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Methylphenidate and MDMA adolescent exposure in mice: Long-lasting consequences on cocaine-induced reward and psychomotor stimulation in adulthood

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Abstract

Pre-exposure to psychostimulants enhances the rewarding and psychomotor stimulating effects of subsequent drug exposure. Currently, there is a prevalence of adolescent exposure to the psychostimulants methylphenidate (MPD) and 3,4-methylenedioxymethamphetamine (MDMA). However, there is a paucity of investigation concerning the long-term behavioral consequences of exposure to these stimulants during adolescence. The aim of the present study was to investigate the effect of MPD and MDMA exposure in adolescence on cocaine-induced reward and psychomotor stimulation in adulthood. Adolescent Swiss-Webster mice received intraperitoneal injections of saline, MPD (10 mg/kg) or MDMA (10 mg/kg) from PD 26 to PD 32. Animal weights were monitored during and after drug administration. One month later, cocaine-induced conditioned place preference (CPP) and locomotor activity (LMA) were investigated. MPD and MDMA inhibited weight increase from PD 28 to PD 39 compared to the saline group, but weights amongst the three groups equalized by PD 46.

MDMA exposure resulted in the same magnitude of cocaine (20 mg/kg)-induced CPP as saline exposure; however, MPD exposure caused significantly less CPP. Two weeks following extinction of CPP and withdrawal from cocaine, a priming injection of cocaine (5 mg/kg) reinstated significantly higher CPP in the MPD and MDMA groups than in the saline group. In the LMA experiments, cocaine (15 mg/kg) was administered for 5 consecutive days. On days 1 and 5, cocaine-induced hyperlocomotion in the MPD group was significantly higher than in the saline and MDMA groups. After a 2-week withdrawal period, cocaine (5 mg/kg) evoked significantly higher LMA responses in the MPD and MDMA groups compared to the saline group. Results suggest that exposure of mice to both MPD and MDMA during adolescence involves long-lasting neural adaptations, manifested as sensitized responses to cocaine-induced reward and psychomotor stimulation following cocaine withdrawal.

Keywords: Psychostimulants; Cocaine; Cross-sensitization; Conditioned place preference; Withdrawal

1. Introduction

Psychostimulants such as methylphenidate (MPD; Ritalin) and 3,4-methylenedioxy-methamphetamine (MDMA; "Ecstasy") are currently used by many adolescents. MPD is the drug of choice for the treatment of adolescents with attention deficit hyperactivity disorder (ADHD). Often, treatment involves years of MPD administration which lasts into adulthood (Schubiner et al., 2002; Wender et al., 2001). MPD exhibits rewarding effects similar to cocaine and amphetamine (Kollins et al., 2001) and thus has potential for abuse. In the US, poison center reports of MPD misuse in youths (10–19 years) showed a sevenfold increase in the frequency of MPD exposures from 1993 to 1999 (Klein-Schwartz and McGrath, 2001). A study of 161 students being treated with MPD found that 16% had been approached to sell, give, or trade their stimulant medication (Musser et al., 1998). In an experiment involving MPD and amphetamine self-administration by non-drug-abusing volunteers, MPD has been shown to function as a reinforcer, a

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strong predictor of abuse potential (Rush et al., 2001). MDMA however, while not a clinically useful drug, is currently the most popular 'club drug' among the youths and its abuse is on the rise. A national survey of over 14,000 college students reported that the prevalence of MDMA use increased by 69% between 1997 and 1999 (Strote et al., 2002). Moreover, a clinical trial of MDMA users between 18 and 37 years of age reported that younger MDMA users might be more vulnerable to MDMA-induced neurotoxicity (Obrocki et al., 2002). Due to the current prevalence of use and abuse of MPD and MDMA, further investigation into the long-term effects of exposure to these psychostimulants during adolescence is needed.

Animal research has provided evidence that repeated exposure to psychostimulants such as cocaine, methamphetamine and MDMA results in sensitization to the rewarding and psychomotor effects of the drugs (Kalivas et al., 1998; Robinson and Berridge, 2000; Robinson et al., 1988). Enhanced responses to stimulants are thought to be the result of neurobiological adaptations occurring predominantly in the mesolimbic dopamine (DA) system. Drug-induced changes in these rewarding circuits, such as changes in gene transcription, RNA and protein synthesis and modifications of neuron morphology are long-lasting (Nestler, 2001). Chronic cocaine exposure increases expression of transcription factors in the nucleus accumbens (NAc), namely delta-FosB, which enhances the responsiveness of an animal to the rewarding and locomotor-activating effects of cocaine through alteration of gene expression (Kelz et al., 1999). Additionally, chronic cocaine administration modifies synaptic strength between the glutamatergic projections on the neurons of the NAc, which may also contribute to behavioral sensitization and addiction (Thomas et al., 2001). It is thus expected that exposure to MPD and MDMA during adolescence produces long-term changes that may perpetuate into adulthood.

Several studies investigated the consequences of adult and adolescent rat exposure to MPD on the responsiveness to psychostimulants. Exposure of adults rats to a high dose of MPD increased vulnerability to cocaine self-administration (Schenk and Izenwasser, 2002). Exposure to MPD in pre-adolescent and adult rats led to aversion or no effect following cocaine administration, respectively, in the conditioned place preference (CPP) paradigm (Andersen et al., 2002). The treatment of adolescent rats with a low dose of MPD caused a subsequent increase in cocaine self-administration, while treatment with a moderate dose of MPD caused sensitization to the motor stimulating effect of cocaine (Brandon et al., 2001). In another study, adolescent rat exposure to MPD subsequently caused a slight decrease in the locomotor stimulating effects of methamphetamine (Kuczenski and Segal, 2002). It is likely that the different results are due to varying schedules of exposure to MPD. Reports on the consequences of rat exposure to MDMA on the responsiveness to psychostimulants are more consistent. MDMA exposure induced locomotor sensitization (Spanos and Yamamoto, 1989; Kalivas et al., 1998) and facilitated the acquisition of cocaine self-administration (Fletcher et al., 2001). However, there is a scarcity of reports on the long-term effects of adolescent exposure to MDMA. Repeated MDMA administration to adolescent rats enhanced the sub-threshold rewarding effect of cocaine in adulthood (Fone et al., 2002). The persistent treatment of ADHD with MPD, its potential for abuse, and the frequent recreational abuse of MDMA during adolescence warrant further investigation into the long-term effects of these stimulants on the susceptibility to drug-seeking behavior in adulthood.

The present study was undertaken to investigate the outcome of adolescent mouse exposure to MPD and MDMA on a) cocaine-induced CPP and b) cocaine-induced psychomotor stimulation, as an indication of long-term responsiveness to psychostimulants. The experimental design was aimed to investigate a) the induction of cocaine-induced CPP and psychomotor stimulation and b) the outcome of cocaine withdrawal and cocaine challenge in adulthood.

2. Materials and methods

2.1. Drugs

(±) MDMA-HCl and (±) MPD-HCl were gifts from the National Institute on Drug Abuse (Washington, DC). Cocaine-HCl was purchased from Sigma (St. Louis, MO). All drug solutions were prepared in saline and injected intraperitoneally (i.p.) in a volume of 0.1 ml per 10 g of body weight.

2.2. Animals

Adolescent male Swiss Webster mice, postnatal day (PD) 21 (15–17g; Charles River Laboratories, Wilmington, MA, USA) ($n = 55$) were habituated for 5 days in the Division of Veterinary Resources (University of Miami School of Medicine) before drug treatments. Mice were maintained on a 12-h light/dark cycle, at a room temperature of 22 ± 0.5 °C, and housed in groups of 3 to 5 mice per cage with free access to food and water. Animal care was in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council, National Academy Press, 1996) and as approved by the University of Miami Animal Care and Use Committee. From PD 26 through PD 32 mice were treated once a day, at 12:00 noon, with either saline (0.9% NaCl solution; $n = 23$), MPD (10 mg/kg; $n = 16$) or MDMA (10 mg/kg; $n = 16$). The doses of MPD and MDMA were based on our previous studies which

showed that 10 mg/kg MPD (Itzhak and Martin, 2002a) and 10 mg/kg MDMA (Itzhak et al., 2003) were pharmacologically relevant for observation of a moderate increase in locomotor activity in Swiss Webster mice. Animals' weights were monitored daily prior to drug administration (PD 26–PD 32), and thereafter once a week until PD 60. Saline, MPD and MDMA pretreated mice were divided into two separate groups to conduct either a) CPP (n = 27) or b) LMA experiments (n = 28).

2.3. Cocaine-induced conditioned place preference (CPP)

Animals' place preference was monitored by the CPP apparatus, Opto-Max Activity Meter v2.16 (Columbus Instruments, Columbus, OH). The CPP cage, 42 × 20 × 20 m, was separated by a removable guillotine door into two zones, one comprising a black floor with 4 black walls and the other a white floor with 4 white walls. The apparatus was covered with a transparent Plexiglas lid perforated to allow adequate ventilation. A transparent, colorless, enclosed Plexiglas waiting chamber (12 × 8 × 8 cm) was affixed to one side of the CPP cage at the junction of the black and white compartments. Mice were placed in the waiting chamber and allowed entry via a guillotine door that matched the black/white walls of the CPP apparatus. The cage was equipped with matching pairs of horizontal sensors mounted alongside opposing lengths (42 cm long). The black and white zones (21 × 20 × 20 cm) were each scanned at a rate of 10Hz by 7 infrared beams, spaced at 2.54 cm intervals. A null zone was assigned at the interface of the black and white zones in the center of the box and was monitored by 2 beams.

Thirty days following pretreatment, saline (n = 9), MPD (n = 9) and MDMA (n = 9) pre-treated groups of adult mice (PD 62) were randomly selected to evaluate cocaine-induced CPP. On the first day, mice were allowed a 30 min habituation period in which they were free to explore the black and white zones of the CPP apparatus. On the second day, during pre-conditioning, mice were placed in a chamber outside the apparatus and allowed unbiased entry through the guillotine door. Time spent in the black, white and null zones was recorded for 30 min. For each group, approximately a third of the mice showed equal preference for the black and white zones, a third showed slight preference for the black and a third showed slight preference for the white zone (see Results 3.2). To utilize the 'unbiased' design, 4–5 mice per group were paired with cocaine injections in the white zone, and the remainder was paired with cocaine injections in the black zone. Routinely, the mice that showed initial preference to one zone were paired with the drug injection in the opposite zone.

The conditioning phase (day 3–10) consisted of 8 days of alternating cocaine (20 mg/kg) and saline injections. This particular dose of cocaine (20 mg/kg) was administered because it was found to be the optimal dose for achieving CPP in Swiss-Webster mice (Itzhak and Martin, 2000, 2002b). Each mouse received 4 cocaine sessions and 4 saline sessions, 30 min each. During these sessions, a guillotine door with black and white walls was placed at the center of the CPP cage in order to separate the two zones. On the 11th day, in the post-conditioning phase, mice received a saline injection and were allowed free access to all zones of the apparatus as in the pre-conditioning phase. Time spent in each zone was recorded for 30 min. The induction of CPP was assessed by the difference in time it spent in drug-paired zone before and after conditioning. Extinction of CPP (day 12–25) was achieved by daily administration of saline and confinement for 30 min in alternating drug- and saline-paired zones as previously described (Itzhak and Martin, 2002b). Each mouse was subjected to seven saline sessions in the previously drug-paired zone and seven saline sessions in the saline-paired zone. On the 26th day, the extinction test was performed immediately following a saline injection as described for the post-conditioning test. Extinction was determined as the difference in time spent in the previously drug-paired zone before induction and after extinction of place preference. On the 27th day, CPP was reinstated by a priming injection of a low dose of cocaine (5 mg/kg). Mice were allowed free access to the two zones of the CPP cage and time spent in each zone was recorded for 30 min. Reinstatement of CPP was determined as the difference in time spent in the previously drug-paired zone after both extinction and reinstatement.

2.4. Locomotor activity

Animal activity was monitored by an activity meter, Opto-Varimex Mini (Columbus Instruments, Columbus, OH). Each test cage was a standard transparent rectangular rodent cage (42 × 24 × 20 cm high), equipped with an array of 15 infrared emitter/detector pairs, spaced at 2.65 cm intervals. Each emitter and detector was mounted alongside the length of the cage (42 cm long).

Both the total counts and the ambulatory counts were recorded and transferred by a computer counter interface to an IBM computer. Ambulatory counts correspond to horizontal activity, while the difference between the total and ambulatory counts corresponds to vertical activity. Based on our previous observations (Itzhak, 1997) and the current results, it appears that the fraction of non-ambulatory counts (25–30% of total counts) increased or decreased in parallel to corresponding changes in ambulatory counts. Because of this relationship, only ambulatory counts are reported.

Thirty days following saline (n = 14), MPD (n = 7) and MDMA (n = 7) pre-treatment (PD 62), the effect of

cocaine (15 mg/kg) on locomotion was investigated. Cocaine (15 mg/kg) was administered since this was found to be the optimal dose for investigating LMA in Swiss-Webster mice (Itzhak, 1997). Routinely, each animal was placed in the test cage and allowed a 30 min habituation period to the new environment. In the first phase of the experiment, MDMA, MPD and half of the saline pretreated mice were given cocaine (15 mg/kg) while the remaining saline pretreated mice received saline. Treatments were administered for 5 consecutive days. LMA was recorded on day 1 and day 5 for 30 min. In the second phase of the experiment, all animals remained drug-free for 14 days and were then challenged with cocaine (5 mg/kg), and their locomotion was recorded for 30 min.

2.5. Statistical analysis

Daily weight averages were analyzed by two-way ANOVA (drug × time effects) with time as the repeated measure, followed by Bonferroni post hoc test for comparison between weights of saline- and drug-treated animals on specific days. The CPP results were analyzed by one-way ANOVA followed by Newman-Keuls post hoc test to compare between the magnitude of CPP in the saline, MPD and MDMA groups following induction, extinction and reinstatement of place preference.

Results of LMA experiments in response to the first and fifth of 5 daily cocaine injections were analyzed by repeated ANOVA, followed by Newman-Keuls post hoc test to determine differences between groups. A two-way ANOVA (drug × time effects) followed by Bonferroni post hoc test was performed for determination of sensitization over time. A one-way ANOVA followed by Newman-Keuls post hoc test was applied to analyze the LMA responses produced by a single challenge cocaine injection given to four different groups of mice. In all analyses, a p value less than 0.05 was considered a statistically significant difference.

3. Results

3.1. Effect of MPD and MDMA on weight gain

Fig. 1 illustrates the weight increase of the mice while undergoing drug or saline treatments and 28 days thereafter. Results show that administration of MPD and MDMA significantly inhibited weight increase compared to the saline group from PD 28–PD 39. A two-way ANOVA comparing saline and MPD treated mice resulted in significant drug effect, $F[1,300] = 42.42$; $p < 0.0001$ and time effect $F[9,300] = 220.2$; $p < 0.0001$ and insignificant interaction $F[9,300] = 1.753$; $p = 0.0769$. On PD 28, 29 and 32 the weights of MPD treated mice were significantly lower than saline controls

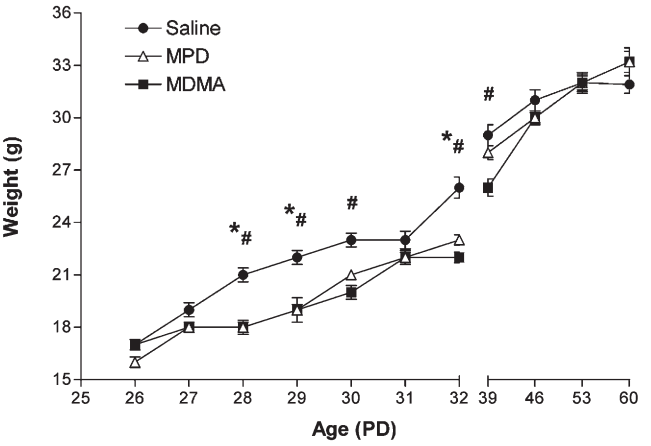

Fig. 1. Effect of MPD and MDMA on weight gain. Swiss-Webster mice, PD26, were given saline, MPD (10 mg/kg) or MDMA (10 mg/kg) for seven days until PD32. Weights were measured daily during drug treatment, and thereafter once a week until PD 60. There was a significant difference in weight between the saline and MPD groups on PD 28, 29, 32 ($*p < 0.01$) and between saline and MDMA groups on PD 28, 29, 30, 32 and 39 ($\#p < 0.01$). Weights amongst the three groups equalized by PD 46.

($t = 2.375$–2.625; $p < 0.01$). A two-way ANOVA comparing saline and MDMA treatment resulted in significant drug effect $F[1,300] = 65.91$; $p < 0.0001$ and time effect $F[9,300] = 207$; $p < 0.0001$ and significant interaction $F[9,300] = 3.671$; $p = 0.0002$. On PD 28, 29, 30, 32 and 39 the weights of the MDMA treated mice were significantly lower than saline controls ($t = 3.187$–4.958; $p < 0.01$). There were no significant differences between drug-treated (MPD or MDMA) and control groups 14 days after the last drug exposure (PD 46; Fig. 1).

3.2. Effect of MPD and MDMA adolescent pretreatment on the induction and reinstatement of cocaine-induced CPP

During the pre-conditioning phase, mice from all 3 groups (saline, $n = 9$; MPD, $n = 9$ and MDMA, $n = 9$) spent an average of 745 ± 28 sec in the black zone of the CPP cage, 712 ± 41 sec in the white zone and 363 ± 34 sec in the null zone. The 3 groups differed by less than 8%. In each group some mice had slight preference to either the black or the white zone (127 ± 18 sec). The times spent in the least preferred zone were 585 ± 51, 619 ± 47 and 605 ± 38 sec for the saline, MPD and MDMA groups, respectively. Accordingly, 4–5 mice from each group were paired with cocaine in the least preferred compartment (black or white).

The CPP experiments consisted of three phases: induction, extinction and reinstatement. Fig. 2(A) illustrates the induction of CPP. Conditioning of the saline, MPD and MDMA pretreated mice with cocaine (20 mg/kg) resulted in an increase of 243 ± 31, 93 ± 25 and

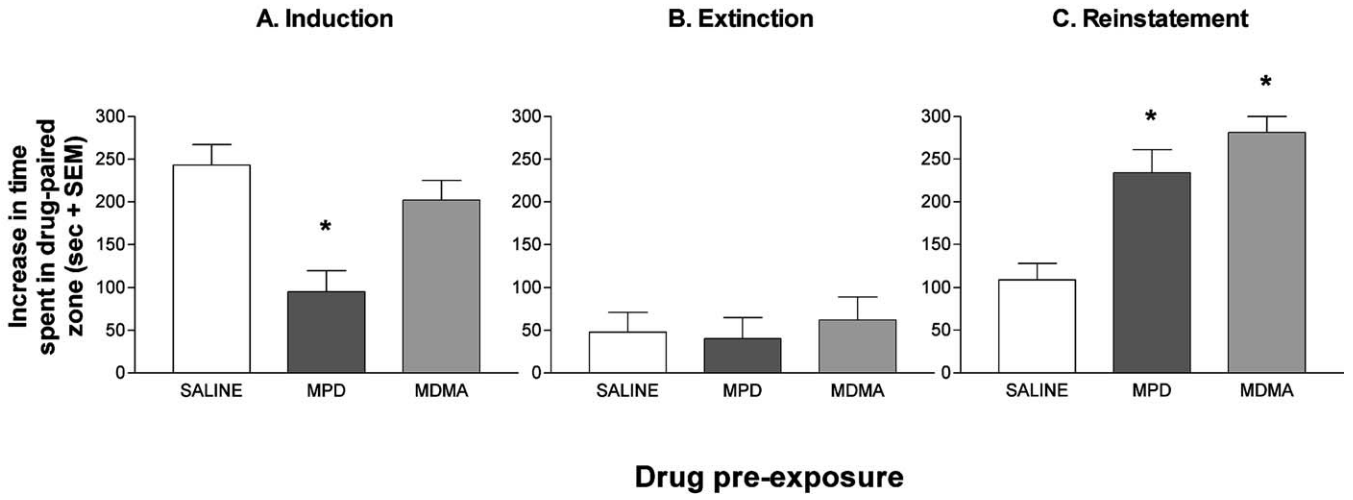

Fig. 2. Effect of MPD and MDMA pretreatment on the induction, extinction and reinstatement of cocaine CPP. Panel A: Induction. Mice were pretreated with saline ($n = 9$), MPD (10 mg/kg; $n = 9$) and MDMA (10 mg/kg; $n = 9$) for 7 days (PD 26–32). From PD 62, mice were given four cocaine (20 mg/kg) and four saline injections on alternating days in their respective cocaine-assigned and saline-assigned zones. Data are presented as the difference in time spent in the drug paired zone before and after the induction of place preference. The MPD group spent significantly less time in the drug paired zone compared to the saline ($*p < 0.05$) and MDMA ($*p < 0.05$) groups. Panel B: Extinction. Mice received repeated saline injections (14 days) in the drug- and saline-paired zones. Data are presented as the difference in time spent in the drug-paired zone before the induction and after the extinction of place preference. The time spent in the drug-paired zone after extinction was not significantly different from the time spent in the same zone before the induction of CPP, and no significant differences between the three groups were observed. Panel C: Reinstatement. Mice were administered a priming injection of cocaine (5 mg/kg), and the time spent in each zone was recorded under the influence of the drug. Results are presented as the difference in time spent in the previously drug-paired zone after the extinction of CPP and following cocaine administration. The magnitude of CPP in the MPD and MDMA groups was significantly higher than in the saline group ($*p < 0.05$).

202 ± 23 sec, respectively, in the time spent in the drug-paired zone, compared to the time spent in the same zone before the conditioning. The magnitude of CPP observed in the saline group is highly similar to the magnitude we observed previously in Swiss Webster mice conditioned with the same dose of 20 mg/kg cocaine (Itzhak and Martin, 2000, 2002b). One-way ANOVA for comparison of the magnitude of CPP induction between the three groups resulted in a significant main effect $F[2,24] = 6.991$; $p = 0.0041$. Post hoc Newman-Keuls test showed a significant difference between saline and MPD groups ($p < 0.05$) and MDMA and MPD groups ($p < 0.05$). No significant difference between the saline and MDMA groups was observed (Fig. 2(A)). These results suggest that the induction of CPP in the MPD group was significantly lower than in the saline and MDMA groups.

Extinction of CPP was accomplished by administration of repeated saline injections for 14 days in both the drug- and saline-paired compartments. This procedure diminished the expression of CPP. The differences in time spent in the previously drug-paired zone before induction and after extinction of place preference were 47 ± 23, 41 ± 25 and 58 ± 27 sec for the saline, MPD and MDMA groups, respectively. The differences between the three groups were insignificant $F[2,24] = 0.201$; $p = 0.819$ (Fig. 2(B)). To investigate the susceptibility of the three groups of mice to a priming injection of cocaine, mice were challenged with a low dose of cocaine (5 mg/kg). Results illustrated in Fig. 2(C) show the difference between the time spent in the previously drug-paired zone after extinction and following reinstatement of CPP in the saline (109 ± 31 sec), MPD (234 ± 27 sec) and the MDMA (281 ± 23 sec) groups. One-way ANOVA for comparison between the three groups resulted in a significant main effect $F[2,24] = 7.231$; $p = 0.0035$. Post hoc Newman-Keuls test showed a significant difference between the saline and MPD groups ($p < 0.05$) and saline and MDMA groups ($p < 0.05$). No significant difference between the MPD and MDMA groups was observed (Fig. 2(C)). These results suggest that the reinstatement of place preference in the drug pre-exposed animals was significantly higher than in the control group.

3.3. Effect of MPD and MDMA adolescent pretreatment on cocaine-induced LMA

To investigate the effects of MPD and MDMA pretreatment during adolescence on cocaine-induced LMA, mice received five daily injections of cocaine (15 mg/kg) and locomotion was recorded on days 1 and 5. An additional group of saline pretreated mice received saline

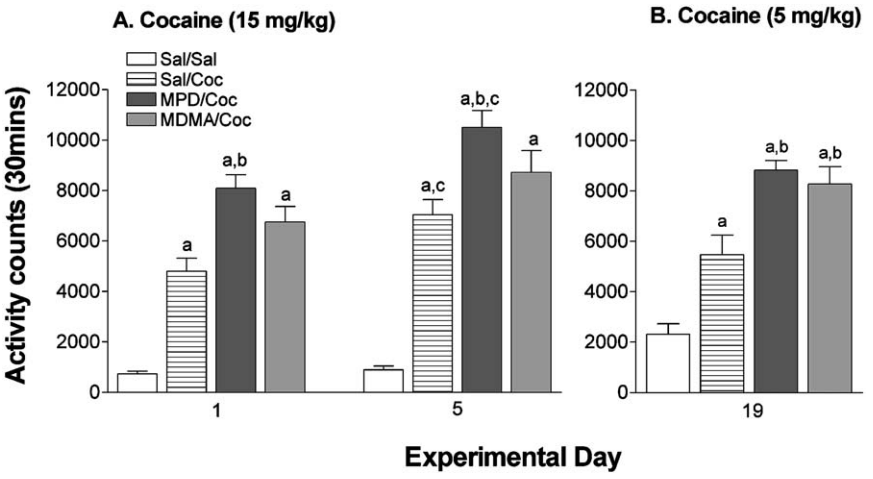

Fig. 3. Effect of MPD and MDMA pretreatment on cocaine-induced LMA. Panel A: Effect of repeated administration of cocaine (15 mg/kg) for 5 days. Mice were pretreated with saline ($n = 14$), MPD (10 mg/kg; $n = 7$) and MDMA (10 mg/kg; $n = 7$) for 7 days (PD 26–32). From PD 62, mice were given either saline (Sal/Sal) or cocaine (Sal/Coc, MPD/Coc and MDMA/Coc) for 5 days. LMA counts for 30 mins are presented for days 1 and 5. On day 1, cocaine induced significantly higher locomotion than saline injection, Sal/Sal vs. Sal/Coc; MPD/Coc; and MDMA/Coc (a), $p < 0.001$. Also, the response of the MPD/Coc group on day 1 was significantly higher than the response of the Sal/Coc group (b), $p < 0.05$. There was no significant difference between the Sal/Coc and MDMA/Coc groups. On day 5, as on day 1, cocaine induced higher locomotion than saline (a), $p < 0.001$, and again the MPD/Coc group was more sensitive to cocaine than the Sal/Coc group (b), $p < 0.05$. No significant difference was observed between the Sal/Coc and the MDMA/Coc groups. Comparison of cocaine effects between days 1 and 5 shows that on day 5, the Sal/Coc group was sensitized to the effect of cocaine compared to day 1 (c), $p < 0.05$, and similarly the MPD/Coc group was sensitized to cocaine compared to day 1 (c), $p < 0.05$. No significant sensitization was observed in the MDMA/Coc group (comparison between days 1 and 5). Panel B: Effect of a challenge injection of cocaine (5 mg/kg). Animals remained drug free for 14 days and on day 19 (from the first cocaine injection) were challenged with a low dose of cocaine (5 mg/kg). The challenge injection of cocaine induced higher locomotion in the Sal/Coc, MPD/Coc and MDMA/Coc groups than in the Sal/Sal group (a), $p < 0.05$, suggesting that all cocaine (15 mg/kg) pre-exposed mice were sensitized to cocaine compared to saline pre-exposed mice. Also, the response of the MPD/Coc and the MDMA/Coc groups was significantly higher than the response of the Sal/Coc group (b), $p < 0.05$, suggesting that MPD and MDMA pre-exposure induced further sensitization to cocaine.

injections for five days. Fig. 3(A) illustrates the results of LMA measured on days 1 and 5 in response to saline (Sal/Sal) and cocaine (15 mg/kg) (Sal/Coc; MPD/Coc; MDMA/Coc). Repeated measure ANOVA for the comparison of LMA between the 4 groups on days 1 and 5 showed the following results: $F[3,56] = 43.84$; $p = 0.0056$. Newman-Keuls multiple comparisons test showed significant differences between the Sal/Sal vs. Sal/Coc ($p < 0.001$), Sal/Sal vs. MPD/Coc ($p < 0.001$) and Sal/Sal vs. MDMA/Coc ($p < 0.001$) groups. In addition, there was a significant difference between Sal/Coc vs. MPD/Coc ($p < 0.05$) on day 1 and day 5. These results suggest that adolescent exposure to MPD resulted in higher sensitivity to the locomotor stimulating effect of cocaine. No significant difference between the saline and MDMA groups was observed for both days 1 and 5. The effect of cocaine on locomotion on days 1 and 5 was analyzed by two-way ANOVA (drug treatment x time). There was a significant drug effect $F[3,48] = 87.32$; $p < 0.0001$, a significant time effect $F[1,48] = 17.38$; $p = 0.0001$, and insignificant interaction $F[3,48] = 1.981$; $p = 0.129$. Bonferroni post-hoc test showed significant differences between days 1 and 5 in Sal/Coc group ($t = 2.810$; $p < 0.05$) as well as in the MPD/Coc group ($t = 3.050$; $p < 0.05$). These findings indicate that saline and MPD pretreated mice developed sensitization to cocaine by day 5. The MDMA group showed a trend towards sensitization on day 5 compared to day 1, but the difference was not statistically significant ($t = 2.475$; $p > 0.05$).

To determine the effect of a challenge injection of cocaine following withdrawal, animals remained drug free for 14 days and were then challenged with a low dose of cocaine (5 mg/kg). Results in Fig. 3(B) depict the effect of the challenge injection of cocaine given to all four groups on day 19. One-way ANOVA resulted in a significant main effect: $F[3,24] = 25.105$; $p < 0.0001$. Post-hoc Newman-Keuls test showed a significant difference between the LMA response of Sal/Sal vs. Sal/Coc; MPD/Coc and MDMA/Coc ($p < 0.05$) suggesting the development of sensitization to cocaine (5mg/kg) in all three cocaine pre-exposed groups compared to the Sal/Sal group. There was also significant difference between Sal/Coc vs. MPD/Coc ($p < 0.05$) and Sal/Coc vs. MDMA/Coc ($p < 0.05$), suggesting that the pre-exposure to MPD and MDMA enhanced the sensitization to challenge injection of cocaine.

4. Discussion

We report that exposure of mice to MPD or to MDMA (10 mg/kg × 7 days) during adolescence ultimately increases cocaine-induced reward and psychomotor stimulation in adulthood. The sensitization to reward and locomotion was observed particularly after a 2-week withdrawal period from cocaine. The relevance of these results to human adolescent exposure to MPD and MDMA should be cautiously interpreted for the following reasons: a) There is no reliable animal model of ADHD. While in ADHD patients MPD suppresses hyperactivity, in rodents MPD acts as other psychostimulants, causing an increase in LMA. b) The route of drug administration used in the present study (i.p.) is different than human drug administration and thus different pharmacokinetics are expected. c) Serum levels of the drug were not determined; therefore we cannot speculate whether the dose of the drug administered to the animals is relevant to the human therapeutic dose of MPD as well as to abused doses of MPD and MDMA. Nevertheless, the findings presented suggest that exposure to both MPD and MDMA may cause similar enduring neural adaptations that impact the reward system in adulthood, causing heightened propensity for drug relapse.

4.1. Drug effects on body weight

The results from the weight measurements during MPD and MDMA administration showed a significant inhibition in weight gain in the drug treated mice compared to saline treated mice from PD 28 through PD 39 (Fig. 1). This finding is in accordance with the known anorectic effect of amphetamines as well as with studies on adolescent patients treated with MPD. A significant suppression of weight gain in MPD-treated ADHD children and adolescents, but no effect on the development in height, has been reported (Spencer et al., 1996; Vincent et al., 1990). The finding that from PD 46 and thereafter the weights of drug treated mice were the same as controls (Fig. 1) suggests that the impairment of weight gain was transient and due to the acute effect of the drug.

4.2. MPD and MDMA adolescent exposure and cocaine-induced reward in adulthood

Evidence suggests that pre-exposure to drugs of abuse increases the propensity to seek further drug reward as indicated by drug self-administration (Horger et al., 1990; Schenk and Partridge, 2000; Schulze et al., 2002) and CPP (Gaiardi et al., 1991; Shippenberg and Heidbreder, 1995) studies. Previous reports on adolescent rat exposure to MPD and cocaine-induced reward are inconclusive. Pretreatment of 4-week old rats with MPD (2 mg/kg) caused an increase in cocaine self-administration two weeks after MPD treatment was terminated (Brandon et al., 2001). In another study, the pretreatment of PD 20 rats with MPD (2 mg/kg) for 15 days reduced the rewarding effect of cocaine as determined 25 days after MPD treatment ceased (Andersen et al., 2002).

The current data suggest that pre-exposure to MPD (10 mg/kg × 7 days) during adolescence initially impaired the acquisition of cocaine-induced CPP in adulthood. This is consistent with the findings by Andersen et al. (2002). The authors reported that pre-exposure of adolescent rats to MPD induced neural plasticity, manifested as an increase in the expression of CREB (cAMP response element binding protein) in the nucleus accumbens, and that this was associated with a decrease in the rewarding effect of cocaine. An additional account for the initial diminished sensitivity of the MPD group to the rewarding effect of 20 mg/kg cocaine may be due to the development of partial aversion to this particular high dose of cocaine as a result of sensitization to the drug (Fig. 3(A)). Cocaine caused an inverse U shape dose-response in CPP experiments, such that high doses no longer produced CPP (Le Pen et al., 1998).

Pre-exposure to MDMA (10mg/kg × 7 days) did not affect the induction of CPP, as the magnitude of CPP in the MDMA group was not different from controls (Fig. 2A). Although a trend in sensitization to the psychomotor stimulating effect of cocaine was observed in this group, there was no statistically significant difference between the saline and MDMA groups in their response to cocaine-induced hyperlocomotion (Fig. 3(A); day 1). Moreover, the MDMA group, unlike the MPD group, did not develop significant sensitization on day 5 compared to day 1 (Fig. 3(A)). Therefore, if pre-exposure to MPD but not MDMA caused an increased sensitivity to cocaine (15 mg/kg) in the LMA study, then the dose of 20 mg/kg given for the induction of CPP may have been less rewarding, thus inhibiting the induction of CPP in the MPD group. The initial difference between the two groups in their response to cocaine may be due in part to the various neurotransmitters involved in the effects of MPD and MDMA. Evidence suggests that acute administration of MPD primarily increases DA but not 5-HT efflux (Kuczenski and Segal, 1997), while MDMA increases extracellular levels of both DA and 5-HT (Logan et al., 1988; O'Shea et al., 2001).

Although the initial responses of the MPD and MDMA groups to cocaine-induced CPP differed, the final response after extinction and withdrawal from cocaine was the same, and significantly different from controls. A priming injection of cocaine (5 mg/kg) following two weeks' withdrawal from cocaine induced marked CPP in both MPD and MDMA groups, which was significantly higher than in the saline group (Fig. 2(C)). It appears that the cocaine withdrawal rendered long-lasting neural adaptations in the reward system of both the MPD and MDMA groups, culminating in

enhanced sensitivity to the rewarding effect of cocaine. The sensitized response of the MDMA group to the priming injection of cocaine is in general agreement with the findings showing that rat pre-exposure to MDMA facilitated the acquisition of cocaine self-administration (Fletcher et al., 2001) and cocaine-induced CPP (Fone et al., 2002).

4.3. MPD and MDMA adolescent exposure and cocaine-induced psychomotor stimulation in adulthood

It is widely accepted that pre-exposure to psychostimulants induces sensitization to subsequent drug exposure (Robinson and Berridge, 2000). In the present study, results of the initial cocaine-induced psychomotor stimulation experiments (Fig. 3; day 1) suggest that adolescent exposure to MPD induced sensitization to cocaine. The sensitized response to cocaine was observed on the first and the fifth day of cocaine administration; subsequently, both the saline and MPD groups developed relative sensitization to cocaine on the fifth day of injections. However, although a trend in sensitization to cocaine effect on day 1 was observed in the MDMA group, there was no significant difference between the responses of the saline and MDMA groups to cocaine. Also, the MDMA group did not develop significant sensitization to cocaine following the 5-day cocaine administration (Fig. 3(A)). The lack of sensitization to locomotion was not due to an increase in vertical activity. Routinely, we observed that administration of 15 or 20mg/kg cocaine for 5 days to Swiss Webster mice did not cause stereotypy (Itzhak, 1997; Itzhak and Martin, 2000). The different outcome from MPD and MDMA administration may be due to the fact that MPD stimulates mainly dopaminergic transmission (Kuczenski and Segal, 1997) while MDMA increases extracellular levels of both DA and 5-HT in mice (Logan et al., 1988; O'Shea et al., 2001). Thus, the initial neural alterations that developed during adolescent MPD treatment may have differed from those resulting from MDMA treatment. Yet, two weeks after withdrawal from cocaine, it appeared that a similar adaptation had developed in both drug pre-exposed groups compared to the saline pretreated group, culminating in an enhanced response to cocaine. As shown in Fig. 3(B), two weeks following the termination of the repeated cocaine injections, the MPD and MDMA groups were significantly more sensitive to cocaine (5 mg/kg) than the saline group.

In a previous study administration of MPD (5 or 10 mg/kg) to 4-week old rats for 7 days resulted in a sensitized response to cocaine challenge that was given after two weeks (Brandon et al., 2001). In a second study, administration of MPD (2 mg/kg) to PD 20 rats for 15 days caused reduced sensitivity to the locomotor stimulating effect of cocaine challenge that was given after 25 days (Andersen et al., 2002). In a third study, administration of MPD (0.75–3.0 mg/kg) to PD 41 rats for 20 days resulted in diminished responsiveness to the motor stimulating effect of methamphetamine that was given after10 days for a period of 16 days (Kuczenski and Segal, 2002). Our results of MPD administration to mice are similar to those of Brandon et al. (2001) probably because of the similar schedule of MPD exposure (e.g., 10 mg/kg for 7 days). The differences between our results and Andersen et al. (2002) and Kuczenski and Segal (2002) studies may depend upon: a) different schedules of MPD pre-exposure, b) age and species of the animals, and c) the period of abstinence between the termination of MPD administration and the first challenge injection of the psychostimulant (methamphetamine or cocaine). These variables may all contribute to the different outcomes from exposure to MPD.

4.4. The withdrawal period from cocaine facilitates sensitization to the rewarding and locomotor stimulating effects of subsequent drug exposure in MPD and MDMA pre-exposed mice

One of the major differences between the present study and previous studies on the outcome of adolescent rodents' exposure to MPD is the cocaine-withdrawal period that we investigated. In the previous studies, the subsequent effects of cocaine (Brandon et al., 2001; Andersen et al., 2002) and methamphetamine (Kuczenski and Segal, 2002) were investigated after acute (cocaine) or repeated (methamphetamine) injections, but the consequences of withdrawal from the drugs, and re-challenge with the psychostimulants were not investigated.

The significance of the withdrawal period from cocaine stems from both the CPP and LMA experiments. Although the initial acquisition of cocaine place preference was low (MPD group) or not different from controls (MDMA group), the withdrawal period from cocaine resulted in enhanced sensitivity to a priming injection of the drug in the MPD and MDMA groups compared with the saline group. Likewise, while the MDMA group did not show psychomotor sensitization following initial cocaine administration, withdrawal from cocaine rendered higher sensitivity in both the MDMA and MPD groups to cocaine challenge compared to controls. Taken together, these findings suggest that exposure of the developing brain to two different psychostimulants, MPD and MDMA, may trigger similar long-lasting neural alterations that are expressed as an increased sensitivity to cocaine challenge following withdrawal from cocaine. Enhanced sensitivity to psychostimulants following withdrawal is thought to be relevant to vulnerability to drug relapse, which may persist for many years. Further molecular work is needed to

ascertain which neural substrates are responsible for the behavioral changes revealed in this study.

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