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Short communication

Periadolescent morphine exposure alters subsequent behavioral sensitivity to morphine in adult rats

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Abstract

Little research has been conducted investigating the long-term impact of opioid exposure during adolescence. These experiments were conducted to determine the behavioral effects of morphine exposure during periadolescence (postnatal days 30–32) versus young adulthood (postnatal days 65–67) on subsequent sensitivity to morphine. Male Sprague–Dawley rats were treated with three days of saline (S-S-S), one day of 10 mg/kg morphine followed by two days of saline (M-S-S), or three days of morphine (M-M-M). Unlike adult-treated counterparts, periadolescent M-M-M-treated rats showed greater locomotor response to morphine compared to S-S-S or M-S-S cohorts five weeks after treatment, suggesting age- and exposure-dependent differences in opioid sensitivity.

Keywords: Opioid; Adolescence; Mu-opioid receptor

1. Introduction

Epidemiological evidence shows a propensity for excessive use and development of addiction to drugs of abuse (e.g., alcohol, nicotine, cocaine) when initial exposure occurs during early adolescence (for review see Spear, 2000). Thus, adolescence appears to be a critical developmental period during which individuals are more "vulnerable" to the deleterious and addictive properties of drugs. The underlying mechanisms for this increased vulnerability are not well understood but probably stem from specific behavioral features as well as a number of hormonal, metabolic, neurochemical and morphological changes associated with the developmental age (Spear, 2000).

Despite the links between age of exposure and development of addiction to drugs of abuse, there have been few preclinical studies exploring the question of whether adolescent drug exposure alters later drug sensitivity. Nevertheless, there is some evidence (Ferris et al., 1998; Adriani et al., 2003). For example, in golden hamsters voluntary ethanol consumption during adolescence significantly enhanced aggression towards smaller intruders in adulthood compared to control subjects (Ferris et al., 1998). In another study, nicotine exposure in periadolescent rats, but not in postadolescent rats, led to increases in nicotine self-administration as adults (Adriani et al., 2003). Taken together, these findings suggest that the effects of drug exposure during adolescence are long-lasting and can alter sensitivity to later drug exposure.

The effects of opioids have only received limited attention in the adolescent preclinical literature (Spear et al., 1982). According to the 2003 Monitoring the Future Report, use of heroin has remained steady among high school students (Johnston et al., 2004). However, there is evidence of increased use of two other potent opiates, OxyContin® and Vicodin®. In fact, after marijuana, Vicodin® was the second most frequently reported drug used among high school seniors (Johnston et al., 2004). The considerable addictive potential of these drugs and upward trends in their use make studies assessing the long-term impact of exposure to opioids of critical importance. Therefore, the purpose of this study was to determine the effects of limited morphine exposure during periadolescence versus adulthood on subsequent (i.e., later in life) morphine-induced locomotion.

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2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats approximately 22 or 57 days old upon arrival (Charles River, Wilmington, NC) were pair-housed in standard polycarbonate cages in a centralized climate-controlled facility. All animals were maintained on a 12-h light:dark cycle and were provided with food ad libitum. Animals were maintained according to the "Guide for the Care and Use of Laboratory Animals" (National Academy of Sciences, 1996), and all procedures were approved by the Institutional Animal Care and Use Committee.

2.2. Locomotor apparatus

Locomotor activity was measured using eight Accuscan Digiscan Activity Monitors (AccuScan Instruments, Inc., Columbus, OH), with the aid of the VersaMax® software (Version 1.30, AccuScan Instruments, Inc.). For locomotor testing, rats were placed individually in one of eight clear acrylic chambers (40×40×30 cm). Each chamber was inside a ventilated, sound attenuating cabinet illuminated by candescent light (approximately 45 Lux). During testing, a number of behaviors were measured, including total movement in the horizontal plane (horizontal activity), with an array of infrared beams surrounding the chambers. Movements were determined by breaks in photobeams and were converted into locomotor activity counts with the aid of the software VersaDat® (Version 1.3; AccuScan Instruments Inc.), which was interfaced with a microcomputer.

2.3. Drug treatment and testing

One week after arrival, on postnatal days 29 (periadolescents) and 64 (adults), basal horizontal activity was measured for 2 h. Beginning the next day (postnatal days 30 and 65 for periadolescent and adults, repectively), rats were treated with one of three injection regimens: three days of saline (S-S-S), one day of morphine (10 mg/kg) followed by two days of saline (M-S-S), or three days of 10 mg/kg morphine (M-M-M). To eliminate the possibility of overdosing, the total morphine dose was given in two 5.0 mg/kg injections (s.c.) administered 10–14 h apart. The first injection each day was given in the laboratory immediately preceding a 2 h locomotor test, which occurred between 0900–1530 h. The second daily injection was given in the vivarium during the dark phase 10–14 h later. Five weeks later, dose-response curves for horizontal activity were determined following saline and morphine (0.1–3.0 mg/kg). Testing occurred over 5 days, and drug doses were given in ascending order.

2.4. Drugs

Morphine sulfate (Penick, Newark, NJ) was dissolved in saline and administered s.c. in a volume of 1.0 ml/kg body weight. All doses are expressed as the free base.

2.5. Statistical analysis

For basal horizontal activity prior to treatment, a two-factor analysis of variance (ANOVA; age × treatment group) was used to determine age-related differences or differences between groups of animals. Differences in horizontal activity during the initial treatment regimens and in response to morphine for the horizontal activity dose-response curves were determined using a mixed, two-factor ANOVA (treatment-repeated × day or dose) for each respective age group. The horizontal activity data from the initial treatment regimens were also normalized as a percentage of within-subject basal horizontal activity. These data were analyzed using a mixed, three-factor ANOVA (age of treatment × treatment-repeated × day). Newman–Keuls post hoc comparison was used to determine significant differences among means. The alpha level chosen was $P<0.05$.

3. Results

3.1. Basal horizontal activity

As shown in Fig. 1, periadolescent and adult rats exhibited comparable levels of basal horizontal activity upon their initial exposure to the locomotor activity chamber. Consequently, there were no significant age-related ($F_{[1,46]}=2.74$) or group-related ($F_{[2,41]}=3.06$) differences in horizontal activity.

3.2. Horizontal activity during treatment

Twenty-four hours after the assessment of basal horizontal activity subjects began treatment with one of three regimens: S-S-S, M-S-S, or M-M-M. Periadolescent rats injected with morphine (M-S-S and M-M-M groups) exhibited significantly greater horizontal activity compared with saline injected rats (S-S-S) (Fig. 2A). However, on the second and third days of treatment, only the rats receiving M-M-M maintained elevated levels of horizontal activity. M-S-S-treated rats were behaviorally similar to S-S-S-treated rats. A mixed two-factor ANOVA (treatment group × day, with

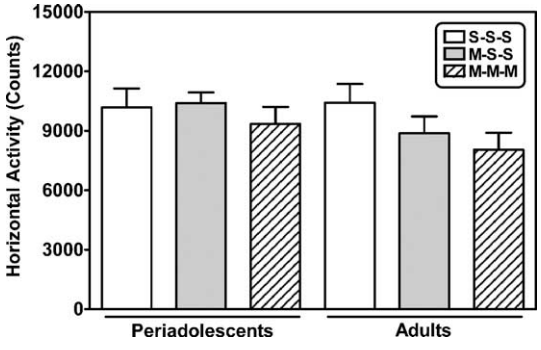

Fig. 1. There is no difference in basal locomotor activity between periadolescent and adult rats. The day before beginning morphine treatment locomotor activity was assessed for 2 h. The locomotor boxes were novel to the test subjects. Periadolescent and adult rats were 29 and 64 days old, respectively. Each data point represents the mean±S.E.M. of 7–8 rats per age group.

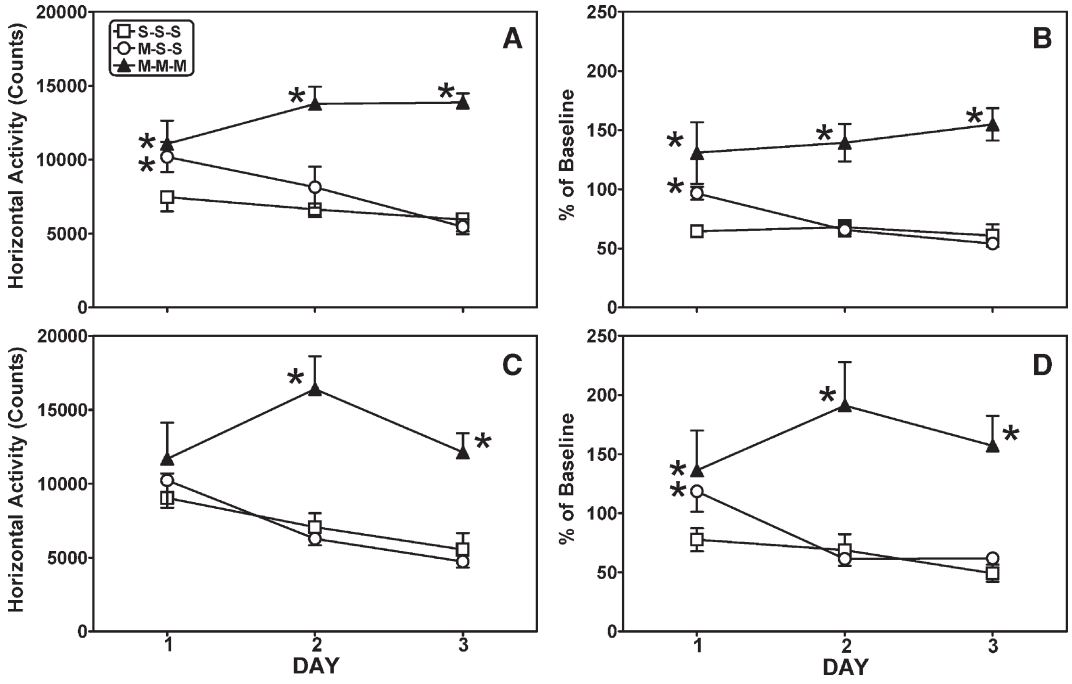

Fig. 2. Acutely administered morphine increases horizontal activity in periadolescent and adult rats. Periadolescent (A, B) and adult (C, D) rats were treated with one of three regimens: three days of saline (S-S-S), one day of morphine (10 mg/kg/day) followed by two days of saline (M-S-S), or three days of 10 mg/kg morphine (M-M-M). The total morphine dose was administered in two 5.0 mg/kg injections given 10–14 h apart. In panels A and C, each data point represents the mean ± S.E.M. (N=7–8) of horizontal counts over 2 h of testing after the first injection each day. In panels C and D, the data are expressed as a percentage of within-subject basal horizontal activity (see Fig. 1). *Indicates a significant difference from S-S-S treated cohorts; mixed two-factor ANOVA, Newman–Keuls post hoc, P<0.05.

repeated measures on treatment group) revealed a significant effect of treatment ($F_{[2,23]}$=23.1) and a significant interaction between treatment group and day ($F_{[4,71]}$=4.79). Unlike the findings with the periadolescent animals, in adult animals all three treatment groups had similar activity on day 1 immediately after being injected with saline or morphine (Fig. 2C). On the second and third days of treatment, the patterns of horizontal activity for the three treatment groups were consistent with those exhibited by their respective periadolescent counterparts as M-M-M-treated rats exhibited significantly greater horizontal activity than saline treated cohorts. Statistical analysis revealed a significant effect of treatment ($F_{[2,22]}$=11.47), a significant effect of day ($F_{[2,46]}$= 3.86) and a significant interaction between treatment group and day ($F_{[4,68]}$=2.91).

Normalizing the data as a percentage of the basal horizontal activity exhibited prior to treatment (see Fig. 1.) revealed similar patterns of behavior between periadolescent and adult rats (Fig. 2B,D). Animals receiving morphine on day one, regardless of age group, were more active than saline-treated cohorts. Again, only those animals receiving M-M-M maintained greater horizontal activity than those receiving saline over the entire treatment regimen. Consistent with these findings, a mixed three factor ANOVA (age of treatment × treatment group × day, with repeated measures on treatment group) failed to show a significant effect of age of treatment ($F_{[1,45]}$=2.40) but did reveal a significant effect of treatment ($F_{[2,92]}$=75.35), a significant effect of day ($F_{[2,92]}$=3.13) and a significant interaction between treatment group and day ($F_{[4,137]}$=9.01).

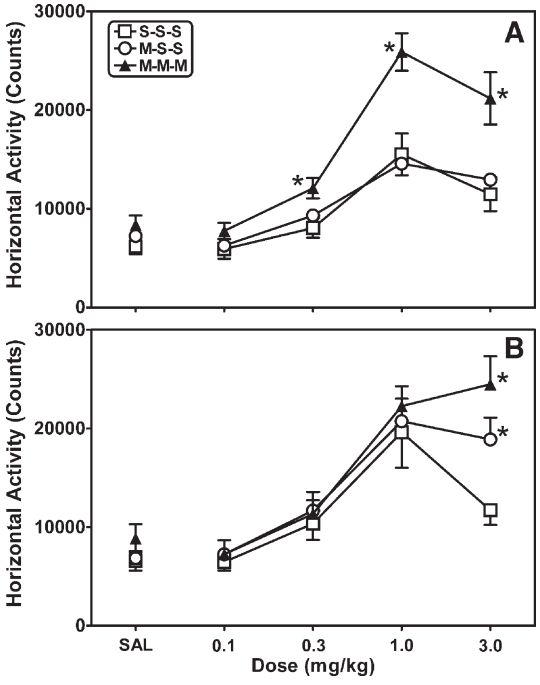

Fig. 3. Morphine exposure differentially impacts subsequent morphine-induced locomotor responses in rats treated as periadolescents or adults. Periadolescent (A) and Adult (B) rats were treated with one of three injection regimens: three days of saline (S-S-S), one day of morphine (10 mg/kg/day) followed by two days of saline (M-S-S), or three days of 10 mg/kg morphine (M-M-M). Five weeks later, horizontal activity was assessed (2 h) immediately following the administration of saline or increasing doses of morphine. Each data point represents the mean ± S.E.M. (N=7–8). *Indicates a significant difference from S-S-S treated cohorts; mixed two-factor ANOVA, Newman–Keuls post hoc, P<0.05.

3.3. Subsequent morphine-induced horizontal activity

Five weeks after treatment, when periadolescent rats had reached adulthood, subjects were tested for motor activity in response to morphine (Fig. 3). As shown in Fig. 3A, morphine dose-dependently and significantly ($F_{[4,96]}=64.92$) increased horizontal activity in all three groups treated during periadolescence, with peak activity occurring following the 1.0 mg/kg dose. M-M-M-treated rats were more active following morphine (0.3–3.0 mg/kg) as compared to their M-S-S-and S-S-S-treated cohorts, revealing a significant effect of treatment ($F_{[2,23]}=11.55$). Adult-treated rats (Fig. 3B) also demonstrated significant dose-dependent increases in locomotion following morphine ($F_{[4,96]}=64.92$). However, regardless of initial morphine treatment, all three groups exhibited comparable levels of activity following morphine up to a dose 1.0 mg/kg. Consistent with this finding a mixed two-factor ANOVA (treatment, repeated x morphine dose) did not reveal a significant effect of treatment ($F_{[2,22]}=2.52$). Following 3.0 mg/kg morphine, horizontal activity was suppressed in S-S-S-treated rats and was significantly less than that of M-M-M- and M-S-S-treated rats. Finally, there were significant interactions between initial treatment and response to morphine for both periadolescent-and adult-treated rats ($F_{[8,119]}=5.1$ and $F_{[8,114]}=2.88$, respectively).

4. Discussion

Our data extend the range of pre-clinical evidence demonstrating adolescent drug exposure alters later drug sensitivity to include opioids. As adults, periadolescent M-M-M-treated rats were more sensitive to morphine-induced locomotion than were S-S-S- or M-S-S-treated cohorts. The impact of morphine exposure on subsequent sensitivity to morphine was less marked in adult-treated rats, in which only highest dose of morphine (3.0 mg/kg) revealed any observable treatment-related differences. Here M-S-S- and M-M-M-treated rats were resistant to the suppressing effects of morphine compared to their S-S-S counterparts. Consistent with the literature (Vanderschuren et al., 2001) these findings demonstrate that the effects of a limited number of exposures to morphine are quite profound and long lasting, regardless of age of initial exposure. These data also suggest that, unlike the situation with adult treatment, there is a minimum number of exposures to morphine during adolescence required to alter subsequent sensitivity. However, once the minimum requirement is achieved, the effects are quite profound. This latter finding is notable given the purported links between sensitivity to drug-induced locomotion and increased vulnerability to drug taking (Vezina, 2004).

We found no age-dependent differences in locomotor activity between periadolescent and adult rats following exposure to a novel environment. Additionally, periadolescent and adult rats had similar behavioral responses to the different treatment regimens (i.e., S-S-S, M-S-S, and M-M-M). These findings are contrary to what has been reported in the literature. Adolescent rats are typically hyperactive and exhibit greater exploration in novel situations as compared behaviorally with adult rats (Spear and Brake, 1983; Spear, 2000). A number of studies also report age-related differences between periadolescent and adult rats in response to several drugs of abuse (Laviola et al., 1995; Markwiese et al., 1998; Faraday et al., 2001; Collins and Izenwasser, 2002, 2004; Schochet et al., 2004), including acutely administered morphine (Spear et al., 1982). In that study, adolescent rats exhibited greater locomotor activity in response to morphine (1.0–10 mg/kg) relative to adults. An obvious difference between that study and ours is the dosing regimen used. However, Spear and colleagues found the greatest group differences at 5.0 and 10 mg/kg-doses comparable to the one used in the present study. Another study investigating the effects of age on the reward value of drugs found that morphine (1.0 and 2.5 mg/kg) conditioned similar degrees of place preference in adolescent and adult rats (Campbell et al., 2000). These findings suggest that both groups of rats found the effects of morphine to be equally rewarding. The issue of age-dependent sensitivity to the acute effects of morphine clearly needs further investigation.

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