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Social influences on morphine sensitization in adolescent females

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Abstract

We recently observed that social interactions influence morphine responsiveness in adolescent males. Given sex-related differences in both social interactions and responses to morphine, the present study examines social influences on morphine sensitization in adolescent female mice. Four experimental groups were examined: [1] morphine-treated mice (twice daily, 10–40 mg/kg, s.c.) housed physically and visually separated from saline-treated mice ('morphine only'), [2] morphine-treated mice housed together with saline-treated mice ('morphine cage-mates (of saline)'), [3] saline-treated mice housed together with morphine-treated mice ('saline cage-mates (of morphine)'), and [4] saline-treated mice housed physically and visually separated from morphine-treated mice ('saline only'). Following the treatment period, mice were tested individually for their locomotor response to 20 mg/kg morphine (s.c.). There were no significant differences in morphine-induced hyper-locomotion between saline only and saline cage-mates (of morphine) female adolescent mice. Notably, morphine only mice exhibited significantly greater morphine sensitization as compared to morphine cage-mates (of saline). Thus, this study demonstrates social influences on morphine sensitization in adolescent females. Drug use during early adolescence is a key predictor of later drug abuse and dependence during adulthood. Thus, understanding the specific vulnerabilities to drug use in this age group may represent a first step in helping develop more effective treatment programs.

Keywords

Opioid; Locomotion; Peer-influences; Drugs of abuse

1. INTRODUCTION

Drug use during adolescence is particularly problematic because it is a major predictor of later drug abuse and dependence during adulthood (Chen et al., 2009; Grant and Dawson, 1998; Hawkins et al., 1997; Odgers et al., 2008). Additionally, an increased rate of comorbidity of anxiety and mood disorders was demonstrated when drug use began at a young age (Caspi et al., 2005; Degenhardt et al., 2007; Gfroerer et al., 2002; Mathers et al., 2006; Tucker et al.,

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2006). A recent survey revealed that nonmedical use of opioid prescription pain relievers (such as hydrocodone, oxycodone, and morphine) is the second most common form of illicit drug use in the United States after marijuana (SAMHSA, 2009). This clearly illustrates the need for more research and a better understanding of the effects of opiate exposure during adolescence.

Peer influences are among the strongest predictors of adolescents' drug use. While most studies focus on humans, some studies suggest that in rodents there may also be a social effect on alcohol preference and consumption from interaction with intoxicated peers (Fernández-Vidal and Molina, 2004; Hunt et al., 2001). Similarly, we recently observed that social interactions with morphine-treated mice resulted in an enhanced hyper-locomotion response to morphine in drug-naïve adolescent male mice (Hodgson et al., 2010).

In both humans and rodents, there are sex-related differences in social interactions, including in play fighting and display of aggression (Meaney, 1989; Pinna et al., 2004). Additionally, there are also sex differences in the response to stress. Female rats exhibit fewer behavioral deficits than males after acute stressors but adapt more slowly to chronic stress (Dalla et al., 2007; Haleem et al., 1988; Mitsushima et al., 2003; Steenbergen et al., 1990). Moreover, there are sex-specific differences in the potency or efficacy of opioids as reinforcers, where females are more vulnerable to the acquisition of opioids, administer larger quantities, and work harder to obtain it (Cicero et al., 2003; Lynch and Carroll, 1999). Furthermore, differences in the emotional response to morphine withdrawal were observed between male and female mice (Hodgson et al., 2009a; Hodgson et al., 2009b). Given sex-related differences in both social interactions and in the responses to morphine, this study examines vulnerabilities to social influences on morphine locomotor sensitization in female adolescent mice.

2. METHODS

2.1 Animals

All procedures were conducted in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals, and were approved by the TAMU Institutional Animal Care and Use Committee. Female C57BL/6 mice, purchased from Harlan Lab (Houston, TX), were housed 4 per cage in a temperature-controlled vivarium with a 12 h/12 h light/dark cycle (light on at 07:00) and food and water ad lib. In this study, mice were injected during what is considered the late phase of their prepubescent period, and were tested during their mid-adolescence/periadolescent period (Spear, 2000). Accordingly, mice arrived at postnatal day 22 (PND 22). They were acclimated to the vivarium until PND 28, when morphine injections began, and behavioral testing was performed on PND 42.

2.2 Morphine treatment regimen

The different experimental groups are summarized in Table 1. Female adolescent (PND 28, n=10–12) mice were treated twice daily (9 a.m. and 5 p.m.) for 6 consecutive days with increasing doses of morphine (10–40 mg/kg, 10 ml/kg, s.c.) or saline for a total of 12 injections. Specifically, on days 1 and 2, the mice were injected with 10 mg/kg morphine or saline. On days 3 and 4, they were injected with 20 mg/kg morphine or saline. On days 5 and 6, they were injected with 40 mg/kg morphine or saline. This morphine regimen was selected based on both our previous studies (Buckman et al., 2009; Eitan et al., 2003; Hodgson et al., 2008; Hodgson et al., 2009a; Hodgson et al., 2010; Hodgson et al., 2009b) as well as other investigators (Contet et al., 2008; el-kadi and Sharif, 1994; Kest et al., 2001; Matthes et al., 1996; Spanagel et al., 1994) demonstrating that such doses induce significant antinociceptive tolerance, sensitization, dependence and withdrawal. Following the injections, mice were returned to their respective home cages. Morphine sulfate was purchased from Sigma (St. Louis, MO).

Mice were group-housed in one of two conditions referred to as 'only' and 'cage-mates'. 'Morphine only' mice are morphine-treated mice housed physically and visually separated from saline-treated mice (i.e. all 4 mice in the cage received morphine). Similarly, 'saline only' mice are saline-treated mice housed physically and visually separated from morphine-treated mice (i.e. all 4 mice in the cage received saline). There was also a group of morphine- and saline-treated mice that were housed together (i.e. 2 mice receiving morphine and 2 mice receiving saline per cage). They represent two different treatment conditions. The saline-treated mice of this group are referred to as 'saline cage-mates (of morphine)' (i.e. each saline cage-mate (of morphine) mouse had 2 morphine-treated and 1 saline-treated cage-mates). The morphine-treated mice are referred to as 'morphine cage-mates (of saline)' (i.e. each morphine cage-mate (of saline) mouse had 2 saline-treated and 1 morphine-treated cage-mates).

2.3 Morphine-induced hyper-locomotion

Nine days following the final pretreatment dose of morphine or saline, locomotion was recorded in the second part of the light phase, which is between 2 pm and 6 pm. Mice were habituated to the room for at least 30 minutes prior to testing and then placed separately (one mouse per apparatus) into an opaque upright cylindrical container (261 mm in diameter and 355 mm high). Each behavioral room contained 4 cylinders, thus 4 mice were individually recorded in the same room at the same time. Note that the mice were physically and visually separated during the locomotion test. Baseline locomotion activity was recorded for 60 minutes by an overhead camera. All mice were then injected with 20 mg/kg morphine and recorded for another 60 minutes. Each cylindrical container was cleaned thoroughly with water, dried completely and aired for at least 24 hours before being used again. Total distance traveled in cm (locomotion) was scored using EthoVision 3.1 (Noldus Information Technology, Leesburg, VA).

2.4 Data analyses

Similar to our previous study (Eitan et al., 2003), the locomotor sensitization study represents a split-plot design. Separate analyses of variance were computed for the total distance traveled scores (sum of baseline 60 minutes, and sum of post-morphine 60 minutes) and a within-group factor of time (1–120 minutes summed in 5 minute intervals). Additional post-hoc contrasts between each treatment group were computed using Bonferroni's post-hoc procedure. Differences with p-values less than 0.05 were deemed statistically significant. Results are presented as mean $\pm$ SEM.

3. RESULTS

The results are presented in Fig 1. For the baseline and post-morphine total distance traveled scores (Fig 1A), two way ANOVA revealed a main effect of experimental group ($F(3, 80) = 13.58, p < 0.0001$), a main effect of treatment ($F(1, 80) = 1059, p < 0.0001$), and a significant interaction between experimental group and treatment ($F(3, 80) = 12.95, p < 0.0001$). Similarly, for the total distance traveled scores summed in 5 minute intervals over the 120 minute test (Fig 1B), two way ANOVA revealed a main effect of experimental group ($F(3, 960) = 103.3, p < 0.0001$), a main effect of time ($F(23, 960) = 469.5, p < 0.0001$), and a significant interaction between experimental group and time ($F(69, 960) = 6.04, p < 0.0001$). Bonferroni's post-hoc comparisons revealed no significant differences in baseline locomotor activity (the first 60 minutes) between the different experimental groups. In all four experimental groups, locomotion increased following morphine administration (for each experimental groups' baseline vs. post-morphine, $p < 0.001$). There were no significant differences in morphine-induced hyper-locomotion between saline only and saline cage-mates (of morphine) female adolescent mice. As expected, both morphine-treated groups exhibited locomotion sensitization (morphine only vs. saline only, $p < 0.001$; and morphine cage-mates (of saline) vs.

saline only, $p < 0.01$). Notably, significant differences in morphine sensitization were found between morphine cage-mates (of saline) and morphine only mice, i.e. a significantly higher hyper-locomotion response to morphine was observed in the morphine only female mice as compared to the morphine cage mate (of saline) mice (morphine cage-mates (of saline) vs. morphine only, $p < 0.01$).

4. DISCUSSION

This study demonstrates social influences on morphine locomotor sensitization in adolescent female mice. Drug responsiveness was studied in group-housed mice. The response of mice housed in mixed treatment cages, where morphine- and saline-treated mice were housed together, was compared to that of mice housed in non-mixed cages, consisting of only morphine-treated or only saline-treated mice. There were no significant differences in morphine-induced hyper-locomotion between saline cage-mates (of morphine) and saline only female mice. As expected, both morphine-treated groups exhibited locomotor sensitization as compared to saline-injected mice. However, there was a significant difference in the response to morphine between the morphine cage-mates (of saline) and morphine only mice.

In this study, four mice were tested at a time. The cylindrical test containers were aired for at least 24 hours between each use. Thus, it is unlikely that significant odor cues remained from a previously tested mouse to affect the behaviors of the next mouse examined in that particular cylinder. The mice were physically and visually separated from each other during the test; nonetheless we cannot rule out the possibility that other cues transferred between the mice during the test period had some effect on their behaviors. As was demonstrated for 'pain empathy' (Langford et al., 2006), it is possible that the cues will have a greater effect on the cage-mates compared to unfamiliar mice. Although odor or vocal cues transferred during the test might play some role, a more likely explanation is that interactions prior to the locomotion test play a significant role in the behavioral outcome observed.

Olfactory cues transmitted during encounters with alcohol-intoxicated peers can affect subsequent alcohol preference and consumption in periadolescent mice (Fernández-Vidal and Molina, 2004; Hunt et al., 2001). Thus, perhaps pheromones secreted by the morphine- and/or saline-treated mice during their interactions (i.e. while housed together) do affect their peers. Each morphine only mouse is exposed to three morphine-treated cage-mates but not to any saline-treated cage-mates, while each morphine cage-mate (of saline) mouse interacts with only one other morphine-treated mouse and is exposed to two saline-treated cage-mates. If pheromones secreted by morphine- and/or saline-treated mice have a modulating effect, this might result in differences in morphine sensitization levels between the morphine cage-mates (of saline) and the morphine only mice.

Housing conditions were previously reported to affect drug responsiveness. Social isolation was demonstrated to enhance morphine sensitization while re-grouping reversed this effect (Frances et al., 2000). This was suggested to be mediated by stress levels, as indicated by the effects of isolation and re-grouping on plasma corticosterone levels. Stress can also be instigated as a result of increased aggressive behaviors between peers. Indeed, morphine withdrawal was demonstrated to increase aggressive behaviors in males (Felip et al., 2000; Rodríguez-Arias et al., 1999; Sukhotina, 2001). Thus, the morphine only mice might be exposed to more incidents of aggressive behaviors from morphine-treated cage-mates as compared to the morphine cage-mates (of saline). However, this study used female mice. Although we cannot rule out the possibility that the females display recordable levels of aggression (resulting in increased stress in their cage-mates), aggressive behaviors are not generally exhibited by non-lactating females (Moyer, 1968). Moreover, if morphine withdrawal indeed results in increased aggression and fighting, it is expected to affect all the

mice in the cage including the saline cage-mates. As mentioned earlier, stress is known to affect the responsiveness to morphine, thus the lack of a significant difference between the saline cage-mates (of morphine) and saline only mice, even though each saline cage-mate (of morphine) was exposed to two morphine-treated mice, suggests that the morphine-treated females do not display significant levels of aggressive behaviors.

In contrast to isolation, environmental enrichment was demonstrated to decrease the rewarding properties of opioids in mice (El Rawas et al., 2009). This effect was suggested to result from blunting of the hypothalamo-pituitary-adrenal (HPA) stress axis (Stairs and Bardo, 2009). Thus, perhaps the exposure to the saline-treated mice by the morphine cage-mates (of saline), but not by morphine only mice, also results in blunting of the HPA stress axis, resulting in a reduced morphine sensitization in the morphine cage-mates (of saline) as compared to the morphine only mice. However, environmental enrichment had no effect on the activating effects of opioids (El Rawas et al., 2009). Thus, further studies of environmentally and/or socially enriched mice as compared to isolated mice are required to better understand specific age and developmental vulnerabilities to drug use.

This study demonstrates social influences on locomotor sensitization in morphine-treated females. Multiple behavioral assays are used to index abuse potential (Ator and Griffiths, 2003; Carter and Griffiths, 2009), including the capacity of a drug to induce hyper-locomotion (Wise and Bozarth, 1987), the capacity to induce conditioned place preference (Bardo and Bevins, 2000), and the capacity to support intravenous drug self-administration (Brady and Griffiths, 1976). Intravenous self-administration is considered to be the “gold-standard” for abuse liability, whereas the capacity of a drug to stimulate locomotion is an indicator of abuse potential. Thus, the interpretation of the present results, namely the extrapolation from a measure of rodent response to humans, should be performed with caution. Further studies are required to identify the nature of the social interactions that cause this phenomenon (i.e. which sensory modality mediates the social effect on morphine sensitization - physical, visual, auditory and/or olfactory), to establish the duration of the effect, to determine possible social effects on other drug-induced behaviors (such as reward and self-administration), and to reveal the generality of the phenomenon to other drugs of abuse. Moreover, the molecular underpinnings of this social effect have yet to be determined. Understanding the mechanisms underlying adolescents’ vulnerabilities to the social influences on drug use may represent a first step in helping develop more effective treatment programs.

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Fig. 1. Morphine locomotor sensitization in adolescent female mice

(A) Total distance traveled (cm) in the 60 minutes prior to morphine administration (Baseline, White bars) and in the 60 minutes following 10 mg/kg morphine injection (Morphine, Gray bars). (§) indicates a significant difference from baseline ($p < 0.001$); (*) indicates a significant difference from saline only mice ($p < 0.01$); (b) indicates a significant difference between the morphine cage-mates (of saline) and morphine only mice ($p < 0.01$). (B) Total distance traveled (cm) during the entire 120 minute test, segmented into 5 minute intervals. Arrow indicates time of morphine administration. (*) indicates a significant difference from saline only mice ($p < 0.05$); (#) indicates a significant difference from saline only mice ($p < 0.001$); (a) indicates a significant difference between the morphine cage-mates (of saline) and morphine only mice ($p < 0.05$); (b) indicates a significant difference between the morphine cage-mates (of saline) and morphine only mice ($p < 0.01$); (c) indicates a significant difference between the morphine cage-mates (of saline) and morphine only mice ($p < 0.001$). Results are presented as mean $\pm$ SEM.

Table 1

Summary of the experimental groups

Experimental group	Housed together with mice	Cage configuration *	Exp days 1-6	Exp days 7-14	Exp day 15**	n		
Saline only	Saline-injected	SSSS	Saline	No treatment	20 mg/kg Morphine	12		
Saline cage-mates (of morphine)	Morphine-injected	SS(MM)	Morphine (10-40 mg/kg)			10		
Morphine cage-mates (of saline)	Saline-injected	MM(SS)				10		
Morphine only	Morphine-injected	MMMM				12		

* S – Saline-injected mouse; M- Morphine-injected mouse

** Locomotion recording