Adolescent exposure to methylphenidate has no effect on the aversive properties of cocaine in adulthood

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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. The present results indicate that changes in adult cocaine self-administration are not likely mediated by changes in the aversive response. The possibility that such changes are a function of reductions in reward threshold is discussed.

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1. Introduction

Methylphenidate (MPH) is among the most widely prescribed pharmacotherapeutic treatments of attention deficit/hyperactivity disorder (AD/HD) in children and teens in the United States (Volkow et al., 2001). In addition to its attentional effects, MPH also has actions that are involved in drug reward and reinforcement and its clinical use has often raised concerns over the possibility that it could potentiate the risk for later drug-related problems (Carlezon and Konradi, 2004; Kuczenski and Segal, 2001). Research involving cocaine (COC) self-administration (SA, a measure of the overall subjective value of a drug; see Schuster and Thompson, 1969) in adult animals preexposed to MPH during adolescence has shown that they tend to achieve stable levels of drug intake more quickly than controls. Low doses of MPH in adolescent rats (2, 5 and 10 mg/kg, intraperitoneally [IP], for 5 to 7 days) significantly increase the acquisition rate of fixed-ratio (FR) COC SA in adulthood (Brandon et al., 2001), an effect which has been replicated in rats preexposed as adults to higher, but still moderate doses of MPH (20 mg/kg, IP, for 9 days, Schenk and Izenwasser, 2002). More recently, adolescent MPH exposure in male rats did not affect SA acquisition rates and produced minimal effects on FR responding, but significantly increased break-points in progressive-ratio responding at all preexposure doses of MPH (2 and 5 mg/kg) and adulthood COC tested (0.25 and 0.75 mg/kg, Crawford et al., 2011).

In spite of evidence for an enhanced response to COC SA, 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 Achte-Mendes et al., 2003; Adriani et al., 2005; Andersen et al., 2001; Augustyniak et al., 2006; Carlezon et al., 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, 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. Attenuation of COC-induced CPP in adult animals preexposed to MPH during adolescence has been interpreted as resulting from MPH-induced neuroplastic changes that result in an elevated reward threshold in adulthood (Andersen et al., 2001; Brandon and Steiner, 2003; Carlezon et al., 2003; Mague et al., 2005). In such a condition, doses of COC that induce CPP in control subjects would be ineffective in the MPH-preexposed subjects, which is further supported by findings that adolescent MPH preexposure in animals may lead to a blunted reward response in adulthood to other experimental stimuli, such as intracranial self-stimulation (Mague et al., 2005), or naturally rewarding stimuli, such as sucrose or sexual activity (Bolaños et al., 2003).
The fact that COC-induced place preferences are reduced following MPH preexposure seems contradictory with the aforementioned increases in COC self-administration in animals with the MPH history. In this context, it is important to note that the overall subjective value of a drug (and, therefore, its abuse potential, as reflected by SA) is thought to be the product of a balance between the rewarding and aversive properties of that drug (Brockwell et al., 1991; Simpson and Riley, 2005; Wise et al., 1976), which raises the question of whether the observed differences in COC SA following adolescent MPH exposure could involve changes (i.e., weakening) in the aversive properties of COC in adulthood. Given that a drug’s aversive effects are thought to limit its reinforcing properties, and thereby have an impact on overall drug acceptability, any attenuation of these effects resulting from drug history might in turn alter the overall affective balance. Such weakening from drug history is a well-documented phenomenon, known as US preexposure effects (see Randich and Lolordo, 1979; Riley and Simpson, 2001 for extensive reviews) and has most recently been reported in a number of adolescent models (see Hutchison and Riley, 2008; Hutchinson et al., 2010; Rinker et al., 2011).

Importantly, the relationship between reward and aversion is orthogonal, rather than linear, allowing them to vary independently, meaning that an individual’s relative sensitivity to a drug’s aversive effects is not dependent on the reward response, yet can potentially influence the propensity to continue taking the drug (Brockwell et al., 1991; Doremus-Fitzwater et al., 2010; Riley, 2011; Wise et al., 1976). Given this balance, it is possible that changes in adult COC SA responding following chronic adolescent exposure to MPH could be the result of changes in the aversive effects of COC. Interestingly, two of the previously noted studies involving COC CPP each found evidence in one group for a COC-induced conditioned place aversion (CPA) with a moderate COC challenge (10 mg/kg, IP) following adolescent exposure to MPH (Andersen et al., 2001; Carlezen et al., 2003). Although the place preference procedure can index aversions in the form of CPA, such effects do not always parallel those in more direct assessments in which aversions can often be detected with the same drugs/doses that also produce place preferences (Riley, 2011; Wise et al., 1976).

One procedure often used in directly indexing a drug’s aversive effects is the conditioned taste aversion preparation (CTA, see Riley and Tuck, 1985; www.CTALearning.com; Riley and 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 and Garcia, 1970; Revusky and Gorry, 1973). It has already been established that MPH is capable of generating CTA (Riley and Zellner, 1978) and that COC produces CTA at doses also known to produce reward (Ferrari et al., 1991; Mayer and 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 and Riley, 2008; Hutchinson et al., 2010; Rinker et al., 2011).

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), there have been no assessments directly testing its effects on the aversive properties of COC. 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, in Experiment 1, adolescent male Sprague–Dawley rats were preexposed to a clinically relevant dose of MPH (i.e., 2 mg/kg, 2× per day) or equivolume vehicle and then were tested as adults for differential responding to COC. In Experiment 2, adolescents were exposed to a higher dose of MPH (i.e., 10 mg/kg once per day) and then tested as adults for differential responding to COC and MPH.

2. Experiment 1

2.1. Material and methods

2.1.1. 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 (26×48×21 cm) located within a colony room maintained on a 12-h light/dark cycle (lights on at 0800 h) 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.

2.1.2. 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 equimolar to the highest dose of the accompanying drug (see procedure below). Lithium chloride (LiCl, Fisher Scientific; used as a control, see CTA procedure below) was prepared as a 0.15 M 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.

2.1.3. Adolescent MPH exposure

Beginning on PND 25, subjects were randomly divided into two groups, one of which received twice-daily injections of MPH (2 mg/kg; IP) at 2-h intervals (beginning at 1200 h) for 15 consecutive days, while the other received twice-daily IP injections of equimolar VEH (final injections were delivered on PND 39). The dose of MPH was based on its equivalence to therapeutic doses in adolescent humans (Kuczenski and Segal, 2002) and is within the range used in previously noted work assessing the effects of adolescent MPH on adult COC CPP and SA (i.e., from 2 mg/kg once per day to 10 mg/kg once per day). The PND window and number of days for preexposure were based on previous research on adolescent MPH preexposure effects on COC reward (see Andersen et al., 2001; Carlezen et al., 2003; Mague et al., 2005) in which rats were preexposed to 2 mg/kg, twice per day, from PND 20–35; whereas other previously noted work has utilized regimens ranging from 6 to 16 days in length, in the range of PND 20–46), 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 et al., 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).

2.1.4. Adult CTA habituation, conditioning and testing

Animals were deprived of water for 23 2/3 h 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 20 min, the bottles were removed and consumption volumes were recorded.


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Subjects were considered habituated to this procedure after 14 days, defined as approaching the water bottle within 2 s of presentation and drinking within 2 mL of the previous day for at least four 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), COC10, COC18 and COC32, such that mean saccharin consumption was comparable across groups. This resulted in 10 total groups, i.e., VEH–VEH, VEH–LiCl, VEH–COC10, VEH–COC18, VEH–COC32, MPH–VEH, MPH–LiCl, MPH–COC10, MPH–COC18 and MPH–COC32 (n = 8 per group except VEH–COC32 and VEH–LiCl, n = 9; and MPH–COC18, n = 10). The first letters denote the drug given during preexposure, and the second letters denote the drug given during conditioning. Within 20 min of saccharin consumption, animals were injected with vehicle or drug. The chosen doses of COC reflect those previously reported to induce taste aversions (Ferrari et al., 1991; Mayer and Parker, 1993). 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; 25.4 mg/kg), an emetic well established as an aversion-inducing agent (Revusky and Gerry, 1973; Riley and Tuck, 1985). The dose of LiCl was based on the fact that it produces intermediate aversions, thus allowing an assessment of any potentiating or attenuating effects of MPH preexposure (Mastropaolo et al., 1986). All injections were delivered subcutaneously (SC).

The 3 days following the initial saccharin presentation day 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.

2.1.5. Collateral assays

To monitor potential developmental effects of exposure to MPH, as exhibited in previous research (Achat-Mendes et al., 2003; Bolaños et al., 2003; Crawford et al., 2011), body weights were recorded to ascertain any differences between groups. Additionally, challenge doses of drug/vehicle 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; Anderssen et al., 2001; Brandon et al., 2001; Guerriero et al., 2006). Testing 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 × 21 cm deep × 34.5 cm high. Each individual chamber was dimly illuminated by three white LED lights and featured a 16 × 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. On PND 106 (15 days following CTA Test day), individual subjects were placed in the chambers for 1 h of habituation and baseline recording, immediately after which they were injected with the exact drug/dose (i.e., COC 10, 18 or 32 mg/kg, LiCl or VEH) that each animal received during CTA conditioning. They were then immediately returned to the chamber for an additional hour of locomotor recording.

2.2. Statistical analyses

The CTA consumption data (mL consumed) were analyzed using a 2 × 5 × 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 × 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 ≤ 0.05/15, or 0.003). Locomotor data were recorded in 15-min segments. For each phase, these data were analyzed using separate 2 × 5 × 4 repeated measures ANOVAs with between-subjects factors of Preexposure Drug and Conditioning Drug and a within-subjects factor of Quarter (four 15-min 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 ≤ 0.05, unless otherwise indicated.

2.3. Results and discussion

The 2 × 5 × 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 × Conditioning Drug interaction [F (16, 296) = 39.531, p < 0.001]. However, there were no effects of, nor significant interactions with, Preexposure Drug (see Fig. 1A). One-way ANOVAs for Trial 1 and CTA Test indicated that saccharin consumption was equivalent between groups when conditioning began, but that significant differences in consumption had developed by the CTA Test [F (9, 74) = 75.72, p < 0.001; see Fig. 1B]. Tukey’s HSD post-hoc for the CTA Test showed that the VEH and COC10 groups consumed the greatest volumes of saccharin, respectively, which were significantly different from each other and significantly higher than all other groups (p < 0.001 for all significant pairwise comparisons). The COC18, COC32 and LiCl groups consumed the least saccharin, respectively, and were not different from each other (p > 0.001 for all 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. However, there were no differences between preexposure groups at any conditioning level, which could indicate that the dose of MPH currently used, although clinically relevant, was too low to alter adult COC-CTA results reliably. Although this outcome agrees with previous work showing no effect of chronic adolescent nicotine exposure on COC CTA (Hutchison and 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).

The 2 × 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 effects of Preexposure Drug, nor significant interactions, 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). Fig. 2 illustrates that animal weights.
increased an average of 6.60 g daily, as is typical of normal development, although there were no effects related to drug. Given no significant differences between groups (consecutive beam breaks) and fine (repeated beam breaks) locomotor activity counts, the two locomotor measures were collapsed to create the dependent variable, Total Activity (total beam breaks). The $2 \times 5 \times 4$ 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 or Conditioning Drug nor any significant interactions. As Fig. 3A 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 [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 × Conditioning Drug interaction [F(12, 222) = 8.377, p < 0.001]. However, there were no significant interactions for Preexposure Drug. Fig. 3B shows that activity increased over time in a drug- and dose-dependent manner and that activity was generally higher among groups preexposed to MPH compared to those that received VEH in adolescence. In contrast, some 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 taken together, locomotor results following chronic MPH have been largely inconclusive (see Kuczenski and Segal, 2001, for a brief review of related literature). Nevertheless, psychostimulants in general are known to sensitize locomotor activation upon repeated administration (i.e., the same doses generate enhanced activation, see Vanderschuren and Kalivas, 2000), thus it should be noted that the previous exposure to COC during CTA conditioning may have influenced the response during subsequent locomotor testing.

3. Experiment 2

Interestingly, 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; Adrían et al., 2005; Andersen et al., 2001; Bolaños et al., 2003; Brandon et al., 2001; Crawford et al., 2011; Guerriero et al., 2006; Kuczenski and Segal, 2001; 2002). 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, Experiment 2 examined the effects of a higher, albeit not clinically relevant, dose of MPH. Specifically, Experiment 2 tested the effects of a higher low dose of MPH (e.g., 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. If the effects of adolescent MPH are long-lasting, it may have an impact on its own aversive properties in adults, which may involve changes in mechanisms that it does not share with COC (see Randich and Lollordo, 1979; Riley and Simpson, 2001 for reviews on cross-drug US preexposure effects).

3.1. Material and methods

The parameters for Experiment 2 were similar to Experiment 1, with the following exceptions: Subjects were 48 experimentally naïve male Sprague–Dawley rats. For preexposure and taste aversion conditioning, MPH 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 equimolar vehicle. 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 and 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 equivalent injections via each route.

3.2. Statistical analyses

The data analyses for Experiment 2 were similar to Experiment 1, with the following exceptions: The CTA data were analyzed using a $2 \times 3 \times 5$ 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 (1–4 and final CTA Test). Locomotor data from the baseline and test phases were broken into 15-min segments of total activity counts (collapsed across fine + gross movements, given no significant differences between the measures) and analyzed using separate $2 \times 3 \times 4$ mixed model ANOVAs with between-subjects factors of Preexposure Drug and Conditioning Drug and a within-subjects factor of Quarter (four 15-min segments). Significance levels for all analyses were $p \leq 0.05$, unless otherwise indicated.

3.3. Results and discussion

The $2 \times 3 \times 5$ 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 $\times$ Conditioning Drug interaction [$F(4, 168) = 220.700, p < 0.001$]. There were no effects of Preexposure Drug, nor any related significant interactions (see Fig. 4A). A one-way ANOVA for Trial 1 indicated that each group consumed comparable volumes of saccharin when conditioning began, while a similar ANOVA revealed significant differences in consumption by CTA Test [$F(5, 42) = 40.901, p < 0.001$; see Fig. 4B]. Tukey's HSD post-hoc for the CTA Test showed that the VEH conditioning groups consumed more saccharin than all other groups ($p < 0.001$ for all significant pairwise comparisons), while the COC and MPH groups consumed the least saccharin, but did not differ from each other. There was no effect of adolescent exposure to MPH on CTA Test day.

The $2 \times 15$ 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 $\times$ Preexposure Drug interaction [$F(14, 644) = 12.908, p < 0.001$]. Fig. 5 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 weighed significantly less than the VEH group (corrected $\alpha = 0.003$). 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). 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.

As with Experiment 1, given no significant differences between gross (consecutive beam breaks) and fine (repeated beam breaks)
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 × Conditioning Drug interaction \(F (6, 126) = 14.364, p < 0.001\), but no main effects of Quarter or Preexposure Drug, nor any other significant interactions. Fig. 6B 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, which 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.

4. General discussion

The present research assessed the effects of chronic adolescent exposure to MPH on COC-induced CTAs in adulthood in order to determine the possible role of these effects in previously reported increases in adult COC SA following similar preexposure. As described, there was no effect of adolescent MPH on CTA in adulthood, which brings into question 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 et al., 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 significant main effect of Preexposure Drug in the locomotor assessment from Experiment 1, indicating that MPH preexposed groups exhibited generally higher levels of activation than their VEH preexposed counterparts. 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 active at both doses 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.

One underlying assumption of the present work is that a history of MPH should impact subsequent aversion learning in general, i.e., produce a US preexposure effect. It should be noted, however, that although preexposure effects are well-documented with drugs such as amphetamine (Cappell and LeBlanc, 1975, 1977) and cocaine (Riley and Diamond, 1998), there has been no research assessing MPH in this preparation. That is, it is not known if such preexposure would impact aversion learning, even in the adult population (where conditioning generally occurs within 96 h of the drug preexposure). It is possible that such effects do not occur in general and thus the failure to see an effect of MPH in adolescents on cocaine-induced aversions in adults may reflect a general inability of such effects with this drug. Work currently underway in our laboratory is examining whether MPH is capable of inducing short-term preexposure effects to itself and cocaine in both adolescent and adult animals. In light of the present findings, the issue remains that adult responding to COC CPP and SA following adolescent MPH appears contradictory, i.e., COC-induced place preferences are decreased while COC SA increases. One possible explanation for this apparent inconsistency concerns changes in reward thresholds that might
accompanied adolescent MPH exposure. 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; Bolaños et al., 2003; Brandon and Steiner, 2003; Carlezon et al., 2003; Mague et al., 2005). Although increases in SA responding are commonly interpreted as resulting from potentiation of reward, it has also been suggested that allostatic elevations in reward thresholds following chronic exposure to drugs of abuse (i.e., MPH in adolescence) might also feasibly explain such increases during protracted abstinence (i.e., for COC in adulthood) as a means of counteracting antireward neuroadaptations (Koob and Le Moal, 2005). In this context, the COC becomes a negative reinforcer that is alleviating the symptoms of a hypofunctioning reward system, which may then require higher doses of the drug to achieve subjective effects. Further, since the neural mechanisms that mediate CTA (Reilly, 2009) are separate from those that mediate reward (Koob and Volkow, 2009), the two constructs can vary independently (Freeman and Riley, 2009; Riley, 2011; Verendeev and Riley, 2011; Wise et al., 1976). By eliminating the possible role of changes in COC’s aversive response, the present results may provide support for the allostatic model and suggest that the most parsimonious explanation for the available preclinical data is that changes in the response to COC following adolescent exposure to MPH are mediated solely by resultant changes within the reward circuit.

Although this account may seem to be the most logical in the preclinical context, it becomes less so when clinical data are considered. Recreational drug use in adolescence is a strong predictor of abuse liability in adulthood (Chen and Kandel, 1995; Lewinsohn et al., 1999; Patton et al., 2004; 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 et al., 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 drug 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 et al., 2004), with high impulsivity prior to first drug use serving as a strong predictor of this behavior (Belin et al., 2008). While these methods yield different prevalence rates, they

**Fig. 6.** Locomotor results for Experiment 2. 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 × Conditioning Drug interaction [F (6, 126) = 14.364, p < 0.001], but no main effects of Quarter or Preexposure Drug, nor any other interactions. Significance for all tests was p < 0.05, n = 8 per group.
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 and 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 (see dela Peña et al., 2011a, 2011b; Harvey et al., 2011; Sagvolden, 2000; Yang et al., 2003, 2006 for extensive reviews). Yet, these patterns are more consistent across doses compared to outbred subjects, while adult SHR display weaker preferences at all doses (dela Peña et al., 2011a). 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 (see Augustyniak et al., 2006; dela Peña et al., 2011b; Harvey et al., 2011; although this fact is not obvious within each study, it becomes so when results are compared to previously mentioned outbred research). 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.

5. Conclusion

The present results demonstrate that previously observed increases in adulthood COC SA following chronic adolescent exposure to MPH are not likely mediated by changes in the aversive response to COC, as measured by CTA, which may provide support for the allostatic model by suggesting that such increases in COC SA are mediated solely by changes in reward. For the moment, the reason why the preclinical results of adolescent exposure to MPH differ from the clinical data is not clear. Further, whether 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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