Prenatal exposure to methylphenidate hydrochloride decreases anxiety and increases exploration in mice

Melanie P. McFadyen-Leussis[a], Stephen P. Lewis[a], Tamara L.Y. Bond[a],
Normand Carrey[b], Richard E. Brown[a,*]

[a]Department of Psychology, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4J1
[b]Department of Outpatient Psychiatry, IWK-Grace Hospital, Halifax, Nova Scotia, Canada B3H 4A1

Abstract

The administration of methylphenidate (MPH) to girls and adults has increased in the last decade. Given the similarity of MPH to cocaine and the increasing possibility of embryonic exposure, the gestational effects of this stimulant on development must be considered. We administered MPH (5 mg/kg) or saline to female CD-1 mice at three different periods during pregnancy [embryonic (E) days 8–10, 12–14, and 16–18]. MPH-exposed pups were compared with the saline-treated pups for changes in physical, motor, and behavioral development at postnatal day (PND) 3–11. In adulthood (>60 days of age) these mice were tested in the open field, elevated plus maze, and water maze, and given an acute MPH challenge. We observed limited effects of MPH exposure on early developmental variables. In adulthood, mice exposed to MPH on E8–10 exhibited a general decrease in anxiety-related behaviors and a concomitant increase in exploratory behavior. Prenatal MPH exposure did not alter water maze performance or the response to an acute MPH challenge. Our data provide an initial overview of the possible effects occurring as a result of prenatal exposure to MPH, and strongly suggest that further studies of the in utero and developmental effects of psychostimulants are needed.
© 2004 Elsevier Inc. All rights reserved.

Keywords: Anxiety; Behavior; Development; Methylphenidate; Mice; Prenatal; Rodent

1. Introduction

Methylphenidate hydrochloride (MPH; Ritalin) is routinely prescribed for the treatment of attention deficit hyperactivity disorder (ADHD; Diller, 1998; Greenhill, 1995, 1998; Kimko et al., 1999). Between 1990 and 1998, there was a 2.8-fold increase in MPH prescriptions for girls (Robison et al., 2002). It is estimated that in up to 70% of ADHD cases, symptoms persist into adulthood (Biederman, 1998). ADHD, although less prevalent in females (Anderson et al., 1987), may actually be more severe in females; as they are more likely to seek treatment (Wender, 1987), they display greater disturbances in cerebral glucose metabolism than males (Ernst et al., 1994), and they exhibit a higher genetic loading for the disorder than males do (Pauls, 1991). For these and other reasons, it has been suggested that

ADHD may be more likely to persist beyond adolescence in females (Andersen and Teicher, 2000). In addition, a sevenfold increase in MPH abuse among 10–19 year olds was reported between 1993 and 1999 (Klein-Schwartz and McGrath, 2003). In other words, since MPH is being increasingly prescribed to women of childbearing age, the incidence of prenatal MPH exposure should also be increasing. It is therefore imperative that we examine the effects of MPH on the developing fetus.

Only one study has investigated the potential effects of intrauterine exposure to MPH in humans. Infants were identified with retrospective measures to assess the occurrence of maternal MPH or pentazocine abuse during pregnancy, and were followed for two years. The results suggested that prenatal MPH exposure is associated with premature birth, growth retardation, and signs of neonatal withdrawal, but not with any teratogenic anomaly or severe developmental delay (Debooy et al., 1993). However, the study did not distinguish between the effects of prenatal MPH or pentazocine and did not include sufficient controls.

MPH is a nonamphetamine stimulant that acts to inhibit catecholamine uptake, predominantly of dopamine, in a manner similar with cocaine (Fowler et al., 2001). In mice, dopamine is first detected in developing embryo at around embryonic day 9.5 (E9.5) and peaks late in the neonatal period, while norepinephrine appears at about E10.5 and epinephrines and 5-HT both appear around E13.5 (Berger-Sweeney and Hohmann, 1997; Miranda-Contreras et al., 1998; Thomas et al., 1995). In contrast, dopaminergic neurons appear in the rat around E12–16 (Ugrumov, 1997). Norepinephrine and 5-HT fibers, both axons and dendrites, begin to appear at approximately E17 (Berger-Sweeney and Hohmann, 1997). The early emergence of catecholamine expression suggests that catecholamines play a critical role in neural development (Pendleton et al., 1998; Thomas et al., 1995). Early manipulations of serotonin or dopamine can cause permanent alterations in these systems (Mazer et al., 1997; Vitiello, 1998). Furthermore, drugs of abuse can significantly impact brain development (for review, see Levitt, 1998). More importantly, prenatal exposure to drugs, such as cocaine, can influence behavior and learning later in life. Prenatal cocaine exposure leads to several changes in behavioral development including decreased ultrasonic vocalizations from 2 to 4 days of age (Hahn et al., 2000), delayed righting reflex ability in infancy (Henderson and McMillen, 1990), deficits in first-order Pavlovian conditioning at 9, but not at 12, days of age (Kosofsky and Wilkins, 1998), and hyperactivity at 30 days of age (Henderson and McMillen, 1990). In adulthood, the enduring behavioral consequences of prenatal cocaine exposure include impoverished social behavior (Johns and Noonan, 1995), higher levels of prepulse inhibition and greater immobility in a forced-swim test (Overstreet et al., 2000), and deficits in a Pavlovian conditioning blocking paradigm (Kosofsky and Wilkins, 1998). It follows that exposure to MPH, an agent that alters normal neurotransmitter levels, could result in organizational changes in the brain, which can lead to long-term neurobiological and behavioral changes in the individual.

Few studies have examined the effects of MPH on development in rodents. Much of the work to date has focused on postnatal MPH neurotoxicity (Teo et al., 2002a,b; Yuan et al., 1997; Zaczek et al., 1989). Several studies have looked at the effects of postnatal administration of MPH, but these have focused on the short-term effects of MPH on behavior (Carrey et al., 2000; Penner et al., 2001) or neurochemistry (Moll et al., 2001; Penner et al., 2002; Sproson et al., 2001). MPH exposure for 14 days in prepubertal rats inhibits c-*fos* expression in the striatum (Chase et al., 2003). Furthermore, MPH exposure in young rats causes a decrease in the density of striatal dopamine transporters, which endures into adulthood, long after drug treatment has ceased (Moll et al., 2001). Prenatal exposure to MPH has not been investigated in rodents. There is a need to do so; first, to establish whether administration of MPH during pregnancy can be deleterious to the fetus, and second, to detail any neurobehavioural changes that result from the administration of MPH at distinct times during embryonic development.

Our research has focused on systematically assessing the effects of MPH exposure during infancy (Penner et al., 2001, 2002) or just prior to puberty (Carrey et al., 2000; McFadyen et al., 2002) on physical and behavioral development. To complement the postnatal work that we have done, we examined the effects of prenatal MPH exposure on development and behaviour in infancy and adulthood. MPH was administered to pregnant females at three different embryonic time points. Pups were tested in a developmental test battery between 3 and 11 days of age. As adults, the same mice were tested in the open field, elevated plus maze, and water maze, and were given an acute MPH challenge and retested in the open field.

2. Methods

2.1. Subjects

Outbred CD-1 mice (Charles River Laboratories, Quebec) were mated in the laboratory at Dalhousie University. The resulting litters were used as subjects. The housing room was on a 12:12 light/dark cycle (lights off at 9:00 am) and the temperature was maintained at 21 +/− 2 °C. Food (Laboratory rodent chow 5001, Agribrand, Strathray, ON) and tap water were available ad libitum in all stages of the study. Mice were treated in accordance with the guidelines set by the Canada Council on Animal Care, and the experimental protocol was approved by The Dalhousie University Committee on Laboratory Animal Care.

2.2. Drugs

Pregnant females were randomly assigned to either the experimental or the control group. Based on our previous results in infants (Penner et al., 2001), the experimental group received 5 mg/kg MPH (Medisca Pharmaceutique, Montreal, Canada) in 0.9% sterile saline, by subcutaneous injection. Given the greater metabolism of MPH in mice as compared with humans (Faraj et al., 1974), a dose of 5 mg/kg should be approximately equivalent to a clinically relevant dose in humans, which ranges from 0.3 up to 1 mg/kg (Sachdev and Trollor, 2000; Solanto, 2000). The control group received an equivalent volume of 0.9% sterile saline. The drug was administered daily over a 3-day embryonic period, from E8 to 10, E12 to 14, or E16 to 18, which mirrors the early appearance of dopamine, dopaminergic neurons, and norepinephrine and 5-HT fibers, respectively. Thus, the last exposure period targets brain development after the dopaminergic system is mostly in place.

2.3. General procedures

Female mice were housed in groups of 12 in clear polypropylene cages ($46 \times 12 \times 16$ cm), with stainless steel wire lids and wood chip bedding, for a period of $10-14$ days prior to the beginning of the experiment, so as to synchronize their estrous cycles, such that timed pregnancies could be achieved. Each female was then individually placed in a clear plastic cage ($32 \times 12 \times 16$ cm) with a single male. Females were checked daily for the presence of a vaginal plug, and the day a plug was detected was counted as E0. Pregnant females were housed individually in opaque white plastic hanging cages ($28 \times 12 \times 16$ cm) until their litter was delivered and weaned. Only when two litters, one from each drug treatment group, were born within 24 h of each other, were they included in the study. At postnatal day 2 (PND 2), litters were culled to four male and four female pups each, and one half of the mice (i.e., two male and two female pups) from each of the matched litters were cross fostered to the litter of the opposite treatment group, to control for the effects of drug treatment on maternal behavior.

Beginning on PND 2, pups were removed individually from the nest, weighed, and marked with a nontoxic marker. Testing in the developmental test battery took place every second day, from PND 3 to 11. The mice were weaned at 21 days of age, and housed in same-sex littermate groups of three or four (two birth siblings and two fostered siblings) in clear plastic cages ($32 \times 12 \times 16$ cm). Beginning at 62 days of age, the mice were tested in the open field, elevated plus maze, and water maze, were challenged with an acute dose of MPH (5 mg/kg), and were retested in the open field. The mice were taken from no less than three litters for each drug exposure group in each embryonic exposure period to minimize litter effects. All mice were tested in each paradigm during development and as adults.

2.4. Developmental test battery

Seven measures (listed below) were used to examine the effects of prenatal MPH on motor and behavioral development. Twenty-four pups (13 male, 11 female) exposed to MPH and 23 pups (13 male, 10 female) exposed to saline at $E8-10$ were tested in the developmental test battery, as were 15 pups (9 male, 6 female) exposed to MPH and 16 pups (6 male, 10 female) exposed to saline on $E12-14$, and 10 pups (5 male, 5 female) exposed to MPH and 12 pups (4 male, 8 female) exposed to saline on $E16-18$.

2.4.1. Body weight

Pups were weighed every test day (PND 3, 5, 7, 9, and 11) before testing began. Their adult weight was measured on PND 78.

2.4.2. Locomotor activity and ultrasonic vocalizations

The ultrasound/activity chamber (UVbox) was made of black plexiglass, with a clear plexiglass panel in the front of the chamber to allow for observation of the animal being tested. The chamber ($40 \times 40 \times 30$ cm) contained an infrared activity monitoring system that detected horizontal locomotor activity in the chamber. The total distance traveled during the 6-min test period was analyzed.

Ultrasonic vocalizations (UVs) were detected by a QMC bat detector (QMC Instruments, London). The microphone was suspended 5 cm above the center of the test chamber. The high-frequency output from the bat detector was digitized using an eight-channel digitizer, with frequencies set at 30, 40, 50, 60, 70, 80, 90, and 100 kHz. A desktop computer recorded locomotor activity and the number of UVs. Locomotion and UVs were recorded every test day (PND 3, 5, 7, 9, and 11).

2.4.3. Surface righting reflex

Pups were placed on a flat surface in a supine position. The time taken to right, defined as all four limbs placed under the body, was recorded to a maximum of 60 s. Two trials were done on each test day (PND 3, 5, and 7), with a 60-s rest period between trials. If a pup failed to complete the task, it was assigned the maximum allotted time (60 s). The mean of the two daily trials was used for analysis.

2.4.4. Negative geotaxis

Pups were placed on $30°$ incline, with their head pointing down the incline. The time for the pup to turn $180°$ was recorded to a maximum of 60 s. Two trials, with a rest period of 60 s between trials, were given on each test day (PND 3, 5, and 7). The mean of the two daily trials was used for analysis.

2.4.5. Forelimb grip strength

The forepaws of the pup were placed on a thin rubber band suspended 6 in. above the soft bedding material. The time to fall was recorded to a maximum of 30 s. Two trials, with a rest period of 60 s between trials, were administered on each test day (PND 7, 9, and 11). The better of the two scores was recorded.

2.4.6. Swimming ability

The pups were placed in a plastic container (20×13 cm), filled to a depth of 6 cm with room temperature tap water. Whether the pups could swim, defined as their ability to keep their nose above the water for 5 s, was recorded on each day. The pups were removed promptly if their nose dropped below the water. A single trial was given on each test day (PND 5 and 7). After the trial, the pups were dried and returned to their home cage.

2.5. Adult behavioral tests

The mice were given four tests in adulthood. Starting at PND 62, we tested 23 mice (12 male, 11 female) exposed to MPH and 22 mice (13 male, 9 female) exposed to saline on $E8-10$; 13 mice (7 male, 6 female) exposed to MPH and 16

mice (6 male, 10 female) exposed to saline on E12–14; and 10 mice (5 male, 5 female) exposed to MPH and 11 mice (4 male, 7 female) exposed to saline on E16–18.

2.5.1. Open field

On PND 62, each mouse was given a 5-min trial in the open field, which provides simultaneous measures of spontaneous locomotion (line crossing), exploration (rearing), and fear or anxiety (center square entries, defecation; Weiss and Greenberg, 1996). A detailed review of the procedure and apparatus has appeared elsewhere (Carrey et al., 2000).

2.5.2. Elevated plus maze

On PND 64, each mouse was individually given a 5-min trial in the elevated plus maze, which provides a measure of anxiety in rodents (Lister, 1987). The procedure and apparatus have been described in detail elsewhere (Carrey et al., 2000).

2.5.3. Water maze

Testing in the water maze took place over a period of 4 days, from PND 67 to 70. A visible platform was used on the first 3 days, and a hidden platform was used on the last day (McDonald and White, 1994). On each of the 4 days, the mice had four trials, each up to a maximum of 60 s in duration, with one trial beginning from each starting location (N, S, E, or W). The procedure and apparatus have been described in more detail elsewhere (McFadyen et al., 2002).

2.5.4. MPH challenge

On PND 78, each mouse was given an injection of MPH (5 mg/kg, subcutaneously), as a challenge to determine whether prenatal exposure altered the response to MPH later in life. One hour postinjection, the mice were tested in the open field for a second time, using the same protocol as described above. These data were analyzed as the difference from the results in the open field on PND 62. This allows us to minimize individual baseline variation and focus on the effect of the acute drug administration.

2.5.5. Statistical analyses

The three embryonic injection periods were analyzed separately because different neural systems are developing during each embryonic period and thus may lead to differential postnatal effects or interactions. A repeated-measures mixed between-within analysis of variance (ANOVA) was used to analyze body weight, locomotor activity, frequency and duration of UVs, and the adult water maze data. A chi-square test was used to analyze swimming ability. Neuromotor developmental data (righting reflex, negative geotaxis, forelimb grip strength) were analyzed by multivariate analysis of variance (MANOVA). One-way ANOVAs were used to analyze the data collected in adulthood in the open field and elevated plus maze. Differences in scores for the acute

MPH challenge in the open field, as they differed from open-field measures at 62 days of age, were analyzed by MANOVA. For all analyses, sex, litter, and cross fostering were analyzed as control variables. Except where otherwise indicated, the data were collapsed across these variables as no differences were found.

3. Results

3.1. Developmental test battery

3.1.1. Body weight

Although body weight increased across days for all treatment groups [F_{E8-10} (4,180) = 2238.57, $P < .001$; $F_{E12-14}(4,108) = 512.36$, $P < .001$; $F_{E16-18}(4,80) = 576.90$, $P < .001$], pups exposed to MPH did not differ in weight from those exposed to saline during any of the embryonic administration periods [$F_{E8-10}(1,45) = 1.87$, $P = .18$; $F_{E12-14}(1,27) < 1.0$; $F_{E16-18}(1,20) < 1.0$; Table 1].

3.1.2. Locomotor activity

The distance traveled in the UVbox increased across testing days for all groups [$F_{E8-10}(4,120) = 85.45$, $P < .001$; $F_{E12-14}(4,88) = 26.08$, $P < .001$; $F_{E16-18}(4,32) = 36.32$, $P < .001$], but pups exposed to MPH did not differ from those exposed to saline for any of the embryonic injection periods [$F_{E8-10}(1,30) = 2.09$, $P = .16$; $F_{E12-14}(1,22) < 1.0$; $F_{E16-18}(1,8) = 1.12$, $P < .32$; Fig. 1].

3.1.3. Ultrasonic vocalizations

The number of UVs changed across days [$F_{E8-10}(4,120) = 4.47$, $P < .002$; $F_{E12-14}(4,88) = 5.30$, $P < .001$; $F_{E16-18}(4,32) = 1.79$, $P = .16$], peaking at PND 7. There were no differences between mice prenatally exposed to MPH or saline for any embryonic exposure period (Table 1).

3.1.4. Surface righting reflex

All mice reduced their latency to right from a supine position as they aged [$F_{E8-10}(2,90) = 91.92$, $P < .001$; $F_{E12-14}(2,54) = 94.86$, $P < .001$; $F_{E16-18}(2,40) = 43.32$, $P < .001$]. There were no differences between those pups exposed to MPH and those exposed to saline at E8–10 or E12–14 (Table 1). There was, however, a significant effect of MPH exposure at E16–18, with MPH-exposed pups righting faster than the saline-exposed pups from the same embryonic exposure period (Table 1).

3.1.5. Negative geotaxis

All mice reduced their latency to show the negative geotaxis response as they aged [$F_{E8-10}(2,90) = 62.28$, $P < .001$; $F_{E12-14}(2,54) = 13.27$, $P < .001$; $F_{E16-18}(2,40) = 9.92$, $P < .001$]. There were no differences between the pups exposed to MPH and those exposed to saline at E8–10 or E12–14 (Table 1). However, for the E16–18 exposure

Table 1
Means (± S.E.M.) for tasks measuring neuromotor development, including righting reflex, negative geotaxis, and forelimb grip strength

Behavior	E8−10			E12−14			E16−18		
	SAL	MPH		SAL	MPH		SAL	MPH	
Body weight (g)			$F(1,45)$			$F(1,27)$			$F(1,20)$
PND 3	2.38 ± 0.1	2.30 ± 0.1	1.87	2.66 ± 0.1	2.80 ± 0.1	<1.0	2.35 ± 0.1	2.35 ± 0.1	<1.0
PND 11	6.73 ± 0.2	6.43 ± 0.1		6.18 ± 0.3	6.52 ± 0.3		6.36 ± 0.2	6.25 ± 0.2	
PND 78	32.38 ± 0.9	32.33 ± 1.0		31.68 ± 1.3	32.38 ± 1.3		31.25 ± 1.1	32.01 ± 1.0	
Neuromotor development			$F(2,90)$			$F(2,54)$			$F(2,40)$
Righting reflex (s)									
PND 3	37.75 ± 3.6	43.90 ± 3.2	<1.0	51.23 ± 2.9	40.40 ± 6.0	2.96	54.56 ± 3.8	38.82 ± 4.6	9.57 *
PND 5	19.66 ± 3.4	17.66 ± 2.2		26.87 ± 4.1	15.64 ± 4.1		35.87 ± 5.4	22.97 ± 5.2	
PND 7	8.30 ± 2.3	9.35 ± 2.1		7.47 ± 1.1	7.75 ± 3.5		13.97 ± 2.6	7.65 ± 1.1	
Negative geotaxis (s)									
PND 3	55.82 ± 1.6	54.51 ± 2.8	<1.0	49.74 ± 3.3	41.47 ± 5.4	1.12	58.49 ± 1.5	41.39 ± 6.9	7.30 *
PND 5	34.39 ± 4.3	31.25 ± 3.6		37.07 ± 4.6	33.70 ± 4.4		41.08 ± 4.5	30.65 ± 5.7	
PND 7	24.43 ± 3.6	23.92 ± 3.3		24.93 ± 4.7	23.23 ± 4.7		30.29 ± 5.6	23.70 ± 6.0	
Forelimb grip strength (s)									
PND 7	8.46 ± 1.6	7.10 ± 1.1	<1.0	7.41 ± 1.3	8.43 ± 1.7	<1.0	3.46 ± 0.7	4.04 ± 0.8	<1.0
PND 9	12.61 ± 1.8	13.45 ± 2.1		13.40 ± 2.2	12.47 ± 2.3		6.94 ± 1.1	9.42 ± 1.4	
PND 11	16.83 ± 1.5	14.83 ± 1.8		15.65 ± 2.3	20.30 ± 1.6		14.60 ± 1.9	11.31 ± 1.3	
Ultrasonic vocalizations (frequency)			$F(1,30)$			$F(1,22)$			$F(1,8)$
PND 3	1.74 ± 0.7	0.68 ± 0.3	<1.0	1.57 ± 0.8	2.73 ± 1.1	2.9	2.33 ± 1.8	2.50 ± 1.8	<1.0
PND 5	4.17 ± 1.7	3.74 ± 1.5		3.88 ± 1.3	9.09 ± 5.3		6.58 ± 2.7	13.56 ± 6.6	
PND 7	12.68 ± 4.9	7.41 ± 2.5		6.94 ± 2.3	15.55 ± 7.2		3.42 ± 1.5	35.57 ± 21.5	
PND 9	6.14 ± 1.8	7.86 ± 2.9		3.38 ± 1.1	4.18 ± 1.7		8.43 ± 2.5	2.86 ± 1.0	
PND 11	1.61 ± 0.9	8.93 ± 8.3		0.56 ± 0.4	0.2 ± 0.2		1.36 ± 0.5	0.38 ± 0.38	

Mice were prenatally exposed to either saline (SAL) or methylphenidate (MPH), at embryonic (E) days 8−10, 12−14, or 16−18, and were then tested at postnatal days (PND) 3−11. Body weight and UV data were analyzed as repeated measures, and F values for between-subject analyses are given. Neuromotor developmental data were analyzed by MANOVA.

* $P < .02$.

period, MPH-exposed pups turned faster on the negative geotaxis than the saline-exposed pups did (Table 1).

3.1.6. Forelimb grip strength

The latency to fall in the forelimb grip strength test increased for all pups as they aged [$F_{E8−10}(2,90) = 15.81$, $P < .001$; $F_{E12−14}(2,54) = 13.85$, $P < .001$; $F_{E16−18}(2,40) = 26.79$, $P < .001$]. There were no differences between pups
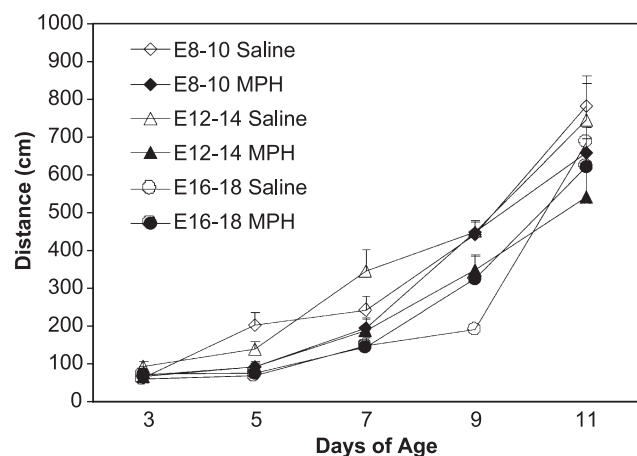

Fig. 1. Mean (± S.E.M.) distance traveled in the developmental open field (UVbox) by pups (3–11 days of age) prenatally exposed to MPH or saline at one of three embryonic time periods.

exposed to MPH and pups exposed to saline at any embryonic exposure period (Table 1).

3.1.7. Swimming ability

There were no significant effects of MPH on swimming ability. At PND 5, for E8−10 treated pups, 66.6% of MPH-exposed and 78.3% of saline-exposed pups could swim [$\chi^2_{E8−10}(1) < 1.0$]; for E12−14 treated pups, 38.5% of MPH-exposed and 56.3% of saline-exposed pups could swim [$\chi^2_{E12−14}(1) < 1.0$]; for E16−18 treated pups, 70.0% of MPH-exposed and 33.3% of saline-exposed pups could swim [$\chi^2_{E16−18}(1) = 2.93$, $P = .086$]. At PND 7, for E8−10 treated pups, 91.7% of MPH-exposed and 95.7% of saline-exposed pups could swim [$\chi^2_{E8−10}(1) < 1.0$]; for E12−14 treated pups, 100% of MPH-exposed pups and 93.8% of saline-exposed pups could swim [$\chi^2_{E12−14}(1) < 1.0$]; and for E16−18 treated pups, 100% of pups in both exposure groups could swim (no variability between groups).

3.2. Adult behavioral tests

3.2.1. Open field

Mice exposed to MPH from E8 to 10 reared more often in the open field than did the mice exposed to saline over the same embryonic period [$F_{E8−10}(1,44) = 4.19$, $P < .05$; $F_{E12−14}(1,27) < 1.0$; $F_{E16−18}(1,19) < 1.0$; Fig. 2]. These same MPH-exposed mice showed a trend toward entering
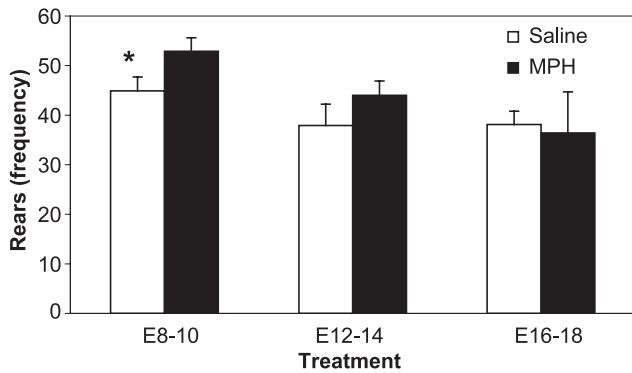
Fig. 2. Mean (+S.E.M.) number of rears exhibited across 5 min in the open field by adult mice that were prenatally exposed to MPH or saline at one of three embryonic time periods (*$P < .05$).

Fig. 3. Mean (± S.E.M.) time spent in the open arms of the elevated plus maze in a 5-min trial by adult mice prenatally exposed to MPH or saline at one of three embryonic time periods (*$P < .05$).

the center square of the open field more often than the saline-exposed mice did [$F_{E8-10}(1,44) = 3.39$, $P < .07$; $F_{E12-14}(1,27) < 1.0$; $F_{E16-18}(1,19) = 2.62$], but there was no difference in the total time they spent in the center square [$F_{E8-10}(1,44) < 1.0$; $F_{E12-14}(1,27) < 1.0$; $F_{E16-18}(1,19) < 1.0$]. Mice exposed to MPH from E8 to 10 spent more time sniffing the floor than saline-exposed mice [$F_{E8-10}(1,44) = 7.91$, $P < .01$; $F_{E12-14}(1,27) = 2.53$; $F_{E16-18}(1,19) = 1.84$] and defecated more frequently in the open field than the saline-exposed mice did [$F_{E8-10}(1,44) = 40.35$, $P < .02$; $F_{E12-14}(1,27) < 1.0$; $F_{E16-18}(1,19) < 1.0$]. Mice exposed to MPH from E16 to 18 sniffed the floor and walls less frequently than did the saline-exposed mice [$F_{E16-18}(1,19) = 8.34$, $P < .01$], but the MPH-exposed mice sniffed the air more frequently than did the saline-exposed mice [$F_{E8-10}(1,44) = 1.21$; $F_{E12-14}(1,27) < 1.0$; $F_{E16-18}(1,19) = 9.36$, $P < .01$]. There were no differences in locomotor activity [$F_{E8-10}(1,44) < 1.0$; $F_{E12-14}(1,27) < 1.0$; $F_{E16-18}(1,19) = 1.12$] and grooming frequency [$F_{E8-10}(1,44) < 1.0$; $F_{E12-14}(1,27) < 1.0$; $F_{E16-18}(1,19) < 1.0$] or duration [$F_{E8-10}(1,44) = 3.04$, $P < .09$; $F_{E12-14}(1,27) < 1.0$; $F_{E16-18}(1,19) = 1.56$].

3.2.2. Elevated plus maze

Mice exposed to MPH from E8 to 10 spent more time in the open arms of the elevated plus maze than did saline-exposed mice [$F_{E8-10}(1,44) = 7.35$, $P = .01$; $F_{E12-14}(1,27) < 1.0$; $F_{E16-18}(1,19) < 1.0$; Fig. 3]. Similarly, mice exposed to MPH from E8 to 10 exhibited a trend towards more frequent open arm entries [$F_{E8-10}(1,44) = 3.27$, $P < .08$; $F_{E12-14}(1,27) = 1.35$; $F_{E16-18}(1,19) < 1.0$]. The total number of arm entries did not differ between MPH and saline-exposed mice at any embryonic exposure period [$F_{E8-10}(1,44) < 1.0$; $F_{E12-14}(1,27) = 2.32$; $F_{E16-18}(1,19) < 1.0$], indicating that there was no difference in the overall locomotor behavior. Mice exposed to MPH from E8 to 10 performed more head dips than saline-exposed controls in the elevated plus maze did, indicating a decrease in fear [$F_{E8-10}(1,44) = 8.63$,
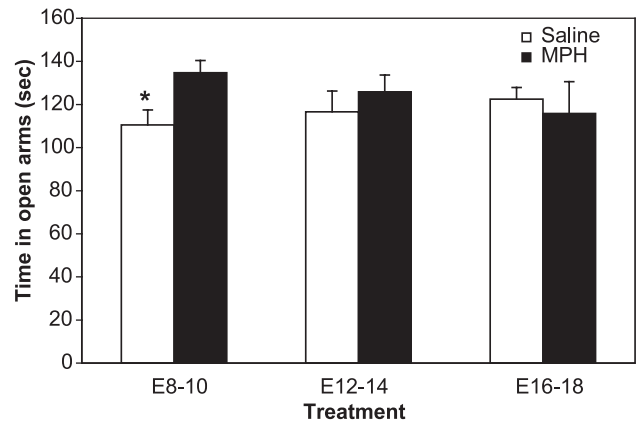
$P < .01$; $F_{E12-14}(1,27) < 1.0$; $F_{E16-18}(1,19) < 1.0$; Fig. 4]. MPH-exposed mice from the E12–14 group reared more often in the elevated plus maze than did the saline-exposed controls [$F_{E8-10}(1,44) < 1.0$; $F_{E12-14}(1,27) = 11.47$, $P < .01$; $F_{E16-18}(1,19) < 1.0$]. Mice exposed to MPH from E16 to 18 spent more time grooming in the elevated plus maze than the saline-exposed mice did [$F_{E8-10}(1,44) < 1.0$; $F_{E12-14}(1,27) < 1.0$; $F_{E16-18}(1,19) = 4.77$, $P = .04$]. The frequency of protected (within the closed arms) stretch attends [$F_{E8-10}(1,44) = 3.06$, $P < .09$; $F_{E12-14}(1,27) = 2.96$; $F_{E16-18}(1,19) = 1.06$] and the frequency of unprotected (within the open arms) stretch attends [$F_{E8-10}(1,44) < 1.0$; $F_{E12-14}(1,27) < 1.0$; $F_{E16-18}(1,19) = 2.98$] did not differ between the drug- and saline-exposed mice for any exposure period. Mice exposed to MPH from E16 to 18 defecated more often than saline-exposed controls in the elevated plus maze [$F_{E8-10}(1,44) = 1.81$; $F_{E12-14}(1,27) = 3.80$, $P = .06$; $F_{E16-18}(1,19) = 4.79$, $P = .04$].
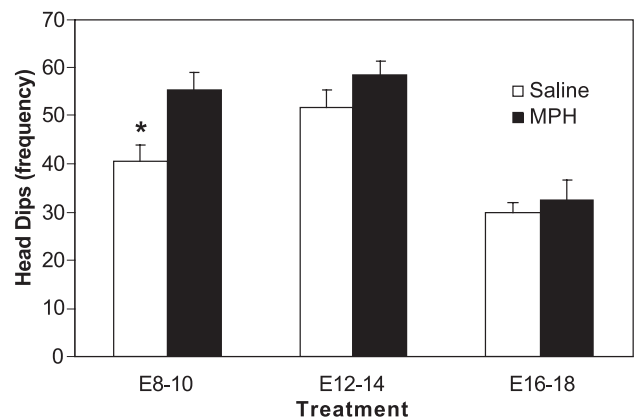

Fig. 4. Mean (± S.E.M.) number of head dips in the elevated plus maze by adult mice prenatally exposed to MPH or saline during one of three embryonic periods (*$P < .05$).

Table 2
Mean latencies (± S.E.M.) to find the visible (Days 1–3) and hidden (Day 4) platform in the water maze for adult mice prenatally exposed to saline (SAL) or methylphenidate (MPH) at embryonic (E) days 8–10, 12–14, or 16–18

Latency	E8–10			E12–14			E16–18		
	SAL	MPH	$F(1,42)$	SAL	MPH	$F(1,27)$	SAL	MPH	$F(1,19)$
Time to find platform (s)									
Day 1	29.80 ± 2.3	28.69 ± 2.4	1.85	32.76 ± 2.0	28.51 ± 2.8	1.08	31.04 ± 3.0	31.50 ± 3.6	<1.0
Day 2	17.71 ± 2.0	15.86 ± 1.7		13.38 ± 1.8	13.86 ± 1.9		13.38 ± 1.7	18.45 ± 3.1	
Day 3	15.00 ± 2.3	10.31 ± 1.0		13.37 ± 2.6	8.86 ± 1.1		8.16 ± 0.7	8.78 ± 1.1	
Day 4	18.86 ± 2.1	17.73 ± 1.5		20.19 ± 2.3	20.55 ± 3.0		20.85 ± 1.3	23.82 ± 4.5	

Data were analyzed as repeated measures, and F values for between-subject analyses are given.

3.2.3. Water maze

Performance in the water maze improved across the first three days, reflecting the learning curve for the task, and was poorer on the fourth day, reflecting the harder task of finding the hidden platform [$F_{E8-10}(3,126) = 30.31$, $P < .001$; $F_{E12-14}(3,81) = 33.27$, $P < .001$; $F_{E16-18}(3,57) = 32.69$, $P < .001$]. However, there were no differences between MPH- and saline-exposed mice for any embryonic exposure period [$F_{E8-10}(1,42) = 1.85$; $F_{E12-14}(1,27) = 1.08$; $F_{E16-18}(1,19) < 1.0$; Table 2].

3.2.4. MPH challenge

Mice exposed to MPH or saline at any of the three embryonic periods did not differ in most of the behaviors examined in the open field when challenged with MPH and when data were analyzed as the change from baseline (Table 3). For all exposure periods, there was no variation between groups in their difference scores for locomotion, rearing, center square entries, time spent in the center square, or in the frequency or duration of grooming. There were no differences between exposure groups in the time spent sniffing the floor and walls. However, mice exposed to

MPH from E12 to 14 sniffed the floor and walls more frequently compared with the saline-exposed mice, but mice exposed to MPH from E8 to 10 or from E16 to 18 showed no differences in this measure. There were no differences between exposure groups in the change in frequency or duration of sniffing the air. Lastly, there were no differences in the defecation between the groups.

4. Discussion

Prenatal MPH exposure resulted in both acute and enduring behavioral modifications. The differences observed in both the pups and adult mice provide evidence that MPH exposure, particularly from E8 to 10, reduced fearful behavior and increased exploration. Pups spent more time in the center of the UVbox and, as adults, they spent more time in the open arms of the elevated plus maze, reared more frequently in the open field, and entered the center square of the open field more frequently. This correlates well with the findings of Sobrian et al. (2003), who reported that the exploration and total time spent in the open arms of

Table 3
Difference scores (+ S.E.M.) for each behavior in the open field (means in open field subtracted from means in open field after MPH challenge) for mice prenatally exposed to either saline (SAL) or methylphenidate (MPH) at embryonic (E) days 8–10, 12–14, or 16–18

Behavior	E8–10			E12–14			E16–18		
	SAL	MPH	$F(1,43)$	SAL	MPH	$F(1,27)$	SAL	MPH	$F(1,19)$
Open field									
Line crossings	51.05 ± 7.7	43.09 ± 6.0	1.03	39.56 ± 10.7	17.85 ± 9.2	2.24	-22.73 ± 12.7	-23.8 ± 12.6	<1.0
Rearing	-3.9 ± 4.3	-4.17 ± 2.0	2.01	-5.44 ± 5.0	-3.69 ± 5.0	<1.0	-9.55 ± 6.2	-5.70 ± 4.5	<1.0
Center square									
Number of entries	1.10 ± 0.6	0.30 ± 0.7	<1.0	-0.50 ± 0.9	-2.08 ± 0.8	1.55	-2.09 ± 0.6	-1.50 ± 1.0	<1.0
Duration (s)	-0.57 ± 1.7	-3.22 ± 2.4	<1.0	-9.69 ± 2.6	-16.62 ± 3.3	2.75	-2.73 ± 2.0	7.70 ± 6.7	2.41
Grooming									
Frequency	-0.67 ± 0.4	-0.43 ± 0.3	1.26	0.19 ± 0.3	0.38 ± 0.5	<1.0	-0.09 ± 0.7	1.40 ± 0.9	1.64
Duration (s)	-0.05 ± 1.1	-2.04 ± 0.8	<1.0	1.75 ± 1.5	2.54 ± 1.1	<1.0	2.45 ± 2.5	8.20 ± 3.1	2.08
Sniffing floor/walls									
Frequency	-3.76 ± 1.1	-2.35 ± 1.3	3.64	1.31 ± 1.0	-1.92 ± 1.1	4.58 *	-6.27 ± 1.4	-3.70 ± 1.35	1.72
Duration (s)	-12.05 ± 3.3	-16.15 ± 3.4	2.44	5.63 ± 3.2	-4.69 ± 5.4	2.89	-7.00 ± 2.8	-6.30 ± 2.9	<1.0
Sniffing air									
Frequency	2.29 ± 0.7	2.39 ± 0.8	2.18	2.13 ± 0.8	-0.23 ± 0.9	3.63	0.18 ± 1.1	-1.00 ± 1.7	<1.0
Duration (s)	3.95 ± 1.4	7.07 ± 3.0	2.25	4.56 ± 1.6	0.46 ± 1.7	2.94	-8.91 ± 7.8	2.20 ± 2.5	1.68
Defecation	3.62 ± 0.9	2.48 ± 0.9	<1.0	2.56 ± 0.9	3.08 ± 1.2	<1.0	3.82 ± 0.9	2.40 ± 1.5	<1.0

Difference scores were analyzed by MANOVA.
 * $P < .05$.

the elevated plus maze was increased in adult rats that had been prenatally exposed to cocaine (40 mg/kg). However, others have reported no effects of gestational cocaine (15 mg/kg) on elevated plus maze behavior (Overstreet et al., 2000). The difference in the dose between these two studies may be the critical factor for the variable effects observed in the elevated plus maze following prenatal cocaine exposure. The predominant behavioral effects we observed occurred following MPH exposure during the E8–10 embryonic period, when dopamine first appears, suggesting an interaction between the dopaminergic effects of the drug and ontogeny. As dopamine is a crucial neurotransmitter, effects at this time point were expected.

Overall, MPH administration at each of our exposure periods during gestation resulted in limited effects on early development. The effect of prenatal cocaine exposure on body weight varies across studies with reports of both decreased weight (Henderson and McMillen, 1990; Middaugh et al., 1996) and no change in weight (Smith et al., 1989). Our results demonstrate that a short period of prenatal exposure to MPH has no effect on pup or adult weight. It is possible that a chronic prenatal exposure, or a higher dose of MPH, would have a more significant effect on growth. We did not follow maternal weight changes in this study, as it has already been shown that MPH (6 mg/kg), chronically administered from E7 through lactation day 20, at doses just slightly higher than those we used in our study, had no effect on maternal body weight (Teo et al., 2002a). Furthermore, chronic prenatal cocaine administration (40 mg/kg) did not alter gestational length, fetal mortality (Choi et al., 1998), litter size, or gender composition (Stewart et al., 1998), thus, we did not record these measures in our study.

We did not find any overall enhancement or detriment to motor ability as a result of prenatal MPH exposure, although there were some inconsistent effects observed for the E16–18 period, including faster righting latencies and slower negative geotaxis turn times. Prenatal cocaine exposure can delay the development of the righting reflex (Henderson and McMillen, 1990); however, this effect was observed when pregnant females received cocaine throughout their pregnancy. There were also no general effects of prenatal MPH exposure on spontaneous activity in pups or adults. The literature examining the influence of prenatal cocaine on spontaneous or novelty-induced activity is divided as to the effects produced in the offspring. For instance, Church and Tilak (1996) reported hypoactivity in both pups and adults following cocaine administration (40 or 80 mg/kg) from E7 to 20. However, Henderson and McMillen (1990) reported hyperactivity in 30-day-old mice following gestational cocaine (15 mg/kg) exposure throughout pregnancy. Lastly, others have reported no effect of prenatal cocaine (40 mg/kg) exposure from E12 to 21 on locomotor activity in offspring (Choi et al., 1998). It seems reasonable that these apparent discrepancies may actually be due to differences in quantity, schedule, and route of stimulant dosing, as well as to the age

at behavioral testing and the specific paradigm used to determine locomotor activity.

MPH exhibits similar pharmacological properties to cocaine (Fowler et al., 2001) and also shares important reinforcing (Volkow et al., 2002) and sensitizing (Gaytan et al., 2001) properties. Prior exposure to MPH can sensitize an organism to the rewarding properties of MPH (Meririnne et al., 2001) or other drugs (Brandon et al., 2001; Schenk and Izenwasser, 2002). Exposure of adolescent rats to MPH results in increased susceptibility to the reinforcing effects of cocaine, as measured by self-administration (Brandon et al., 2001; Schenk and Izenwasser, 2002). However, preadolescent exposure to MPH did not enhance the reinforcing effects of cocaine, and even increased the aversive properties of a moderate dose of cocaine in a conditioned place preference test (Andersen et al., 2002). The method of cocaine administration (passive vs. self-administration) differed across these studies, as did the age of exposure to MPH, which could account for these results. It is clear that postnatal MPH exposure can enhance both the aversive and reinforcing effects of other drugs via cross sensitization. However, in our study, prenatal MPH and saline-exposed mice responded similarly to an acute MPH challenge, suggesting that prenatal MPH exposure did not influence reaction to the drug later in life. Similar results have been reported in studies where prenatal cocaine exposure was followed by a later cocaine or amphetamine challenge (Glatt et al., 2000; Sobrian et al., 2003). Our finding that prenatal MPH exposure did not interact with the response to a later MPH challenge may be due to the very short intrauterine exposure period. Further experiments are needed to determine the potential for cross sensitization after longer prenatal MPH exposure.

In addition to the direct effects of MPH administration on development, we need to consider other factors which might have influenced early development in our mice. Early postnatal experiences, such as brief separation from the dam (for up to 15 min), can modulate stress reactivity (Caldji et al., 2000; Francis et al., 1999) and fearfulness (Caldji et al., 1998) in offspring. The deleterious effects of prenatal cocaine exposure can be exacerbated by stress or be minimized by environmental enrichment, in the form of early handling (Spear et al., 1998). This may provide a compensatory mechanism through which detrimental effects of prenatal MPH exposure are nullified or decreased during development. Future studies should include several doses of MPH, with a focus on chronic administration periods during gestation. Our study aimed to evaluate the effect of MPH administration during specific embryonic developmental periods. However, it is critical that chronic prenatal MPH administration also be evaluated because fetal exposure will be long term in pregnant women who are prescribed with MPH. Future studies should also further evaluate neuromotor development, exploration, and anxiety-related behaviors, as well as focus on other aspects of behavior, including learning and memory, social behavior, and drug self-admin-

istration. In addition, interactions between prenatal and postnatal exposure to MPH need to be examined.

Prenatal cocaine exposure has been reported to delay righting reflex, alter locomotor activity (Choi et al., 1998; Church and Tilak, 1996; Henderson and McMillen, 1990), and disrupt Pavlovian conditioning (Kosofsky and Wilkins, 1998). Furthermore, the prenatal administration of Haloperidol, a neuroleptic that antagonizes dopamine receptors, increases anxiety-related behaviors in the open field and elevated plus maze (Singh and Singh, 2002). The reductions in anxiety-related behaviors in adult mice and the sporadic developmental effects in infancy that we have observed are similar with the findings with cocaine. In addition, there are similar effects observed following prenatal cocaine exposure in both rats and humans, including an enduring increase in activity levels (Henderson and McMillen, 1990; Nulman et al., 2001). This suggests caution in the administration of MPH to women who are pregnant, or are considering becoming pregnant, and highlights another important factor in the growing concern about increasing MPH abuse.

Acknowledgements

References

Andersen SL, Teicher MH. Sex differences in dopamine receptors and their relevance to ADHD. Neurosci Biobehav Rev 2000;24:137–41.

Andersen SL, Arvanitogiannis A, Pliakas AM, LeBlanc C, Carlezon Jr WA. Altered responsiveness to cocaine in rats exposed to methylphenidate during development. Nat Neurosci 2002;5:13–4.

Anderson JC, Williams S, McGee R, Silva PA. DSM-III disorders in preadolescent children: prevalence in a large sample from the general population. Arch Gen Psychiatry 1987;44:69–76.

Berger-Sweeney J, Hohmann CF. Behavioral consequences of abnormal cortical development: insights into developmental disabilities. Behav Brain Res 1997;86:121–42.

Biederman J. Attention-deficit/hyperactivity disorder: a lifespan perspective. J Clin Psychiatry 1998;59:4–16.

Brandon CL, Marinelli M, Baker LK, White FJ. Enhanced reactivity and vulnerability to cocaine following methylphenidate treatment in adolescent rats. Neuropsychopharmacology 2001;25:651–61.

Caldji C, Tannenbaum B, Sharma S, Francis D, Plotsky PM, Meaney MJ. Maternal care during infancy regulates the development of neural systems mediating the expression of fearfulness in the rat. Proc Natl Acad Sci U S A 1998;95:5335–40.

Caldji C, Diorio J, Meaney MJ. Variations in maternal care in infancy regulate the development of stress reactivity. Biol Psychiatry 2000;48:1164–74.

Carrey N, McFadyen MP, Brown RE. Effects of sub-chronic methylphenidate hydrochloride administration on the locomotor and exploratory behavior of prepubertal mice. J Child Adolesc Psychopharmacol 2000;10:277–86.

Chase TD, Brown RE, Carrey N, Wilkinson M. Repeated methylphenidate attenuates c-fos expression in the striatum of prepubertal rats. NeuroReport 2003;14:769–72.

Choi SJ, Mazzio E, Soliman KF. The effects of gestational cocaine exposure on pregnancy outcome, postnatal development, cognition and locomotion in rats. Ann NY Acad Sci 1998;844:324–35.

Church MW, Tilak JP. Differential effects of prenatal cocaine and retinoic acid on activity level throughout day and night. Pharmacol Biochem Behav 1996;55:595–605.

Debooy VD, Seshia MM, Tenenbein M, Casiro OG. Intravenous pentazocine and methylphenidate abuse during pregnancy. Maternal lifestyle and infant outcome. Am J Dis Child 1993;147:1062–5.

Diller L. Running on ritalin: a physician reflects on children, society, and performance in a pill. Toronto: Bantam Books; 1998.

Ernst M, Liebenauer LL, King AC, Fitzgerald GA, Cohen RM, Zametkin AJ. Reduced brain metabolism in hyperactive girls. J Am Acad Child Adolesc Psych 1994;33:858–68.

Faraj BA, Israili ZH, Perel JM, Jenkins ML, Holtzman SG, Cucinell SA, et al. Metabolism and disposition of methylphenidate-14C: studies in man and animals. J Pharmacol Exp Ther 1974;191:535–47.

Fowler JS, Volkow ND, Wang GJ, Gatley SJ, Logan J. [11]Cocaine: PET studies of cocaine pharmacokinetics, dopamine transporter availability and dopamine transporter occupancy. Nucl Med Biol 2001;28:561–72.

Francis D, Diorio J, Liu D, Meaney MJ. Nongenomic transmission across generations of maternal behavior and stress responses in the rat. Science 1999;286:1155–8.

Gaytan O, Sripada S, Swann A, Daffy N. Blockade of sensitization to methylphenidate by MK-801: partial dissociation from motor effects. Neuropharmacology 2001;40:298–309.

Glatt SJ, Bolanos CA, Trksak GH, Crowder-Dupont C, Jackson D. Prenatal cocaine exposure alters behavioral and neurochemical sensitization to amphetamine in adult rats. Neuropharmacology 2000;39:599–610.

Greenhill LL. Attention-deficit hyperactivity disorder: the stimulants. Child Adolesc Psychiatr Clin N Am 1995;4:123–68.

Greenhill LL. The use of psychotropic medication in preschoolers: indications, safety, and efficacy. Can J Psychiatry 1998;43:576–81.

Hahn ME, Benno RH, Schanz N, Phadia E. The effects of prenatal cocaine exposure and genotype on the ultrasonic calls of infant mice. Pharmacol Biochem Behav 2000;67:729–38.

Henderson MG, McMillen BA. Effects of prenatal exposure to cocaine or related drugs on rat development and neurological indices. Brain Res Bull 1990;24:207–12.

Johns JM, Noonan LR. Prenatal cocaine exposure affects social behavior in Sprague–Dawley rats. Neurotoxicol Teratol 1995;17:569–76.

Kimko HC, Cross JT, Abernathy DR. Pharmacokinetics and clinical effectiveness of methylphenidate. Clin Pharmacokinet 1999;37:457–70.

Klein-Schwartz W, McGrath J. Poison center's experience with methylphenidate abuse in pre-teens and adolescents. J Am Acad Child Adolesc Psych 2003;42:288–94.

Kosofsky BE, Wilkins AS. A mouse model of transplacental cocaine exposure. Clinical implications for exposed infants and children. Ann NY Acad Sci 1998;846:248–61.

Levitt P. Prenatal effects of drugs of abuse on brain development. Drug Alcohol Depend 1998;51:109–25.

Lister RG. The use of a plus-maze to measure anxiety in the mouse. Psychopharmacology 1987;92:180–5.

Mazer C, Muneyyirci J, Taheny K, Raio N, Borella A, Whitaker-Azmitia PM. Serotonin depletion during synaptogenesis leads to decreased synaptic density and learning deficits in the adult rat: a possible model of neurodevelopmental disorders with cognitive deficits. Brain Res 1997;760:68–73.

McDonald RJ, White NM. Parallel information processing in the water maze: evidence for independent memory systems involving dorsal striatum and hippocampus. Behav Neural Biol 1994;61:260–70.

McFadyen MP, Carrey N, Brown RE. Subchronic methylphenidate administration has no effect on locomotion, emotional behavior, or water maze learning in prepubertal mice. Dev Psychobiol 2002;41:123–32.

Meririnne E, Kankaanpaa A, Seppala T. Rewarding properties of methylphenidate: sensitization by prior exposure to the drug and effects of

dopamine D1- and D2-receptor antagonists. J Pharmacol Exp Ther 2001;298:539–50.

Middaugh LD, Boggan WO, Bingel SA, Patrick KS, Xu W. A murine model of prenatal cocaine exposure: effects on the mother and the fetus. Pharmacol Biochem Behav 1996;55:565–74.

Miranda-Contreras L, Mendoza-Briceno RV, Palacios-Pru EL. Levels of monoamine and amino acid neurotransmitters in the developing male mouse hypothalamus and in histotypic hypothalamic cultures. Int J Dev Neurosci 1998;16:403–12.

Moll GH, Hause S, Ruther E, Rothenberger A, Huether G. Early methylphenidate administration to young rats causes a persistent reduction in the density of striatal dopamine transporters. J Child Adolesc Psychopharmacol 2001;11:15–24.

Nulman I, Rovet J, Greenbaum R, Loebstein M, Wolpon J, Pace-Asciak P, et al. Neurodevelopment of adopted children exposed in utero to cocaine: the Toronto Adoption Study. Clin Invest Med 2001;24:129–37.

Overstreet DH, Moy SS, Lubin DA, Gause LR, Lieberman JA, Johns JM. Enduring effects of prenatal cocaine administration on emotional behavior in rats. Physiol Behav 2000;70:149–56.

Pauls DL. Genetic factors in the expression of attention-deficit hyperactivity disorder. J Child Adolesc Psychopharmacol 1991;1:353–60.

Pendleton RG, Rasheed A, Roychowdhury R, Hillman R. A new role for catecholamines: ontogenesis. Trends Pharmacol Sci 1998;19:248–51.

Penner MR, McFadyen MP, Carrey N, Brown RE. Effects of chronic and acute methylphenidate hydrochloride (Ritalin) administration on locomotor activity, ultrasonic vocalizations, and neuromotor development in 3- to 11-day-old CD-1 mouse pups. Dev Psychobiol 2001;36:216–28.

Penner MR, McFadyen MP, Pinaud R, Carrey N, Robertson HA, Brown RE. Age-related distribution of c-*fos* expression in the striatum of CD-1 mice after acute methylphenidate administration. Brain Res Dev Brain Res 2002;135:71–7.

Robison LM, Skaer TL, Sclar DA, Galin RS. Is attention deficit hyperactivity disorder increasing among girls in the US? Trends in diagnosis and the prescribing of stimulants. CNS Drugs 2002;16:129–37.

Sachdev PS, Trollor JN. How high a dose of stimulant medication in adult attention deficit hyperactivity disorder? Aust NZ J Psychiatry 2000;34:645–50.

Schenk S, Izenwasser S. Pretreatment with methylphenidate sensitizes rats to the reinforcing effects of cocaine. Pharmacol Biochem Behav 2002;72:651–7.

Singh KP, Singh M. Effect of prenatal haloperidol exposure on behavioral alterations in rats. Neurotoxicol Teratol 2002;24:497–502.

Smith RF, Mattran KM, Kurkjian MF, Kurtz SL. Alterations in offspring behavior induced by chronic prenatal cocaine dosing. Neurotoxicol Teratol 1989;11:35–8.

Sobrian SK, Marr L, Ressman K. Prenatal cocaine and/or nicotine exposure produces depression and aging in rats. Prog Neuropsychopharmacol Biol Psychiatry 2003;27:501–18.

Solanto MV. Clinical psychopharmacology of AD/HD: implications for animal models. Neurosci Biobehav Rev 2000;24:27–30.

Spear LP, Campbell J, Snyder K, Silveri M, Katovic N. Animal behavior models. Increased sensitivity to stressors and other environmental experiences after prenatal cocaine exposure. Ann NY Acad Sci 1998;846:76–88.

Sproson EJ, Chantrey J, Hollis C, Marsden CA, Fonel KC. Effect of repeated methylphenidate administration on presynaptic dopamine and behavior in young adult rats. J Psychopharmacol 2001;15:67–75.

Stewart CW, Scalzo FM, Valentine J, Holson RR, Ali SF, Slikker Jr W. Gestational exposure to cocaine or pharmacologically related compounds: effects on behavior and striatal dopamine receptors. Life Sci 1998;63:2015–22.

Teo SK, Stirling DI, Thomas SD, Hoberman AM, Christian MS, Khetani VD. The perinatal and postnatal toxicity of D-methylphenidate and D,L-methylphenidate in rats. Reprod Toxicol 2002a;16:353–66.

Teo S, Stirling D, Thomas S, Hoberman A, Kiorpes A, Khetani V. A 90-day oral gavage toxicity study of D-methylphenidate and D,L-methylphenidate in Sprague–Dawley rats. Toxicology 2002b;179:183–96.

Thomas SA, Matsumoto AM, Palmiter RD. Noradrenaline is essential for mouse fetal development. Nature 1995;374:643–6.

Ugrumov MV. Hypothalamic monoaminergic systems in ontogenesis: development and functional significance. Int J Dev Neurosci 1997;41:809–16.

Vitiello B. Pediatric psychopharmacology and the interaction between drugs and the developing brain. Can J Psychiatry 1998;43:582–4.

Volkow ND, Fowler JS, Wang GJ, Ding YS, Gatley SJ. Role of dopamine in the therapeutic and reinforcing effects of methylphenidate in humans: results from imaging studies. Eur Neuropsychopharmacol 2002;12:557–66.

Weiss E, Greenberg G. Open field procedures. In: Greenberg G, Haraway MH, editors. Comparative psychology: a handbook. New York: Garland Publishers; 1996. p. 603–20.

Wender PH. The hyperactive child, adolescent, and adult: attention deficit disorder through the lifespan. New York: Oxford Univ. Press; 1987.

Yuan J, McCann U, Ricaurte G. Methylphenidate and brain dopamine neurotoxicity. Brain Res 1997;767:172–5.

Zaczek R, Battaglia G, Contrera JF, Culp S, De Souza EB. Methylphenidate and pemoline do not cause depletion of rat brain monoamine markers similar to that observed with methamphetamine. Toxicol Appl Pharmacol 1989;100:227–33.