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Morphine Deprivation Increases Self-Administration of the Fast- and Short-Acting μ -Opioid Receptor Agonist Remifentanyl in the Rat

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Abstract

Opiate dependence and withdrawal have long been hypothesized to enhance the reinforcing effects of opiates; however, opiate agonist self-administration in these states has yet to be systematically assessed. To address this issue, the reinforcing property of the short-acting μ -opioid agonist, remifentanyl, was assessed in morphine-dependent (MD), morphine-dependent and -withdrawn (MW), and nondependent, control (C) rats. Dependence was established by twice daily administration of increasing doses of morphine for 4 days (10, 20, 30, and 40 mg/kg s.c.) and then maintained with a daily injection of the large dose. Morphine deprivation-induced withdrawal (defined by weight loss and hyperalgesia) was apparent 24, but not 12, h after morphine treatment. Remifentanyl self-administration (0.4, 0.8, 1.6, 3.2, or 6.4 μ g/kg/infusion) was assessed over 20 successive, daily, 1-h sessions, either 12 or 24 h after the maintenance dose of morphine. Compared with the control group, the MD group demonstrated suppressed remifentanyl self-administration, whereas the MW group exhibited enhanced responding for every dose of remifentanyl. The increased responding observed in the MW group compared with the control and MD groups resulted in an upward shift in the remifentanyl dose-response curve, an effect that was expressed only after repeated exposure to the contingency, demonstrating that morphine withdrawal ultimately enhances the reinforcing effects of remifentanyl.

μ -Opiate agonists are abused by humans, are reinforcing in nonhuman primates and rodents, and can produce physiologic dependence upon repeated administration. The withdrawal syndrome that occurs upon termination of chronic drug taking and/or administration is reportedly aversive and is alleviated by further opiate administration. Although the reinforcing effects of opiates have been clearly established in the absence of observable dependence (for review, see Woods and Schuster, 1971), some have hypothesized that continued opioid abuse and development of opiate addiction is primarily because of the termination of the aversive withdrawal state (e.g., Solomon and Corbit, 1974; Koob and Le Moal, 2001; Colpaert et al., 2006). An alternative and conceptually appealing notion is to argue that withdrawal enhances the positive-reinforcing effects of μ -opioid agonists, with repeated exposure to opiate self-administration in the presence of the withdrawn state to be vital to the observable enhancement (Hutcheson et al., 2001).

Although evidence exists that dependence enhances opiate self-administration, the conditions under which the reinforcing effects of opioids change in the withdrawn state have

not been explored extensively. Variables that potentially affect the observed enhancement include strength of dependence, withdrawal state, and reinforcement history in the presence of these states. To assess directly the extent to which these variables contribute to the observed enhancement, opiate-maintained responding in dependent (experimental) and nondependent (control) subjects was compared in situations in which the state of dependence and consequent withdrawal was “held constant” during acquisition and maintenance of opiate self-administration.

One challenge that faces such an investigation is to reduce the impact of the opioid self-administration on the level of dependence in both the dependent and control groups. Dependence has been reported to occur with the self-administration of long-acting agonists, e.g., heroin or morphine (Woods et al., 1973; Naruse and Asami, 1987; Dai et al., 1989; Kenny et al., 2006), high doses of agonists (Dai et al., 1989), and under extended access conditions (Chen et al., 2006; Kenny et al., 2006) in both rodents and nonhuman primates. Thus, acquisition of opioid self-administration occurs in a non-dependent state but continues into a “developing” dependent state. The effect of self-induced dependence can be reduced markedly by limiting access to the opioid and/or by using a very short-acting opioid (Dai et al., 1989; Kenny et al., 2006). Remifentanyl, a fast- and short-acting μ -opioid agonist, is reinforcing in rodents (Panlilio and Schindler, 2000; Panlilio et al., 2003) and monkeys (Ko et al., 2002; Woods and Winger, 2002; Winger et al., 2006) and is hypothesized to not induce dependence because of its short duration of action ($t_{1/2}$ of 42 s when administered i.v. in the rat; Haidar et al., 1997). Remifentanyl is thus an interesting reinforcer to use when investigating differences in opiate-maintained responding in dependent versus nondependent subjects.

Understanding how opiate self-administration changes as a function of dependence, deprivation, and reinforcement history in the presence of these states requires multiple exposures to the contingency. Given the evidence that repeated exposure to both μ -opioid agonist administration (Bläsing et al., 1973; Bhargava, 1977) and antagonist-precipitated morphine withdrawal (Harris et al., 2004) increases the apparent strength of dependence and withdrawal severity, the possibility that the strength of dependence would not be maintained across observation periods (i.e., self-administration sessions) in the currently described study was a concern. Thus, it was important to develop and characterize a morphine dosing regimen that maintained a stable level of opiate dependence while allowing for repeated behavioral observations during the deprived state.

To determine the effects of opiate dependence on the reinforcing effects of remifentanyl, dependence was initiated using an intermittent, noncontingent morphine dosing paradigm previously shown to produce dependence in the rat (Houshyar et al., 2003). After 4 days of twice daily administration of escalating doses of morphine, dependence was maintained for 20 days by single daily injections of morphine. Behavioral and physiologic markers of deprivation, including hyperalgesia (Kayan et al., 1971; Tilson et al., 1973), decreased exploration (Schulteis et al., 1998; Espejo et al., 2001), and weight loss (Gellert and Holtzman, 1978; Houshyar et al., 2001, 2003), were assessed 12 and 24 h after morphine administration. These measures were used to verify dependence, to estimate the time after the morphine injections at which withdrawal occurred, and to assess the severity of withdrawal over many episodes.

Effects of morphine dependence on remifentanyl self-administration were assessed under morphine-dependent, nondeprived (MD) and withdrawn (MW) conditions by investigating responding maintained by a range of doses of remifentanyl (0.4, 0.8, 1.6, 3.2, or 6.4 μ g/kg/infusion; each dose studied in a separate group of rats) over 20 daily sessions and compared with self-administration behavior of a nondependent, control (C) group. Thus, remifentanyl

self-administration was assessed as a function of dependence and deprivation, dose of remifentanyl, and reinforcement history (i.e., session number).

Materials and Methods

Drugs

Morphine sulfate (National Institute on Drug Abuse, Rockville, MD) was dissolved in saline and administered s.c. in a volume of 1 ml/kg. Remifentanyl hydrochloride was purchased as Ultiva (Glaxo-SmithKline, Uxbridge, Middlesex, UK) from the University of Michigan Hospital pharmacy and was diluted in sterile water and i.v. self-administered in a volume of 100 μ l/kg. Acetic acid was diluted in sterile water and administered i.p. in a volume of 1 ml/kg.

Subjects

Male Sprague-Dawley rats weighing ~300 g before experimentation were maintained on a 12-h light/dark cycle with light on at 7:00 A.M. at an average temperature of 21°C. Standard laboratory chow and water were provided freely throughout the experiment. All studies were carried out in accordance with the Guide for Care and Use of Laboratory Animals as adopted by the National Institutes of Health. Experimental protocols were approved by the University of Michigan University Committee on the Use and Care of Animals.

Morphine Dosing Procedure and Evaluation of Dependence

One week after arrival, three groups of rats were treated with escalating doses of morphine twice daily every 12 h (± 2 h) for 4 days (10, 20, 30, and 40 mg/kg s.c. two times per day) a dosing regimen shown to establish dependence in rats (Houshyar et al., 2003). The first group was evaluated for deprivation-induced withdrawal on the morning of the 5th day, 12 h after the final morphine injection. The second group was treated with an additional dose of 40 mg/kg morphine on the 5th morning, given saline (1 ml/kg s.c.) that evening, and observed on the 6th morning, 24 h after the final morphine injection. To determine whether strength of dependence changed as a function of repeated exposures to morphine deprivation, a third group of rats was treated identically to the first two groups; however, this group was maintained on a single A.M. injection of 40 mg/kg morphine and saline injection in the evening for an additional 20 days and was observed on the morning of the 25th day (24 h after the last morphine injection). An additional group of rats was treated with saline twice daily and observed on the morning of the 5th day, 12 h after the final saline injection.

Acetic Acid-Induced Writhing—To determine the time course of withdrawal after morphine treatment, acetic acid-induced writhing was monitored 12 h after morphine treatment on the 5th day of the morphine dosing regimen ($n = 5$ –6 for each acetic acid concentration), 24 h after morphine treatment on the 6th day of the dosing regimen ($n = 5$ –8 for each acetic acid concentration), and 24 h after morphine treatment on the 25th day of the dosing regimen ($n = 5$ –6 for each acetic acid concentration). The nondependent group was monitored for acetic acid-induced writhing 12 h after saline treatment on the 5th day of the dosing regimen ($n = 5$ –6 for each acetic acid concentration). Rats received injections i.p. with acetic acid (0, 0.32, 1.0, or 3.2% in a volume of 1 ml/kg) and placed in an observation chamber. Vocalizations during injections were recorded. Writhes, characterized as abdominal stretching (for reference, see Giamberardino et al., 1995), were observed 15 min after acetic acid treatment for 30 min. Number of writhes and total time spent writhing (measured to the 10th of a second using a stopwatch) during the observation period were recorded. Rears during the session were also counted.

Weight Change—Weight change was determined from rats that were used for the self-administration studies. Every 12 h (± 2 h), rats were weighed before injections (either saline or morphine). Rats were given morphine as described above, with two groups receiving escalating doses of morphine twice daily for 4 days. On the 5th day, one group was given saline in the morning and the highest dose of morphine (40 mg/kg) in the evening for 20 days. The second group continued to receive the highest dose of morphine (40 mg/kg) in the mornings and saline in the evenings for 21 days. The last group received saline every 12 h. Recorded weights from the experimental period (up to 26 days) were subtracted from baseline weights for individual rats.

Remifentanyl Self-Administration

Intravenous Catheterization—For long-term i.v. drug administration, rats were anesthetized with ketamine (100 mg/kg i.m.) and xylazine (10 mg/kg i.m.) and surgically implanted with i.v. catheters made of Micro-Renathane tubing (Braintree Scientific, Inc., Braintree, MA). In brief, a longitudinal incision was made on the inner leg, exposing the femoral vein. The catheter was inserted into the femoral vein and passed s.c. to an incision made between the shoulder blades. The tubing was connected to metal tubing attached to a metal back plate (Lomir Biomedical Inc., Malone, NY) that was secured s.c. where the metal tubing exited the skin (Baird et al., 2000). Catheters were flushed daily with 0.5 ml (100 U/ml) of heparinized saline to maintain patency. After surgery, rats were singly housed and fed standard rat chow (Purina, St. Louis, MO) and water ad libitum.

After a 1-week recovery period, rats were treated with morphine (MD and MW groups) or saline (C group) as described previously. The MD group was allowed to self-administer remifentanyl on the 5th day of the morphine dosing schedule, 12 ± 2 h after the last morphine injection ($n = 6$ per dose of remifentanyl). Approximately 15 min after the experimental session, rats were weighed and received injections with saline. This group was treated with morphine (40 mg/kg) each evening, 12 ± 2 h before the next session. The MW group first self-administered remifentanyl on the 6th morning of the dosing schedule, 24 ± 2 h after the last morphine injection ($n = 5$ –6 per dose of remifentanyl). This group was treated with morphine (40 mg/kg) ~15 min after the self-administration session. Each evening, these rats were treated with saline. The nondependent group ($n = 6$ per dose of remifentanyl) was allowed to self-administer remifentanyl beginning on the 5th morning, 12 h after a saline injection. Saline was then administered ~15 min after the session and each evening.

Self-Administration Apparatus—Drug was administered i.v. by attaching the i.v. catheter to a tether (MED Associates, St. Albans, VT) joined to a pneumatic syringe (IITC, Woodland Hills, CA) by a swivel (Instech Laboratories Inc., Plymouth Meeting, PA) held in place by a counter-balanced arm (MED Associates). The operant chamber (12.0-inch length $\times$ 9.5-inch width $\times$ 8.25-inch height; MED Associates) was placed in a sound-attenuating cubicle and equipped with a single nose poke (2.5 cm; MED Associates) located 6 cm above the stainless steel grid floor. The aperture was bisected by an infrared photo beam that, when broken, sent an output signal to the computer. Response-contingent drug was delivered in a volume of 35 μ l over 0.1 s.

Self-Administration Procedure—Remifentanyl administration was contingent upon a fixed-ratio 1, no time-out schedule of reinforcement, a schedule that has been shown previously to maintain remifentanyl self-administration in rats (Panlilio and Schindler, 2000). The beginning of the 60-min session was signaled by illumination of the house light, and infusions were signaled by illumination of lights in and above the nose poke. Rats were allowed to self-administer a single dose of remifentanyl (0.4, 0.8, 1.6, 3.2, or 6.4 μ g/kg/

infusion) for 20 daily sessions. Some rats were not able to complete the study because of loss of catheter patency or illness, and data from these rats were omitted.

Data Analysis—To determine differences in acetic acid-induced writhing behavior (number of writhes and duration), a two-way ANOVA was used to assess main effects of morphine treatment and concentration of acetic acid. Differences according to concentration were assessed using Bonferroni's post hoc analysis. Vocalizations and rearing were analyzed similarly. Changes in weight from baseline were averaged, and S.E.M. values were derived for control, MD, and MW groups. A two-way ANOVA with Bonferroni's post hoc analysis was used to determine the main effects of morphine dependence and time on weight change.

Average responses (with S.E.M.) for each treatment condition (MD, MW, and C) for each dose of remifentanyl were determined according to self-administration session and dose of remifentanyl. To determine the interactions and main effects of treatment condition, remifentanyl dose, and session number on responding, a repeated-measures multivariate analysis of variance was applied determining significant effects with Pillai's Trace (Coombs et al., 1996). Bonferroni's post hoc analyses were implemented to determine at what remifentanyl dose, session, and treatment group differences occurred. To assess the main effects of remifentanyl dose and treatment condition on remifentanyl self-administration and intake during the final (20th) session, a two-way ANOVA with Bonferroni's post hoc analysis was implemented (SPSS 14.0; SPSS Inc., Chicago IL).

To assess how dose-response functions changed across sessions, individual dose-response curves for remifentanyl self-administration were constructed for each session (1–20) for each treatment condition (MD, MW, and C). Areas under the curve (GraphPad Prism; Graph-Pad Software Inc., San Diego, CA) for each dose-response curve (session $\times$ deprivation condition) were calculated. A repeated-measures two-way ANOVA, using the Greenhouse-Geisser correction for within-subject analysis, and Bonferroni's post hoc analysis were then implemented to determine main effects of deprivation and session number on area under the curve.

Quarter-life, time to complete the first 25% of responses, was evaluated to determine differences between the control and MW groups in pattern of intrasession responding. This measure reflects consistency of self-injection responding through individual sessions. For each rat, latency to make the first response was subtracted from the time it took to complete 25% of the total responses (x) and from the total session time (y). Quarter-life was then calculated as x/y and then averaged, according to dose of remifentanyl and dependence condition.

Results

Characterizing Morphine Dependence and Deprivation

No differences in writhing behavior, vocalization, or rearing were apparent in the two MW groups (1st and 20th episodes); therefore, the data for these groups were pooled for statistical purposes. Data from the two groups are noted in Tables 1 and 2.

Acetic Acid-Induced Writhing: Total Writhes and Duration of Writhes—Number of writhes increased with increasing concentration of acid [$F(3,82) = 24.7, p \leq 0.001$] (Table 1). Furthermore, number of writhes varied as a function of treatment condition [$F(2,82) = 13.4, p \leq 0.001$], such that the MD group demonstrated significantly less writhing than the control ($p \leq 0.01$) and MW ($p \leq 0.001$) groups. For the highest concentration of acetic acid, the MW group exhibited significantly fewer writhes than the control group ($p \leq 0.05$). Total

number of acetic acid-induced writhes varied as a function of an interaction between the acetic acid concentration and treatment condition [$F(2,82) = 4.2, p \leq 0.01$].

Duration of writhes increased with increasing concentrations of acetic acid [$F(3,82) = 64.8, p \leq 0.001$] and differed among treatment conditions [$F(2,82) = 82.2, p \leq 0.001$] (Table 1). Post hoc analysis revealed that duration of time spent writhing differed among all three groups, with the MW group exhibiting a longer duration of writhing compared with the control and MD groups ($p \leq 0.001$) and the MD group exhibiting shorter writhing duration than the control group ($p \leq 0.05$). Duration of time spent writhing varied as a function of an interaction between concentration of acetic acid and treatment condition [$F(5,82) = 13, p \leq 0.001$].

Vocalizations—Vocalizations after i.p. injections of sterile water or acetic acid were dependent upon treatment condition [$F(2,82) = 12.4, p \leq 0.001$] and acetic acid concentration [$F(3,82) = 4.3, p \leq 0.01$] (Table 2). Control and MD groups vocalized less than the MW group ($p \leq 0.01$).

Rearing—Rearing varied as a function of treatment condition [$F(2,82) = 17.1, p \leq 0.001$], acetic acid concentration [$F(3,82) = 6.7, p \leq 0.001$], and the interaction of these two variables [$F(5,82) = 3.4, p \leq 0.01$] (Table 2). The control group reared more than both the MD and MW groups ($p \leq 0.001$), demonstrating decreased exploratory behavior in both dependent groups.

Weight Change—Over the 20 experimental sessions, body weight continued to increase in the control group, whereas weight fluctuated in groups treated with morphine (Fig. 1). Weight loss was observed 12 to 24 h after the maintenance dose of morphine, and weight gain occurred 0 to 12 h after morphine treatment. The net effect was that control rats gained 77.5 ± 10.2 g over the 20 experimental sessions, whereas the dependent rats did not (0.8 ± 8.7 g). Thus, the morphine dosing regimen produced a daily weight change, which fluctuated according to the time morphine was administered, and a longer term effect that resulted in an overall, cumulative weight deficit.

To determine whether robust remifentanyl self-administration affected weight gain in the control group, weights were compared between the control group allowed to self-administer a dose that maintained robust responding ($0.8 \mu\text{g/kg/infusion}$) and the control group allowed to self-administer a dose of remifentanyl that maintained insignificant self-administration ($0.4 \mu\text{g/kg}$, results discussed in the following section) (Fig. 1). After the 20th session, no significant difference in weight was apparent between the control groups that were allowed to respond for $0.4 \mu\text{g/kg/infusion}$ (68.8 ± 8.2 g) and control rats responding for $0.8 \mu\text{g/kg/infusion}$ (82.7 ± 15.0 g), demonstrating that robust remifentanyl self-administration of $0.8 \mu\text{g/kg}$ had no effect on weight gain.

Effects of Morphine Dependence and Deprivation on Remifentanyl Self-Administration

Overall Effects of Session Number, Remifentanyl Dose, and Treatment Group on Remifentanyl Self-Administration—Univariate analysis revealed main effects of session number [$F(19,51) = 9.8, p \leq 0.001$], remifentanyl dose [$F(4,69) = 7.7, p \leq 0.001$], and treatment condition [$F(2,69) = 13.6, p \leq 0.001$] (Fig. 2). Remifentanyl self-administration was also observed to vary as a function of the two-way interactions between session and remifentanyl dose [$F(76,216) = 1.6, p \leq 0.01$] and between session and treatment condition [$F(38,104) = 2.1, p \leq 0.01$]. Finally, multivariate analysis demonstrated remifentanyl self-administration to vary as a function of a three-way interaction among session number, remifentanyl dose, and treatment condition [$F(133,399) = 1.5, p \leq 0.01$].

Thus, remifentanyl self-administration increased with session number and varied with remifentanyl dose, such that high and low doses of remifentanyl maintained little behavior, and intermediate doses maintained larger amounts of behavior. In addition, MW animals responded more than the control ($p \leq 0.05$) and MD ($p \leq 0.001$) groups, and the MD group responded less than the control group ($p \leq 0.05$).

The lowest dose of remifentanyl self-administered by the MW group (0.4 $\mu\text{g/kg/infusion}$) did not maintain responding for the first 10 sessions; however, some responding was maintained during the final 10 sessions. This dose of remifentanyl maintained no responding in the control group (Fig. 2a). The lowest dose of remifentanyl that maintained responding in the control group (0.8 $\mu\text{g/kg/infusion}$) did not maintain responding in the MD group but maintained robust responding in the MW group. Responding seemed to plateau by the 9th session for the control group, with some variability across sessions, whereas responding observed in the MW group was variable throughout the 20 sessions, ultimately resulting in twice as much responding as observed in the control group (193.5 ± 51.3 versus 89.0 ± 27.2 responses) (Fig. 2b). This increase in responding across the 20 sessions was observed for all doses tested in the MW group (1.6 $\mu\text{g/kg/infusion}$, 88.0 ± 18.0 versus 188.8 ± 38 responses; 3.2 $\mu\text{g/kg/infusion}$, 134.6 ± 45.6 versus 242.0 ± 53.6 responses; 6.4 $\mu\text{g/kg/infusion}$ 55.0 ± 17.8 versus 95.3 ± 13.3 responses). The MD group exhibited suppressed responding compared with the control and MW group for the two highest doses of remifentanyl tested on the 10th session (3.2 $\mu\text{g/kg/infusion}$, 45.0 ± 15.1 ; 6.4 $\mu\text{g/kg/infusion}$, 20.3 ± 13.7). However, similar to the MW group, response output continued to increase across the 20 sessions until self-administration did not significantly differ from the control group on the 20th session (3.2 $\mu\text{g/kg/infusion}$, 92.8 ± 40.6 ; 6.4 $\mu\text{g/kg/infusion}$, 38.8 ± 17.9) (Fig. 2, d and e).

Self-Administration and Intake among Dependent and Control Groups on the Final Session—Responding for remifentanyl during the final (20th) session was dependent upon dose of remifentanyl [$F(4,69) = 9.7$, $p \leq 0.001$] and treatment condition [$F(2,69) = 17.1$, $p \leq 0.001$] (Fig. 3a). The remifentanyl dose-response curve had an inverted U shape, with the smallest and largest doses maintaining less behavior and intermediate doses maintaining greater amounts of behavior. Overall, the MW group exhibited increased responding compared with the control ($p \leq 0.001$) and MD ($p \leq 0.001$) groups. Although not statistically reliable, the MD group responded somewhat less than the control group at lower doses of remifentanyl; additionally, the lowest dose of remifentanyl that maintained responding in control rats (0.8 $\mu\text{g/kg/infusion}$) failed to reinforce responding in the MD group.

Remifentanyl intake during the last sessions was dependent upon remifentanyl dose [$F(4,69) = 20.0$, $p \leq 0.001$] and treatment condition [$F(2,69) = 14.0$, $p \leq 0.001$] (Fig. 3b). Intake of remifentanyl was greatest in the MW group compared with the control ($p \leq 0.001$) and MD ($p \leq 0.001$) groups. Remifentanyl intake in the MW group was greater than twice the intake of the control group for 0.4 (15.0 ± 11.0 versus 0.3 ± 0.1 $\mu\text{g/kg}$), 0.8 (154.8 ± 41.1 versus 71.2 ± 21.8 $\mu\text{g/kg}$), and 3.2 (774.4 ± 171.7 versus 296 ± 42.2 $\mu\text{g/kg}$) $\mu\text{g/kg/infusion}$ and more than twice the intake of the MD group for all doses.

General Analysis of Intersession Changes in Behavior—Analyzing dose-response functions for each treatment group for each session according to AUC collapsed the data presented in Fig. 2, and depicted dose-dependent responding for each treatment group according to session (Fig. 4). Within-subject analysis revealed dose-dependent responding to change as a function of session [$F(5,68) = 26.0$, $p \leq 0.001$] and an interaction between treatment condition and session number [$F(9,68) = 3.9$, $p \leq 0.001$]. Between-subject analysis revealed responding to also be a function of treatment condition [$F(2,15) = 10.9$, $p \leq 0.001$]. For all groups, AUC increased with session; however, this effect differed

according to treatment condition. AUC derived from the control group was greater than the area generated from the MD group. The control and MW groups demonstrated equivalent AUC until the 15th session, when the groups diverged as the AUC for the MW group continued to increase, whereas the AUC for the C group remained constant, reflecting overall increases in responding throughout the 20 sessions in the MW group but not in the C group.

Intrasession Responding—Quarter-life reflects general intrasession response patterns and indicates whether responding is linear throughout the session. To further understand the nature of the differences in responding observed between the MW and control rats, quarter-life was determined for 0.8 and 3.2 µg/kg/infusion remifentanyl, two doses that maintained significantly different responding between the two groups, and a dose that engendered similar responding between the two groups (6.4 µg/kg/infusion). Latencies to emit the first response were nearly equivalent between the two groups and doses of remifentanyl, ranging between 2.5 and 3.5 min. These long latencies were unexpected given that the three doses of remifentanyl maintained responding that exceeded 60 responses/h in both treatment groups. However, the long latency to first response may indicate impaired recall of the operant response and may be explained within the framework of state-dependent memory retrieval (Bruins Slot and Colpaert, 1999a,b). Quarter-life varied as a function of treatment condition [$F(1,27) = 20.0, p \leq 0.001$] but not dose of remifentanyl. Control rats emitted the first quarter of total responses in almost one fifth of the total session time, whereas the MW rats required over 33% of the session to emit the first quarter of the responses for the lowest dose of remifentanyl (0.8 µg/kg/infusion) and slightly more than 25% of the session for the two higher doses. Disregarding initiation of responding at the beginning of the session, linear responding throughout the session is suggested by a quarter-life of 25%. The modest deviation from 25% observed in both groups indicates that both exhibited a nonlinear response pattern, and the difference in quarter-life between the two groups demonstrates differences in response pattern. Thus, negatively accelerated responding was observed in the control group and positively accelerated responding in the deprived group. These data demonstrate that although robust intersession patterns of responding differed between the control and MW groups, the pattern of intrasession responding between the two groups varied only slightly.

Discussion

Opiate dependence and withdrawal has long been thought to enhance opiate self-administration in humans, monkeys, and rats (e.g., Spragg, 1940; Thompson and Schuster, 1964; Weeks and Collins, 1964; Nichols, 1968; Dole, 1972; Solomon and Corbit, 1974; Koob and Le Moal, 2001). However, opiate self-administration has never been systematically compared in both dependent and nondependent organisms. In the present study, remifentanyl self-administration was compared in morphine-dependent, morphine-dependent and -deprived, and nondependent rats. Dose-dependent remifentanyl self-administration was observed under all conditions. However, compared with nondependent conditions, morphine dependence in the absence of deprivation suppressed remifentanyl responding, whereas morphine deprivation enhanced remifentanyl self-administration for all doses tested, a robust effect that emerged only after several sessions. These findings provide evidence that morphine deprivation-induced withdrawal increases the reinforcing properties of remifentanyl, with self-administration history being an integral component of this effect.

Characterization of Morphine Dependence and Deprivation

The morphine dosing regimen devised for the present study is the first to be characterized to rapidly initiate and maintain dependence while allowing for repeated observation of the

effects of deprivation-induced withdrawal. After initiation of morphine dependence, indications of withdrawal were observed at 24 h after morphine administration. These indicators of withdrawal included hyperalgesia, vocalizations, changes in exploratory behavior, and weight loss. They are in accordance with previous reports of both morphine deprivation- and antagonist-induced withdrawal in rats (Kayan et al., 1971; Tilson et al., 1973; Gellert and Holtzman, 1978; Schulteis et al., 1998; Espejo et al., 2001; Houshyar et al., 2001, 2003).

Morphine deprivation-induced withdrawal was apparent 24, but not 12, h after morphine treatment as evidenced by an enhanced writhing response to acetic acid. The total number of acetic acid-induced writhes was limited by the duration of writhes, such that higher, presumably more noxious concentrations of acetic acid produced longer, more sustained writhes than lower concentrations that produced more writhes of shorter durations. Although both dependent groups exhibited significantly fewer writhes than the control group for some concentrations of acetic acid, the MW group demonstrated a significantly greater duration of writhing compared with the control group, indicating that although fewer number of writhes were observed, this group remained in the writhing posture for a greater portion of the observation period than the control group. Furthermore, the MW group also exhibited increased vocalizations during acetic acid injections compared with the other groups, providing further evidence of hyperalgesia and morphine deprivation-induced withdrawal 24 h after morphine treatment. The MD group exhibited a fewer number of writhes and shorter duration of writhing during the observation period than the control group, demonstrating that morphine retained some analgesic effectiveness 12 h after its administration. This observed analgesia was expected because the half-life of s.c. administered morphine is reported to be 5 h in the rat (Mullis et al., 1979). Thus, a little less than 10 mg/kg remained in the circulation 12 h after a 40 mg/kg treatment, a concentration that is reported to exceed morphine's antinociceptive ED₅₀ to a variety of noxious stimuli (Morgan et al., 2006). These findings may reflect the bidirectional first and second order effects of opioid signal transduction (e.g., Colpaert, 1996). The analgesia observed in the MD group reflects morphine's direct activation of the opioid receptor (the first order effect), whereas the hyperalgesia observed in the MW group demonstrates the second order effect that is of the opposite direction of the initial response after direct activation (Colpaert, 1996; Bruins Slot and Colpaert, 1999c; Bruins Slot et al., 2002; Colpaert et al., 2006).

Although previous findings have demonstrated increased withdrawal severity after multiple exposures to antagonist-induced opiate withdrawal (Harris et al., 2004), intensity of the deprivation syndrome, as indicated by acetic acid writhing measures of hyperalgesia, remained unchanged from the 1st through 20th deprivation episodes. The earlier findings may indicate an increasing sensitivity to repeated administration of the opioid antagonist rather than a general increase in withdrawal severity. Furthermore, the earlier study assessed potentiation of acoustic startle responding as a function of repeated antagonist-precipitated withdrawal. Exposure to anxiogenic and stressful stimuli increases acoustic startle reflex, suggesting that startle behavior may be an additional, interesting endpoint to determine the "affective" effects of drug withdrawal (Fendt and Mucha, 2001). Thus, increases in acoustic startle may represent a behavioral characteristic of withdrawal that is distinct from deprivation-induced hyperalgesia. Furthermore, it is possible that startle behavior is a more sensitive measure of behavior compared with the acetic acid-induced writhing and is therefore able to detect subtle variations in withdrawal severity.

Both dependent groups (MD and MW) lost or gained very little weight across sessions, indicating that dependence was maintained throughout the experiment. Control groups consistently gained weight throughout the experiment, despite differences in daily intake of

remifentanil, indicating, by this measure, that opiate dependence did not develop in the rats that did not receive morphine.

Weights of the dependent groups fluctuated over each 24-h period, such that weight loss was observed 24 h after morphine treatment, and weight was regained 12 h after morphine treatment. It is interesting to note that weight gain above baseline values was observed in the MD group 12 h after morphine treatment, an effect that was not observed in the MW group. This difference may be because the MD group received morphine at the beginning of the dark cycle, when normal feeding occurs. The MW group received morphine in the early part of the light cycle so that maximal morphine deprivation occurred during the dark, active cycle for these rats. Because withdrawal has been shown to suppress feeding behavior (Langerman et al., 2001), overall caloric intake may have been compromised because withdrawal was attenuated only during the light cycle, when food intake is usually minimal.

Effects of Morphine Dependence and Deprivation on Remifentanil Self-Administration: Remifentanil Self-Administration as a Function of Operant History

Dose-dependent remifentanil self-administration was observed in the three groups of rats (control, MD, and MW). It should be noted that initiation of remifentanil self-administration was rapid and did not require behavioral shaping or operant experience with nondrug reinforcers (e.g., food), which are commonly used aids for initiation of self-administration. In this respect, remifentanil is a novel reinforcer and can be exploited in cases where the lack of other reinforcement exposure is important.

Responding for remifentanil varied as a function of remifentanil dose, session, and state of dependence. The contribution of these three factors is reflected in the 0.8 µg/kg/infusion-maintained responding in the control and MW groups, but not in the MD group. Responding for this dose of remifentanil in the control group did not vary much after the 10th session, whereas behavior continued to increase throughout the 20 sessions in the MW group, ultimately resulting in greater self-administration than the control group. This effect was also observed for 3.2 µg/kg/infusion, a dose of remifentanil that was an effective reinforcer in all groups. It is interesting to note that 0.4 µg/kg/infusion, a dose of remifentanil that failed to maintain responding in the control group, did not maintain behavior in the MW group over the first 10 sessions. However, after the 10th session, some behavior was generated and maintained subsequently in MW rats, clearly demonstrating that morphine deprivation increased the sensitivity to the reinforcing effects of remifentanil.

Remifentanil-reinforced behavior, as assessed in this study, was complexly determined as a function of a three-way interaction among treatment group (MD, MW, and C), remifentanil dose, and operant history (session number). Area under the curve analysis allowed for the interaction between operant history and treatment condition to be more clearly delineated. In the control group, remifentanil-maintained behavior and, therefore, AUC values, did not vary much after the 10th session. The MD group initially exhibited lower rates of self-administration compared with the control group and, therefore, generated smaller AUCs for the majority of the sessions. However, with repeated exposures to the contingency, responding increased for all doses except for the lowest two tested and thus resulted in similar AUC values for the two groups for the final session. The gradual enhancement of the reinforcing effects of remifentanil that ultimately resulted in an upward shift in the dose-response function in the MW group compared with the C group is reflected in the difference in AUC between the two groups observed over the last five sessions.

Establishing to what degree the extended morphine history and multiple exposures to the contingency affected operant responding would be helpful in understanding why increased responding was observed in the deprived group over time. The increases in remifentanil self-

administration observed under deprived conditions may be directly related to changes in the negative-reinforcing properties (withdrawal-reducing effects) of the drug. The increased intersession responding exhibited by the morphine-deprived group was independent of any observable changes in withdrawal intensity as measured by acetic acid-induced writhing. However, it is possible that although the hyperalgesia assay detected the presence or absence of morphine withdrawal, it may have lacked the sensitivity to distinguish subtle differences in withdrawal magnitude induced by withdrawal. Subtle increases in withdrawal severity not detected by changes in acetic acid-induced writhing may be hypothesized to enhance the negative-reinforcing properties of remifentanyl, resulting in increased remifentanyl self-administration (Colpaert et al., 2006). Regardless of changes in withdrawal severity, the enhanced responding observed across the 20 sessions may be because of the acquisition ("learning") of the operant response in relation to the negative-reinforcing properties of the drug.

Although withdrawal did not seem to vary in intensity across time, it is likely that prolonged noncontingent morphine-exposure produced a variety of possible receptor adaptations such as changes in μ -receptor density (Viganò et al., 2003), function (Sim-Selley et al., 2000), and changes in the opioid signaling transduction system, including modification of regulatory proteins ($G_i\alpha$ and $G\beta\gamma$) and effector molecules (adenylyl cyclase) (for review, see Gintzler and Chakrabarti, 2006). Therefore, molecular changes produced by prolonged morphine exposure should be considered when interpreting the shift in the remifentanyl self-administration dose-effect curve observed under deprivation conditions.

Decreased remifentanyl self-administration observed in the MD group was probably because of prolonged direct (motor-suppressing) effects of morphine. The presence of morphine's antinociceptive effects 12 h after administration as demonstrated by a suppressed writhing response to acetic acid indicated that morphine retained some of its behavioral effects at this time point. The observed decrease in operant behavior is consistent with previous findings demonstrating opiate suppression of opiate-reinforced responding in rodents and nonhuman primates (Woods et al., 1973; Mello and Mendelson, 1985; Young, 1986; Winger et al., 1992; Winger and Woods, 2001; Negus and Mello, 2002; Stevenson et al., 2003). Although this provides a plausible explanation for why the MD group exhibited low response rates compared with the control and MW groups, it is unknown whether the time courses for the antinociceptive and response-suppressing effects of morphine are identical. An alternative explanation for the decreased self-administration observed in the MD group may be the decreased sensitivity to the discriminative stimulus effects of remifentanyl because of the presence of behaviorally active morphine levels. However, the behavioral effects that morphine exerted on self-administration behavior were overridden with the highest dose of remifentanyl tested (6.4 μ g/kg/infusion). In addition, decreased self-administration compared with the control group was observed for the majority of the sessions; however, by the 20th session, behavior approximated that of the control group, with the exception of the lowest dose of remifentanyl, which maintained responding in the control group (0.8 μ g/kg/infusion). Thus, the pattern of increased intersession responding may reflect behavioral tolerance to the effects of morphine over time.

Effects of Morphine Dependence and Deprivation on Remifentanyl Self-Administration: Implications of Withdrawal-Induced Increases in Remifentanyl Self-Administration

Morphine deprivation ultimately enhanced rates of remifentanyl-maintained responding and increased intake of remifentanyl compared with the nondependent and nondeprived conditions. This increase was dose-dependent and yielded an upward shift of the remifentanyl dose-response curve. The fact that morphine deprivation increased remifentanyl-maintained responding on both the ascending and descending limbs of the remifentanyl dose-effect curve raises some interesting issues. Behavior on the ascending

limb of the dose-effect curve is thought to be controlled by the reinforcing effects of the drug, whereas the descending limb is thought to be controlled both by the reinforcing and response-suppressing effects of the self-administered drug (for review, see Zernig et al., 2004). A leftward shift in the entire remifentanyl dose-effect curve would imply sensitization to both the reinforcing effects (smaller doses) and rate-suppressing effects (larger doses) of remifentanyl. A rightward shift would suggest tolerance to both of these effects. Therefore, the present findings suggest that opiate deprivation increases sensitivity to remifentanyl's reinforcing effects, exemplified by behavior maintained by 0.4 $\mu\text{g/kg/infusion}$ only in the MW group while decreasing the drug's rate-suppressing effects, as represented by increased rates of responding maintained by 6.4 $\mu\text{g/kg/infusion}$ in the MW compared with the control group.

Withdrawal-induced changes in remifentanyl self-administration are similar to the upward shift in cocaine self-administration observed when rats are exposed to long-access conditions (Ahmed and Koob, 1998). Increased intake of methamphetamine (Kitamura et al., 2006) and heroin (Ahmed et al., 2000; Kenny et al., 2006) also have been observed under these conditions. This effect has been described previously as being indicative of the transition from normal reinforced behavior to a state of excessive drug-taking behavior and has been identified as a potential animal model of "addiction" (Ahmed and Koob, 1998). Individual differences among animals demonstrated by vertical shifts in the self-administration dose-response curve for cocaine are also suggested to be predictive of a behavioral phenotype with increased proclivity to drug addiction (Piazza et al., 2000).

The current findings demonstrate that opiate deprivation-induced withdrawal increases opiate self-administration in the rat. However, these conclusions are limited to the reinforcing effects of remifentanyl under a fixed ratio 1 schedule of reinforcement. Direct drug effects are prominent under simple schedules of reinforcement (Seiden and Dykstra, 1977). Therefore, the increase in behavior observed in the MW group may be due in part to tolerance to the response-suppressing effects in addition to an increase in the reinforcing effects of remifentanyl. Furthermore, speculating whether operant behavior is maintained by the positive- or negative-reinforcing effects of remifentanyl is difficult under this contingency. In addition, investigating how opiate deprivation affects behavior maintained by multiple classes of stimuli (e.g., cocaine, alcohol, nicotine, and food) would clarify whether the increased self-administration behavior observed with remifentanyl under deprivation conditions is selective. Finally, determining whether deprivation alters the choice of opiate agonist when other reinforcers are available would verify changes in the relative reinforcing value of opiates because of deprivation, an effect that has been established in dependent monkeys preferring heroin to food under some conditions (Negus, 2006).

Conclusion

The current findings are the first to demonstrate increases in opiate-reinforced responding as a function of well defined and well characterized repeated opioid deprivation compared with nondeprived and control conditions. These increases were obtained across a range of self-administered doses of remifentanyl comprising both the ascending and descending limbs of the dose-reinforcer relationship. The behavior was derived from experimental groups that had been exposed to identical operant training and experimenter handling, as opposed to other models that have reported increased drug self-administration after manipulation of drug history. This between-subject design prevents some covariates that may arise from differences in operant history and prior exposure to various reinforcers (or doses). The conclusions derived from these studies enhance the understanding of the consequences of dependence and withdrawal on opioid-reinforced behavior. Opiate deprivation-induced

enhancement of remifentanyl self-administration provides a model of excessive drug self-administration that warrants further studies to elucidate the contributing variables that mediate the development and expression of this behavior.

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ABBREVIATIONS

MD	morphine dependent
MW	morphine withdrawn
C	control
ANOVA	analysis of variance
AUC	area under the curve

References

- Ahmed SH, Koob GF. Transition from moderate to excessive drug intake: change in hedonic set point. *Science*. 1998; 282:298–300. [PubMed: 9765157]
- Ahmed SH, Walker JR, Koob GF. Persistent increase in the motivation to take heroin in rats with a history of drug escalation. *Neuropsychopharmacology*. 2000; 22:413–421. [PubMed: 10700660]
- Baird TJ, Deng SX, Landry DW, Winger G, Woods JH. Natural and artificial enzymes against cocaine: I. Monoclonal antibody 15A10 and the reinforcing effects of cocaine in rats. *J Pharmacol Exp Ther*. 2000; 295:1127–1134. [PubMed: 11082449]
- Bhargava HN. Rapid induction and quantitation of morphine dependence in the rat by pellet implantation. *Psychopharmacology*. 1977; 52:55–62. [PubMed: 403558]
- Bläsing J, Herz A, Reinhold K, Zieglgänsberger S. Development of physical dependence on morphine in respect to time and dosage and quantification of the precipitated withdrawal syndrome in rats. *Psychopharmacologia*. 1973; 33:19–38. [PubMed: 4797644]
- Bruins Slot LA, Colpaert FC. Recall rendered dependent on an opiate state. *Behav Neurosci*. 1999a; 113:337–344. [PubMed: 10357458]
- Bruins Slot LA, Colpaert FC. Opiate states of memory: receptor mechanisms. *J Neurosci*. 1999b; 19:10520–10529. [PubMed: 10575048]
- Bruins Slot LA, Colpaert FC. Experimental realization of a signal transduction algorithm. *J Theor Biol*. 1999c; 200:39–48. [PubMed: 10479538]
- Bruins Slot LA, Pauwels PJ, Colpaert FC. Sign-reversal during persistent activation in mu-opioid signal transduction. *J Theor Biol*. 2002; 215:169–182. [PubMed: 12051972]
- Chen SA, O'Dell LE, Hoefler ME, Greenwell TN, Zorrilla EP, Koob GF. Unlimited access to heroin self-administration: independent motivational markers of opiate dependence. *Neuropsychopharmacology*. 2006; 31:2692–2707. [PubMed: 16452993]
- Colpaert FC. System theory of pain and of opiate analgesia: no tolerance to opiates. *Pharmacol Rev*. 1996; 48:355–402. [PubMed: 8888306]
- Colpaert FC, Deseure K, Stinus L, Adriaenssens H. High-efficacy 5-hydroxy-tryptamine 1A receptor activation counteracts opioid hyperalldynia and affective conditioning. *J Pharmacol Exp Ther*. 2006; 316:892–899. [PubMed: 16254131]

- Coombs WT, Algina J, Oltman DO. Univariate and multivariate omnibus hypothesis tests selected to control type I error rates when population variances are not necessarily equal. *Rev Educ Res.* 1996; 66:137–179.
- Dai S, Corrigan WA, Coen KM, Kalant H. Heroin self-administration by rats: influence of dose and physical dependence. *Pharmacol Biochem Behav.* 1989; 32:1009–1015. [PubMed: 2798525]
- Dole VP. Narcotic addiction, physical dependence and relapse. *N Engl J Med.* 1972; 286:988–992. [PubMed: 4622638]
- Espejo EF, Serrano MI, Caillé S, Stinus L. Behavioral expression of opiate withdrawal is altered after prefrontocortical dopamine depletion in rats: monoaminergic correlates. *Neuropsychopharmacology.* 2001; 25:204–212. [PubMed: 11425504]
- Fendt M, Mucha RF. Anxiogenic-like effects of opiate withdrawal seen in the fear-potentiated startle test, an interdisciplinary probe for drug-related motivational states. *Psychopharmacology.* 2001; 155:242–250. [PubMed: 11432686]
- Gellert VF, Holtzman SG. Development and maintenance of morphine tolerance and dependence in the rat by scheduled access to morphine drinking solutions. *J Pharmacol Exp Ther.* 1978; 205:536–546. [PubMed: 566320]
- Giamberardino MA, Valente R, de Bigontina P, Vecchiet L. Artificial ureteral calculosis in rats: behavioural characterization of visceral pain episodes and their relationship with referred lumbar muscle hyperalgesia. *Pain.* 1995; 61:459–469. [PubMed: 7478690]
- Gintzler AR, Chakrabarti S. Post-opioid receptor adaptations to chronic morphine; altered functionality and associations of signaling molecules. *Life Sci.* 2006; 79:717–722. [PubMed: 16581089]
- Haidar SH, Moreton JE, Liang Z, Hoke JF, Muir KT, Eddington ND. Evaluating a possible pharmacokinetic interaction between remifentanyl and esmolol in the rat. *J Pharm Sci.* 1997; 86:1278–1282. [PubMed: 9383740]
- Harris AC, Hanes SL, Gewirtz JC. Potentiated startle and hyperalgesia during withdrawal from acute morphine: effects of multiple opiate exposures. *Psychopharmacology.* 2004; 176:266–273. [PubMed: 15164157]
- Houshyar H, Cooper ZD, Woods JH. Paradoxical effects of chronic morphine treatment on the temperature and pituitary-adrenal responses to acute restraint stress: a chronic stress paradigm. *J Neuroendocrinol.* 2001; 13:862–874. [PubMed: 11679055]
- Houshyar H, Gomez F, Manalo S, Bhargava A, Dallman MF. Intermittent morphine administration induces dependence and is a chronic stressor in rats. *Neuropsychopharmacology.* 2003; 28:1960–1972. [PubMed: 12915862]
- Hutcheson DM, Everitt BJ, Robbins TW, Dickinson A. The role of withdrawal in heroin addiction: enhances reward or promotes avoidance? *Nat Neurosci.* 2001; 4:943–947. [PubMed: 11528427]
- Kayan S, Woods LA, Mitchell CL. Morphine-induced hyperalgesia in rats tested on the hot plate. *J Pharmacol Exp Ther.* 1971; 177:509–513. [PubMed: 5568805]
- Kenny PJ, Chen SA, Kitamura O, Markou A, Koob GF. Conditioned withdrawal drives heroin consumption and decreases reward sensitivity. *J Neurosci.* 2006; 26:5894–5900. [PubMed: 16738231]
- Kitamura O, Wee S, Specio SE, Koob GF, Pulvirenti L. Escalation of methamphetamine self-administration in rats: a dose-effect function. *Psychopharmacology.* 2006; 186:48–53. [PubMed: 16552556]
- Ko MC, Terner J, Hursh S, Woods JH, Winger G. Relative reinforcing effects of three opioids with different durations of action. *J Pharmacol Exp Ther.* 2002; 301:698–704. [PubMed: 11961075]
- Koob GF, Le Moal M. Drug addiction, dysregulation of reward, and allostasis. *Neuropsychopharmacology.* 2001; 24:97–129. [PubMed: 11120394]
- Langerman L, Piscoun B, Bansinath M, Shemesh Y, Turndorf H, Grant GJ. Quantifiable dose-dependent withdrawal after morphine discontinuation in a rat model. *Pharmacol Biochem Behav.* 2001; 68:1–6. [PubMed: 11274701]
- Mello NK, Mendelson JH. Behavioral pharmacology of buprenorphine. *Drug Alcohol Depend.* 1985; 14:283–303. [PubMed: 3888577]

- Morgan MM, Fossum EN, Stalling BM, King MM. Morphine antinociceptive potency on chemical, mechanical, and thermal nociceptive tests in the rat. *J Pain*. 2006; 7:358–366. [PubMed: 16632325]
- Mullis KB, Perry DC, Finn AM, Stafford B, Sadée W. Morphine persistence in rat brain and serum after single doses. *J Pharmacol Exp Ther*. 1979; 208:228–231. [PubMed: 762653]
- Naruse T, Asami T. Intravenous self-administration of diazepam in rats. *Eur J Pharmacol*. 1987; 135:365–373. [PubMed: 3582499]
- Negus SS, Mello NK. Effects of mu-opioid agonists on cocaine- and food-maintained responding and cocaine discrimination in rhesus monkeys: role of mu-agonist efficacy. *J Pharmacol Exp Ther*. 2002; 300:1111–1121. [PubMed: 11861822]
- Negus SS. Choice between heroin and food in control and heroin-dependent rhesus monkeys: effects of naloxone, buprenorphine, and methadone. *J Pharmacol Exp Ther*. 2006; 317:711–723. [PubMed: 16456085]
- Nichols JR. Morphine as a reinforcing agent: laboratory studies of its capacity to change behavior. *Res Publ Assoc Res Nerv Ment Dis*. 1968; 46:299–310. [PubMed: 5723799]
- Panlilio LV, Katz JL, Pickens RW, Schindler CW. Variability of drug self-administration in rats. *Psychopharmacology*. 2003; 167:9–19. [PubMed: 12644888]
- Panlilio LV, Schindler CW. Self-administration of remifentanyl, an ultra-short acting opioid, under continuous and progressive-ratio schedules of reinforcement in rats. *Psychopharmacology*. 2000; 150:61–66. [PubMed: 10867977]
- Piazza PV, Deroche-Gamonet V, Rouge-Pont F, Le Moal M. Vertical shifts in self-administration dose-response functions predict a drug-vulnerable phenotype predisposed to addiction. *J Neurosci*. 2000; 20:4226–4232. [PubMed: 10818158]
- Schulteis G, Yackey M, Risbrough V, Koob GF. Anxiogenic-like effects of spontaneous and naloxone-precipitated opiate withdrawal in the elevated plus-maze. *Pharmacol Biochem Behav*. 1998; 60:727–731. [PubMed: 9678657]
- Seiden, LS.; Dykstra, LA. *Psychopharmacology: A Biochemical and Behavioral Approach*. Van Nostrand Reinhold Company; New York: 1977. Drugs as reinforcers; p. 359-396.
- Sim-Selley LJ, Selley DE, Vogt LJ, Childers SR, Martin TJ. Chronic heroin self-administration desensitizes mu opioid receptor-activated G-proteins in specific regions of rat brain. *J Neurosci*. 2000; 20:4555–4562. [PubMed: 10844025]
- Solomon RL, Corbit JD. An opponent-process theory of motivation: I. Temporal dynamics of affect. *Psychol Rev*. 1974; 81:119–145. [PubMed: 4817611]
- Spragg SD. Morphine addiction in chimpanzees. *Comparative Psychology Monographs*. 1940; 15:1–132.
- Stevenson GW, Folk JE, Linsenmayer DC, Rice KC, Negus SS. Opioid interactions in rhesus monkeys: effects of delta + mu and delta + kappa agonists on schedule-controlled responding and thermal nociception. *J Pharmacol Exp Ther*. 2003; 307:1054–1064. [PubMed: 14557380]
- Thompson T, Schuster CR. Morphine self-administration, food-reinforced, and avoidance behaviors in rhesus monkeys. *Psychopharmacologia*. 1964; 5:87–94. [PubMed: 14137126]
- Tilson HA, Rech RH, Stolman S. Hyperalgesia during withdrawal as a means of measuring the degree of dependence in morphine dependent rats. *Psychopharmacologia*. 1973; 28:287–300. [PubMed: 4734848]
- Viganò D, Rubino T, Di Chiara G, Ascari I, Massi P, Parolaro D. Mu opioid receptor signaling in morphine sensitization. *Neuroscience*. 2003; 117:921–929. [PubMed: 12654343]
- Weeks JR, Collins RJ. Factors affecting voluntary morphine intake in self-maintained addicted rats. *Psychopharmacologia*. 1964; 6:267–279. [PubMed: 5890552]
- Winger G, Skjoldager P, Woods JH. Effects of buprenorphine and other opioid agonists and antagonists on alfentanil- and cocaine-reinforced responding in rhesus monkeys. *J Pharmacol Exp Ther*. 1992; 261:311–317. [PubMed: 1560376]
- Winger G, Woods JH. The effects of chronic morphine on behavior reinforced by several opioids or by cocaine in rhesus monkeys. *Drug Alcohol Depend*. 2001; 62:181–189. [PubMed: 11295322]

- Winger G, Galuska CM, Hursh SR, Woods JH. Relative reinforcing effects of cocaine, remifentanyl, and their combination in rhesus monkeys. *J Pharmacol Exp Ther*. 2006; 318:223–229. [PubMed: 16571623]
- Woods, JH.; Downs, DA.; Villarreal, JH. Changes in operant behavior during deprivation- and antagonist-induced withdrawal states. In: Hoffmeister, F.; Goldberg, L., editors. *Psychic Dependence*. Springer-Verlag; New York: 1973. p. 114-121.
- Woods, JH.; Schuster, CR. Opiates as reinforcing stimuli. In: Thompson, T.; Pickens, RW., editors. *Stimulus Properties of Drugs*. Appleton-Century-Crofts; New York: 1971. p. 163-175.
- Woods JH, Winger GD. Observing responses maintained by stimuli associated with cocaine or remifentanyl reinforcement in rhesus monkeys. *Psychopharmacology*. 2002; 163:345–351. [PubMed: 12373435]
- Young AM. Effects of acute morphine pretreatment on the rate-decreasing and antagonist activity of naloxone. *Psychopharmacology*. 1986; 88:201–208. [PubMed: 3081932]
- Zernig G, Wakonigg G, Madlung E, Haring C, Saria A. Do vertical shifts in dose-response rate-relationships in operant conditioning procedures indicate “sensitization” to “drug wanting”? *Psychopharmacology*. 2004; 171:349–351. [PubMed: 14530895]

Fig. 1.

Average change in body weight from baseline weight ($\pm$ S.E.M.) measured every 12 h in control ($\circ$, baseline weight = 289 ± 10 g), MD ($\bullet$, baseline weight = 336.5 ± 19 g), and MW ($\blacksquare$, baseline weight = 344 ± 7 g) rats self-administering 0.8 μ g/kg/infusion remifentanyl and control rats ($\square$, baseline weight = 315 ± 5 g) self-administering 0.4 μ g/kg/infusion. Weights of the dependent groups were significantly less than the control group after the 4th self-administration session ($p \leq 0.05$), an effect that persisted until the termination of the experiment. Weights from the two control groups did not differ.

Fig. 2.

Average ($\pm$ S.E.M.) responding for control ($\square$), MD ($\bullet$), and MW ($\blacksquare$) groups for 0.4 (a), 0.8 (b), 1.6 (c), 3.2 (d), and 6.4 (e) $\mu\text{g/kg}$ /infusion over 20 self-administration sessions. #, difference between control and MD (#, $p \leq 0.05$; ##, $p \leq 0.01$; ###, $p \leq 0.001$). *, difference between control and MW (*, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.001$).

Fig. 3.

Average ($\pm$ S.E.M.) responding for (a) and intake of (b) remifentanyl in control ($\square$), MD ($\bullet$), and MW ($\blacksquare$) groups on the final self-administration session. Dose-dependent responding and intake were observed for all three groups. Suppressed responding was observed in the MD group, whereas enhanced responding and intake were observed in the MW rats. *, difference between control and MW (*, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.001$). \$, difference between MD and MW groups (\$\$, $p \leq 0.01$; \$\$\$, $p \leq 0.001$).

Fig. 4. Average AUC ($\pm$ S.E.M.) analysis of remifentanyl self-administration dose-response functions generated from control ($\square$), MD ($\bullet$), and MW ($\blacksquare$) groups according to session. #, difference between control and MD groups (#, $p \leq 0.05$; ##, $p \leq 0.01$). *, difference between control and MW groups (*, $p \leq 0.01$; ***, $p \leq 0.001$).

Writhing behavior indicated as average number of writhes ($\pm$ S.E.M.) during the observation period and average total duration of time spent writhing ($\pm$ S.E.M.) in control, MD, and MW groups during the 1st and 20th episodes of withdrawal as a function of vehicle or acetic acid (0.32, 1.0, or 3.2%) injection

TABLE 1

Percentage Acetic Acid	Control		MD		MW (1st episode)		MW (20th episode)	
	Number	Duration <i>min</i>	Number	Duration <i>min</i>	Number	Duration <i>min</i>	Number	Duration <i>min</i>
Vehicle	1.7 $\pm$ 1.2	0.1 $\pm$ 0.1	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.6 $\pm$ 0.4	0.0 $\pm$ 0.0	2.2 $\pm$ 0.9	0.2 $\pm$ 0.0
0.32	1.5 $\pm$ 0.6	0.1 $\pm$ 0.1	Not done	Not done	3.6 $\pm$ 1.6	0.2 $\pm$ 0.2	8.3 $\pm$ 2.4	0.7 $\pm$ 0.4
1	9.83 $\pm$ 5.3	0.9 $\pm$ 0.6	0.2 $\pm$ 0.2	0.0 $\pm$ 0.0	13.0 $\pm$ 4.7	9.5 $\pm$ 4.6	21.5 $\pm$ 5.4	11.5 $\pm$ 2.0
3.2	29.0 $\pm$ 3.8	15.5 $\pm$ 2.5	7.3 $\pm$ 3.5	2.8 $\pm$ 1.7	23.2 $\pm$ 3.0	22.4 $\pm$ 1.8	15.8 $\pm$ 1.7	25.1 $\pm$ 0.5

Exploratory behavior as measured by average rears ($\pm$ S.E.M.) and vocalization (percentage of group) during the acetic acid-induced writhing assay in control, MD, and MW groups during the 1st and 20th episodes of withdrawal

TABLE 2

Percentage Acetic Acid	Control		MD		MW (1st episode)		MW (20th episode)	
	Rears	Vocalize %	Rears	Vocalize %	Rears	Vocalize %	Rears	Vocalize %
Vehicle	18.2 $\pm$ 4.4	16.7 $\pm$ 15.2	2.2 $\pm$ 1.4	33.3 $\pm$ 19.3	5.3 $\pm$ 3.1	75.0 $\pm$ 17.9	3.8 $\pm$ 1.5	83.0 $\pm$ 17.9
0.32	18.2 $\pm$ 3.1	0.0 $\pm$ 0.0	Not done	Not done	5.2 $\pm$ 2.2	66.7 $\pm$ 19.2	14.2 $\pm$ 4.0	83.0 $\pm$ 17.9
1	21.2 $\pm$ 2.9	66.7 $\pm$ 19.3	2.8 $\pm$ 2.3	16.7 $\pm$ 15.2	1.8 $\pm$ 1.4	66.7 $\pm$ 19.2	4.7 $\pm$ 2.2	100.0 $\pm$ 0.0
3.2	2.6 $\pm$ 1.5	83.3 $\pm$ 15.2	2.2 $\pm$ 1.4	50.0 $\pm$ 20.4	0.4 $\pm$ 0.4	80.0 $\pm$ 17.8	1.2 $\pm$ 1.0	100.0 $\pm$ 0.0