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Spontaneous withdrawal in intermittent morphine administration in rats and mice: effect of clonidine coadministration and sex-related differences

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Background/aim: Treating animals repeatedly with intermittent and increasing morphine doses has been suggested to allow some withdrawal during each dosing interval, which causes repeated stress. The present study aimed to test this hypothesis and assess sex-related differences in withdrawal signs and their suppression by clonidine.

Materials and methods: Male and female rats and mice were administered with increasing doses of morphine twice daily at different dosing intervals. Rats were given clonidine in drinking water (5 μg/mL). Spontaneous and naloxone-precipitated withdrawal signs and novelty-induced grooming were evaluated.

Results: Male rats and male and female rats displayed manifestations of morphine withdrawal at the end of 14-h and 24-h dosing intervals, respectively. Clonidine attenuated the severity of the withdrawal signs. Male but not female mice displayed withdrawal signs at the end of 12-h and 17-h dosing intervals. Female mice exhibited less pronounced naloxone-precipitated withdrawal syndrome. Grooming did not reflect a "stress-like state" in morphine-treated animals.

Conclusion: These findings indicate intermittent morphine treatment-induced spontaneous withdrawal in rats and mice and sex-related differences in spontaneous and naloxone-precipitated withdrawal signs in mice. Since the treatment protocol closely parallels the drug use pattern in opioid addicts, further experiments are needed to clarify the stress associated with the treatment and the efficacy of sedatives.

Key words: Morphine, intermittent treatment, clonidine, spontaneous withdrawal, grooming, sex, dependence

1. Introduction

The effectiveness of morphine and other opiates in the management of pain is limited by the development of tolerance and physical dependence with prolonged or repeated administrations (1). Following long-duration opiate intake, abrupt drug withdrawal results in multiple aversive somatic signs and behavioral changes that may play a key role in maintaining continued drug use (2). The alpha 2-adrenergic agonist clonidine alleviates most of the somatic signs of opiate withdrawal syndrome (3).

Experimental studies in animals have shown that long-term exposure to morphine induces physical dependence manifested by somatic withdrawal symptoms when the drug is withheld or soon after the administration of naloxone or other opiate antagonists. Several studies have demonstrated sex-related differences in the expression of withdrawal signs (4–8). Many techniques were developed to render rodents rapidly dependent on morphine. These include constant treatments provided by infusions (9,10) or implanted slow-release pellets (11,12) and daily injections of constant or escalating doses (6,8,13–16). Treating animals repeatedly with intermittent and increasing morphine doses is suggested to mimic opiate use patterns in humans (8). In contrast to constant treatments, injections of morphine in escalating doses at 12-h intervals result in elevated basal plasma adrenocorticotropic hormone and corticosterone concentrations. A "stress-like" state is suggested to occur in response to repeated cycles of partial withdrawal associated with intermittent treatment regimen (17,18). It is well known that stress contributes to the development and course of addictive behavior in humans (19). Stressors in animals also reinstate heroin self-administration (20) or increase morphine intake (21).

The aims of the present study were to assess the "stress-like" state and spontaneous withdrawal associated with intermittent morphine treatment in male and female

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rats and mice, the sex-related differences in spontaneous as well as naloxone-precipitated morphine withdrawal symptoms, and the effect of clonidine on withdrawal signs associated with intermittent treatment. Novelty-induced grooming behavior was used to evaluate the stress response. Clonidine was given during dose intervals. Because repeated injections may cause additional stress, the drug was added to the drinking water of the animals (22–24).

2. Materials and methods

2.1. Animals

Inbred male (280–380 g) and female (220–255 g) Wistar albino rats and male and female (25–30 g) BALB/c mice were obtained from the Animal Care Unit of the Institute for Experimental Medicine of İstanbul University. Sixty-three rats and 60 mice were used in the study. The animals were housed 5 (rats) and 6 or 7 (mice) per cage under a regular light/dark schedule (lights on at 0700 hours) for at least 1 week prior to experimentation and were allowed free access to both food and water. All studies were approved by the Local Ethics Committee on Animal Experiments (İstanbul University, 08/2009, 66-67/2010).

2.2. Drugs

Morphine (Verenigde Pharmaceutische Fabriken BV, Holland) and naloxone hydrochloride (Sigma, St. Louis, MO, USA) were dissolved in saline. Clonidine hydrochloride (Sigma) was added to tap water. Rats were injected with morphine and naloxone subcutaneously (s.c.) in a volume of 1 mL/kg body weight. Mice were injected with morphine intraperitoneally (i.p.) and with naloxone s.c. in a volume of 10 and 1 mL/kg body weight, respectively. Drug solutions were prepared fresh every day. Clonidine dose was chosen according to our previous study (24). Naloxone dose was chosen according to the studies referred to for the induction of morphine dependence in rats and mice, as indicated below.

2.3. Induction of morphine dependence in male and female rats and mice

In Experiment I, development of spontaneous withdrawal associated with intermittent morphine treatment and effect of clonidine administration on spontaneous and naloxone-precipitated withdrawal signs were assessed in rats. The intermittent treatment regimen employed was based on studies by Caille et al. (25) and Houshyar et al. (17,18). In this regimen, morphine was given twice daily at 12-h intervals with increasing doses each day. A modification was made in the injection schedule that provided interdose intervals of 10 and 14 h. Because extension of the time interval between doses may induce more withdrawal, clonidine was given during the 14-h time period. Male rats were randomly divided into 4 groups (n = 10 animals): control (saline; tap water), clonidine (saline; tap water with clonidine), morphine (morphine; tap water), and morphine + clonidine (morphine; tap water with clonidine). For 4 days, the animals received saline twice a day (at 0830 and 1830 hours) or increasing doses of morphine as follows (in mg/kg of body weight): day 1 (10, 10), day 2 (20, 20), day 3 (30, 30), and day 4 (40, 40). The final saline and morphine (40 mg/kg) injections were delivered at 0830 hours on day 5. Throughout the experiment, control and morphine groups received plain tap water. Clonidine and morphine + clonidine groups received tap water containing clonidine (5 μg/mL) from 1830 to 0830 hours (14-h withdrawal) and plain tap water from 0830 to 1830 hours. Water consumption during 14-h dosing intervals was measured every morning in all groups. Mean daily water consumption per rat was calculated by dividing the total daily water consumption by the number of animals present in the cage. Two animals died (one in the morphine group and one in the morphine + clonidine group) during the course of the experiments.

In Experiment II, development of spontaneous withdrawal associated to intermittent morphine treatment and sex-related differences in spontaneous and naloxone-precipitated withdrawal signs were assessed in rats. The intermittent treatment regimen employed in the present study was based on a study by Streel et al. (26). Male and female rats were randomly divided into control (saline) and morphine groups (n = 5–9 animals). For 3 days, the animals received saline twice a day (at 1000 and 1600 hours) or increasing doses of morphine as follows (in mg/kg of body weight): day 1 (10, 20), day 2 (30, 40), and day 3 (50, 50). During the next 3 days, the rats received saline or a regular dose of morphine once a day (at 1200 hours). The doses were as follows (in mg/kg of body weight): day 4 (50), day 5 (50), and day 6 (50).

Intermittent morphine treatment is widely used in mice for the induction of dependence (8,14,16). Therefore, in Experiment III, development of spontaneous withdrawal associated with intermittent morphine treatment and sex-related differences in spontaneous and naloxone-precipitated withdrawal signs were also assessed in mice. The intermittent treatment regimens employed were based on studies conducted by Papaleo and Contarino (8) and Li et al. (14). Male and female mice were randomly divided into control (saline) and morphine groups (n = 6–10 animals). In the first regimen (M-10/50), the animals received saline twice a day for 5 days (at 0800 and 2000 hours) or increasing doses of morphine as follows (in mg/kg of body weight): day 1 (10, 10), day 2 (20, 20), day 3 (30, 30), day 4 (40, 40), and day 5 (50, 50). The final saline and morphine (50 mg/kg) injections were delivered at 0800 hours on day 6. In the second regimen (M-20/100), the animals received saline twice a day for 5 days (at 0900 and

1600 hours) or increasing doses of morphine as follows (in mg/kg of body weight): day 1 (20, 20), day 2 (40, 40), day 3 (60, 60), day 4 (80, 80), and day 5 (100, 100). The final saline or morphine (100 mg/kg) injections were delivered at 0900 hours on day 6.

2.4. Measurement of stress response and spontaneous withdrawal signs

Animals were placed individually into wire mesh observation cages (width and length 30 cm, height 25 cm) just before the final injections (at the end of a 14-h dosing interval in Experiment I, 24-h dosing interval in Experiment II, and 12- and 17-h dosing intervals in Experiment III). Following a 1-min habituation period, rats were observed for 5 min and mice for 10 min for grooming behavior and spontaneous withdrawal signs. Grooming activities were considered as scratching of body and face and licking of body fur, tail, limbs, and genital area. Total time spent grooming was also measured in rats. Grooming frequency was recorded in mice by giving a score for the presence of grooming behavior every 15 s (27). Teeth chattering, ptosis, urination, cheek tremors, freezing, eye blinking, sniffing, and wet dog shakes were assessed as somatic signs of spontaneous morphine withdrawal in rats. Ptosis, urination, cheek tremors, freezing, eye blinking, and diarrhea were assessed as somatic signs of spontaneous morphine withdrawal in mice. One point was attributed for each observed sign and a global withdrawal score was calculated for each animal by summing up all the checked signs (28).

2.5. Measurement of naloxone-precipitated withdrawal signs

Animals were treated with 1 mg/kg naloxone 2 h after the final injections and were then placed individually into observation cages. The occurrence of escape jumps (jumping frequency) was recorded for 15 min. Animals were weighed before and after the naloxone challenge to determine body weight loss. On test days, the animals were acclimatized to the experimental room 1 h prior to experimentation. Experiments took place under quiet conditions and in dim light. The observation cages were cleaned thoroughly with wet and dry cloths between each test. A trained observer who was blinded to the treatment given to each group carried out all behavioral measurements.

2.6. Statistical analysis

All data analyses were performed using SPSS 21.0. One-way analysis of variance (ANOVA) was used to compare global withdrawal score, duration and frequency of grooming, body weight loss, and jumping frequency data collected from the animals. The Bonferroni test was used for group comparisons. Significance was accepted at the $P < 0.05$ level. For the estimation of the statistical power of the study, post hoc power analysis was performed using G*Power 3.1.9.2 Statistical Analysis. Power analysis for prediction of water intake, grooming duration, global withdrawal score, weight loss, and jumping in Experiment I and II, and for global withdrawal score and jumping in the M-10/50 group and grooming frequency, global withdrawal score, and jumping in the M/20-100 group in Experiment III revealed a statistical power of more than 80% for our sample size, with an α error of 0.05. Power of grooming frequency was 50% in the M-10/50 group. Power of body weight loss was 8% and 13% in the M-10/50 and M-20/100 groups, respectively.

3. Results

In Experiment I, average water consumption for each rat during the 14-h time period was calculated as 22.4 ± 0.86, 13.1 ± 1.85, 10.8 ± 2.74, and 6.5 ± 1.35 mL in the control, clonidine, morphine, and morphine + clonidine groups, respectively. One-way ANOVA revealed significant differences among the groups in terms of water intake [F (3,34) = 12.704; $P < 0.001$]. Clonidine, morphine, and morphine + clonidine groups had lower water consumption compared to the control group ($P < 0.001$). Approximate average dose of clonidine consumed daily by each rat was 65.5 ± 8.59 and 26.6 ± 4.77 μg/mL in the clonidine and morphine + clonidine groups, respectively. Novelty-induced grooming behavior and spontaneous and naloxone-precipitated withdrawal in rats determined on day 5 are shown in Figure 1. At the end of the 14-h dosing interval, one-way ANOVA revealed significant differences among the groups in terms of grooming duration [$F(3,34) = 6.023$; $P < 0.01$] and withdrawal signs [$F(3,34) = 8.282$; $P < 0.001$]. Total time spent on grooming was less in the morphine ($P < 0.01$) and morphine + clonidine ($P < 0.05$) groups compared to the control group. Animals in the morphine ($P < 0.001$) and morphine + clonidine ($P < 0.01$) groups showed marked global withdrawal scores compared to the control animals. Withdrawal was less severe in the morphine + clonidine group compared to the morphine group ($P < 0.05$). One-way ANOVA revealed significant differences between the groups in body weight loss [$F(3,34) = 3.785$; $P < 0.05$] and jumping frequency [$F(3,34) = 9.362$; $P < 0.001$] after naloxone administration. Animals in the morphine ($P < 0.05$; $P < 0.001$) and morphine + clonidine ($P < 0.05$; $P < 0.01$) groups exhibited marked weight loss and number of jumps compared to the control animals.

In Experiment II, novelty-induced grooming behavior and spontaneous and naloxone-precipitated withdrawal signs in male and female rats determined on day 6 are shown in Figure 2. At the end of the 24-h dosing interval, one-way ANOVA revealed significant differences between the groups in terms of withdrawal signs [$F(3,21) = 19.083$; $P < 0.001$], although not in grooming duration [$F(3,21) =$

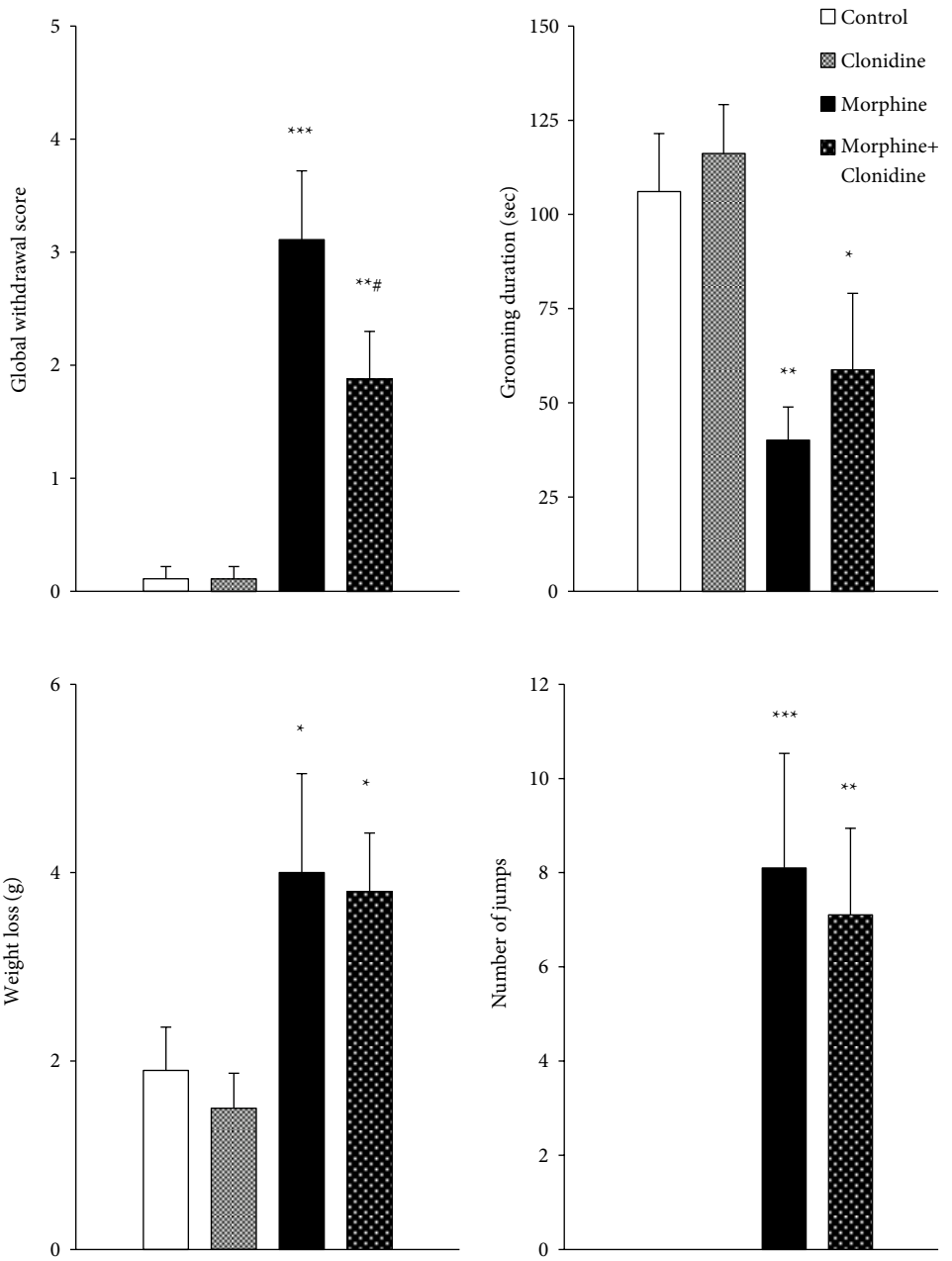

Figure 1. Spontaneous withdrawal and grooming activity observed at the end of the 14-h dosing interval, and naloxone-precipitated weight loss and jumping observed 2 h after the final injections in intermittent morphine administration in rats. Animals were injected s.c. with saline or morphine (10–40 mg/kg) for 5 days (twice daily on days 1, 2, 3, and 4 and once daily on the morning of day 5). Clonidine (5 µg/mL) was given in drinking water during the 14-h time period. Withdrawal signs and calculation of global withdrawal score were described in the text. Results are presented as means ± SEM (n = 9–10 rats for each group). Number of jumps in the control and clonidine groups are 0.0 ± 0.00.

*: P < 0.05, **: P < 0.01, ***: P < 0.001 difference from the control group; #: P < 0.05 difference from the morphine group.

2.183; P > 0.05]. Both male and female rats treated with morphine showed marked global withdrawal scores compared to their relative control groups (P < 0.01). One-way ANOVA also revealed significant differences between the groups in body weight loss [$F(3,21) = 9.017$; P < 0.001] and jumping frequency [$F(3,21) = 10.795$; P < 0.001]. Male (P < 0.01; P < 0.05) and female (P < 0.05) rats in the morphine groups exhibited marked weight loss and number of jumps compared to the same-sex control animals.

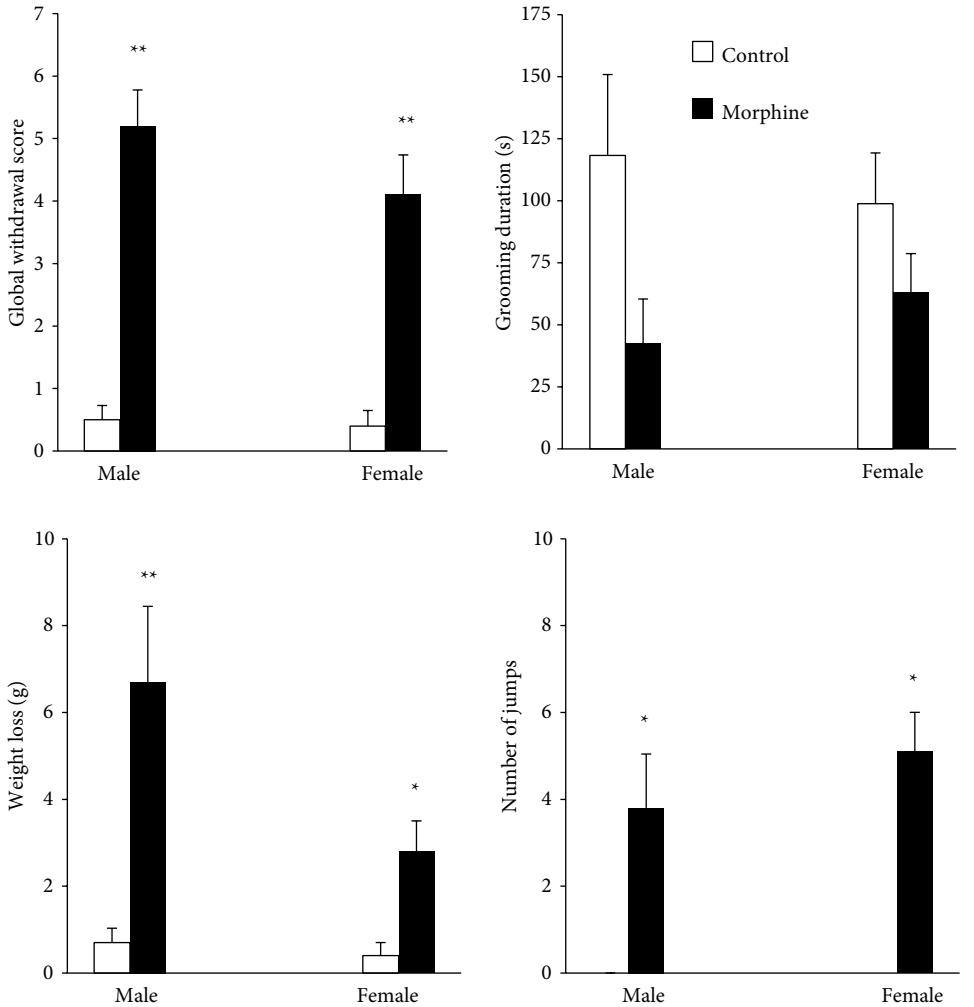

Figure 2. Spontaneous withdrawal and grooming activity observed at the end of 24-h dosing interval, and naloxone-precipitated weight loss and jumping observed 2 h after the final injections in intermittent morphine administration in male and female rats. Animals were treated s.c. with saline or increasing doses of morphine (10–50 mg/kg) for 6 days (twice daily on days 1, 2, and 3 and once daily on days 4, 5, and 6). Withdrawal signs and calculation of global withdrawal score were described in the text. Results are presented as means ± SEM (n = 5–9 rats for each group). Number of jumps in male and female control animals are 0.0 ± 0.00.
*: $P < 0.05$, **: $P < 0.01$ difference from the same-sex control groups.

In Experiment III, novelty-induced grooming behavior and spontaneous and naloxone-precipitated withdrawal in male and female mice determined on day 6 are shown in Figure 3. At the end of the 12-h dosing interval in the M-10/50 dose regimen, one-way ANOVA revealed significant differences between the groups in the global withdrawal score [$F(3,28) = 7.377$; $P < 0.001$] and grooming frequency [$F(3,28) = 3.366$; $P < 0.05$]. Male mice but not female mice treated with morphine showed a marked global withdrawal score compared with the same-sex control animals ($P < 0.01$). Post hoc analysis did not reveal significant differences between morphine-treated and control animals for the grooming activity. One-way ANOVA also revealed significant differences between the groups in the body weight loss [$F(3,28) = 3.055$; $P < 0.05$] and jumping frequency [$F(3,28) = 7.275$; $P < 0.001$] after naloxone administration. Male animals but not females treated with morphine had higher body weight loss ($P < 0.05$) and number of jumps ($P < 0.01$) compared to same-sex control animals. At the end of the 17-h dosing interval in the M-20/100 dose regimen, one-way ANOVA revealed significant differences among the groups both in global withdrawal score [$F(3,24) = 9.779$; $P < 0.001$] and grooming frequency [$F(3,24) = 5.979$; $P < 0.01$]. Male mice but not female mice treated with morphine showed higher global withdrawal score ($P < 0.001$), and both male and female morphine-treated mice showed a lower number of grooming actions ($P < 0.05$) compared to their

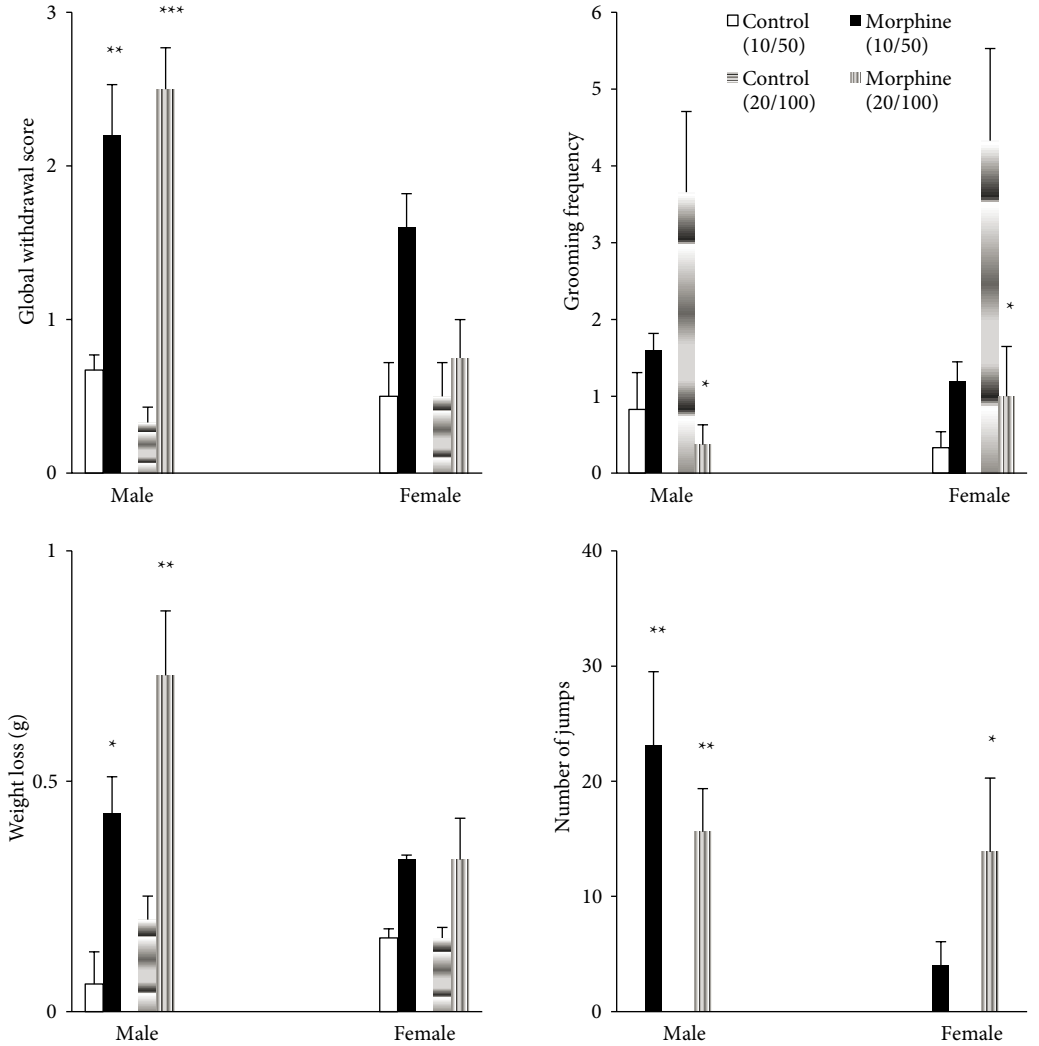

Figure 3. Spontaneous withdrawal and grooming activity observed at the end of 12-h (M-10/50) or 17-h (M-20/100) dosing intervals, and naloxone-precipitated weight loss and jumping observed 2 h after the final injections in intermittent morphine administration in male and female mice. Animals were treated s.c. with saline or increasing doses of morphine (10–50 or 20–100 mg/kg) for 6 days (twice daily on days 1, 2, and 3, and once daily on days 4, 5, and 6). Withdrawal signs and calculation of global withdrawal score were described in the text. Results are presented as means ± SEM (n = 6–10 mice for each group). Number of jumps in M-10/50 and M-20/100 male and female control animals is 0.0 ± 0.00.
*: P < 0.05, **: P < 0.01, ***: P < 0.001 difference from the same-sex control groups.

relative control groups. One-way ANOVA also revealed significant differences between the groups in body weight loss [$F(3,24) = 6.944$; $P < 0.01$] and jumping frequency [$F(3,24) = 8.830$; $P < 0.001$] after naloxone administration. Morphine-treated male mice had higher body weight loss and number of jumps ($P < 0.01$), and female mice had higher number of jumps ($P < 0.05$) compared to the same-sex control animals.

4. Discussion

This study was designed for the following purposes: first, to investigate the hypothesis that induction of morphine dependence by intermittent injections may induce stress and allow some withdrawal during treatment; and, second, to evaluate the sex-related differences in withdrawal symptoms. Our results showed that novelty-induced grooming behavior could not imply a "stress-like state" in rats and mice treated intermittently with increasing doses of morphine. The treatment protocols, however, produced marked manifestations of spontaneous withdrawal at the end of the dosing interval. Administration of clonidine during dose intervals attenuated the withdrawal signs. The expression of spontaneous and naloxone-precipitated withdrawal syndrome, on the other hand, did not

significantly differ in male and female rats, but appeared somewhat less pronounced in female mice compared to males.

The degree and severity of physical dependence on opioids is influenced by dose, frequency (dosing interval), and duration of drug administration (9,29). It has been suggested that frequency is the most important factor (29). In studies with daily morphine injections, animals receive each dose at equal (6,18,25,30) or unequal dosing intervals (6,13,15,31). In the present study, animals were given intermittent morphine injections with different interdose intervals for a better assessment of spontaneous withdrawal associated to the treatment regimen.

Following long-term morphine administration, abrupt withdrawal elicits spontaneous withdrawal in rats as expressed by wet dog shakes, jumping, paw tremor, diarrhea, ptosis, teeth chattering, piloerection, eye blinking, salivation, grooming, body weight loss, and reduction in food consumption and water intake (9,15,25,28,32). Somatic signs of spontaneous morphine withdrawal in mice are diarrhea, body weight loss, jumping, wet dog shake, ptosis, paw tremor, and head shaking (8,14,30). In the present study, spontaneous withdrawal evaluated just before the final morphine dose was manifested as teeth chattering, ptosis, wet dog shakes, urination, cheek tremors, freezing, eye blinking, and sniffing in rats and freezing, diarrhea, tremors, eye blinking, and ptosis in mice. The variety of withdrawal signs appears to be similar to those observed in chronic morphine use in rodents. Male and female rats treated intermittently with increasing doses of morphine displayed higher global withdrawal scores at the end of the 14-h and 24-h dosing intervals, respectively (Figures 1 and 2). Intermittent morphine treatment also produced higher global withdrawal scores in male mice at the end of the 12-h and 17-h dosing intervals (Figure 3). These results support the suggestion that intermittent injections of morphine allow withdrawal during dosing intervals (18). The withdrawal symptoms observed in both species are obviously less pronounced than those reported in previous studies. This indicates that partial withdrawal is associated with intermittent morphine administration. The severity of the withdrawal score between male and female rats is inconsequential. This finding is not in agreement with the finding that male rats have more severe spontaneous withdrawal syndrome than females (7). In contrast to rats, mice showed sex-related differences in withdrawal scores in both treatment regimens. These results contradict previous observations that M-10/50 and M-20/100 morphine regimens allow spontaneous withdrawal in both sexes (8). This discrepancy between the two studies may be due to the differences in testing times (just before the last dose vs. several hours after the last dose) and/or test session durations (10 min vs. 30 min).

Several withdrawal signs have been observed following naloxone challenge in morphine-dependent animals (5,6,11,16,30,31). Weight loss and jumping are considered predominant signs for quantification of naloxone withdrawal in both rats (33,34) and mice (6,11,35). Accordingly, naloxone precipitated withdrawal weight loss and jumping in morphine-treated male and female rats (Figures 1 and 2). Both M-10/50 and M-20/100 male mice also displayed significant weight loss and jumping, and M-20-100 female mice exhibited significant jumping following naloxone administration (Figure 3). There were no sex-related differences in the expression of naloxone-precipitated withdrawal signs in rats. In contrast, M-10/50 female mice did not show withdrawal signs, and M-20/100 female mice had less severe withdrawal (only jumping) in respect to M-20/100 males. Studies on sex-related differences in the expression of morphine withdrawal syndrome precipitated by naloxone have produced inconsistent results both in rats (4,7) and in mice (5,6,11,35). Our results agree with the previous finding showing that naloxone-precipitated withdrawal syndrome is similar in male and female rats (7). Moreover, data obtained from mice support the previous findings that female mice are less prone to developing signs of naloxone withdrawal syndrome than male animals (5,35).

Noradrenergic neurons from the locus coeruleus are thought to be an essential component of opiate withdrawal (36). Clonidine has been shown to alleviate most of the somatic signs of opiate withdrawal with its ability to reduce firing of these neurons via presynaptic stimulation of α_2-adrenoceptors (3,36). Acute or repeated clonidine administration before (25,26,32,37,38) or after (28,36,39) the cessation of morphine treatment effectively decreases both spontaneous and naloxone-precipitated withdrawal symptoms. Accordingly, in the present study, self-administration of clonidine in the drinking water for 4 days during the dosing interval attenuated the spontaneous withdrawal associated with intermittent morphine treatment in rats. Reduced water consumption in the clonidine groups suggests achievement of adequate amounts of clonidine, as the drug was shown to elicit marked reduction in water intake (23,40). It has been hypothesized that clonidine coadministration during long-term morphine treatment might diminish noradrenergic hyperactivity and consequently the development of dependence and withdrawal signs (37). Distinct from the spontaneous withdrawal, the severity of naloxone-precipitated weight loss and jumping did not differ between morphine and clonidine + morphine groups. This finding implies that clonidine self-administration during dosing interval could not affect the development of dependence on morphine. Repeated coadministration of clonidine with morphine has been shown to either decrease (38)

or potentiate (37) naloxone-precipitated withdrawal symptoms. Neither of these contradictory findings was supported by this study.

Considered a response to stress, novelty-induced grooming has long been studied in neurobehavioral stress research in mice and rats (41,42). Stress-evoked alterations in grooming activity were reported with dramatic increases in its frequency and duration (43,44). It has been shown that withdrawal syndrome in morphine-dependent rats is combined with the development of stress, reflected by increased duration of grooming behavior (45,46). Our results, however, do not imply a "stress-like" state associated with spontaneous withdrawal syndrome in animals treated intermittently with morphine (18). We found that neither the duration (Figures 1 and 2) nor the frequency (Figure 3) of grooming activity increases in the novel environment in morphine-treated male and female rats and mice. Instead, rats had reduced grooming duration at the end of the 14-h dosing interval (Figure 1), and both M-20/100 male and female mice had reduced grooming frequency at the end of the 7-h dosing interval (Figure 3). These results suggested sufficient blood morphine levels after dosing intervals to affect grooming, since grooming decreases in the first week of a repeated morphine treatment regimen after animals receive daily morphine injections (47). On the other hand, it has been shown that injection of clonidine into the brains of rats decreases grooming duration (48). Its administration in drinking water, however, could not produce a similar effect (Figure 1). Interestingly, grooming duration did not differ between the morphine + clonidine and control groups. Since clonidine has a sedative effect (48), this finding might suggest an antistress activity during dose intervals. Further experimentation using a detailed analysis of grooming behavior, such as its 'qualitative' or patterning characteristics (49,50), or other measures of anxiety, such as elevated plus-maze behavior (51), may provide a better assessment of stress and antistress effects associated with the intermittent morphine treatment regimen.

In conclusion, intermittent injections of increasing doses of morphine produce manifestations of spontaneous withdrawal at the end of the dosing interval in rats and mice, attenuated by administration of clonidine during dose intervals. Novelty-induced grooming behavior in the animals, however, could not reflect a withdrawal-induced stress associated with the treatment regimens. Absence of significant spontaneous withdrawal signs and presence of somewhat less pronounced naloxone-precipitated withdrawal signs in female mice appear as sex- and species-related differences in the development of physical dependence on morphine. On the other hand, opiate withdrawal syndrome powerfully motivates drug-seeking and abuse in humans (2). Therefore, spontaneous withdrawal associated with intermittent morphine treatment in animals needs further evaluation for its possible role in maintaining continued drug use and response to drugs possessing sedative activity.

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