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Research report

Sex differences, learning flexibility, and striatal dopamine D1 and D2 following adolescent drug exposure in rats

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HIGHLIGHTS

- Adolescent prescription drug exposure may impact learning and emotion in adulthood.
- We found sex differences in discrimination learning and anxiety, assessed in adulthood.
- Methylphenidate-pretreated animals showed attenuated reversal learning.
- Rats that underwent learning and drug exposure showed changes in dopamine receptors.

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ABSTRACT

Corticostriatal circuitry supports flexible reward learning and emotional behavior from the critical neurodevelopmental stage of adolescence through adulthood. It is still poorly understood how prescription drug exposure in adolescence may impact these outcomes in the long-term. We studied adolescent methylphenidate (MPH) and fluoxetine (FLX) exposure in rats and their impact on learning and emotion in adulthood. In Experiment 1, male and female rats were administered MPH, FLX, or saline (SAL), and compared with methamphetamine (mAMPH) treatment beginning in postnatal day (PND) 37. The rats were then tested on discrimination and reversal learning in adulthood. In Experiment 2, animals were administered MPH or SAL also beginning in PND 37 and later tested in adulthood for anxiety levels. In Experiment 3, we analyzed striatal dopamine D1 and D2 receptor expression in adulthood following either extensive learning (after Experiment 1) or more brief emotional measures (after Experiment 2). We found sex differences in discrimination learning and attenuated reversal learning after MPH and only sex differences in adulthood anxiety. In learners, there was enhanced striatal D1, but not D2, after either adolescent MPH or mAMPH. Lastly, also in learners, there was a sex x treatment group interaction for D2, but not D1, driven by the MPH-pretreated females, who expressed significantly higher D2 levels compared to SAL. These results show enduring effects of adolescent MPH on reversal learning in rats. Developmental psychostimulant exposure may interact with learning to enhance D1 expression in adulthood, and affect D2 expression in a sex-dependent manner.

1. Introduction

The adolescent period is characterized by increased risk-taking, reward-seeking, and an enhanced need for environmental stimulation [34,37,38]; characteristics that likely evolved to promote skills for independence [50]. Changes in mesocorticolimbic dopamine (DA) signaling provide much of the basis for this behavioral phenotype. DA D1 receptor (D1) and D2 receptor (D2) densities in the striatum peak at the onset of the rat adolescent period (postnatal day, PND 28) but decrease with maturity [54,15,55]. We recently reported reduced D1 expression, and unaltered D2 expression, in the striatum of animals that had experiences with reward learning during adolescence, when compared to animals that went through the same learning in adulthood [52]. This suggests that learning and the experience of cognitive training may interact with neural

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maturation processes to shape long-lasting expression profiles of, in particular, D1 receptors [62]. Exposure to psychostimulants may also cause robust changes to DA receptors in the developing brain that manifest in long-lasting effects on learning and behavior in adulthood. Adolescent rats treated for 2 months with ADHD medication methylphenidate (MPH, 1 and 2 mg/kg) beginning on PND 30 show significantly reduced D2 binding compared to vehicle-treated rats, as measured by microPET [56]. This is likely meaningful to behavior since low striatal D2 availability has been associated with poor reversal learning and addiction vulnerability [31].

The administration of prescription drugs to adolescents is at an all time high [65,41]. Some of the most commonly-prescribed are MPH for ADHD [48,8,10,20], and fluoxetine (FLX) for the treatment of Major Depression [27,23]. MPH exhibits a similar pharmacological profile to amphetamines and cocaine, may modulate neurodevelopment [21,56,2], and by extension, may impact learning and behavior mediated by corticostriatal circuitry. There is evidence for long-term effects of adolescent MPH on adult locomotor behavior and addiction vulnerability. These effects include increased sensitization to later mAMPH [48], increased cocaine abuse risk [32], increased alcohol intake [19], and increased cocaine-induced reward and behavioral sensitization [1] in adulthood (cf. [20]. The effects of adolescent FLX, conversely, appear limited to significant increases in anxious responding to emotion-eliciting stimuli [24,27]; [23]; cf. [42]. There is relatively little known about the long-lasting effects of adolescent FLX exposure on later adult learning and behavior, though in the adult, FLX results in fewer errors in the early phase of reversal learning [6].

To our knowledge there has not yet been a systematic comparison of the long-term effects of MPH and FLX on adulthood learning flexibility and associated striatal D1 and D2 receptor expression. Additionally, females have not typically been included in these investigations. Therefore, in the present experiments we investigated the effects of adolescent MPH and FLX on later adulthood learning flexibility, and we compared these effects with those following treatment with escalating doses of illicit drug methamphetamine (mAMPH; Experiment 1). In a separate group of animals, we then studied the effects of MPH on anxiety in adulthood (Experiment 2), to ascertain differential effects of MPH on learning experiences vs. emotional reactivity. And finally, in Experiment 3, we assessed striatal dopamine D1 and D2 expression in adulthood following drug treatment and either extensive learning (late adulthood, PND 140) or a more brief emotion test (early adulthood, PND 64). We predicted that adolescent exposure to MPH and mAMPH, but not FLX, would interact with DA receptor expression, to produce enduring effects in learning flexibility measures in adulthood.

2. Methods

2.1. Subjects

Male and female Long–Evans rats (Charles River Laboratories, Inc.) arrived at post-natal day (PND) 28, early rat adolescence [50], weighing between 76 and 100 g, and were socially housed two per cage with both males and females housed in the same room. All rats were habituated to the vivarium from PND 28–31. The rats had unrestricted access to food until behavioral testing began and were provided water *ad libitum*. Rats in Experiment 1 were maintained on a restricted diet during learning (85% of free-feeding weight). We have previously shown that this food scheduling does not compromise the healthy development of young animals: the rats stay within vendor-provided weight ranges for normal growth [52]. Rats in Experiment 2 did not undergo food restriction and were assessed for social play behavior during drug treatment (data not reported here). The vivarium maintained a 12 h light/12 h dark cycle, with the temperature held constant at 22 °C. All drug treatment and behavioral testing took place between 0700 and 0900 h. All procedures were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals and were approved by the Chancellor's Animal Research Committee of UCLA. Order of drug treatment, testing, and euthanasia for Experiments 1–3 are outlined in Fig. 1.

2.2. Handling and drug treatment

Each rat was handled for a minimum of 10 min once per day for 5 consecutive days starting on PND 32, prior to drug treatment. Following handling, rats began treatment at PND 37. Injections were administered once per day for 15 consecutive days. For Experiment 1, drug treatments consisted of MPH (methylphenidate hydrochloride, Sigma, St. Louis, MO; 3 mg/kg, or 1 mg/kg, s.c.), FLX (fluoxetine hydrochloride, Sigma, St Louis, MO; 5 mg/kg or 10 mg/kg, s.c.), each dissolved in physiological saline (SAL) solution, mAMPH (D-methamphetamine hydrochloride, Sigma, St. Louis, MO; 0.1–3.0 mg/kg s.c., increasing in 0.3 mg/kg increments between days) administered as an escalating dose to more closely resemble human recreational consumption, or SAL. The mAMPH group was treated until day 10 of injections and received SAL for the remaining 5 days of injections. This was done to ensure the treatment reached its maximum escalating dose (3 mg/kg) at day 10, to remain more consistent with the duration of treatment in our previous-published study [63]. However, mAMPH treatment in the present experiment was initiated 5 days earlier than in [63]. MPH doses were selected to remain consistent with the range of doses previously published, which are known to produce clinically relevant levels of drug in the plasma [10,17]. For Experiment 2, MPH dosing was selected based on Experiment 1, which showed no significant differences between high and low dose MPH groups on learning. Rats for Experiment 2 were randomly assigned to one of two groups: MPH (3 mg/kg, n = 8) or SAL (n = 8). Injections for both experiments began at 0700 h. The order of injections was counterbalanced by rat identification number, treatment, and sex, and left/right placements of injections were rotated daily. During drug or SAL treatment, rats had access to food and water *ad libitum*.

2.3. Experiment 1

2.3.1. Subjects

Thirty two male (n = 16) and female (n = 16) Long-Evans rats were randomly assigned to one of six groups: MPH high dose (3 mg/kg, n = 6; 3 male, 3 female), MPH low dose (1 mg/kg, n = 4; 2 male, 2 female), FLX high dose (10 mg/kg, n = 6; 3 male, 3 female), FLX low dose (5 mg/kg, n = 4; 2 male, 2 female), mAMPH escalating dose (.3 mg- 3 mg/kg, n = 6; 3 male, 3 female) and SAL (n = 6; 3 male, 3 female).

2.4. Behavioral testing apparatus

Behavioral testing was conducted in eight operant conditioning chambers (Model 80604 Lafayette Instrument Co., Lafayette, IN) that were housed within sound- and light- attenuating cubicles. Each chamber was equipped with a house light, tone generator, video camera, and LCD touchscreen opposing the pellet dispenser. The pellet dispenser delivered single 45 mg dustless precision sucrose pellets. Modified software (ABET II TOUCH) controlled touchscreen stimuli presentation, tone generation, tray- and house-light illumination and pellet dispensation.