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Reinforcer-dependent enhancement of operant responding in opioid-withdrawn rats

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Abstract

Rationale and objective—Opioid withdrawal increases the reinforcing effectiveness of the μ -opioid agonist remifentanyl in rodents. The current study explored the selectivity of this effect by assessing operant behavior maintained by drug and non-drug reinforcers, remifentanyl, cocaine, a palatable liquid food, and standard food pellets, as a function of opioid dependence and withdrawal.

Materials and methods—Operant responding exhibited by nondependent, morphine-naïve groups was compared with responding exhibited by morphine-dependent and withdrawn groups. Dependence was established using a noncontingent morphine dosing procedure that has been previously verified to maintain dependence while allowing for daily behavioral observation during a withdrawn state. Behavior maintained by remifentanyl (0.10–10.0 μ g/kg/infusion), cocaine (0.032–1.0 mg/kg/infusion), a palatable liquid food reinforcer (3.2–100.0% Vanilla Ensure® and water), or food pellets was assessed in dependent and nondependent groups.

Results—Morphine withdrawal enhanced remifentanyl self-administration, resulting in an upward and rightward shift of the descending limb of the dose–response curve, and increased operant responding for both food reinforcers. However, opioid withdrawal did not affect cocaine self-administration, nor did it affect responding for water.

Conclusions—Enhanced operant responding observed under opioid-dependent and withdrawn conditions, while selective, is generalized to some nonopioid reinforcers.

Keywords

Self-administration; Dependence; Opioid; Morphine; Remifentanyl; Cocaine; Operant behavior; Food

Introduction

Physiological withdrawal and tolerance are hallmark characteristics of opioid dependence (American Psychiatric Association 1994), factors hypothesized to be associated directly with a pattern of increased opioid use over time among the heroin-dependent population (Goldstein 1972). The degree to which these variables contribute to escalating opioid use in humans has not been clearly elucidated. However, recent findings have demonstrated increases in the reinforcing effects of opioids in laboratory animals rendered dependent with noncontingent morphine. Importantly, this effect was contingent upon withdrawal state, with enhanced opioid self-administration emerging after behavioral expression of withdrawal was observed (Negus 2006; Cooper et al. 2008; Gerak et al. 2009). The current study was designed to investigate the extent to which this withdrawal-associated increase in operant responding is reinforcer dependent.

Opioid dependence has been postulated to augment the reinforcing effects of opioid agonists as they alleviate or postpone the opioid-withdrawal syndrome (Dole 1972; Solomon and Corbit 1974; Frenois et al. 2005), thus predicting a selective increase in opioid self-administration in a dependent organism specifically in a withdrawn state. However, in opioid-dependent nonhuman primates, increases in opioid self-administration observed during withdrawal were maintained several weeks after the behavioral manifestations of withdrawal dissipated (Gerak et al. 2009). Furthermore, administration of nonopioid agents that decreased withdrawal (10 mg/kg/day antalarmin and 0.032 mg/kg/h clonidine) did not reverse withdrawal-associated increases in the relative reinforcing effects of heroin (Negus and Rice 2009). Similarly, during protracted abstinence in opioid-dependent humans, the *N*-methyl-D-aspartate receptor antagonist memantine decreased withdrawal severity without affecting heroin self-administration (Bisaga et al. 2001; Comer and Sullivan 2007). These findings call into question (1) the significance of the negative reinforcing effects of withdrawal in contributing to the observed withdrawal-associated increases in opioid self-administration and (2) the generalizability of opioid-withdrawal increases in operant responding to stimuli that do not serve to directly decrease withdrawal (i.e., nonopioid reinforcers).

Among humans, opioid dependence and withdrawal increases the use of nonopioid drugs in specific contexts (decreased availability of heroin, increased cost of heroin, pharmacological blockade with antagonist pharmacotherapy treatment for opioid dependence) (Degenhardt et al. 2005; Raby et al. 2009), and anecdotal evidence suggests increased desire for sweet, palatable foods, such as candy, during opioid withdrawal (Kleber, unpublished observations). Similarly, there is some evidence in laboratory animals of opioid-withdrawal-associated changes in responding for nonopioid reinforcers. For instance, withdrawal increased the potency of cocaine as a reinforcer (Gerak et al. 2009) but decreased food intake and food-maintained responding for standard food chow or food pellets (Ford and Balster 1976; Steinfels and Young 1981; Langerman et al. 2001).

To further understand the effects of opioid withdrawal on operant responding for opioid and nonopioid reinforcers, behavior maintained by an opioid (remifentanyl), stimulant (cocaine), and two non-drug reinforcers (palatable liquid food and standard food pellets) was compared between opioid-dependent, withdrawn rats and nondependent rats. An intermittent morphine dosing regimen previously shown to reliably induce dependence while allowing for behavioral observations during both withdrawn and non-withdrawn states was implemented for the current study. Dependence was initiated with twice daily injections of increasing doses of morphine and was then maintained with single daily injections of morphine. Thus, withdrawal-associated behavioral changes were evaluated repeatedly in the same rat while maintaining the magnitude of dependence across observation periods. Opioid withdrawal

was reliably observed 23 (but not 12) hours after the daily maintenance morphine dose. The withdrawal was indicated by hyper-algesia, distress vocalizations, and weight loss, with no observable changes in severity of withdrawal over multiple observation periods (i.e., withdrawal episodes) (Cooper et al. 2008). This exact morphine dosing regimen was implemented to repeatedly measure operant behavior maintained by remifentanyl, cocaine, a palatable liquid food, and standard food pellets during the withdrawn state. A between-groups design was used to directly compare operant responding maintained by these reinforcers between a morphine-dependent, withdrawn group and a nondependent group. Given the recent evidence in nonhuman primates demonstrating that withdrawal-associated enhancement of opioid self-administration generalized to cocaine, a nonselective increase in operant responding was expected for all reinforcers tested in the current study.

Methods

Subjects

Experimentally naive male Sprague–Dawley rats weighing approximately 300 g prior to experimentation were obtained from Harlan Sprague Dawley (Indianapolis, IN). Standard laboratory chow (Purina, St. Louis, MO) and water were provided freely throughout the experiment. Rats were maintained on a 12-h light/dark cycle with light on at 7 a.m. The housing room was maintained at an average temperature of 21°C. All studies were carried out in accordance with the guidelines of the Committee on Care and Use of Laboratory Animal Resources, National Research Council (Department of Health, Education, and Welfare, publication No. (NIH) 85–23, 1996), and experimental protocols were approved by the University Committee on Use and Care of Animals of the University of Michigan.

Drugs

Morphine sulfate (NIDA, Rockville, MD) was dissolved in saline and administered subcutaneously in a volume of 1 ml/kg. Remifentanyl hydrochloride was purchased as Ultiva (GlaxoSmithKline) from the University of Michigan Hospital pharmacy and diluted in sterile water. Cocaine hydrochloride (NIDA, Rockville, MD) was dissolved in sterile water. Both remifentanyl and cocaine were delivered intravenously in a volume of about 35 µl.

Operant conditioning chamber configuration

Remifentanyl and cocaine were administered intravenously by attaching the intravenous catheter to a tether (Med Associates Inc, St. Albans, VT) joined to a pneumatic syringe (IITC, Woodland Hill, CA) by a swivel (Instech Laboratories Inc., Plymouth Meeting, PA) held in place by a counter-balanced arm (Med Associates Inc., St. Albans, VT). The operant conditioning chamber (30.5 cm. L×24 cm. W×21 cm. H, Med Associates Inc.) was placed in a sound-attenuating cubicle and equipped with a single nosepoke aperture (2.5 cm, Med Associates Inc.) located 6 cm above the stainless steel grid floor. The aperture was bisected by an infrared photobeam, the interruption of which sent an output signal. Response-contingent drug was delivered in a volume of approximately 100 µl/kg over 0.1 s. The pneumatic syringe and counter-balanced arm were removed from the chamber during the food self-administration experiments. A pellet dispenser (Med Associates Inc.) or a liquid dipper was placed 8.1 cm. from the nosepoke, 6 cm above the stainless steel floor. The dipper provided 50 µl of fluid, and the pellet dispenser delivered 45 mg standard laboratory pellets (PJ Noyes Company Inc., Lancaster, NH).

Surgery

For long-term intravenous drug administration, rats were surgically implanted with intravenous catheters. Rats were anesthetized with ketamine (100 mg/kg, i.m.) and xylazine

(10 mg/kg, i.m.) and implanted with intravenous catheters made of Micro-Renathane® tubing (Braintree Scientific, Inc, Braintree, MA). Briefly, a longitudinal incision was made on the inner leg, exposing the femoral vein. The catheter was inserted into the femoral vein and passed subcutaneously to an incision made between the shoulder blades. The tubing was then connected to metal tubing attached to a metal back plate (Lomir Biomedical Inc., Malone, NY) and secured subcutaneously, where the metal tubing exited the skin. Catheters were flushed daily with 0.5 ml (100 units/ml) of heparinized saline to maintain patency. Following surgery, rats were singly housed and given 1 week to recover prior to experimentation.

Experimental design: drug- and food-maintained behavior

Induction and maintenance of morphine dependence

As depicted in Fig. 1, all rats were first acclimated to the laboratory for 7 days. About 60% of the group underwent surgery for intravenous catheterization to assess remifentanyl and cocaine self-administration, and 40% were used to measure food-maintained responding and did not require catheterization. The number of rats used to measure drug self-administration was larger than those used to assess food-maintained responding in order to account for anticipated attrition due to loss of catheter patency during the experimental period. Following recovery from surgery, rats were divided into dependent and nondependent groups and further divided into groups assigned to respond for remifentanyl or cocaine (completers in dependent group for remifentanyl, $n=7$ and cocaine, $n=6$; completers in the nondependent group for remifentanyl, $n=6$ and cocaine, $n=6$). Rats used to measure food-maintained responding were divided into dependent and nondependent groups and further divided into groups assigned to respond for liquid food or food pellets (completers in dependent group for liquid food, $n=6$ and food pellets, $n=5$; completers in the nondependent group for liquid food, $n=5$ and food pellets, $n=5$). The dependent groups were treated with escalating doses of morphine twice a day for 4 days (10, 20, 30, and 40 mg/kg, s.c.) every 12 ± 2 h, a dosing regimen previously used to establish dependence in rats (Cooper et al. 2008). On subsequent days, these rats were treated with a single daily injection of 40 mg/kg morphine to maintain dependence throughout the experiment. The nondependent group was injected twice a day with saline for the first 4 days and a single saline injection the fifth day. Operant conditioning sessions commenced the morning of the sixth day after initiation of the injection schedule for all groups of animals, 22–24 h after the dependent groups received the previous morphine injection, a time point marked by opioid withdrawal. The dependent group received morphine (to maintain dependence), and the nondependent group received a saline injection approximately 30 min after the completion of the operant conditioning session.

Remifentanyl self-administration

Remifentanyl administration was contingent upon a single response (fixed-ratio 1 (FR1); no time-out). The 60-min sessions were signaled by illumination of the house light, and infusions were signaled by illumination of lights in and above the nosepoke aperture (0.1 s). During the infusion (<0.1 s), nosepoke responses were recorded but had no programmed consequences. Remifentanyl self-administration was initiated with a large dose (10.0 $\mu\text{g/kg}$ /infusion). After a pattern of stable responding was established (three sessions with less than 30% deviation from mean responding with no increasing or decreasing trend), self-administration of the next lower dose was tested (5.6 $\mu\text{g/kg}$ /infusion). Self-administered dose/injection was successively decreased (3.2, 1.0, 0.32, and 0.10 $\mu\text{g/kg}$ /infusion) once stable responding was observed at each dose. Each rat was exposed to at least five of the six doses tested.

Cocaine self-administration

Cocaine administration was delivered on an FR1, 5 s time-out (TO5) schedule of reinforcement. The 60-min sessions were signaled by illumination of the house light, and infusions were signaled by illumination of lights in and above the nosepoke aperture (0.1 s). The house light was extinguished to signal the timeout (5 s) and occurred immediately after the drug infusion (<0.1 s). This time-out period was implemented for cocaine but not for remifentanyl self-administration sessions to accommodate the relatively long half-life of cocaine [18.11 ± 3.22 min (Barbieri et al. 1992)] compared with remifentanyl [45 s (Haidar et al. 1997)]. During the infusion and time-out, nosepoke responses were recorded but had no programmed consequences. Cocaine self-administration was initiated with 0.56 or 1.0 mg/kg/infusion. The dose available for self-administration was decreased successively (0.32, 0.1, 0.032 mg/kg/infusion) when stable responding was observed. Each rat was exposed to at least four of the five doses tested.

Food-maintained responding, liquid food

Availability of liquid food was delivered on an FR1, TO5, schedule of reinforcement, with 10-s access to a dipper of liquid food (50 μ l) serving to maintain responding. The 60-min sessions were signaled by illumination of the house light, and reinforced nosepokes were signaled by illumination of lights in and above the nosepoke aperture (0.1 s). During presentation of the food dipper, a light inside the receptacle was illuminated, and nosepokes were not reinforced. The time-out was signaled by the absence of the house light. Liquid food-maintained responding was initiated with 100% Vanilla Ensure® and was decreased successively once stable behavior was observed (32%, 10%, 3.2%, and tap water).

Food-maintained responding, pellets

A single pellet was dispensed into a receptacle according to the same schedule FR1 TO5 used for liquid food-maintained responding and was accompanied by a 10-s illumination of a light in the receptacle. During this time, additional nosepokes were recorded with no programmed consequences.

Data analysis

Body weights were recorded each morning and subtracted from an individual rats' baseline weight (weight at the time of the first injection). Values from the first, eighth, and 15th days of morphine maintenance or 'withdrawal episodes' (representing the first, second, and third weeks of the experiment, the minimum amount of time required for an animal to exhibit stable responding for remifentanyl, cocaine, or food) were averaged according to day, morphine-dependent condition, and reinforcer. Nose-poke responses and drug intake for remifentanyl and cocaine self-administration were averaged across morphine-dependent, withdrawn condition and dose. Average values according to group and dose were substituted for missing values. Responding for liquid food was averaged according to morphine-dependent, withdrawn condition and concentration of Ensure®. Responding for food pellets was averaged over blocks of four consecutive sessions according to morphine-dependent condition (total of five blocks).

All statistical analyses were performed with SPSS 14.0 (SPSS Inc., Chicago IL). A mixed-model ANOVA was implemented to determine within-subject effects (day, dose of drug, concentration of food, and session block) with Huynh-Feldt correction for sphericity and between-subject effects (morphine-dependent condition) for each dependent variable (net weight gain, responding for remifentanyl, intake of remifentanyl, responding for cocaine, intake of cocaine, responding for liquid food, and responding for food pellets). When significant main effects for within- and between-subject effects were detected, pairwise

comparisons were performed using the Bonferroni adjustment for multiple comparisons to determine further differences between dependent, withdrawn groups and nondependent groups. Within- and between-subject effects and pairwise comparisons were considered statistically significant when p values were equal to or less than 0.05.

Results

Weight change

As portrayed in Fig. 2, net weight gain varied as a function of time across groups (main effect of day: remifentanyl, $F(2, 22) = 9.4$, $p \leq 0.01$; cocaine, $F(2, 20) = 3.7$, $p \leq 0.05$; liquid food, $F(2, 18) = 41.5$, $p \leq 0.0001$; food pellets, $F(2, 16) = 31.6$, $p \leq 0.0001$). Morphine-dependent, withdrawn groups lost weight, whereas the nondependent groups gained weight over the experimental period (main effect of morphine-dependent condition: remifentanyl, $F(1, 11) = 24.9$, $p \leq 0.0001$; cocaine, $F(1, 10) = 44.5$, $p \leq 0.0001$; liquid food, $F(1, 9) = 93.9$, $p \leq 0.0001$; food pellets, $F(1, 8) = 24.5$, $p \leq 0.01$). Additionally, differences in net weight gain between the treatment conditions increased over time such that nondependent groups consistently gained weight, whereas dependent, withdrawn groups either continued to lose weight or approached baseline weights (interaction between morphine-dependent condition and day: remifentanyl, $F(2, 22) = 4.9$, $p \leq 0.05$; cocaine, $F(2, 20) = 45.2$, $p \leq 0.01$; liquid food, $F(2, 18) = 9.8$, $p \leq 0.01$; food pellets, $F(2, 16) = 12.9$, $p \leq 0.01$).

Remifentanyl self-administration

Remifentanyl maintained self-administration behavior in both nondependent and dependent, withdrawn groups as depicted in Fig. 3. Operant responding varied as a function of dose such that low and high doses maintained little behavior, whereas the intermediate doses maintained a higher response rate (main effect of dose: $F(5, 55) = 37.3$, $p \leq 0.0001$). Remifentanyl self-administration differed between the nondependent and dependent, withdrawn groups (main effect of morphine-dependent condition: $F(1, 11) = 6.1$, $p \leq 0.05$). Specifically, the nondependent group maintained greater responding for the lowest dose tested compared with the dependent, withdrawn group, whereas higher rates of responding were observed in the dependent, withdrawn group for the four highest remifentanyl doses tested compared with the nondependent group. Thus, the descending limb of the dose–response curve for the dependent group was shifted to the right of the descending limb of the dose–response curve for the nondependent group (interaction between remifentanyl dose and morphine-dependent, withdrawn condition: $F(5, 55) = 5.8$, $p \leq 0.01$).

Remifentanyl intake increased with dose ($F(5, 55) = 67.7$, $p \leq 0.0001$), with greater consumption observed in the opioid-dependent, withdrawn group compared with the nondependent group (main effect of morphine-dependent condition, $F(1, 11) = 24.3$, $p \leq 0.0001$). Although intake did not differ between the groups for the two lower doses tested (0.32 and 0.10 $\mu\text{g/kg/infusion}$), a robust increase in remifentanyl intake was observed for all other doses tested in the dependent, withdrawn group compared with the nondependent group. For the highest three doses tested, intake was stable in the nondependent group, whereas remifentanyl intake at these doses was variable and ultimately increased with the highest dose tested (interaction between remifentanyl dose and morphine-dependent, withdrawn condition, $F(5, 55) = 9.6$, $p \leq 0.0001$).

Cocaine self-administration

As demonstrated in Fig. 4, cocaine maintained dose-dependent self-administration in both dependent, withdrawn and nondependent groups (main effect of cocaine dose, $F(4, 40) = 6.7$, $p \leq 0.01$) and did not vary as a function of morphine-dependent, withdrawn condition. The highest dose tested (1.0 mg/kg/infusion) maintained the least amount of behavior

compared with the intermediate doses (0.32 and 0.1 mg/kg/infusion). There was substantial variability in the number of responses maintained by the lowest dose per injection of cocaine (0.032 mg/kg/infusion) in both groups. Cocaine intake increased with self-administered dose ($F(4, 40) = 26.6, p \leq 0.0001$) and did not vary as a function of morphine-dependent, withdrawn condition or interaction between morphine withdrawal and cocaine dose.

Liquid food-maintained responding

As shown in Fig. 5, palatable liquid food maintained operant responding in a concentration-dependent manner in both dependent, withdrawn and nondependent groups resulting in a monotonic increasing function for increasing concentrations of liquid food (main effect of concentration, $F(4, 36) = 73.9, p \leq 0.0001$). The dependent, withdrawn group exhibited greater responding for the liquid food compared with the nondependent group ($F(1, 9) = 25.6, p \leq 0.001$) for every concentration evaluated with the exception of water. An interaction between concentration and treatment condition ($F(4, 36) = 10.4, p \leq 0.01$) indicated that both treatment and concentration of food contributed to the differences observed in responding.

Food pellet-maintained responding

Figure 6 depicts response rates for food pellets as a function of opioid dependence and session block. While the entire nondependent group acquired responding for the food pellets, one rat from the deprived, withdrawn group failed to respond for food pellets (data from the one rat that did not respond for pellets were omitted from analysis). Responding for food pellets increased over sessions in both groups (main effect of session block, $F(4, 28) = 12.2, p \leq 0.0001$), with greater responding observed in the morphine-dependent, withdrawn group compared with the nondependent group (main effect of morphine-dependent condition, $F(1, 7) = 8.5, p \leq 0.05$). An interaction effect between session block and morphine-dependent, withdrawn condition on responding maintained by food pellets was not detected.

Discussion

The experiments described herein were designed to investigate the effects of opioid withdrawal on operant behavior maintained by an opioid (remifentanyl), a stimulant (cocaine), a palatable liquid food, and standard food pellets. The between-groups design allowed for the comparison of behavior maintained by these reinforcers while controlling, to some extent, for operant conditioning and drug histories under morphine-dependent and nondependent conditions. For example, all reinforcers maintained behavior in both groups of rats. However, morphine withdrawal enhanced high-dose remifentanyl self-administration and responding for the two distinct food reinforcers while having no effect on cocaine reinforcement. These findings complement and extend previous reports of withdrawal-associated increases in opioid-maintained responding in nonhuman primates and rats and demonstrate that, though these increases are not selective for opioid agonists, they are reinforcer dependent.

The robust increase in remifentanyl intake observed under morphine-withdrawn conditions for the moderate and high doses may indicate tolerance (loss of potency) to the rate-suppressing effects of remifentanyl, which may in part explain the enhanced responding observed for the higher doses relative to the nondependent condition. Additionally, although intake did not differ as a function of dependence for the lowest dose of remifentanyl tested, decreased responding for this dose was observed in the dependent group relative to the nondependent group, suggesting tolerance to remifentanyl's reinforcing effects. Behavioral cross-tolerance to opioid agonist effects following acute or repeated exposure to an agonist has been well established. However, tolerance is usually observed with agonists of equal or lower efficacy than the agonist initially administered. For example, monkeys treated

repeatedly with daily morphine exhibited tolerance to the reinforcing effects of the lower-efficacy agonists, nalbuphine and buprenorphine, but not to the higher efficacy agonist, alfentanil (Winger and Woods 2001). Similarly, rats treated daily with small doses of the low-efficacy agonist butorphanol (3.0 mg/kg) exhibited tolerance to the rate-suppressing effects of low-efficacy agonists (buprenorphine and butorphanol) but not to mid- and high-efficacy agonists (morphine, fentanyl, and sufentanil). By increasing the daily butorphanol dose by tenfold, tolerance to the rate-suppressing effects of both low- and high-efficacy agonists was observed (Smith and Picker 1998). Thus, it is plausible that morphine-induced behavioral cross-tolerance to both the rate-suppressing and reinforcing effects of remifentanil accounts for some of the observed differences in remifentanil self-administration between the two groups.

In a previous study, remifentanil self-administration was compared among five pairs of morphine-dependent and nondependent groups using the morphine dosing schedule described in the current study. For 20 consecutive sessions, each pair of groups self-administered a single dose of remifentanil (0.4, 0.8, 1.6, 3.2, or 6.4 $\mu\text{g/kg/infusion}$) (Cooper et al. 2008). In the current study, remifentanil self-administration was initiated with the highest dose (10 $\mu\text{g/kg/infusion}$) and decreased incrementally, such that each rat was exposed to at least five remifentanil doses in a successively decreasing order. Relative to previous findings, the method employed in the current study produced a wider range of reinforcing doses, with lower doses of remifentanil maintaining operant responding compared with the previous report (0.10 $\mu\text{g/kg/infusion}$ vs 0.4 $\mu\text{g/kg/infusion}$). Additionally, maximum responding for remifentanil was about 30% higher in both dependent and nondependent groups in the current study relative to the previous report. Greater responding maintained by lower remifentanil doses observed in the current study relative to the previous findings may reflect the enhanced conditioned reinforcing effects of the stimuli associated with remifentanil infusions. Two factors that likely contributed to the hypothesized increase in the conditioned reinforcing effectiveness of these stimuli under the current method are (1) the association of the stimuli with a primary reinforcer of larger magnitude (dose) and (2) increased exposure to pairings of the stimuli and primary reinforcer (Kelleher and Gollub 1962). Although the evaluation of remifentanil self-administration differed across the two studies, withdrawal-associated increases in the reinforcing effects of remifentanil were conserved. In both studies, deprivation shifted the dose that maintained peak responding to the right and increased behavioral output maintained by all doses that fell on the descending portion of the dose-response curve relative to the nondependent group. The preservation of this effect demonstrates the robust nature of morphine deprivation-induced increases in remifentanil self-administration across procedural variation.

The similarity in cocaine-maintained responding and intake between the dependent and nondependent groups was somewhat unexpected given the evidence that opioid history augments some of cocaine's behavioral effects. For example, morphine treatment for five consecutive days increased the positive conditioning effects of cocaine, as measured in a conditioned place preference model in rats (Shippenberg and Heidbreder 1995). History of opioid dependence increased cocaine reinforcement as determined with a progressive-ratio schedule in rats 4 days after discontinuation of a constant, 1-week infusion of morphine; however, responding on an FR1 schedule of reinforcement for cocaine (0.32 mg/kg/infusion) during the first 3 days after discontinuation of morphine administration was unaffected (He and Grasing 2004), findings that are consistent with the current results. The findings from the current study are most surprising given the recent reports of increased potency of cocaine's reinforcing effects in opioid-dependent and withdrawn monkeys (Gerak et al. 2009). The discrepancy between these results is likely due to the divergent methods used between the two studies. The most fundamental difference between the studies

was the within-subject design used in the previous report; the same animals exhibited increased heroin self-administration during withdrawn states. Because the discriminative stimuli used for both heroin and cocaine self-administration were identical, the change in cocaine self-administration observed under withdrawn conditions may be, in part, due to the conditioned stimulus effects associated with heroin infusions. The current study used experimentally naive animals and different groups to assess responding for each reinforcer. Therefore, operant experience (history of contingencies and exposure to discriminative stimuli) associated with primary reinforcers that maintain different response topographies based on state of dependence (heroin, remifentanyl) may be necessary to observe withdrawal-associated increases in cocaine's reinforcing effects.

Morphine withdrawal produced an upward and leftward shift of the concentration–response function for behavior maintained by the palatable liquid food but did not affect behavior maintained by water. Thus, the withdrawal-associated increase in liquid food-maintained behavior observed under deprived conditions was not due to thirst but rather due to the nutritive and palatability qualities of the liquid food. The observed withdrawal-associated increase in food pellet-maintained responding is consistent with this interpretation. These findings are quite different from the large body of literature indicating that opioid deprivation decreases both food consumption and schedule-controlled behavior maintained by food presentation (e.g., Ford and Balster 1976; Langerman et al. 2001; Houshyar et al. 2004) and therefore raise questions regarding these seemingly incompatible, opposing effects of opioid withdrawal on food-maintained responding observed in the current study. Although food was provided ad libitum in the home cage, the dependent groups consistently lost weight relative to the nondependent groups across the experimental period. Food and water intake in the home cage was not recorded throughout this study; therefore, decreased caloric intake as a function of morphine dependence under the current morphine dosing regimen cannot be verified. However, under other dosing regimens, morphine intoxication has been documented to suppress feeding behavior (Langerman et al. 2001; Ford and Balster 1976), and weight loss in morphine-withdrawn rats was verified to be a function of decreased caloric efficiency, defined as body weight gained (grams)/food intake (calories) (Houshyar et al. 2003). Thus, the enhanced food-maintained responding observed in the dependent group suggests a means to compensate for the caloric deficit and decreased caloric efficiency. Indeed, morphine deprivation-induced withdrawal has been shown to initially decrease food-maintained behavior, followed by responding that exceeded baseline (non-intoxicated and non-deprived) values (Ford and Balster 1976; Babbini 1976).

That opioid withdrawal increased responding for remifentanyl and both food reinforcers but not for cocaine demonstrates that the effect was reinforcer dependent. The most appealing explanation for this effect is that, though all three reinforcers directly stimulate the opioid system, the neurobiological factors that contribute to food and remifentanyl reinforcement are more closely related than those that contribute to cocaine reinforcement. As of yet, there is no one study demonstrating direct evidence for a neurobiological component essential for both food and opioid reinforcement that is not required for cocaine-maintained behavior. However, a recent study reported that, while rates of responding for remifentanyl and food were similar between mice lacking the dopamine D1 receptor and wild types, knock-out mice failed to self-administer cocaine, thus providing evidence for divergent mechanisms regulating cocaine reinforcement compared with opioid and food reinforcement (Caine et al. 2007). A second hypothesis for reinforcer-selective increases in operant behavior as a function of opioid withdrawal relates to the deprivation state of the dependent group. The weight loss observed over the experimental period in the dependent group in conjunction with reports that morphine intoxication directly suppresses feeding behavior (Babbini et al. 1976; Ford and Balster 1976; Houshyar et al. 2003) suggest that this group was likely food deprived. Thus, opioid dependence and withdrawal resulted in deprived states (food and

morphine deprivation), which elicited increases in behavior maintained by reinforcers that effectively eliminated the deprivation state (liquid food, food pellets, and remifentanyl). Given that opioid dependence does not render the animal in a state of deprivation that can be directly reversed by stimulant administration, the similarity in cocaine self-administration observed between the experimental and control groups is not surprising.

Limitations

The conclusions from this study are limited to the FR1 schedule of reinforcement that was used as the contingency for all reinforcers tested. Under this schedule, it cannot be concluded how withdrawal-associated increases in remifentanyl self-administration reflects changes in the reinforcing efficacy of remifentanyl considering that behavioral tolerance to the rate-suppressing effects of the reinforcer likely contributed to the enhancement. Because direct drug effects affect behavior prominently under simple schedules of reinforcement (Seiden and Dykstra 1977), assessing operant behavior using a progressive-ratio schedule of reinforcement or observing behavior with contingencies that allow for behavioral economic analysis would further clarify how opioid dependence and withdrawal alters the reinforcing effectiveness of reinforcers tested in this study. Furthermore, assessing choice between reinforcers would determine withdrawal-associated changes in the relative reinforcing value of opioids, cocaine, and food. For instance, although withdrawal increased operant responding for food in the current study, opioid-withdrawn monkeys prefer an opioid agonist to a food reinforcer in choice paradigms (Spragg 1940; Negus 2006). However, free access to food during operant conditioning sessions blunted the increased reinforcing effects of heroin observed in rats with extended heroin self-administration history, suggesting the substitutability of food for opioids under certain conditions (Lenoir and Ahmed 2008). Importantly, because operant behavior was only compared between morphine-naïve, nondependent groups and morphine-dependent, withdrawn groups, the effects of opioid dependence and withdrawal on operant responding cannot be dissociated. Finally, recent evidence has demonstrated that food deprivation alters neurobiological substrates that contribute to cocaine self-administration (Collins et al. 2008) and may extend to the reinforcing effects of remifentanyl and food. Thus, the neurobiological consequences of decreased caloric intake and weight loss may have impacted operant behavior in the dependent group. Controlling for caloric intake in dependent and nondependent groups would help determine to what extent the continued weight loss, independent of chronic morphine exposure, may have been a contributing factor to the observed differences in behavior between the two groups.

Conclusion

The results from the current study indicate that the withdrawal-associated increase in operant responding observed formerly for opioid agonists is generalized to selective reinforcers. The evidence suggesting that many of the reinforcers tested in this study decrease withdrawal directly (remifentanyl) or indirectly [cocaine (Hermann et al. 2005; Kosten and Kosten 1989; Kosten 1990) and sweetened food (Blass et al. 1991; Jain et al. 2004; Reboucas et al. 2004)] did not predict withdrawal-associated increases in operant responding; these increases were not observed for cocaine but were observed for standard, unsweetened food pellets. Therefore, these findings add to the current literature challenging the role of negative reinforcement in withdrawal-associated enhancement of operant responding for select reinforcers.

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Fig. 1.
Flowchart illustrating experimental design. Rats were divided into eight groups, with one pair of morphine-dependent and nondependent groups tested for each reinforcer

Fig. 2. Net weight gain ($\pm$ SEM) in dependent and nondependent groups responding for each reinforcer. Differences in net weight gain between groups during each week as determined by post hoc analysis is represented as follows: * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$

Fig. 3.

Average ($\pm$ SEM) responding for (a) and intake of (b) remifentanil in dependent and nondependent groups. Differences in response number and intake between groups for each dose as determined by post hoc analysis is represented as follows: * $p \leq 0.05$ and ** $p \leq 0.01$

Fig. 4. Average responding for (a) and intake of (b) cocaine in dependent and nondependent groups

Fig. 5.

Average responding ($\pm$ SEM) for liquid food in dependent and nondependent groups according to concentration of liquid food. Differences in response number between groups for each concentration as determined by post hoc analysis is represented as follows:

* $p \leq 0.05$; *** $p \leq 0.001$

Fig. 6.

Average responding ($\pm$ SEM) for food pellets in dependent and nondependent groups according to session block. Differences in response number between groups during each session block as determined by post hoc analysis is represented as follows: $*p \leq 0.05$