Pharmacology, Biochemistry and Behavior 96 (2010) 166–174

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

Intermittent methylphenidate during adolescent development produces locomotor hyperactivity and an enhanced response to cocaine compared to continuous treatment in rats

Richard Griggs, Corey Weir, Wesley Wayman, Timothy E. Koeltzow *

Drug Abuse Research Laboratory Department of Psychology Bradley University, USA

ARTICLE INFO

Article history:
Received 25 November 2009
Received in revised form 18 April 2010
Accepted 30 April 2010
Available online 9 May 2010

Keywords:
Attention-deficit/hyperactivity disorder
Cocaine
Methylphenidate
Self-administration
Locomotion
T-maze
Light/dark chamber
Open field
Adolescence
Osmotic minipump
Extinction
Reinstatement

ABSTRACT

The consequences of chronic methylphenidate (MPH) administration in adolescents for the treatment of attention-deficit/hyperactivity disorder (ADHD) remain to be fully understood. Studies in rats indicate that the pharmacokinetics of psychostimulant administration can powerfully influence the behavioral and neural consequences of chronic treatment. The purpose of the present study was to assess the effects of intermittent (0.8 or 1.6 mg/kg, s.c., twice daily) versus continuous (1.6 or 3.2 mg/kg/day via osmotic minipump) MP administration across four weeks of adolescent development in rats. Results indicate that intermittent treatment produced hyperactivity in a novel open field and increased sensitivity to both the reinforcing and locomotor-activating effects of cocaine. In contrast, continuous MPH resulted in a hypoactive response to the novel open field and a reduced sensitivity to both operant and non-contingent cocaine. To the extent that the continuous release condition models the sustained-release formulations utilized in human ADHD treatment, we interpret these data to indicate that sustained-release formulations are less likely to advance a risk of subsequent substance abuse.

1. Introduction

Methylphenidate (MPH) is among the most commonly prescribed medications for attention-deficit/hyperactivity disorder (ADHD) and exhibits high efficacy in the management of symptoms (Greenhill et al., 2002; Faraone, 2009). In addition to the signature symptoms of inattentiveness, impulsivity and hyperactivity, individuals with ADHD exhibit an increased prevalence of substance use disorders (Schubiner, 2005). Despite a mechanism of action similar to cocaine, i.e., blockade of the dopamine (DA) transporter and increased synaptic concentrations of DA in reward-relevant regions of the brain (Volkow et al., 2005), studies indicate that MPH may confer protection against substance abuse among ADHD populations (Faraone and Wilens, 2003; Mannuzza et al., 2008). Nevertheless, due to persistent concerns about diagnosis (Jensen, 2000), the potential for diversion and misuse (Darredeau et al., 2007; Poulin, 2007), and a limited understanding of the long-term consequences of chronic MPH exposure during development, its use remains controversial (Kollins, 2008).

In rodent models of addiction, it is well-established that repeated, intermittent exposure to psychomotor stimulants results in a progressive enhancement of the locomotor-activating and DA-releasing effects of these drugs (Robinson and Berridge, 2001). This ability to promote sensitization has been linked to increased acquisition and reinstatement of operant drug self-administration, has been shown to persist for prolonged periods (Paulson et al., 1991) and may parallel the neural adaptations responsible for drug craving (Vanderschuren et al., 1999). Similar to amphetamine and cocaine, MPH is readily self-administered by rats (Botly et al., 2008), and repeated intermittent MPH administration in adult rats produces sensitized locomotor responses (Kuczenski and Segal, 2001; Yang et al., 2006) and conditioned place preference (Sellings et al., 2006).

The consequences of repeated MPH administration during adolescence have been mixed. Measures of spontaneous locomotor activity have reported enhanced activity (Carlezon et al., 2003), no effect (Valvassori et al., 2007) or decreased activity (Gray et al., 2007; Wiley et al., 2009). Although sensitized locomotor responses to cocaine challenge have been reported in both mice and rats (Brandon et al., 2001; Achat-Mendes et al., 2003; Adriani et al., 2006), others have

* Corresponding author. Department of Psychology 1501 W. Bradley Ave. Peoria, IL 61625, USA. Tel.: +1 309 677 2590; fax: +1 309 677 3763.
E-mail address: tkoeltzow@bradley.edu (T.E. Koeltzow).

0091-3057/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2010.04.026

failed to identify a sensitized locomotor response to methylphenidate challenge (McFadyen et al., 2002; Valvassori et al., 2007). Likewise, whereas adolescent administration of MPH was reported to result in enhanced operant cocaine self-administration and increased reinstatement of extinguished conditioned place preference (Brandon et al., 2001; Achat-Mendes et al., 2003), others have found that adolescent MPH results in a diminished sensitivity to cocaine on measures of conditioned place preference and intracranial self-stimulation (Andersen et al., 2002; Mague et al., 2005; Augustyniak et al., 2006). Variability between studies likely results from important methodological differences in the timing, duration and dosing parameters of adolescent drug administration, but also potentially represents the complex behavioral profile of adolescents to psychomotor stimulant drugs (Spear and Brake, 1983).

In recent years, sustained-release formulations of MPH have been increasingly utilized to enhance patient compliance (Pelham et al., 2001; Wolraich and Doffing, 2004). Although similar in treatment efficacy, sustained-release formulations appear to exert a more limited abuse profile and are more difficult to divert for illicit use (Parasrampuria et al., 2007b). In this regard, it is noteworthy that the ability of cocaine to elicit behavioral sensitization in rats is determined by the administration protocol utilized during the pre-exposure period. Thus, whereas repeated, intermittent cocaine administration is associated with an increased sensitivity to subsequent cocaine challenge, continuous cocaine infusion results in the development of tolerance to cocaine and a decreased sensitivity to subsequent cocaine exposure (King et al., 1993). Yet, with few exceptions (Thanos et al., 2007), the vast majority of studies conducted to date have utilized intermittent injection procedures to model chronic MPH administration in adolescent rats.

The purpose of the present experiments was to characterize the consequences of chronic adolescent administration of continuous versus intermittent MPH in rats across a battery of behavioral tests. Dependent variables included spontaneous locomotor activity, behavioral anxiety in a light/dark chamber, acquisition of a T-maze task maintained by food reward, and operant cocaine self-administration. It was hypothesized that intermittent MPH administration would produce behaviors consistent with sensitization (enhanced spontaneous locomotor activity and enhanced cocaine-seeking behavior). By contrast, continuous MPH administration was hypothesized to promote diminished locomotor activity and decreased sensitivity to cocaine reward.

2. Methods

2.1. Subjects

Forty-two male Sprague–Dawley rats (Harlan, Indianapolis, IN) weighing 100–124 g (postnatal day (PND) 32–35) were individually housed and maintained on a 12 h light/dark cycle with constant temperature and humidity and with food and water available *ad libitum*. Following one week of habituation to the vivarium, rats were randomly assigned to one of five conditions: intermittent saline (SAL; $n=8$); low-dose intermittent MPH (IL; $n=8$); high-dose intermittent MPH (IH; $n=8$); low-dose continuous MPH (CL; $n=8$); high-dose continuous MPH (CH; $n=10$). In rodent models, given that the onset of puberty typically occurs around PND 40, adolescence can be differentiated into Early (PND 24–35), Middle (PND 37–48) and Late (PND 50–61; (Spear and Brake, 1983; Adriani et al., 2002). For the current study, drug administration persisted throughout Middle and Late adolescence. All behavioral tests were conducted during the light phase of the light/dark cycle. All procedures were performed in accordance with an approved Institutional Animal Care and Use Committee protocol and in compliance with NIH guidelines.

2.2. Drug administration

Previous research indicates that systemic MPH injections below 5 mg/kg effectively model clinically-relevant doses (Carlezon et al., 2003; Bolanos et al., 2003). The current study is the first to utilize osmotic minipumps as a mechanism to model the sustained-release pharmacokinetics of MPH administration. Because there is no precedent in the literature for establishing dosing parameters, pilot studies were conducted to assess blood MPH concentrations during the fourth week of MPH administration. Rats ($n=4$) were anesthetized with ketamine (80 mg/kg) and xylazine (12 mg/kg) and an Alzet osmotic minipump (Model 2ML4; Durect Corporation, Cupertino, CA) was implanted subdermally in the midscapular region. For pilot testing, minipumps were loaded with 2 ml of 5.6 mg/ml MPH HCl (Sigma, St. Louis, MO) dissolved in sterile physiological saline. Given that the flow rate for pumps = 2.5 µl/h (60 µl/day) over 28 days, the 5.6 mg/ml concentration results in the release of 0.34 mg MPH/day. For a rat weighing 200 g, this translates to 1.68 mg/kg/day. On average, rats weighed approximately 170 g at the time of surgery (2.00 mg/kg/day) and 225 g (1.49 mg/kg/day) on Day 24 post-implantation. At that time, rats were terminally anesthetized with ketamine/xylazine, the rib cage was opened and 1 ml blood was extracted from the cardiac chamber using a 1 cc syringe. MPH blood concentrations from frozen samples were determined by National Medical Services (Willow Grove, PA) using liquid chromatography-mass spectrometry. Results indicate that minipumps loaded with 5.6 mg/ml produced MPH blood concentrations that averaged 5.8 +/− 0.9 ng/ml and ritalinic acid concentrations of 38.3 +/− 5.1 ng/ml during the fourth week of treatment (a lower dose (2.8 mg/ml) fell below the threshold (4 ng/ml) of detecting MPH concentrations in blood).

To establish dose–effect parameters, minipumps were loaded with 2.0 ml of either 5.6 (CL) or 11.2 (CH) mg/ml MPH (Table 1). At the time of surgery, rats weighed an average of 169 mg (CL = 2.01 mg/kg/day; CH = 4.02 mg/kg/day). By the fourth week of treatment, as rats gained body mass (224 g), effective dosages decreased (CL = 1.52 mg/kg/day; CH = 3.04 mg/kg/day). Because behavioral testing was initiated at the beginning of the fourth week of testing, the intermittent doses were selected to approximate the estimated daily cumulative doses of the continuous group (approximately 1.6 (CL) and 3.2 (CH) mg/kg/day) at that time. Thus, IL = 0.8 mg/kg twice

Table 1
Depicts the average daily cumulative dosing for each treatment group as a function of age. Weight of rats is based on averages of all treatment groups, typically representing the beginning of each week. MPH (mg) represents the average daily MPH delivered to each group independent of weight, whereas dose values represent MPH dosage expressed in mg/kg. Asterisk denotes that rats were food-restricted beginning during the third week of treatment prior to T-maze training.

		IL		IH		CL		CH	
Week	Weight (g)	MPH (mg)	Dose (mg/kg)	MPH (mg)	Dose (mg/kg)	MPH (mg)	Dose (mg/kg)	MPH (mg)	Dose (mg/kg)
1	169	0.27	1.6	0.54	3.2	0.34	2.01	0.68	4.02
2	198	0.32	1.6	0.64	3.2	0.34	1.72	0.68	3.44
3	229	0.36	1.6	0.72	3.2	0.34	1.49	0.68	2.99
4*	224	0.36	1.6	0.72	3.2	0.34	1.52	0.68	3.04

daily; IH = 1.6 mg/kg twice daily. All injections were dissolved in physiological saline (1 ml/kg body weight) and delivered 3 h apart, beginning at the onset of the active phase of the diurnal cycle (20:00 h; see Gray et al., 2007).

2.3. Apparatus

The T-maze (91 cm runway with two 51 cm arms) was constructed of wood and painted black. At the beginning each trial, rats were placed in a start box with a removable door that permitted access to the runway. The presence of reward in either arm was obscured by a small barrier that represented the boundary of the goal box at the terminal end of each arm.

Spontaneous locomotor activity was measured in a white open field arena (122 cm × 122 cm). The field contained a grid (each quadrant measured 20.3 cm × 20.3 cm) and line crosses were counted when all four paws crossed from one quadrant to another. Additional dependent variables included rearing bouts, crosses into the center quadrants and time spent grooming.

The light/dark activity chamber (46 cm W × 91 cm L × 46 cm H) was painted white on one half and black on the other. Dependent variables included chamber crosses (defined as all four paws crossing midline), time spent on each side of the chamber and rearing behavior.

Operant chambers (30.5 cm L × 24.1 cm W × 21.0 cm H) are commercially available (ENV-008, Med Associates, St. Albans, VT). Each chamber was equipped with two nose poke holes 10 cm apart, 1.5 cm above floor level. Along the longitudinal axis were four infrared beams, 6 cm apart for detection of locomotor activity. Measurement of behavioral responses and drug delivery was controlled by commercially available infusion pumps (PHM-100), SoftCR software (SOF-721-2) and computer interface (Med PC-IV, SG-65100, DIG-770F, DIG-716B, Med Associates).

2.4. Procedures

Rats exhibit a natural tendency to alternate arm selections in a T-maze in a manner that is thought to model foraging behavior (Deacon and Rawlins, 2006) and is conceptualized as a measure of working memory (Wenk, 2001). We utilized a modified T-maze procedure in order to assess motivation to seek a non-drug reward and to assess the ability of rats to detect a change in contingency. Beginning during the third week of treatment, rats were food-deprived to 85% free-feeding body weight and given access to an appetitive cereal to prevent neophobic responses to reward (note that due to a failure to adequately food restrict one flight of subjects, CL $n = 4$; CH $n = 4$ for T-maze data). At the beginning of the fourth week of treatment, rats were given 90 s for each of 7 trials to explore the maze. Across all 6 days of training, both arms were baited with food reward and alternation behavior was recorded. On the test day (Day 7), only one arm (the less preferred arm from the previous training day to maximize the probability that rats would sample the unbaited arm and detect the change in contingency) was baited across 12 60 s trials. To optimize reward acquisition, this change in contingency would require rats to inhibit any tendency to spontaneously alternate arm selections. Dependent variables included arm selection, latency to arm selection, rewards consumed, and alternation behavior. In addition, the average latencies following rewarded vs. unrewarded trials were compared.

Prior to the culmination of drug treatment, locomotor activity was assessed in an open field arena for 30 min, followed by a 5 min test in a light/dark activity chamber. This test utilizes the natural tendency of rats to avoid brightly lit, open spaces and is an established measure of anxiety in rodents (File et al., 2004).

At the conclusion of MPH treatment, rats were anesthetized (ketamine/xylazine), osmotic minipumps were removed and custom catheters were implanted into the right jugular vein, as previously described (Koeltzow and Vezina, 2005). Rats were given seven days recovery, and catheters were flushed twice daily with heparinized saline. During training (7 days), rats were placed in operant chambers and allowed to self-administer intravenous cocaine (1.0 mg/kg/infusion) maintained on a FR-1 schedule of reinforcement for 1 h. In response to each reinforced nose poke response, a light was activated (CS) for ten seconds, during which additional responses were recorded, but without consequence (time out). Acquisition was defined as consecutive days of at least 4 mg/kg cocaine and at least 75% selectivity for the active versus inactive nose poke hole (4 rats failed to achieve this criterion). On Day 8, rats were subjected to a 1 h extinction session, followed immediately by a priming injection of cocaine (10 mg/kg, i.p.) and testing continued for one additional hour (reinstatement). During both extinction and reinstatement, the CS was presented in response to drug-paired nose-pokes (responding for conditioned reinforcement), but cocaine was not delivered.

2.5. Statistical analysis

Comparisons between treatment conditions were analyzed using one way analysis of variance (ANOVA). For each measure, there were five levels of treatment (SAL, IL, HL, CL and CH), except for self-administration extinction data, in which high and low dose conditions were pooled (between subjects factors = SAL, intermittent and continuous). Repeated measures ANOVA was utilized to assess time course data: latency to arm selection during T-maze training (within-subjects factors = average latency for each training day); locomotor activity counts in the open field (six 5 min intervals). All post-hoc analyses were performed utilizing Fisher's LSD. The level of significance was $p < 0.05$. Statistical analyses were made using SPSS 15.0 for Windows.

3. Results

3.1. T-Maze

The average latency to arm selection across 6 days of T-maze training is depicted in Fig. 1. Repeated measures ANOVA revealed a statistically significant main effect of Training Day ($F_{(5, 135)} = 54.29$, $p < 0.001$) and of Treatment ($F_{(4, 27)} = 4.12$, $p < 0.05$). Post-hoc analyses indicated that IL and IH rats exhibited significantly decreased latencies compared to SAL rats (LSD $p < 0.005$). Analysis of time course data indicate that treatment differences were most pronounced on Day 1 (main effect $F_{(4, 27)} = 2.83$, $p < 0.05$), Day 2 ($F_{(4, 27)} = 3.51$, $p < 0.05$), and Day 3 ($F_{(4, 27)} = 3.26$, $p < 0.05$). ANOVA on the total

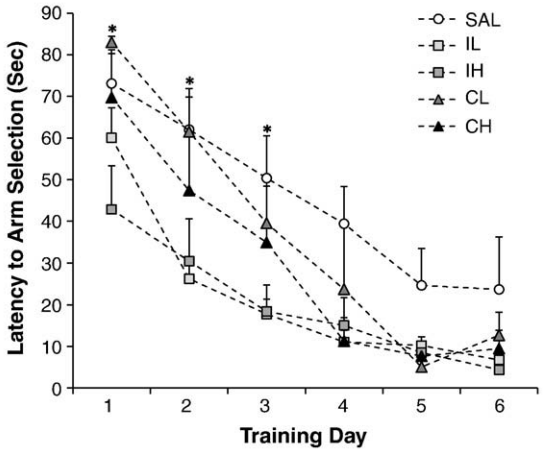

Fig. 1. Depicts the mean latency in seconds to select an arm across 6 days of T-Maze training. Significant main effects of treatment were observed on Days 1, 2 and 3. Asterisks denote statistically significant differences between IH vs. SAL on Day 1 and between IH and IL vs. SAL on Days 2 and 3 (LSD $p < 0.05$). Error bars represent SEM.

number of arm selections during training revealed a statistically significant main effect of treatment ($F_{(4, 27)} = 3.06$, $p<0.05$). Thus, the decreased latencies, particularly during the early portion of training, at least partially reflect an increased number of arm selections among rats in the intermittent treatment groups whereas SAL rats were more likely to have a trial time out prior to selecting an arm of the maze.

To assess the ability of rats to detect a change in contingency and to inhibit any tendency to alternate between arms of the maze, only one arm was baited with food reward across 12 trials on Test Day. Consistent with previous reports, alternation rates observed during training ranged from 60 to 70% (Moustgaard et al., 2008). As depicted in Fig. 2, paired *t*-tests revealed that only rats in the IL, IH and CL MPH conditions exhibited statistically significant decreases in alternation rates during testing. Because rats must sample both arms in order to be exposed to the change in contingency, it was anticipated that rewarded arm selections would be more prevalent during later compared to earlier test trials. Analysis of the number of non-rewarded arm selections during the first 6 trials of testing compared to the last 6 trials indicated that both the IL and CL rats exhibited a significant decrease in errors during the final 6 trials of testing (Fig. 2). These data would seem to indicate that MPH treatment served to enhance adaptive responding during testing. However, one way ANOVAs on latency to arm selection ($F_{(4,27)} = 1.67$, n.s.), reward-appropriate arm selections ($F_{(4,27)} = 0.82$, n.s.), and reward consumption ($F_{(4,27)} = 1.92$, n.s.) failed to identify statistically significant differences among groups (Fig. 3).

Taken together, these results indicate that intermittent MPH administration produced enhanced acquisition of the T-maze task, manifest primarily as more rapid arm selections. Neither MPH administration regimen had a significant impact on reward consumption or on the number of rewarded decisions made in response to the change in contingency on test day, though it's striking that only MPH-treated rats exhibited a decrease in alternation behavior, particularly during the last 6 trials of testing.

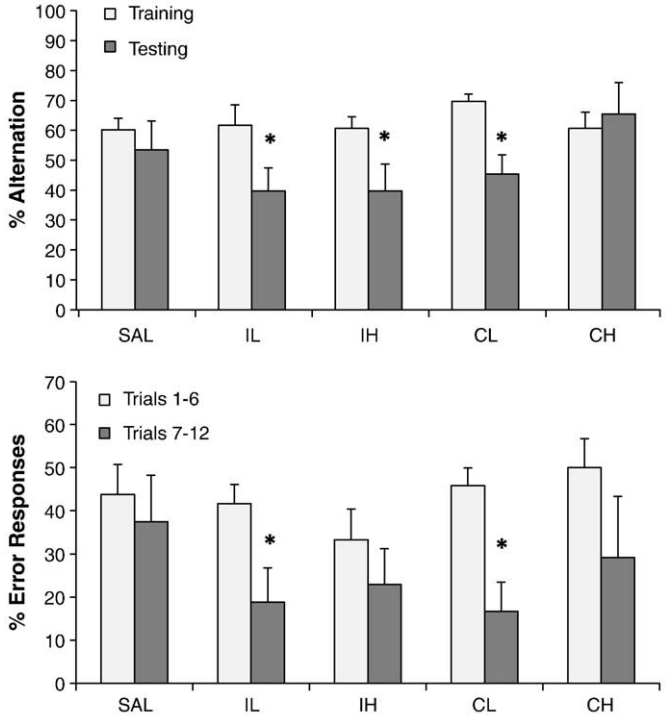

Fig. 2. *Top*: mean alternation behavior expressed as a percentage of total arm selections. *Bottom*: arm selection errors (non-rewarded arm selections) expressed as a percentage of all selections during the first 6 or last 6 trials of T-maze testing. Asterisks indicate statistical significance between training versus Test day. Error bars represent SEM.

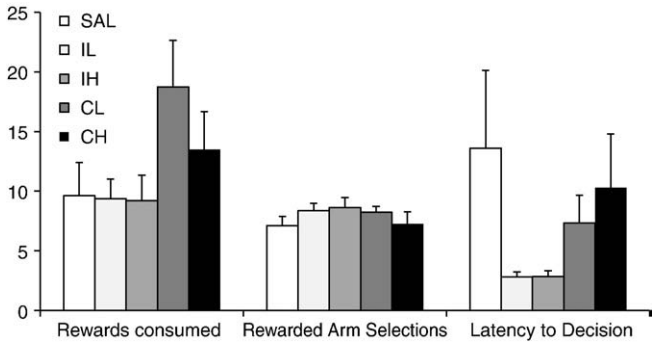

Fig. 3. The mean number of rewards consumed (*left*), mean number of rewarded arm decisions (*middle*) and mean latency to arm selection (*right*) during the T-maze test are depicted. Error bars represent SEM.

3.2. Spontaneous locomotor activity

Forward locomotor activity, defined as grid crosses within the open field, is depicted in Fig. 4. Repeated measures ANOVA revealed a statistically significant main effect of interval ($F_{(5, 185)} = 42.1$, $p<0.001$) and treatment ($F_{(4, 37)} = 3.1$, $p<0.05$), and a statistically significant interval x treatment interaction ($F_{(20, 246)} = 2.4$, $p<0.001$). Post-hoc analysis indicated that IH rats exhibited statistically significantly higher levels of locomotor activity compared to SAL rats (LSD $p<0.05$). By contrast, analysis of time course data indicate that rats from both continuous treatment groups were less active compared to SAL rats during the first 5 min of testing. As depicted in Fig. 5 (*top*), repeated measures ANOVA indicated a similar effect of treatment on rearing behavior ($F_{(4, 37)} = 3.9$, $p<0.01$). Although no statistically significant differences were observed between SAL-treated rats and any of the other treatment conditions, both IL and IH rats exhibited more rearing behavior than either the CL or CH rats (LSD $p<0.05$). Taken together, these data indicate that rats in the intermittent treatment conditions were hyperactive in the open field.

3.3. Behavioral anxiety

A statistically significant main effect of treatment was observed for light/dark chamber crosses ($F_{(4, 37)} = 3.7$, $p<0.05$). Post-hoc analyses indicate that IL (LSD $p<0.05$) and IH (LSD $p<0.005$) rats were more

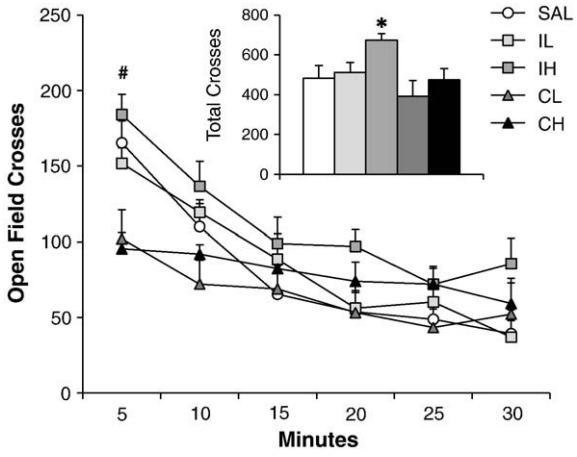

Fig. 4. Depicts the mean locomotor activity counts in 5 min intervals across 30 min of open field testing. Pound (#) denotes that both CL and CH treatment groups exhibited significantly fewer activity crosses during the first 5 min of testing compared to SAL treatment ($p<0.05$). Inset: cumulative locomotor activity counts. Asterisk denotes IH rats exhibited more total activity counts compared to SAL rats ($p<0.05$). Error bars represent SEM.

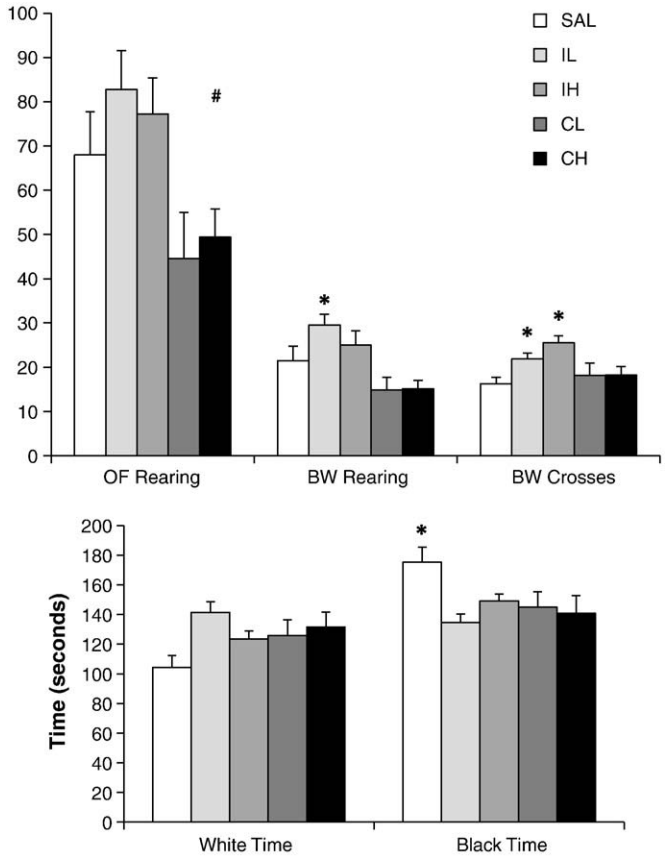

Fig. 5. *Top*: Mean rearing events in both the open field (OF rearing) and light/dark chamber (BW rearing), and the mean number of transitions between black and white compartments. Pound (#) denotes statistically significant differences between intermittent treatment groups compared to either continuous treatment group. Asterisk denotes statistically significantly different from SAL ($p<0.05$). *Bottom*: Mean time spent in the black versus white compartments of the light/dark chamber, expressed in seconds. Asterisk denotes SAL significantly different from IL, CL and CH ($p<0.05$). Error bars represent SEM.

active in the light/dark chamber compared to rats in the SAL condition. As depicted in Fig. 5 (*top*), similar results were observed with regard to rearing in the light/dark chamber ($F_{(4, 37)}=5.6$, $p<0.001$), as IL rats reared significantly more than rats in the SAL condition (LSD $p<0.05$). Analysis of the preference for the light vs. dark compartments of the chambers indicated that only rats in the SAL condition exhibited robust behavioral anxiety (Fig. 5, *bottom*). For example, one way ANOVA on time spent in the dark compartment identified a significant main effect of treatment ($F_{(4, 37)}=2.8$, $p<0.05$), and post-hoc analysis indicated a statistically significant increase in time spent in the dark compartment by SAL rats compared with IL, CL and CH rats (LSD $p<0.05$). In summary, these data indicate that the differences in exploratory locomotor activity exhibited by intermittent versus continuous treatment groups did not result from group differences in behavioral anxiety. Rather, these data seem to indicate that chronic MPH administration, regardless of route of administration, results in an anxiolytic behavioral response to the light/dark chamber.

3.4. Cocaine self-administration

ANOVA on nose poke responses during acquisition (Fig. 6 *top*) revealed a statistically significant main effect of treatment ($F_{(4, 29)}=2.71$, $p<0.05$). In particular, IH executed more drug-paired nose poke responses compared to either group of continuous treatment rats (LSD $p<0.05$). However, the difference between IH and SAL approached, but failed to achieve statistical significance (LSD $p<0.08$). CH rats appeared to exhibit decreased responding compared to SAL-treated rats, though this effect also failed to achieve statistical significance (LSD $p=0.17$). No statistically significant effects of treatment on inactive nose poke responses ($F_{(4, 29)}=0.73$, n.s.) or on locomotor activity ($F_{(4,29)}=1.85$, n.s.) during acquisition were observed (Fig. 6, *middle and bottom*). Extinction and reinstatement data are depicted in Fig. 7. No statistically significant main effects of treatment on drug-paired nose poke responses (*top*) were observed during either extinction ($F_{(4, 29)}=2.24$, $p=0.09$) or reinstatement ($F_{(4,29)}=0.19$, n.s.). However, when data were pooled based on route of administration (SAL=7, intermittent=15, continuous=12), a statistically significant main effect of treatment was observed during extinction ($F_{(2, 31)}=4.79$, $p<0.05$). Consistent with the acquisition data, the treatment effect during extinction resulted from increased drug-paired nose poke responses by rats in the intermittent conditions compared to those in the continuous conditions (LSD $p<0.005$). Analysis of the locomotor responses (*bottom*) failed to identify a statistically significant main effect of treatment during extinction ($F_{(4, 29)}=1.52$, n.s.). During reinstatement, however, such an effect was observed ($F_{(4, 29)}=3.31$, $p<0.05$), resulting from an enhanced locomotor response by both IL and IH rats compared to SAL (LSD $p<0.05$). In summary, the self-administration data indicate that rats treated with intermittent MPH achieved enhanced acquisition of the operant response and increased drug-seeking behavior during extinction compared to either SAL or continuous MPH treatment groups. Although cocaine challenge failed to reinstate drug-paired nose poke responding, the enhanced locomotor response of rats in the intermittent conditions during reinstatement suggests these rats may be more sensitive to the behavioral effects of cocaine compared to rats treated with continuous MPH.

4. Discussion

The purpose of the present study was to examine the behavioral consequences of intermittent versus continuous administration of physiologically relevant concentrations of MPH across 4 weeks of adolescent development in rats. Results indicate that intermittent MPH administration produces hyperactivity across a variety of measures, including exploratory activity in a novel open field and in a light/dark activity chamber, latency to arm selection during a T-maze task, and in response to non-contingent cocaine administration. By contrast, continuous MPH treatment was associated with a hypoactive response to the novel open field, particularly during the first 10 min of testing and a reduced apparent sensitivity to both operant and non-contingent cocaine. Finally, both treatment conditions appeared to attenuate the anxiogenic properties of the light/dark chamber. These data are interpreted to indicate that continuous MPH treatment in rats does not advance hyperlocomotor activity or confer an enhanced vulnerability to addiction.

The present study is the first to utilize osmotic minipumps as a delivery system to model sustained-released MPH pharmacokinetics. Pilot studies demonstrated that the low dose used in the current study was sufficient to produce physiologically-relevant blood MPH concentrations (5.8 ng/ml) 24 days post-implantation. In humans, plasma concentrations of MPH have been reported to peak at 6 ng/ml 6 h after administration of a low dose of sustained-release MPH (18 mg Concerta®), whereas non-time release formulations (20 mg Ritalin®) produce much higher peak drug concentrations (9 ng/ml; (Markowitz et al., 2003). Although we did not measure blood MPH concentrations as a consequence of the high continuous dose, it seems likely that the CH treatment represents a moderate drug dose typical of human treatment conditions (e.g., see Wolraich and Doffing, 2004; Parasrampuria et al., 2007a).

Previous studies of the effects of repeated MPH administration during adolescent development in rats have reported mixed findings

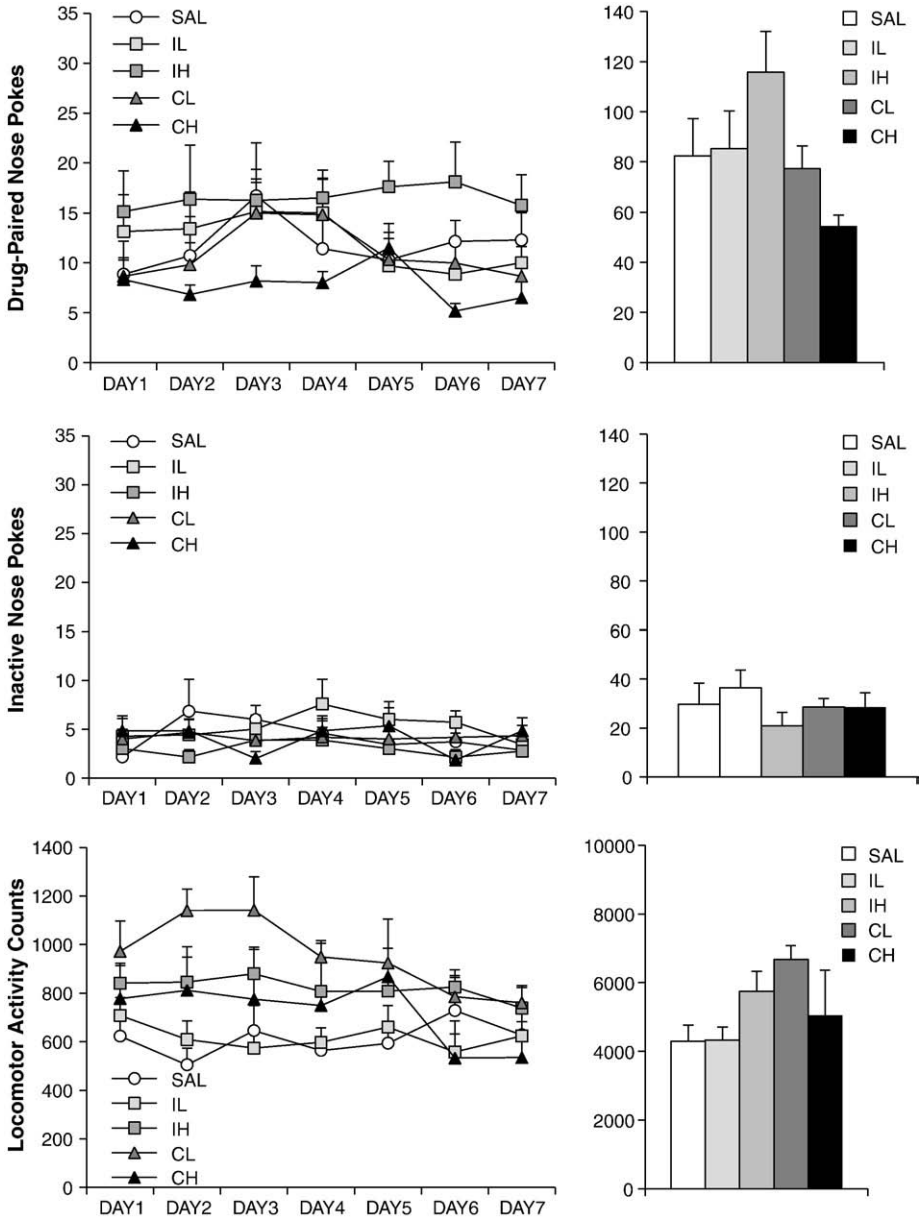

Fig. 6. Mean drug-paired nose poke responses are depicted across 7 days of training (*top*). For purposes of comparison, inactive nose poke responses are depicted utilizing identical axes (*middle*). Mean locomotor activity counts across training day are depicted (*bottom*). Insets represent mean cumulative totals across all 7 days of training. Pound (#) denotes increased nose poke responding by IH compared to both CL and CH rats (LSD $p<0.05$). Error bars represent SEM.

across a number of measures. Although drug dose may play a contributing role to this variability, as dosing has ranged from less than 1.0 mg/kg to as much as 10 mg/kg, the majority of studies have utilized a daily dose of about 2 mg/kg (Mague et al., 2005; Adriani et al., 2006; Augustyniak et al., 2006). In this regard, it is perhaps more likely that variability results from differences in both the timing and duration of treatment. For example, whereas some studies have utilized administration regimens targeting developmental windows as narrow as one week (Brandon et al., 2001), others have sustained treatment for 4 weeks or more (Gray et al., 2007; Valvassori et al., 2007; Thanos et al., 2007; Britton and Bethancourt, 2009). Similarly, whereas at least one study initiated treatment as early as PND 7 (Gray et al., 2007), most studies have targeted either early (Carlezon et al., 2003; Wiley et al., 2009) or middle (Adriani et al., 2006; Augustyniak et al., 2006) adolescence.

In terms of locomotor activity, Valvassori et al. (2007) reported no differences in spontaneous activity after 4 weeks of daily intermittent MPH administration (1, 2 or 10 mg/kg). However, their test duration was limited to 5 min and may not have sufficed to observe the attenuated rates of habituation reported by Carlezon et al., (2003). Analysis of the time course of the locomotor response to the open field is intriguing for several reasons. It is well-established that DA is elevated by novel stimuli (Schultz, 1998) and that individual differences in the locomotor activation elicited by novelty can be linked to variations in DA function (Marinelli and White, 2000; Chefer et al., 2003). Because repeated, intermittent psychostimulant administration has been shown to result in sensitized DA activity (Vezina et al., 2002), it could be expected that intermittent MPH administration would result in a heightened locomotor response to novelty. Interestingly, however, rats treated with intermittent MPH in the current study did not exhibit a robust initial locomotor response in the open field compared to SAL rats. Rather, the elevated response by IH rats predominantly manifested during the second half of testing, suggesting that intermittent MPH disrupted habituation processes.

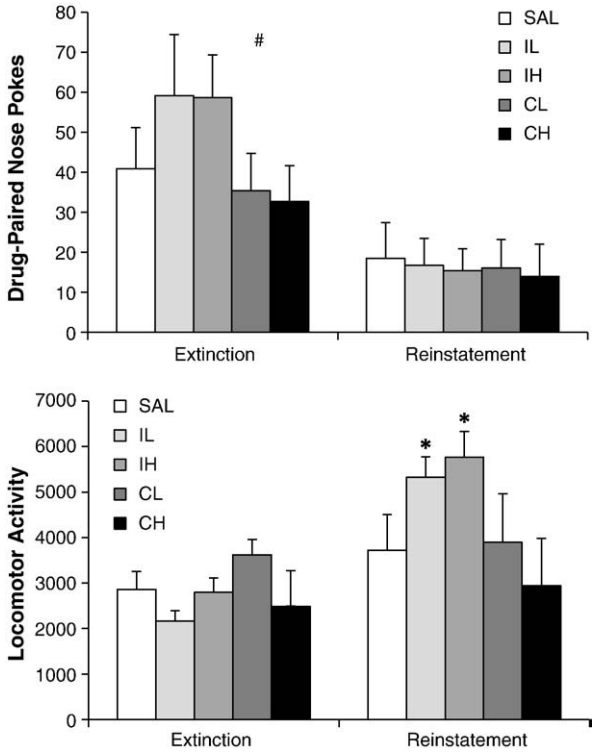

Fig. 7. Means during extinction and reinstatement training are depicted for both drug-paired nose poke responses (*top*) and locomotor activity counts (*bottom*). Pound (#) denotes significance difference between intermittent and continuous treatments when data were pooled ($p<0.05$). Asterisk denotes statistically significantly different from SAL ($p<0.05$). Error bars represent SEM.

Similarly, although the initial locomotor response of CH rats was decreased compared to SAL rats, there was little evidence of habituation exhibited by these rats across the duration of testing. Given that DA transporter knockdown mice have been reported to exhibit substantially impaired locomotor habituation (Zhuang et al., 2001), the current data indicate that both MPH administration protocols utilized in the present study may have disrupted DA transporter activity sufficient to impair habituation processes.

One possible explanation for the differential levels of spontaneous activity in the open field could be related to the anxiogenic properties of this paradigm. However, when behavioral anxiety was explicitly measured in the light/dark chamber, only SAL rats exhibited a robust preference for the dark compartment of the chamber. It therefore seems unlikely that alterations in spontaneous locomotor activity can be explained in terms of variability in the processing of anxiogenic stimuli as a consequence of either repeated MPH administration or as a response to the acute withdrawal thereof. Nevertheless, the decreased expression of behavioral anxiety in the light/dark chamber is consistent with the reported effects of acute MPH on the elevated plus maze (Gray et al., 2007). However, one recent report indicates that a long-term consequence of adolescent, intermittent MPH administration (2 mg/kg/day) is an enhanced reactivity to anxiety- and stress-provoking stimuli in adulthood (Wiley et al., 2009) whereas another identified no substantive long-term effects following 4 weeks of treatment (Britton and Bethancourt, 2009). It is unclear whether continuous infusion protocols can mediate this effect, and is an appropriate question for further research.

Previous studies that assessed the long-term consequences of intermittent MPH administration during adolescence have reported both facilitation (Adriani et al., 2007) and impairment (Crawford et al., 2007) on behavioral tasks maintained by food, and the reasons for these discrepant findings are not obvious. The modified T-maze task was intended to assess the sensitivity of treated rats to a non-drug reward condition, but also to measure the ability of rats to detect a change in contingency as individuals with ADHD exhibit deficits across a number of measures of executive function, including those sensitive to working memory (Schmitz et al., 2002). The ability to effectively adjust to the change in reward availability during the T-maze test putatively required an ability to maintain the contingency change in memory as a guide to behavior. During training, intermittent MPH produced reduced latencies to arm selection without increased reward consumption, suggesting that this effect occurred secondary to diminished behavioral anxiety or hyperactivity. On test day, intermittent MPH rats better inhibited alternation behavior and exhibited a trend towards enhanced reward-appropriate arm selections. At the very least, neither MPH treatment condition resulted in substantive impairment on this task. This is in contrast to a recent report showing impaired object recognition following chronic oral MPH administration during adolescence (LeBlanc-Duchin and Taukulis, 2007). Given the hallmark features of inattentiveness and impulsivity among individuals with ADHD, additional characterization of the consequences of adolescent MPH on measures of impulsivity and working memory are warranted.

Previous studies have reported mixed effects of repeated MPH during adolescence on the subsequent response to drugs of abuse. Studies reporting sensitization to the locomotor-activating effects of cocaine (Adriani et al., 2006; Brandon et al., 2001) appear at odds with reports of reduced sensitivity to cocaine on intracranial self-stimulation thresholds and aversive effects in the conditioned place preference paradigm (Andersen et al., 2002; Mague et al., 2005). Consistent with the findings of Brandon et al. (2001), we found that IH rats exhibited increased operant cocaine self-administration across training compared to SAL rats, and exhibited an elevated (sensitized) locomotor response to the cocaine priming injection during reinstatement testing. Nevertheless, the failure of cocaine priming to reinstate extinguished cocaine-seeking behavior prohibits definitive conclusions regarding the potential consequences of adolescent MPH administration on cocaine-induced drug-seeking behavior. First, the dose of cocaine (1 mg/kg/infusion) was too high to yield high rates of operant self-administration during acquisition, as rats in all treatment conditions typically failed to perform more than 20 drug-paired nose poke responses per day. Consequently, extinction training may have been sufficient to render the challenge dose of cocaine (10 mg/kg) too low to promote robust reinstatement, even in SAL-treated rats. Moreover, increased self-administration behavior during training could be construed to represent a diminished sensitivity to cocaine. Dose–effect curves for cocaine self-administration would be useful in addressing this question. Nevertheless, the relative insensitivity to continuous MPH-treated rats to cocaine across both acquisition and reinstatement indicates that this treatment protocol does not advance a pronounced vulnerability to addiction, consistent with reports on the subjective effects of sustained-release MPH in humans (Parasrampuria et al., 2007b).

The current study utilized a relatively unusual extinction/reinstatement paradigm (for review, see Shalev et al., 2002). More commonly, a within-session design is utilized, whereby self-administration, extinction and reinstatement take place within a single session. This design is ideal for repeated measures of reinstatement, though it is difficult to rule out the effects of residual drug intake. Alternatively, between-sessions designs are typically characterized by repeated daily extinction sessions that persist until drug-paired responding falls reliably under a certain threshold. While this method is perhaps best suited to the identification of stimuli capable of eliciting robust reinstatement, it is relatively labor intensive. The hybrid between/within-session paradigm used presently was selected because it may allow the most direct comparison of reinstatement after varying periods of withdrawal, including those persisting well into adulthood. However, the failure of cocaine to reinstate drug-paired nose poke responses in the current

study, even among SAL-treated rats, likely limits the extrapolation of the current findings to those utilizing more protracted withdrawal time points.

Several substantive methodological considerations are worth noting. First, one limitation of the continuous treatment regimen used presently is that drug is delivered across the 24 h circadian cycle whereas human dosing typically occurs only during waking hours. Unfortunately, we had no quantitative mechanism by which to assess the potential extent to which either treatment regimen disrupted circadian rhythmicity. This is not a trivial concern, as previous studies have reported that intermittent MPH administration produces diurnal disruptions in both rats (Algahim et al., 2009) and humans (Corkum et al., 2008). Moreover, emerging evidence indicates that psychostimulants powerfully interact with circadian genes, perhaps substantively contributing to the addiction process (McClung et al., 2005; Lynch et al., 2008). In the present study, it is possible that the attenuated locomotor response by rats in the continuous condition resulted from chronic disruptions of the diurnal cycle. However, the relative insensitivity of continuous MPH rats to both the rewarding and locomotor-activating effects of cocaine indicates that this potential disruption did not facilitate cocaine sensitivity.

Second, whereas daily dosing delivered via osmotic minipumps varied as a function of body weight, dosing in the intermittent conditions did not, as depicted in Table 1. That is, in the intermittent conditions, the volume of drug delivery increased commensurate with body weight. Functionally, given the sharp increase in body weight over the course of adolescent development, continuous treatment rats were exposed to more total drug compared to intermittent rats, particularly during the first week of treatment. Thus, differences among rats in the intermittent versus continuous treatment conditions could potentially have resulted from differences in cumulative dosing across the duration of treatment. Additionally, the current study did not utilize a saline control for the continuous treatment condition. Consequently, because SAL rats in the current study were handled twice daily for injection delivery, it's possible that the observed effects in the continuous treatment conditions result partly from an absence of daily handling or injection stress. Given the established ability of either stress or enrichment to modulate DA in reward-relevant brain areas (Segovia et al., 2009), incorporating a sham control condition is an important consideration for future studies. Nevertheless, studies generally indicate that isolation results in increased, rather than decreased responses to cocaine or novelty (Solinas et al., 2009), as was observed among continuous treatment groups in the present study.

Another feature of the current experimental design is that behavioral testing was performed during the inactive phase of the diurnal cycle whereas intermittent drug administration was delivered at the onset of the active phase. Although this was intended to mitigate the impact of acute MPH treatment on spontaneous activity, it introduced the possibility that rats in the intermittent condition would be experiencing acute withdrawal at the time of testing. In this regard, it is interesting to note that the spontaneous activity of rats in these conditions did not resemble the crash phase of psychostimulant withdrawal (Koeltzow and White, 2003). Instead, IL and IH rats routinely exhibited elevated activity responses.

In conclusion, the purpose of the present study was to assess the behavioral consequences of intermittent versus continuous MPH administration during adolescence in rats. Whereas intermittent MPH was associated with hyperactivity and an increased sensitivity to the effects of cocaine, rats in the continuous MPH condition did not exhibit these effects. Collectively, these data indicate that sustained-release formulations of MPH used in the treatment of ADHD may yield fewer problematic effects compared to the immediate release formulations. Future research, however, must clarify the potential long-term consequences of these treatment conditions, and it will be important to extend the present findings to rat models natively characterized by hyperactivity and/or impulsivity.

Acknowledgements

This work was supported by Undergraduate Research Grants from Psi Chi (RG and WW) and by Bradley University Special Emphasis and Research Excellence Awards (TEK). Thanks to Dianna K. Taylor, Leora Gomez, Nina L. Tiberi and Marybeth Southard for excellent technical support.

References

Achat-Mendes C, Anderson KL, Itzhak Y. Methylphenidate and MDMA adolescent exposure in mice: long-lasting consequences on cocaine-induced reward and psychomotor stimulation in adulthood. Neuropharmacology 2003;45:106–15.
Adriani W, Canese R, Podo F, Laviola G. 1H MRS-detectable metabolic brain changes and reduced impulsive behavior in adult rats exposed to methylphenidate during adolescence. Neurotoxicol Teratol 2007;29:116–25.
Adriani W, Leo D, Greco D, Rea M, Di PU, 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.
Adriani W, Macri S, Pacifici R, Laviola G. Peculiar vulnerability to nicotine oral self-administration in mice during early adolescence. Neuropsychopharmacology 2002;27:212–24.
Algahim MF, Yang PB, Wilcox VT, Burau KD, Swann AC, Dafny N. Prolonged methylphenidate treatment alters the behavioral diurnal activity pattern of adult male Sprague–Dawley rats. Pharmacol Biochem Behav 2009;92:93–9.
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.
Augustyniak PN, Kourrich S, Rezazadeh SM, Stewart J, Arvanitogiannis A. Differential behavioral and neurochemical effects of cocaine after early exposure to methylphenidate in an animal model of attention deficit hyperactivity disorder. Behav Brain Res 2006;167:379–82.
Bolanos 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.
Botly LC, Burton CL, Rizos Z, Fletcher PJ. Characterization of methylphenidate self-administration and reinstatement in the rat. Psychopharmacology (Berl) 2008;199:55–66.
Brandon CL, Marinelli M, Baker LK, White FJ. Enhanced reactivity and vulnerability to cocaine following mehtylphenidate treatment in adolescent rats. Neuropsychopharmacology 2001;25:651–61.
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.
Carlezon Jr WA, Mague SD, Andersen SL. Enduring behavioral effects of early exposure to methylphenidate in rats. Biol Psychiatry 2003;54:1330–7.
Chefer VI, Zakharova I, Shippenberg TS. Enhanced responsiveness to novelty and cocaine is associated with decreased basal dopamine uptake and release in the nucleus accumbens: quantitative microdialysis in rats under transient conditions. J Neurosci 2003;23:3076–84.
Corkum P, Panton R, Ironside S, Macpherson M, Williams T. Acute impact of immediate release methylphenidate administered three times a day on sleep in children with attention-deficit/hyperactivity disorder. J Pediatr Psychol 2008;33:368–79.
Crawford CA, Villafranca SW, Cyr MC, Farley CM, Reichel CM, Gheorghe SL, et al. 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.
Darredeau C, Barrett SP, Jardin B, Pihl RO. Patterns and predictors of medication compliance, diversion, and misuse in adult prescribed methylphenidate users. Hum Psychopharmacol 2007.
Deacon RM, Rawlins JN. T-maze alternation in the rodent. Nat Protoc 2006;1:7-12.
Faraone SV. Using meta-analysis to compare the efficacy of medications for attention-deficit/hyperactivity disorder in youths. P. T. 2009;34:678–94.
Faraone SV, Wilens T. Does stimulant treatment lead to substance use disorders? J Clin Psychiatry 2003;64(Suppl 11):9-13.
File SE, Lippa AS, Beer B, Lippa MT. Animal tests of anxiety. Curr Protoc Neurosci 2004 Chapter 8: Unit.
Gray JD, Punsoni M, Tabori NE, Melton JT, Fanslow V, Ward MJ, et al. Methylphenidate administration to juvenile rats alters brain areas involved in cognition, motivated behaviors, appetite, and stress. J Neurosci 2007;27:7196–207.
Greenhill LL, Pliszka S, Dulcan MK, Bernet W, Arnold V, Beitchman J, et al. Practice parameter for the use of stimulant medications in the treatment of children, adolescents, and adults. J Am Acad Child Adolesc Psychiatry 2002;41:26S–49S.
Jensen PS. Current concepts and controversies in the diagnosis and treatment of attention deficit hyperactivity disorder. Curr Psychiatry Rep 2000;2:102–9.
King GR, Kuhn C, Ellinwood Jr EH. Dopamine efflux during withdrawal from continuous or intermittent cocaine. Psychopharmacology (Berl) 1993;111:179–84.
Koeltzow TE, Vezina P. Locomotor activity and cocaine-seeking behavior during acquisition and reinstatement of operant self-administration behavior in rats. Behav Brain Res 2005;160:250–9.
Koeltzow TE, White FJ. Behavioral depression during cocaine withdrawal is associated with decreased spontaneous activity of ventral tegmental area dopamine neurons. Behav Neurosci 2003;117:860–5.

Kollins SH. ADHD, substance use disorders, and psychostimulant treatment: current literature and treatment guidelines. J Atten Disord 2008;12:115–25.
Kuczenski R, Segal DS. Locomotor effects of acute and repeated threshold doses of amphetamine and methylphenidate: relative roles of dopamine and norepinephrine. J Pharmacol Exp Ther 2001;296:876–83.
LeBlanc-Duchin D, Taukulis HK. Chronic oral methylphenidate administration to periadolescent rats yields prolonged impairment of memory for objects. Neurobiol Learn Mem 2007;88:312–20.
Lynch WJ, Girgenti MJ, Breslin FJ, Newton SS, Taylor JR. Gene profiling the response to repeated cocaine self-administration in dorsal striatum: a focus on circadian genes. Brain Res 2008;1213:166–77.
Mague SD, Andersen SL, Carlezon Jr WA. Early developmental exposure to methylphenidate reduces cocaine-induced potentiation of brain stimulation reward in rats. Biol Psychiatry 2005;57:120–5.
Mannuzza S, Klein RG, Truong NL, Moulton III JL, Roizen ER, Howell KH, et al. Age of methylphenidate treatment initiation in children with ADHD and later substance abuse: prospective follow-up into adulthood. Am J Psychiatry 2008;165:604–9.
Marinelli M, White FJ. Enhanced vulnerability to cocaine self-administration is associated with elevated impulse activity of midbrain dopamine neurons. J Neurosci 2000;20:8876–85.
Markowitz JS, Straughn AB, Patrick KS, Devane CL, Pestreich L, Lee J, et al. Pharmacokinetics of methylphenidate after oral administration of two modified-release formulations in healthy adults. Clin Pharmacokinet 2003;42:393–401.
McClung CA, Sidiropoulou K, Vitaterna M, Takahashi JS, White FJ, Cooper DC, et al. Regulation of dopaminergic transmission and cocaine reward by the Clock gene. Proc Natl Acad Sci USA 2005;102:9377–81.
McFadyen MP, Brown RE, Carrey N. Subchronic methylphenidate administration has no effect on locomotion, emotional behavior, or water maze learning in prepubertal mice. Dev Psychobiol 2002;41:123–32.
Moustgaard A, Hau J, Lind NM. Effects of dopamine D4 receptor antagonist on spontaneous alternation in rats. Behav Brain Funct 2008;4:49.
Parasrampuria DA, Schoedel KA, Schuller R, Gu J, Ciccone P, Silber SA, et al. Assessment of pharmacokinetics and pharmacodynamic effects related to abuse potential of a unique oral osmotic-controlled extended-release methylphenidate formulation in humans. J Clin Pharmacol 2007a;47:1476–88.
Parasrampuria DA, Schoedel KA, Schuller R, Silber SA, Ciccone PE, Gu J, et al. Do formulation differences alter abuse liability of methylphenidate? A placebo-controlled, randomized, double-blind, crossover study in recreational drug users. J Clin Psychopharmacol 2007b;27:459–67.
Paulson PE, Camp DM, Robinson TE. Time course of transient behavioral depression and persistent behavioral sensitization in relation to regional brain monoamine concentrations during amphetamine withdrawal in rats. Psychopharmacology (Berl) 1991;103:480–92.
Pelham WE, Gnagy EM, Burrows-Maclean L, Williams A, Fabiano GA, Morrisey SM, et al. Once-a-day Concerta methylphenidate versus three-times-daily methylphenidate in laboratory and natural settings. Pediatrics 2001;107:E105.
Poulin C. From attention-deficit/hyperactivity disorder to medical stimulant use to the diversion of prescribed stimulants to non-medical stimulant use: connecting the dots. Addiction 2007;102:740–51.
Robinson TE, Berridge KC. Incentive-sensitization and addiction. Addiction 2001;96: 103–14.
Schmitz M, Cadore L, Paczko M, Kipper L, Chaves M, Rohde LA, et al. Neuropsychological performance in DSM-IV ADHD subtypes: an exploratory study with untreated adolescents. Can J Psychiatry 2002;47:863–9.
Schubiner H. Substance abuse in patients with attention-deficit hyperactivity disorder: therapeutic implications. CNS Drugs 2005;19:643–55.
Schultz W. Predictive reward signal of dopamine neurons. J Neurophysiol 1998;80: 1-27.
Segovia G, Del AA, Mora F. Environmental enrichment, prefrontal cortex, stress, and aging of the brain. J Neural Transm 2009;116:1007–16.
Sellings LH, McQuade LE, Clarke PB. Characterization of dopamine-dependent rewarding and locomotor stimulant effects of intravenously-administered methylphenidate in rats. Neuroscience 2006;141:1457–68.
Shalev U, Grimm JW, Shaham Y. Neurobiology of relapse to heroin and cocaine seeking: a review. Pharmacol Rev 2002;54:1-42.
Solinas M, Thiriet N, El RR, Lardeux V, Jaber M. Environmental enrichment during early stages of life reduces the behavioral, neurochemical, and molecular effects of cocaine. Neuropsychopharmacology 2009;34:1102–11.
Spear LP, Brake SC. Periadolescence: age-dependent behavior and psychopharmacological responsivity in rats. Dev Psychobiol 1983;16:83-109.
Thanos PK, Michaelides M, Benveniste H, Wang GJ, Volkow ND. Effects of chronic oral methylphenidate on cocaine self-administration and striatal dopamine D2 receptors in rodents. Pharmacol Biochem Behav 2007;87:426–33.
Valvassori SS, Frey BN, Martins MR, Reus GZ, Schimidtz F, Inacio CG, et al. Sensitization and cross-sensitization after chronic treatment with methylphenidate in adolescent Wistar rats. Behav Pharmacol 2007;18:205–12.
Vanderschuren LJ, Schoffelmeer AN, Mulder AH, De Vries TJ. Dopaminergic mechanisms mediating the long-term expression of locomotor sensitization following pre-exposure to morphine or amphetamine. Psychopharmacology (Berl) 1999;143: 244–53.
Vezina P, Lorrain DS, Arnold GM, Austin JD, Suto N. Sensitization of midbrain dopamine neuron reactivity promotes the pursuit of amphetamine. J Neurosci 2002;22: 4654–62.
Volkow ND, Wang GJ, Fowler JS, Ding YS. Imaging the effects of methylphenidate on brain dopamine: new model on its therapeutic actions for attention-deficit/hyperactivity disorder. Biol Psychiatry 2005;57:1410–5.
Wenk GL. Assessment of spatial memory using the T maze. Curr Protoc Neurosci 2001 Chapter 8: Unit.
Wiley MD, Poveromo LB, Antapasis J, Herrera CM, Bolanos Guzman CA. Kappa-opioid system regulates the long-lasting behavioral adaptations induced by early-life exposure to methylphenidate. Neuropsychopharmacology 2009;34:1339–50.
Wolraich ML, Doffing MA. Pharmacokinetic considerations in the treatment of attention-deficit hyperactivity disorder with methylphenidate. CNS Drugs 2004;18:243–50.
Yang PB, Swann AC, Dafny N. Chronic methylphenidate modulates locomotor activity and sensory evoked responses in the VTA and NAc of freely behaving rats. Neuropharmacology 2006;51:546–56.
Zhuang X, Oosting RS, Jones SR, Gainetdinov RR, Miller GW, Caron MG, et al. Hyperactivity and impaired response habituation in hyperdopaminergic mice. Proc Natl Acad Sci USA 2001;98:1982–7.