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Nicotinic Receptors Differentially Modulate the Induction and Expression of Behavioral Sensitization to Methylphenidate in Rats

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Abstract

Rationale—Nicotinic acetylcholine receptors (nAChRs) regulate sensitization to stimulant drugs such as *d*-amphetamine and cocaine.

Objectives—The current study determined if nAChRs modulate the induction and/or expression of behavioral sensitization to high methylphenidate doses.

Methods—In Experiment 1, rats received saline or mecamylamine (3 mg/kg, sc), followed by saline or methylphenidate (5.6 or 10 mg/kg, sc) during 10 daily sessions; the effect of methylphenidate (1–17 mg/kg, sc) alone was determined 14 days later. In Experiment 2, rats received saline or dihydro- β -erythroidine (DH β E; 3 mg/kg, sc), followed by saline or 5.6 mg/kg of methylphenidate. In Experiment 3, rats received saline or methylphenidate (5.6 or 10 mg/kg, sc) alone for 10 days; the effect of acute mecamylamine (3 mg/kg, sc) on the response to methylphenidate (1–17 mg/kg, sc) was determined 14 days later. Locomotor activity, sniffing, rearing, grooming and stereotypy ratings were dependent measures.

Results—Methylphenidate produced dose-dependent increases in locomotor activity, sniffing and stereotypy on Day 1 and these effects were enhanced on Day 10, indicative of sensitization. Mecamylamine attenuated methylphenidate-induced stereotypy only on Day 1, but reduced locomotor activity, sniffing, rearing and stereotypy on Day 10 and during the methylphenidate challenge phase; similar results were obtained with DH β E. However, acute mecamylamine did not alter the effects of the methylphenidate challenge following the induction of sensitization to methylphenidate alone.

Conclusions—Although nAChRs do not appear to regulate the expression of methylphenidate-induced behavioral sensitization, inhibition of high affinity β 2 subunit nAChRs attenuates the induction of behavioral sensitization to high doses of methylphenidate.

Keywords

methylphenidate; mecamylamine; DH β E; rat; locomotor activity; sensitization; antagonist; nicotinic receptor

Introduction

The behavioral effects of stimulant drugs such as *d*-amphetamine and cocaine are mediated in large part by monoamine and amino acid neurotransmitters. However, there is also evidence that acetylcholine (ACh) is involved (Smith et al. 2004; Williams and Adinoff 2007). Nicotinic ACh receptor (nAChR) antagonists, including the nonselective, noncompetitive nAChR ion

channel blocker mecamylamine (Papke et al. 2001) and the high-affinity $\beta 2$ subunit-containing competitive nAChR antagonist dihydro- β -erythroidine DH β E; (Whiteaker et al. 2000), attenuate the induction of locomotor sensitization to *d*-amphetamine, cocaine and ephedrine in mice and rats (Karler et al. 1996; Miller and Segert 2005; Schoffelmeer et al. 2002). Knockout mice lacking the $\beta 2$ nAChR subunit show less cocaine-conditioned place preference than wild-type mice (Zachariou et al. 2001). Cocaine self-administration also increases ACh turnover rates in various mesocorticolimbic nuclei in rats (Smith et al. 2004), while mecamylamine reduces cocaine self-administration acutely (Blokhina et al. 2005; Levin et al. 2000) and prevents the escalation of cocaine self-administration during extended access with repeated administration (Hansen and Mark 2007). These findings indicate that nAChRs are involved in the abuse-related behavioral effects of stimulant drugs in preclinical animal models of drug abuse.

Studies examining interactions between nAChRs and stimulant drugs have focused primarily on *d*-amphetamine and cocaine; thus, it is unclear whether such findings generalize to other stimulants. Methylphenidate is a dopamine (DA) and norepinephrine reuptake inhibitor that produces prototypical stimulant-like drug effects, but is perhaps best known for its prominent role in the treatment of attention-deficit/hyperactivity disorder (ADHD; Solanto et al. 2001). The purpose of the present investigation was to determine if mecamylamine and DH β E would alter the induction of behavioral sensitization to repeated methylphenidate (Experiments 1 and 2, respectively), and/or the expression of methylphenidate-induced sensitization in rats treated previously with methylphenidate alone (Experiment 3). In these experiments, relatively high doses of methylphenidate were used because we sought to examine the abuse-related effects, rather than therapeutic effects, of methylphenidate.

Methods and Materials

Animals

A total of 128 adult male Sprague-Dawley rats (Harlan Industries, Indianapolis, IN) was used. Rats were housed 2 per cage in standard plastic cages in a temperature- and humidity-controlled facility set to a 14:10 hr light/dark cycle (lights on at 0600 hr). Rats were handled and acclimated to the colony for 1 week prior to the beginning of the experiment, which was conducted during the light phase. Rats had *ad libitum* access to food and water while in the home cage. Experimental protocols were in accordance with the 1996 NIH Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee at the University of Kentucky.

Apparatus

Locomotor activity was recorded using an automated Digiscan animal activity monitoring system (AccuScan Instruments, Columbus, OH). The system consisted of 12 clear chambers (42 $\times$ 42 $\times$ 30 cm). The outer surface of each side and rear wall chamber was made opaque by attaching sheets of white plastic to its outer surface; however, no plastic was attached to the front wall in order to permit behavioral assessment. Each chamber incorporated a horizontal 16 $\times$ 16 grid of photo beam sensors connected to a personal computer operating Digipro System software (v. 1.40, AccuScan Instruments); each beam was spaced 2.5 cm apart and 7.0 cm above the chamber floor. Horizontal activity was measured as photo beam interruptions and expressed as distance traveled (cm).

Experiment 1: Effect of Mecamylamine on Induction of Behavioral Sensitization to Repeated Methylphenidate

Rats ($n=48$) were assigned randomly to a 2 $\times$ 3 factorial design in which each rat received pretreatment with mecamylamine (3 mg/kg, sc) or saline 15 min prior to saline or

methylphenidate (5.6 or 10 mg/kg, sc) across 10 repeated days ($n=8$ per group). The mecamylamine dose was chosen based on a previous report (Schoffeleer et al. 2001) and the methylphenidate doses were chosen based on our previous work (Wooters et al. 2006).

On the day immediately prior to the start of drug treatment, each rat was placed individually in an activity monitor for a 1-hr habituation session; no injections were given on this day. Over the following 10 consecutive days (Days 1 - 10), rats received their respective pretreatment 15 min prior to administering methylphenidate or saline; locomotor activity was then monitored for 60 min immediately following the second injection. In addition, on Day 1 and Day 10, a blind observer scored the total bouts of sniffing, rearing and grooming: (1) sniffing was defined as flaring of the nostrils with simultaneous movement of vibrissae; (2) rearing was defined as lifting both front paws off of the floor for at least 1 sec; and (3) grooming was defined as repetitive movements of the front paws around the snout and face. Each of these measures was scored for each rat during 3 separate 1-min intervals beginning 12, 30 and 48 min after the start of the session; the total number of observations for each measure was then summed together across the 3 observation periods. In addition, a stereotypy rating was also scored for each rat during the final observation period on Day 1 and Day 10, using a 5-point rating scale. The rating scale was as follows: (0) asleep or inactive; (1) normal locomotor activity; (2) locomotor activity combined with brief bursts of stereotyped sniffing, rearing or head movement; (3) brief bursts of stereotyped sniffing, rearing or head movement without locomotor activity; and (4) continuous stereotyped sniffing, rearing or head movement without locomotor activity.

Once the 10-day repeated treatment phase was completed, all rats remained undisturbed in the home cage for a 14-day drug-free period (Days 11 - 24). Beginning on Day 25, saline and a series of incrementing methylphenidate challenge doses were administered across days to examine the expression of behavioral sensitization using an ascending-dose protocol modified from previous work (Kalinichev et al. 2004). First, on Day 25, all rats were challenged with saline to assess for conditioned hyperactivity, and on Days 26-30, rats were challenged each day with one of a series of progressively increasing doses of methylphenidate (1, 3, 5.6, 10 and 17 mg/kg, sc, respectively); no pretreatment injections were administered. On each of the saline and methylphenidate challenge days, locomotor activity was assessed for 60 min immediately following each challenge injection. In addition, sniffing, rearing, grooming and stereotypy scores were also determined using the same procedure as described for Days 1 and 10.

Experiment 2: Effect of DH β E on Induction of Behavioral Sensitization to Repeated Methylphenidate

A separate group of rats ($n=32$) was assigned randomly to a 2×2 factorial design in which each rat received pretreatment with DH β E (3 mg/kg, sc) or saline 15 min prior to 5.6 mg/kg of methylphenidate or saline ($n=8$ per group). The DH β E dose was chosen based on a previous report (Schoffeleer et al. 2001), and the methylphenidate dose was selected based on the results of Experiment 1. All other procedures, including the 10-day repeated drug treatment phase, the 14-day drug-free period and the subsequent saline and methylphenidate (1-17 mg/kg) challenge dose phase, were similar to those described for Experiment 1.

Experiment 3: Effect of Mecamylamine on Expression of Behavioral Sensitization to Repeated Methylphenidate

A separate group of rats ($n=48$) was assigned randomly to receive saline, 5.6 mg/kg of methylphenidate or 10 mg/kg of methylphenidate ($n=16$ per group) alone for 10 consecutive locomotor test days (Days 1-10). Once the 10-day repeated treatment phase was completed, each rat remained undisturbed in the home cage for a 14-day drug-free period (Days 11 - 24), and then was assessed for sensitization to methylphenidate using the ascending-dose protocol described previously (Days 25-30). Half of the rats from each treatment group were assigned

randomly to receive pretreatment with 3 mg/kg of mecamlamine ($n=8$) 15 min prior to the methylphenidate challenge doses, whereas the remaining rats from each treatment group received pretreatment with saline ($n=8$). Locomotor activity was monitored for 60 min during each session, and the other dependent measures were assessed during each day of the methylphenidate challenge phase.

Drugs

Methylphenidate HCl (Mallinckrodt, St. Louis, MO), DH β E (Tocris Bioscience, Ellisville, MO) and mecamlamine HCl (Sigma, St. Louis, MO) were prepared in 0.9% NaCl (saline) and administered subcutaneously in a volume of 1 ml/kg of body weight. All drug doses represent the salt weights.

Data Analysis

For each dependent measure, separate analyses of variance (ANOVA) for repeated measures were used to assess group differences. Mecamlamine or DH β E pretreatment and methylphenidate dose were between-subject variables and time and/or day were within-subject variables. Planned post hoc comparisons (Tukey's HSD or Dunnett's test) were conducted when ANOVA revealed significant main effects or interactions. Results were deemed significant at $P<0.05$.

Results

Experiment 1: Effect of Mecamlamine on Induction of Behavioral Sensitization to Repeated Methylphenidate

For locomotor activity on Day 1 and Day 10, ANOVA revealed a significant time $\times$ day $\times$ pretreatment $\times$ treatment interaction [$F(27,462)=9.88$, $P<0.001$]. On Day 1 (Fig. 1a), 5.6 and 10 mg/kg of methylphenidate produced significantly greater activity than saline alone during the latter portion of the session; mecamlamine had no significant effect given alone or in combination with methylphenidate. On Day 10 (Fig. 1b), 5.6 and 10 mg/kg of methylphenidate alone produced significantly greater activity than saline alone, with the onset of hyperactivity occurring earlier in the session following 10 mg/kg of methylphenidate than following 5.6 mg/kg of methylphenidate. Each methylphenidate dose produced greater activity on Day 10 than on Day 1, an effect indicative of sensitization. Mecamlamine attenuated the effects of both 5.6 and 10 mg/kg of methylphenidate, with the attenuation occurring early in the session following 10 mg/kg of methylphenidate and later in the session following 5.6 mg/kg of methylphenidate.

The effects of methylphenidate on the sniffing, rearing, grooming and stereotypy measures during Days 1 and 10 in saline- or mecamlamine-pretreated rats are presented in Table 1. For sniffing, ANOVA revealed significant day $\times$ pretreatment [$F(1,42)=6.82$, $P<0.05$] and day $\times$ treatment [$F(2,42)=31.09$, $P<0.001$] interactions. On Day 1, 10 mg/kg of methylphenidate produced significantly greater sniffing than saline in each pretreatment group. On Day 10, 5.6 and 10 mg/kg of methylphenidate produced significantly greater sniffing than saline in each pretreatment group; however, mecamlamine attenuated the effect of methylphenidate. For rearing, ANOVA revealed significant day $\times$ pretreatment [$F(1,42)=10.08$, $P<0.001$] and day $\times$ treatment [$F(2,42)=26.64$, $P<0.001$] interactions. On Day 10, 5.6 and 10 mg/kg of methylphenidate produced significantly greater rearing than saline or mecamlamine alone; however, mecamlamine attenuated the effect of methylphenidate. For grooming, ANOVA revealed a significant day $\times$ pretreatment $\times$ treatment interaction [$F(2,42)=5.92$, $P<0.01$]. On Day 1, mecamlamine produced significantly less grooming than saline. For stereotypy ratings, ANOVA revealed significant day $\times$ treatment [$F(2,42)=6.13$, $P<0.01$] and pretreatment $\times$ treatment [$F(2,42)=3.87$, $P<0.05$] interactions. On Day 1, each dose of methylphenidate

produced significantly greater stereotypy ratings than saline. On Day 10, each methylphenidate dose produced significantly greater stereotypy ratings than saline; however, mecamlamine attenuated the effect of methylphenidate.

Analysis of locomotor activity results from the incrementing methylphenidate (1-17 mg/kg) challenge days revealed a significant prior treatment $\times$ challenge dose interaction [$F(10,210)=3.09, P<0.01$]. In rats pretreated previously with mecamlamine or saline alone (Fig. 2a), ANOVA revealed a significant main effect of methylphenidate challenge dose [$F(5,70)=74.09, P<0.001$]; however, there was no significant effect of prior mecamlamine pretreatment. In rats pretreated previously with mecamlamine or saline prior to 5.6 mg/kg of methylphenidate (Fig. 2b), ANOVA revealed a significant prior pretreatment $\times$ methylphenidate challenge dose interaction [$F(5,70)=4.37, P<0.01$]. Post hoc comparisons indicated that 10 and 17 mg/kg methylphenidate challenge doses produced significantly less activity in the mecamlamine pretreatment group compared to the saline pretreatment group. In rats pretreated previously with mecamlamine or saline prior to 10 mg/kg of methylphenidate (Fig. 2c), ANOVA revealed a significant main effect of methylphenidate challenge dose [$F(5,70)=32.75, P<0.001$]; however, there was no significant effect of prior mecamlamine pretreatment.

The effects of the incrementing methylphenidate (1-17 mg/kg) challenge doses on sniffing, rearing, grooming and stereotypy measures in are presented in Table 2. For sniffing, ANOVA revealed significant prior pretreatment $\times$ challenge dose [$F(5,210)=2.32, P<0.05$] and prior treatment $\times$ challenge dose [$F(10,210)=3.19, P<0.01$] interactions. Overall, methylphenidate challenge doses dose-dependently increased sniffing more in rats treated previously with methylphenidate than rats treated previously with saline; however, prior mecamlamine pretreatment attenuated this sensitization. For rearing, ANOVA revealed significant main effects of prior pretreatment [$F(1,42)=4.16, P<0.05$], prior treatment [$F(2,42)=9.14, P<0.01$] and methylphenidate challenge dose [$F(5,210)=118.95, P<0.001$]. Overall, methylphenidate challenge doses dose-dependently increased rearing compared to saline challenge and this effect was increased by prior methylphenidate treatment; however, prior mecamlamine pretreatment attenuated the effect of the methylphenidate challenge. For grooming, ANOVA revealed a significant main effect of methylphenidate challenge dose [$F(5,210)=4.28, P<0.01$]; mecamlamine pretreatment did not alter this effect. For stereotypy ratings, ANOVA revealed significant main effects of prior pretreatment [$F(1,42)=17.12, P<0.001$], prior treatment [$F(2,42)=8.12, P<0.01$] and methylphenidate challenge dose [$F(5,210)=63.79, P<0.001$]. Overall, methylphenidate challenge doses dose-dependently increased stereotypy ratings compared to saline challenge and this effect was increased by prior methylphenidate treatment; however, prior mecamlamine pretreatment attenuated the effect of the methylphenidate challenge.

Experiment 2: Effect of DH β E on Induction of Behavioral Sensitization to Repeated Methylphenidate

For locomotor activity, ANOVA revealed a significant time $\times$ day $\times$ pretreatment $\times$ treatment interaction [$F(11,308)=2.13, P<0.05$]. On Day 1 (Fig. 3a), methylphenidate produced significantly greater activity than saline or DH β E alone during the latter portion of the session; DH β E had no significant effect given alone or in combination with methylphenidate. On Day 10 (Fig. 3b), methylphenidate produced significantly greater activity than saline or DH β E alone during the latter portion of the session. Methylphenidate also produced greater activity on Day 10 than on Day 1, an effect indicative of sensitization. Further, DH β E attenuated the effect of methylphenidate during the latter portion of the session.

The effects of methylphenidate on the sniffing, rearing, grooming and stereotypy measures in saline- or DH β E-pretreated rats on Day 1 and Day 10 were also determined. Similar to the effects of mecamlamine, DH β E pretreatment attenuated the effects of repeated

methylphenidate on sniffing and rearing; however, there was no effect of DH β E pretreatment on either grooming or stereotypy ratings (results not shown).

Analysis of results from the incrementing methylphenidate (1-17 mg/kg) challenge days revealed significant prior pretreatment $\times$ challenge dose [$F(5,140)=2.79$, $P<0.05$] and prior treatment $\times$ challenge dose [$F(5,140)=2.43$, $P<0.05$] interactions. In rats pretreated previously with DH β E or saline alone (Fig. 4a), ANOVA revealed a significant main effect of methylphenidate challenge dose [$F(5,70)=54.16$, $P<0.001$]; however, there was no significant effect of prior DH β E pretreatment. In rats pretreated previously with DH β E or saline prior to methylphenidate (Fig. 4b), ANOVA revealed a significant prior pretreatment $\times$ methylphenidate challenge dose interaction [$F(5,70)=3.33$, $P<0.01$]. Post hoc comparisons indicated that 5.6, 10 and 17 mg/kg methylphenidate challenge doses produced significantly greater activity than saline challenge. Further, prior pretreatment with DH β E significantly attenuated the effects of 3, 10 and 17 mg/kg methylphenidate challenge doses.

The effects of incrementing methylphenidate (1-17 mg/kg) challenge doses on the sniffing, rearing, grooming and stereotypy measures were also determined. Similar to the effect of mecamylamine, prior DH β E pretreatment attenuated the effect of methylphenidate challenge on sniffing; however, there was no significant effect of prior DH β E pretreatment for rearing, grooming or stereotypy ratings (results not shown).

Experiment 3: Effect of Mecamylamine on Expression of Behavioral Sensitization to Repeated Methylphenidate

For locomotor activity, the overall ANOVA revealed a significant prior treatment $\times$ challenge dose interaction [$F(10,210)=2.47$, $P<0.01$]. In rats treated previously with saline (Fig. 5a), ANOVA revealed a significant main effect of methylphenidate challenge dose [$F(5,70)=15.17$, $P<0.001$]; post hoc comparisons indicated that 10 and 17 mg/kg methylphenidate challenge doses produced significantly greater activity than the saline challenge. In rats treated previously with 5.6 mg/kg of methylphenidate (Fig. 5b), ANOVA revealed a significant main effect of methylphenidate challenge dose [$F(5,70)=59.49$, $P<0.001$]; post hoc comparisons indicated that 10 and 17 mg/kg methylphenidate challenge doses produced significantly greater activity than the saline challenge. In rats treated previously with 10 mg/kg of methylphenidate (Fig. 5c), ANOVA revealed a significant main effect of methylphenidate challenge dose [$F(5,70)=35.27$, $P<0.001$]; post hoc comparisons indicated that 10 and 17 mg/kg methylphenidate challenge doses produced significantly greater activity than the saline challenge. No significant main effects or interactions containing the acute pretreatment factor were obtained for any dependent measure, indicating that mecamylamine did not alter the expression of methylphenidate-induced sensitization.

Discussion

The results of the present study indicate that the noncompetitive, nonselective nAChR antagonist mecamylamine attenuates the induction, but not the expression, of behavioral sensitization to methylphenidate in rats. High-affinity $\beta 2$ subunit nAChRs appear to mediate this effect since DH β E produced a similar pattern of results. Consistent with previous reports (Torres-Reveron and Dow-Edwards 2005; Wooters et al. 2006; Yang et al. 2007), methylphenidate dose-dependently increased locomotor activity on Day 1, regardless of pretreatment. Methylphenidate also increased locomotor activity on Day 10, but to a greater extent than on Day 1, indicating that sensitization was obtained. In contrast to Day 1, the effects of methylphenidate were significantly attenuated by mecamylamine and DH β E on Day 10. After the 14-day drug-free period, rats pretreated previously with mecamylamine or DH β E in combination with 5.6 mg/kg of methylphenidate also exhibited a reduced response to the methylphenidate challenge. In contrast to these findings, acute mecamylamine pretreatment

did not alter the expression of sensitization to methylphenidate challenge doses in rats treated previously with 5.6 mg/kg or 10 mg/kg of methylphenidate alone. These results are concordant with previous reports showing that nAChR antagonists attenuate the induction of sensitization to *d*-amphetamine, cocaine and ephedrine with repeated administration, without altering their acute effects or the expression of sensitization following administration of these drugs alone (Champtiaux et al. 2006; Miller and Segert 2005; Schoffelmeer et al. 2002). Thus, it appears that nAChRs are involved in the mechanism(s) regulating the induction of stimulant-induced behavioral sensitization, but not the mechanism(s) regulating acute hyperactivity or the expression of sensitization.

In addition to locomotor activity, methylphenidate also increased other behavioral measures. Similar to previous reports, acute methylphenidate produced dose-dependent increases in sniffing (Fog 1969; Kleven et al. 1996; McDougall et al. 1999; Torres-Reveron and Dow-Edwards 2005), rearing (Bryan and Ellison 1975; Koek and Colpaert 1993; Luthman et al. 1989) and stereotypy ratings (Kuczenski and Segal 1999; Roffman and Raskin 1997; Torres-Reveron and Dow-Edwards 2005). These observed effects were enhanced with repeated administration, indicative of sensitization. Pretreatment with mecamylamine did not alter the acute effects of methylphenidate, but attenuated sniffing, rearing and stereotypy following repeated co-administration. Pretreatment with DH β E also attenuated sniffing and rearing following repeated co-administration with methylphenidate. In contrast to the present results, the cholinesterase inhibitor and indirect nAChR agonist physostigmine attenuates methylphenidate-induced stereotypy in rats (Gonzalez and Ellinwood 1984; Janowsky et al. 1972); however, physostigmine also inhibits nAChR ion channels at higher concentrations (Albuquerque et al. 1984) and thus some caution is needed when comparing results across these studies. Nonetheless, the current findings using classical nAChR antagonists show that, similar to locomotor activity, nAChR blockade does not alter the acute effects of methylphenidate, but does attenuate sniffing, rearing and stereotypy with repeated administration.

Stimulant-induced behavioral sensitization has been linked to alterations in dopaminergic and glutamatergic neurotransmission in the mesocorticolimbic DA system (Vanderschuren and Kalivas 1997). Afferent projections arising from ventral tegmental area (VTA) DA neurons are believed to be a critical substrate for the induction of behavioral sensitization to *d*-amphetamine and cocaine, whereas pre- and post-synaptic DA receptors in the nucleus accumbens (NAcc) are important for the expression of stimulant-induced behavioral sensitization (Brebner et al. 2005; Kalivas 1995; Pierce and Kalivas 1995). Similar to *d*-amphetamine and cocaine, DA also plays an important role in the acute stimulant and repeated sensitization effects of methylphenidate. Acute methylphenidate decreases sensory-evoked responses in the VTA and NAcc, an effect that is augmented with repeated methylphenidate (Yang et al. 2006). The DA synthesis inhibitor α -methyl-para-tyrosine, and the DA receptor antagonists haloperidol and *cis*-flupenthixol, attenuate the acute stimulant effects of methylphenidate (Breese et al. 1975; Mithani et al. 1986; Sellings et al. 2006). Finally, the time course of methylphenidate-induced hyperactivity parallels the rise in extracellular NAcc DA levels following systemic administration (Gerasimov et al. 2000a).

Although DA is a critical mediator of the stimulant effects of methylphenidate, there is recent evidence of serotonergic involvement. Pretreatment with the serotonin (5-hydroxytryptamine; 5-HT) uptake inhibitor fluoxetine or the 5-HT_{1B} receptor agonist CP 94253 augments the stimulant effect of methylphenidate, an effect that is reversed by the 5-HT_{1B} receptor antagonist GR 55562 (Borycz et al. 2008). The 5-HT_{2C} receptor antagonist SB242084 also potentiates methylphenidate-induced hyperactivity (Fletcher et al. 2006), suggesting that multiple 5-HT receptor subtypes may be involved. Since mecamylamine inhibits basal 5-HT release (Kenny et al. 2000), it is possible that a serotonergic mechanism(s) may have played a role in the present findings.

While increased ACh release does not appear to be critical for the expression of stimulant-induced sensitization, there is evidence that the cholinergic impact of these drugs is altered with repeated stimulant exposure. *d*-Amphetamine elicits greater striatal ACh efflux in *d*-amphetamine-treated rats relative to saline-treated rats (Bickerdike and Abercrombie 1997). Repeated co-administration of mecamlamine or DH β E also inhibits sensitization of *d*-amphetamine- and cocaine-induced [3 H]DA release in the NAcc relative to treatment with these drugs alone (Schoffmeier et al. 2001). Therefore, it is possible that interactions between nAChR antagonists and methylphenidate in the VTA may be involved in the attenuated response to repeated methylphenidate observed in the present study, whereas acute mecamlamine pretreatment failed to alter the expression of sensitization regulated primarily by DA receptors in the NAcc.

Similar to mecamlamine, DH β E also attenuated the induction of methylphenidate-induced sensitization, suggesting that DA-regulating β 2 subunit nAChRs are involved in the present findings. Heteromeric β 2 nAChRs are expressed on VTA DA neurons (Keath et al. 2007; Klink et al. 2001). Activation of these receptors increases VTA neuron firing rates (Pidoplichko et al. 1997), whereas mecamlamine reduces VTA neuron activity (Grenhoff et al. 1986). Several presynaptic nAChR subtypes (i.e., α 4 α 6 β 2 β 3*, α 6 β 2 β 3*, α 6 β 2*, α 4 β 2* and α 4 α 5 β 2*) have also been identified on striatal DA terminals in mouse (Grady et al. 2007; Salminen et al. 2007) and rat (Wonnacott 1997), where they regulate a large portion of DA release (Exley and Cragg 2008). Interestingly, recent evidence suggests that DH β E-sensitive α 6 β 2* nAChRs control the majority of nAChR-mediated DA release in rat NAcc (Exley et al. 2008). In addition to heteromeric nAChRs, homomeric α 7 nAChRs are localized on glutamatergic terminals projecting to the VTA (Mansvelder and McGehee 2000). Stimulation of α 7 nAChRs excites VTA neurons via enhanced glutamate activation of local ionotropic glutamate receptors (Grillner and Svensson 2000; Mansvelder et al. 2002), suggesting that α 7 nAChR blockade could result in reduced VTA DA output. However, this seems unlikely since the α 7 nAChR antagonist methyllycaconitine does not affect VTA neuron firing rates (Linderholm et al. 2007). Regardless of the precise localization and subunit composition of the relevant nAChR subtypes, methylphenidate increases extracellular NAcc DA levels by preventing the reuptake of DA release that is dependent on basal VTA neuron activity (Grace 2001). Thus, the most parsimonious explanation for the present findings is that inhibition of β 2-containing nAChRs decreases basal DA release, thereby decreasing the extracellular DA content available to reuptake inhibition by methylphenidate and attenuating methylphenidate-induced behavioral sensitization.

Stimulant-induced behavioral sensitization also includes an associative conditioning component (Badiani et al. 1997; Badiani and Robinson 2004). After administration of a single high dose of cocaine, rats exhibit greater hyperactivity in response to a subsequent cocaine challenge when tested in the same environment compared to a different environment (Weiss et al. 1989). *d*-Amphetamine and cocaine also induce greater levels of early-immediate gene expression when administered in a novel environment compared to the home cage (Uslaner et al. 2001). Saline injections administered in an environment paired previously with repeated administration of *d*-amphetamine, methamphetamine, nicotine or morphine can elicit conditioned hyperactivity (Anagnostaras and Robinson 1996; Bevins and Bardo 1998; Bevins and Peterson 2004; Bevins et al. 2005). Similar to our previous work with methylphenidate (Wooters et al. 2006), evidence of conditioned hyperactivity was noted in the present study. On the saline challenge test day, rats treated previously with methylphenidate were significantly more active than saline-treated rats (cf. saline challenge data across Figs. 2a-2c);

*Denotes possible inclusion of additional nAChR subunits.

however, no evidence was obtained to suggest that nAChRs modulate the expression of methylphenidate-conditioned hyperactivity.

Finally, the present results may have clinical implications. Although the methylphenidate doses chosen for the current study exceed the suggested therapeutic dose range in rats (Kuczenski and Segal 2005), the present findings extend previous work indicating that acute administration of low, therapeutically-relevant methylphenidate doses (1 and 3 mg/kg) alter cholinergic transmission in rats (Tzavara et al. 2006) by demonstrating that behavioral sensitization with repeated exposure to higher doses of methylphenidate involves nAChRs. It is well-documented that ADHD patients smoke at higher rates than the general population (Fuemmeler et al. 2007; McClernon and Kollins 2008; Rodriguez et al. 2007; Upadhyaya et al. 2005). The greater incidence of smoking in this population may represent a form of “self-medication” (Gehricke et al. 2007), as nicotine and the $\alpha 4\beta 2$ nAChR agonist ABT-418 are effective in alleviating the symptoms of ADHD (Levin et al. 1996; Wilens and Decker 2007). Alternatively, clinically-relevant doses of methylphenidate have also been shown to increase nicotine self-administration in rodents (Wooters et al. 2007) and cigarette smoking in human participants (Rush et al. 2006; Vansickel et al. 2007); thus, co-administration of nicotine and methylphenidate may have an additive/synergistic effect on behavior similar to the effects of these drugs on extracellular DA levels in rat NAcc (Gerasimov et al. 2000b). Regardless of the underlying mechanism(s), the present findings suggest that repeated exposure to high doses of methylphenidate for therapeutic or abuse-related purposes may have a “priming” effect that alters the subsequent response of DA release-regulating nAChRs to nicotine, which may increase susceptibility to tobacco dependence. Future studies should attempt to disentangle the beneficial therapeutic effects of methylphenidate as a primary treatment option for ADHD from its abuse liability, although nAChRs apparently contribute to each action. Such research would likely increase our knowledge of the neurobiology of ADHD, while potentially aiding the development of novel medications which retain therapeutic efficacy without promoting abuse.

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Abbreviations

nAChR, nicotinic acetylcholine receptor; ADHD, attention-deficit/hyperactivity disorder; DH β E, dihydro- β -erythroidine; ACh, acetylcholine; DA, dopamine; 5-HT, serotonin; NAcc, nucleus accumbens; VTA, ventral tegmental area..

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Figure 1.

Effects of saline (open symbols) or 3 mg/kg of mecamylamine (filled symbols) pretreatment on locomotor activity in rats given saline or methylphenidate (5.6 or 10 mg/kg) on Day 1 (panel a) and Day 10 (panel b). Data points reflect the mean ($\pm$ SEM) distance traveled (cm) per 5-min interval. Asterisk (*) indicates a significant difference from saline treatment at the corresponding time point ($p < 0.05$); asterisk above the line indicates a significant difference from saline at each corresponding time point for all methylphenidate treatment groups ($p < 0.05$). Hash mark (#) indicates a significant difference relative to the corresponding saline pretreatment group ($p < 0.05$). $N = 8$ rats/group.

Figure 2.

Effects of saline and methylphenidate (1-17 mg/kg) challenge injections alone on locomotor activity 14 days after the repeated treatment phase in rats treated previously with saline (open circles) or 3 mg/kg of mecamylamine (filled circles) in combination with saline (panel a), 5.6 mg/kg of methylphenidate (panel b) or 10 mg/kg of methylphenidate (panel c). Data points reflect the mean ($\pm$ SEM) total distance (cm) traveled across the entire session. Asterisk (*) indicates a significant difference compared to the saline challenge injection ($p < 0.05$). Hash mark (#) indicates a significant difference compared to the corresponding saline pretreatment group ($p < 0.05$). N=8 rats/group.

Figure 3.

Effects of saline (open symbols) or 3 mg/kg of DHβE (filled symbols) pretreatment on locomotor activity in rats given saline or 5.6 mg/kg of methylphenidate on Day 1 (panel a) and Day 10 (panel b). Data points reflect the mean ($\pm$ SEM) distance traveled (cm) per 5-min interval. Asterisk (*) indicates a significant difference from saline treatment at the corresponding time point ($p < 0.05$); asterisk above the line indicates a significant difference from saline at each corresponding time point for all methylphenidate treatment groups ($p < 0.05$). Hash mark (#) indicates a significant difference relative to the corresponding saline pretreatment group ($p < 0.05$). $N=8$ rats/group.

Figure 4.

Effects of saline and methylphenidate (1-17 mg/kg) challenge injections on locomotor activity 14 days after the repeated treatment phase in rats treated previously saline (open circles) or 3 mg/kg of DHβE (filled circles) in combination with saline (panel a) or 5.6 mg/kg of methylphenidate (panel b). Data points reflect the mean ($\pm$ SEM) total distance (cm) traveled across the entire session. Asterisk (*) indicates a significant difference compared to the saline challenge injection ($p < 0.05$). Hash mark (#) indicates a significant difference compared to the corresponding saline pretreatment group ($p < 0.05$). $N = 8$ rats/group.

Figure 5.

Effect of acute pretreatment with saline (open symbols) or 3 mg/kg of mecamylamine (filled symbols) prior to saline and methylphenidate (1-17 mg/kg) challenge injections on locomotor activity beginning 14 days after repeated treatment with saline alone (panel a), 5.6 mg/kg of methylphenidate alone (panel b) or 10 mg/kg of methylphenidate alone (panel c). Data points reflect the mean ($\pm$ SEM) total distance (cm) traveled across the entire session. Asterisk (*) indicates a significant difference compared to the saline challenge injection ($p < 0.05$). $N=8$ rats/group.

Table 1

Effects of pretreatment with saline or mecamylamine (3 mg/kg) prior to saline, 5.6 mg/kg of methylphenidate (MPH 5.6 mg/kg) or 10 mg/kg of methylphenidate (MPH 10 mg/kg) on mean ($\pm$ SEM) bouts of sniffing, rearing, grooming and stereotypy ratings during Day 1 and Day 10 of the repeated drug treatment phase. $N=8$ rats/group.

Day	Measure	Saline Pretreatment						Mecamylamine (3 mg/kg) Pretreatment					
		Saline			MPH 5.6 mg/kg			MPH 10 mg/kg			Saline		
		<i>M</i>	<i>SEM</i>		<i>M</i>	<i>SEM</i>		<i>M</i>	<i>SEM</i>		<i>M</i>	<i>SEM</i>	
Day 1	Sniffing	11.5	1.57		14.8	1.49		17.8 ^a	1.35		12.0	0.63	
	Rearing	5.63	1.82		7.00	1.16		9.00	1.09		7.50	1.80	
	Grooming	3.00	1.02		1.38	0.46		1.13	0.55		0.75 ^b	0.41	
	Stereotypy	0.25	0.16		1.00 ^a	0.27		1.75 ^a	0.16		0.32	0.22	
	Sniffing	5.88	1.14		21.3 ^a	1.91		25.9 ^a	1.51		7.00	1.30	
Day 10	Rearing	2.50	0.78		14.8 ^a	1.47		19.0 ^a	1.95		1.88	0.48	
	Grooming	0.50	0.27		1.75	0.41		2.00	0.57		1.25	0.37	
	Stereotypy	0.38	0.18		2.00 ^a	0.38		2.88 ^a	0.40		0.12	0.10	
	Sniffing										14.1	1.11	
	Rearing										8.00	1.16	
	Grooming										1.13	0.35	
	Stereotypy										0.63	0.26	
	Sniffing										16.0 ^a	1.10	
	Rearing										11.3 ^a	1.01	
	Grooming										1.38	0.38	
	Stereotypy										1.50 ^a	0.27	
	Sniffing										18.0 ^d	1.56	
	Rearing										8.50	1.63	
	Grooming										2.25	0.62	
	Stereotypy										1.00 ^{ab}	0.19	
	Sniffing										20.6 ^{ab}	1.12	
	Rearing										12.4 ^{ab}	1.46	
	Grooming										1.13	0.35	
	Stereotypy										1.88 ^{ab}	0.23	

^a Significant difference relative to the corresponding saline treatment group ($p<0.05$)

^b Significant difference relative to the corresponding saline pretreatment group ($p<0.05$)

Table 2

Effects of saline and the methylphenidate (1-17 mg/kg) challenge doses alone on mean ($\pm$ SEM) total bouts of sniffing, rearing, grooming and stereotypy ratings in rats previously given pretreatments of saline or 3 mg/kg of methylphenidate prior to saline, 5.6 mg/kg of methylphenidate or 10 mg/kg of methylphenidate during the saline and methylphenidate challenge phase. $N=8$ rats/group.

MPH Challenge Dose	Measure	Prior Treatment											
		Saline Pretreatment						Mecamylamine (3 mg/kg) Pretreatment					
		Saline			MPH 5.6 mg/kg			Saline			MPH 5.6 mg/kg		
		<u>M</u>	<u>SEM</u>		<u>M</u>	<u>SEM</u>		<u>M</u>	<u>SEM</u>		<u>M</u>	<u>SEM</u>	
Saline	Sniffing	8.50	2.26		7.75	1.28		5.88	1.22		6.88	1.01	
	Rearing	2.00	1.45		1.25	0.45		1.38	0.50		2.25	0.59	
	Grooming	0.88	0.30		1.00	0.38		1.63	0.46		0.88	0.40	
	Stereotypy	0.13	0.10		0.20	0.12		0.14	0.07		0.22	0.08	
1.0	Sniffing	10.6	1.80		18.9 ^a	1.16		10.9	1.08		15.9 ^a	1.64	
	Rearing	4.00	0.98		5.88	1.16		1.88	0.52		4.38	0.82	
	Grooming	1.25	0.31		1.38	0.53		1.38	0.53		1.50	0.53	
	Stereotypy	0.50	0.19		0.38	0.18		0.25	0.16		0.13	0.13	
3.0	Sniffing	20.1	0.83		22.4	1.73		16.4	2.16		19.5	0.78	
	Rearing	8.25	1.46		9.38	0.78		6.75	1.74		8.88	1.59	
	Grooming	1.25	0.41		1.38	0.53		2.38	0.82		1.63	0.46	
	Stereotypy	0.63	0.18		0.50	0.27		0.45	0.25		0.32	0.11	
5.6	Sniffing	20.4	1.39		28.6 ^a	1.31		19.5	1.15		22.6 ^b	1.64	
	Rearing	9.13	2.16		12.1	1.96		7.75	1.33		10.4	2.15	
	Grooming	2.25	0.45		0.88	0.40		1.23	0.48		1.38	0.53	
	Stereotypy	1.00	0.19		1.25	0.31		0.75	0.25		1.13	0.35	
10.0	Sniffing	25.4	1.67		26.4	1.59		20.3 ^b	0.84		23.6	1.79	
	Rearing	14.9	1.75		17.1	2.07		14.0	2.02		16.1	1.64	
	Grooming	0.50	0.27		0.88	0.40		0.75	0.22		1.26	0.40	
	Stereotypy	1.50	0.27		1.50	0.33		1.25	0.37		1.00	0.38	
17.0	Sniffing	33.6	1.56		34.1	1.65		31.1	0.93		31.5	1.79	
	Rearing	17.9	1.08		21.8	1.85		19.0	1.77		20.9	1.78	
	Grooming	0.88	0.40		0.63	0.26		0.75	0.31		1.13	0.61	

^a Significant difference relative to the corresponding saline treatment group ($p < 0.05$)