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Methylphenidate potentiates morphine-induced antinociception, hyperthermia, and locomotor activity in young adult rats

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ABSTRACT

The goal of this study was to determine if the exaggerated morphine-induced conditioned place preference (CPP) response seen in adult rats after preweanling methylphenidate exposure is unique to reward-mediated behaviors or is indicative of generalized changes in opioid-mediated behaviors. Rats were exposed to saline or methylphenidate (2.0 or 5.0 mg/kg) for 10 consecutive days starting on postnatal (PD) 11 with testing beginning on PD 60. In Experiment 1, morphine-induced (0, 2.5, 5.0 or 10.0 mg/kg) antinociception was assessed using the tail immersion and hot plate tasks. In Experiment 2, morphine-induced (0, 2.5, 5.0, or 10.0 mg/kg) hyperthermia and locomotor activity were measured. Morphine caused an increase in antinociception, with early methylphenidate (5.0 mg/kg) exposure potentiating the effects of 5.0 mg/kg morphine. Rectal temperatures were elevated after morphine, with the greatest increase occurring in male rats. Methylphenidate potentiated the hyperthermic effects of morphine (10.0 mg/kg) but only in males. Moderate doses (2.5 and 5.0 mg/kg) of morphine increased the locomotor activity of adult rats, while a higher dose (10.0 mg/kg) decreased locomotion. Interestingly, methylphenidate-pretreated females showed increased locomotor activity relative to controls. These results suggest that early methylphenidate exposure induces general changes in opioid system functioning that are not specific to reward-mediated behaviors.

1. Introduction

Each year millions of school-aged children (i.e., 6–17 year olds) who are diagnosed with attention deficit hyperactivity disorder are effectively treated with psychostimulant medications, such as methylphenidate, with limited adverse side effects (for reviews, see Biederman and Faraone, 2005; Brown et al., 2005; CDC, 2005; King et al., 2006). While repeated exposure to psychostimulants can be addictive in adults, studies suggest that treating school-aged children with methylphenidate does not increase the likelihood of later illicit drug use and may reduce the probability of substance abuse disorder (Hechtman and Greenfield, 2003; Mannuzza et al., 2003, 2008; Wilens et al., 2003; but see Lambert and Hartsough, 1998). Increasingly, methylphenidate is being used to treat a small but growing percentage of preschool-aged children (i.e., 3–5 year olds), although very limited information exists on the efficacy and safety of psychostimulant use in this age group (Greenhill et al., 2008; Vaughan et al., 2008). The available clinical trials, however, suggest there are age-dependent differences in response to methylphenidate, with older children showing greater symptom reduction and fewer adverse effects than preschool-aged children (Gleanson et al., 2007; Kratochvil et al., 2004).

A number of developmental animal studies have assessed the long-term effects of early methylphenidate exposure on cocaine-rewarded behavior (Achat-Mendes et al., 2003; Andersen et al., 2002; Brandon et al., 2001; Carlezon et al., 2003). Interestingly, early methylphenidate exposure is consistently found to alter cocaine's reinforcing potential; however, the reported effects are in opposing directions. Specifically, one study found that early methylphenidate exposure increased cocaine self-administration (Brandon et al., 2001), while other studies reported that early methylphenidate exposure attenuated cocaine-induced CPP (Achat-Mendes et al., 2003; Andersen et al., 2002; Carlezon et al., 2003). While these studies vary in numerous ways (e.g., self-administration versus CPP), age at methylphenidate exposure may be an important factor influencing the reward value of cocaine. Specifically, rats exposed to methylphenidate during adolescence (PD 35–PD 42) showed a long-term increase in cocaine self-administration (Brandon et al., 2001), while methylphenidate exposure during preadolescence (PD 20–PD 35) decreased the later preference for cocaine (Andersen et al., 2002; Carlezon et al., 2003). Thus, it is possible that methylphenidate exposure differentially affects cocaine's reward value depending on the age at which it is administered.

Because methylphenidate is being increasingly given to preschool-aged children, we recently examined whether exposing rats to methylphenidate during the preweanling period (PD 11–PD 20)

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would have long-term impact on reward system functioning. Specifically, we found that methylphenidate exposure during the preweanling period increased the reinforcement value of morphine and sucrose in young adult rats (Crawford et al., 2007), while the reinforcing properties of cocaine were unaltered (Crawford, unpublished observations). Although the cause of these methylphenidate-induced behavioral changes has not been determined, it is possible that they are due to changes in opioid receptor sensitivity. Support for this hypothesis is two-fold: (1) exposure to methylphenidate during the preweanling period enhances the rewarding effects of morphine but not cocaine (Crawford et al., 2007), and (2) repeated treatment with psychostimulants (i.e., methamphetamine and cocaine) causes an up-regulation of μ-opioid receptors and peptides (Chiu et al., 2006; Hammer, 1989; Unterwald et al., 1992, 1994). Importantly, it is uncertain whether methylphenidate's ability to alter μ-opioid functioning is restricted to reward circuitry or is a general phenomenon that impacts a variety of opioid-mediated behaviors. The goal of the present study, therefore, was to determine whether exposing rats to methylphenidate during the preweanling period would cause long-term changes in the antinociceptive, hyperthermic, and locomotor activating properties of morphine (the prototypical μ-opioid agonist). We hypothesized that preweanling methylphenidate exposure would alter opioid receptor sensitivity and increase morphine-induced antinociception, hyperthermia, and locomotor activity.

2. Experimental procedures

2.1. Animals

Subjects were 535 (N=10–12) male and female rats of Sprague–Dawley descent (Charles River), born and raised at California State University, San Bernardino. Different groups of rats were used for each experiment. Litters were culled to 10 pups (5 male and 5 female) at 3 days of age. The day of parturition was considered PD 0. Rats were kept with their dam until PD 25, at which time they were weaned and placed in group cages (2–3 rats per cage) with same-sex litter mates. The colony room was maintained at 22–24 °C and kept under a 12-hr light/dark cycle. Behavioral testing was done during the light cycle, at approximately the same time each day. Subjects were treated according to the "Guide for the Care and Use of Mammals in Neuroscience and Behavioral Research" (National Research Council, 2003) under a research protocol approved by the Institutional Animal Care and Use Committee of CSUSB.

2.2. Drugs

Methylphenidate hydrochloride and morphine sulfate salt were obtained from Sigma-Aldrich (St. Louis, MO). Methylphenidate was dissolved in saline and injected intraperitoneally (IP) at a volume of 5 ml/kg. Morphine was dissolved in saline and injected subcutaneously (SC) at a volume of 1 ml/kg. Drug doses were expressed in the forms listed above.

2.3. In vivo drug treatment

Starting on PD 11, rats were injected with saline or methylphenidate (2.0 or 5.0 mg/kg, IP) for 10 consecutive days. This injection period was chosen because this age span is roughly comparable to early childhood in humans (Andersen, 2003, 2005; Andersen and Navalta, 2004). After methylphenidate pretreatment, rats were left undisturbed until behavioral testing.

2.4. Apparatus

A water bath (Model 182, Precision Scientific, Chicago, IL), maintained at 52.0 °C (±1 °C), and a hot plate analgesia meter (IITC Life Science Inc, Woodland Hills, CA), maintained at 54.0 °C (±0.1 °C), were used to measure tail immersion and hot plate nociception. Locomotor activity was assessed in activity monitoring chambers (Coulbourn Instruments, Allentown, PA). The chambers (41×41×41 cm) consisted of Plexiglas walls, a plastic floor, and an open top. Each chamber included an X–Y photobeam array, with 16 photocells and detectors, which was used to determine distance traveled. Rectal temperatures were assessed using a BAT-12 microprobe thermometer (Physitemp Instruments, Piscataway, NJ).

2.5. Experiment 1: tail immersion and hot plate procedure

On PD 60, tail immersion nociception was assessed by holding rats vertically over the heated water bath with their tails immersed (5 cm) in the water. Latency (s) until tail-withdrawal was recorded. Hot plate nociception was assessed by placing the rats on the heated plate and latency to lick one of the hind paws or jumping (with all four paws leaving the plate) was recorded. Paw-lick latencies were assessed immediately after tail immersion testing. A cut-off time of 30 s was used for both procedures to prevent tissue damage. Three baseline tail immersion and hot plate trials were conducted for each rat with a 20-min interval between each trial. Immediately after the third baseline trial, rats were injected with morphine (0, 2.5, 5.0, or 10.0 mg/kg, SC) and returned to their home cage for 20 min. Tail-withdrawal and paw-lick latencies were then measured three additional times, again with a 20-min interval between each trial.

2.6. Experiment 2: rectal temperature and locomotor activity procedure

Rectal temperatures and locomotor activity were assessed over a two-day period. On PD 60 (i.e., the saline test day), rectal temperatures were taken at the beginning of the testing session and rats were immediately injected (SC) with saline and returned to their home cages. After 20 min, rectal temperatures were recorded and rats were placed into the locomotor activity chambers for 80 min. After conclusion of locomotor activity testing (i.e., 100 min after initial injection), rectal temperatures were measured again. On PD 61 (i.e., the morphine test day), the same procedure was used with the exception that rats were injected with morphine (0, 2.5, 5.0, or 10.0 mg/kg, SC) instead of saline.

2.7. Statistics

Body weights during the drug pretreatment phase were analyzed using a 2×3×10 (sex×pretreatment dose×day) repeated measures analysis of variance (ANOVA). Body weights of adult rats were analyzed using a 2×3 (sex×pretreatment dose) ANOVA. Baseline latencies for the tail immersion and hot plate tasks (collapsed across the three trials) were analyzed using separate 2×3 (sex×pretreatment dose) ANOVAs. The percent of the maximal analgesic response [defined as (test latency−baseline latency)/(cut-off time−baseline latency)×100] was calculated for each rat on both nociception tasks, with separate 2×3×4×3 (sex×pretreatment×post-treatment×time block) repeated measures ANOVAs being used. Analgesic response did not vary according to time block, so that factor was not included in the final statistical analyses.

Basal rectal temperatures on PD 60 (the saline test day) were collapsed across the three time points and analyzed using a 2×3 (sex×pretreatment dose) ANOVA. Rectal temperatures on PD 60 (the morphine test day) were converted into difference scores (i.e., basal temperatures [pre-morphine administration] were subtracted from temperatures recorded 20 min and 100 min post-morphine administration). For clarity, the difference scores of the 0 mg/kg morphine group were set at zero. Difference scores were analyzed by a 2×3×4×2 (sex×pretreatment×post-treatment×time) repeated measures ANOVA. Basal locomotor activity on PD 60 (the saline test day)

was assessed using a 2×3×16 (sex×pretreatment×time block) repeated measures ANOVA; whereas, locomotor activity on PD 61 (the morphine test day) was analyzed using a 2×3×4×16 (sex×pretreatment×post-treatment×time block) repeated measures ANOVA. For all analyses, significant higher-order interactions were further analyzed using two- or one-way ANOVAs. Post hoc analysis was done using Newman–Keuls tests ($p<0.05$).

3. Results

3.1. Body weight

On PD 11–20, male and female rats exhibited a progressive increase in body weight [Day main effect, $F_{9,\ 4770}=626.94$; $p<0.001$; Newman–Keuls tests, $p<0.05$] that was not altered by methylphenidate exposure (data not shown). Overall, male rat pups weighed significantly more than female pups [Sex main effect, $F_{1,\ 530}=7.20$; $p<0.01$], with the differences between sexes not varying according to postnatal day. Body weights of the methylphenidate- and saline-pretreated rats did not differ on PD 60, although male rats (x =383 g, SE±2) weighed significantly more than females (x =238 g, SE±1) [Sex main effect, $F_{1,\ 529}=3687.62$; $p<0.001$].

3.2. Nociception assays

3.2.1. Hot-plate task

Basal responsiveness to the hot plate was not altered by early methylphenidate treatment, although male rats (x =12.66 s, SE±0.60) had longer paw-lick latencies than female rats (x =9.93 s, SE±0.42) [Sex main effect, $F_{1,\ 259}=7.40$; $p<0.001$]. As expected, morphine increased paw-lick latencies on the hotplate task (Fig. 1) [Post-treatment main effect, $F_{3,\ 241}=88.89$; $p<0.001$; Newman–Keuls tests, $p<0.05$]. In male rats, morphine (2.5, 5.0 and 10.0 mg/kg) induced a dose-dependent increase in paw-lick latencies, while only the two higher doses of morphine significantly increased paw-lick latencies of female rats [Post-treatment×Sex interaction, $F_{3,\ 241}=5.45$; $p<0.001$; Newman–Keuls tests, $p<0.05$]. Males treated with 5.0 or 10.0 mg/kg morphine had longer paw-lick latencies than similarly treated female rats, thereby suggesting that morphine induced a greater analgesic response in male rats. Importantly, early methylphenidate (5.0 mg/kg) exposure enhanced the analgesic effects of a submaximal dose of morphine (Fig. 2), because methylphenidate-pretreated male and female rats injected with 5 mg/kg morphine had longer paw-lick latencies than control rats given the same dose of morphine [Pretreatment×Post-treatment interaction, $F_{3,\ 241}=2.77$; $p<0.05$; Newman–Keuls tests, $p<0.05$).

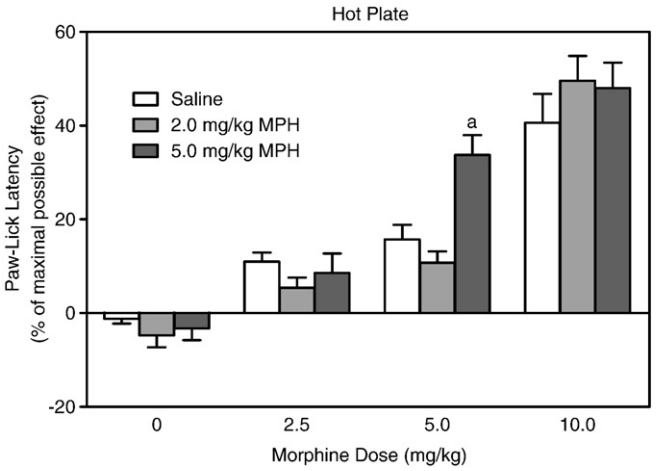

Fig. 2. Mean (±SE) paw-lick latency of morphine (0, 2.5, 5.0, or 10.0 mg/kg, SC) treated male and female rats (N=265) on the hot plate task (data expressed as % of maximal possible effect). Rats were pretreated with saline or methylphenidate (2.0 or 5.0 mg/kg) from PD 11 to PD 20 and tested with morphine on PD 60. Data in this figure are from the same animals as presented in Fig. 1 and are collapsed across sex. [a]Significantly different from rats pretreated with saline or 2.0 mg/kg methylphenidate and given 5.0 mg/kg morphine.

3.2.2. Tail immersion task

Similar to the hot-plate task, basal tail-withdrawal latencies were longer for male rats (x =14.68 s, SE±0.73) than female rats (x =10.33 s, SE±0.54) [Sex main effect, $F_{1,\ 259}=22.20$; $p<0.001$], while basal performance on the tail immersion task was not affected by early methylphenidate treatment. Although all doses of morphine increased the tail-withdrawal latencies of male and female rats [Sex main effect, $F_{1,\ 241}=49.75$; $p<0.001$], the analgesic effects of morphine were more pronounced in males (Fig. 3) [Sex×Post-treatment interaction, $F_{3,\ 241}=6.92$; $p<0.001$]. Specifically, male rats injected with 2.5, 5.0, or 10.0 mg/kg morphine exhibited significantly longer tail-

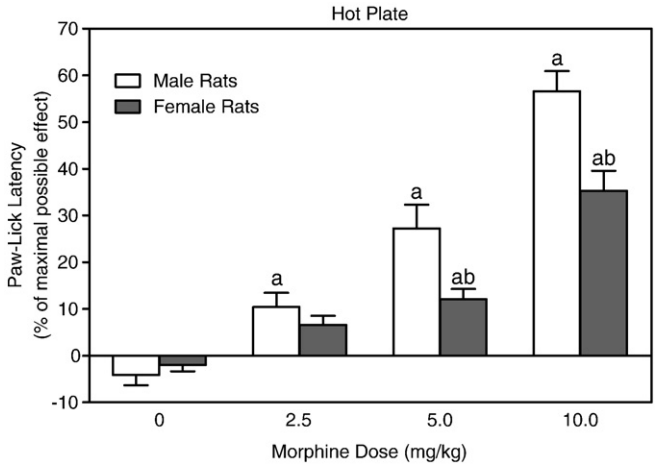

Fig. 1. Mean (±SE) paw-lick latency of morphine (0, 2.5, 5.0, or 10.0 mg/kg, SC) treated male and female rats (N=265) on the hot plate task (data expressed as % of maximal possible effect). Rats were pretreated with saline or methylphenidate (2.0 or 5.0 mg/kg, IP) from PD 11 to PD 20 and tested with morphine on PD 60. Data in this figure are collapsed across the early methylphenidate exposure condition. [a]Significantly different from saline-treated rats of the same sex. [b]Significantly different from similarly treated male rats.

Fig. 3. Mean (±SE) tail-withdrawal latency of morphine (0, 2.5, 5.0, or 10.0 mg/kg, SC) treated male and female rats (N=265) on the tail immersion task (data expressed as % of maximal possible effect). Rats were pretreated with saline or methylphenidate (2.0 or 5.0 mg/kg) from PD 11 to PD 20 and tested with morphine on PD 60. Data in this figure are collapsed across the early methylphenidate exposure condition. [a]Significantly different from saline-treated rats of the same sex. [b]Significantly different from similarly treated male rats.

withdrawal latencies than female rats treated with the identical doses of morphine (Newman–Keuls tests, $p<0.05$). Methylphenidate pretreatment did not alter the analgesic response to morphine on the tail immersion task.

3.3. Rectal temperature

On PD 60 (i.e., the saline test day), female rats (x =38.23 °C, SE±0.05) had higher rectal temperatures than male rats (x =37.24 °C, SE±0.06) [Sex main effect, $F_{1,\ 264}=158.61$; $p<0.001$]. Basal rectal temperatures were not altered by methylphenidate pretreatment.

On PD 61 (i.e., the morphine test day), rectal temperatures varied according to time [Time main effect, $F_{1,\ 246}=264.83$; $p<0.001$], therefore data from the 20- and 100-min time points were analyzed separately. When measured 20 min after morphine treatment, male rats (x =+0.26 °C, SE±0.06) exhibited a greater absolute increase in rectal temperatures than female rats (x =0 °C, SE±0.05) [Sex main effect, $F_{1,\ 246}=5.32$; $p<0.05$]. This sex difference was largely restricted to the high-dose methylphenidate condition, because rectal temperatures of rats pretreated with 5.0 mg/kg methylphenidate (x =+0.30 °C, SE±0.06) were significantly greater than rats pretreated with 0 mg/kg methylphenidate (x =+0.03 °C, SE±0.07) [Pretreatment main effect, $F_{2,\ 246}=10.53$; $p<0.001$; Newman–Keuls tests, $p<0.05$].

When assessed 100 min after morphine administration, a separate ANOVA showed that saline-pretreated male rats given 5.0 mg/kg morphine had a greater increase in rectal temperatures than saline controls (upper graph, Fig. 4) [Post-treatment main effect, $F_{3,\ 38}=3.96$; $p<0.05$; Newman–Keuls tests, $p<0.05$]. Saline-pretreated female rats given morphine also showed enhanced rectal temperatures, but this effect was evident after 2.5, 5.0, and 10.0 mg/kg morphine (lower graph, Fig. 4) [Post-treatment main effect, $F_{3,\ 43}=4.43$; $p<0.01$; Newman–Keuls tests, $p<0.05$]. An omnibus ANOVA showed that among male rats pretreated with 2.0 methylphenidate only 10.0 mg/kg morphine increased rectal temperatures [Sex×Pretreatment×Post-treatment interaction, $F_{6,\ 246}=2.22$; $p<0.05$; Newman–Keuls tests, $p<0.05$]. Likewise, among male rats pretreated with 5.0 mg/kg methylphenidate both 5.0 and 10.0 mg/kg morphine increased rectal temperatures (upper graph, Fig. 4). Interestingly, methylphenidate-pretreated male rats injected with 10.0 mg/kg morphine had significantly greater rectal temperatures than saline-pretreated rats given the identical dose of morphine (compare the black bars in the upper graph of Fig. 4). Post hoc analysis of the same omnibus ANOVA showed that female rats pretreated with 2.0 mg/kg methylphenidate and tested with 5.0 mg/kg morphine exhibited a greater increase in rectal temperatures than controls pretreated with 2.0 mg/kg methylphenidate (lower graph, Fig. 4) [Sex×Pretreatment×Post-treatment interaction, $F_{6,\ 246}=2.22$; $p<0.05$; Newman–Keuls tests, $p<0.05$]. Among female rats pretreated with 5.0 mg/kg methylphenidate, both 2.5 and 5.0 mg/kg morphine increased rectal temperatures relative to controls.

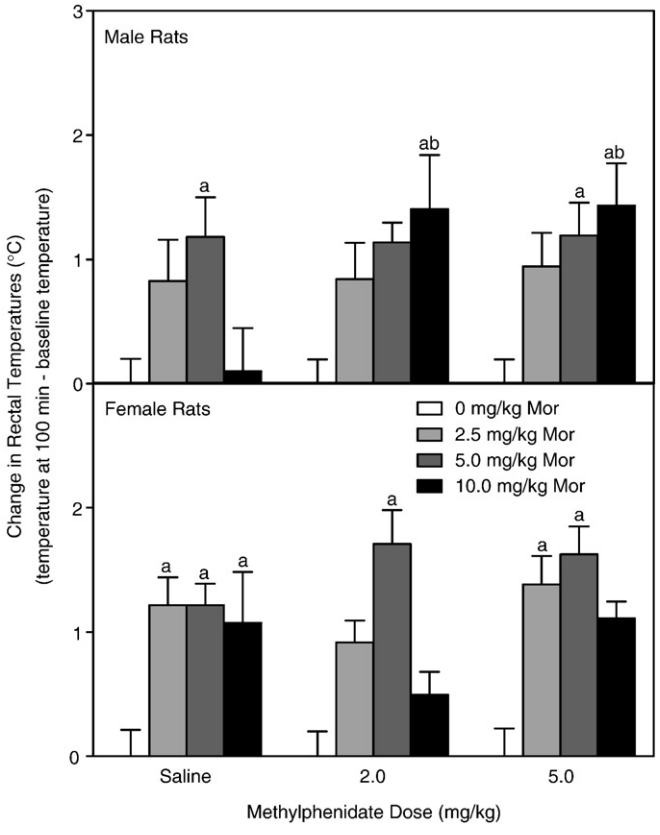

Fig. 4. Mean (±SE) change in rectal temperature 100 min after male and female rats (N=270) were treated with morphine (0, 2.5, 5.0, or 10.0 mg/kg, SC). Rats were pretreated with saline or methylphenidate (2.0 or 5.0 mg/kg) from PD 11 to PD 20 and injected with morphine on PD 60. [a]Significantly different from similarly pretreated rats given 0 mg/kg morphine. [b]Significantly different from rats pretreated with saline and treated with the 10.0 mg/kg morphine.

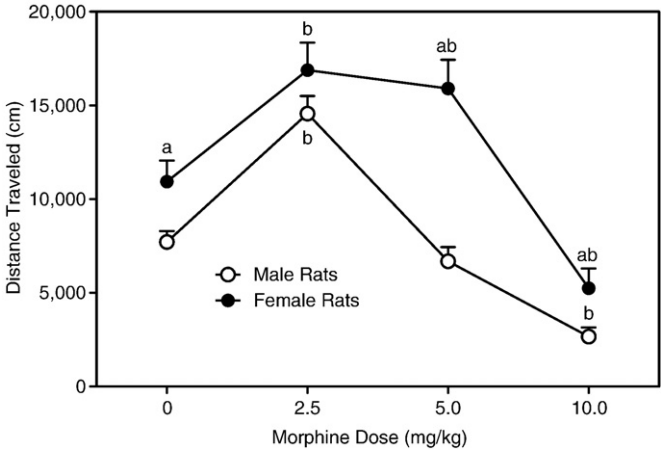

Fig. 5. Mean (±SE) distance traveled of morphine (0, 2.5, 5.0, or 10.0 mg/kg, SC) treated male and female rats (N=270). Activity testing lasted for 80 min. Rats were pretreated with saline or methylphenidate (2.0 or 5.0 mg/kg) from PD 11 to PD 20 and tested with morphine on PD 60. Data in this figure are collapsed across the early methylphenidate exposure condition. [a]Significantly different from female rats given the same dose of morphine. [b]Significantly different from similarly treated rats given 0 mg/kg morphine.

3.4. Locomotor activity

On PD 60 (i.e., the saline test day), female rats (x =16,356 cm, SE±834) had greater distance traveled values than males rats (x =11,684 cm, SE±345) [Sex main effect, $F_{1,\ 246}=27.34$; $p<0.001$]. Methylphenidate pretreatment did not alter locomotor activity on the saline test day.

On PD 61 (i.e., the morphine test day), female rats again had greater distance traveled values than male rats [Sex main effect, $F_{1,\ 246}=34.01$; $p<0.001$]: an effect that was evident after saline or morphine (5.0 or 10.0 mg/kg) treatment (Fig. 5) [Sex×Post-treatment interaction, $F_{3,\ 246}=4.58$; $p<0.01$; Newman–Keuls tests, $p<0.05$]. The two lower doses of morphine (2.5 and 5.0 mg/kg) increased the distance traveled values of female rats, while only 2.5 mg/kg morphine increased the locomotion of male rats [Newman–Keuls tests, $p<0.05$]. The highest dose of morphine (10.0 mg/kg) significantly decreased locomotor activity in both males and females.

Methylphenidate pretreatment altered locomotor responsiveness in only female, but not male, rats (Fig. 6). Specifically, female rats pretreated with 2.0 mg/kg methylphenidate displayed greater distance traveled values than saline-pretreated females on time blocks 1–5 [Sex×Pretreatment×Time Block interaction, $F_{30,\ 3690}=1.51$; $p<0.05$;

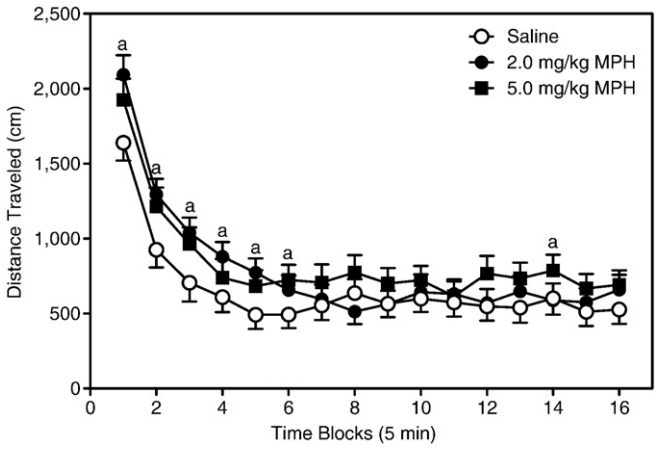

Fig. 6. Mean (±SE) distance traveled of morphine (0, 2.5, 5.0, or 10.0 mg/kg, SC) treated female rats (N=135). Activity testing lasted for 80 min. Rats were pretreated with saline or methylphenidate (2.0 or 5.0 mg/kg) from PD 11 to PD 20 and tested with morphine on PD 60. Data in this figure are from the same animals as presented in Fig. 5 and are collapsed across morphine condition. [a]Significantly different from rats pretreated with saline.

Newman–Keuls tests, $p<0.05$]. The higher dose of methylphenidate (5.0 mg/kg) also caused a persistent increase in the locomotor activity of female rats, but only on time blocks 1, 6, and 14 [Newman–Keuls tests, $p<0.05$].

4. Discussion

Exposing rats to methylphenidate during the preweanling period causes a long-term enhancement in the reward value of both morphine and sucrose that persists into adulthood (Crawford et al., 2007). To determine whether these methylphenidate-induced changes are specific to reward circuitry or are a more general phenomenon affecting multiple opioid receptor systems, we assessed the effects of morphine on nonreward-related tasks. In general, our results indicate that exposing rats to methylphenidate on PD 11–PD 20 cause long-term changes in opioid receptor sensitivity that affect a broad range of behavioral and physiological responses. For example, early methylphenidate treatment potentiated morphine-induced paw-lick latencies and hyperthermia, while causing small, but measurable, increases in the locomotor activity of female rats.

In terms of nociception, early methylphenidate treatment did not affect baseline responses to thermal stimuli, although male rats did have significantly longer paw-lick and tail-withdrawal latencies than female rats. Sex differences in nociceptive responsiveness have been reported before but the data are often inconsistent, with female rats showing both increased and decreased thermal sensitivities when compared to males (for a review, see Mogil et al., 2000). As expected, morphine caused a dose-dependent increase in antinociception when tested on both the hot plate and tail immersion tasks. The magnitude of morphine's analgesic effects differed according to sex, because each dose of morphine induced significantly greater antinociception in male rats. Early methylphenidate exposure potentiated the analgesic effects of 5.0 mg/kg morphine, thus suggesting a leftward shift in the dose–response relationship. A similar pattern of effects was not observed when rats were treated with a higher dose of morphine (10.0 mg/kg).

Curiously, the ability of methylphenidate to enhance morphine-induced antinociception was only observed using the hot plate task, but not the tail immersion task. A possible reason for this difference is that the paw-lick response is mediated by supraspinal mechanisms, while the tail-withdrawal response is a spinal reflex (Caggiula et al., 1995; Espejo and Mir, 1993; Le Bars et al., 2001). Spinal and supraspinal μ-opioid receptors are pharmacologically distinct, because the μ-opioid antagonist, naloxoanzine, blocks systemic and supraspinal antinociception but not spinal antinociception (Paul et al., 1989). In terms of the present findings, supraspinal opioid receptors may be uniquely sensitive to the effects of early methylphenidate exposure, thus potentially explaining why methylphenidate was able to potentiate morphine-induced antinociception on only the hot plate task. Alternatively, early methylphenidate treatment may affect performance on the hot plate task by altering the sensitivity of dopamine receptors in the periaqueductal gray (PAG) of the midbrain. Reducing dopaminergic transmission in the PAG, via dopamine receptor blockade or dopamine depletion, has been shown to attenuate morphine-induced antinociception on the hot plate task but not the tail immersion task (Flores et al., 2004).

As with nociception, basal rectal temperatures differed according to sex, with female rats having higher rectal temperatures than male rats (see also Kest et al., 2000; Quock et al., 1985). Morphine altered rectal temperatures, but these effects were complex as the dose–response relationship varied according to time. Specifically, 10.0 mg/kg morphine produced greater hyperthermia after 20 min then after 100 min, while 5.0 mg/kg morphine produced a slight increase in body temperature across the two time points. In terms of body temperature, these results suggest that higher doses of morphine (e.g., 10.0 mg/kg) have a faster onset and offset than lower doses. In agreement with this idea, Rawls et al. (2003) showed that hyperthermia peaked 45–60 min after treatment with 4.0 mg/kg morphine, whereas 15.0 mg/kg morphine caused peak hyperthermia only 15–30 min after injection. Early methylphenidate exposure did not affect basal rectal temperatures, but methylphenidate did potentiate morphine-induced hyperthermia. Once again this effect was sex-dependent, because methylphenidate exposure enhanced the hyperthermic effects of 10.0 mg/kg morphine in only male rats.

Opioid receptor stimulation affected locomotor activity in a characteristic manner, because morphine treatment produced an inverted "U" shaped dose–response curve. Specifically, 2.5 and 5.0 mg/kg morphine increased locomotor activity, while 10.0 mg/kg morphine decreased locomotion. The effects of opioid receptor stimulation were sex-dependent, because morphine caused more locomotor activity in female rats. The dose- and sex-dependent effects of morphine have been reported previously (Craft et al., 2006; Kalinichev et al., 2004; Vanderschuren et al., 1999). Similar to its ability to potentiate morphine-induced antinociception and hyperthermia, early methylphenidate exposure enhanced the locomotor activating effects of morphine in female, but not male, rats. It is unclear why this effect was restricted to female rats, but it may be due to sex-dependent differences in dopaminergic activity. More precisely, morphine stimulates locomotor activity by indirectly altering the firing rate of nigrostriatal and mesolimbic neurons and, in this way, increasing dopamine release in the striatum and nucleus accumbens (Cadoni and Di Chiara, 1999; Di Chiara and Imperato, 1988; Vanderschuren et al., 2001). These dopaminergic neurons show enhanced responsiveness in female rats (Becker, 1999; Walker et al., 2000), perhaps explaining why methylphenidate only potentiated morphine-induced locomotor activity in the females.

Regardless of the behavior or physiological response being measured, the effects of morphine often varied according to sex. Some of these effects are obscured by sex-related differences in basal responding, but morphine differentially affected the antinociceptive responses and rectal temperatures of male and female rats when data were analyzed as change from baseline. The underlying cause of these sex differences is unclear but it is possible that circulating sex hormones may have modulated the actions of morphine. Prior studies have demonstrated that estrous phase and gonadal removal can modulate morphine-induced nociception and locomotor activity (Bernal et al., 2007; Craft et al., 2006; Krzanowska and Bodnar, 1999), although inconsistent findings, especially regarding antinociception, have been reported (Mogil et al., 2000; Peckham et al., 2005). Interestingly, morphine-induced sex differences appear to be the result of either

organizational effects of sex hormones or direct effects of X- or Y-linked genes (Cicero et al., 2002; Gioiosa et al., 2008; Mogil et al., 2000).

As with sex, developmental stage of the animal also alters the impact of early methylphenidate treatment. Specifically, methylphenidate exposure during adolescence increases the rewarding effects of cocaine, while preadolescent treatment decreases cocaine's reward value (Achat-Mendes et al., 2003; Brandon et al., 2001; Carlezon et al., 2003). Nondrug-induced behaviors are also differentially affected by methylphenidate depending on age at treatment onset. For example, rats exposed to methylphenidate from PD 20–PD 35 spend less time in the open arms of an elevated plus maze than controls, whereas greater time is spent in the open arms if methylphenidate was administered during an earlier developmental period (PD 7–PD 35) (Bolaños et al., 2003, 2008; Gray et al., 2007). Given these past findings, it is very possible that the long-term effects of early methylphenidate exposure on morphine-induced behaviors also vary according to the developmental stage in which the drug is given. Although the neural mechanisms responsible for this age-dependent effect are unknown, dopamine systems undergo substantial changes during the postnatal period. For example, dopamine content, dopamine transporter sites, and dopamine D_1 and D_2 receptor sites increase linearly from birth and reach adult-like levels around the fourth postnatal week (Broaddus and Bennett, 1990; Coyle and Campochiaro, 1976; Murrin and Zeng, 1986, 1990; Rao et al., 1991). At the time of adolescence, there is additional neural reorganization in which D_1 and D_2 receptors are dramatically overproduced (Andersen et al., 1997; Giorgi et al., 1987; Teicher et al., 1995), with dopamine receptor numbers gradually declining to adult levels (for reviews, see Andersen, 2003; Tarazi and Baldessarini, 2000).

In general, the present results are in agreement with our hypothesis that early methylphenidate exposure causes alterations in opioid receptor functioning that are not limited to reward circuitry. The neural mechanisms responsible for this effect may involve methylphenidate-induced changes in the level of endogenous opioid peptides, coupling of opioid receptors with their G-proteins, or opioid receptor densities. Indeed, repeated treatment with other psychostimulants (cocaine and methamphetamine) can alter the density and sensitivity of μ-opioid receptors in adult rats (Chiu et al., 2006; Hammer, 1989; Schroeder et al., 2003; Unterwald et al., 1992, 1994). Regardless of the mechanism, methylphenidate-induced changes in opioid system functioning are of potential importance, because opioid systems also modulate affective behavior and impulse control (Filliol et al., 2000; Ognibene et al., 2007; Vergura et al., 2008; Waldhoer et al., 2004). For example, pretreating very young rats with methylphenidate decreases anxiety-like behavior when measured in adulthood (Gray et al., 2007).

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