Published in final edited form as:

Behav Brain Res. 2013 June 1; 246: 29–35. doi:10.1016/j.bbr.2013.02.031.

Novelty-induced conditioned place preference, sucrose preference, and elevated plus maze behavior in adult rats after repeated exposure to methylphenidate during the preweanling period

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Abstract

Early treatment with methylphenidate has a persistent effect on the affective (i.e., anxiety- and depressive-like) behaviors of adult rats and mice. Interestingly, age at methylphenidate exposure appears to be a critical determinant influencing the expression of affective behaviors. In the present study, we exposed rats to methylphenidate during the preweanling period (i.e., PD 11-PD 20) because this ontogenetic period is analogous to early childhood in humans (an age associated with increasing methylphenidate usage). Rats were injected with methylphenidate (0, 2, or 5 mg/kg) from PD 11 to PD 20 and reactivity to rewarding and aversive stimuli were measured in early adulthood. Specifically, novelty-induced CPP, sucrose preference, and elevated plus maze behavior were assessed on PD 60. Early treatment with 2 or 5 mg/kg methylphenidate increased total time spent in the white compartment of the CPP chamber. This methylphenidate-induced effect occurred regardless of exposure condition. Performance on the elevated plus maze was also impacted by early methylphenidate exposure, because rats treated with 5 mg/kg methylphenidate spent more time in the closed compartment of the elevated plus maze than vehicle controls. Early methylphenidate exposure did not alter sucrose preference. These data indicate that exposing rats to methylphenidate during the preweanling period differentially affects anxiety-like behavior depending on the type of anxiety-provoking stimulus. Specifically, early methylphenidate exposure decreased aversion to a bright white room when measured on a novelty-induced CPP task, whereas methylphenidate caused a long-term increase in anxiety when measured on the elevated plus maze.

Keywords

methylphenidate; novelty-seeking; sucrose preference; elevated plus maze; ontogeny; affective behavior

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1. Introduction

In the last decade, a number of studies have investigated the long-term effects of early methylphenidate exposure on the behavior of adult rats [1-11]. A majority of this research has examined the effects of early methylphenidate on cocaine-rewarded behavior [1, 3, 6, 7]. Interestingly, all of these studies found that early methylphenidate exposure altered the reinforcing potential of cocaine; however, the reported effects were often in opposing directions. While procedural variations (e.g., different reward paradigms and rat strains) could be responsible for the inconsistent outcomes, an alternative explanation is that the differential actions of early methylphenidate treatment may be a consequence of age at drug exposure. Specifically, rats that began methylphenidate treatment during adolescence showed enhanced cocaine self-administration and more robust cocaine-induced behavioral sensitization [2, 6, 10]. In contrast, when methylphenidate treatment began during preadolescence the strength of cocaine-induced conditioned place preference (CPP) and intracranial self-stimulation was reduced [1, 3, 7, 11]. Thus, it is possible that methylphenidate exposure during the adolescent period increases the rewarding potential of cocaine, while methylphenidate exposure during earlier time periods decreases cocaine's reward value.

The effects of methylphenidate exposure on nondrug-induced behavior also appears to vary according to age at drug exposure. Rats exposed to methylphenidate during the preadolescent period [i.e., postnatal day (PD) 20-PD 35] were less responsive to nondrug rewards, such as sucrose and novelty, and showed more anxiety-like behaviors in adulthood [4,5]. In contrast, rats treated with methylphenidate from infancy to the end of the preadolescent period (PD 7-PD 35) were less anxious (i.e., they spent more time in the open arms of an elevated plus maze) when tested as adults [9]. Lastly, exposure to methylphenidate during adolescence (PD 29-PD 50) did not alter depressive-like behaviors when measured using the saccharin preference paradigm [8]. These data suggest that methylphenidate has substantially different long-term effects depending on when it is administered during ontogeny.

Recently, the preweanling period (PD 11-PD 20) in rats has been studied in a number of different contexts [12-14], because it is roughly analogous to early childhood in humans (an age group increasingly being prescribed methylphenidate) [15, 16]. Interestingly, administering methylphenidate during the preweanling period produces a pattern of effects that differ from older age groups [17, 18]. Specifically, methylphenidate exposure during the preweanling period increases cocaine self-administration and has no effect on cocaine-induced CPP [18]. Moreover, administering methylphenidate to preweanling rats enhances morphine-induced CPP and sucrose-reinforced lever pressing when tested in adulthood [17]. Taken together, these data suggest that exposing rats to methylphenidate during the preweanling period enhances the long-term reward value of both drug and non-drug stimuli. The goal of the current study was to examine whether early methylphenidate exposure would alter the affective responses of adult rats to appetitive and aversive stimuli. To this end, we assessed novelty-induced place preference, sucrose preference, and elevated plus maze behavior of young adult rats (i.e., PD 60) that had been exposed to methylphenidate from PD 11 to PD 20. We hypothesized that methylphenidate treatment during the preweanling period would enhance the reward value of novelty and sucrose, while decreasing the aversiveness of the elevated plus maze.

2. Material and methods

2.1. Subjects

Subjects were 293 male and female rats of Sprague-Dawley descent (Charles River, Hollister, CA), born and raised at California State University, San Bernardino (CSUSB). Litters were culled to 10 pups at 3 days of age and kept with the dam until PD 25 in clear polycarbonate maternity cages (56 × 34 × 22 cm) with wire lids. Beginning on PD 11, each rat was given a distinctive tail mark using colored nontoxic markers. Tails were remarked every day until weaning and every third day after weaning. On PD 25, rats were weaned and placed in group cages with same-sex litter mates. The colony room was maintained at 21–23°C and kept under a 12-h light/dark cycle. Behavioral testing was done during the light cycle, at approximately the same time each day. Rats in the sucrose preference experiment were single caged in clear polycarbonate cages (43 × 23 × 22 cm) on PD 58 for the duration of testing. Food and water were freely available except for the sucrose preference experiment, in which rats were water deprived for 24 h prior to the preference test. Subjects were treated according to the “Guide for the Care and Use of Mammals in Neuroscience and Behavioral Research” [19] under a research protocol approved by the Institutional Animal Care and Use Committee of CSUSB.

2.2. Apparatus

Novelty-induced CPP conditioning and testing were conducted in rectangular wooden chambers that had three compartments: two large compartments and one small placement chamber, arranged in a truncated T-shape. The two large compartments (37 × 30 × 45 cm) were adjacent to each other and separated by a removable partition. The smaller placement chamber (18 × 18 × 45 cm) was located at the side of the junction between the two larger compartments and was connected to them by a removable partition. When opened, rats were able to enter either large compartment from the placement chamber. The color, flooring, and odor of the large compartments varied, with one having white walls, wire mesh flooring, and pine bedding; while the other compartment had black walls, metal rod flooring, and cedar bedding. The small placement chamber had gray walls and a solid wood floor.

The plus maze was made of black plastic and was elevated 50 cm above the floor (San Diego Instruments, San Diego). The apparatus consisted of four arms, 50 cm long and 10 cm wide aligned perpendicularly. Two arms were enclosed by 30 cm high walls and the other two arms were exposed. The exposed or “open” arms had a 0.9 cm lip to help prevent rats from falling off. The maze was placed in the center of a quiet, dimly lit room, with a video camera located above the maze.

2.3. Drugs and in vivo drug treatment

Methylphenidate hydrochloride was obtained from Sigma-Aldrich (St. Louis, MO). Methylphenidate was dissolved in saline and injected intraperitoneally (ip) at a volume of 5 ml/kg. Saline or methylphenidate (2 or 5 mg/kg, ip) were administered daily on PD 11–PD 20.

2.4. CPP procedure

On PD 60, rats were randomly assigned to one of four conditioning groups: No Exposure, Equal Exposure, Biased Exposure–Black, or Biased Exposure–White. Rats in the No Exposure group were individually placed in Plexiglas maternity cages in the testing room. Rats in the Equal Exposure group received alternating daily placements in the end compartments. Rats in the Biased Exposure groups were exposed to only the black or white compartment. There were 8 daily conditioning sessions lasting 30 min each. On the 9th day, rats were put in the placement chamber and given free access to the large end compartments

for 15 min. The CPP procedure was videotaped on days 1, 8, and 9. The number of line crosses within the conditioning compartment was scored on days 1 and 8, while time spent in each compartment was scored on day 9. Behavior was assessed by experimenters blind to treatment conditions.

2.5. Sucrose preference procedure

On PD 60, rats were tested for sucrose preference over a five day period using a two-bottle choice test. On the first day, rats were singly housed and habituated to drinking from two bottles, both of which contained water. On the following three days, rats were trained on the sucrose choice procedure, in which one of the water bottles was replaced with a bottle containing a 0.125% sucrose solution. Rats were allowed to drink freely from both bottles for 24 h. The bottles were weighed and refilled each day at the same time in the morning. The position of the bottles was switched daily to avoid position preferences. After removing the bottles at the end of the last habituation day, rats were water deprived for 24 h and then given a 1 h preference test. Sucrose consumption and preference [defined as (weight of sucrose ingested) / (weight of water ingested + weight of sucrose ingested) $\times$ 100] were calculated for the habituation days and the test day.

2.6. Elevated plus maze procedure

Immediately following the sucrose test, rats were individually brought into the test room and placed in the center of the elevated plus maze facing an open arm. Rats were left on the maze for 5 min. Time spent in the open and closed arms, as well as the center area, were scored from the videotapes. Entry into an open or closed arm was defined as all four paws crossing the threshold, whereas entry into the center of the maze was defined as two paws crossing the threshold.

2.7. Statistical analyses

ANOVAs were used to analyze both body weight and behavioral data. Body weight during the preweaning period was analyzed with a 3 2 10 (pretreatment condition sex day) repeated measures ANOVA, while body weight on PD 60 was analyzed using a 3 2 (pretreatment condition sex) ANOVA. When the assumption of sphericity was violated, as determined by Mauchly's test of sphericity, the Greenhouse-Geisser epsilon statistic was used to adjust the degrees of freedom [20]. Corrected degrees of freedom were rounded to the nearest whole number. Line-cross data from the CPP experiment were analyzed by 3 2 2 (pretreatment sex compartment color) ANOVA; whereas, data from the CPP test day were analyzed by a 3 2 4 2 (pretreatment sex compartment pre-exposure condition compartment color) mixed factors ANOVA. Sucrose consumption and preference data from the habituation days were analyzed using 3 2 2 (pretreatment condition sex day) repeated measures ANOVAs. Sucrose consumption and preference did not vary according to day, so that factor was not included in the final statistical analyses. Sucrose consumption and preference on the test day were analyzed with 3 2 (pretreatment condition sex) between factors ANOVAs. Data from the elevated plus maze were analyzed using 3 2 (pretreatment condition sex) ANOVAs. Post hoc data analysis was made using Tukey tests ($p < 0.05$).

3. Results

3.2. Body weight

The mean body weights of male and female rats (see Table 1) increased progressively across PD 11-PD 20 [pretreatment day main effect, $F(3,790) = 5576.80$, $p < 0.001$]. Body weights did not differ by sex during the pretreatment phase, although rats treated with the higher dose of methylphenidate (5 mg/kg) weighed significantly less on the last two pretreatment

injection days (i.e., PD 19 and PD 20) [day $\times$ pretreatment interaction, $F(6,790) = 6.05$, $p < 0.001$]. The effects of methylphenidate on body weight did not persist into adulthood, but male rats ($M = 382.9$ g, $SEM 2.3$) did weigh more than female rats ($M = 238.4$ g, $SEM 1.1$) on PD 60 [sex main effect, $F(1,298) = 1513.05$, $p < 0.001$].

3.3. Novelty-Induced CPP

Conditioning Day—On conditioning days 1 and 8, rats in the two Biased Exposure groups (i.e., Biased Exposure-Black and Biased Exposure-White) exhibited more line-crosses in the white compartment than the black compartment (see Fig. 1) [exposure condition main effect, $F(1,107) = 14.44$, $p < 0.001$]. For both exposure groups, line-crosses decreased from day 1 to day 8 [conditioning day main effect, $F(1,107) = 106.12$, $p < 0.001$]. Overall neither sex nor methylphenidate pretreatment altered the number of line-crosses; however, a separate ANOVA examining vehicle-pretreated rats showed that female rats were more active than male rats on day 1 in the black compartment [sex $\times$ exposure condition interaction, $F(1,34) = 4.38$, $p < 0.05$, Tukey Tests, $p < 0.05$] (see Fig 1, top right panel).

Test Day—Overall, there was no preference for either the black or white compartment on the test day; however, time spent in the two end compartments was significantly altered by exposure condition. Specifically, rats in the Biased Exposure-Black and Biased Exposure-White groups spent more time in the novel compartment than in the familiar compartment (see Fig. 2) [exposure condition $\times$ compartment interaction, $F(3,214) = 26.08$, $p < 0.001$, Tukey tests, $p < 0.05$]. In addition, rats in the two Biased Exposure groups spent relatively more time in the novel compartment than did the rats in the two control exposure conditions (i.e., the Equal Exposure and No Exposure groups) [Tukey tests, $p < 0.05$]. That is, rats in the Biased Exposure-Black group spent more time in the white compartment when compared to rats given equal exposure or no exposure to the chamber; whereas, rats in the Biased Exposure-White group spent more time in the black compartment than the two control exposure groups. Equal Exposure group had a small, albeit significant, preference for the black compartment, while rats in the No Exposure group showed no preference for either compartment [Tukey tests, $p < 0.05$]. Methylphenidate pretreatment altered time spent in the end compartments, because rats pretreated with 5 mg/kg methylphenidate spent more time in the white compartment than rats pretreated with saline [pretreatment $\times$ compartment interaction, $F(2,214) = 4.83$, $p < 0.01$, Tukey tests, $p < 0.05$] (see Fig 2, bottom panel).

3.3. Sucrose Preference

Neither sucrose consumption nor sucrose preference varied significantly over the three-day habituation period, hence data were collapsed across days. During the habituation period, male rats consumed a greater amount of sucrose than female rats (see Table 2) [sex main effect, $F(1,58) = 36.03$, $p < 0.001$]. This effect was most likely due to body weight differences, since sucrose preference did not vary according to sex. Early methylphenidate exposure did not alter sucrose preference. Similar to the habituation period, male rats consumed more sucrose than female rats during the 1 h sucrose test [sex main effect, $F(1,58) = 17.17$, $p < 0.001$], while neither sucrose consumption nor sucrose preference were altered by methylphenidate pretreatment (see Table 2).

3.4. Elevated Plus Maze

Percent time in the open arms and time in the center of the elevated plus maze during the 5 min test are shown in Figure 3. Overall, female rats spent more time in the open arms of the maze than male rats [sex main effect, $F(1,57) = 5.81$, $p < 0.05$]. Methylphenidate treatment altered time in the open arms of the elevated plus maze, because rats pretreated with 5 mg/

kg methylphenidate spent less time in the open arms than rats treated with saline or 2 mg/kg methylphenidate [pretreatment main effect, $F(2,57) = 6.71$, $p < 0.005$]. Neither the pretreatment or sex variables affected time spent in the center of the maze.

4. Discussion

Administering methylphenidate during the preweanling period causes a number of long-term changes in drug responsiveness, including enhanced sensitivity to the reinforcing effects of cocaine and increased opioid receptor sensitivity [18, 21,22]. In the present study, we assessed whether early methylphenidate treatment would also have long-lasting effects on non-drug related responses. Specifically, we examined whether administering methylphenidate on PD 11-PD 20 would decrease the anxiolytic responses of young adult rats when tested on the elevated plus maze or enhance preferences for novelty and sucrose on the novelty CPP and sucrose preference tasks.

In terms of the novelty paradigm, young adult rats preferred a novel environment over a familiar environment (see also [23-26]). This novelty preference was apparent in both male and female adults, and occurred regardless of whether rats were habituated to the white or black compartment (control subjects exhibited a preference for the black compartment). Interestingly, methylphenidate (5 mg/kg) pretreatment altered the preference for the white compartment. More specifically, rats exposed to 5 mg/kg methylphenidate spent more time in the white compartment than saline controls, and this color preference was apparent in rats habituated to either the black (Biased Exposure-Black) or white (Biased Exposure-White) compartment.

This result suggests that early methylphenidate exposure did not enhance novelty preference or novelty-seeking *per se* but, instead, decreased the natural fear or anxiety for the white compartment. While novelty-induced CPP is not typically used as a measure of anxiety, our procedure is very similar to the light-dark box exploration test. The latter test is a standard measure of anxiety in which rats are allowed to move between a black (dark) and bright white (light) compartment [27,28]. That our methylphenidate-treated rats preferred the white compartment suggests an anxiolytic response, and is consistent with data showing that an early methylphenidate treatment regimen (starting at PD 7) reduces anxiety on the elevated plus maze [9]. In contrast, exposing rats to methylphenidate during preadolescence increases anxiety-like behaviors when measured using the elevated plus maze or fear conditioning paradigm [4, 5, 29, 30]. Notably, one study has reported that exposing rats to methylphenidate on PD 11-PD 20 did not alter anxiety responses on the elevated zero maze [31]. Therefore, the most parsimonious conclusion is that the effects of early methylphenidate exposure on anxiety-like behaviors may be dependent on both the paradigm used and age at drug treatment. Alternatively, these discrepancies could also result from procedural difference (e.g., animal handling, time of day, lighting conditions, etc.) between laboratories.

The sucrose preference test is frequently used to measure anhedonia and is predictive of antidepressant effects in humans [32]. Data from both the sucrose habituation phase and the test session (Tables 2 and 3) showed that preweanling methylphenidate exposure did not alter the hedonic value of sucrose in young adult rats. This result was somewhat surprising because we have previously shown that administering methylphenidate during the preweanling period enhances the reinforcing properties of sucrose on a sucrose-reinforced lever press task [17]. Moreover, Bolaños et al. [4, 5] reported that rats treated with methylphenidate during adolescence exhibited a reduced sucrose preference. In the present study, male rats consumed more sucrose solution than female rats, but no sex differences were apparent if preferences were calculated as percentage of total fluid intake.

Our elevated plus maze data were in general agreement with those of previous studies showing that repeated methylphenidate treatment causes a long-term increase in anxiety [4, 5]. Specifically, we found that exposing rats to methylphenidate (5 mg/kg) during the preweanling period decreased total time spent in the open arms of the elevated plus maze. Methylphenidate's actions were apparent in both male and female adult rats, although females did spend more time in the open arms than males. Similar sex differences have been observed before using the elevated plus maze [33-37]. Interestingly, this sex effect may be age dependent, because older rats (over PD 105) do not typically exhibit sex differences when tested on the elevated plus maze [38, 39]. Nonetheless, when considered together with the Bolaños studies [4, 5], it appears that anxiety responses, as measured on the elevated plus maze, are enhanced after exposure to methylphenidate during either the preweanling or adolescent periods (but see [9]). This methylphenidate-induced effect was apparent in both sexes, although young adult female rats exhibit less anxiety than male rats.

If our interpretation of the novelty CPP and elevated plus maze data is correct, then methylphenidate had opposing effects on anxiety depending on the task employed. Specifically, methylphenidate treatment increased the time spent in the white compartment (i.e., the novelty CPP experiment), thus indicating a decrease in anxiety; whereas, methylphenidate treatment reduced the time spent in the open arms (i.e., the elevated plus maze experiment), thus indicating an increase in anxiety. While the elevated plus maze and light-dark box exploration test typically provide consistent results, [27, 28] such congruent findings are not universal. For instance, exposure to male chemical cues is anxiolytic in sexually receptive females when measured on the elevated plus maze, but the same cues modestly increase anxiety in the light-dark box exploration test [40]. In addition, phencyclidine (PCP) induces anxiety in young adult rats when assessed on the elevated plus maze, but PCP decreases anxiety in the light-dark box test [39]. Thus, it is possible that procedural differences could be responsible for the diverse outcomes in the various studies. Alternatively, it has been argued that these discrepancies reveal the multidimensional nature of emotional behaviors like anxiety (for a review, see [41]). Therefore, it is quite possible that tasks and procedures designed to measure anxiety only quantify a particular idiosyncratic domain of that emotion. It is likely that multiple tests and experimental approaches will be required to fully evaluate the effects of early methylphenidate exposure on affective behavior.

The translational relevance of the present findings is difficult to determine, as relating preclinical data from young rats to children is fraught with complications [42]. In particular, dose comparisons between humans and rats are difficult to make because of species differences in the pharmacokinetics of methylphenidate and other psychostimulants (see [43]). In the present study, we used a 2 mg/kg dose of methylphenidate, which is generally considered to be within the therapeutic dose range of school-aged humans [44]. However, our results showed that 5 mg/kg methylphenidate, but not 2 mg/kg, altered novelty CPP and behavior on the elevated plus maze. Even so, we believe that these results have translational relevance because preschool-aged children are typically given the same treatment doses as older children but have significantly lower clearance rates of methylphenidate [45]. Thus, preschool-aged children, which are the focus of our early methylphenidate exposure condition, have relatively greater circulating levels of methylphenidate than other age groups. For this reason, we believe a broader dose range is necessary to accurately translate the effects of methylphenidate to younger human populations.

In summary, we found that preweanling methylphenidate treatment has persistent effects on affective behaviors. Specifically, exposing rats to methylphenidate during the preweanling period alters anxiety-like behaviors, but not depressive-like behaviors, in young adult rats. We found evidence of methylphenidate-induced anxiolytic effects using a novelty CPP task,

which is similar to the light-dark box exploration test, while data obtained using the elevated plus maze indicates that early methylphenidate exposure increases anxiety.

Acknowledgments

This work was supported by PHS grants DA027683 and DA025319

References

1. Achat-Mendes C, Anderson KL, Anderson YI. Methylphenidate and MDMA adolescent exposure in mice: Long-lasting consequences on cocaine-induced reward and psychomotor stimulation in adulthood. *Neuropharmacology*. 2003; 45:106–15. [PubMed: 12814663]
2. Adriani W, Leo D, Greco D, Rea M, di Porzio U, Laviola G, Perrone-Capano C. Methylphenidate administration to adolescent rats determines plastic changes on reward-related behavior and striatal gene expression. *Neuropsychopharmacology*. 2006; 31:1946–56. [PubMed: 16319916]
3. Andersen SL, Arvanitogiannis A, Pliakas AM, LeBlanc C, Carlezon WA Jr. Altered responsiveness to cocaine in rats exposed to methylphenidate during development. *Nat Neurosci*. 2002; 5:13–4. [PubMed: 11731802]
4. Bolaños CA, Barrot M, Berton O, Wallace-Black D, Nestler EJ. Methylphenidate treatment during pre- and periadolescence alters behavioral responses to emotional stimuli at adulthood. *Biol Psychiatry*. 2003; 54:1317–29. [PubMed: 14675795]
5. Bolaños CA, Wiley MD, Maffeo ML, Powers KD, Kinka DW, Grausam KB, Henderson RP. Antidepressant treatment can normalize adult behavioral deficits induced by early-life exposure to methylphenidate. *Biol Psychiatry*. 2008; 63:309–16.
6. 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. [PubMed: 11682248]
7. Carlezon WA Jr, Mague SD, Andersen SL. Enduring behavioral effects of early exposure to methylphenidate in rats. *Biol Psychiatry*. 2003; 54:1330–37. [PubMed: 14675796]
8. Ferguson SA, Boctor SY. Cocaine responsiveness or anhedonia in rats treated with methylphenidate during adolescence. *Neurotoxicol Teratol*. 2010; 32:432–42. [PubMed: 20347964]
9. Gray JD, Punsoni M, Tabori NE, Melton JT, Fanslow V, Ward MJ, Zupan B, Menzer D, Rice J, Drake CT, Romeo RD, Brake WG, Torres-Reveron A, Milner TA. Methylphenidate administration to juvenile rats alters brain areas involved in cognition, motivated behaviors, appetite, and stress. *J Neurosci*. 2007; 27:7196–207. [PubMed: 17611273]
10. Harvey RC, Sen S, Deaciuc A, Dwoskin LP, Kantak KM. Methylphenidate treatment in adolescent rats with an attention deficit/hyperactivity phenotype: cocaine addiction vulnerability and dopamine transporter function. *Neuropsychopharmacology*. 2011; 36:837–47. [PubMed: 21150910]
11. Mague SD, Andersen SL, Carlezon WA Jr. Early developmental exposure to methylphenidate reduces cocaine-induced potentiation of brain stimulation reward in rats. *Biol Psychiatry*. 2005; 57:120–5. [PubMed: 15652869]
12. McDougall SA, Kozanian OO, Greenfield VY, Horn LR, Gutierrez A, Mohd-Yusof A, Castellanos KA. One-trial behavioral sensitization in preweanling rats: differential effects of cocaine, methamphetamine, methylphenidate, and D-amphetamine. *Psychopharmacology*. 2011; 217:559–71. [PubMed: 21537939]
13. Molina JC, Pautassi RM, Truxell E, Spear N. Differential motivational properties of ethanol during early ontogeny as a function of dose and postadministration time. *Alcohol*. 2007; 41:41–55. [PubMed: 17452298]
14. Smith KS, Morrell JI. Behavioral responses during the initial exposures to a low dose of cocaine in later preweanling and adult rats. *Neurotoxicol Teratol*. 2008; 30:202–12. [PubMed: 18276106]
15. Andersen SL. Trajectories of brain development: point of vulnerability or window of opportunity. *Neurosci Biobehav Rev*. 2003; 27:3–18. [PubMed: 12732219]

16. Andersen SL, Navalta CP. Altering the course of neurodevelopment: a framework for understanding the enduring effects of psychotropic drugs. *Int J Dev Neurosci*. 2004; 22:423–40. [PubMed: 15380841]
17. Crawford CA, Villafranca SW, Cyr MC, Farley CM, Reichel CM, Gheorghe SL, Krall CM, McDougall SA. Effects of early methylphenidate exposure on morphine- and sucrose-reinforced behaviors in adult rats: relationship to dopamine D2 receptors. *Brain Res*. 2007; 1139:245–53. [PubMed: 17274963]
18. Crawford CA, Baella SA, Farley CM, Herbert MS, Leslie LR, Campbell RH, Zavala AR. Early methylphenidate exposure enhances cocaine self-administration but not cocaine-induced conditioned place preference in young adult rats. *Psychopharmacology*. 2011; 213:43–52. [PubMed: 20848087]
19. National Research Council. Guidelines for the care and use of laboratory animals. 8th ed.. National Academy Press; Washington: 2010.
20. Geisser S, Greenhouse SW. An extension of Box's results on the use of the *F* distribution in multivariate analysis. *Ann Mat. Statist*. 1958; 29:885–91.
21. Cyr MC, Morgan MM. Early methylphenidate exposure enhances morphine antinociception and tolerance in adult rats. *Neuropharmacology*. 2009; 57:673–77.
22. Halladay LR, Iñiguez SD, Furqan F, Previte MC, Chisum AM, Crawford CA. Methylphenidate potentiates morphine-induced antinociception, hyperthermia, and locomotor activity in young adult rats. *Pharmacol Biochem Behav*. 2009; 92:190–6. [PubMed: 19100281]
23. Klebaur JE, Bardo MT. The effects of anxiolytic drugs on novelty-induced place preference. *Behav Brain Res*. 1999; 101:51–7. [PubMed: 10342399]
24. McDougall SA, Zavala AR, Karper PE, Abbott DL, Figueroa S, Crawford CA. Chronic amphetamine exposure during the preweanling period does not affect avoidance learning or novelty-seeking of adult rats. *Neurobiol Learn Mem*. 2001; 75:338–45. [PubMed: 11300739]
25. Molander AC, Mar A, Norbury A, Steventon S, Moreno M, Caprioli D, et al. High impulsivity predicting vulnerability to cocaine addiction in rats: some relationship with novelty preference but not novelty reactivity, anxiety or stress. *Psychopharmacology*. 2011; 215:721–31. [PubMed: 21274702]
26. Pierce RC, Crawford CA, Nonneman AJ, Mattingly BA, Bardo MT. Effect of forebrain dopamine depletion on novelty-induced place preference behavior in rats. *Pharmacol Biochem Behav*. 1990; 36:321–5. [PubMed: 2113297]
27. Calabrese EJ. An assement of anxiolytic drug screening tests: hermetic dose responses predominate. *Crit Rev Toxicol*. 2008; 38:489–542. [PubMed: 18615308]
28. Kliethermes CL. Anxiety-like behaviors following chronic ethanol exposure. *Neurosci Biobehav Rev*. 2005; 28:837–50. [PubMed: 15642625]
29. Britton GB, Bethancourt JA. Characterization of anxiety-related responses in male rats following prolonged exposure to therapeutic doses of oral methylphenidate. *Pharmacol Biochem Behav*. 2009; 93:451–9. [PubMed: 19540871]
30. Britton GB, Segan AT, Sejour J, Mancebo SE. Early exposure to methylphenidate increases fear responses in an aversive context in adult rats. *Dev Psychobiol*. 2007; 49:265–75. [PubMed: 17380504]
31. Amos-Kroohs RM, Williams MT, Vorhees CV. Neonatal methylphenidate does not impair spatial learning in the Morris water maze in rats. *Neurosci Lett*. 2011; 502:152–6. [PubMed: 21798318]
32. Papp M, Moryl E, Wilner P. Phamacological validation of the chronic mild stress model of depression. *Eur J Pharmacol*. 1996; 296:129–136. [PubMed: 8838448]
33. Doremus-Fitzwater TL, Varlinskaya EI, Spear LP. Social and non-social anxiety in adolescent and adult rats after repeated restraint. *Physiol Behav*. 2009; 97:484–94. [PubMed: 19345235]
34. Huynh TN, Krigbaum AM, Hanna JJ, Conrad CD. Sex differences and phase light cycle modify chronic stress effects on anxiety and depressive-like behavior. *Behav Brain Res*. 2011; 222:212–22. [PubMed: 21440009]
35. Simpson J, Ryan C, Curley A, Mulcaire J, Kelley JP. Sex differences in baseline and drug-induced behavioral responses in classical behavioral tests. *Prog Neuropsychopharmacol Biol Psychiatry*. 2012; 37:227–36. [PubMed: 22353173]

36. Verma P, Hellemans KGC, Choi FY, Yu W, Weinberg J. Circadian phase and sex differences on depressive/anxiety-like behaviors and HPA axis responses to acute stress. *Physiol Behav.* 2010; 99:276–85. [PubMed: 19932127]
37. Xiang X, Huang W, Haile CN, Kosten TA. Hippocampal GluR1 associates with behavior in the elevated plus maze and shows sex differences. *Behav Brain Res.* 2011:326–31. [PubMed: 21497621]
38. Imhof JT, Coelho ZM, Schmitt ML, Morato GS, Carobrez AP. Influence of gender and age on performance of rats in the elevated plus maze apparatus. *Behav Brain Res.* 1993; 56:177–80. [PubMed: 8240712]
39. Turgeon SM, Kim D, Pritchard M, Salgado S, Thaler A. The effects of phencyclidine (PCP) on anxiety-like behavior in the elevated plus maze and the light-dark exploration test are age dependent, sexually dimorphic, and task dependent. *Pharmacol Biochem Behav.* 2011; 100:191–8. [PubMed: 21889525]
40. Behr GA, da Motta LL, de Oliveira MR, Oliveira MWS, Hoff MLM, Silvestrin RB, Moreira JCF. Decreased anxiety-like behavior and locomotor/exploratory activity, and modulation in hypothalamus, hippocampus, and frontal cortex redox profile in sexually receptive female rats after short-term exposure to male chemical cues. *Behav Brain Res.* 2009; 199:263–70. [PubMed: 19110005]
41. Ramos A. Animal models of anxiety: do I need multiple tests? *Trends Pharmacol Sci.* 2008; 29:493–8. [PubMed: 18755516]
42. Roulet, FI; Lai, JK.; Foster, JA. In utero exposure to valproic acid and autism – a current review of the clinical and animal studies. *Neurotoxicol Teratol.* 2013 doi: 10.1016/j.ntt.2013.01.004.
43. Kuczenski R, Segal DS. Stimulant actions in rodents: implications for attention-deficit/hyperactivity disorder treatment and potential substance abuse. *Biol Psychiatry.* 2005; 57:1391–6. [PubMed: 15950013]
44. Gerasimov MR, Franceschi M, Volkow ND, Gifford A, Gatley SJ, Marsteller D, Molina PE, Dewey SL. Comparison between intraperitoneal and oral methylphenidate administration: a microdialysis and locomotor activity study. *J Pharmacol Exp Ther.* 2000; 295:51–7. [PubMed: 10991960]
45. Wigal SB, Gupta S, Greenhill L, Posner K, Lerner M, Steinhoff K, Wigal T, Kapelinski A, Martinez J, Modi NB, Stehli A, Swanson J. Pharmacokinetics of methylphenidate in preschoolers with attention-deficit/hyperactivity disorder. *J Child Adolesc Psychopharmacol.* 2007; 17:153–64. [PubMed: 17489710]

Highlights

Early methylphenidate exposure has persistent effects on drug responsiveness.

We tested the response to nondrug stimuli after preweanling methylphenidate exposure.

Preweanling exposure to methylphenidate did not affect sucrose preference.

Preweanling exposure to methylphenidate did alter anxiety-like behaviors.

Fig 1.

Mean line-crosses ($\pm$ SEM) of rats ($n=9-10$) in the black and white compartments on conditioning days 1 and 8. Rats were given eight daily placements in the black (Biased Exposure-Black) or white (Biased Exposure-White) compartment. Rats had been pretreated with saline or methylphenidate (2 or 5 mg/kg, ip) for 10 consecutive days starting on PD 11. ^aSignificantly different from day 1. ^bSignificantly different from male rats tested on day 1 in the same compartment. ^ASignificantly different from rats conditioned in the black compartment.

Fig 2.

Mean time spent in the black (filled bar) and white (open bar) compartments on the test day. Rats ($n=9-10$) had previously received eight daily placements in the black compartment (Biased Exposure-Black), the white compartment (Biased Exposure-White), both compartments (Equal Exposure), or a holding cage (No Exposure). Rats had been pretreated with saline or methylphenidate (2 or 5 mg/kg) for 10 consecutive days starting on PD 11. ^aSignificantly different from time spent in the familiar compartment. ^bSignificantly different from rats in the Equal Exposure and No Exposure groups. ^cSignificantly different from time spent in the white compartment. ^ASignificantly different from saline-pretreated rats.

Fig. 3.

Mean percent time ($\pm$ SEM) spent in the open arms of the elevated plus maze. Rats are the same as those in the sucrose preference experiment (Table 2 and 3). Rats had been pretreated with saline or methylphenidate (2 or 5 mg/kg) for 10 consecutive days starting on PD 11. ^aSignificantly different from male rats. ^bSignificantly different from rats pretreated with saline or 2 mg/kg methylphenidate.

Table 1

Mean ($\pm$ SEM) body weight (g) of male and female rats ($N=293$) exposed to saline or methylphenidate on PD 11-PD 20.

Treatment	Postnatal Day (PD)									
	PD 11	PD 12	PD 13	PD 14	PD 15	PD 16	PD 17	PD 18	PD 19	PD 20
Saline	25.3 $\pm$.4	27.9 $\pm$.4	30.4 $\pm$.5	32.8 $\pm$.5	34.9 $\pm$.6	37.6 $\pm$.6	40.0 $\pm$.6	42.7 $\pm$.7	45.9 $\pm$.7	49.9 $\pm$.8
2 mg/kg MPH	25.7 $\pm$.4	28.4 $\pm$.5	30.8 $\pm$.5	33.5 $\pm$.5	35.8 $\pm$.6	38.2 $\pm$.6	40.2 $\pm$.6	43.0 $\pm$.6	45.8 $\pm$.7	49.9 $\pm$.8
5 mg/kg MPH	25.4 $\pm$.4	27.9 $\pm$.5	30.1 $\pm$.5	32.7 $\pm$.2	34.8 $\pm$.6	36.9 $\pm$.6	39.1 $\pm$.6	41.4 $\pm$.6	44.0 $\pm$.7 ^a	47.9 $\pm$.8 ^a

^aSignificantly different from rats exposed to saline

Table 2

Mean ($\pm$ SEM) sucrose consumption and sucrose preference in male and female rats ($n=10-11$) during the three-day habituation period. Rats had received daily injections of saline or methylphenidate (2 or 5 mg/kg) for 10 consecutive days starting on PD 11.

Pretreatment	Sucrose Consumption		Sucrose Preference	
	Male	Female	Male	Female
Saline	27.3 (± 1.7) ^a	21.7 (± 1.8)	61.2 (± 6.8)	63.9 (± 4.9)
2 mg/kg MPH	33.9 (± 1.2) ^a	18.9 (± 1.5)	71.9 (± 3.1)	59.1 (± 3.9)
5 mg/kg MPH	29.7 (± 2.6) ^a	21.3 (± 1.0)	65.3 (± 4.6)	63.1 (± 3.3)

Sucrose consumption is presented as the amount of 0.125% sucrose solution consumed (g) and sucrose preference is calculated as (weight of sucrose consumed / (weight of water consumed + weight of sucrose consumed) $\times$ 100).

^aSignificantly different from female rats.

Table 3

Mean ($\pm$ SEM) sucrose consumption and sucrose preference in male and female rats on the test day. Rats had received daily injections of saline or methylphenidate (2 or 5 mg/kg) for 10 consecutive days starting on PD 11.

Pretreatment	Sucrose Consumption		Sucrose Preference	
	Male	Female	Male	Female
Saline	14.0 ($\pm$ 2.6) ^a	8.7 ($\pm$ 1.5)	66.6 ($\pm$ 6.8)	64.1 ($\pm$ 11.2)
2 mg/kg MPH	15.0 ($\pm$ 1.7) ^a	7.4 ($\pm$ 1.5)	70.7 ($\pm$ 7.2)	46.6 ($\pm$ 6.9)
5 mg/kg MPH	13.7 ($\pm$ 1.8) ^a	8.9 ($\pm$ 1.0)	65.4 ($\pm$ 8.4)	60.5 ($\pm$ 7.0)

Sucrose consumption is presented as the amount of 0.125% sucrose solution consumed (g) and sucrose preference is calculated as (weight of sucrose consumed / (weight of water consumed + weight of sucrose consumed) $\times$ 100).

^aSignificantly different from female rats.