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Acute and long-term effects of adolescent methylphenidate on decision-making and dopamine receptor mRNA expression in the orbitofrontal cortex

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Abstract

Though commonly used as a treatment for ADHD, the psychostimulant methylphenidate (MPH) is also misused and abused in adolescence in both clinical and general populations. Although MPH acts via pathways activated by other drugs of abuse, the short- and long-term effects of MPH on reward processing in learning and decision-making are not clearly understood. We examined the effect of adolescent MPH treatment on a battery of reward-directed behaviors both in adolescence during its administration and in adulthood after its discontinuation. We further measured whether MPH had lasting effects on dopamine receptor mRNA expression in orbitofrontal cortex (OFC) that may correspond with behavior. Long-Evans rats were injected with MPH (0, 1, 2.5, or 5 mg/kg IP) twice daily from middle to late adolescence (PD38-57). During adolescence, the high dose of MPH reduced preference for large rewards in a Reward Magnitude Discrimination task, but did not affect preference for smaller-sooner rewards in a Delay Discounting task. In adulthood, after discontinuation of MPH, animals previously treated with the moderate dose of MPH showed improved acquisition, but not reversal, in a Reversal Learning task. MPH exposure did not increase preference for large-risky rewards in a Risk task in adulthood. We then quantified mRNA expression of D1, D2, and D3 receptors in the OFC using qPCR. MPH increased mRNA expression of dopamine D3 receptor subtype, but not D1 or D2. Overall, these results indicate that MPH has both immediate and lasting effects on reward-dependent learning and decisions, as well as dopaminergic function in rodents.

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Keywords

Orbitofrontal; Adolescent; Rat; Methylphenidate; Decision-making; Reward

1. Introduction

Methylphenidate (MPH) has been globally used for over 50 years for the medical treatment of children and adolescents with attention-deficit/hyperactivity disorder (ADHD) [1]. This treatment has been shown to improve attention and response inhibition, and reduce hyperactivity in patients with ADHD [2], as well as in non-clinical human populations and animals [3–5]. MPH has a neuropharmacological profile similar to that of cocaine or amphetamine, in that it is thought to indirectly increase dopamine levels by blocking the dopamine active transporter (DAT) [6]. Additionally, MPH has an affinity for blocking norepinephrine and serotonin transporters, albeit less than for dopamine [7,8]. Due to its pharmacological profile and ease of access, MPH is also recreationally misused in non-clinical adolescent populations [9]. While the most common motive for nonmedical use of stimulant medications is to enhance cognitive and/or academic functioning among college students, they are also misused for their ability to produce a characteristic “high” at larger doses [10].

MPH has been shown to have wide ranging effects on behavior. Rodent models have elucidated decreased sensitivity to natural and drug reward [11,12], increased anxiety-like behavior [11], reductions in social interactions [13], improved acquisition of T-maze discrimination [14], and reduced risk-preference on a gambling-like task in rodents [15]. There is a growing body of literature that suggest that MPH can have differing effects depending on the dose [16]. Animal behavioral studies have shown that at lower doses (<3 mg/kg), MPH can facilitates acquisition and attention [17–19] and higher doses (>5 mg/kg) can result I cognitive impairments[16]. Further, higher doses can also elicit robust conditioned place preference in rats [20,21], an effect not seen at a lower doses (1 mg/kg). While much work has investigated the effects of stimulant medications on reward sensitivity and basic learning in adulthood after adolescent exposure, less is understood about their effects on more complex decision-making tasks. Far less is known about the effect of MPH on adolescent behavior during concurrent treatment.

Normal neuronal development requires a precise orchestration of maturation in a temporally specific manner [22,23]. Therefore, persistent changes in monoaminergic transmission during development due to chronic MPH could profoundly affect synaptogenesis, myelination, gliogenesis, and ultimately behavior [22–24]. In particular, the prefrontal cortex (PFC) continues to develop throughout adolescence, making it vulnerable to environmental insult, which may lead to long-lasting neural changes. For example, the effects of a therapeutically relevant dose of MPH during adolescence and then cessation of treatment in adulthood showed lasting changes in DAT function in the orbitofrontal cortex (OFC) [25]. These results suggest that nonmedical adolescent use of MPH could result in persistent changes in the OFC dopamine system. We have previously shown that the OFC plays a role

in reward-directed behavior during risk preference [26] and behavioral flexibility tasks [27], thus suggesting that developmental exposure to MPH may alter these behaviors.

Given the accessibility of MPH and its widespread use by adolescents, it is important to understand the more general effects of MPH on decision-making processes, both during adolescence and subsequently in adulthood, even once use has discontinued. The goal of this study is to examine the short- and long-term effects on cognitive processing associated with adolescent MPH treatment and the impact it may have on dopamine receptor expression in the OFC. We administered a range of doses of MPH to rats during late adolescence and used a battery of tasks to model the illicit use of lower doses for cognitive enhancement and higher doses for the “high”. We also investigated whether MPH altered mRNA expression of dopamine receptors in the OFC in adulthood, which may indicate long-lasting changes in the prefrontal dopaminergic system that could underlie behavioral changes.

2. Method

2.1. Subjects

Subjects were 52 male Long-Evans rats (Charles River Laboratories, Chicago, IL). Rats arrived in the laboratory on postnatal day (PD) 22 and were housed in littermate pairs in standard polycarbonate cages. Of the 52 rats, 34 underwent behavioral testing in both adolescence and adulthood, while the remaining 18 only performed behavioral tasks as adults. The colony room was kept under a 12-h light/dark cycle (lights on at 07:00) with behavioral testing conducted during the light phase. Lab chow was restricted during testing to at least 90% of free-feeding weight during testing periods. Subjects were cared for in accordance with the guidelines issued by the National Institutes of Health and approved by the University of Illinois at Chicago Animal Care Committee.

2.3. Apparatus

Behavioral testing was conducted in standard rat operant chambers (30.5 × 40 × 29cm) housed inside sound-attenuating boxes (Med Associates, St. Albans, VT). Each chamber was equipped with two retractable levers located on either side of a central port (pellet magazine) and a cue light located above each lever, which functioned as ‘wait-cue’ lights during the Delay Discounting task. Additionally, two nosepoke ports (2.5 cm diameter) with three colored lights were located on the back wall, directly opposite of the left and right levers. A house light was positioned at the rear of the chamber on the same wall as the nosepoke ports, and infrared beams were used to mark entries into the pellet dispenser and both nosepoke ports.

2.2. Methylphenidate Treatment

MPH hydrochloride was obtained from Sigma-Aldrich (St. Louis, MO). MPH (1, 2.5 or 5 mg/kg) was dissolved in 0.9% saline and injected intraperitoneally (ip) at a volume of 1 ml/kg during middle to late adolescence (PD 38–57; [28]). This time period was chosen to replicate the high level of use in late high school and early college years [29–31]. Rats received chronic treatment of one dose of MPH twice daily, with the first injection 30min prior to behavioral testing and second injection occurring just before the onset of the dark

cycle, around 8h after the initial injection. This injection schedule was used to replicate school-day doses and taking into account the higher metabolic rates of rats [32]. Control animals received saline injections (1ml/kg) on the same schedule. Figure 1 shows a timeline of drug administration and behavioral testing.

2.4. Behavioral Procedure

2.4.1. Magazine Training—Rats first underwent magazine training (PD 24–37), where a nosepoke at the center food receptacle resulted in illumination of the port-cue lights. A nosepoke into either port resulted in delivery of one 45mg sucrose pellet. Once rats reached a criterion of at least 10 trials, they performed two additional days of training in which only one port choice was illuminated at a time, presented in alternation. This was done to ensure that rats had equal experience with both port choices and to minimize the development of a side bias.

2.4.2. Reward Magnitude Discrimination and Delay Discounting in Adolescence—After magazine training, rats were trained to perform the Magnitude Discrimination task for five days (PD 38–42). In this task, one port was designated as smaller-shorter (SS) and the other as larger-later (LL) to correspond with how they would be identified in the Delay Discounting task. A nosepoke into the SS port resulted in the immediate offset of cue lights and illumination of the ‘wait-cue’ lights followed 1s later by offset of cue lights and delivery of one pellet. A nosepoke into the LL port resulted in the same events except that a larger reward was delivered (3 pellets). The houselight remained off for a varying ITI (10s, 13s, or 15s) before turning back on, indicating that the next trial could begin. All sessions lasted 30 minutes or 50 trials.

Beginning on PD 43, rats performed the Delay Discounting task. Here, the delay between nosepokes into the LL port and delivery of 3 sucrose pellets increased each day. Delays of 5s, 10s, 15s, 20s, 30s, 50s, 70s, and 90s were presented subsequently on each day, until PD 50. Nosepokes to either port or at the central food receptacle during the delay had no programmed response. The ‘wait-cue’ lights remained on for the entire delay period, turning off with the delivery of the reward. Performance was measured as the percentage of LL choices, defined as $[\text{number of LL choices} / \text{total active responses}] * 100$. Performance on the Magnitude Discrimination and Delay Discounting tasks was analyzed separately using two-way (treatment $\times$ session) repeated measures ANOVAs followed by posthoc Tukey HSD tests where appropriate.

2.4.3. Reversal Learning in Adulthood—After discontinuation of MPH and a withdrawal period lasting until adulthood, rats were trained to lever press an FR1 schedule. Criterion for training was met when the animal made at least 50 presses on two consecutive sessions. Reversal Learning was then tested in two phases: Acquisition and Reversal. During Acquisition, a nosepoke in the central port resulted in the extension of the two spatially distinct levers and onset of the cue lights above them. A press of the ‘correct’ lever resulted in cue light extinction, lever retraction, and the delivery of two sucrose pellets. A press of the opposite, ‘incorrect’ lever resulted in cue light extinction, lever retraction, and reward omission. Following a 10s or 15s ITI, the house light turned on to denote that the rat could

begin the next trial. ‘Correct’ and ‘incorrect’ sides were counterbalanced across subjects. Criterion was met when the animal made 10 sequential ‘correct’ choices, which resulted in the termination of the test program. If the criterion was not met, the program ended after 1h or 100 trials, and animals were given additional sessions, 24h apart, until criterion was met. Following Acquisition, Reversal began 24h later. During Reversal, the locations of the ‘correct’ and ‘incorrect’ levers were reversed and the learning criterion was the same. Acquisition and Reversal criteria were separately analyzed using one-way ANOVA to evaluate differences in performance as a function of prior MPH treatment.

An analysis of errors during the Reversal session(s) was conducted to determine whether their MPH treatment affected the ability to inhibit the previously rewarded choice (perseverative error) or maintain a new choice pattern (regressive error). To determine the number of perseverative errors, trials were separated into consecutive blocks of four trials. The first block composed of a majority of lever presses (3 of 4) on the new correct spatial location was considered a successful block. When a rat chose the previously correct spatial location before this successful block, it was scored as a ‘perseverative error’. All subsequent trials that resulted in an incorrect response were scored as ‘regressive errors’. Separate one-way ANOVAs for perseverative and regressive errors made during reversal were conducted to determine if the number of errors depended on treatment.

2.4.4. Risk task in Adulthood—Rats then performed a Risk task, in which each daily session was comprised of two blocks of trials: Forced Response and Free Choice. In the block of 20 Forced Response trials, levers designated as ‘certain’ or ‘risky’ were presented one at a time, in alternation. The ‘certain’ lever response resulted in a small-certain reward, one sucrose pellet delivered on 100% of trials. Presses of the ‘risky’ lever would result in a large-risky reward, the probabilistic delivery of either 3 sucrose pellets or reward omission (detailed below). Each trial was initiated a center nosepoke resulting in the illumination of a cue light(s) followed by extension of the lever(s). Once one lever was pressed, the cue light(s) extinguished, lever(s) retracted, and the reward (or omission) immediately followed. After a variable ITI (8s, 10s, or 12s), the house light re-illuminated to indicate the start of the next trial. Reward omissions were denoted by the absence of the reward and no other discerning cues.

Rats were first trained for 4–6 sessions with a 100% payoff on the ‘risky’ lever, similar to the Magnitude Discrimination task of adolescence. Once rats learned to discriminate reward magnitudes (at least a 60% preference for the larger reward), one level of risk (16%, 33%, or 67%) was randomly assigned to the risky lever and held constant for two consecutive sessions. These values were chosen due to their expected values (risky vs certain: 0.5 vs 1 on 16%, 1 vs 1 on 33%, and 2 vs 1 on 67%). A Free Choice block then followed, in which both options were presented simultaneously on each trial, and rats were allowed to choose freely between the two options for a maximum of 1h or 100 trials. Preference for the large-risky reward option in the Risk task was calculated as [number of risky choices / all free choice trials in the session] * 100. Differences in risk-preference across probabilistic sessions (16%, 33%, or 67%) and prior treatment group (0, 1, 2.5 or 5mg/kg MPH) were assessed using two-way (treatment × probability) repeated measures ANOVA, followed by Tukey posthoc analysis when appropriate.

After completion of the Risk task, rats were given both a 2-session block in which ‘risky’ lever presses were never rewarded (0% reward probability; Extinction) and a block in which the ‘risky’ lever was rewarded every time it was chosen (100% reward probability; Magnitude Discrimination). These trial types (0% or 100%) were presented in random order across animals. Preference for the large-risky reward option during these sessions was also assessed using a two-way (treatment $\times$ probability) repeated measures ANOVA.

2.5. Quantitative Real-Time Polymerase Chain Reaction Procedure

At the conclusion of behavioral testing in adulthood, 27 randomly selected rats from MPH treatment groups (0, 2.5, 5 mg/kg) received a lethal dose of sodium pentobarbital (100 mg/kg; i.p.), deeply anesthetizing the animal. Rats were decapitated and a 2 mm coronal slice, including the OFC, was removed using a brain matrix. OFC tissue was punched out of slices using a 2 mm diameter brain tissue punch (Stoelting Co., Wood Dale, IL, USA). Tissue punches were immediately frozen in dry ice and stored at -80°C .

mRNA was isolated and purified using the GeneJET Purification Kit (Thermo Fisher Scientific Inc.). The resulting RNA concentrations were calculated using the Epoch Gen 5 Microplate Reader (BioTek Corporation, Broadview, IL). Complimentary DNA (cDNA) was synthesized using the Maxima First Strand cDNA Synthesis Kit with dsDNase (Thermo Fisher Scientific Inc.) and the Bio-Rad PTC 200 Thermo Cycler System (Global Medical Instruments, Ramsey, MN). A master mix of Maxima SYBR Green/ROX qPCR Master Mix (Thermo Fisher Scientific Inc.) and predesigned forward and reverse primers were created for (a) dopamine D1 receptor, forward 5' $\rightarrow$ 3' CACCTGAGGTCCAAGGTGAC and reverse 5' $\rightarrow$ 3' AAGGACCCAAAGGGCCAAAA, (b) D2 receptor, forward 5' $\rightarrow$ 3' CTGGAAGCCTCGAGCAGC and reverse 5' $\rightarrow$ 3' TCTGCCGCCTCTCCAGATCGTCA, (c) D3 receptor, forward 5' $\rightarrow$ 3' TCTGCCGCCTCTCCAGATCGTCA, (c) D3 receptor, forward 5' $\rightarrow$ 3' GTCTGAGGCTGCATCCCAT and reverse 5' $\rightarrow$ 3' GTCTGAGGCTGCATC-CCATT and reverse 5' $\rightarrow$ 3' GCTGCAGGTGTGACAAAAGG (Integrated DNA Technologies, Coralville, IA). Similarly, a master mix of FAM-labeled GCTGCAGGTGTGACAAAAGG (Integrated DNA Technologies, Coralville, IA). Similarly, a master mix of FAM-labeled Rat ACTB Endogenous Control and 2 $\times$ Maxima Probe/ROX qPCR Master Mix (Thermo Fisher Scientific Inc.) was created to quantify the housekeeping gene, β -actin. The qPCR analyses were carried out using the Thermo Scientific PikoReal Real-time PCR System.

Gene transcription of D1, D2, and D3 were normalized to β -actin and comparative Cq (Cq) was calculated to quantify relative gene expression. Relative gene expression was expressed in arbitrary units using the following formula: $R = 2^{-Cq}$ (Cq = Cq Sample - Cq calibrator, Cq = Cq Target gene - Cq β -actin). Individual one-way ANOVAs were conducted to determine if relative gene expression varied across treatment groups. Additionally, Pearson correlations between significant behavioral indices and dopamine receptor mRNA expression were calculated.

3. Results

3.1. Weight gain across MPH treatment

During the period of MPH treatment (i.e., PD 38–57), adolescent rats demonstrated a progressive increase in body weight (Figure 1) [$F(34,1632)=1245$, $P<0.0001$]. However weight gain was not altered by MPH treatment. Body weight was also not significantly different after 0 d (PD57), 18 d (PD 75), 28 d (PD 85), or 38 d (PD 95) following the final MPH treatment.

3.2. Reward Magnitude Discrimination and Delay Discounting test in adolescence

During chronic administration of MPH, 34 (8–9 per group) adolescent rats performed the Reward Magnitude Discrimination task to determine whether treatment affected the ability to reliably choose a large (3 sucrose pellets) over small (1 sucrose pellet) reward. Overall, adolescent rats demonstrated a progressive increase in preference for the larger reward [$F(4,28)=5.299$, $p<0.05$] with a greater preference for the LL option on all subsequent days compared to the first day (Figure 2A). There was also a significant effect of treatment [$F(3,21) = 5.61$, $p<0.05$], which was due to lower preference for LL by rats treated with the high MPH dose (5 mg/kg: mean = 50.2%) compared with each of the lower doses (1 mg/kg: mean = 66.2% or 2.5 mg/kg: mean = 66.4%, $p<0.05$). There were no differences in total rewards earned or number of trials performed during Magnitude Discrimination. While adolescent rats reliably discriminated reward magnitudes, exposure to high levels of MPH disrupted this reward learning.

To determine whether MPH treatment affected the evaluation of delayed rewards, rats were then tested on a Delay Discounting task in which the delay to delivery of the larger reward progressively increased over subsequent sessions. The proportion of LL choices depended session [$F(8,240) = 14.72$, $p<0.05$], such that preference shifted from the LL choice to the SS choice as delay increased [1s vs. 50s, 70s, or 90s; all $p<0.05$], (Figure 2B). The overall proportion of LL choices depended on MPH treatment [$F(3,30) = 3.29$, $p<0.05$]. Rats treated with 5 mg/kg MPH showed a lower preference for LL (mean = 45.2%) compared to the 2.5 mg/kg treatment group (mean = 63.0%; $p<0.05$). While all groups discounted the larger reward as its delivery was delayed, MPH did not alter temporal discounting compared to controls. However, the high dose of MPH impaired discrimination learning and subsequently produced a leftward shift in temporal discounting compared to rats that received the moderate dose.

3.3. Reversal Learning and Risk Preference in adulthood

To assess the longitudinal effect of MPH on rats' ability to learn and adapt to changing reward contingencies, rats treated with MPH during adolescence performed Reversal Learning and Risk tasks. An additional set of 18 rats was added to the adult behavioral analysis resulting in a total of 52 rats (13 per group). Overall, two rats were excluded from the Reversal Learning analysis; one rat in the 5 mg/kg group ($n=12$) failed to complete the Acquisition phase of Reversal Learning and the other in the 2.5 mg/kg group ($N=12$) was excluded due to a computer error; however, these rats performed and were included in the analysis of the Risk task.

Prior treatment with MPH in adolescence affected learning performance in adulthood. In Reversal Learning, the average number of trials to reach the Acquisition criterion depended on adolescent MPH treatment [$F(3,46)=4.413$, $p<0.05$], (Figure 3A left panel). Rats treated with 2.5 mg/kg MPH acquired the initial discrimination between rewarded and unrewarded levers at a faster rate (mean = 35.67 trials) than controls (mean = 66.62 trials; $p<0.05$). However, there were no differences in the number of trials needed to reach criterion between groups during Reversal performance (overall mean = 76.41 trials; Figure 3A right panel). MPH did not affect perseverative (responding to previously rewarded option) or regressive (errors made after adopting the new choice pattern) errors (Figure 3B right panel). These patterns of behavior on during Reversal Learning show an enhancement in initial learning in adults following adolescent MPH exposure, but no differences in the ability to flexibly adapt to changing reward contingencies.

To determine whether adolescent MPH treatment affected the evaluation of probabilistic rewards, rats performed a Risk task where they chose between two levers yielding different contingencies that depended on reward size and probability. The ‘certain’ option resulted in the delivery of the small reward (1 sucrose pellet, 100%) and the ‘risky’ option resulted in either no reward or a large reward (3 sucrose pellets), depending on the reward schedule. Regardless of treatment group, all rats modified preference for the risky option as the probability of payoff decreased [$F(2,92)=47.37$, $p<0.05$; Figure 4A]. Rats thus discriminated the different probabilities of large-risky reward across sessions, but adolescent MPH exposure did not alter risk-preference in adulthood.

During sessions in which the large reward was always delivered (100%) or omitted (0%), rats showed a strong discrimination of outcomes. When large reward was certain to be delivered, rats demonstrated an overall strong preference for the large-risky lever (Magnitude Discrimination task; mean = 83.5%) and shifted their preference to the small-certain lever when the large-risky reward was always omitted (Extinction; mean = 34.5%). Although there was a difference in preference for the ‘risky’ lever during Magnitude Discrimination and Extinction [Figure 4B; $F(1,47)=727.2$, $p<0.05$], there were no differences in performance between treatment groups.

3.4. Dopamine receptor expression after MPH treatment in adolescence

To determine whether adolescent MPH treatment causes lasting effects on dopamine receptor expression in the OFC, qPCR was conducted from OFC tissue samples of a subset of adult rats treated with MPH 2.5 mg/kg ($n = 9$), 5 mg/kg ($n = 11$) or vehicle ($n = 7$) in adolescence. Animals who received the 1 mg/kg dose were not included because no behavioral differences were observed in group. There were no differences in expression of D1 [$F(2,20) = 0.387$, ns] or D2 mRNA [$F(2,20) = 0.582$, ns; Figure 5 left and middle panels respectively]. However, D3 receptor expression in the OFC depended on MPH treatment history [$F(2,24) = 5.324$, $p<0.05$; Figure 5 right panel]. Rats that had received chronic 2.5 mg/kg MPH treatment showed increased D3 expression compared to controls and 5 mg/kg MPH treatment ($p<0.05$ s). As this dose was also related to improved Acquisition in the Reversal Learning task, we measured whether there was a correlation between learning rate and D3 mRNA expression and (not shown), however this correlation was not significant.

4. Discussion

Psychostimulants, such as MPH, are widely available for nonmedical use in adolescent populations, who take these drugs for their perceived cognitive enhancing effects. This age also marks a period of extensive development of prefrontal cortex [33], a region of cortex implicated in reward processing and executive control of behavior. To determine how MPH treatment during adolescence affects this type of cognitive function, we examined the effect of MPH on behavioral tasks that rely on prefrontal function [34–37], both during adolescence concurrent with treatment, and in adulthood after treatment was discontinued. Additionally, we examined a potential mechanism for how MPH might affect cognitive function by measuring dopamine receptor expression in the OFC, a subregion of prefrontal cortex important for value-based decision-making that receives a dense dopaminergic input. We found that higher doses of MPH during late adolescence altered discrimination of different sized rewards, but did not impact the processing of delayed rewards. Long-term, a moderate dose of MPH in adolescence enhanced learning and increased D3 receptor mRNA expression in adulthood. Risk-preference in adulthood was not affected by prior MPH treatment, though. Overall, these results indicate that MPH can have both immediate and lasting effect on OFC-dependent cognitive performance and dopaminergic function in rodents.

More specifically, we found that the high dose of MPH, a dose chosen to mimic illicit use, impaired reward discrimination learning on the Magnitude Discrimination task. This suggests that MPH can affect reward sensitivity and choice behavior. However, these changes in discrimination learning were not exhibited in adulthood. This is consistent with experimental evidence demonstrating a decreased preference for natural rewards such as sucrose preference and novelty seeking [11], as well as diminished sensitivity to drugs of abuse [38]. Although discrimination of reward size depended on MPH treatment, we did not find that concurrent MPH treatment altered impulsivity related to immediate versus delayed rewards (Delay Discounting) in normal non-hyperactive adolescent rats. This is contrary to prior literature, which found that MPH can increase preference for the larger delayed rewards in adolescent rats [39]. However, in this latter study, MPH was effective only in adolescent Wistar rats treated with MPH 3 mg/kg or 5 mg/kg tested in a T-maze apparatus, which may be sensitive to locomotor effects of MPH. While we measured a discounting curve across a wide range of delays, this previous study used only a single delay with an acute treatment of the drug. These substantial differences may have contributed to the overall differences seen in performance. MPH has been shown to increase locomotor activity at higher doses ($5 < \text{mg/kg}$; [40,41]) which could potentially interfere with the subjects' ability to complete the task. However, we did not observe any differences in number of trials completed or number of rewards during Magnitude Discrimination.

Pretreatment with a moderate dose of MPH in adolescence enhanced initial acquisition of a simple spatial Reversal Learning task in adulthood, but did not affect the animals' ability to flexibly adapt to changing reward contingencies within the task. While consistent with some previous work, the effects of MPH on learning rate seem to vary depending on task complexity and dosage. Some studies report improved radial arm maze performance after sub-chronic exposure to 1 mg/kg MPH [42] and 3 mg/kg MPH [19]. However, other studies

have also found no effect of lower doses and deterioration in performance after higher doses (5 mg/kg) [42], or that 0.5 mg/kg MPH, but not 2 mg/kg, improved performance [16].

MPH is widely used as a performance enhancing drug in humans in late adolescence/early adulthood (high school/college-aged) to help focus attention and maintain concentration to enhance academic performance [9,10]. While there is evidence that MPH can improve declarative memory [43] and cognitive control [44] in college students, it is important to note that nonmedical users tend to have lower grade point averages [45], calling into question the long term success of such use. Regardless, the results presented here seem to be consistent with findings that MPH exposure can improve learning performance under specific circumstances even when administered in late adolescence.

Although MPH had a lasting effect on learning, risk-preference was not affected by prior MPH treatment. We measured rats' preferences for probabilistic rewards/tolerance for reward omission using a Risk task in which they chose between small-certain and large-risky options. Rats treated with MPH in adolescence showed a trend for increased risk preference in adulthood; however, this was not significantly different from controls on any probability payoff. Overall, all rats showed the ability to discriminate between varying probabilities of large reward delivery. When the probability of large reward was set at 0% (Extinction), rats were able to shift their preference to the small-certain option, although they never completely extinguished lever-pressing for the risky option. This is constant with foraging research that indicates that optimal choice strategy included 'checks' and reassessment of probabilities throughout the learning event [46]. In a similar risk task that used a larger risky reward (5 pellets), saline-treated animals maintained their choice strategy for the larger reinforcement option in spite of adverse consequences on total long-term success, while adolescent-treated MPH rats displayed reduced responding for the large-risky option [15]. This is contrary to the current finding, which indicated that MPH rats maintained responding for the larger reward. This discrepancy might be due to the difference in reward magnitudes, the order of presentation of risk sessions (random vs. progressive), or the developmental window of MPH treatment (later (PD 38–57) vs. earlier (PD 30–46)). However, the lack of difference in risk-preference seen here is likely not caused by a deficit in behavioral flexibility or difference in perceived reinforcement value, as results from the Reversal Learning and Magnitude Discrimination task demonstrate. Trending differences between groups occurred only at the lowest probability (16%) where the expected value of the risky option was less than that of the certain option. It is possible that the lasting effects of MPH are more evident at probabilities that tend to elicit more "risk-averse" behavior.

Significant neurodevelopmental changes in the dopaminergic system during adolescence might underlie age-related cognitive differences. As chronic adolescent MPH can have effects on behaviors that rely on prefrontal signaling, we sought to examine potential changes in OFC that may underlie the behavioral changes. We found that mRNA expression of dopamine D3 receptors was substantially increased after MPH (2.5 mg/kg), but not other doses. The lack of effect of the 5 mg/kg dose on D3 expression is surprising, given the change following the 2.5 mg/kg dose. As a member of the D2-like receptor family, D3 receptors maintain a high affinity for dopamine leading to spontaneous receptor activation [47,48], and may serve as critical modulators due to small changes in receptor number

and/or function [49]. While the role of D3 receptors in cognitive processing in normal humans has not been well-characterized [50,51], D3 receptor seems to play an important role in cognitive performance in rodents [52–54] and monkeys [55,56]. We expected MPH to alter expression of message for dopamine receptors due to prior studies demonstrating the effect of stimulants administered during adolescence on D1 subtypes [20,57] and D2 [58–60]. While there were no differences in D1 or D2 receptor mRNA concentrations, levels of message for D3 receptors were heightened in the same group of animals (MPH 2.5 mg/kg) that demonstrated enhanced acquisition in adulthood; however, we did not find a significant correlation between D3 mRNA expression and learning ability. While we would initially expect blockade of DAT to increase dopamine concentration and reduce receptor levels, long-term MPH administration should eventually lead to an increase in receptors to counter reduced levels of dopamine. Previously, juvenile administration (PD20–35) of MPH has resulted in a decrease in mRNA expression of D3 in the medial PFC [38]. While paradoxical to our results, mPFC and OFC regions have very different functions and are impacted behaviorally by MPH in different ways.

5. Conclusions

Overall, we found that adolescent MPH had short-and long-term effects on learning based on reward processing. The moderate dose of MPH that was associated with long-term improvements in learning also resulted in increased expression of dopamine D3 receptor mRNA expression. Given the widespread nonmedical use of MPH, for either its cognitive enhancement or euphoric effects, it is critical to understand its potential consequences developmentally from adolescence into adulthood. There is a disproportionate number of young males diagnosed and medicated with ADHD [61]. Therefore, while we initially focused on male subjects because of the higher propensity for males to be prescribed this drug, further work needs to be done to determine the effects MPH may cause on cognitive development in females.

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Highlights

- Adolescent methylphenidate (MPH) impaired reward size discrimination
- Delay discounting in adolescence was not affected by MPH
- In adulthood, adolescent MPH improved learning a spatial discrimination
- Expression of D3 receptor message in orbitofrontal cortex was altered by adolescent MPH

Figure 1.

Rats received MPH twice daily for 20 days beginning on PD 38. Behavioral training began on PD 25. Five days of Magnitude Discrimination occurred on PD38–42 followed by the Delay Discounting test (PD39–57). After discontinuation of the drug, rats were trained to lever press and tested on a Reversal Learning paradigm and Risk task which included Magnitude Discrimination and Extinction probe trials. Treatment did not affect normal weight gain across adolescent development or after discontinuation in adulthood.

Figure 2.

Adolescent performed a reward Magnitude Discrimination task subsequently followed by a Delay Discounting task. (A) All groups, except MPH 5 mg/kg, showed an increased preference for LL on at least one other day compared to the initial session. The 5 mg/kg MPH treatment seemed to disrupted reward discrimination learning, with LL preference significantly reduced in this group compared to the 1 and 2.5 mg/kg dose on the 5th session. (B) While all groups discounted the larger reward as its delivery was delayed, there were no differences between groups during the Delay Discounting.

Figure 3.

In Adulthood, rats performed a simple Reversal Learning task with two spatially distinct levers. A ‘correct’ lever press resulted in delivery of two sucrose pellets whereas an ‘incorrect’ response resulted in an omission of the reward. (A) Rats that had been treated with 2.5 mg/kg MPH in adolescence showed enhanced learning performance during Acquisition (left panel). All groups performed similarly in the Reversal test of behavioral flexibility (right panel). * $p < 0.05$ from VEH group. (B) Prior MPH exposure did not alter the type of errors made in adulthood during Reversal Learning. The two types of errors analyzed were perseverative (left panel) and regressive (right panel) errors.

Figure 4.

(A) Rats' preference for probabilistic rewards/tolerance for reward omission was measured using a Risk task in which they chose between small-certain and large-risky options. Rats treated with MPH in adolescence showed a trend for increased risk preference in adulthood however this was not significantly different from controls on any probability payoff (16%, 33% or 67% risk probabilities). (B) Adolescent treatment with MPH also did not cause lasting effects on reward Magnitude Discrimination (100% risk payoff) or Extinction (0% risky payoff).

Figure 5.

Real-time qPCR was conducted on OFC tissue samples of adult rats pretreated with MPH in adolescence to determine whether MPH causes lasting effects on dopamine receptor expression. MPH (2.5 mg/kg; gray) exposure increased D3 mRNA expression in the OFC compared to vehicle controls (white). While D3 mRNA in the 5 mg/kg dose (black) was slightly elevated, there was no difference. There was also no significant difference in expression of D1 or D2 mRNA. * $p < 0.05$ from VEH group.