific shift of naloxone potency to increase brain reward

thresholds under conditions of acute morphine treatment, because naloxone up to 1.0 mg/kg was without effect on brain reward thresholds under Morphine–Naive conditions, even upon repeated administration (Vehicle–Repeat NAL). This is consistent with previous reports that repeated treatment with naloxone at doses as high as 16 mg/kg are without effect on brain reward thresholds (Perry et al., 1981).

Differences in the strain of rats (Sprague–Dawley vs. Wistar) or opioid antagonist (naltrexone vs. naloxone) used could account for the differing magnitude of threshold elevations noted by Easterling and Holtzman (1997), Easterling et al. (2000), and in the current study. However, an intriguing alternative explanation offered by Easterling et al. (2000) was that the reinforcement animals obtained from the remaining brain stimulation behavior in their procedures acted as a “hedonic buffer” that prevented the measurement of a more profound underlying state of reward deficit. For example, in the progressive ratio procedure (Easterling et al., 2000) naltrexone suppressed responding by as much as 60–70%, but the high baseline response rates resulted in residual response rates of 0.75/s even under most naltrexone treatment conditions. The current study demonstrated significantly greater reward deficits in a discrete-trial current–intensity threshold procedure with very low response requirements and a limited availability of reinforcement opportunities, and we suggest that the hedonic buffer may have exerted a more limited effect on thresholds in this paradigm.

The current study also demonstrated that repeated treatment with morphine and naloxone at daily intervals (morphine–repeat NAL) results in a further shift in the potency of naloxone to elevate brain reward thresholds (Fig. 1, lower panel). The increase in reward threshold produced by the highest dose of naloxone tested (1.0 mg/kg) after 4 days of daily morphine treatment was comparable to the maximal threshold elevations observed in rats made chronically dependent on morphine through pellet implantation (Schulteis et al., 1994). It must be noted, however, that lower doses of naloxone (0.03 mg/kg) are capable of eliciting this maximal response under conditions of chronic morphine dependence (Schulteis et al., 1994).

Moreover, when brain reward thresholds were determined before morphine injection on a given treatment day, and hence 23.5 h after the morphine treatment on the previous day, brain reward thresholds increased modestly (10–15%) but significantly by Treatment Day 3 (Fig. 1, upper panel). This increase was not specific to any particular naloxone treatment condition, but rather likely reflected an emerging spontaneous threshold increase evident at 23.5 h but not 4 h postmorphine. Thus, repeated treatment with a moderate dose of morphine (5.6 mg/kg) induces an emerging state of opioid dependence that is accompanied by brain reward deficits, and can be measured through either naloxone-precipitated withdrawal at 4 h postmorphine, or spontaneous withdrawal at 23.5 h postmorphine.

Comparison of the threshold elevations produced by naloxone on Treatment Day 4 in the Morphine–Single NAL and Morphine–Repeat NAL groups indicates that repeated experience with naloxone at daily intervals contributes to the shift in the naloxone dose-effect function. Indeed, the response to 1.0 mg/kg naloxone on Treatment Day 4 in the Morphine–Single NAL group was no greater than the response to this dose of naloxone seen after a single morphine pretreatment (i.e., Day 1 in Morphine–Repeat NAL group). These findings were not attributable to a direct “sensitizing” effect of repeated naloxone itself, since repeated treatment with 1.0 mg/kg of naloxone without prior morphine exposure failed to alter reward thresholds (Vehicle–Repeat NAL group). Thus, our findings are more reminiscent of similar findings with the suppression of operant responding model of acute opioid dependence, in which repeated naloxone experience in the presence of morphine pretreatment results in a greater potentiation of suppression of responding than either repeated naloxone or repeated morphine alone (Schulteis et al., 1999, 2003, 2004).

This earlier work with suppression of operant responding has indicated an apparent involvement of conditioning mechanisms early in the development of opioid dependence (e.g., Adams and Holtzman, 1990; Amitai et al., 2004; Schulteis et al., 1999, 2003, 2004). For example, repeated naloxone experience in the presence of morphine pretreatment resulted in potentiation of naloxone potency to elicit suppression of operant responding only when said repeated experience was associated with the operant context (Schulteis et al., 2004). We have argued that unique elements or cues provided by antagonist administration, which includes the injection regimen itself as well as the interoceptive drug cues, resulted in the formation of a new episodic context within an otherwise familiar operant environment. “This novel contextual representation reliably predicted the onset of an aversive motivational state of opioid withdrawal, with a corresponding shift to withdrawal-related behaviors upon subsequent exposure to this withdrawal-predictive context” (Schulteis et al., 2004). More recently (Amitai et al., 2004) we have demonstrated that a discrete tone/light stimulus in addition to context could, through repeated pairings with naloxone in acutely dependent rats, come to elicit a conditioned-withdrawal response (suppression of responding). Based on the results of the current study, we postulate that brain reward thresholds are similarly capable of becoming associated with the “withdrawal-predictive context” when the naloxone administration regimen is consistently applied 4 h postmorphine and immediately before a brain reward threshold determination session.

In summary, our current data along with that of Easterling and Holtzman (1997) and Easterling et al. (2000) clearly demonstrate that acute opioid dependence is characterized by brain reward deficits when an opioid antagonist is administered several hours postmorphine. Moreover, repeated treatment at daily intervals results in a

potentiation of the antagonist-precipitated reward deficit measured 4 h postmorphine, perhaps in part due to context-specific conditioning processes, and results in the gradual emergence of a modest spontaneous withdrawal-induced reward deficit, measured 23.5 h postmorphine. Thus, affective signs of opioid withdrawal may be experienced very early on in the development of dependence on opioids. In individuals predisposed to such rapid neuroadaptation in brain reward systems, such affective withdrawal states may therefore contribute to the transition from casual use of opioids to loss of control over use (i.e., compulsive use or addiction).

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