The Functional Consequences of Early Methylphenidate Exposure in Adolescent Rats
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Introduction

Attention deficit/hyperactivity disorder (ADHD) affects 5-12% of the children population (Faraone et al., 2005; Bloom et al., 2010). Treatment for this disorder is primarily via methylphenidate (MPH) administration (Faraone et al., 2005). The latter is worrisome as psychostimulants are being used to treat children 6 years of age or less diagnosed with ADHD (Zito et al. 2000), despite the lack of approval by the Food and Drug Administration for this particular age group (Greenhill et al., 2006). There is also a concern about the functional consequences of early and extended use of MPH on a developing nervous system. (Teicher et al., 1996; Marshall, 2000). In particular, there is concern as to whether early exposure to MPH can increase the rewarding effects of drugs of abuse later in life. One drug in particular, methamphetamine, is highly abused drug and its rate of use has been increasing.

Studying the long-term effects of the early use of methylphenidate in humans can be complicated because of time and ethical issues. In contrast, animal models provide a useful approach to understanding the functional consequences of early exposure to MPH as the brain of rodents exhibit similar brain maturation as a human brain, but at a much rapid rate. For example, it is thought that childhood (~3-5 years) occurs between postnatal days (PD) 10-20 in rats, whereas adolescence occurs between PD 28-58 (Anderson, 2003). Preclinical studies with animals suggest that the age of exposure to psychostimulants alters the rewarding properties of drugs when animals are tested as adults (Andersen et al. 2002; Brandon et al. 2001). Specifically, rats pretreated with MPH
during preadolescence (PD 20-35) showed decreased cocaine sensitivity when animals were tested as adults (PD 60) (Andersen et al., 2002). On the other hand, repeated exposure to MPH during adolescence (PD 35-41) was shown to increase cocaine-seeking behavior in the self-administration paradigm (Brandon, 2001). Similarly, exposure to MPH during postnatal days (PD) 11-20, a period of rat development that resembles early childhood in humans, resulted in increased cocaine self-administration when animals were tested as adults (PD 90) (Crawford et al. 2011). To date, no studies have examined the functional consequences of early MPH exposure (PD 11-20) on the behavioral and neurochemical effects during adolescence (PD 27-50 to determine if there is an increased risk for drug addiction during adolescence.

Exposure to psychoactive drugs during childhood is critical to study, since brain development during the first years of life is sensitive to harmful neurological adaptations caused by external factors that may increase an individual’s likelihood of developing an addiction (Attridge et al., 2011). Moreover, it is important to assess the rewarding effects of drugs of abuse during adolescence, as it is a critical period of development during which there is an increased risk for drug abuse. Adolescence has been found as the age where humans begin experimenting with drugs, and that early drug use is associated with greater consumption and difficulty in quitting later in life (Chen and Kandel 1995; Kandel and Logan 1984).

Therefore, in the present study we examined the behavioral long-term effects of early exposure to MPH on methamphetamine reward during adolescence. Specifically, we investigated whether early (PD 11-20) exposure to MPH increases methamphetamine-induced reward during early (PD 27-36) and late (PD 42-48) adolescence in male and
female rats using conditioned place preference (CPP), an animal model commonly used to study the rewarding effects of drugs (Markou et al. 1993; Tzschentke 1998). We hypothesized that early MPH treatment during PD 11-20 would increase the rewarding effects of methamphetamine in early, but not late adolescent rats.

Methods

**Animals.** Male and female rats \( (N = 101) \) of Sprague-Dawley descent (Charles Rives, Hollister, CA) were obtained from the breeding colony at CSU Long Beach. Rats were housed under standard housing conditions and given food and water *ad libitum*.

**Apparatus.** CPP was assessed in a truncated T-maze, composed of two large equal-size compartments divided by a removable solid partition and a small adjacent start compartment located to the side of the junction of the two large compartments (see Figure 1). The three chambers were distinguished with different visual and tactile cues.

**Procedures.** Beginning on PD 11, rats were randomly assigned to receive twice-daily injections of clinically relevant doses of MPH (0, 2 or 4 mg/kg, IP) for 10 days (ie., PD 11-20). Following the pretreatment phase, all rats were tested for methamphetamine-induced CPP using a 10-day CPP procedure during early (PD 27-36) or late (PD 39-48) adolescence. During days 1 and 10 of the CPP procedure, rats were tested for their preconditioning and postconditioning place preference, respectively in 15-minute sessions. During preference tests, rats were placed into a CPP box and allowed to move freely between two chambers. The side in which rats spent less than 50% of the total test time was designated as the nonpreferred side and the other as the preferred side. During days 3-8, rats underwent 30-min conditioning sessions, where they received an injection
of methamphetamine and were immediately confined into their initially nonpreferred compartment; and on alternate days, they received an injection of saline and were immediately confined to their initially preferred compartment. Males received a methamphetamine dose of .1 mg/kg, and females received a dose of .05 mg/kg based on previous studies suggesting sex differences in sensitivity in response to methamphetamine (Milesi-Halle et al., 2007). This two-day conditioning cycle was repeated once more over the next four consecutive days. Days 2 and 9 were rest days.

**Statistical Analysis**

CPP was defined as a significant increase in the time spent on the methamphetamine-paired compartment during the postconditioning day versus the preconditioning day. Planned comparisons were thus used to determine if there was a increase in time spent in the methamphetamine-paired compartment between the pre- and post-conditioning days among the different pretreatment groups.

**Results**

In early adolescent males (see Figure 2), planned comparisons revealed CPP was evident in rats pretreated with 0 mg/kg of MPH, \( t(8) = -3.79, p = .005 \), 2 mg/kg of MPH, \( t(7) = -2.77, p = .028 \), and 3 mg/kg of MPH, \( t(8) = -2.40, p = .043 \). In late adolescent males, CPP was evident only in the group of rats pretreated with 0.0 mg/kg of MPH, \( t(8) = -2.759, p = .025 \) but not those pretreated with 2 or 4 mg/kg of MPH. For early adolescent females (see Figure 3), planned comparisons revealed that CPP was evident in rats pretreated with 2 mg/kg of MPH, \( t(8) = -1.97, p = .05 \). For late adolescent females, CPP was evident only in the group of rats pretreated with 4.0 mg/kg of MPH, \( t(8) = -4.72, p < .05 \), but not in rats pretreated with 0 or 2 mg/kg.
Discussion

The purpose of this experiment was to examine how exposure to MPH during postnatal days 11-20 would alter the rats’ sensitivity to the rewarding effects of methamphetamine during early (PD 27) and late (PD 42) adolescence. Contrary to our hypothesis, in both male and female rats, the late adolescent rats appeared to be most affected by MPH (4 mg/kg) pretreatment. Specifically, late adolescent male rats in this group did not show an increase in time spent on the methamphetamine-paired side, whereas the rats pretreated with saline and 2 mg/kg did. Interestingly, the opposite was observed for females, where late adolescent females pretreated with MPH (4 mg/kg) showed an increase in time spent on the methamphetamine-paired side, but not females pretreated with saline and 2 mg/kg MPH.

These findings suggest that early MPH exposure increases the risk to methamphetamine abuse in females, but not males. This appears to be consistent with previous literature that showing that females are more sensitive to the rewarding effects of drugs of abuse. However it remains unclear whether the absent CPP response seen in late adolescent male rats pretreated with MPH is the result of an aversive experience, or an attenuated reward experience. The finding that MPH is causing an aversive response to drugs of abuse has previously been observed (Achat-Mendes, Anderson, & Itzhak, 2003; Andersen et al., 2002), but studies also suggest that this may be due to MPH exposure producing anhedonia, a depressive-like phenotype (Bolaños et al., 2003; 2008; Warren et al., 2011). At the very least, this study adds to a growing body of literature showing that the early and extended use of MPH alters the rewarding properties of drugs later in development and recommends precaution on the use of MPH on children.
Appendix

Figure 1. Sketch of the apparatus that will be used to assess CPP in adolescent rats.

FIGURE 2. Average time spent in the methamphetamine-paired compartment for male rats ($N = 50$) during the preconditioning and postconditioning days. Rats were pretreated with 0.0, 2.0 or 4.0 mg/kg of MPH and conditioned with 0.1 mg/kg of methamphetamine during early (PND 29-38) or late (PND 39-48) adolescence in Experiment 1. Based on planned comparisons, $\emptyset$ signifies a significant difference between preconditioning and postconditioning time as examined by MPH pretreatment.
FIGURE 3. Average time spent in the methamphetamine-paired compartment for female rats ($N = 51$) during the preconditioning and postconditioning days. Rats were pretreated with 0.0, 2.0 or 4.0 mg/kg of MPH and conditioned with 0.05 mg/kg of methamphetamine during early (PND 29-38) or late (PND 39-48) adolescence in Experiment 1. Based on planned comparisons, $\omega$ signifies a significant difference between preconditioning and postconditioning time as examined by MPH pretreatment.
References


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