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Chronic Methylphenidate Administration in Mice Produces Depressive-Like Behaviors and Altered Responses to Fluoxetine

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Abstract

Methylphenidate (MPH) is a psychostimulant used in the treatment of attention-deficit/hyperactivity disorder in children and adults. Increasing abuse rates of this drug have raised questions regarding the effects of chronic, high-dose MPH administration. Although the effects of chronic MPH exposure have been well-documented in regard to reward-related behaviors in adolescent and adult animals, there are few studies of the effects of MPH on depressive-like behaviors and antidepressant responses, particularly in adult models. We examined the effects of chronic (14 days) high-dose (20 mg/kg i.p.) MPH exposure on locomotor activity and forced swim test behavior in C57Bl/6J mice. We show that MPH treatment ameliorates the locomotor suppression seen in response to fluoxetine. In addition, chronic MPH treatment produces depressive-like effects in the forced swim test, with decreased latency to first immobility and a trend toward increased immobility. These effects are reversed with acute fluoxetine administration, in contrast to saline-treated animals, which show no response to fluoxetine. The induction of depressive-like behaviors after chronic MPH treatment in adult mice is in agreement with previous studies in adolescent rats, and the marked alterations in fluoxetine responses implicate alterations in the serotonin system and possibly the dopamine system produced by MPH.

Keywords

forced swim test; methylphenidate; fluoxetine; depressive-like behaviour; locomotor activity

Methylphenidate (MPH) is a stimulant used for the treatment of attention-deficit/hyperactivity disorder in children and adults that is gaining in popularity as a drug of abuse (Biederman and Spencer, 2002; McCabe et al., 2005; Teter et al., 2003; Teter et al., 2006). It is similar in structure to amphetamine (Gatley et al., 1996) and inhibits dopamine (DA) and norepinephrine (NE) transporters, but has almost no affinity for serotonin (5-HT) transporters (Davies et al., 2004; Kuczenski and Segal, 1997; Markowitz, 2006). The effects of acute and chronic MPH exposure on reward-related behaviors and drug self-administration have been extensively studied (Adriani et al., 2006; Brandon and Steiner, 2003; Brandon et al., 2001; Valvassori et al., 2007; Yang et al., 2010), but there are only a few studies, in rats, on the effects of chronic MPH on depressive-like behaviors (Bolanos et al., 2003; Bolanos et al., 2008; Carlezon et al., 2003).

We hypothesized that chronic, high-dose administration of MPH in mice would produce depressive-like behaviors during withdrawal, similar to results found after chronic exposure

to reinforcers (Barr et al., 2002; Moreau et al., 1995), as well as increased antidepressant-like effects of fluoxetine. Accordingly, we tested the effects of 2 weeks of MPH administration (20 mg/kg i.p., once daily for 14 days, National Institute on Drug Abuse, Bethesda, MD) on locomotion and forced swim test (FST) responses in male and female adult mice (C57Bl/6J, no differences observed by *t* test between sexes). We chose a high dose of MPH to mimic abused doses of this drug in humans (Coetzee et al., 2002; Klein-Schwartz, 2002). A dose of 20 mg/kg i.p. in mice has been shown to produce locomotor activation associated with greater than 75% occupancy of the DAT, which has been shown to produce a subjective high in human subjects (Gatley et al., 1999). All behavioral testing took place 48 h following the last administration of MPH or saline, during acute withdrawal from MPH, similar to previous studies of withdrawal from reinforcers (Barr et al., 2002; Moreau et al., 1995). Animal care was in accordance with Wake Forest University's Institutional Animal Care and Use Committee and National Institutes of Health guidelines.

Following MPH treatment, we assessed locomotor activity responses to increasing doses of fluoxetine (1–15 mg/kg i.p., Sigma-Aldrich, St. Louis, MO). Animals were allowed to habituate to the locomotor chambers (Med Associates, St. Albans, VT) for 2 h before drug administration, and no differences were observed between groups in response to a novel environment ($P > 0.05$, Fig. 1A). After fluoxetine injection, activity was recorded for another 2 h, and data were analyzed for distance traveled in cm by repeated measures ANOVA. Fluoxetine decreased locomotor activity in saline-treated animals at the three highest doses, but MPH-treated animals showed no changes ($F_{1,108} = 5.080$, $P < 0.05$, Fig. 1B).

Depressive-like behaviors and responses to fluoxetine were assessed in the FST over 2 days of testing, with the 2nd day used for analysis. A separate group of animals were treated chronically with MPH as described above and were injected with 15 mg/kg fluoxetine or saline 20 min before testing. During the test period, animals were placed in a clear cylindrical beaker (diameter 15 cm, height 22 cm) partially filled with water (23°C, depth 17.5 cm), and activity was observed and scored for 6 min. The latency to first immobility episode (immobility lasting > 5 s) and the total immobility time over the 6 min period were recorded, and data were analyzed using Student's *t* test. At baseline, MPH-treated animals exhibited a significantly shorter latency to first immobility episode compared to saline-treated animals ($t = 2.755$, $P < 0.05$, Fig. 2A). MPH-treated animals also showed a nonsignificant trend toward increased total time spent immobile ($t = 1.475$, $P = 0.1$, Fig. 2C). Following administration of fluoxetine, saline-treated animals showed no differences in latency to first immobility episode compared to baseline ($P > 0.05$, Fig. 2B). In contrast, MPH-treated animals exhibited a significant increase in latency to first floatation episode in comparison to baseline measures ($t = 3.320$, $P < 0.01$, Fig. 2B), resulting in a significant difference between saline- and MPH-treated groups after fluoxetine ($t = 3.357$, $P < 0.01$, Fig. 2B). In measures of total immobility time following fluoxetine administration, saline-treated animals showed no difference compared to baseline ($P > 0.05$, Fig. 2D), whereas MPH-treated animals exhibited a trend toward decreased time spent immobile ($t = 1.954$, $P = 0.06$, Fig. 2D) in comparison to baseline immobility. This resulted in a significant difference in fluoxetine response between the two groups ($t = 2.917$, $P < 0.01$, Fig. 2D).

The results of this study indicate that chronic, high-dose MPH administration and subsequent withdrawal in mice produces depressive-like behaviors and an increased antidepressant-like response to fluoxetine administration. These results are in agreement with similar studies examining the effects of adolescent MPH administration (2 mg/kg i.p. postnatal day 20–36) on adult FST behavior (Bolanos et al., 2003; Bolanos et al., 2008; Carlezon et al., 2003). These findings are not limited to MPH treatment; withdrawal from other psychostimulants and intracranial self-stimulation have been shown to produce

depressive-like behaviors (Barr et al., 2002; Moreau et al., 1995). Our results indicate that the depressive-like behaviors seen following chronic, low-dose MPH treatment in adolescent rats can be produced by high-dose MPH treatment in adult mice. Our studies also show that this depressive-like phenotype following MPH treatment is accompanied by decreased locomotor sensitivity to fluoxetine and increased antidepressant like effects. This study shows for the first time that MPH treatment produces resistance to the locomotor-decreasing effects of fluoxetine, an effect that may influence responses in the FST. The strain of mice utilized in this study, C57Bl/6J (C57), is known to be resistant to the effects of fluoxetine in tests of depressive-like behaviors (Crowley et al., 2005; Lucki et al., 2001) and exhibits decreases in locomotor activity following acute fluoxetine exposure (Brookshire and Jones, 2009, Fig. 1B). We show a decrease in locomotor activity and no response to fluoxetine administration in the FST in saline-treated animals, an effect that could be due to the locomotor suppression we have seen at this dose. After chronic MPH treatment, however, C57 mice show a lack of locomotor suppression, accompanied by increases in latency to first immobility episode at baseline and decreased time spent immobile following acute fluoxetine. This suggests that MPH treatment may unmask antidepressant effects in this mouse model, effects that may normally be reduced due to the locomotor attenuating effects of the drug in this strain (Crowley et al., 2005; Lucki et al., 2001), and indicates that MPH treatment may render mice more susceptible to antidepressant effects. Similarly, people who abuse MPH may have altered clinical responses to antidepressant, which could be important in determining dosages and treatment regimens.

Because MPH is a psychostimulant with little affinity for the 5-HT transporter, the primary target of fluoxetine, the effects of MPH treatment on subsequent fluoxetine responses are surprising. It is possible that MPH-induced elevations in DA and NE alter baseline FST responses during withdrawal, as DA in particular has been shown to affect FST behaviors (see Willner, 1997 for review). It is also possible that chronic MPH treatment may produce changes in 5-HT systems, because there are extensive interactions between all of the monoamines. In particular, the 5-HT_{1B} receptor, located in the ventral midbrain (Hoyer et al., 1994), shows increased expression following chronic psychostimulant exposure (Hoplight et al., 2007; Przegalinski et al., 2003). The alternations seen in this study following chronic MPH could be due to similar receptor changes. Further studies will be necessary to determine how MPH treatment alters 5-HT system responses to produce these effects.

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Fig. 1.

Effects of chronic MPH treatment on locomotor responses. **A:** No differences observed between MPH and saline-treated mice in locomotor habituation ($P > 0.05$). **B:** Locomotor response to fluoxetine (0–15 mg/kg, i.p., $n = 8$ males, 4 females per group). Saline-treated animals showed a decrease in locomotor activity at the three highest doses tested (2.5, 5, 15 mg/kg); MPH-treated animals showed no effect of fluoxetine on locomotor activity. * $P < 0.05$ difference from saline locomotor response, $P < 0.05$ difference between MPH and saline-treated animals.

Fig. 2.

Effects of chronic MPH on FST measures. **A:** MPH-treated animals show decreased latency to immobility compared to saline-treated animals ($n = 12/\text{treatment}$, 6 males and 6 females per group). $*P < 0.05$ compared to saline-treated animals. **B:** MPH-treated mice show increased latency to immobility compared to baseline following administration of 15 mg/kg i.p. fluoxetine. Saline-treated animals showed no differences in comparison to baseline ($n = 6$ males and 6 females/group). $**P < 0.01$ compared to baseline, $++P < 0.01$ compared to saline-treated group. **C:** MPH-treated animals show a trend ($P = 0.1$) toward increased time spent immobile at baseline compared to saline-treated animals ($n = 6$ males and 6 females/group). **D:** MPH-treated animals show a trend ($P = 0.06$) toward decreased immobility compared to baseline in total time spent immobile following administration of 15 mg/kg i.p. fluoxetine ($n = 6$ males and 6 females/treatment). $**P < 0.01$ difference from saline-treated animals.