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Preadolescent *drd1*-EGFP mice exhibit cocaine-induced behavioral sensitization

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HIGHLIGHTS

- *Drd1*-EGFP mice exhibit higher basal locomotor activity than C57BL/6 mice.
- *Drd1*-EGFP mice express higher levels of D1 and D2 receptors than C57BL/6 mice.
- Preadolescent mice of both strains exhibit cocaine-induced behavioral sensitization.
- Termination of cocaine-induced response is different between the two mice strains.
- Sensitization does not persist in *drd1*-EGFP mice after a 2-week cocaine withdrawal.

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ABSTRACT

In adult mice, repeated cocaine administration induces behavioral sensitization measured as increased horizontal locomotor activity. Cocaine-induced locomotor sensitization has been well characterized in adult mice. In adult animals, the D1 dopamine receptor is important for mediating effects of cocaine. The effect of cocaine on D1 receptor expression and function in preadolescent animals is less understood. The recently described *drd1*-enhanced green fluorescent protein (*drd1*-EGFP) reporter mouse is a useful model for performing such mechanistic studies; however, preadolescent *drd1*-EGFP mice have not been characterized previously. Here we studied cocaine-induced locomotor sensitization in preadolescent *drd1*-EGFP reporter mice. We administered 15 mg/kg cocaine three times daily at 1 h intervals for seven consecutive days beginning on postnatal day 23 to *drd1*-EGFP reporter mice and the commonly used C57BL/6 mice. Under this regimen, preadolescent mice of both strains exhibited cocaine-induced locomotor sensitization; however, by day 7 the cocaine-induced locomotor activity in the *drd1*-EGFP mice was maintained for a longer duration compared to the C57BL/6 mice. The preadolescent *drd1*-EGFP mice also exhibited elevated basal locomotor activity in a novel environment and had higher D1 and D2 dopamine receptor mRNA levels in the caudate nucleus compared to the C57BL/6 mice. The cocaine-induced locomotor sensitization was not retained when the *drd1*-EGFP mice were maintained cocaine-free for two weeks suggesting that in preadolescent *drd1*-EGFP mice the cocaine-induced changes do not persist.

1. Introduction

Drugs that affect the dopaminergic system elicit age dependent effects on behavioral and neurochemical responses [16,35]. In preadolescent mice, the cortico-striatal-thalamo-cortical pathway, which is the primary circuit involved in decision making and motivation, undergoes postnatal developmental maturation [7,13,24,25]. In adult mice this pathway is modulated by the dopaminergic system, which undergoes drugs of abuse-induced long-term neurochemical adaptations [1,23]. While numerous studies have investigated mechanisms underlying cocaine addiction in adult animals and in utero, few studies have investigated the effects of cocaine on preadolescent mice [23, reviewed in 27]. It is of particular interest, given the postnatal developmental maturation that occurs in the brain during the preadolescent period [6,7,17]. Cocaine-induced behavior sensitization is a progressive augmentation of horizontal locomotor activity in response to repeated cocaine administration. Behavior sensitization has been postulated to underlie the neural basis of drug addiction [20,26].

Dopamine receptors are divided into two classes, the D1-like (D1 and D5) and the D2-like (D2, D3 and D4), based on their stimulatory and inhibitory effects on adenylate cyclase, respectively [3].

Abbreviations: EGFP, enhanced green fluorescent protein; PAS, photobeam activity system; ANOVA, analysis of variance; RT-PCR, reverse transcriptase-polymerase chain reaction.

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Using pharmacological and genetic tools it has been demonstrated that the D1 receptor is necessary for cocaine-mediated neural and behavioral responses [5,9,11,32]. The dopaminergic system also undergoes postnatal developmental maturation; in particular, the D1 receptor protein expression levels are low at birth and peak at postnatal day 21 [10,28]. The molecular mechanisms underlying D1 receptor expression and function at the post-weanling, preadolescent developmental stage and the effect of cocaine on these mechanisms are not known. Such studies would be facilitated by the *drd1*-EGFP reporter mouse model characterized in this paper. The *drd1*-EGFP transgenic mice are a relatively new transgenic mouse line developed by the Gene Expression Nervous System Atlas project. This reporter mouse model expresses the enhanced green fluorescent protein (EGFP) in cells that endogenously express the D1 receptor which facilitates the identification and characterization of D1 receptor expression and function in vivo. These mice have been used recently to study D1 receptor function in adult animals [12,15,30]. Given the role of D1 receptor in mediating the effects of cocaine, the *drd1*-EGFP mouse strain is a valuable in vivo model for studying the underlying molecular mechanisms [21]. In this study our primary goal was to determine the effects of cocaine on preadolescent *drd1*-EGFP mice.

2. Experimental procedures

2.1. Animals

All experiments described in this paper were performed with male mice. Two breeding pairs of *drd1*-EGFP mice (Tg(Drd1-a-EGFP)X60Gsat/Mmmh MMRRC:000297) were obtained from the Mutant Mouse Regional Resource Center at University of Missouri, Columbia, Missouri, USA and a local breeding colony established at Rutgers-New Jersey Medical School. The *drd1*-EGFP transgenic mice have a mixed Swiss Webster/FVB genetic background. Male C57BL/6 mice were purchased from Charles River Laboratories (Kingston, NY). The mice were weaned at P21 and used for experiments on P23 or P30. Animals were housed in individual cages on a 12 h light/dark cycle (lights on at 0700), and provided food and water ad lib. All procedures were approved by the IACUC committee at Rutgers-New Jersey Medical School.

2.2. Cocaine administration

Beginning on P23, mice received three daily intraperitoneal (i.p.) injections of saline or 15 mg/kg of cocaine HCl (Medisca, Plattasburgh, NY), 1 h apart, for seven consecutive days in the locomotor arena. The injection volume was 0.2 mL. The dose of cocaine and the binge administration protocol have been previously described [21,22,33].

2.3. Activity measurement

Horizontal locomotor activity was recorded on each of the 7 treatment days using the open field photobeam activity system (PAS; SD Instruments, San Diego, CA). The PAS recording software was programmed to collect data over 4 phases with 12 intervals per phase. Each interval was 300 s long. The animals were placed in the open field for half an hour prior to injections for habituation. Photobeam breaks were collected in 5 min bins for half an hour prior to injections and 1 h after each of three injections for a total recording time of 3.5 h. In some experiments the locomotor activity of naïve non-injected P30 mice were recorded for 30 min. Photobeam breaks were converted to total distance traveled in cm using the PAS reporter software (version 2). The resting time parameter in the software was set at 4 s.

2.4. Brain tissue harvest

Brains were harvested for mRNA analysis from naïve non-injected mice on P30. Whole brain was isolated and immersed in ice-cold saline. Brain sections (300 μm thick) were obtained using a refrigerated Vibratome® 1500 sectioning system (Vibratome, St. Louis, MO) maintained at 3 °C. The nucleus accumbens and caudate brain regions were micro-punched (2 mm) from 300 μm coronal sections obtained from following coordinates – interaural 5.4 mm/bregma 1.94 mm to interaural 3.70 mm/bregma −1.10 mm. The micro-punches for RNA isolation were stored in RNAlater® (Ambion) and stored at −80 °C.

2.5. Real-time reverse transcriptase PCR

RNA isolation and RT-PCR was performed as described previously [18,31]. D1, D2 and D3 dopamine receptor cDNA levels were measured using TaqMan® gene expression assays Mm0135211, Mm00438545 and Mm00432887, respectively. The internal control GAPDH cDNA was detected using Mm99999915 TaqMan® gene expression assay. Appropriate negative and positive controls were included in the RT-PCR experiments [18,28].

2.6. Statistics

One-way, two-way, two-way repeated measure analysis of variance (ANOVA), post hoc multiple comparison tests and two-tailed Student's *t*-test were performed with the SigmaPlot® 11 (SPSS Inc.). For the two-way ANOVA tests, the main factors were treatment and time. Data were considered statistically significant when the probability value (P) was less than 0.05. The number of animals used in each experiment is indicated in the figure legends.

3. Results

3.1. Preadolescent drd1-EGFP and C57BL/6 mice exhibits cocaine-induced locomotor sensitization

To determine if preadolescent mice exhibit cocaine-induced locomotor sensitization, we treated male *drd1*-EGFP and C57BL/6 mice beginning at P23 with saline or cocaine as described in the Experimental procedure section. Statistical analysis of the results in Fig. 1 using two-way repeated measure ANOVA with time and treatment (saline and cocaine) as main factors, suggests that the preadolescent *drd1*-EGFP and C57BL/6 mice exhibits significant cocaine-induced locomotor sensitization commencing day 4 (Fig. 1b and f; $F_{1,123} = 2.918$, $p < 0.001$ and $F_{1,205} = 10.782$, $p < 0.001$, respectively). Two-way ANOVA analysis with days (day 1 and day 7) and treatment (saline and cocaine) groups as main factors, followed by Holm–Sidak post hoc multiple comparison test revealed that the total cocaine-induced locomotor activity of *drd1*-EGFP and C57BL/6 mice is significantly higher on day 7 than on day 1 (Fig. 1d and h; $F_{1,12} = 13.93$, $p = 0.003$ and $F_{1,20} = 18.003$, $p < 0.001$). Initially, in both strains, peak cocaine-induced locomotor activity is observed 5–10 min after each cocaine injection with the locomotor activity returning to baseline in 45–60 min; however, by day 7 the cocaine-induced locomotor activity of the C57BL/6 mice (Fig. 1g) returned to base line faster ($\tau = 0.168 \pm 0.04$) than the *drd1*-EGFP mice ($\tau = 0.0339 \pm 0.01$; Fig. 1c). The decay constant (τ) for cocaine-induced locomotor activity in C57BL/6 mice was significantly different compared to *drd1*-EGFP mice ($p = 0.008$, Student's *t*-test).