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Assessment of acute and chronic morphine dependence in male and female mice

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Abstract

The present study compared male and female mice for frequency of naloxone-precipitated jumping and naloxone ED_{50} values, two common indices of physical dependence, following acute and chronic morphine administration. Both sexes displayed a positive dose–response relationship between acute morphine and naloxone doses and jumping frequency. There was a significant main effect of sex, with mean jumping frequencies greater in males. The naloxone ED_{50} estimate was also fourfold lower in males, indicating greater withdrawal sensitivity than females. Jumping frequencies were similar in male and female saline-treated control mice, discounting initial sex differences as a significant factor in the unequal magnitude and sensitivity in acute morphine dependence between sexes. In contrast, males and females displayed similar mean withdrawal jumping frequencies and naloxone ED_{50} values after 3 days of morphine injections. Sex difference in withdrawal jumping was also not observed when morphine treatment was increased to 7 days via daily injection or continuous subcutaneous infusion. The present study demonstrates the development of greater physical dependence in male relative to female mice following acute but not chronic morphine administration.

Keywords: Sex differences; Naloxone-precipitated withdrawal; Morphine dependence

1. Introduction

Physical dependence is an unwanted side effect of morphine administration that is manifested by a characteristic withdrawal syndrome of multiple aversive behavioral and physiological signs in a wide variety of species. Withdrawal is typically observed following abrupt termination of morphine intake or precipitated by administration of a narcotic antagonist such as naloxone. Among withdrawal behaviors in rodents, jumping is widely considered the most sensitive and reliable index of withdrawal intensity and is the most commonly used (El-Kadi and Sharif, 1994; Blum et al., 1976; Ritzmann, 1981; Smits, 1975; Saelens et al., 1971; Blasig et al., 1973; Way et al., 1969; Marshall and Weinstock, 1969; Fernandes et al., 1977; Miyamoto and Takemori, 1993a). A comparison of morphine time–effect curves for jumping frequency and locomotor activity in mice indicate that the withdrawal jumping response is not an artifact of the stimulatory effects of morphine (Smits, 1975). Furthermore, jumping frequencies in response to opiate withdrawal in mice correlates well with the known physical dependence capacity of these drugs in man (Saelens et al., 1971), lending validity to this measure of morphine dependence. In addition to jumping frequency, a jumping response to various doses of naloxone has also been previously used to determine a median effective dose, or naloxone ED_{50} estimate, for naloxone-precipitated withdrawal (Way et al., 1969; Miyamoto and Takemori, 1993b; Fernandes et al., 1977; Huidoboro et al., 1963). These studies demonstrate an inverse relationship between the degree of dependence and the dose of an opioid antagonist to precipitate a critical threshold of withdrawal symptoms. Thus, whereas jumping frequency indexes the magnitude of the dependent state, the sensitivity to undergo withdrawal per se is reflected by naloxone ED_{50} values. Chronic morphine treatments of longer duration and/or greater cumulative dose are associated with both greater frequencies of jumping (El-Kadi and Sharif, 1994) and lower naloxone ED_{50} estimates (Fernandes et al., 1977; Way et al., 1969).

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Although physical dependence is commonly associated with chronic morphine use, symptoms of withdrawal can be precipitated by naloxone following a single exposure to morphine (Schulteis et al., 1997; Wiley and Downs, 1979; McLemore et al., 1997; Smits, 1975; Ritzmann, 1981). As is observed for chronic dependence, there is a positive dose–response relationship between acute morphine or naloxone dose and magnitude of withdrawal (e.g., frequency of jumping) (Smits, 1975; Fernandes et al., 1977; Ritzmann, 1981). Based on differences in the presence or temporal order of withdrawal signs, it has been suggested that acute and chronic morphine dependence are not unitary phenomena (Ritzmann, 1981). Acute and chronic morphine treatment also produces different effects on synaptosomal Ca^{2+} levels (Yamamoto et al., 1978), calmodulin activity (Nehmad et al., 1982), and catecholamine utilization (Kovacs et al., 1983). Furthermore, selective antagonists of the AMPA and NMDA excitatory amino acid receptor subtypes have been demonstrated to attenuate acute but not chronic morphine dependence in mice (McLemore et al., 1997; Marquis et al., 1991). Collectively, these data indicate that the neural substrates contributing to dependence after acute and chronic morphine administration, as well as the adaptive physiological effects they engender, is to some extent distinct.

Morphine has been demonstrated to produce similar effects — but of different magnitude and at different doses — in males and females on a variety of acute measures, including analgesia (see review: Kest et al., 2000c), respiration (Kest et al., 1998), hypothermia (Kest et al., 2000a; Quock et al., 1985; de-la-Cruz et al., 1987), activity (Li et al., 1990) and place conditioning (Cicero et al., 2000). The consequences of chronic morphine treatment may also differ between sex, as suggested by the differential development of tolerance to its analgesic (Kest et al., 2000b; Craft et al., 1999; Badillo-Martinez et al., 1984) and hypothermic (Kest et al., 2000a) effects in male and female rodents. Although the mechanism underlying sex differences has been the subject of many investigations (see review: Kest et al., 2000c), a cogent explanation of these differences that incorporates the documented contribution of widely disparate variables has yet to emerge. Centrally located pharmacodynamic processes have been implicated since sex differences on behavioral measures are not simply related to sex differences blood levels of morphine and its metabolites in rats (Cicero et al., 1997) and humans (Sarton et al., 2000), but also persist following acute and chronic CNS morphine microinjection (Kest et al., 1999; Boyer et al., 1998; Krzanowska and Bodnar, 1999). Despite evidence for sex differences in morphine effects on several measures, studies comparing males and females on morphine dependence are relatively scarce. In mouse studies, sex differences in dependence were not observed after 3 or 7 days of morphine treatment (Blum et al., 1976; El-Kadi and Sharif, 1994). Studies in rats, by contrast, report greater dependence in females than males after 5 and 6 days of multiple daily morphine injections (Craft et al., 1999; Ali et al., 1995). However, only limited conclusions regarding sex differences in morphine dependence may be drawn from these studies for the following reasons. First, and perhaps most importantly, sex comparisons in all of the above studies were made using only a single morphine and naloxone dose. The absence of dose–response data is of concern since the magnitude of withdrawal symptoms is morphine and naloxone dose-dependent (Gellert and Holtzman, 1978; Way et al., 1969; Marshall and Grahame-Smith, 1971; Blasig et al., 1973; Smits, 1975; Fernandes et al., 1977; Ritzmann, 1981; Miyamoto and Takemori, 1993a). Single doses also preclude calculation of a naloxone ED_{50} estimate, and thus possible sex differences in morphine withdrawal sensitivity are unknown. Lastly, despite the demonstration that acute and chronic morphine treatment produces different physiological adaptations (Yamamoto et al., 1978; Kovacs et al., 1983; Nehmad et al., 1982) and each elicits dependence that is mediated by distinct neurochemical substrates (McLemore et al., 1997; Marquis et al., 1991), males and females have only been compared for chronic morphine dependence. Thus, the present study compared male and female mice for naloxone-precipitated jumping frequency and naloxone ED_{50} estimates in both acute and chronic dependence models using a range of morphine and naloxone doses.

2. Methods

2.1. Subjects and drugs

Adult male and female CD-1 mice (Charles River, Wilmington, MA) were housed four to a cage with same-sex littermates and maintained on a 12/12 h light/dark cycle in a temperature-controlled environment with unrestricted food and water. Animals were housed at least 1 week prior to testing, which was conducted near midphotophase to minimize circadian fluctuations in morphine sensitivity (Kavaliers and Hirst, 1983). Each dose of every condition was comprised of at least 10 mice/sex and each mouse was used only once. Morphine sulfate was obtained through the Drug Supply System of the National Institute on Drug Abuse (Bethesda, MD). Naloxone hydrochloride was purchased from Sigma-Aldrich (St. Louis, MO). Both drugs were dissolved in 0.9% physiological saline and injected subcutaneously in a volume of 10 ml/kg.

2.2. Morphine and naloxone doses

Acute dependence was induced by a single injection of morphine across a range of doses (25, 50, and 100 mg/kg) on Day 1, followed 3 h later by naloxone (50 mg/kg). Previous studies have reported dose-dependent increases in jumping frequencies using naloxone doses as high, or even higher than 50 mg/kg (Smits, 1975; Fernandes et al., 1977), and we have previously obtained reliable and robust levels of responding at this naloxone dose in CD-1 mice as well

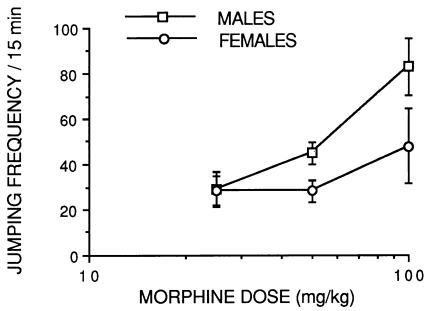

Fig. 1. Morphine dose–response curves for jumping frequency in male and female mice following acute morphine treatment. Points illustrate mean jumping frequency (±S.E.M.) precipitated by naloxone (50 mg/kg) injected 3 h after morphine ($n=10-20$/sex/dose). Analysis of variance revealed a significant main effect of morphine dose ($P<.001$) and sex ($P=.01$).

(McLemore et al., 1997). Thus, a relatively high maximum naloxone dose of 50 mg/kg was used in the present studies in order to maximize any possible sex differences. Acute dependence was also assessed by measuring withdrawal jumping after a single morphine injection (50 mg/kg) on Day 1 and followed 3 h later with various doses of naloxone. Chronic dependence was induced by three daily injections (t.i.d.: 08:00, 14:00, and 20:00) of morphine for 3 or 7 days according to an escalating dose schedule. For the 3-day chronic treatment paradigm, three different dosing regimens were used. In one group, mice were injected with 10, 20, and 40 mg/kg of morphine on treatment Days 1, 2, and 3, respectively. Other dose groups received 20, 40, and 80 mg/kg or 40, 80, and 120 mg/kg on Days 1, 2, and 3, respectively. Mice received a final morphine injection (40 mg/kg) 3 h prior to naloxone on the day of testing on Day 4. Chronic treatment for 7 days was accomplished using two morphine administration protocols. The first consisted of morphine injections (dose on Days 1–7: 10, 20, 40, 40, 80, 80, and 100 mg/kg, respectively). A second group received continuous subcutaneous morphine infusion (3.4 mg/kg/24 h) for 7 days via osmotic minipump (Model 2001, Alza, Palo Alto, CA). This dose was the maximum morphine dose possible given the solubility of morphine ($\sim$60 mg/ml) and the infusion rate of the pumps (1 μl/h).

Table 1
Naloxone ED_{50} estimates following acute and chronic morphine treatment in male and female mice

	Naloxone ED_{50} (95% CI)		
Morphine treatment/dose	Males	Females	Potency ratio
Acute			
50 mg/kg	1.7 (0.7–3.6)	7.4 (4.3–15.1)	0.23*
Chronic			
10, 20, and 40 mg/kg	0.58 (0.2–1.6)	0.85 (0.3–2.1)	0.68
20, 40, and 80 mg/kg	0.51 (0.4–0.7)	0.49 (0.4–0.6)	1.04

Values are naloxone dose (mg/kg) to precipitate jumping ($\geq$10 jumps/15 min) in 50% of subjects. Acute treatment was comprised of a single morphine dose injected 3 h prior to naloxone. Chronic treatment was comprised of t.i.d. morphine injections for three consecutive days with the daily escalating doses indicated. A final morphine (40 mg/kg) injection was made 3 h prior to naloxone on the day of testing (Day 4). 95% CI: 95% confidence interval. Potency ratio is male ED_{50} estimate/female ED_{50} estimate.

* Significant sex difference, $P<.05$.

Pumps were implanted and removed under oxygen/isoflurane inhalant anesthesia. Pumps were removed 3 h prior to assessment of dependence. Mice undergoing chronic morphine treatment for 7 days also received a final 40 mg/kg morphine injection 3 h prior to naloxone on the day of testing on Day 8. Control mice in acute and chronic treatment conditions were injected with saline instead of morphine throughout.

2.3. Assessment of dependence

As noted above, subjects were injected with naloxone 3 h following a single (acute condition) or final (chronic condition) morphine injection on the day of testing. This morphine–naloxone interval has been shown to yield maximal jumping responding in mice (El-Kadi and Sharif, 1994). Immediately after naloxone injection, mice were placed into individual Plexiglas observation cylinders (25 cm high × 11 cm wide). Among withdrawal measures, only the jumping response, defined as the simultaneous removal of all four paws from the horizontal surface over the next 15 min, reliably displayed morphine and/or naloxone dose-dependency in our

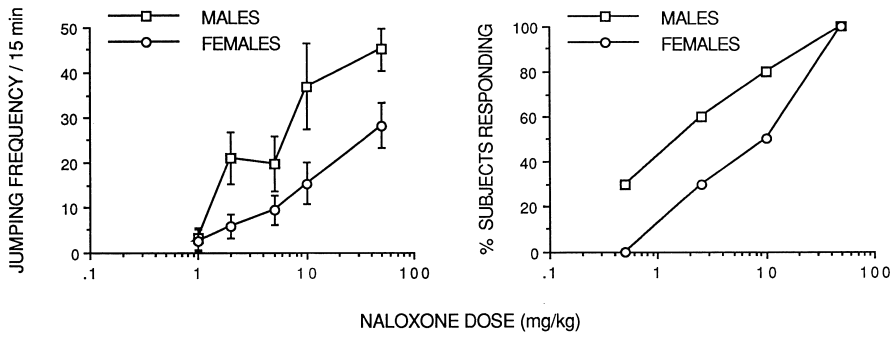

Fig. 2. Naloxone dose–response curves in male and female mice following acute morphine treatment. Graphs illustrate mean (±S.E.M.) jumping frequency (left) and percent mice displaying jumping response (right) precipitated by naloxone ($n=9-17$/sex/dose) injected 3 h after a single morphine (50 mg/kg) dose. Analysis of variance revealed a significant main effect of naloxone dose ($P<.001$) and sex ($P=.05$) on jumping frequencies. Naloxone ED_{50} estimates for precipitating a jumping response also differed between sexes.

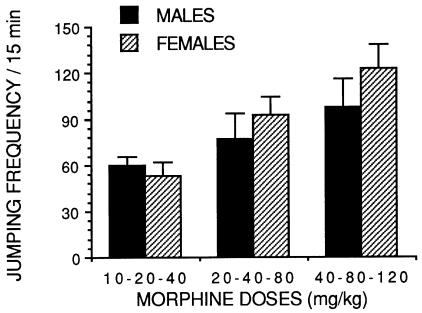

Fig. 3. Chronic morphine dose–response curves in male and female mice. Repeated (t.i.d.) morphine injections were made for 3 days according to an escalating dosing schedule. Doses administered for each group on Days 1, 2, and 3, respectively, are indicated ($n=10-18$/sex/dose). Data illustrate mean jumping frequency (±S.E.M.) precipitated by naloxone (50 mg/kg) injected 3 h after a final morphine (40 mg/kg) dose on Day 4. Analysis of variance revealed a significant effect of dose ($P=.01$) only.

pilot studies. Other symptoms, such as diarrhea, ptosis, wet-dog shakes, lacrimation, although initially tallied, were thus subsequently excluded from further study.

Mean frequency of jumps was used to index magnitude of dependence for each group. Naloxone ED_{50} estimates, indicating the naloxone dose required to elicit a positive jumping response in 50% of subjects, was adapted from a method first described by Way et al. (1969). In the present study, various doses of naloxone were injected ($n \geq 8$ mice/dose) and jumping responses were counted for 15 min after the acute and chronic morphine treatment conditions described above. A positive response was operationally defined as a jumping frequency ≥ 10, a value ≥ 3 standard deviations above the mean jumping frequency obtained for saline-treated control mice injected with naloxone (5 and 50 mg/kg) in pilot studies. A minimum of three naloxone doses was used in obtaining ED_{50} values.

2.4. Data analysis

Two-way (Sex × Dose) ANOVA was used to compare mean jumping frequencies, followed by Student–Neuman–Keuls tests where appropriate. Naloxone ED_{50} values, 95% confidence intervals (CIs), and estimates of relative potency were determined using BLISS-21 computer program. This program maximizes the log-likelihood function to fit a parallel set of Gaussian sigmoid curves to the dose–response data as previously described (Umans and Inturrisi, 1981). For all comparisons, $\alpha=.05$.

3. Results

3.1. Acute dependence

As illustrated in Fig. 1, acute morphine injection precipitated jumping in a dose-dependent manner following naloxone (50 mg/kg) in both sexes ($P<.001$). There was also a significant ($P=.01$) main effect of sex, with greater mean jumping frequencies, indicative of greater dependence, in males relative to females across morphine doses (Fig. 1). There was no significant interaction between morphine dose and sex. There was no sex difference in naloxone-precipitated jumping frequencies in saline-treated control mice.

Varying naloxone dose following a single acute morphine (50 mg/kg) injection also revealed significant main effects of dose ($P<.001$) and sex ($P=.05$). Fig. 2 (left) illustrates the positive dose–response relationship between naloxone dose and jumping for both sexes, and the greater jumping frequencies observed in males relative to females across a range of naloxone doses. Fig. 2 (right) presents dose–response curves illustrating percentage of animals of each sex positively responding to various doses of naloxone. As presented in Table 1, lower naloxone ED_{50} estimates were obtained for males (1.7 mg/kg) relative to females (7.4 mg/kg). Nonoverlapping 95% CIs between sexes indicate that this difference was significant. The calculated potency ratio (male ED_{50}/female $ED_{50}=0.23$) reveals an approximately fourfold greater sensitivity to naloxone-precipitated withdrawal in males relative to females.

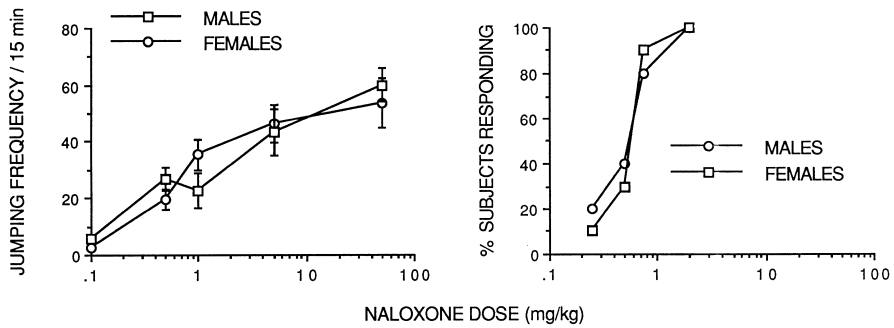

Fig. 4. Naloxone dose–response curves in male and female mice following chronic morphine treatment. Mice received repeated (t.i.d.) and chronic morphine (10, 20, and 40 mg/kg on Days 1, 2, and 3, respectively) injections. Jumping responses were assessed 3 h after a final morphine (40 mg/kg) dose on Day 4. Graphs illustrate mean (±S.E.M.) jumping frequency (left) and percent mice displaying jumping response (right) precipitated by naloxone ($n=10-17$/sex/dose). Analysis of variance revealed a significant effect of dose ($P=.01$) only. Naloxone ED_{50} estimates for precipitating a jumping response did not differ between sexes.

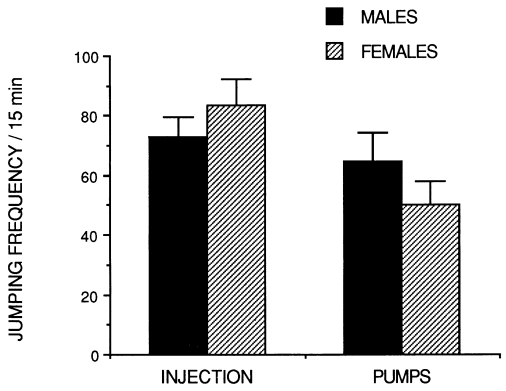

Fig. 5. Withdrawal jumping frequencies in male and female mice following 7 days of morphine treatment. Bars illustrate mean jumping frequencies (±S.E.M.). Morphine was delivered by repeated (t.i.d.) daily injection (left) according to an escalating dose schedule (see text) or via continuous delivery via an osmotic minipump (3.4 mg/kg/24 h). Naloxone (50 mg/kg) was injected 3 h after a final morphine (40 mg/kg) dose on Day 8.

3.2. Chronic dependence

Morphine treatment of increasing dosing schedules for 3 days produced significant dose-related increases in jumping frequencies in mice of both sexes ($P<.01$), but no significant difference between males and females (Fig. 3). Increasing naloxone dose resulted in significantly ($P<.01$) greater jumping frequencies when mice were treated with morphine doses of 10, 20, and 40 mg/kg (Fig. 4, left) or 20, 40, and 80 mg/kg (not shown) on Days 1, 2, and 3, respectively. Males and females did not differ in jumping frequency following either dosing schedule. Dose–response curves for percent responders to various naloxone doses are illustrated in Fig. 4 (right). No significant sex difference in naloxone ED_{50} estimates was observed for either of the two chronic dosing conditions (Table 1).

Naloxone-precipitated jumping was also observed after 7 days of morphine injection (Fig. 5, left) or chronic subcutaneous infusion via osmotic minipump (Fig. 5, right), but sex differences in jumping frequencies were not observed. Testing greater morphine doses than those presently reported was not possible since mice receiving 7 days of t.i.d. injection displayed uncharacteristic behavior starting on Day 5, and we had reached maximum morphine solubility in the pump condition. Furthermore, since the pattern of sex differences for morphine and naloxone dose–response curves were identical, in the interest of minimizing animal use and cost naloxone dose–response curves were not determined for 7-day treated mice.

4. Discussion

The present study demonstrates naloxone-precipitated withdrawal jumping in male and female mice after both acute and chronic morphine treatments. Jumping frequencies in the two treatment conditions displayed a positive dose–response relationship with morphine across a range of doses. Jumping was similarly dose-dependently increased with naloxone doses as high as 50 mg/kg. In CD-1 mice, naloxone doses as high as 50 mg/kg yield consistent and reliable levels of withdrawal jumping (McLemore et al., 1997; Kest et al., 1996). The present data confirms these prior findings, and additionally demonstrates that naloxone-precipitated jumping is increased in a morphine and naloxone dose-dependent manner in mice of both sexes, a finding previously demonstrated for males only (Fernandes et al., 1977; Smits, 1975; Miyamoto and Takemori, 1993a).

Using the jumping response, the present study is the first to compare male and female mice for acute morphine dependence. Following a single morphine injection, mean jumping frequencies were significantly greater for males than females as morphine doses were increased to its highest levels. The sex difference in jumping frequencies remained relatively uniform across the dose range, but was not evident at the lowest dose. These data suggest that sex differences in morphine dependence may be more apparent at some doses. There was also a significant effect of sex on naloxone ED_{50} values derived from naloxone dose–response curves, and based on the proportion of subjects responding. Males required 1.7 mg/kg naloxone to elicit withdrawal jumping, an ED_{50} estimate approximately fourfold lower than the 7.4 mg/kg obtained for females, and indicative of their greater sensitivity to naloxone-precipitated withdrawal. Together, the two indices demonstrate significant sex differences in acute morphine dependence in mice, with greater sensitivity and magnitude of morphine dependence in males relative to females. It should be noted that distinct neural substrates mediate various withdrawal symptoms (see review: Koob et al., 1992). Thus, findings using naloxone-precipitated jumping do not easily extrapolate to other indices of dependence. Since other dependence-related behaviors did not display dose-dependency and were therefore excluded from our study, it remains unknown whether they too are sensitive to subject sex. Future studies need to address this issue.

In contrast, there were no apparent sex differences on either measure of dependence following chronic morphine treatment of varied duration or route of delivery. Mean withdrawal-jumping frequencies and naloxone ED_{50} values did not differ between males and females after 3 days of morphine injection. Morphine treatment of longer duration, by daily injection or chronic subcutaneous infusion for 7 days, also produced withdrawal jumping of equal magnitude in both sexes. Morphine was delivered for 7 days using two administration protocols since incidence of withdrawal symptoms, including jumping frequency, may depend on method of dependence induction (Way et al., 1969; Cerletti et al., 1976). Use of minipumps for periods less than 7 days did not reliably produce dependence (data not shown), thus we were unable to compare this method of morphine administration with 3 days of chronic injection. Subcutaneously implanted pumps also minimizes possible

contextual conditioning due to repeated handling, injection of drug, and drug effect, which can influence withdrawal behavior (Azorlosa et al., 1994; Deffner-Rappold et al., 1996) in a sex-dependent manner (Van Haaren et al., 1990). Our data thus indicate that sex does not impact chronic morphine dependence in mice.

Our findings on chronic morphine dependence are consistent with those from previous sex comparisons. Using only a single morphine and naloxone dose, jumping responses in male and female mice were of equal magnitude after 3 days of subcutaneous morphine pellet implantation (Blum et al., 1976) or 7 days of morphine injection with daily escalating doses (El-Kadi and Sharif, 1994). In the latter study, sex differences were not found for naloxone-precipitated withdrawal measures such as hypothermia, weight loss, or wet-dog shakes, although males did burrow more than females at the one dose tested. The similarity between their findings and those presently reported using morphine and naloxone dose–response curves convincingly demonstrates that chronic morphine dependence develops in equal measure in male and female mice. In studies performed with rats, however, sex differences in chronic morphine dependence are observed, with males displaying greater dependence than females after either 5 (Craft et al., 1999) or 6 days of morphine injection (Ali et al., 1995). This is not the first instance where a different pattern of sex differences after chronic morphine treatment has emerged between mice and rats. Whereas female mice displayed greater tolerance to the analgesic effects of morphine than their male counterparts after 3 or 7 days of chronic morphine injection (Kest et al., 2000b), male rats were more tolerant than females on two different nociceptive assays (Craft et al., 1999; Badillo-Martinez et al., 1984). Although there is generally high concurrence between rat and mouse studies regarding sex differences in acute morphine-related measures (Kest et al., 2000a,c), findings from the analgesic tolerance and dependence studies suggests that the sex differences to chronic morphine may be species-dependent. We are not, however, aware of any sexual dimorphism exclusively present in rats or mice that would indeed yield a pattern of sex differences unique to only one species.

Alternatively, there are substantial methodological differences between studies of sex in dependence using rats and mice. As noted above, conclusions from the use of single morphine and naloxone doses in prior studies may be misleading. A particularly salient difference is the indexing of withdrawal intensity using the weighted scale of Gellert and Holtzman (1978), based on weighted values for different withdrawal symptoms, in the rat study by Craft et al. (1999). In contrast to the use of jumping frequency in the mouse studies, contributions to the withdrawal score in rats are from several graded and checked symptoms, the latter of which are assigned different values not related to the frequency of their appearance. Since only a single withdrawal intensity score for males and females is reported, it is not possible to ascertain jumping frequencies of either sex, or whether jumping per se was even an integral part of their withdrawal response. Comparisons between the rat and mouse studies are especially difficult since in CD-1 mice withdrawal symptoms such as diarrhea, ptosis, wet-dog shakes, and lacrimation are only occasionally observed, whereas abdominal constrictions, facial fasciculation, teeth chattering, swallowing movements, profuse salivation, and penile grooming/erection/ejaculation have not been observed at all (McLemore et al., 1997; Kest et al., 1996). In the Sprague–Dawley rats studied by Craft et al. (1999), such symptoms may have been more prominent in one sex, and significantly contributed to the sex difference in overall withdrawal score. Indeed, Ali et al. (1995) reported that in Sprague–Dawley rats, withdrawal jumping was not observed at the low single dose of naloxone used by Craft et al. (1999). Although the frequencies of different withdrawal measures were reported in the mouse study of El-Kadi and Sharif (1994), the use of weighted withdrawal scores precludes comparisons with symptoms in the rat. It is not known whether withdrawal symptoms observed in the rat are part of the withdrawal repertoire of any mouse strain. Additional comparisons of male and female mice and rats, on a variety of acute and chronic withdrawal measures and across a range of morphine and naloxone doses, are clearly needed to clarify possible species differences. Such data are vital as long as mice and rats are almost exclusively used as rodent models of sex differences of chronic opiate use.

In the present study, a sex difference was observed in acute, but not chronic, morphine dependence. One possible explanation may be the increased handling in the chronic treatment conditions. That is, there may have been differences between male and female in response to initial handling that presumably impacts withdrawal jumping, but which acclimates after several days of handling. We suggest this is unlikely since jumping responses in mice of both sexes were similar when morphine was delivered chronically via minipumps. The extent of handling in this condition is very similar to that experienced during acute treatment, in that mice were injected with only a single morphine and naloxone injection on the test day. In fact, the only difference between acute and chronic treatment via infusion was the implantation and removal of minipumps. The extra handling afforded by this minor and brief procedure (pumps are implanted and later removed in under 45 s) is negligible. Nonetheless, sex differences were observed in the acute, but not chronic infusion, treatment. Furthermore, we do not believe that the sex difference in acute dependence is easily attributable to morphine pharmacokinetic parameters since there is no positive relationship between plasma morphine levels and acute withdrawal severity (Kishioka et al., 1995). Additionally, pharmacokinetic differences between sexes after acute administration would be reasonably expected to also impact morphine disposition after chronic delivery and thus chronic dependence. For these reasons, we believe the present data reveal a sexually dimorphic neuroadaptive mechanism in mice

apparent after a single prior morphine exposure. Given the absence, however, of sex differences in dependence after chronic morphine treatment, only neural variables that are both sexually dimorphic and differentially participate in acute and chronic morphine dependence have explanatory power. Given the paucity of studies comparing males to females in either acute or chronic dependence, any proposed mechanism would be highly speculative at this time. Nonetheless, we consider findings from studies on the AMPA subtype of excitatory amino acid receptor to be of interest. The AMPA antagonist LY293558 has been demonstrated to block naloxone-precipitated withdrawal following acute, but not chronic, morphine injection using the same mouse strain and morphine and naloxone dose as those reported here (McLemore et al., 1997). Furthermore, the B_{max} of low affinity [^{3}H]AMPA binding sites in synaptosomal membranes prepared from mouse forebrain has been demonstrated to be greater in males than females (Akinci and Johnston, 1994). It is therefore conceivable that, by virtue of presenting with a greater population of AMPA receptors, males may undergo increased AMPA receptor activation following morphine administration relative to females. Accordingly, the subsequent adaptive neural changes mediated by AMPA receptors and underlying acute but not chronic morphine dependence would be greater in this sex too. Our laboratory is currently testing AMPA receptor antagonists on acute morphine dependence in mice of both sexes.

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