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Research report

Methylphenidate place conditioning in adolescent rats: An analysis of sex differences and the dopamine transporter

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HIGHLIGHTS

- Methylphenidate produced conditioned place preference in adolescent rats.
- Methylphenidate decreased the dopamine transporter in brain areas of reward.
- Conditioning every second day did not alter methylphenidate conditioned place preference.
- There were no sex differences in methylphenidate conditioned place preference.

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ABSTRACT

In two experiments, we analyzed the effects of methylphenidate (MPH) on conditioned place preference (CPP) in adolescent male and female rats, and the effects of MPH on the dopamine transporter (DAT). In Experiment 1, male and female rats were conditioned for 5 consecutive days from postnatal day (P)44 to P48 with saline, 1, or 5 mg/kg MPH. On the post conditioning preference test, the group administered the 1 mg/kg dose of MPH resulted in no significant preference compared to controls, whereas the 5 mg/kg dose of MPH produced a robust significant preference for the paired context, but there were no sex differences. Analysis of the DAT revealed that animals conditioned with the 5 mg/kg dose of MPH demonstrated a significant decrease of the dopamine transporter (DAT) in the nucleus accumbens and striatum compared to controls. In Experiment 2, animals were conditioned using an every second day paradigm from P33–41 to model a previous MPH treatment regimen that had revealed sex differences in behavioral sensitization. MPH produced an increased preference for the paired context on a post-conditioning preference test in Experiment 2, but as in Experiment 1, no sex differences were observed. These data show that a relatively high dose of MPH has rewarding associative effects in both adolescent male and female rats reliably across two different conditioning paradigms and ages in adolescence, but no sex difference. In addition, MPH results in a significant decrease of the DAT in drug reward brain areas which has implications toward plasticity of the brain's reward system.

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1. Introduction

While the cause of ADHD is still actively being investigated, there is fairly good consensus that stimulant medications can alleviate some of the most common symptoms. One of the most commonly used stimulants for the treatment of ADHD is methylphenidate (MPH) [1]. In addition to its use in the treatment of ADHD, MPH also has potential for abuse due to its induction of reward pathways in the brain, which is attributed to MPH action on the dopamine (DA) system. MPH inhibition of DA reuptake results in increases in extracellular DA levels in the striatum and nucleus accumbens, similar to cocaine [2,3]. In fact, MPH is slightly more potent than cocaine at blocking the DAT both in vitro [4] and in vivo [5]. Although MPH is not abused as frequently as cocaine, recent reports demonstrate an increasing incidence of MPH abuse. For example, reports of MPH misuse in youth (10–19 years of age) showed a seven-fold increase in frequency from 1993–1999 [2], and it is estimated that 5% of college students have used MPH for non-medical use [6]. It is also important to point out that when MPH is recreationally used, it is typically self-administered through

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non-oral routes of administration. Past studies have reported, approximately 75% of MPH abusers are administering the drug through inhalation [7,8].

The conditioned place preference (CPP) behavioral paradigm is a common and procedurally simple behavioral test for the associative rewarding effects of drugs in rodents. In a typical CPP paradigm, animals are injected with a drug temporally paired with one context, with saline temporally paired with a different context over several consecutive days of conditioning. These contexts are distinctive in terms of color and tactile surface, and separated by removable dividers. A drug-free test is given after conditioning, with dividers removed, to test for preference. Animals typically demonstrate a stronger preference for the drug-paired context (for review, see [9]). Past work has shown that MPH, administered across a range of doses and routes of administration, produced CPP in adult male rats [10–12]. Regarding adolescent animals, de la Pena et al. [13] administered 1.25, 5 or 20 mg/kg of MPH to adolescent Wistar or spontaneously hyperactive rats (SHR) for 14 days from P21 to P35 before commencing conditioning from P39 to P45. This study demonstrated that Wistar rats, but not SHR animals, demonstrated CPP to all three doses of MPH. Importantly, this past study analyzed MPH after pre-exposure to the drug. In the present study, animals will not be pre-exposed to MPH, and there have been not been any studies to analyze sex differences on the effects of MPH on CPP in adolescent rats.

The primary neurobiological mechanism for MPH is blockade of the DAT, and there have been several studies to analyze the effects of MPH on DAT protein. In general, MPH has been shown to produce a significant decrease of DAT protein in both younger animals [14] and in adults [15], and reverse the increase in striatal DAT in an animal model of ADHD [16]. Ultimately, a decrease in DAT should result in increased dopaminergic activity at the synapse. Further, in adult male rats, in vivo quantification of the DAT using small animal SPECT discovered a dose-dependent decrease of striatal DAT after iv administration of MPH (3 and 10 mg/kg) 2 h post drug treatment, but nucleus accumbens was not analyzed [17]. Finally, one study analyzed 0.75 and 1.5 mg/kg MPH given for 7 days reported no changes in the DAT of several brain areas, including the nucleus accumbens core, shell, ventral tegmental area (VTA), substantia nigra, and dorsal striatum, although a slight increase (10–15%) in the nucleus accumbens shell was reported [18]. Therefore, although in general it appears that MPH results in a significant decrease of DAT, most of these past studies have analyzed the striatum and not the nucleus accumbens, which is the primary brain center for drug reinforcement and reward. However, and important to the analysis of DAT in the current study, there are no data on whether there may be sex differences in DAT density in response to MPH. Although there are no data to suggest females abuse MPH more readily than males, there are known developmental sex differences in the dopamine system during adolescence [19,20], known sex differences in the conditioned rewarding effects of MPH [21], as well as sex differences in the behavioral and dopaminergic response to psychostimulants (for review, see [22,23]). Based on these past data, we decided to analyze sex differences in the response to MPH in the current study.

The focus of the present study was to analyze the effects of MPH on CPP, a behavioral test of drug reward, in adolescent male and female rats as well as the effects of MPH on the DAT. In the first experiment, we compared a dose consistent with the brain concentration of a clinically therapeutic dose [24,25] to a dose that may be more relevant to abuse (5 mg/kg) in animals behaviorally tested using CPP, and predicted that only the higher dose would result in CPP. In addition, the dorsal striatum and nucleus accumbens were analyzed for DAT protein 24 h after the post-conditioning preference test in animals given saline and the higher dose of MPH (5 mg/kg). In the second experiment, we altered the drug administration paradigm to an every second day MPH administration using the higher dose. We expected that in this experiment, females would demonstrate a more robust CPP based on recent past work from our laboratory demonstrating more robust sensitization to the 5 mg/kg dose of MPH in adolescent female as compared adolescent male rats using an every second day administration paradigm [26]. Further, although we realize that sensitization and CPP test different behavioral mechanisms of drug abuse, we expected a more robust CPP in adolescent females based on past findings that have shown increases in sensitization to psychostimulants typically indicates a more robust dopamine response in brain areas mediating drug reward [27–29]. We expected that placing a non-treatment day in between MPH treatments may result in a plasticity change within the brain's reward system that would ultimately result in a more robust CPP in adolescent female rats in the present study.

2. Experiment 1: methods

2.1. Subjects

A total of 48 Sprague–Dawley rats, 24 males and 24 females were ordered from a commercial breeder (Harlan, Inc., Indianapolis, IN) and used as subjects. All animals were 21 days of age on arrival (P21) and socially housed, 2–3 per cage in a climate-controlled vivarium with a 12 h on/off light/dark cycle. Animals were raised to P43 before behavioral testing. All procedures employed in this study were approved by the East Tennessee State University Committee on Animal Care which is consistent with all procedures and guidelines provided by the NIH Guide on Care and Use of Animals.

2.2. Apparatus

A three chambered CPP apparatus was used, which measured 84 cm × 33 cm. Each of the three contexts was identical in size, measuring 28 cm × 33 cm and was separated by removable dividers and each context was distinct in both visual and tactile cues. One context was painted in vertical stripes, one in horizontal stripes, and the middle context was painted flat gray. Also, the tactile surface was different was across all three contexts with dowel rods in the vertical context, a painted wooden floor in the gray context, and a tin wire mesh floor was used in the horizontal context.

2.3. Initial preference test

All animals were administered an initial preference test on P43. This was conducted to determine if there was an initial context or side preference for any subject. All animals were ip administered saline, and 10 min later placed into the apparatus with dividers removed, and allowed to freely explore the apparatus for a 10 min period.

2.4. Conditioning

Conditioning began the day after the initial preference test on P44 and removable dividers were placed into the apparatus. The assignment of each context was randomized across subjects, based on the finding that no initial preference was shown for any context (see Figs. 1a and 4a). In animals given MPH, the unpaired context was the context which was not temporally paired with MPH, and the paired context was the context which was temporally paired with MPH. Controls were given saline in both contexts. On each conditioning day, in the morning session, all animals were given saline and placed into their assigned context for a 10 min trial. In the afternoon session, animals in the MPH group were administered MPH (1 mg/kg or 5 mg/kg) and 10 min later, placed into the