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Sex differences in locomotor effects of morphine in the rat

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Abstract

Sex differences in reinforcing, analgesic and other effects of opioids have been demonstrated; however, the extent to which sex differences in motoric effects of opioids contribute to apparent sex differences in their primary effects is not known. The goal of this study was to compare the effects of the prototypic mu opioid agonist morphine on locomotor activity in male vs. female rats. Saline or morphine (1–10 mg/kg) was administered s.c. to adult Sprague–Dawley rats, which were placed into a photobeam apparatus for 3–5 h to measure activity. Modulation of morphine's effects by gonadal hormones and by handling (either during the test session or for 4 days before the test session) were examined. Morphine initially suppressed and later increased locomotor activity in both sexes relative to their saline-injected controls, but males were more sensitive than females to the initial locomotor suppressant effect of morphine. Intermittent, brief handling during the 3-h test session blunted morphine-induced locomotor activation in both sexes. Females in proestrus were the most sensitive to morphine's locomotor-stimulant effect, with females in estrus showing the least response to morphine. Gonadectomized (GDX) males with or without testosterone were equally sensitive to morphine's effects, whereas GDX females treated with estradiol showed a blunted response to morphine's effects, similar to intact females in estrus. Brief handling on each of 4 consecutive days pre-test attenuated morphine's locomotor suppressant effect in males but had no effect in females, thereby eliminating the sex difference. These data suggest that sex differences in morphine's effects on locomotor activity can be attributed to gonadal hormones in females, and to differential stress-induced modulation of morphine's effects in males vs. females.

Keywords: Gender; Opioids; Estrous cycle; Estradiol; Testosterone; Activity

1. Introduction

Sex differences in behavioral effects of opioids are widely reported in animal studies. For example, morphine and other opioids may be more potent, and in some cases more efficacious, in male than female rodents in tests of antinociception (for review, see Craft, 2003). Sex differences in the effects of morphine or other mu opioid agonists also have been shown in tests of drug reinforcement (Klein et al., 1997; Cicero et al., 2000, 2003; Craft et al., 2001a; Lynch and Carroll, 1999; Carroll et al., 2002), feeding (Marrazzi et al., 1996), drug discrimination (Craft et al., 1996, 1999), and urinary retention (Craft et al., 2000). The behaviors measured in some of these studies may be influenced by opioid effects on motor activity. For example, we have found that morphine is more potent in males than females in suppressing operant responding, which may underlie apparent sex differences in morphine's discriminative (Craft et al., 1998) and rewarding (Craft et al., 2001b) effects. Because some analgesia assays also involve supraspinally integrated behavioral responses (e.g., hotplate and formalin tests), it is important to determine to what extent sex differences in motoric effects of mu opioids may contribute to sex differences in their other behavioral effects. Additionally, if mu opioids are more potent or efficacious in one sex or the other in producing both therapeutic effects and undesirable side-effects such as sedation or agitation (hyperactivity), then they may not be preferentially useful in one sex over the other.

The present study was designed to characterize sex differences in the effects of morphine on locomotor activity in the rat. Initially, gonadally intact, adult male and female rats were given saline or one of several doses of morphine, and locomotor activity was assessed for 3–5 h post-injection using a standard photobeam apparatus. Because there is substantial evidence for sex differences in stress responses (to handling and other stressors) (Faraday, 2002; Kudielka and Kirschbaum,

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2005), half of the rats of each sex were handled intermittently during testing, and the other half were not (Experiment 1). Because some laboratories routinely handle subjects before experimentation and others do not, we also examined whether 4 days of handling *before* testing would alter the observed sex difference in morphine's effect on locomotor activity (Experiment 4). We used two approaches to determine whether morphine's locomotor effects were influenced activationally by gonadal steroid hormones. First, gonadally intact females were tested in different stages of the estrous cycle (Experiment 2). Then a hormone manipulation study was conducted to examine the effects of testosterone (T) in males, and estradiol (E2) in females (Experiment 3); several previous studies have shown that these hormones can modulate morphine's antinociceptive effects in adult rats (e.g., Berglund et al., 1988; Ratka and Simpkins, 1991; Stoffel et al., 2003; but see Cicero et al., 2002).

2. Materials and methods

The research described herein complies with the guidelines for conducting animal research at Washington State University (LARC #2354, 3085) and as outlined in the "Guide for the Care and Use of Laboratory Animals" (http://www.nap.edu/readingroom/books/labrats).

2.1. Subjects

Subjects were Sprague–Dawley rats (bred in-house from Taconic stock or purchased from Taconic, Germantown, NY). Rats were 3–6 months old at the time of testing. Rats were housed in same-sex pairs in the vivarium, which was maintained at 21±2 °C, on a 12:12 h light:dark cycle (lights on at 0600 h).

2.2. Apparatus

Locomotor activity was measured using a clear Plexiglas cage (20×40×23 cm) placed into a photobeam apparatus (Opto-Varimex, Columbus, OH); the 15 photobeams that crossed the width of the cage were 2.5 cm apart and 8 cm above the cage floor.

2.3. Procedure

All tests were conducted during the light part of the cycle, between approximately 0800–1500 h (2–7 h after lights on).

2.3.1. Surgery

All rats in Experiment 3 were gonadectomized (GDX). Rats were anesthetized with Equithesin i.p. (active ingredients: sodium pentobarbital, 28.2 mg/kg and chloral hydrate, 123.3 mg/kg), and gonadectomies were conducted as described previously (Stoffel et al., 2003).

2.3.2. Steroid hormone replacement

Chronic hormone replacement (Experiment 3) was achieved via Silastic® capsules implanted s.c. between the shoulder blades immediately following gonadectomy. Males received one 2-mm, blank capsule or one 2-mm, T-filled capsule/100 g of body weight; females received one 1-mm, blank or E2-filled capsule. Plasma hormone levels after Silastic capsule implantation (unincubated capsules) reach steady-state within a few days, and are relatively stable when measured approximately 3 and 10 weeks later (Smith et al., 1977). In the present study, behavioral testing occurred 4 weeks after capsule implantation, to ensure that gonadal steroid hormone levels and resultant behavioral changes were stable. The specific hormone replacement protocol used in the present study yields rats that are similar in reproductive behavior and physiology to gonadally intact rats (Stoffel et al., 2003).

2.3.3. Experimental protocol

In Experiment 1 (morphine dose- and time-effect curves), gonadally intact rats were brought from the vivarium to the testing room immediately before testing. Each rat was weighed and injected s.c. with saline or a single dose of morphine (1.0, 3.2 or 10 mg/kg), and immediately placed into the locomotor apparatus. The number of photobeams broken every 30 min for 3 h was recorded. Half of the male and half of the female rats were briefly handled every 30 min ("handled" groups): immediately after recording photobeam breaks, rats were removed from the locomotor chambers, injected s.c. with saline (1 ml/kg), and then returned to the locomotor chambers, whereupon the next 30-min measurement period began. The other half of the rats were left in the locomotor chambers undisturbed for the entire 3 h ("unhandled" groups). Because 10 mg/kg suppressed locomotor activity for over 2 h in most groups, a subset of rats in each group that were initially injected with saline or 10 mg/kg morphine were tested out to 5 h post-injection (with photobeam breaks recorded every hour for the last 2 h-long periods), to determine whether hyperactivity would be observed at later time points. A vaginal smear was obtained immediately after testing each female.

In Experiment 2 (locomotor effects of morphine in cycling females), gonadally intact female rats were used. Vaginal smears were obtained to determine estrous stage; rats in proestrus, estrus or diestrus-1 were injected s.c. with saline or 10 mg/kg morphine, and immediately placed into locomotor chambers. Locomotor activity was recorded every 30 min for 3 h; thus, these females were "unhandled" as in Experiment 1, except for the fact that many were handled briefly for more than one day before testing, in order to obtain vaginal smears. A second vaginal smear was obtained immediately after post-test; data from rats that had switched estrous stages from before to after testing were not included in analyses. Females in diestrus-2 were not tested because hormonally they are very similar to females in diestrus-1 (Feder, 1981).

In Experiment 3 (effect of gonadal steroid hormones on locomotor effects of morphine), all rats were GDX as described above, and implanted with either blank or hormone-filled capsules. Twenty-eight days later, rats were brought to the testing room, weighed, injected with saline or morphine (10 mg/kg), and immediately placed into the locomotor chambers. Locomotor activity was recorded every 30 min for 3 h.

In Experiment 4 (effect of pre-test handling on locomotor effects of morphine), gonadally intact rats were used. Half of the male and half of the female rats were assigned to the "naive" group, and the other half of rats of each sex were assigned to the "handled" group. Rats in the "naive" group were brought to the testing room, weighed, injected with saline or morphine (10 mg/kg) and immediately placed into the locomotor chambers (similar to "unhandled" rats in the Experiment 1). Locomotor activity was recorded every 30 min for 3 h. Rats in the "handled" group were handled for 4 consecutive days before testing, as follows: each day at the same time, rats were brought to the testing room, weighed, injected with saline, and returned to their home cages but left in the testing room. Three hours later the home cages were returned to the vivarium. On the fifth day, rats in the "handled" group were brought to the testing room, weighed, injected s.c. with saline or morphine (10 mg/kg), and tested for locomotor activity in the same way as the "naive" rats.

2.4. Vaginal cytology

To assess gonadal steroid hormone state in female rats, vaginal smears were taken after (Experiments 1 and 4) or before and after (Experiment 2) measuring locomotor activity. Proestrus was identified by the prevalence (approximately 75% or more of epithelial cells in sample) of nucleated epithelial cells; estrus was identified by the prevalence of dense sheets of cornified epithelial cells; diestrus-1 was identified by the presence of leukocytes and scattered nucleated and cornified epithelial cells, and diestrus-2 was identified by a relative lack of cells in the sample (Freeman, 1988). To keep the stages highly distinct in Experiment 2, females whose vaginal cytology indicated that they were in transition from one estrous stage to another were not tested. In order to represent a "normal" sample of females, testing was not limited to 4-day cyclers. Thus, the designation of diestrus-1 is based on vaginal cytology alone, and does not necessarily indicate the day after estrus. Locomotor activity was tested only once in each rat, but vaginal smears were taken an average of three times (on three days, usually consecutive) before a given rat was tested in Experiment 2.

2.5. Drugs

Morphine sulfate was obtained from the National Institute on Drug Abuse (Bethesda, MD) and from Sigma Chemical Co. (St. Louis, MO), and dissolved in 0.9% physiological saline. Injections were given s.c. in a volume of 1 ml/kg. Steroid hormones (Steraloids, Newport, RI) were administered via Silastic® capsule (inner diameter=0.062 in.; outer diameter=0.125 in.) prepared as previously described (Stoffel et al., 2003).

2.6. Data analysis

In each experiment, the number of photobeam breaks was recorded once every 30 min (or once/h for time points beyond 3 h, in Experiment 1). Because there were some group differences in activity in the absence of drug (after saline injection), morphine data were converted to % control at each time point before analyzing: (# photobeam breaks after morphine / mean # photobeam breaks after saline) × 100. Saline and morphine data were analyzed by repeated measures ANOVA followed by independent samples *t*-tests using the Bonferroni correction, to determine at what time points group differences occurred. In Experiments 1 and 4, the two sexes were directly compared (entered as a main factor in the ANOVA), whereas in Experiment 3, data from each sex were analyzed separately, as the effect of the sex-specific gonadal hormone manipulation was the primary variable of interest. Significance level was $p<0.05$.

3. Results

3.1. Morphine dose- and time-effect curves: effect of within-session handling (Experiment 1)

Fig. 1 shows locomotor activity over 3 h in gonadally intact, adult male and female rats injected with saline. Females were significantly more active than males, primarily within the first 30 min (sex × time: $F(5,180)=4.75$, $p<0.001$). Not surprisingly, rats that were briefly handled every 30 min showed more activity than rats that were left undisturbed during the 3-h session (handling: $F(1,36)=8.96$, $p=0.005$). Although handling increased activity in both males and females, this effect was statistically significant in males ($p=0.002$) but not females ($p=0.15$).

Because handling did not differ before the first 30-min sampling period, data from the "handled" and "unhandled" groups were pooled for dose–response analysis of morphine's early, locomotor-suppressant effect. Fig. 2 shows morphine's effect during the first 30 min in males vs. females, plotted as % of saline-treated controls. Morphine dose-dependently decreased locomotion in both sexes, but this effect was

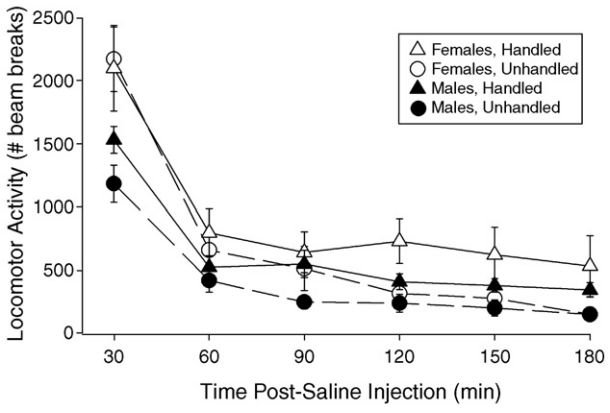

Fig. 1. Locomotor activity in gonadally intact male (closed symbols) and female (open symbols) rats treated with s.c. saline. Half of the rats of each sex were briefly handled every 30 min ("handled", triangles) or left undisturbed ("unhandled", circles) during the 3-h session. The number of photobeam breaks was recorded every 30 min for 3 h. Each point is the mean ± 1 S.E.M. of 8–10 female or 12 male rats.

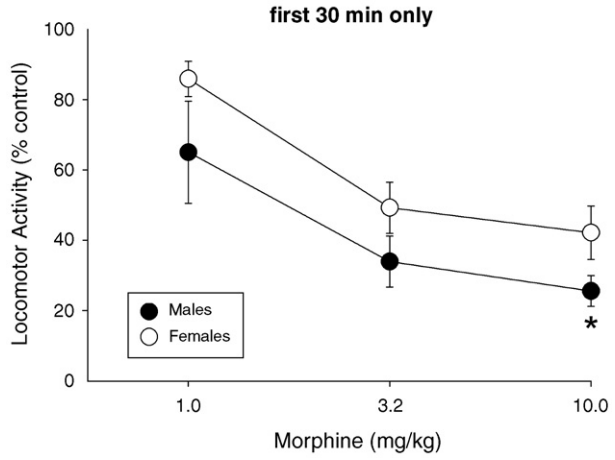

Fig. 2. Morphine-induced suppression of locomotor activity during the first 30 min post-injection in gonadally intact male and female rats, plotted as % of same-sex, saline-treated controls. Because all rats were handled the same way before the first 30-min sampling period, data from rats in "unhandled" and "handled" groups were pooled for this analysis. Thus, each point is the mean ± 1 S.E.M. of 16–18 female or 16–22 male rats. *Significant sex difference, $p<0.05$.

significantly greater in males than in females ($F(1,99)=8.16$, $p=0.005$). This sex difference does not reflect a decrease in absolute number of photobeam breaks that was the same between males and females; rather, the mean absolute difference between saline- and morphine (10 mg/kg)-treated rats was 982 beam breaks in males and 1237 beam breaks in females.

Fig. 3 shows locomotor activity over the entire 3-h session in morphine-treated male and female rats that were briefly handled every 30 min ("handled") or not ("unhandled"), plotted as % of their respective saline-treated controls. Morphine initially suppressed and then increased locomotion in both sexes; the magnitude and duration of morphine's biphasic locomotor effects increased as the dose of morphine increased. Sex differences in morphine's effect on locomotion were time- and handling-dependent (sex × time × handling: $F(5,465)=7.05$, $p<0.001$), with handling effects emerging at 90–120 min post-injection, and being greater in males than females at 1.0 mg/kg, but greater in females than in males at 3.2 and 10 mg/kg.

The high dose of morphine, 10 mg/kg, suppressed locomotion longer in unhandled males than females; thus, it is possible that sex differences in hyperactivity observed at later time points were simply due to a delayed hyperactivity effect in males. To examine a more complete time course of effect after 10 mg/kg morphine, locomotor activity was also recorded at the 4-h and 5-h time points in approximately half of the rats in each of the groups injected with 10 mg/kg morphine. At these later time points, activity in unhandled females began to return towards baseline levels (from approximately 1100% of control at 3 h to approximately 400% of control at 5 h). In contrast, activity in unhandled males continued to climb (from approximately 400% of control at 3 h to approximately 1200% of control at 5 h post-injection). Thus, within a 5-h sampling period, 10 mg/kg morphine produced approximately the same maximal hyperactivity in unhandled males as in unhandled females, but it occurred 2 h later in males. Activity in handled males and females injected with 10 mg/kg morphine did not differ at 4–5 h post-injection (data not shown), and did

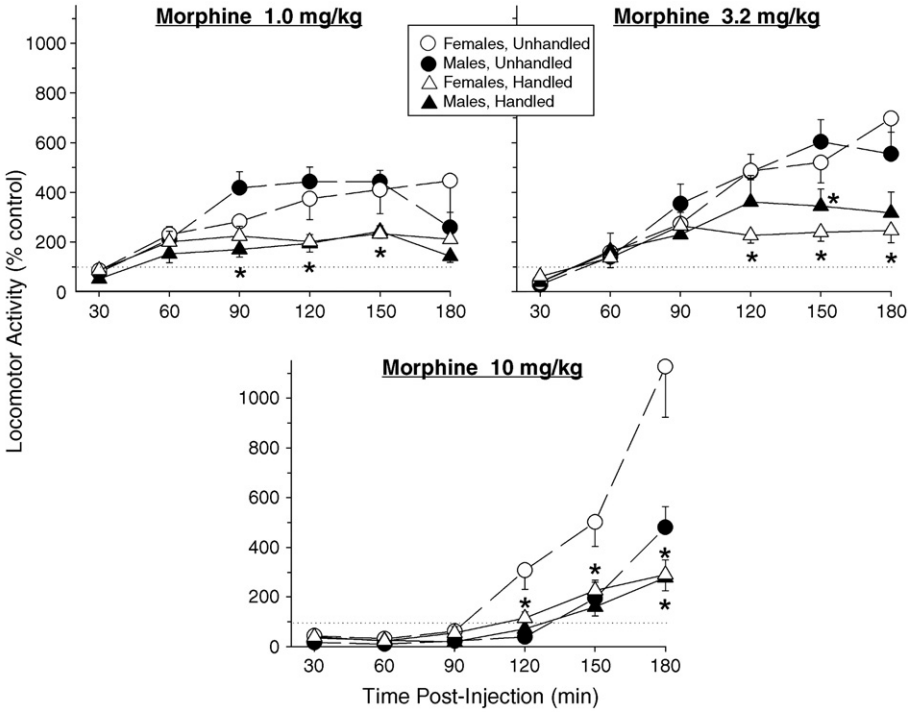

Fig. 3. Locomotor activity in gonadally intact male and female rats treated with s.c. morphine, plotted as % of same-sex, same-handling condition, saline-treated controls. Each point is the mean ± 1 S.E.M. of 8–10 female or 8–12 male rats. *Significant handling effect, within-sex, $p<0.05$; at the 1.0 mg/kg dose, *indicates a significant handling effect in males only.

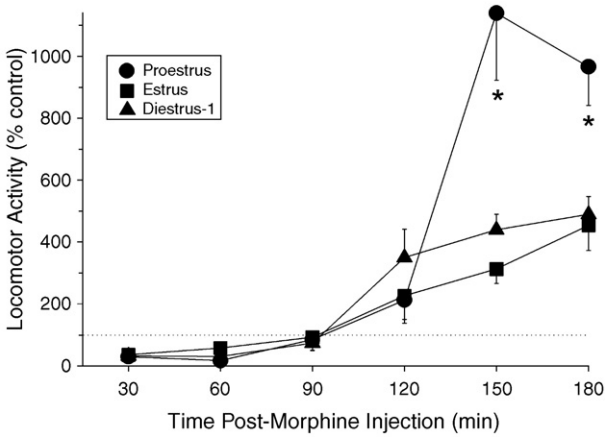

Fig. 4. Locomotor activity in gonadally intact females tested during various stages of the estrous cycle. Activity in rats tested during proestrus, estrus or diestrus with 10 mg/kg s.c. morphine is plotted as percent of same-stage, saline-treated controls. Each point is the mean±1 S.E.M. of 8–9 rats. *Activity significantly greater than rats tested in estrus or diestrus-1, $p<0.05$.

not change appreciably from that observed at 3 h post-injection (approximately 300% of control, Fig. 3). Therefore, the "blunting" effect of handling on hyperactivity produced by the 10 mg/kg dose of morphine that was clearly observed in females by 2–3 h post-injection was also observed in males, but not until 4–5 h post-injection.

When examined immediately after testing, the distribution of females among estrous stages was not equivalent across all groups, precluding analysis of estrous stage effects on activity in Experiment 1. For example, the "unhandled" and "handled" groups tested with 10 mg/kg morphine had 3 females each in proestrus, whereas the groups tested with lower doses of morphine each had 0 or 1 female in proestrus. Thus, a separate experiment was conducted to address the question of whether morphine's locomotor effects varied with estrous stage in females.

3.2. Effect of estrous stage on morphine's locomotor effects (Experiment 2)

Among saline-treated females, locomotor activity did not vary significantly by estrous stage (data not shown). Fig. 4 shows the effects of 10 mg/kg morphine in gonadally intact females tested in various stages of the estrous cycle, plotted as percent of same-stage, saline-treated controls. Proestrus females were significantly more sensitive to the locomotor-activating effects of morphine at 150–180 min post-injection than females tested in either estrus or diestrus-1 (estrous stage × time: $F(5,105)=12.81$, $p<0.001$). Estrus females were the least sensitive to morphine's locomotor-suppressing and locomotor-activating effects, although the differences between estrus and diestrus females were not statistically significant.

3.3. Effect of gonadal steroid manipulation on morphine's locomotor effects (Experiment 3)

Among saline-treated rats, locomotor activity was greater in GDX females treated with E2 than in GDX females without hormone replacement ($F(1,16)=7.50$, $p=0.015$), whereas T treatment did not affect activity in GDX males (data not shown). Fig. 5 shows the effects of 10 mg/kg morphine on locomotor activity in GDX rats with and without E2 (females, top) or T (males, bottom) replacement, plotted as percent of same-sex/hormone condition, saline-treated controls. E2 treatment in GDX females slightly attenuated morphine's initial suppressant effect and significantly attenuated morphine's later stimulant effect (E2 × time: $F(5,75)=15.33$, $p<0.001$), whereas T treatment in GDX males did not significantly modulate morphine's effect on locomotion. Thus, E2-treated GDX females responded to morphine similarly to intact females tested during estrus (compare Figs. 4 and 5).

3.4. Effect of pre-test handling on morphine's locomotor effects (Experiment 4)

Pre-test handling for 4 days had no significant effect on activity in saline-treated female or male rats (data not shown). Fig. 6 shows the effects of 10 mg/kg morphine on locomotor activity in rats that were handled for 4 days before testing ("handled") vs. rats that were tested immediately ("naïve"), plotted as percent of saline-treated, same-sex controls. Four days of pre-test handling modulated morphine's effect on locomotor activity in males but not females, in a time-dependent manner (sex × handling × time: $F(5,140)=10.74$, $p<0.001$).

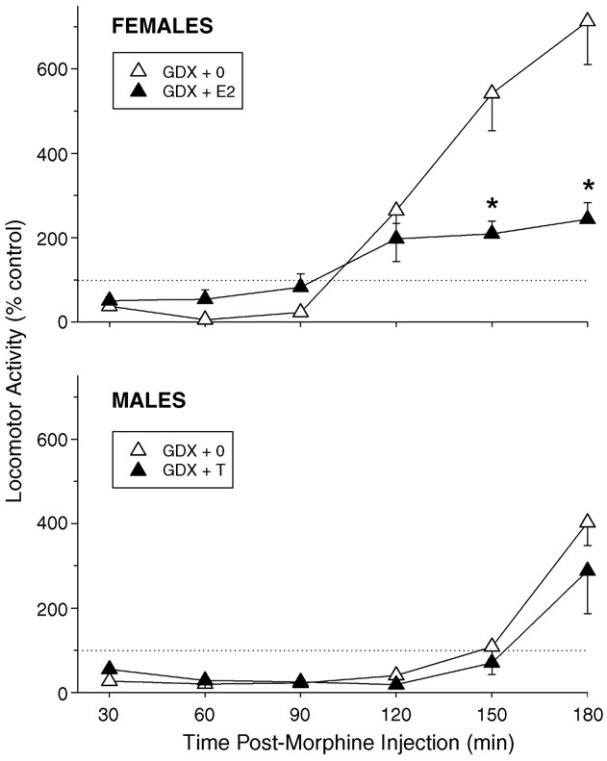

Fig. 5. Spontaneous locomotor activity in gonadectomized (GDX) rats treated with no hormone (0) or estradiol (E2) or testosterone (T) for 28 days before testing. Activity in rats injected s.c. with 10 mg/kg morphine is plotted as percent of same-sex/hormone group, saline-treated controls. Each point is the mean±1 S.E.M. of 8–9 rats. *Activity significantly different from GDX+0 rats of same sex, $p<0.05$.

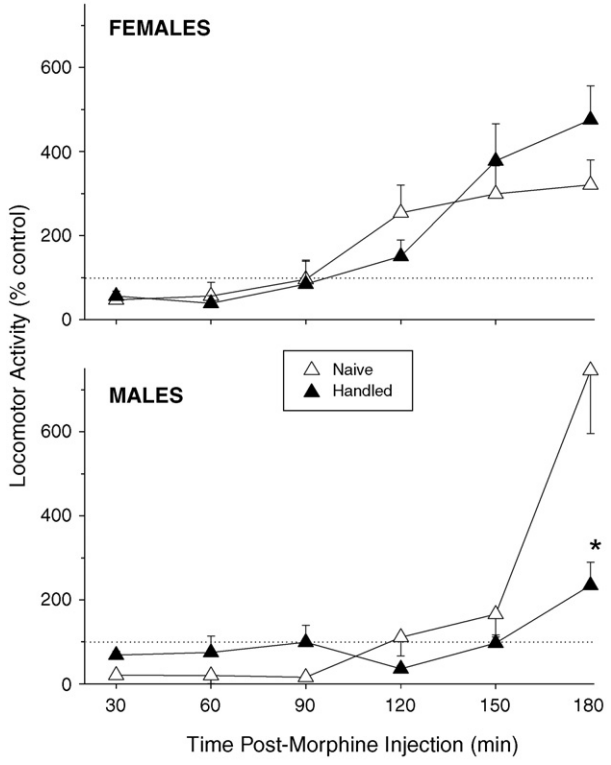

Fig. 6. Spontaneous locomotor activity in female and male rats that were handled for four days before testing ("handled"), or tested immediately upon removal from the vivarium ("naïve"). Activity in rats injected s.c. with 10 mg/kg morphine is plotted as percent of same-sex/handling group, saline-treated controls. Each point is the mean ± 1 S.E.M. of 8 rats. *Activity significantly different from "naïve" rats of same sex, $p<0.05$.

Specifically, morphine produced similar effects in handled vs. naïve females, whereas handling in males blunted morphine's early suppressant and later stimulant effects (males, handling × time: $F(5,70)=12.72$, $p<0.001$).

When examined immediately post-test, females in this experiment were in the following estrous stages: in each female group there was 1 proestrus, 0–1 estrus, 4–5 diestrus-1, and 2 diestrus-2. Thus, there were fewer proestrous females in this experiment than those tested with 10 mg/kg in Experiment 1.

4. Discussion

The primary findings of the present study are: (1) morphine produced greater and longer-lasting locomotor suppression in males than in females; (2) females in proestrus were more sensitive than females in estrus or diestrus-1 to morphine's locomotor-activating effect; (3) chronic E2 treatment decreased GDX females' sensitivity to morphine's locomotor-activating effect, whereas chronic T treatment did not affect GDX males' response to morphine; and (4) handling on each of several days *before* testing blunted the biphasic effects of morphine in male but not female rats; however, handling *during* testing blunted morphine's hyperactivity effect in both sexes.

Under baseline (saline) conditions, females were significantly more active than males. Sex differences in basal activity are sometimes but not always reported in rodents; when a sex difference is reported, females are found to be more active than males (e.g., Blizard et al., 1975; Beatty, 1979; Jones et al., 1990; van Haaren and Meyer, 1991; Craft et al., 1996; Craft and Bernal, 2001; but see Forgie and Stewart, 1994; Pearl et al., 1997; Sell et al., 2000; Schindler and Carmona, 2002). Although estrous cycle-related fluctuations in activity have been reported previously (e.g., Scimonelli et al., 1999), other laboratories report no significant differences (e.g., Quiñones-Jenab et al., 1999; Sell et al., 2000; present study). Becker et al. (1987) has shown that sensorimotor performance (coordination) rather than simply the amount of movement per se changes across the estrous cycle; another study also showed that estrous-related changes in movement depend on the type of movement measured, and may not be seen when only horizontal activity is assessed (Steiner et al., 1981). It should be noted that a limitation of the present study was the use of a single measure of activity, horizontal locomotion; whether sex differences exist in other measures of activity such as spatial pattern, rearing, duration of activity bouts, etc., remains to be determined.

The present study demonstrates some significant sex differences in the effects of morphine on locomotor activity. In both sexes, morphine produced the expected biphasic effects on activity — early decreases (suppression) and later increases (hyperactivity); however, the early locomotor suppression was more pronounced and longer-lasting in males. Although morphine-induced hyperactivity was greater in unhandled females than males at the 10 mg/kg dose when assessed out to 3 h post-injection, examination of later time points suggested that morphine-induced hyperactivity was simply delayed in males. Presumably the prolonged morphine-induced locomotor suppression in males at this dose delayed the emergence of hyperactivity until 4–5 h post-injection. Sex differences in both locomotor suppression and stimulation observed in the present study generally agree with those observed in previous studies. For example, 10 mg/kg morphine decreased activity more in male than female rats during the first hour post-injection, and only increased activity during the second hour in females (Stewart and Rodaros, 1999). Morphine was also more potent in male than female rats in decreasing operant responding for food (Craft et al., 1996, 1998) and for brain stimulation (Craft et al., 2001a), when measured within an hour after injection. Sex differences in morphine's locomotor-suppressant effects are likely mediated by central mechanisms. When administered directly into the rostral ventromedial medulla, morphine produced greater locomotor suppression in male than in female rats, measured 15–20 min post-injection (Boyer et al., 1998). Similarly, when administered into the periaqueductal gray (PAG), β-endorphin was more potent in suppressing locomotion in male than in (estrous) female rats during the first 30 min post-injection (Krzanowska and Bodnar, 2000).

The present results suggest that gonadal hormones modulate morphine's effect on locomotor activity in females but not males. First, morphine's effects on locomotor activity fluctuated across the estrous cycle in gonadally intact females. Proestrous and estrous females were the most and least sensitive, respectively, to morphine-induced hyperactivity. In fact, the substantial hyperactivity observed in "unhandled" females

tested with 10 mg/kg in Experiment 1 may be due in part to the fact that 3 of 10 females were in proestrus during testing, whereas those tested at the lower morphine doses were primarily in other estrous stages. Conversely, the *lack* of extreme hyperactivity in females tested in Experiment 4 (both "naïve" and "handled" groups) may be attributed to the fact that most were in stages other than proestrus at the time of testing. Second, GDX females treated chronically with E2 were significantly less sensitive to morphine's effects than GDX females without E2 replacement. We have shown previously that the 28-day E2 treatment used in the present study produces a female that, in terms of reproductive behavior/physiology and sensitivity to morphine antinociception, is more similar to a cycling female in estrus than one in proestrus (Stoffel et al., 2003). This appeared to be true in the present study as well: E2 treatment in GDX females slightly attenuated morphine-induced locomotor suppression and significantly attenuated morphine-induced hyperactivity; among the cycling females, those in estrus were the least sensitive to morphine's biphasic locomotor effects. In a previous study, the locomotor-activating effect of intra-PAG β-endorphin was also blunted in estrous females compared to males (females in other stages were not tested: Krzanowska and Bodnar, 2000). In contrast to females, testosterone treatment in GDX males did not significantly affect their response to morphine. A previous study also showed that testosterone treatment only slightly attenuated morphine's sedative effect in the first hour (Day 1: Stewart and Rodaros, 1999), whereas E2 treatment has been shown to enhance or blunt morphine's stimulant effect in GDX female rats, depending on the timing of testing relative to E2 treatment, and perhaps the dose of E2 (Nomikos et al., 1987; Stewart and Rodaros, 1999). The present results in cycling females suggest that morphine's locomotor effects change rapidly as ovarian hormone state changes, similar to the effects of estrous cycle on sensitivity to morphine-induced antinociception (Berglund and Simpkins, 1988; Stoffel et al., 2003).

Handling of subjects had different effects depending on whether the handling occurred before or during locomotor testing with morphine. Brief handling on each of the 4 days preceding testing did not affect activity in saline-treated rats, yet altered morphine's locomotor effects in a sexually dimorphic manner: pre-test handling dampened the locomotor-suppressant and -activating effects of morphine in males but had no effect in females (Experiment 4). In contrast, handling rats intermittently during testing (Experiment 1) blunted the locomotor-activating effects of morphine in both sexes. These results suggest that acute stress responses significantly modulate morphine's locomotor effects, and these stress responses can be sex-specific. Previous reports provide additional evidence for this hypothesis. For example, footshock stress has been found to increase freezing behavior in male but not female rats, and this behavior appears to be opioid receptor-mediated (Klein et al., 1998). Sex differences in stress-induced enhancement of opioid analgesia are also widely reported. For example, conditioned fear-induced enhancement of morphine analgesia was greater in male than in female rats (Stock et al., 2001), repeated vehicle injections eliminated the sex difference in swim stress-induced analgesia in rats (Romero et al., 1988), and a variety of stressors, including novel environment (Kavaliers and Innes, 1988), predator (Kavaliers and Colwell, 1991) and biting fly (Kavaliers et al., 1998) exposure all produced greater opioid receptor-mediated analgesia in male than in female deer mice. Taken together, these studies suggest that males are more sensitive than females to acute stress, and that such stress can enhance behavioral effects of opioids. Stress-induced enhancement of opioid analgesia has been shown to be mediated by leu-enkephalin activation of delta opioid receptors in mice (Kavaliers and Innes, 1993; Vanderah et al., 1993). To determine whether a similar mechanism might underlie sex differences in stress-induced enhancement of morphine's locomotor-suppressant effect in males, we pretreated rats with the delta receptor-selective antagonist naltrindole, but did not observe sexually dimorphic antagonism (unpublished results). There are also well-documented sex differences in hypothalamic-pituitary–adrenal (HPA) activation in the rat, with females generally showing greater adrenocorticotropic hormone and corticosterone responses to stressors than males (e.g., Aloisi et al., 1994; Jezˇová et al., 1996; Kuhn and Francis, 1997; Ogilvie and Rivier, 1997). It is not known whether the sexually dimorphic HPA response to stress contributes to the observed sex difference in morphine's locomotor-suppressant effect observed in the present study. Furthermore, estrous cycle may influence females' stress reactivity, and this was not assessed in the present study. A further limitation of the present results was the use of only one strain of rat. It has been shown previously that sex differences in stress responses may differ across strains; for example, Faraday (2002) reported that whereas Long–Evans females and males habituated to the locomotor-suppressant effect of repeated restraint stress, Sprague–Dawley females did not, remaining significantly different from Sprague–Dawley males throughout the weeks-long experiment.

A likely mechanism underlying sex differences in morphine's locomotor-activating effect is sex differences in dopaminergic function. Opioid-induced locomotion is due to mu opioid receptor-mediated increases in striatal dopamine (DA) release (e.g., Kalivas and Duffy, 1987; Johnson and Glick, 1993; Manzanedo et al., 1999; Piepponen et al., 1999), and females show enhanced striatal dopaminergic activity compared to males, particularly after administration of another class of locomotor-activating drugs, the psychostimulants (Becker, 1999; Laakso et al., 2002). Furthermore, the nigrostriatal DA system is known to be enhanced by estradiol in females, and not hormonally modulated in males (e.g., Becker, 1990, 1999); thus, the failure of T to modulate morphine's effects in males, and the ovarian hormone modulation of morphine's hyperactivity effects in females in the present study likely reflect these underlying differences in the dopaminergic system.

Sex differences in locomotor effects of morphine observed in the present study, and their apparent modulation by handling suggest that sex differences in other behavioral effects of mu opioids (e.g., reinforcing and analgesic effects) are likely influenced, at least in part, by differential motoric effects of

opioids in males and females as well as sex-specific responses to handling (stress). Specifically, the present results suggest that acute behavioral tests conducted without much handling of the animals before testing, in which mu opioid effects are evaluated within a short period post-injection, may be confounded by the greater sedative effect of these drugs in males than in females. Conversely, when opioid effects are evaluated over longer periods of time (depending on the duration of action of the particular opioid), the greater locomotor activation observed in proestrous females compared to males could contribute to sex differences in other behaviors. For example, it is possible that both greater sedation in males and greater hyperactivity in females could explain the findings of greater reinforcing effects of mu opioids in females compared to males (Klein et al., 1997; Cicero et al., 2000, 2003; Craft et al., 2001a; Lynch and Carroll, 1999; Carroll et al., 2002). In this regard, when we eliminated the sex difference in morphine's effect on rates of responding in a morphine discrimination procedure through use of a variable interval schedule, the apparent sex difference in morphine discrimination disappeared (Craft et al., 1996, 1998). Although we have not found that sex differences in opioid antinociception are readily explained by sex differences in the effects of these drugs on locomotion (Craft and Bernal, 2001), in this previous study we examined locomotion only at 30–50 min post-injection. We are currently conducting studies to determine whether pre-test handling alters sex differences in morphine's antinociceptive effects.

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