ZOOLOGICAL RESEARCH

Sex differences in morphine-induced behavioral sensitization and social behaviors in ICR mice

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ABSTRACT

Gender and genetic strain are two prominent variants that influence drug abuse. Although certain sex-related behavioral responses have been previously characterized in ICR mice, little is known about the effects of sex on morphine-induced behavioral responses in this outbred strain. Therefore, in this study, we investigated the sex differences of morphine-induced locomotion, anxiety-like and social behaviors in ICR mice. After morphine or saline exposure for four consecutive days (twice daily), increased locomotion, more time spent in the central area, as well as attenuated rearing and self-grooming behaviors were found in morphine-treated females in an open field; no differences were found in locomotion and the time spent in the central area between male and female controls. When interacting with the same-sex individuals, female controls were engaged in more social investigation, following, body contacting and self-grooming behaviors than controls; morphine exposure reduced contacting and self-grooming behaviors in females; in contrast, these effects were not found in males. These results indicate that female ICR mice are more prosocial and are more susceptible to morphine exposure than males.

Keywords: Morphine; ICR mice; Locomotion; Social behavior

INTRODUCTION

Gender and genetic strain are two prominent variants that influence drug abuse (Belzung & Barreau, 2000; Orsini et al, 2005; Phillips et al, 2008). It has been reported that females are more sensitive to the addictive properties of drugs compared with males (Anker, et al, 2011; Becker & Hu, 2008; Carroll & Anker, 2010). Besides, differences in behavioral responses to abused drugs may reflect differences in genetic vulnerability to drug abuse (Grabus et al, 2004). Due to the high genetic homogeneity, many inbred strains have been used in drug-related behavioral tests (Eisener-Dorman et al, 2011; Kennedy et al, 2012). For example, C57BL/6 and BALB/c mice, the two of the most commonly used inbred mouse strains (or sometimes their sublines) have been widely implicated in drug abuse research. In fact, outbred mice with genetically variable compositions, vigorous physiques and economical prices have also been considered for neuroethological studies (Dell'omo et al, 1993; Ge et al, 2013). For example, in addition to applications in immunology, pathology and pharmacology, the ICR mouse is also employed in behavioral neuroscience (Ge et al, 2013; Li et al, 2014; Liang et al, 2014) including drug-related studies (Kitanaka et al, 2014; Mao et al, 2011). Recent research has revealed that the ICR males display distinct paternity, including father-pup social interaction, and shed light on parental behaviors (Liang et al, 2014). They spent less time to get used to a new environment and have better environment memory capacity as compared to inbred C57BL/6 and BALB/c mice (Shi et al, 2008). The female ICR mice showed superior learning and memory abilities than males, but there were no sex differences in locomotion (Ge et al, 2013). Although some sex-related responses have been previously characterized in this strain (Aoki et al, 2010; Ge et al, 2013; Yamaura et al, 2013), to our knowledge, little is known about the sex differences of ICR mice in drug-related behaviors. [1]

Behavioral sensitization is a phenomenon in which neural changes are elicited by exposure to repeated intermittent administration of psychostimulants or opioids (Stewart & Badiani, 1993; Wang et al, 2010). These neuroadaptations are responsible for drug-induced increases in locomotor activity, reinforcing and rewarding effects (Francès et al, 2000; Wang et al, 2010). The endogenous opioid systems are related to changes in social emotional responsivity and social behavior (Kennedy et al, 2011; Nocjar & Panksepp, 2007). The effects of

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morphine on rewarding effects, locomotion or social play behaviors have been explored in some other mice strains (Cunningham et al, 1992; Campbell et al, 2000; Cole et al, 2013; Vanderschuren et al, 1995). Although morphine-induced conditioned place preference (CPP) has been conducted in male ICR mice (Mao et al, 2011), some studies have proposed the possibility of a disassociation between rewarding effects and behavioral sensitization (Miner, 1997; Wang et al, 2012; Zhang et al, 2002). In this study, we explore the sex differences in morphine-induced behavioral sensitization, anxiety and sociability in ICR mice.

MATERIALS AND METHODS

Animal subjects

Male and female ICR mice (18-22 g) were purchased from Ningxia Medical University Laboratory Animal Center (Yinchuan, China). The animals were housed in groups of four in standard transparent Makrolon cages (32 cm×21.5 cm×17 cm). The colony room was illuminated on a 12:12 light-dark cycle (lights on 2000h) and the temperature was maintained at 23±2 ℃. Food and water were available *ad libitum*. Mice were allowed to adapt to housing conditions for one week and handled daily by the same experimenter for three days prior to testing. All experiment procedures were performed strictly in accordance with the guidelines published in the NIH Guide for the Care and Use of Laboratory Animals.

Drug administration

Morphine-hydrochloride (Northwest Pharmaceutical Co., Ltd. Sinopharm, Xi'an, China) was diluted in saline. Mice received subcutaneously a daily binge injection of morphine or saline for four consecutive days. The daily binge pattern was consisted of two injections of morphine (10 mg/kg) or euqal vulume of saline at 0800h and 1400h, respectively. The dose of morphine applied in this study was according to the previous studies on behavioral sensitization, social motivated behaviors and CPP performance in mice (Mao et al, 2011;Nocjar & Panksepp, 2007; Xu et al, 2007).

Open-field test

Animal subjects were randomly assigned to one of the four treatment groups: male mice receiving physiological saline (MS, n=8) and morphine administration (MC, n=8), and female mice receiving physiological saline (FS, n=8) and morphine administration (FC, n=8). Twenty minutes following the last injection, motor activity and anxiety-like behavior were assessed in an open-field chamber. The chamber is a brightly and evenly illuminated square arena (50 cm×50 cm×25 cm) made of white glacial polyvinyl chloride and illuminated with four 60 W lamps mounted 1.5 m above the arena. The area was divided into 16 quadrants (four central and 12 peripheral) (Fiore & Ratti, 2007). Mice were placed individually in the center of the open-field and left to explore for 5 min and videotaped under white illumination. After the test trial was completed, the open-field was thoroughly cleaned with 70% ethanol solution. To assess anxiety-like behavior, the time spent in the center of the open-field was measured. The number of crossings between quadrants was used to assess locomotion. Additionally, rearing (raising on the hind legs and sniffing into the air or the wall of the box) and self grooming (licking own fur, sometimes using forepaws, passing them over the nose with a series of brief, horizontal movements) were recorded. All focal animals were videotaped during experiments using a Sony camera. The frequency and total duration of these behaviors were later scored by a researcher blind to experimental treatment using Jwatcher 1.0.

Same-sex social interaction test

After the open-field test, social interaction test was conducted. The interaction test was performed using only same-sex dyads to eliminate the possibility that even a low frequency of sexually motivated behavior might confound the results of tests. Testing was conducted in a neutral plastic cage (46 cm×31.5 cm×20 cm), with approximately 2 cm of wood shavings covering the floor and a removable opaque divider in the middle. One mouse was placed on each side of the arena with the divider still in place and given 5 min to adapt to the arena. Once the divider was removed a video-recorder mounted 70 cm above the apparatus monitored the arena for 15 min.

The behavior of the individual was classified as social investigation (sniffing the face, body or anogenital area of an individual), following (moving in the direction of or pursuing the test partner who moves away), contact behavior (contact with another individual including staying together or amicable grooming), aggressive behavior (pouncing (jumps or lunges), fighting (tumbling and biting) and chasing), self-grooming (cephalocaudal progression that begins with rhythmic movements of the paws around the mouth and face, ears, descending to the ventrum, flank, anogenital area and tail). Since the aggressive behaviors were rarely observed during social interaction, the related data were not shown.

Statistical analysis

Statistical analyses were carried out using SPSS 10.0 (SPSS Inc., Chicago, Illinois, USA). Data were checked for normality using the one-sample Kolmogorov–Smirnov test. Data from the open-field test and social interaction test were compared using two-way ANOVA with sex and morphine treatment as factors. Group differences were compared using post-hoc tests. All data are presented here as mean±*SE*. Statistical significance was taken at $P \leq 0.05$.

RESULTS

Behaviors in open-field

Two-way ANOVA revealed the effect of sex on transition ($F_{(3,28)}$=4.479, P=0.043) and rearing (duration: $F_{(3,28)}$=5.272, P=0.029; frequency: $F_{(3,28)}$=9.810, P=0.004). Significant effects of morphine were found on the time spent in the central area ($F_{(3,28)}$=6.039, P=0.020), rearing (duration: $F_{(3,28)}$=5.307, P=0.029; frequency: $F_{(3,28)}$=0.016, P=0.692) and self-grooming (duration: $F_{(3,28)}$=4.748, P=0.038; frequency: $F_{(3,28)}$=7.136, P=0.012). The effect of interactions between sex and morphine was found in self-grooming frequency ($F_{(3,28)}$= 9.017, P=0.006).

Specifically, no differences were found in total transition

(*mean difference*=12.71, *P*=0.195) or time spent in the central area *(mean difference*=1.75, *P*=0.667) between female and male controls. Morphine-treated females showed more transitions (*mean difference*=19.663, *P*=0.049) and spent more time in the central area than the saline-treated females (*mean difference*=9.789, *P*=0.021) (Figure 1 A, B).

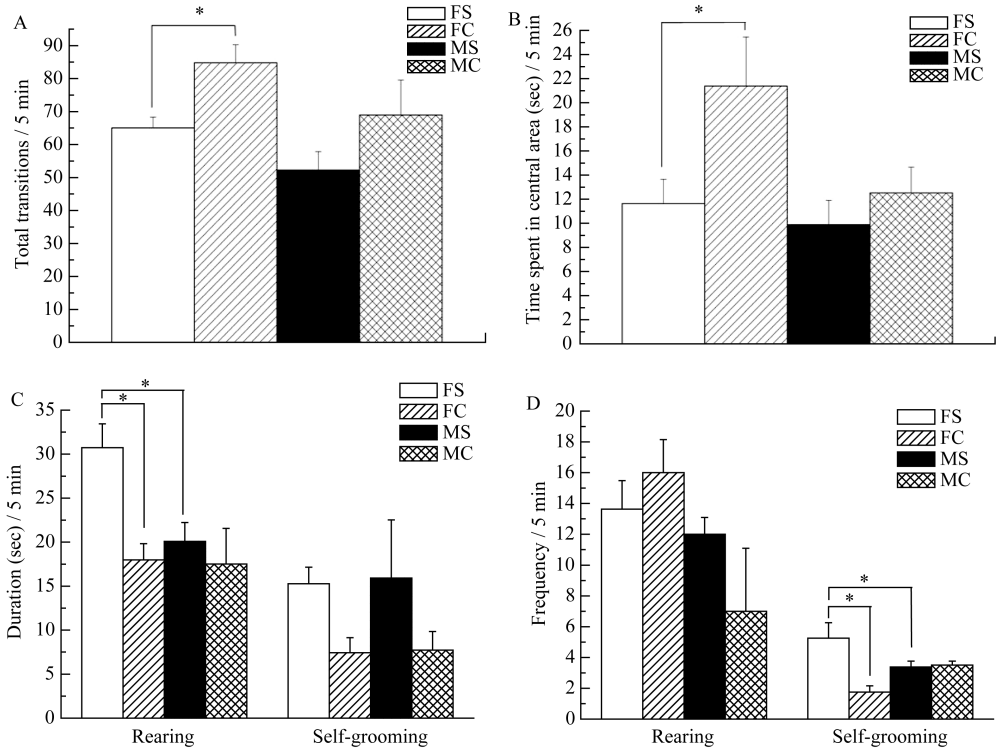

Figure 1 Behaviours of ICR mice during an open-field test

A: Total transitions; B: Total time spent in the central area; C: Duration of rearing and self-grooming behaviors; D: Frequency of rearing and self-grooming behaviors; *: *P*≤0.05.

Compared with male controls, female controls have higher levels of rearing (duration: *mean difference*=10.633, *P*=0.012; frequency: *mean difference=1.625, P=0.530*) and self-grooming behaviors (duration: *mean difference*=–0.655, *P*=0.901; frequency*: mean difference*=1.875, *P*=0.032)). Moreover, morphine-treated females showed less rearing (duration: *mean difference*=–12.743, *P*=0.003; frequency: *mean difference*=2.375, *P*=0.361) and self-grooming behaviors (duration: *mean difference*=–7.84, *P*=0.146; frequency: *mean difference*=–3.500, *P*<0.001) than the saline-treated females (Figure1 C, D). No morphine-induced differences were found in the transition numbers, the time spent in the central area, the rearing and self-grooming behaviors in males (*P*>0.05, data not shown) (Figure 1).

Social interaction

The effects of sex and morphine on the frequency of social investigation (sex: $F_{(3,28)}$=22.846, *P*<0.001; morphine: $F_{(3,28)}$=4.892, *P*=0.035) and self-grooming (sex: $F_{(3,28)}$=23.086, *P*<0.001; morphine: $F_{(3,28)}$=10.024, *P*=0.004) were revealed by two-way ANOVA test. The significant effects of sex on both the frequency of following ($F_{(3,28)}$=4.904, *P*=0.035) and contact behavior ($F_{(3,28)}$=5.931, *P*=0.022) as well the self-grooming duration ($F_{(3,28)}$=4.753, *P*=0.038) were also found. The frequency of contact behavior ($F_{(3,28)}$=7.026, *P*=0.013) and duration of self-grooming behavior ($F_{(3,28)}$=4.281, *P*=0.048) were significantly affected by the interactions between sex and morphine.

Specifically, in saline groups, compared with male controls, female controls showed higher frequencies of social investigation (*mean difference*=6.375, *P*=0.004), following (*mean difference*=3.125, *P*=0.041), contact behaviors (*mean difference*= 3.625, *P*=0.004) and self-grooming behaviors (duration: *mean difference*=52.94, *P*=0.005; frequency: *mean difference*=5.500, *P*<0.001) (Figure 2). Compared with saline controls, morphine-treated female mice exhibited lower frequencies of contact behaviors (*mean difference*=–3.750, *P*=0.003) and self-grooming behaviors (duration: *mean difference*=-48.61, *P*=0.009; frequency: *mean difference*=–4.750, *P*=0.001) (Figure 2C, D). However, no influences of morphine were found in the social investigation, following, contact and self-grooming behaviors in male mice (*P*>0.05, data not shown) (Figure 2).

DISCUSSION

Morphine-induced locomotion and anxiety-like behaviors

In the present study, no sex differences were observed in locomotion or anxiety levels under saline treatment, which are

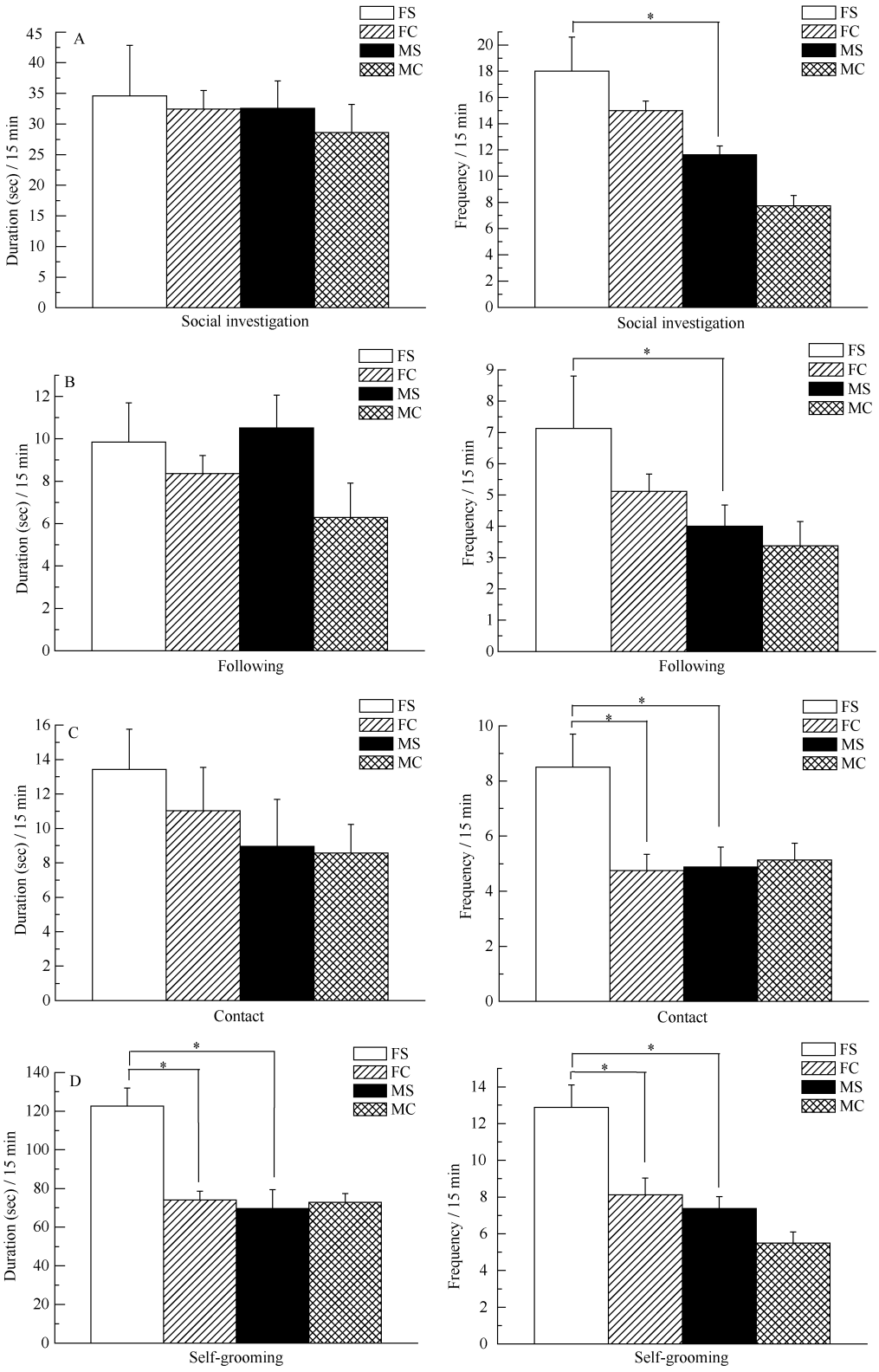

Figure 2 Behaviors of ICR mice in the same-sex social interaction test

A: Social investigation; B: Following behavior; C: Contact behavior; D: Self-grooming; *: $P \leq 0.05$.

consistent with previous findings in ICR mice (Ge et al, 2013). According to An et al (2011), similar phenomena in C57BL/6J and BALB/cJ mice were found in the open field and elevated plus maze tests as well. Here, female ICR mice exhibited more rearing and self-grooming behaviors than males. Since rearing behaviors of mice, to a certain degree, reflected its exploratory and cognitive ability (Edsbagge et al, 2004; Shi et al, 2008), our results indicate that the female ICR mice have higher levels of exploratory abilities than males. In addition to the time spent in the central zone, the changes in rearing and self-grooming are generally considered as indices of emotional behavioral processes (Carey et al, 2005; To & Bagdy, 1999; To & Bagdy, 1999). Thus, the differences between males and females in rearing and self-grooming behaviors suggest their different emotional changes in an open-field.

Several studies have shown that mice exhibit a heightened sensitivity to the effects of morphine (Niu et al, 2013; Xu et al, 2009). In this study, morphine increased the locomotion whereas attenuated the levels of anxiety, rearing and self-grooming behaviors in females; the time spent in the central area of an open field implies the morphine-induced anxiolytic effects in females. Similarly, cocaine treatments also decrease levels of rearing and grooming behaviors (Carey et al, 2005). These findings illustrate the importance of rearing and self-grooming phenotypes in drug-related behavioral researches. Additionally, our results showed that the locomotion, the time spent in the central zone or the rearing and self-grooming behaviors in males were not altered by morphine, indicating that female and male ICR mice respond differently to repeated morphine exposure.

Morphine-induced social behaviors

When interacting with the same-sex individuals, we found that under saline treatment, females were engaged in more social investigation, following, body contact and self-grooming behaviors than males. And these differences between males and females were inconsistent with their patterns in locomotion and anxiety-like behaviors. Interestingly, similar phenomena of high levels of sociability in females were also found in C57BL/6J and BALB/cJ mice (An et al, 2011; Wang et al, 2014). In addition, the decreased contact and self-grooming behaviors in morphine-treated females showed that morphine changed the social behaviors in females, whereas, morphine did not alter any social behaviors in males, combining our findings that morphine increased locomotor activity in females, indicating that female ICR mice are more susceptible to morphine than males.

Addition to ovarian hormones, sensitivity to the effects of drugs has been proposed to be associated with the sociability, emotion and memory (Curtis & Wang, 2007; Niigaki et al, 2010; Perrine et al, 2008). Compare with males, female ICR mice exhibit more sociability and better memory ability (Ge et al, 2013). These traits may influence behavioral responses to morphine in females. Besides, it has been reported that female ICR mice exhibit higher responses to stress and higher levels of serum corticosterone under both basal and stressed conditions as compared with males (Aoki et al, 2010; Yamaura et al, 2013). Sex differences in stress may contribute to sexually dimorphic patterns in morphine-induced behavioral sensitization (Holly et al, 2012; Wang et al, 2010).

In conclusion, this study compared the morphine-induced behavioral sensitization and social behaviors in female and male ICR mice. Their different behavioral responses to repeated morphine exposure indicate that female ICR mice are more susceptible to morphine than males.

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