Methylphenidate Treatment during Pre- and Periadolescence Alters Behavioral Responses to Emotional Stimuli at Adulthood

Carlos A. Bolan˜os, Michel Barrot, Olivier Berton, Deanna Wallace-Black, and Eric J. Nestler

Background: Methylphenidate (MPH) is a psychomotor stimulant medication widely used for the treatment of attention-deficit/hyperactivity disorder (ADHD). Given the extent of prescribed use of MPH, and because MPH interacts with the same brain pathways activated by drugs of abuse, most research has focused on assessing MPH’s potential to alter an individual’s risk for adult drug addiction. Data examining other potential long-term behavioral consequences of early MPH administration are lacking, however.

Methods: We investigated the long-term behavioral consequences of chronic administration of MPH (2.0 mg/kg) during pre- and peradolescent development in adult rats by assessing their behavioral reactivity to a variety of emotional stimuli.

Results: The MPH-treated animals were significantly less responsive to natural rewards such as sucrose, novelty-induced activity, and sex compared with vehicle-treated control animals. In contrast, MPH-treated animals were significantly more sensitive to stressful situations, showed increased anxiety-like behaviors, and had enhanced plasma levels of corticosterone.

Conclusions: Chronic exposure to MPH during development leads to decreased sensitivity to rewarding stimuli and results in enhanced responsivity to aversive situations. These results highlight the need for further research to improve understanding of the effects of stimulants on the developing nervous system and the potential enduring effects resulting from early-life drug exposure.

Key Words: Attention-deficit/hyperactivity disorder, methylphenidate, development, depression, emotion

Introduction

Attention-deficit/hyperactivity disorder (ADHD) is a neuropsychiatric disorder, commonly diagnosed during childhood, characterized by excessive levels of inattentiveness, impulsivity, and hyperactivity (Goldman et al. 1998; Kirby et al. 2002; Miller and Castellanos 1998; Swanson et al. 1998). Although reported figures vary considerably depending on diagnosis criteria, socioeconomic status, geographic source of sample, and gender, it is estimated that up to 15%–20% of elementary school–age children meet the criteria for diagnosis of ADHD (Miller and Castellanos 1998; Wilens et al. 2002; Zito et al. 2000), with a reported population prevalence of up to 12% (Brown et al. 2001; Goldman et al. 1998; LeFever et al. 1999; Swanson et al. 1998). Stimulant medications have been effective for the treatment of ADHD (Findling and Dogin 1998; Spencer et al. 1996; Wolraich et al. 1990), and methylphenidate (MPH) is the most prescribed therapeutic agent (Findling and Dogin 1998; Kirby et al. 2002; Miller and Castellanos 1998), accounting for more than 90% of stimulants used for the management of ADHD in the United States (Goldman et al. 1998; Zito 2002; Zito et al. 2000). Treatment duration for the disorder can last for years (Greenhill et al. 2002; Silver 2000; Spencer et al. 1996), because longitudinal studies report that ADHD symptoms can continue into adulthood at an estimated rate of persistence of up to 75% of diagnosed children (Biederman et al. 1996; Faraone et al. 2000; Silver 2000; Wilens et al. 2002) and because stimulants remain the preferred treatment for the management of adult ADHD (Taylor and Russo 2001).

Similar to the cellular and behavioral actions of the psychomotor stimulants cocaine and amphetamine (Koob et al. 1998; Wise and Bozarth 1987), basic and clinical studies have shown that MPH has psychomotor stimulant–like properties: it blocks the dopamine transporter (Schweri et al. 1985; Volkow et al. 1998), induces dosedependent increases in dopamine levels in brain pathways activated by drugs of abuse (Gerasimov et al. 2000b; Kuczenski and Segal 1997; Volkow et al. 1999, 2001), and produces heightened motor responses after its repeated

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Despite MPH’s potential role in drug abuse liability and the fact that affected children may be treated with MPH for years, it is surprising that little is known about the long-term neuronal and behavioral adaptations resulting from MPH exposure during the juvenile period. In this context, it is well-documented that neurobiological systems still undergo substantial neuronal adaptations during developmental periods before adulthood (Seeman et al. 1987; Spear 2000; Teicher et al. 1995); these age-dependent neuroadaptations have been correlated, at least in part, with changes in cognitive processes and responsivity to emotional stimuli (Hyman 2001; Spear 2000), as well as altered responsiveness to psychopharmacologic intervention (Bolanos et al. 1998; Emslie and Mayes 2001; Laviola et al. 1999). In addition, it has been demonstrated that environmental, emotional, and pharmacologic experiences during development can dramatically influence neurobehavioral functioning later in life (Hyman 2001; Ladd et al. 2000; Spear 2000; Teicher et al. 2002).

Given that MPH interacts with brain structures known to control responses to rewarding as well as aversive stimuli (Kelley and Berridge 2002; Robbins and Everitt 1996; Volkow et al. 1998), we assessed the reactivity of vehicle- (VEH-) and MPH-treated animals to a range of emotional stimuli. This approach was taken given increasing evidence that mesolimbic reward pathways are part of the neural circuitry that controls mood under normal conditions (Barrot et al. 2002; Bolanos et al., in press; Naranjo et al. 2001; Nestler et al. 2002; Newton et al. 2002; Pliakas et al. 2001; Yadid et al. 2001). Thus, to gain some insight into the long-term consequences of early-life MPH treatment, we designed the following study to assess potential long-term behavioral alterations associated with repeated exposure to MPH. We treated developing rats during the pre- and periadolescence period (postnatal day 20–35) with twice-daily injections of a low dose of MPH (2.0 mg/kg), which results in plasma and brain levels of MPH within the range achieved under clinical conditions (Gerasimov et al. 2000a; Volkow et al. 1998; Wargin et al. 1983). After VEH or MPH treatment and early behavioral assessment of play behavior, animals were left undisturbed until they became adults (90+ days old), at which point their behavioral responses to a battery of emotional stimuli were assessed.

Methods and Materials

Subjects and Drug Treatment

Litters containing Sprague–Dawley male and female rat pups with their respective dams were obtained from Harlan (Indianapolis, Indiana) at postnatal day (PD) 14. After a 5-day acclimation period, MPH hydrochloride (2.0 mg/kg, intraperitoneal [IP]; U.S. Pharmacopeia, Rockville, Maryland) or saline vehicle injections were given to pups starting on PD 20 twice daily (4 hours apart, first injection at 9 AM) for 16 days (last injections on PD 35). Rat pups were weaned and separated with same-sex littermates in groups of four animals per cage at PD 23, and double housed in clear polypropylene boxes containing wood shavings at PD 50 in an animal colony maintained at 23°–25°C on a 12-hour light–dark cycle in which lights were on between 7 AM and 7 PM. Only male offspring were used for behavioral testing. With the exception of the play behavior experiments that were conducted on PD 40, behavioral testing started 6 weeks after the last MPH injection. All behaviors, except for locomotor activity to a novel environment, were recorded with a video camera located on the ceiling of each testing room. Raters unaware of treatment conditions scored the videotapes. Behavioral observations were recorded by an observer with no knowledge of the treatment conditions of each animal. With the exception of the subjects used for the corticosterone levels experiment (i.e., they were part of the locomotor responses to novel environment experiment only), animals were tested in the different behavioral paradigms culminating with the forced swim test. There was a period of at least 2 weeks before each behavioral test. Animals were provided with food and water ad libitum. Experiments were conducted in compliance with the National Institute of Health and University of Texas Southwestern’s institutional animal review committee.

Play Behavior

We assessed the short-term effects of MPH treatment by measuring behavioral responses to a natural reward, namely, social play behavior, during periadolescence (PD 40, 5 days after the last MPH injection), an ontogenetic period in which social play still is at its peak (Pankepp et al. 1984). Social play is a critical aspect of rodent development. In addition, play is a powerful reward to young rodents, and access to “play time” in briefly
isolated rodents activates brain reward pathways (Gordon et al 2002; Panksepp et al 1984; Vanderschuren et al 1997). Animals were singly housed for 24 hours before testing. All testing occurred within 2 hours after the start of the dark phase, under red light conditions. Pairs of rats from the same experimental group (i.e., control VEH or MPH), with no previous common social experience, were placed in an arena similar to their home cage, and were allowed to interact freely for 5 min. Interactions were videotaped and later analyzed using The Observer software, version 4.1 (Noldus Information Technology, Leesburg, Virginia). For each pair, the scored parameters were the number of pins (scored when one animal has its dorsal surface toward the ground with the other animal above) and the number of dorsal contacts (scored when one animal touches the dorsal surface of the other animal with its paws) as previously described by Panksepp et al (1984).

Sucrose Preference

The sucrose preference test consisted of a two-bottle choice paradigm. In this behavioral test, animals are given the choice between consuming water versus sucrose. This paradigm has been used extensively in animal models of depression to assess the effects of stress-induced anhedonia (Papp et al 1991; Sampson et al 1992; Willner et al 1987). Additionally, we have successfully used this paradigm to assess changes in appetitive behavior induced by rewarding stimuli after discrete molecular manipulations of reward pathways in brain (Barrot et al 2002; Bolanos et al, in press). Animals were habituated to drink water from two bottles for 5 days. At the start of the experiment, rats were exposed to ascending concentrations of sucrose (.0, .125, .25, .5 and 1%) for 2 days per sucrose concentration. Water and sucrose consumption were measured at 8 AM and 5 PM each testing day, at which time the position of the sucrose bottle (left or right) was balanced between the MPH and VEH groups. The preference for sucrose over water was used as a measure for animals’ sensitivity to reward.

Locomotor Activity Responses to a Novel Environment

Animals were also tested to examine if MPH or VEH treatment during development affects general motor activity as indexed by locomotor responses to a novel environment. Rats were placed in automated (75 cm diameter × 15 cm wide, four photocell beams) circular activity chambers (Med Associates, St. Albans, Vermont; Bolanos et al, in press; Carlezon et al 1997), and their locomotor reactivity to the novel environment (i.e., the circular activity chambers) were assessed for 2 hours divided into 10-min intervals.

Elevated Plus Maze and Self-Grooming Behavior

MPH- and VEH-treated rats were tested for 5 min on the elevated plus maze, a behavioral model of anxiety-like behavior. The maze was made of gray plastic and consisted of two perpendicular, intersecting runways (12 cm wide × 100 cm long; Barrot et al 2002; Bolanos et al, in press). One runway had tall walls (40 cm high) or “closed arms,” and the other one had no walls or “open arms.” The arms were connected together by a central area, and the maze was elevated 1 m from the floor. Testing was conducted between 9 AM and 1 PM under controlled light conditions (~90 lux). At the beginning of the 5-min observation, animals were placed in the central area, facing one of the open arms, and the cumulative time spent in the open arms was videotaped from a video camera placed on the ceiling of the testing room. Raters unaware of the treatment conditions scored the videotapes. Another behavioral response that rodents exhibit in response to stressful and or anxiogenic stimuli is self-grooming (Gispen et al 1988; Gispen and Isaacson 1981; Spruijt et al 1988). Thus, we also assessed time spent grooming in the “closed arms” during the 5-min test.

Social Interaction in an Aversive Environment

In this test, rats face a conflict between social interacting and their innate fear of an aversive environment. In this context, time spent performing social behaviors varies depending on how aversive the testing environment is (File and Hyde 1978; Ramos and Mormede 1998). The MPH- and VEH-treated animals were tested for social interaction in an open-field arena (70 cm square) that was divided into nine virtual squares (23 × 23 cm). To increase the aversiveness of the environment, the light intensity was raised to 250 lux. After social isolation of 1 week, rats were placed in pairs (from same drug treatment, but no previous common social experience) in two opposite corners of the test arena and were then allowed to interact freely for 5 min. Interactions were videotaped and analyzed using The Observer version 4.1. For each animal, the scored parameters were the number of crossed squares and the total duration of active social interactions. The latter dependent variable comprised the following behavioral classes: sniffing, following, grooming, crawling over, and crawling under as described by File (1980).

Sexual Behavior

For the sexual behavior experiments, animals were housed in a separate room maintained on a 12-hour light–dark cycle (lights on between midnight and 12 noon). Sexual behavior was assessed under red light conditions between 1:30 PM and 6 PM in a circular arena (60 cm) containing wood chips on the floor. Each male was given a 5-min acclimation period to the testing arena. Testing started at the end of the acclimation period by the introduction of a receptive female to the arena. Testing sessions lasted 90 min unless the male failed to initiate sexual behavior (at least 1 mount) within 30 min. Behaviors recorded were mounts, intromissions, and ejaculation as previously described (Sodersten et al 1977). More specifically, we measured mount latency (from the start of the test to the first mount with or without intromission), intromission latency (from the start of the experiment to the first intromission), ejaculation latency (calculated from the first intromission), and postejaculatory interval (time from ejaculation to the first following intromission). For statistical analyses, a mount latency of 30 min was attributed to animals that failed to initiate the behavior within this time period. Sprague–Dawley ovariectomized female rats (Charles River, Kingston,
New York) were used in these experiments. Receptivity of the females was induced by injection of estradiol benzoate (50 μg, subcutaneous) and progesterone (500 μg, subcutaneous) 48 and 4–6 hours before testing, respectively. One week before the experiment, the females were tested for one intercourse with an experienced male. Before testing, female receptivity was verified by the exhibition of lordosis, in the presence of the experienced male, and accepted intromission. Each female was used to test only one experimental male.

Forced Swimming

The forced swim test is a 2-day procedure in which rats are forced to swim under conditions in which they cannot escape. On the first day, rats engage initially in a variety of escape-like behaviors but eventually adopt a posture of immobility in which they make only the movements necessary to maintain their head above water. When retested 24 hours later, rats become immobile more quickly; antidepressant treatment between the forced swim exposures can significantly increase their escape-like behaviors, an effect that has been correlated with antidepressant activity in humans (Cryan et al 2002; Porsolt et al 1977). At the start of the experiment, rats were placed in plastic cylinders (30 × 45 cm) filled to a depth of 30 cm (so that their paws and tail do not touch the bottom) with 25°C water and forced to swim for 15 min. At the end of this period, rats were removed from the water, dried with towels, and kept in a warm enclosure for 30 min. All cylinders were emptied and cleaned between rats. Twenty-four hours after the forced swim, rats were retested for 5 min under identical conditions, and sessions were videotaped by a video camera attached to the ceiling of the testing room. Raters unaware of the treatment conditions scored the videotapes. In this study, the latency to become immobile was the dependent variable. Latency to immobility was defined as the time at which the rat first initiated a stationary posture that did not reflect attempts to escape from the water (Bolanos et al, in press; Lucki 1997; Pliakas et al 2001). To qualify as immobility, this posture had to be clearly visible and maintained for ≥ 2 sec.

Plasma Corticosterone (CORT) Levels

A separate group of animals (n = 16; VEH = 8, MPH = 8) were treated with MPH (2.0 mg/kg) or VEH during development for the assessment of CORT levels after restraint stress. Tail blood samples were obtained at four time points: 0, 15, 40, and 90 min. Collection of each blood sample was made in about 3 min. Initially we restrained the animals for 20 min, and blood samples were taken at time 0 and 15 min. After 20 min of restraint, animals were returned to their home cage and were briefly restrained again (twice, for approximately 5 min each time) to obtain blood samples at the 40 and 90 min time points. Blood was collected in ice-cold heparin coated tubes and centrifuged (1000 × g, 15 min, 4°C). Aliquots of plasma were stored at –20°C until assayed. Plasma CORT levels were determined by competitive enzyme immunoassay (EIA) according to the manufacturer’s specifications (ALPCO Diagnostics, Windham, New Hampshire). Data are expressed as ng/mL. The sensitivity of the assay was .23 ng/mL of plasma. Twelve of the animals used for the assessment of CORT levels (VEH = 6, MPH = 6) had previously been part of the locomotor activity responses to novelty experiment only.

Statistical Analysis

Significance was measured using mixed-design (between and within variables) repeated analysis of variance (ANOVA) followed by Scheffé post hoc test. When appropriate, Student’s t, chi-square, and F tests were used to determine statistical significance of preplanned comparisons. Data are expressed as the mean ± SEM. Statistical significance was defined as p < .05.

Results

Effect of MPH on Weight Gain, Water Intake, and Play Behavior in the Developing Rat

Rats were weighed once daily (9 AM) during the MPH or VEH treatment (n = 30). Figure 1A shows that a clinically relevant dose of MPH (2.0 mg/kg, IP twice daily [b.i.d.]) had no effect on weight gain in pre- and peradolescent rats (p > .05). Water intake (total intake per cage) was also monitored during, starting at weaning, and 5 days following the last MPH injection. No changes in water intake were observed (data not shown).

To assess short-term influence on behavior by drug treatment, we measured the ability of MPH treatment to affect play behavior 5 days after the last MPH injection (PD 40; n = 30). Figure 1B shows the average number of pins and dorsal contacts, behaviors characteristic of play in young rodents, indicating that MPH treatment did not affect play behaviors in these animals (p > .05).

Effect of MPH on Sucrose Preference in the Adult

Given previous findings that MPH treatment during preadolescence can alter the rewarding effects of cocaine in the adult (Andersen et al 2002), we first assessed the long-term effects of MPH by measuring animals’ responses to sucrose, a natural reward. We used a sucrose preference test, which is free of associative memory. Overall repeated-measures ANOVAs indicated that chronic exposure to MPH did not significantly affect the rats’ total fluid intake (water + sucrose; Figure 2 lower panel) at any of the sucrose concentrations tested (p > .05); however, sucrose preference varied as a function of drug treatment and sucrose concentration (drug treatment × sucrose concentration interaction: F(7,406) = 9.37; p = .0002). Prior MPH treatment decreased sucrose preference at .125 (p = .003), .25 (p = .005), and .5% (p = .055) sucrose when compared with the preference exhibited by the VEH control group (Figure 2 upper panel). Differences in sucrose preference between groups were not present at the 1% sucrose concentration (n = 30).
Effect of MPH on Locomotor Reactivity to a Novel Environment in the Adult

As can be seen in the inset of Figure 3, animals treated with MPH (2.0 mg/kg, b.i.d.) during preadolescence had significantly less spontaneous ambulatory counts in response to a novel environment (i.e., the locomotor chambers) during the 2-hour session than the VEH-treated control animals (t[40] = -2.9; p = .006; n = 42). Additional analysis indicates that locomotor activity also varied as a function of drug-treatment and time spent in the activity chambers (drug treatment × time interaction: F(11,440) = 2.52; p = .004). Thus, a subsequent Scheffé post hoc test revealed that VEH-treated animals were significantly more active during the first hour of testing (p < .05) than the MPH-treated group (Figure 3). In contrast,

given that locomotor activity of MPH- and VEH-treated animals did not differ in the second hour of testing, it is likely that baseline locomotor activity is not different between the two groups of animals.

Effect of MPH on Elevated Plus Maze and Self-Grooming Behavior in the Adult

We also studied the effect of MPH on anxiety-like behavior using the elevated plus maze (Figure 4A and 4B; n = 30). Drug treatment affected time spent in the open arms of the plus maze (a measure of anxiety-like behavior); animals
receiving MPH during preadolescence spent significantly less time in the open arms than VEH-treated rats (t(28) = 2.51; p < .05; Figure 4A). In addition, the MPH-treated group spent significantly more time engaged in self-grooming behavior than the VEH-treated control animals (t(28) = 3.44; p < .001; Figure 4B). These results suggest an increase in anxiety-like behavior in the MPH-exposed animals.

**Effect of MPH on Social Interaction in an Aversive Environment in the Adult**

We assessed the ability of preadolescence MPH treatment to affect social interaction in adult rats (n = 30). Figure 4C and 4D shows the average number of square crosses, duration of interaction, and time noninteracting, all of which show no difference between the MPH and VEH groups. These findings indicate that MPH did not affect animals’ behavior in this measure of anxiety (p > .05).

**Effect of MPH on Sexual Behavior in the Adult**

Animals treated with MPH during adolescence showed a deficit in sexual behavior at adulthood (Table 1; n = 29). Compared with the VEH-treated control animals, a smaller proportion of MPH-treated animals displayed intromission (55.5% vs. 90.9%; χ² = 13.56; p = .0001) and reached ejaculation (27.7% vs. 72.7%; χ² = 7.8; p = .001) within 30 min after their first contact with a receptive female. This deficit appears to be accounted by a delay in latency to the first mount (p = .015) and to the first intromission (p = .015); however, when considering the subpopulation of MPH-treated animals that successfully completed an intercourse, a tendency for a deficit was still observed as indicated by a larger number of intromissions necessary to reach ejaculation (p = .08; see Table 1). Among those animals that successfully completed the first intercourse, the latency to initiate a second intercourse showed no further deficits (p = .38).

**Effect of MPH on Forced Swimming Behavior in the Adult**

Given the increasing evidence that the mesolimbic dopamine system regulates responses to aversive stimuli as
well as rewarding ones (Barrot et al 2002; Bolanos et al, in press), it was of interest to study the effect of MPH treatment during development on behavioral tests of aversion \((n = 30)\). We used the forced swim test to study an animal’s responses to stressful conditions (Cryan et al 2002; Pliakas et al 2001). In this test, animals initially struggle, apparently trying to escape, but within a minute or two they become immobile. The amount of time rats engaged in escape-directed behaviors (i.e., latency to immobility) in the forced swim test was dependent on drug treatment; animals treated with MPH during development had significantly shorter times to become immobile (treatment main effect: \(F_{(1,28)} = 5.2; \ p = .0009\)) than the VEH-treated control group (Figure 5A).

**Effect of MPH on CORT Levels during Restraint Stress in the Adult**

Given our findings of increased anxiety-like behavioral responsiveness in MPH-treated animals, we assessed plasma CORT levels in a separate group of VEH- and MPH-pretreated adult rats \((n = 16)\). Repeated-measure ANOVA indicated that MPH treatment had a significant effect on CORT levels in response to restraint stress (treatment \(\times\) time interaction: \(F_{(3,42)} = 40.227; \ p = .0001\); Figure 5B). More specifically, subsequent post hoc tests revealed that MPH-treated animals had significantly higher CORT levels at 15 min \((p = .04)\) and were relatively higher than the VEH-treated control animals at 90 min \((p = .09); \) Figure 5B).

**Discussion**

Psychostimulants have proven to be an effective pharmacotherapy for ADHD, and MPH is the preferred medication for the treatment and management of this neuropsychiatric disorder. Given the prevalence of prescribed use of MPH in pediatric populations, it is surprising that few studies have used developmental animal models to analyze the long-term neurobiological and behavioral effects of MPH treatment. The purpose of this study was to assess potential enduring behavioral alterations in adult animals as a consequence of repeated MPH exposure during their juvenile period. We show that repeated exposure to a therapeutic dose of MPH (2.0 mg/kg b.i.d.) during the juvenile period results in altered behavioral responses to a variety of rewarding and aversive stimuli during adulthood.

More specifically, our findings show that adult animals pretreated with MPH during the juvenile period are less sensitive to the rewarding effects of natural rewards such as sucrose, are less responsive with respect to the motor activation exhibited by animals when first exposed to a novel environment, and show deficits in the initiation and performance of sexual behavior. In contrast, these animals show greater sensitivity to several types of aversive stimuli, including swim stress and anxiogenic challenges. Together, these data suggest that MPH treatment during development (i.e., pre- and periadolescence periods) results in decreased responsiveness to rewarding stimuli while enhancing a negative emotional state characterized by increased sensitivity to aversive stimuli.

The findings that adult animals treated with MPH during development are less sensitive to sucrose (i.e., concentrations lower than 1%) are likely due to MPH’s ability to alter an animal’s responsiveness to the rewarding effects of sucrose, because the overall liquid intake during sucrose testing did not differ between the treatment groups. Moreover, no alterations in liquid intake were observed during or after MPH treatment. Our findings further show that MPH-treated animals have deficits in sexual behavior, although in this study it was difficult to differentiate between “interest” versus “efficiency.” It is well-established that the brain’s reward pathways, such as the nucleus accumbens (NAc) and its dopaminergic input from the ventral tegmental area, are involved in regulating motivated behavior and responses to drugs of abuse and natural rewards (Di Chiara and North 1992; Kelley and Berridge 2002; Robbins and Everitt 1996; Wise and Bozarth 1987). Experimental evidence indicates that exposure to sweet solutions such as sucrose (Datla et al 2002; Hajnal and Norgren 2001) and to the opposite sex (Becker et al 2001; Damsma et al 1992; Fiorino et al 1997) are rewarding; they activate the mesolimbic dopamine system and cause increases in dopamine release in the NAc, whereas lesions to these reward pathways block sucrose preference (Shimura et al 2002) and impair sexual behavior (Everitt 1990; Robbins et al 1989). Together, our results are in agreement with recent findings indicating that early exposure to MPH leads to long-lasting alterations in brain dopamine systems that result in decreased sensitivity to cocaine reward and diminished cocaine-seeking behavior (Andersen et al 2002), and we now extend these findings to natural rewards.

We also assessed the short-term effects of MPH treatment by measuring behavioral responses to a natural reward, namely, social play behavior, during periadolescence (PD 40, 5 days after the last MPH injection), an ontogenetic period in which social play is still at its peak (Panksepp et al 1984). Our results indicate that MPH pretreatment did not affect play behavior in these animals. Social play is a critical aspect of rodent development. It has been shown that play is a powerful reward to young rodents and that access to “play time” in briefly isolated rodents activates brain reward pathways (Gordon et al 2002; Panksepp et al 1984; Vanderschuren et al 1997).

\[F_{(1,28)} = 5.2; \ p = .0009\]
Table 1. Measures of Copulatory Behavior

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Methylphenidate</th>
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<tr>
<td></td>
<td>(n = 11)</td>
<td>(n = 18)</td>
</tr>
<tr>
<td>Mount Latency (sec)</td>
<td>526 ± 155</td>
<td>1131 ± 152*</td>
</tr>
<tr>
<td>Intromission Latency (sec)</td>
<td>654 ± 191</td>
<td>1269 ± 145*</td>
</tr>
<tr>
<td>Ejaculation Latency (sec)</td>
<td>653 ± 110 (n = 10)</td>
<td>865 ± 150 (n = 11)</td>
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<tr>
<td>Number of Mounts</td>
<td>6.3 ± 1.2</td>
<td>13.1 ± 4.4</td>
</tr>
<tr>
<td>Number of Intromissions</td>
<td>14.9 ± .9</td>
<td>20.4 ± 2.7h</td>
</tr>
<tr>
<td>Mounts + Intromissions</td>
<td>21.2 ± 1.8</td>
<td>33.4 ± 6.0h</td>
</tr>
<tr>
<td>Intromission Ratio</td>
<td>72% ± 4.1</td>
<td>68% ± 5.3</td>
</tr>
<tr>
<td>PEI (sec)</td>
<td>338 ± 12</td>
<td>325 ± 9</td>
</tr>
<tr>
<td>% I 30</td>
<td>90.9 (10/11)</td>
<td>55.5 (10/18)^a</td>
</tr>
<tr>
<td>% E 30</td>
<td>72.7 (8/11)</td>
<td>27.7 (5/18)^a</td>
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Methylphenidate treatment during development affects sexual behavior during adulthood. Data are presented as mean ± SEM. The ejaculation latency was calculated from the first intromission. PEI, postejaculatory interval; % I 30, proportion of animals (10/18) displaying intromission within 30 min; % E 30, proportion of animals (5/18) reaching ejaculation within 30 min.

* p < .015 (t test).

h p < .08 (t test).

^a p < .001 (χ² test).  
^p < .001 (χ² test).

Our findings are in agreement with previous research showing that exposure to relatively low doses of MPH does not affect play or social interaction in young adult rats (Sproson et al 2001). It is interesting to note, however, that although in this previous study MPH pretreatment did not affect social interaction, it did affect mesolimbic neuronal functioning because presynaptic dopamine release was significantly attenuated by repeated MPH administration during development (Sproson et al 2001). In addition, research has shown that social interaction and play behavior are not entirely dopamine-dependent because nondopaminergic mechanisms are also involved in mediating this behavior (Panksepp et al 1980; Vander- schuren et al 1995). Thus, it is conceivable that whereas repeated MPH exposure affects mesolimbic dopamine systems, it does not affect social play in periadolescent animals.

Our findings also indicate that MPH treatment during development alters an animal’s sensitivity to aversive stimuli. Given that MPH’s primary sites of action are located within limbic structures, these findings lend further support to the notion that the mesolimbic reward pathway may play a role in the symptoms of depression and other stress-related syndromes (Bolanos et al, in press; Kapur and Mann 1992; Naranjo et al 2001; Nestler et al 2002; Pliakas et al 2001; Yadid et al 2001). Thus, animals pretreated with MPH during development exhibited shorter latency to immobility in the forced swim test, an effect opposite to that of antidepressant treatments (Cryan et al 2002; Porstl et al 1977). Further studies with the elevated plus maze found that MPH exposure increased responses to anxiogenic stimuli, because these animals spend significantly less time in the “open arms” and significantly more time engaged in grooming behavior, a behavioral response to stressful or anxiogenic stimuli (Gispen et al 1988; Gispen and Isaacson 1981; Spruijt et al 1988). Another behavioral measure of anxiety, namely, adult social interaction under increased light conditions, did not reveal deficits, because there were no differences in social interaction responding between the treatment groups. In this test, rats face a conflict between social interaction, a behavioral measure of natural reward, and their innate fear of an aversive environment. Thus, the time spent performing social behaviors varies as a function of how aversive the testing environment is or is perceived by the animal (File 1980; File and Hyde 1978; Ramos and Mormede 1998). In this context, it is conceivable that the opportunity to “interact” is an incentive stimulus with positive motivational valance that is powerful enough to mask potential MPH-induced alterations. Additionally, it is likely that the lack of an effect of MPH on social interaction may be due to repeated handling (i.e., same group of animals used for the locomotor activity and the elevated plus maze test) because it is well-established that prior handling has anxiolytic effects in several testing situations (Britton and Britton 1981; Ferre et al 1995; Hogg 1996). Conversely, and similarly to our findings during periadolescence, it is possible that MPH pretreatment does not affect the positive motivational aspects of play and social interaction. Despite the lack of effect on this measure of anxiety, our results of increased CORT levels in response to restraint stress further suggest that MPH treatment during development results in enhanced reactivity to stressful situations at adulthood.

In our attempt to develop a general profile of behavioral responsivity to emotional stimuli after early-life MPH treatment, we also assessed the motor activity induced by exposure to a novel environment (Hooks and Kalivas 1995; Kabbaj et al 2000). In this test, MPH-treated animals showed significantly lower behavioral reactivity to the novel test environment compared with the VEH-treated control animals. Novelty has positive incentive valence (Bardo et al 1996; Pierce et al 1990), and exposure to novel environments increases dopamine release in the NAc (Rebec et al 1997). Accordingly, it is conceivable that the hypophoexicity exhibited by the MPH group was due to decreased sensitivity to the rewarding aspects associated with exploring a novel environment. On the other hand, novelty also has some aversive-stressful components (Bardo et al 1996; Kabbaj et al 2000); thus, the lower reactivity to the novel environment exhibited by the MPH group could be an indication of lesser sensitivity to stress, not to reward. Nevertheless, it has been established that novelty-elicited behavioral activation is largely dependent on the integrity of the mesolimbic dopamine system (Bardo et al 1990; Fink and Smith 1980; Koob et al 1981).
and that the level of behavioral responding is correlated with the activity of ventral tegmental area dopamine neurons (Marinelli and White 2000). In this context, regardless of whether the reduced behavioral reactivity displayed by the MPH group is an indication of alterations in their ability to respond to a rewarding or stressful stimulus, it is likely that the attenuated reactivity to novelty is an indication of deficits in the mesolimbic dopamine system induced by early-life MPH exposure.

The mechanisms underlying these enduring behavioral effects induced by repeated MPH exposure during development are unknown. Based on previous research showing that changes in the transcription factor cAMP response element-binding protein (CREB) within the mesolimbic dopamine system regulates responsiveness to rewarding and aversive stimuli, we hypothesize that changes in this transcription factor may underlie these MPH-induced behavioral adaptations. For example, activation of CREB or its overexpression in the NAc or ventral tegmental area decreases the rewarding effects of drugs of abuse (Carlzon et al 1997, 1998; Self et al 1998) and natural rewards (Barrot et al 2002), while enhancing an animal’s sensitivity to aversive stimuli (Pliakas et al 2001). In addition, recent findings by Andersen et al (2002), indicating that treatment with a similar dose of MPH (2.0 mg/kg) during the same developmental period as in our study causes a sustained increase in CREB levels in the NAc and a subsequent decrease in cocaine-induced reward, lends further support to this hypothesis.

Although our findings are in general agreement with those suggestive of MPH-induced dysregulation of reward substrates leading to decreased cocaine reward (Andersen et al 2002), our results must also be viewed in light of those obtained by Brandon et al (2001), which show increased sensitivity to cocaine reward after MPH treatment. In the latter study, the authors administered MPH (2.0 mg/kg) for a relatively short period (7 days, once daily), whereas in this study we used a longer treatment regimen (15 days) that included twice-daily injections of the drug. In addition, we treated our animals between 20 to 35 days postnatal, whereas in the Brandon et al study animals were exposed to the drug between postnatal days 35 to 42. Accordingly, it is conceivable that whereas shorter MPH treatment during adolescence causes sensitized responses to rewards (Brandon et al 2001), other treatment regimens, involving longer treatment duration and earlier developmental stages (Andersen et al 2002; this study) may yield different results. In this ontogenetic context, it is not surprising to encounter different and, at times, conflicting results given that qualitative and quantitative differences frequently emerge when manipulating the nervous system during a particular maturational stage (Bolanos et al 1996; McDougall and Bolanos 1995; McDougall et al 1992; Spear 2000; Spear and Brake 1983; Zavala et al 2002).

To summarize, results of our study indicate that treatment with MPH during development can significantly alter responsiveness to natural rewards and aversive stimuli in adulthood. These findings provide further impetus for the development of effective, nonstimulant treatments for ADHD; however, it is imperative to note that the MPH-induced long-term behavioral effects described in this study were derived from “normal” animals, and similar MPH treatment using established animal models for ADHD might yield different, even contrary, results. Indeed, the results of this study should not be overinterpreted, because MPH remains a safe and effective treatment for ADHD. Still, basic and human developmental psychopharmacology is an area of study that has received relatively little attention. Thus, little is known about how psychotropic drugs may influence the developmental processes of the central nervous system or the enduring effects during adulthood. Our findings show that developmental administration of MPH results in aberrant behavioral adaptations during adulthood, and these results underscore the need for further developmental research geared toward a better understanding of the mechanisms

Figure 5. Methylphenidate (MPH) exposure during development regulates responses to forced swim stress. (A) Latencies to become immobile varied as a function of drug treatment. Latencies were significantly decreased in rats treated with MPH (2.0 mg/kg, twice daily) during development compared with the vehicle (VEH)-treated control animals. Data are presented as latencies (mean ± SEM, in sec) to become immobile (*p = .0009; n = 30). (B) Corticosterone levels at several time points during restraint stress. Corticosterone levels were generally higher in adult rats treated with MPH during development. It took less than 3 min to collect each blood sample. Initially, animals were retrained for 20 min, and blood samples were taken at times 0 and 15 min. After the first restraint period, animals were returned to their home cage and were briefly restrained again (twice, approximately 5 min each time) to take blood samples at the 40 and 90 min time points. Data are presented as ng/mL. *p = .04; †p = .09; n = 16.

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underlying MPH-induced behavioral plasticity. Research examining the effects of psychotropic drugs during development and their potential enduring effects will lead to a better understanding of the neural and molecular basis for better psychopharmacologic therapies aimed at pediatric populations.

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References


