THE EFFECTS OF ADOLESCENT EXPOSURE TO METHYLPHENIDATE ON THE AVERSIVE PROPERTIES OF COCAINE IN ADULTHOOD

By

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ABSTRACT

Methylphenidate is the most widely prescribed pharmacotherapeutic treatment of AD/HD in children and teens and has actions that are also involved in drug reward and reinforcement. Its clinical use has often raised concerns over the possibility that it could potentiate the risk for later drug-related problems. Animals exposed to methylphenidate during adolescence exhibit attenuated cocaine-induced conditioned place preference, but tend to self-administer cocaine more quickly than controls. A drug's abuse potential, as reflected by self-administration, is thought to be the product of a balance between its rewarding and aversive properties, thus the present research assessed the effects of adolescent exposure to methylphenidate on conditioned taste aversions induced by cocaine in adulthood in 132 male Sprague Dawley rats. Although cocaine induced robust dose-dependent taste aversions in accordance with previous research, there were no effects of adolescent exposure to methylphenidate in spite of evidence that it was behaviorally active.

TABLE OF CONTENTS

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

Attention deficit/hyperactivity disorder (AD/HD), which is characterized by debilitating inattention, hyperactivity and impulsivity, is among the most frequently diagnosed neuropsychological disorders in children (Swanson et al., 1998). According to the Centers for Disease Control and Prevention (CDC, 2010), 4.5 million children between the ages of 5 and 17 in the United States were diagnosed with AD/HD by 2006, with 56% of those receiving pharmacotherapy for the disorder. Further, approximately one-third of individuals diagnosed with AD/HD as children continue to meet the diagnostic criteria as adults, although the hyperactive and impulsive components tend to fade (CDC, 2010; Goldman, Genel, Bezman & Slanetz, 1998; Swanson et al., 1998). With a wide array of geographic and socioeconomic populations affected, there is currently no consensus on the etiology of AD/HD, but there is strong evidence for genetic and neurophysiological components (Swanson et al., 1998; Goldman et al., 1998). In particular, it has been demonstrated that patients diagnosed with AD/HD may have structural deficits in the striatum, as well as in the dopaminergic pathways responsible for mediating and integrating communication within this region (Swanson et al., 1998).

Pharmacotherapeutic treatment of AD/HD is most often achieved with psychostimulants, among which methylphenidate (MPH, marketed as Ritalin®) is the most widely prescribed in the United States (Volkow et al., 2001). The precise action of these drugs with regard to attention disorders has yet to be fully characterized, but they remain the most overwhelmingly effective medications available, with some estimates attributing up to a 90% success-rate for symptom relief (Goldman et al., 1998; Swanson et al., 1998; Nestler, Hyman & Malenka, 2009; Volkow et al., 2001). The psychostimulants, which also include cocaine (COC) and amphetamine, are indirect dopamine (DA) agonists that are known to increase the availability of extracellular DA phasically, particularly in the striatum, through drug-dependent manipulation of a variety of local mechanisms, including stimulation of release and inhibition of reuptake (Koob & LeMoal, 2006; Nestler et al., 2009; Volkow et al., 2001). Stimulants are also known to impact serotonin and norepinephrine (NE), but their subjective and therapeutic effects arise mainly from their interaction with striatal DA, and possibly NE, pathways (Koob & LeMoal, 2006; Nestler et al., 2009).

Interestingly, some of these same DA pathways, particularly in the ventral striatum [more commonly referred to as the nucleus accumbens (NAc)] are also involved in drug reward and reinforcement and the abuse potential of a given drug is directly linked to similar DA activity in this region (Koob & LeMoal, 2006; Nestler, 2001; Nestler et al., 2009). Further, prospective studies have shown that recreational drug use in adolescence greatly enhances the risk of addictive

behaviors in adulthood (Schramm-Sapyta, Walker, Caster, Levin & Kuhn, 2009; Spear, 2000), and animal research suggests that this impact may be due to increased plasticity related to normal development in the adolescent brain (Smith, 2003; Spear, 2000; Stanwood & Levitt, 2004). Hence, the use of MPH for treating AD/HD in children and teens has often spurred controversy over the possibility that it may lead to lasting changes that could potentiate the risk for later drug-related problems (Carlezon & Konradi, 2004; Kuczenski & Segal, 2001). As will be illustrated, preclinical research related to its effects on COC suggests that MPH does indeed modulate drug reward.

Adolescent MPH and COC Reward

Animals exposed to MPH during adolescence have consistently demonstrated an attenuated COC-induced conditioned place preference (CPP, a measure of the incentive properties of a given drug; see Adriani et al., 2005; Andersen, Arvanitogiannis, Pliakas, LeBlanc & Carlezon, 2001; Carlezon, Mague & Andersen, 2003; Augustyniak, Kourrich, Rezazadeh, Stewart & Arvanitogiannis, 2006; Achat-Mendes, Anderson & Itzhak, 2003), with only one known exception, in which MPH had no effect (Crawford et al., 2011). In one such assessment, adolescent rats were injected with MPH [2.5 mg/kg, intraperitoneally (IP)] or equivolume vehicle for 10 days and then allowed to mature to adulthood before place preference conditioning with COC (at one of four doses, e.g., 1, 5, 10 and 20 mg/kg, IP, respectively; see Augustyniak et al., 2006). Rats pretreated with MPH consistently failed to display a preference for the COC-paired chamber, except at the 20 mg/kg dose, which

produced a preference equivalent to the vehicle pretreatment group. Interestingly, the same study found no differences in COC-induced DA levels in the NAc between the two preexposure groups, suggesting that the behavioral differences may be related to changes in the DA response rather than DA availability.

This reduction in COC-induced CPP in animals preexposed to MPH during adolescence has been interpreted as resulting from MPH-induced neuroplastic changes that result in a generalized anhedonia in adulthood (Andersen et al., 2001; Carlezon et al., 2003; Mague, Andersen & Carlezon, 2005; Brandon & Steiner, 2003). In such a condition, doses of COC that produce reward in control subjects would be ineffective in the preexposed subjects due to reduced sensitivity of the reward system. This interpretation is supported by findings that adolescent MPH øìøøâÈ(摧掊VÈ摧¿ È(摧 Ā摧 ĀÈ 摧M& uli, such as intracranial self-stimulation (Mague et al., 2005), or naturally rewarding stimuli, such as sucrose or sexual activity (Bolaños, Barrot, Berton, Wallace-Black & Nestler, 2003). Further evidence suggests that preexposure to MPH may elevate CREB (cAMP response element binding protein) levels in the NAc, which has been linked to clinical depression (Carlezon & Konradi, 2004; Andersen et al., 2001).

Adolescent MPH and the Subjective Value of COC

Research involving COC self-administration (SA, a measure of the overall subjective value of a drug, see Schuster & Thompson, 1969) in animals preexposed

to MPH during adolescence has shown that they tend to achieve stable levels of drug intake more quickly than controls. Low (2 mg/kg, IP for 7 days) to moderate doses (5 and 10 mg/kg, IP, for 5 to 7 days) of MPH in young rats significantly increase the acquisition rate of fixed-ratio (FR) COC self-administration in adulthood (Brandon, Marinelli, Baker & White, 2001), and this effect has been replicated in rats preexposed as adults to higher, but still moderate doses of MPH (20 mg/kg, IP, for 9 days, Schenk & Izenwasser, 2002). More recently, it was demonstrated that MPH preexposure in male rats does not affect SA acquisition rate and produces minimal effects on FR responding, but significantly increases break-points in progressive-ratio responding at all doses of MPH (2 and 5 mg/kg) and COC tested (0.25 and 0.75 mg/kg, Crawford et al., 2011). Interestingly, no effect of MPH preexposure was found for similarly treated female rats in the same assessment.

Although these findings might seem to conflict with CPP research, they in fact may further support the idea of a MPH-induced hedonic shift: An elevated reward threshold could indeed explain why these animals display an attenuated reward response in fixed-dose COC-induced CPP tests, yet work harder to receive more of the drug when given the opportunity. This scenario could indicate that, although MPH preexposure appears to attenuate the rewarding effects of COC, it may still increase its abuse potential by increasing the dosage necessary to produce subjective effects.

The Relationship between Reward and Aversion

The overall subjective value of a drug (and, therefore, its abuse potential, as reflected by SA in the preclinical model) is thought to be the product of a balance between the rewarding and aversive properties of that drug (Brockwell, Eikelboom & Beninger, 1991; Wise, Yokel & DeWitt, 1976; Simpson & Riley, 2005). The aversive effects serve to limit the reinforcing value of the drug and are known to be altered by several factors, including drug-history (Riley & Simpson, 2001; Riley & Diamond, 1998). Figure 1 (page 7) illustrates the relationship between the rewarding and aversive properties of a given drug in terms of a standard SA dose-response function (represented by the 'Subjective Value (SA)' line). The characteristic 'inverted U' shape of this function demonstrates that as the dose increases, responding for a given drug typically plateaus and then decreases, in spite of a relatively stable reward-state (Riley, 2011). This phenomenon is often interpreted as resulting from receptor saturation, diminished availability of neurotransmitter or some other mechanism of habituation to the rewarding effects of the drug.

However, the shape of this function is also likely influenced by the aversive properties of the drug, which become more influential as the dose continues to rise (Riley, 2011). Importantly, the relationship between these factors is orthogonal, rather than linear, allowing them to vary independently. This means that an individual's relative sensitivity to a drug's aversive effects can potentially influence the propensity to continue taking the drug (as would be illustrated by alterations in the shape of the dose-response function in Figure 1, should the position of the

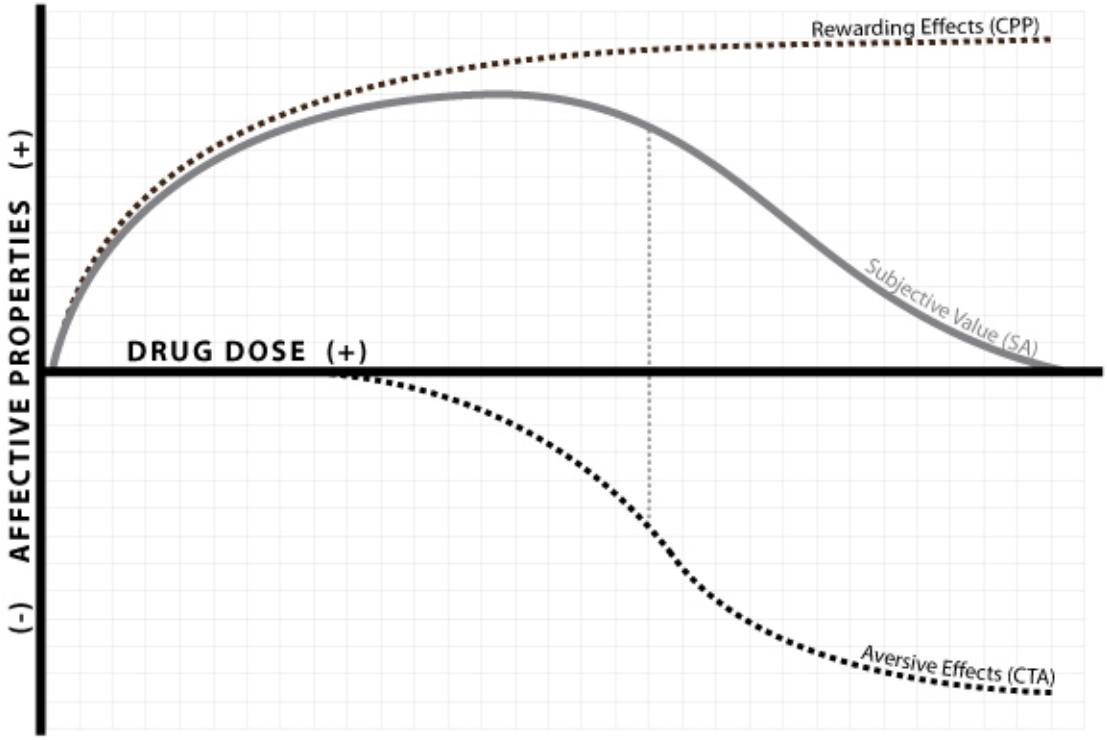

Figure 1. Illustration of the Relationship Between Reward and Aversion in the Abuse Potential of a Given Drug. Represented in terms of a typical dose-response function (depicted by the 'Subjective Value (SA)' line). While the descending limb of the function is typically interpretted only in terms of the rewarding effects of a drug (i.e., receptor saturation, reduced availability of neurotransmitter, etc.), it is also likely influenced by the aversive effects. (Adapted from Riley, 2011)

aversive effects curve shift left or right). Therefore, the overall subjective value of a given drug is as likely to be influenced by its aversive, as by its rewarding effects. Thus, another crucial component of the consequence of adolescent MPH preexposure may be in how it alters the aversive effects of COC in adulthood. That is, the increases in COC SA could be the result of a reduced reward response (as discussed) *or* a reduction in the sensitivity to COC's aversive properties—each of which could simultaneously exert marked effects on the dose-response function for COC. Interestingly, two of the previously noted studies involving COC-induced CPP found evidence for a COC-induced conditioned place *aversion* with a moderate COC

challenge (10 mg/kg, IP) following adolescent preexposure to MPH (Andersen et al., 2001; Carlezon et al., 2003).

Adolescent MPH and the Aversive Properties of COC

While it is impossible to know whether any behavioral assay purely represents a given construct, CPP results are thought to mostly represent the *rewarding effects* in this model of abuse liability (Tzschentke, 1998, 2007). A procedure believed to dominantly index a drug's *aversive effects* is the conditioned taste aversion preparation (CTA, see Riley & Tuck, 1985; www.CTALearning.com; Riley & Freeman, 2004). The CTA procedure exploits the tendency of animals to reduce consumption of an ordinarily preferred novel substance (e.g., saccharin water) after it is paired with a given drug over multiple trials, thus indicating a learned association between the novel taste of the substance and the aversive effects of that drug (Revusky & Garcia, 1970; Revusky & Gorry, 1973). It has already been established that MPH is capable of generating a CTA (Riley & Zellner, 1978) and that COC produces CTAs at doses also known to produce reward (Ferrari, O'Connor & Riley, 1991; Mayer & Parker, 1993). Further, it has recently been shown that adolescent preexposure to substances such as nicotine and ethanol can alter the aversive effects of COC and other substances as measured by CTA (Hutchison & Riley, 2008; Rinker et al., 2011; Hutchison, Albaugh & Riley, 2010).

Although the effects of adolescent preexposure to MPH have been well established for COC reward (e.g., CPP) and its overall subjective value (e.g., SA),

assessments of its effects on the aversive properties of COC have yet to be undertaken. Thus, the present research examined the effects of adolescent exposure to MPH on the aversive properties of COC in adulthood using the CTA procedure. Specifically, adolescent male Sprague Dawley rats were preexposed to a clinically relevant dose of MPH or equivolume vehicle and then were tested as adults for differential responding to COC.

CHAPTER 2

EXPERIMENT ONE

Method

Subjects

Subjects were 84 experimentally naïve male Sprague-Dawley rats (Harlan® Laboratories, Inc., Indianapolis, IN), which arrived at the laboratory on postnatal day 20 (PND 20) and were allowed to acclimate for 5 days. The animals were housed in groups of four or five in Plexiglas bins (26 x 48 x 21 cm) located within a colony room maintained on a 12-h light/dark cycle (lights on at 0800h) and at an ambient temperature of 23 °C. Unless otherwise indicated, food and water were available *ad libitum*. In order to monitor the health of the subjects and limit the effects of stress from handling, the subjects were weighed daily beginning on PND 20. All procedures were conducted between 1000 and 1400 h and were approved by American University's Institutional Animal Care and Use Committee (IACUC). Additionally, guidelines recommended by the National Research Council's Guide for the Care and Use of Laboratory Animals (1996) and the Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research (2003) were followed.

Drugs and Solutions

Methylphenidate hydrochloride and cocaine hydrochloride (both generously supplied by NIDA) were dissolved in 0.9% saline (vehicle, VEH) as 1 and 10 mg/mL solutions, respectively. VEH injections were equivolume to the highest dose of the accompanying drug (see procedure below). Lithium chloride (LiCl, Fisher Scientific; used as a positive control, see procedure below) was prepared as a 0.15M solution in VEH. All drug weights are expressed as the salt form, and all drug solutions were prepared daily. Saccharin (0.1% sodium saccharin solution) was prepared as a 1 g/L solution in tap water.

Procedure

Adolescent MPH Preexposure

Beginning on PND 25, subjects were randomly divided into two groups (n = 42 in each group), one of which received twice-daily injections of MPH (2 mg/kg; IP) at 2-h intervals (beginning at 1200h) for 15 consecutive days, while the other received twice-daily equivolume IP injections of the saline vehicle (VEH) (final injections were delivered on PND 39). The dose of MPH was based on its equivalence to therapeutic doses in adolescent humans (Kuczenski & Segal, 2002). The PND window and number of days for preexposure were based on previous research on adolescent MPH preexposure effects on COC reward (Andersen et al., 2001; Carlezon et al., 2003; Mague et al., 2005) as well as reviews that establish them to be within the period when most rat breeds exhibit developmental

characteristics similar to those of periadolescent to adolescent humans (Spear, 2000; Yang, Swann & Dafny, 2006). During and immediately following preexposure, animals were housed such that bins only contained subjects from the same preexposure group. On PND 50, subjects were separated into individual hanging wire-mesh cages, where they remained for the duration of the study. Following preexposure, animals continued to receive daily handling and weighing, but no further injections were delivered until PND 75 (see below).

CTA Habituation, Conditioning and Testing

Animals were deprived of water for 23 2/3 hours prior to the start of habituation on PND 61. Beginning that day, subjects were permitted 20-min daily access to water presented in graduated 50 mL Nalgene tubes affixed to the front of the hanging cages. At the end of 20min, the bottles were removed and consumption volumes were recorded. Subjects were considered habituated to this procedure once they approached and drank from the water bottle within 2 sec of its presentation and drank within 2 ml of the previous day for at least 4 days with no consistent upward or downward trend.

Conditioning began on PND 75 (chosen based on previous reviews establishing it as the approximate beginning of adulthood in most rat breeds; see Spear, 2000; Yang et al., 2006). On this day, subjects were presented with a novel saccharin solution instead of water during their scheduled 20-min access. Immediately following consumption, animals from each preexposure condition were rank-ordered based on saccharin consumption and assigned to one of five

groups: VEH, LiCl (see below), or COC10, COC18 and COC32 (see below), such that mean saccharin consumption was equivalent across groups. This resulted in 10 total groups, where the first letters denote the drug given during preexposure and the second letters denote the drug given during conditioning: VEH-VEH, VEH-LiCl, VEH-COC10, VEH-COC18, VEH-COC32, MPH-VEH, MPH-LiCl, MPH-COC10, MPH-COC18 and MPH-COC32. Each group contained 8 animals, except VEH-COC32 and VEH-LiCl, which each received one additional animal resulting from delivery surpluses for *n*=9; and MPH-COC18, which received two additional, for *n*=10. Within 20 min of saccharin consumption, animals were injected with vehicle or drug. The chosen doses of COC (e.g., 10, 18 and 32 mg/kg, respectively) are previously reported to induce taste aversions (Mayer & Parker, 1993; Ferrari et al., 1991). To assess whether the effects of MPH preexposure were specific to COC, two groups (one from each preexposure condition) were injected with LiCl (0.6 mEq/kg), an emetic well established as an aversion-inducing agent (Revusky & Gorry, 1973; Riley & Tuck, 1985). The dose of LiCl was chosen based on the fact that it indexes intermediate aversions, thus allowing an assessment of any potentiating or attenuating effects of MPH preexposure (Mastropaolo, Dacanay & Riley, 1986). All injections were delivered subcutaneously (SC).

The 3 days following the initial saccharin presentation were water-recovery days in which the subjects received 20-min access to water with no injections afterward. This complete 4-day cycle (conditioning followed by 3 days of water recovery) was repeated four times (a total of 16 days), and fluid consumption was

recorded each day. On PND 91, subjects were given 20-min access to the saccharin solution in a final one-bottle CTA test with no injections following this presentation. From PND 92 to 105, daily handling and weighing continued and the subjects had *ad lib* access to food and water.

Collateral Assays

To monitor potential developmental effects of exposure to MPH, as exhibited in previous research (Crawford et al., 2011; Bolaños et al., 2003; Achat-Mendes et al., 2003), body weights were recorded to ascertain any differences between groups. Additionally, challenge doses of COC or VEH were followed by locomotor assessments in adulthood to investigate possible variations in locomotor activation effects from COC that have been reported following adolescent exposure to MPH, (Achat-Mendes et al., 2003; Adriani et al., 2005; Andersen et al., 2001; Brandon et al., 2001; Guerriero, Hayes, Dhaliwal, Ren & Kosofsky, 2006). Testing began on PND 106 and was conducted using eight individual automated apparatuses constructed of gray opaque Plexiglas (San Diego Instruments Place Preference System, San Diego, CA), the inner dimensions of which were 70 cm wide x 21 cm deep x 34.5 cm high. Each individual chamber was dimly illuminated by three white LED lights and featured a 16 x 4 photo-beam array for recording gross locomotor activity (consecutive beam breaks) and fine motor activity (repeated breaks of the same beam). The room that housed the chambers was illuminated by an 85-watt red light mounted to the ceiling in the center of the room, and background noise was masked by a white-noise generator. Individual subjects were placed in each chamber for 1 h

of habituation and baseline recording followed by injections of drug/vehicle matching those that each animal received during CTA. They were then returned to the chamber for an additional hour of locomotor recording.

Statistical Analyses

The CTA consumption data (ml consumed) were analyzed using a 2 x 5 x 5 repeated measures analysis of variance (ANOVA) with between-subjects factors of Preexposure Drug (VEH, MPH) and Conditioning Drug (VEH, LiCl, COC10, COC18, COC32) and a within-subjects factor of Trial (1-4 and final CTA test). Where indicated by appropriate interactions, differences in saccharin consumption between groups for individual trials were tested using one-way ANOVAs followed by Tukey's HSD *post-hoc* tests.

Body weights between groups during the preexposure period were analyzed using a 2 x 15 mixed model ANOVA with a between-subjects factor of Preexposure Drug (VEH, MPH) and within-subjects factor of Post Natal Day (25 – 39). Where indicated by appropriate interactions, differences between groups for each day were tested using one-way ANOVAs with Bonferroni corrections ($p \leq 0.05/15$, or 0.003). Locomotor data from the baseline and test phases were broken into 15-min segments of total activity counts (fine + gross movement) and analyzed using separate 2 x 5 x 4 repeated measures ANOVAs with between-subjects factors of Preexposure Drug and Conditioning Drug and a within-subjects factor of Quarter (four 15-minute segments). Where indicated by appropriate interactions, differences in activity between groups for individual time segments were tested

using one-way ANOVAs followed by Tukey's HSD *post-hoc* tests. Significance levels for all analyses were $p \leq 0.05$, unless otherwise indicated.

Results

Conditioned Taste Aversions

The 2 x 5 x 5 mixed model ANOVA revealed significant main effects of Trial [$F(4, 296) = 57.668$, $p < 0.001$] and Conditioning Drug [$F(4, 74) = 71.098$, $p < 0.001$], as well as a significant Trial x Conditioning Drug interaction [$F(16, 296) = 39.531$, $p < 0.001$; data not shown]. However, there were no effects of Preexposure Drug [$F(1, 74) = 0.963$, *n.s.*], Preexposure Drug x Conditioning Drug [$F(4, 74) = 0.208$, *n.s.*] or Trial x Preexposure Drug [$F(4, 296) = 1.454$, *n.s.*] interactions, nor a three-way Trial x Preexposure Drug x Conditioning Drug interaction [$F(16, 296) = 0.486$, *n.s.*]. A one-way ANOVA for Trial 1 indicated that saccharin consumption was equivalent between groups when conditioning began [$F(9, 74) = 0.331$, *n.s.*; see Figure 2A, page 17], while a similar ANOVA revealed significant differences in consumption by the CTA Test [$F(9, 74) = 75.72$, $p < 0.001$; see Figure 2B, page 17]. Tukey's HSD *post-hoc* for the CTA Test showed that, although there were no differences between preexposure groups at any conditioning level, the VEH and COC10 groups consumed the greatest volumes of saccharin, respectively, which were different from each other and all other groups ($p < 0.001$ for all significant pairwise comparisons). The COC18, COC32 and LiCl groups consumed the least saccharin, respectively, and were different from the VEH and COC10 groups, but not from each other ($p < 0.001$ for all

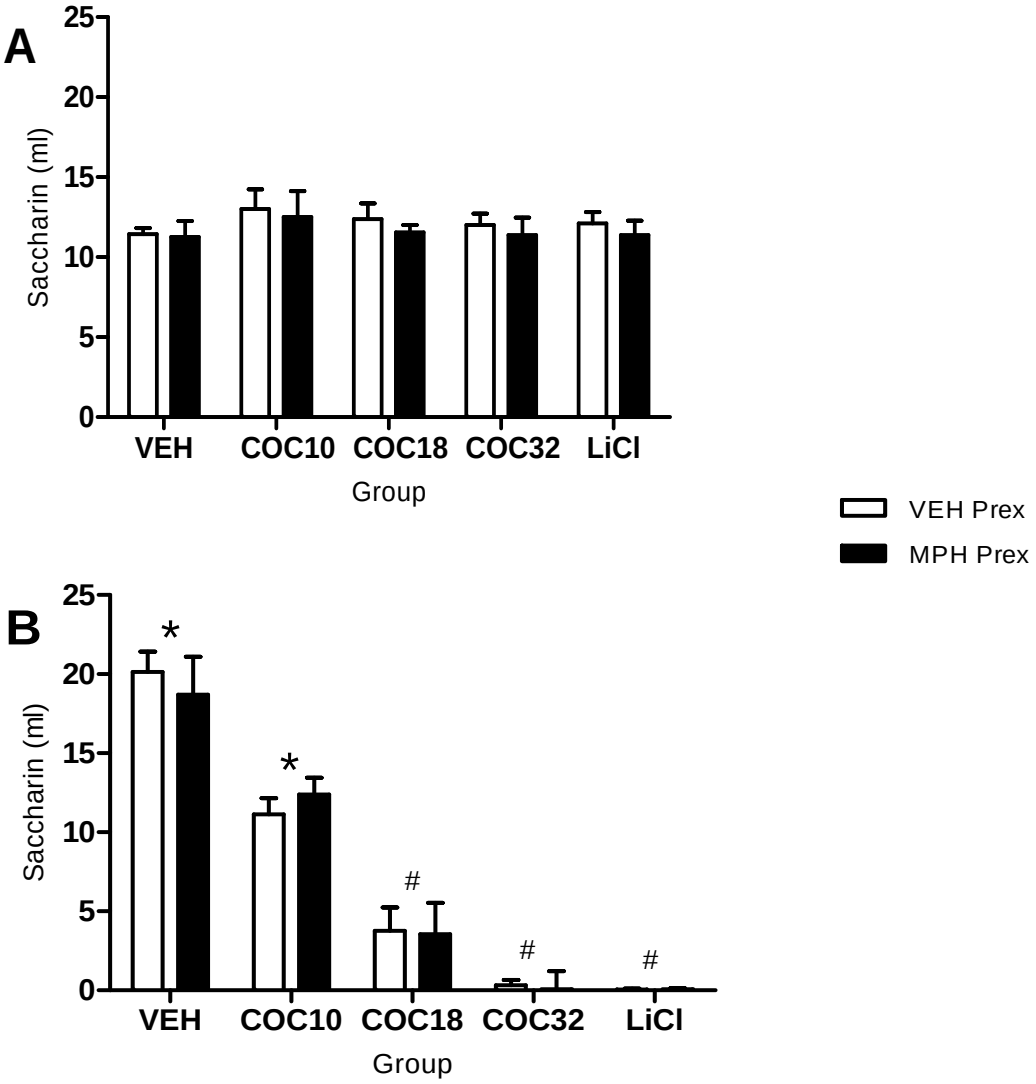

Figure 2**.** Mean Saccharin Consumption in Adulthood (SEM) by Preexposure Group During Trial 1 (A) and Test Day (B) in Experiment 1. Following adolescent exposure to 2 mg/kg MPH or VEH twice per day for 15 days, there were no significant differences in consumption between groups at Trial 1, but a one-way ANOVA revealed significant differences in consumption by Test day [F (9, 74) = 75.72, p < 0.001]. A Tukey's HSD *post-hoc* for Test showed that, although there were no differences between preexposure groups at any conditioning level, (*) VEH groups and COC10 conditioning groups were different from each other and all other groups (p < 0.001 for all significant pairwise comparisons). (#)The COC18, COC32 and LiCl conditioning groups were different from the VEH and COC10 conditioning groups (p < 0.001), but not from each other. Significance for all tests was $p \leq 0.05$, n = 8 per group except as indicated on page 13.

significant pairwise comparisons). Thus, COC induced dose-dependent reductions in saccharin consumption while similar conditioning with vehicle did not. Additionally, LiCl produced aversions similar to the highest doses of COC.

Collateral Assays

The 2 x 15 mixed model ANOVA on body weights during preexposure revealed a main effect of Post Natal Day [F (14, 1148) = 13,529.252, $p < 0.001$], but no effect of Preexposure Drug [F (1, 82) = 0.953, *n.s.*] or Post Natal Day x Preexposure Drug interaction [F (14, 1148) = 1.225, *n.s.*]. Figure 3 (page 19) illustrates that animal weights increased an average of 6.69 g daily, as is typical of normal development, and there were no effects related to drug.

The 2 x 5 x 4 mixed model ANOVA for baseline locomotor activity revealed a significant main effect of Quarter [F (3, 222) = 375.153, $p < 0.001$], but no effects of Preexposure Drug [F (1, 74) = 1.080, *n.s.*] and Conditioning Drug [F (4, 74) = 1.331, *n.s.*] nor any significant interactions [Quarter x Preexposure Drug, F (3, 222) = 2.111, *n.s.;* Quarter x Conditioning Drug, F (12, 222) = 0.908, *n.s.;* Preexposure Drug x Conditioning Drug, F (4, 74) = 0.794, *n.s.;* and Quarter x Conditioning Drug x Preexposure Drug, F (12, 222) = 0.447, *n.s.*]. As Figure 4A (page 20) illustrates, overall activity during baseline decreased as time elapsed and subjects became acclimated to the chambers; however, the decreases were not dependent on either Preexposure Drug or Conditioning Drug. Thus, subjects began locomotor testing with comparable levels of baseline activity. A similar mixed model ANOVA for activity during the test phase indicated significant main effects for Quarter

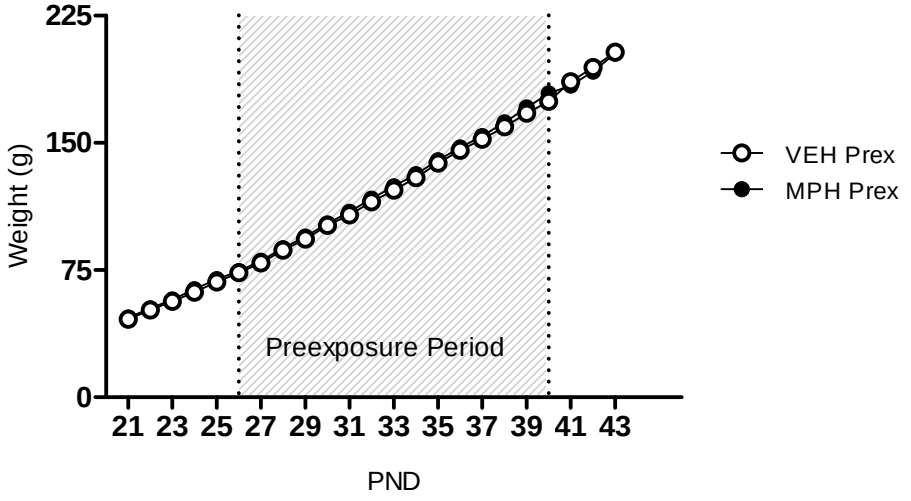

Figure 3**.** Mean Animal Weights (SEM) by Preexposure Group for Period Immediately Before, During and Following Adolescent Preexposure Injections in Experiment 1. Subjects received 2 mg/kg MPH or VEH, twice per day for 15 consecutive days. There was a main effect of Day [F (14, 1148) = 13,529.252, $p < 0.001$], but no effect of Preexposure Drug [F (1, 82) = 0.953, *n.s.*] or Day x Preexposure Drug interaction [F (14, 1148) = 1.225, *n.s.*]. Significance for all tests was $p \leq 0.05$; n = 42 per group.

[F (3, 222) = 9.356, $p < 0.001$], Preexposure Drug [F (1, 74) = 4.618, $p = 0.035$] and Conditioning Drug [F (4, 74) = 12.336, $p < 0.001$], as well as a significant Quarter x Conditioning Drug interaction [F (12, 222) = 8.377, $p < 0.001$]. There were no interactions for Quarter x Preexposure Drug [F (3, 222) = 0.789, *n.s.*], Preexposure Drug x Conditioning Drug [F (4, 74) = 1.673, *n.s.*], or Quarter x Preexposure Drug x Conditioning Drug [F (12, 222) = 0.877, *n.s.*]. Figure 4B (page 20) shows that activity increased over time in a drug- and dose-dependent manner and there were no significant differences between preexposure groups. Although there were no significant interactions related to preexposure, the COC10 and COC18 groups that were preexposed to MPH trended toward higher levels of activity than their VEH

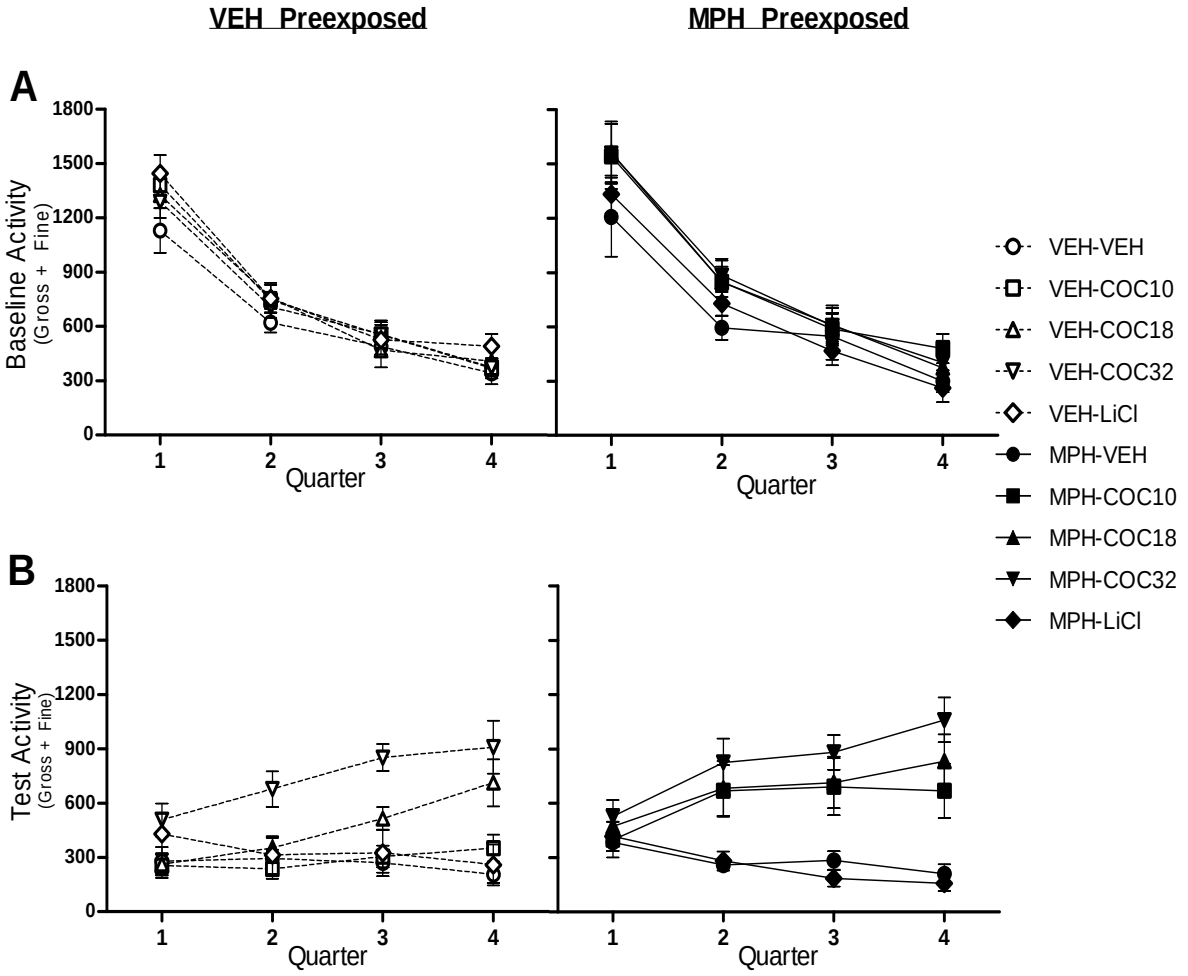

Figure 4**.** Locomotor Results for Experiment 1. Includes total baseline activity (A, gross + fine activity) and total test activity (B, gross + fine activity). An ANOVA for baseline activity revealed a significant main effect of Quarter [F (3, 222) = 375.153, $p < 0.001$], but no effects of Preexposure Drug or Conditioning Drug, nor any significant interactions; indicating that activity decreases were not dependent on drug conditions. Thus, subjects began locomotor testing with comparable levels of baseline activity. A similar ANOVA for activity during the test phase indicated significant main effects for Quarter [F (3, 222) = 9.356, $p < 0.001$], Preexposure Drug [F (1, 74) = 4.618, $p = 0.035$] and Conditioning Drug [F (4, 74) = 12.336, $p < .001$], as well as a Quarter x Conditioning Drug interaction [F (12, 222) = 8.377, $p < 0.001$]. There were no interactions for Quarter x Preexposure Drug [F (3, 222) = 0.789, *n.s.*], Preexposure Drug x Conditioning Drug [F (4, 74) = 1.673, *n.s.*], or Quarter x Preexposure Drug x Conditioning Drug [F (12, 222) = 0.877, *n.s.*]. Thus, activity increased over time in a drug- and dose-dependent manner and there were no significant differences between preexposure groups. Significance for all tests was $p \leq 0.05$, $n = 8$ per group except as indicated on page 13.

preexposed counterparts, such that they reached and maintained slightly elevated activity counts by Quarter 2 in a range that was eventually matched only by the VEH-COC18 group and only in Quarter 4.

Discussion

Experiment 1 investigated the effects of adolescent exposure to a clinically relevant dose of MPH on the aversive properties of COC in adulthood in order to assess the role of such effects in the previously reported increases in COC SA following similar adolescent exposure. As described, COC produced robust dose-dependent CTA that are consistent with previous research (for comparisons, see Ferrari et al., 1991; Mayer & Parker, 1993; Revusky & Gorry, 1973), the greatest of which was mirrored by the emetic LiCl. However, adolescent exposure to MPH had no effect on COC or LiCl CTA in adulthood, which parallels research that has demonstrated no effect of chronic adolescent nicotine on adult COC CTA (Hutchison & Riley, 2008), but contradicts others that have reported attenuated ethanol CTA in adulthood following adolescent nicotine exposure (Rinker et al., 2011) or attenuated adult COC CTA following similar ethanol preexposure (Hutchison et al., 2010). This result could indicate that the dose of MPH currently used, although clinically relevant, was too low to alter adult COC CTA results reliably.

Supporting this assertion, animal weights during preexposure showed no effects of MPH, which is inconsistent with some research using a dose of 2 mg/kg only once per day (Crawford et al., 2011), but not others (Bolaños et al., 2003). Additionally, although locomotor activity increased in a drug- and dose-dependent

manner following challenge injections of 10, 18 and 32 mg/kg of COC, or 0.6 mEq/kg of LiCl, there were no omnibus effects of MPH preexposure. However, a trend toward enhanced activation from the 10 and 18 mg/kg doses of COC among animals preexposed to MPH suggests that this dose of the drug remained influential to some degree in adulthood. Other research has reported definitive reductions in locomotor activation following similar preexposure regimens to MPH and doses of COC in adulthood (Andersen et al., 2001), but when taken together, these results have been largely inconclusive. Further, behavioral alterations in the response to cocaine have been demonstrated following adolescent doses of MPH as high as 10 mg/kg (Achat-Mendes et al., 2003; Adriani et al., 2005; Brandon et al., 2001; Guerriero et al., 2006; Kuczenski & Segal, 2001, 2002; Bolaños et al., 2003; Andersen et al., 2001; Crawford et al., 2011).

Thus, to test whether the results of Experiment 1 were a function of dose or truly reflect no influence from adolescent exposure to MPH on the aversive properties of COC in adulthood, it is necessary to assess the effects of a higher, albeit not clinically relevant, dose of MPH. Further, if MPH remains behaviorally active in adulthood, it should have an impact on its own aversive properties in adults, which may involve changes in mechanisms that it does not share with COC. Therefore, to further explore the potential long-term impact of adolescent exposure to MPH, Experiment 2 tested the effects of a higher low dose of MPH (i.e., 10 mg/kg) in adolescence on the aversive properties of intermediate doses of both COC (e.g., 18 mg/kg) and MPH (e.g., 30 mg/kg) in adulthood.

CHAPTER 3

EXPERIMENT TWO

Method

The parameters for Experiment 2 were similar to Experiment 1, with the following exceptions: Subjects were 48 experimentally naïve male Sprague-Dawley rats (Harlan® Laboratories, Inc., Indianapolis, IN). For preexposure and taste aversion conditioning, methylphenidate hydrochloride (MPH, generously supplied by NIDA) was dissolved in 0.9% saline (vehicle, VEH) as a 10 mg/mL solution. Beginning on PND 25, the subjects were randomly divided into two groups (n = 24 per group), one of which received once-daily injections of MPH (10 mg/kg; IP) for 15 days, while the other received simultaneous IP injections of equivolume vehicle (VEH). Following consumption-based rank ordering for CTA conditioning on PND 75, subjects from each preexposure group were randomly assigned to one of three groups [VEH, COC (18 mg/kg) and MPH (30 mg/kg, see below)]. This resulted in six total groups: VEH-VEH, VEH-COC, VEH-MPH, MPH-VEH, MPH-COC, MPH-MPH; n=8 per group. The first letters denote the drug received during preexposure and the second letters indicate the drug received during CTA conditioning. The doses of COC and MPH were chosen as doses inducing intermediate aversion, based on results from Experiment 1 for COC and previous CTA research for MPH (Riley & Zellner,

1978). All COC injections were delivered SC and MPH injections were delivered IP. To control for possible effects of route of administration, VEH animals were randomly subdivided, with half receiving equivolume injections via each route.

Statistical Analyses

The data analyses for Experiment 2 were similar to Experiment 1, with the following exceptions: The CTA data were analyzed using a 2 x 3 x 5 repeated measures ANOVA with between-subjects factors of Preexposure Drug (VEH, MPH) and Conditioning Drug (VEH, COC and MPH) and a within-subjects factor of Trial. Locomotor data from the baseline and test phases were broken into 15-min segments of total activity counts (fine + gross movement) and analyzed using separate 2 x 3 x 4 mixed model ANOVAs with between-subjects factors of Preexposure Drug and Conditioning Drug and a within-subjects factor of Quarter (four 15-minute segments). Significance levels for all analyses were $p \leq 0.05$, unless otherwise indicated.

Results

Conditioned Taste Aversions

The 2 x 3 x 5 mixed model ANOVA indicated significant main effects of Trial [F (4, 168) = 12.259, $p < 0.001$] and Conditioning Drug [F (2, 42) = 2524.795, $p < 0.001$], as well as a Trial x Conditioning Drug interaction [F (4, 168) = 220.700, $p < 0.001$]. There were no effects of Preexposure Drug [F (1, 42) = 0.684, *n.s.*],

Preexposure Drug x Conditioning Drug [F (2, 42) = 0.043, *n.s.*], Trial x Preexposure Drug [F (4, 168) = 0.883, *n.s.*] or Trial x Preexposure Drug x Conditioning Drug [F (8, 168) = 0.464, *n.s.*] interactions. A one-way ANOVA for Trial 1 indicated that each group consumed comparable volumes of saccharin when conditioning began [F (5, 42) = 0.131, *n.s.*; see Figure 5A, page 26], while a similar ANOVA revealed significant differences in consumption by CTA Test [F (5, 42) = 40.901, $p < 0.001$; see Figure 5B, page 26]. Tukey's HSD *post-hoc* for the CTA Test showed that there were no differences between preexposure groups at any conditioning level and the VEH groups were different from all other groups, but not each other ($p < 0.001$ for all significant pairwise comparisons). The COC and MPH groups did not differ from each other. Thus, COC and MPH induced significant and comparable reductions in saccharin consumption compared to VEH controls and there were no effects of adolescent exposure to MPH.

Collateral Assays

The 2 x 15 mixed model ANOVA on weights during preexposure revealed a main effect of Post Natal Day [F (14, 644) = 5971.012, $p < 0.001$] and Preexposure Drug [F (1, 46) = 6962.668, $p = 0.006$] as well as a Post Natal Day x Preexposure Drug interaction [F (14, 644) = 12.908, $p < 0.001$]. Figure 6 (page 27) illustrates that daily weights in the VEH and MPH groups increased an average of 6.81 g and 6.62 g, respectively, with an average daily difference of 6.22 g between them. One way ANOVAs with Bonferroni corrections showed that by PND 35 the MPH group

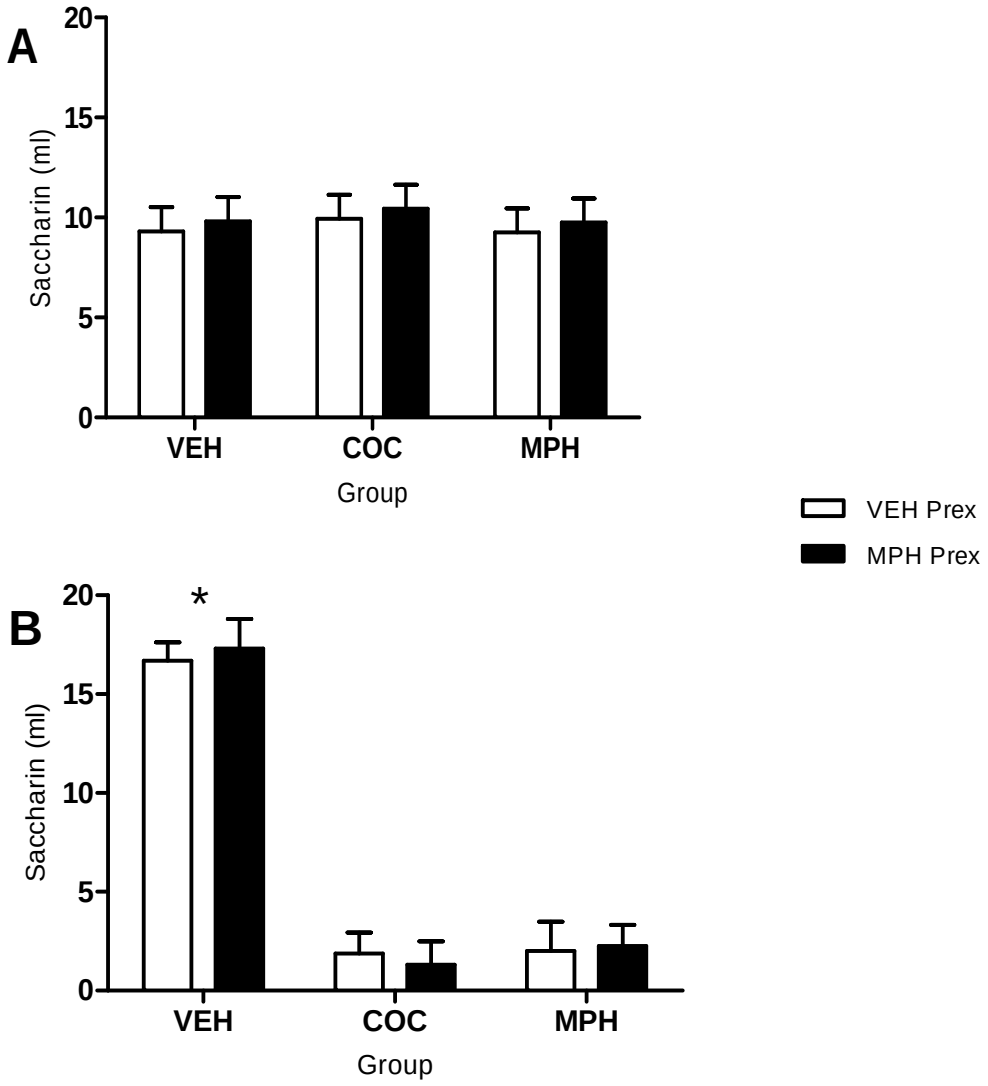

Figure 5**.** Mean Saccharin Consumption in Adulthood (SEM) by Preexposure Group During Trial 1 (A) and Test Day (B) in Experiment 2. Following adolescent exposure to 10 mg/kg MPH or VEH, once per day for 15 consecutive days, there were no significant differences in consumption between groups at Trial 1, but a one-way ANOVA revealed significant differences in consumption by Test day [F (5, 42) = 40.901, $p < 0.001$]. A Tukey's HSD *post-hoc* for Test showed that there were no differences between preexposure groups at any conditioning level and the VEH groups (*) were different from all other groups (but not each other, $p < 0.001$ for all significant pairwise comparisons). The COC and MPH groups did not differ from each other. Significance for all tests was $p \leq 0.05$, $n = 8$ per group.

weighed significantly less than the VEH group (corrected α= 0.003). Although these growth rates are within the limits of normal development, as previously observed in our laboratory, a mean daily difference of 8.16 g persisted between the two groups for the duration of the study (such that the MPH group consistently weighed less than VEH, data not shown).

The 2 x 3 x 4 mixed model ANOVA for baseline locomotor activity revealed a significant main effect of Quarter [F (3, 126) = 273.781, p < 0.001], but no effects of Preexposure Drug [F (1, 42) = 0.042, *n.s.*] and Conditioning Drug [F (2, 42) = 1.198, *n.s.*], nor any significant interactions [Quarter x Preexposure Drug, F (3, 126) = 1.980, *n.s.;* Quarter x Conditioning Drug, F (6, 126) = 1.233, *n.s.;* Preexposure Drug x Conditioning Drug, F (2, 42) = 0.324, *n.s.;* and Quarter x Conditioning Drug x

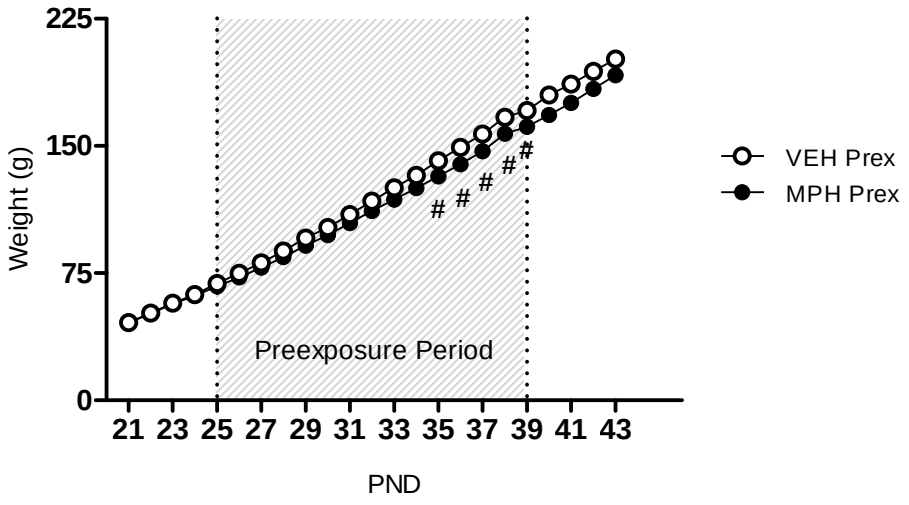

Figure 6. Mean Animal Weights (SEM) by Preexposure Group for Period Immediately Before, During and Following Adolescent Preexposure Injections in Experiment 2. Subjects received 10 mg/kg MPH or VEH, once per day for 15 consecutive days in Experiment 2. There was a main effect of Post Natal Day [F (14, 644) = 5971.012, p < 0.001], Preexposure Drug [F (1, 46) = 6962.668, p = 0.006] and a Post Natal Day x Preexposure Drug interaction [F (14, 644) = 12.908, p < 0.001]. One way ANOVAs with Bonferroni corrections revealed that (#) were significantly different than VEH (corrected α= 0.003); n = 24 per group.

Preexposure Drug, F (6, 126) = 1.787, *n.s.*]. As Figure 7A (page 29) illustrates, as time elapsed and the subjects became acclimated to the chambers, overall activity during baseline recording decreased; however, the changes in activity were not dependent on preexposure or conditioning drugs. Thus, subjects exhibited similar levels of baseline activity as locomotor testing began. The mixed model ANOVA for activity during the test phase revealed a main effect for Conditioning Drug [F (2, 42) = 14.921, $p < 0.001$] and a Quarter x Conditioning Drug interaction [F (6, 126) = 14.364, $p < 0.001$], but no main effects of Quarter [F (3, 126) = 0.422, *n.s.*] or Preexposure Drug [F (1, 42) = 0.381, *n.s.*], nor any Quarter x Preexposure Drug [F (3, 126) = 0.082, *n.s.*], Preexposure Drug x Conditioning Drug [F (2, 42) = 0.294, *n.s.*], or Quarter x Preexposure Drug x Conditioning Drug [F (6, 126) = 1.017, *n.s.*] interactions. Figure 7B (page 29) illustrates that there were no effects of preexposure to MPH, but groups receiving a MPH challenge dose exhibited higher levels of activity throughout testing, with COC conditioning groups escalating to comparable levels by Quarters 3 and 4. Further, similar to Experiment 1, although there was no significant effect of preexposure, the MPH-COC group trended toward higher levels of activation than the VEH-COC group in Quarters 3 and 4, exhibiting about 1.5 times the level of activity.

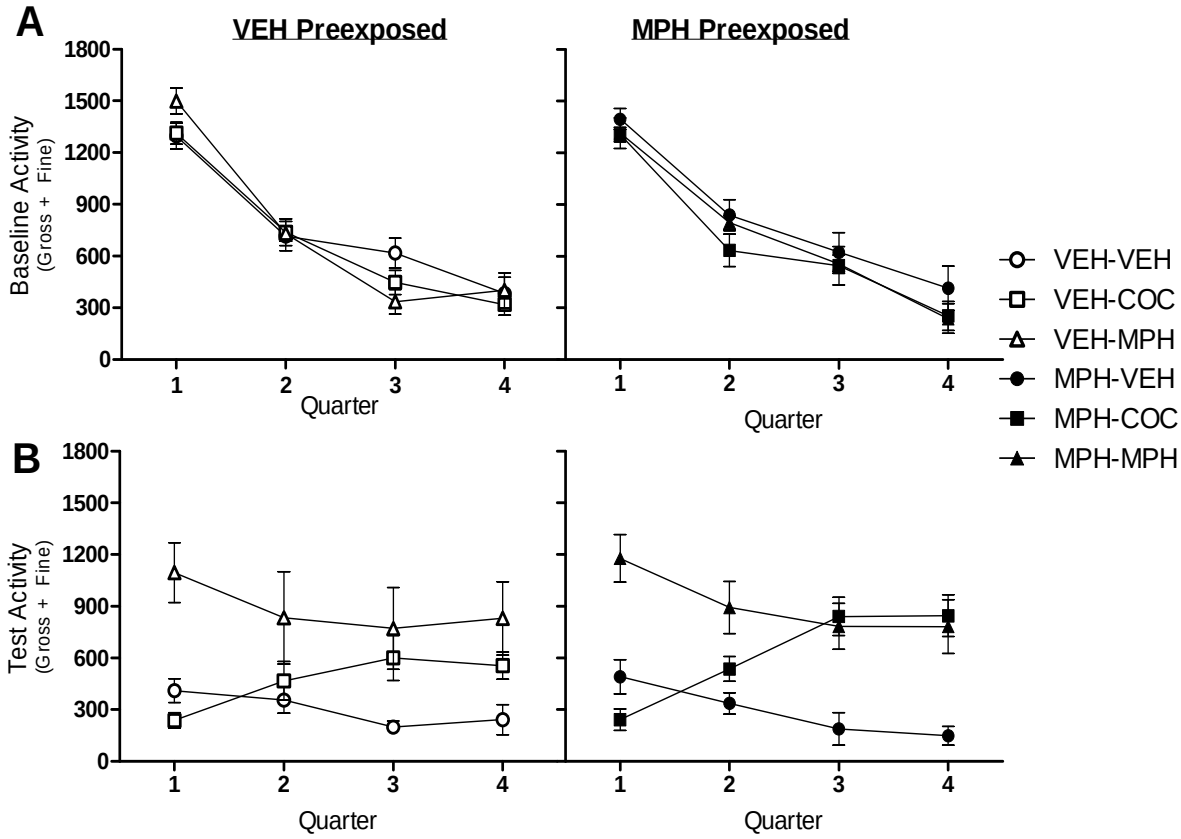

Figure 7. Locomotor results for Experiment 2. Includes total baseline activity (A, gross + fine activity) and total test activity (B, gross + fine activity). An ANOVA for baseline activity revealed a significant main effect of Quarter [F (3, 126) = 273.781, $p < 0.001$], but no effects of Preexposure Drug or Conditioning Drug, nor any significant interactions. Thus, subjects began locomotor testing with comparable levels of baseline activity. A similar ANOVA for activity during the test phase revealed a main effect for Conditioning Drug [F (2, 42) = 14.921, $p < 0.001$] and a Quarter x Conditioning Drug interaction [F (6, 126) = 14.364, $p < 0.001$], but no main effects of Quarter or Preexposure Drug, nor any Quarter x Preexposure Drug, Preexposure Drug x Conditioning Drug or Quarter x Preexposure Drug x Conditioning Drug interactions. Significance for all tests was $p \leq 0.05$, n = 8 per group.

Discussion

Experiment 2 demonstrated the effects of a higher daily dose of MPH (i.e., 10 mg/kg, once per day) during adolescence on the aversive properties of intermediate doses of COC (18 mg/kg) and MPH (30 mg/kg) in adulthood to assess whether the lack of preexposure effects in Experiment 1 were dose- or mechanism-dependent. Presently, COC and MPH produced robust and comparable taste aversions that are in line with expectation, based on the results of Experiment 1 and previous research (Riley & Zellner, 1978). However, similar to Experiment 1, there were no effects of MPH preexposure on COC-induced reductions in saccharin consumption in adulthood. Further, there was no effect on MPH-induced aversions.

Contrasting Experiment 1, significant changes in growth rates were observed during and following adolescent administration of this higher dose of MPH, such that MPH-treated animals exhibited slight decreases in weight gain and chronically lower weights than their VEH counterparts for the duration of the study. Such reductions in weight are supported by other research with the same dose of MPH in mice (although the weights were reported to rebound by adulthood, see Achat-Mendes et al., 2003) or lower doses in rats (2 and 5 mg/kg, see Crawford et al., 2011). The present result confirms that MPH at this dose was physiologically active at the time of administration.

Similar to Experiment 1, locomotor activity reflected no overall effect of MPH preexposure but the MPH-COC group exhibited a trend toward higher activation than the VEH-COC group, thus further suggesting that MPH remained influential to

some degree in adulthood to produce some degree of cross-sensitization to low and intermediate doses of COC in adulthood. Additionally, the VEH-MPH and MPH-MPH groups showed no major differences from each other, but exhibited remarkably increased activation throughout all four Quarters of testing compared to the VEH-COC and MPH-COC groups, which did not reach comparable levels of activity until Quarter 3. This result could reflect variations resulting from route of administration (as MPH was administered IP), but may also be evidence that, although MPH and COC share stimulant properties, they may do so via different mechanisms.

CHAPTER 4

GENERAL DISCUSSION

The present research assessed the effects of chronic adolescent exposure to MPH on adulthood conditioned taste aversions (CTA) induced by COC, MPH and LiCl in order to determine the possible role of these effects in previously reported increases in adult COC self-administration (SA) following similar preexposure. In Experiment 1, adolescents were administered a clinically relevant dose of MPH (i.e., 2 mg/kg, twice per day) for 15 consecutive days and then conditioned as adults with 10, 18 and 32 mg/kg of COC, as well as 0.6 mEq/kg of LiCl. Consistent with expectation and previous research, COC induced dose-dependent CTA in the VEH-preexposed subjects, such that groups consumed decreasing volumes of saccharin as the dose of COC increased, as opposed to increases in consumption throughout conditioning observed in vehicle groups (VEH; see Ferrari et al., 1991; Mayer & Parker, 1993; Revusky & Gorry, 1973). Although it is possible that variations in saccharin consumption may be influenced by other factors (i.e., relative satiety, among others), these results add to a large body of evidence that COC is capable of inducing CTA at doses also known to be rewarding (Riley, 2011). Additionally, CTA in the highest-dose COC groups were mirrored by the emetic LiCl, a well-established CTA-inducing agent (Revusky & Gorry, 1973; Riley & Tuck, 1985). However, there

was no effect of adolescent MPH preexposure in that MPH groups exhibited consumption patterns that were similar to their VEH-preexposed counterparts. Although this outcome agrees with previous work showing no effect of chronic adolescent nicotine exposure on COC-CTA (Hutchison & Riley, 2008), it is inconsistent with evidence that adolescent nicotine attenuates ethanol-CTA in adulthood (Rinker et al., 2011) and that CTA induced by COC is weakened by an adolescent history of ethanol (Hutchison et al., 2010).

To test whether the clinically relevant dose of MPH was too low to produce reliable effects on CTA, Experiment 2 tested a higher dose of MPH (i.e., 10 mg/kg, once per day) on adulthood CTA induced by mid-range doses of COC (i.e., 18 mg/kg) and MPH (i.e., 30 mg/kg). VEH-preexposed animals conditioned with COC and MPH reduced consumption of saccharin, compared to increased intake in VEH controls and, in accordance with expectation, MPH induced CTA in a manner comparable to that induced by COC (Riley & Zellner, 1978). As with Experiment 1, however, there remained no effect of preexposure to MPH in adolescence, as evidenced by similar consumption patterns between groups.

Since there was no effect on CTA, the question remains whether MPH was behaviorally active during preexposure. Several lines of evidence suggest that it was. First, previous research has established that while MPH is not behaviorally active in adolescent male Sprague-Dawley rats at 0.6 mg/kg, it becomes so between that dose and 2.5 mg/kg (Yang, Amini, Swann & Dafny, 2003; Yang et al., 2006). Further, although there was no evidence of an effect on body weights during the

preexposure period at the clinically relevant dose of MPH in Experiment 1, MPH groups exhibited significantly slower growth rates at the 10 mg/kg dose in Experiment 2. These differences persisted throughout the study, thus indicating that the drug was physiologically active at least at this dose. Additionally, there was a trend toward enhanced levels of locomotor activation in response to the 10 and 18 mg/kg challenge doses of COC given in Experiment 1, as well as the 18 mg/kg dose of COC in Experiment 2, two weeks after adulthood CTA testing among animals that were pretreated with MPH as adolescents, suggesting that the preexposure doses were effective in impacting subsequent behavior. Given these observations, and the fact that similar doses, routes of administration and preexposure windows with MPH have produced alterations to the rewarding properties of COC (Andersen et al., 2001; Bolaños et al., 2003; Bolaños et al., 2008), it seems likely that the drug was behaviorally active and would have been adequate to produce a demonstrable effect on CTA, should it exist. Of course, it remains plausible that higher doses of MPH administered during adolescence may have amplified these effects and had an impact on CTA, but exceeding clinical relevance to such a degree would arguably compromise ecological validity.

An alternate interpretation of the current results may be that MPH fails to produce a *US preexposure effect,* as is commonly observed in CTA research. A great deal of previous work has established that prior exposure to an unconditioned stimulus (US) attenuates the ability of that stimulus to induce CTA during subsequent presentations (for a comprehensive review, see Riley & Simpson, 2001).

Additionally, many drugs (but not all) are capable of inducing US preexposure effects with other drugs, whether or not they belong to the same class (known as *cross-drug effects*). The mechanisms that mediate this phenomenon remain to be fully elucidated, but are often interpreted in terms of tolerance or adaptation to their shared aversive effects (see Riley & Simpson, 2001; Randich & LoLordo, 1979 for this and other interpretations). The relative strength of the US preexposure effect is dependent on several important factors, including the dose used during preexposure (i.e., higher doses produce stronger effects) and the amount of time between preexposure and subsequent conditioning (i.e., effect is weakened with longer delays). US preexposure effects typically disappear with delays of greater than 96 hours (Cannon, Berman, Baker & Atkinson, 1975), but there are exceptions. For example, morphine preexposure attenuates morphine-CTA as many as 28 days later, whereas similar treatment with amphetamine has no apparent effect on amphetamine CTA in as few as 7 days (Cappell & LeBlanc, 1975, 1977; see also Barker & Johns, 1978).

Nonetheless, there is plausible evidence for a critical period during development in which the temporal dependencies of the US preexposure effect might be overcome. While animals prenatally preexposed to ethanol display attenuated ethanol-induced CTA when conditioning begins on PND 15 (Riley, Barron, Driscoll & Chen, 1984), no such effect from prenatal COC is evident when CTA conditioning with COC begins on PND 53 (Ferrari & Riley, 1994). These results suggest that US preexposure effects are capable of carrying over from the womb, but

do not acquire longer-term influence during this particular developmental period. In contrast, mice exposed to ethanol vapor for only 64 hours during adolescence exhibited suppressed CTA to ethanol 42 days later as adults, compared to naïve subjects, while mice preexposed as adults showed no differences in ethanol-induced CTA following the same delay (Diaz-Granados & Graham, 2007). Interestingly, this effect was enhanced when the preexposure was intermittent over the 64 hours, rather than constant, suggesting that the pattern of preexposure may have important implications for its long-term effects. For cross-drug preexposure effects, previously mentioned research has demonstrated attenuation of COC-CTA in early adulthood following a 26-day delay from adolescent exposure to ethanol (Hutchison et al., 2010), as well as similar effects on ethanol CTA 46 days after exposure to nicotine during adolescence (Rinker et al., 2011). Additionally, recent evidence points to the possibility that sex differences observed in ethanol-induced CTA learning following adolescent exposure to ethanol (i.e., males are more sensitive to US preexposure effects than females) are mediated by the surge in gonadal hormones during puberty (Sherrill, Berthold, Koss, Juraska & Gulley, 2011; Vetter-O'Hagen & Spear, 2011). Taken together, all of these data may indicate that the adolescent period of development presents a unique environment in which the effects of drug preexposure have a far-reaching impact on subsequent presentations of the same or other drugs. In this context, the reason why adolescent exposure to MPH produced no effect on adult COC-CTA, or at least on MPH-CTA, remains unexplained. Future research will need to test whether MPH is specifically capable

of inducing a US preexposure effect within conventional timeframes (i.e., less than 96 hours) both in adolescence and adulthood, whether the profile of the effect is consistent across both developmental periods and whether it is capable of inducing long-term effects following adolescent administration.

Possibly the most parsimonious explanation for the current results may be that changes in the response to COC following adolescent exposure to MPH are mediated solely by resultant changes within the reward circuit. As mentioned previously, attenuated COC-CPP in adulthood is likely the result of elevated reward thresholds following adolescent MPH exposure, which is supported by evidence that such preexposure limits reward from intracranial stimulation, as well as natural reinforcers such as sucrose and sex (Andersen et al., 2001; Carlezon et al., 2003; Mague et al., 2005; Brandon & Steiner, 2003; Bolaños et al., 2003). Attenuation of reward can also feasibly explain reported increases in COC-SA by escalating the dose necessary to achieve subjective effects and since the neural mechanisms that mediate CTA are separate from those that mediate reward, the two constructs can vary independently (Riley, 2011; Wise et al., 1976; Freeman & Riley, 2009; Verendeev & Riley, 2011). Therefore, a potential reason that adolescent MPH does not affect adult COC-CTA is that there is no shared mechanism.

Although this account may seem to be the most logical in the preclinical context, it becomes less so when clinical data are considered. As mentioned previously, recreational drug use in adolescence is a strong predictor of abuse liability in adulthood (Schramm-Sapyta et al., 2009), which fits well with the

interpretation that the rewarding effects are weakened, thereby creating the need to increase intake to achieve the desired effects and enhancing the risk of abuse. Yet, children receiving pharmacotherapeutic treatment following an AD/HD diagnosis are *less* likely than normative controls to present with substance abuse problems as adults, while those who remain untreated are more likely to do so (Biederman, Wilens, Mick, Spencer & Faraone, 1999). Further, the relative risk for addiction in adulthood is positively correlated with the age of treatment initiation (Mannuzza et al., 2008). These data present strong evidence for a mechanistic overlap between AD/HD and drug reward, but are contrary to the observed escalations in preclinical research involving COC-SA following adolescent exposure to drugs like MPH.

A potential cause for this discrepancy lies within a fundamental problem in translational research: Often, preclinical samples are selected from an entire outbred population, whereas clinical samples are typically drawn from a subset of individuals who are diagnosed with the disorder in question. The potential consequences of this disparity have recently become more evident through research in which rats are given a choice to self-administer COC or consume a saccharin solution (see Ahmed, 2010 for an extensive review of related research). In this setting, approximately 90% of the animals choose the potential nourishment over the COC, unless the concentration of saccharin is dropped to an extremely low level, i.e., 0.0016%, suggesting that COC is of little subjective value to the majority of the rats. Moreover, this pattern holds true even if the rats have an extensive history of COC use. Naturally, this inversely means that about 10% of the animals choose COC

over nourishment. Other research, using parameters designed specifically to emulate clinical diagnostic criteria for addiction, has identified a consistent 15-20% subset of outbred rats that exhibit addictive behavior regardless of imposed conditions (Deroche-Gamonet, Belin & Piazza, 2004), with high impulsivity prior to first drug use serving as a strong predictor of this behavior (Belin, Mar, Dalley, Robbins & Everitt, 2008). While these methods yield different prevalence rates, they demonstrate in the animal model what we know to be true in humans: Most individuals who use drugs of abuse do not become addicted to them, but a consistent subset are at an enhanced risk for a shift to compulsive drug use (Koob & LeMoal, 2006). Furthermore, they establish that there are factors that can influence whether an individual belongs to this high-risk group and emphasize the need to focus preclinical research on models that do so. Since there is clear commonality between AD/HD and drug reward, as well as a connection between addiction and symptoms of AD/HD, such as impulsivity, a more valid preclinical model may be necessary to replicate accurately the conditions observed in humans.

However, it is inherently difficult to know whether a particular animal model accomplishes this goal. To this end, several validation studies have established that the *spontaneously hypertensive* (SHR) inbred rat strain exhibits most of the basic behavioral correlates of AD/HD, including hyperactivity, enhanced impulsivity, increased novelty seeking and attenuated attention (see Sagvolden, 2000; but also dela Peña, Ahn, et al., 2011; dela Peña, Yoon, et al., 2011; Harvey, Sen, Deaciuc, Dwoskin & Kantak, 2011; Yang et al., 2003; Yang et al., 2006 for additional reviews).

Additionally, adolescent SHR display decreased performance compared to outbred strains in a two-choice visual discrimination test (thought to indicate deficits in early-developing attentional circuits mediated by DA in the prefrontal cortex), which is significantly improved with daily administration of 1.5 mg/kg MPH (administered orally via injection into an oyster cracker, see Harvey et al., 2011). SHR also respond uniquely to MPH in a variety of research paradigms, displaying resistance to locomotor sensitization and tolerance from MPH compared to outbred animals following chronic administration (Yang et al., 2006; Yang, Cuellar III, Swann & Dafny, 2011).

Yet, young adult SHR acquire MPH SA comparably to outbred subjects in a fixed-ratio 1 (FR1) schedule, but respond more than outbred strains when the requirements are raised in FR2 and FR3 schedules (dela Peña, Ahn, et al., 2011). Further, although adolescent SHR exhibit MPH-CPP similar to outbred animals when conditioned with low doses of MPH, their preference is attenuated at mid-range doses compared to outbred subjects, while adult SHR display weakened preferences at all doses (dela Peña, Ahn, et al., 2011). These patterns mirror previously mentioned preexposure research with outbred animals and support the attenuated reward interpretation, but importantly, this attenuation is presumably the result of the disorder, not drug preexposure, which may indicate that SHR (and possibly the AD/HD clinical subset) begin with a baseline response to drugs of abuse that is similar to that of preexposed outbred animals. Nonetheless, even when SHR are exposed to MPH as adolescents, their responses to COC in SA and CPP paradigms are

quite similar to those of outbred animals (Augustyniak et al., 2006; dela Peña, Yoon, et al., 2011; Harvey et al., 2011). Thus, although the data are currently limited, they suggest that SHR exhibit similar discrepancies with the clinical results as outbred strains and illustrate that further research is needed to establish whether these differences are parametric or simply reflective of an inadequate model.

For the moment, the reason why the preclinical results of adolescent exposure to MPH differ from the clinical data is not clear. Further, why the clinical subset of AD/HD patients exhibit an enhanced risk for compulsive drug use that is not present in AD/HD patients following chronic adolescent administration of psychostimulants remains unknown, but could be related to a multitude of factors beyond the pharmacodynamics of stimulants themselves, such as concomitant drug therapies or behavioral interventions in the treated population. More work is needed to establish the genetic and epigenetic factors that contribute to AD/HD in humans and how they interact with the subjective value of drugs of abuse to facilitate the shift from impulsive to compulsive drug use. In this way, AD/HD may present a unique and underutilized opportunity to further explain drug addiction and shed light on how it might be prevented or treated more effectively.

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