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Importance of D1 and D2 receptor stimulation for the induction and expression of cocaine-induced behavioral sensitization in preweanling rats

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Abstract

The behavioral manifestations of psychostimulant-induced sensitization vary markedly between young and adult rats, suggesting that the neural mechanisms mediating this phenomenon differ across ontogeny. In this project we examined the importance of D1 and D2 receptors for the induction and expression of cocaine-induced behavioral sensitization during the preweanling period. In the behavioral experiments, rats were injected with reversible D1 and/or D2 antagonists (SCH23390 and/or raclopride) or an irreversible receptor antagonist (EEDQ) either before cocaine administration on the pretreatment day (induction) or before cocaine challenge on the test day (expression). In the EEDQ experiments, receptor specificity was assessed by using selective dopamine antagonists to protect D1 and/or D2 receptors from inactivation. Receptor binding assays showed that EEDQ caused substantial reductions in dorsal striatal D1 and D2 binding sites, while SCH23390 and raclopride fully protected D1 and D2 receptors from EEDQ-induced alkylation. Behavioral results showed that neither D1 nor D2 receptor stimulation was necessary for the induction of cocaine sensitization in preweanling rats. EEDQ disrupted the sensitization process, suggesting that another receptor type sensitive to EEDQ alkylation was necessary for the induction process. Expression of the sensitized response was prevented by an acute injection of a D1 receptor antagonist. The pattern of DA antagonist-induced effects described for preweanling rats is, with few exceptions, similar to what is observed when the same drugs are administered to adult rats. Thus, it appears that maturational changes in D1 and D2 receptor systems are not responsible for ontogenetic differences in the behavioral manifestation of cocaine sensitization.

Keywords

Cocaine; SCH23390; raclopride; behavioral sensitization; EEDQ; ontogeny

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1. Introduction

The abuse and misuse of psychostimulant drugs (e.g., cocaine, amphetamine, and methamphetamine) continues to be a major public health concern. In the United States, an estimated 1.6 million people illicitly use psychostimulants [1], with initial exposure often occurring before the age of 18 [2]. Although many of these individuals first sample psychostimulants during middle and late adolescence, there is a significant subpopulation that begins experimenting with psychostimulants during late childhood [1–3]. This early-onset drug use has important implications, since the risk of drug dependence increases when first exposure occurs at younger ages [3–5].

Although there is a large and growing addiction literature based on adolescent rodent models [6,7], there are relatively few translational drug studies focusing on the late preweanling period, which is the developmental epoch approximately analogous to late childhood in humans [8]. One of the most frequently employed addiction paradigms is behavioral sensitization, which affords a means of studying the attribution of incentive salience [9–10]. Both cocaine and amphetamine-like compounds produce behavioral sensitization, which is manifested as an augmented behavioral response occurring after repeated drug exposure [11,12]. In the case of adult rats and mice, behavioral sensitization is a robust phenomenon that persists for months after drug discontinuation, and is stronger if drug pretreatment and testing occur in the same environmental context [13–17]. When the pretreatment phase consists of only a single drug exposure (i.e., one-trial behavioral sensitization), the sensitized responding of adult rodents is context-dependent [18–21]. During early ontogeny, however, the sensitized responding of preweanling rats is weaker than in adults, and persists for only days to weeks rather than months [22]. In terms of the one-trial paradigm, the behavioral sensitization exhibited by preweanling rats is context-independent (i.e., it does not rely on the formation of Pavlovian associations) and persists for just a few days [23–26]. These age-dependent differences suggest that the neural mechanisms underlying behavioral sensitization undergo substantial modification across ontogeny.

When considering the mechanisms responsible for behavioral sensitization it is important to distinguish between induction (i.e., development of behavioral sensitization) and expression, as these processes are mediated by distinct patterns of neuronal modifications occurring in multiple brain regions [27,28]. In adult animals, the neuroadaptations governing the expression of the sensitized response also differ depending on whether drug challenge occurs days (short-term) or weeks (long-term) after drug discontinuation [28]. In terms of the induction process, neither D1 nor D2 receptor stimulation is necessary for the initial development of cocaine-induced behavioral sensitization in adult rats or mice [29–32]. The major exception involves the one-trial paradigm, since induction is disrupted if D1 or D2 receptor antagonists are administered before cocaine pretreatment [18,19; but see 33]. A different pattern of results is evident when dopamine (DA) antagonists are administered to adult animals on the test day, because selective antagonism of the D1 receptor, but not the D2 receptor, blocks the expression of multi-trial cocaine sensitization [32,34]. If a one-trial procedure is used, neither D1 nor D2 receptor antagonists prevent the expression of cocaine sensitization in adult rats [18,19]. Therefore, it appears that different neural mechanisms, at least in terms of DA receptor involvement, mediate the one-trial and multi-trial behavioral

sensitization of adult animals. It is also the case that different neural mechanisms mediate cocaine- and amphetamine-induced behavioral sensitization [28,32].

Although relatively few DA antagonist studies have been conducted using preweanling rats, available evidence suggests that DA receptor blockade may affect the cocaine-induced sensitized responding of young rats differently than adults. The most notable ontogenetic finding is that D1 receptor blockade does not prevent the induction of cocaine-induced one-trial behavioral sensitization during the late preweanling period [35]. This result is in direct contrast to findings from adult rat and mouse studies showing that D1 receptor antagonism prevents the induction of one-trial cocaine sensitization in adult animals [19,33]. No other published studies have examined the effects of DA receptor antagonists on cocaine sensitization in young animals; therefore, the role that D2 receptors play in the induction of cocaine sensitization has not been reported, nor has the potential importance of D1 and D2 receptors for the expression of one-trial cocaine-induced behavioral sensitization been examined in rats from the preweanling period.

The purpose of the present study was to determine whether D1 and/or D2 receptor stimulation is necessary for the induction and expression of cocaine-induced behavioral sensitization in preweanling rats. To this end, selective D1 and/or D2 receptor antagonists (SCH23390 and raclopride, respectively) were administered either before cocaine pretreatment (induction) or cocaine challenge (expression). These experiments employed a one-trial sensitization procedure in order to minimize the effects of tolerance and dependence, while permitting an unbiased measure of induction and expression [33]. In separate experiments, the irreversible receptor antagonist *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ), which has a high affinity for D1 and D2, 5-HT_{1A} and 5-HT_{2A}, α_2 -adrenergic, and GABA_A receptors [36–42], was administered 24 h before the induction or expression of behavioral sensitization. To determine the relative involvement of DA receptors in these processes, selective DA antagonists were used to protect D1 and/or D2 receptors from EEDQ-induced receptor alkylation [43–46].

2. Materials and Methods

2.1. Subjects

Subjects were 426 male and 426 female rats of Sprague-Dawley descent (Charles River, Hollister, CA) that were born and bred in the university vivarium. Litters were culled to ten pups at PD 3 (day of parturition is PD 0). Preweanling rats were housed with their dam and littermates in large polycarbonate maternity cages (30.5 × 43 × 19 cm) on ventilated racks. Food and water were freely available. The colony room was maintained at 22–24 °C and kept under a 12:12 light-dark cycle. Behavioral testing was conducted in a separate experimental room during the light phase of the cycle. Subjects were cared for according to the “Guide for the Care and Use of Laboratory Animals” [47] under a research protocol approved by the university’s Institutional Animal Care and Use Committee.

2.2. Apparatus

Behavioral testing was done in activity monitoring chambers (25.5 × 25.5 × 41 cm) that consisted of acrylic walls, a plastic floor, and an open top (Coulbourn Instruments, Allentown, PA). Each chamber included an X–Y photobeam array, with 16 photocells and detectors, that was used to determine distance traveled (a measure of horizontal locomotor activity). Photobeam resolution was 0.76 cm, with the position of each rat being determined every 100 ms.

2.3. Drugs

(–)-Cocaine hydrochloride, S(–)-raclopride (+)-tartrate salt, and R(+)-SCH23390 hydrochloride were dissolved in saline; whereas, EEDQ was dissolved in a 50% DMSO solution [1:1 (v/v) in distilled water]. Drugs were purchased from Sigma-Aldrich (St. Louis, MO) and injected intraperitoneally (ip) at a volume of 5 ml/kg.

2.4. Experiments 1a, 1b, and 1c: Effects of D2 or D1/D2 receptor antagonism on the induction of cocaine-induced behavioral sensitization

On the pretreatment day (PD 20) of Experiment 1a, rats ($N=48$) were injected with raclopride (0, 0.1, 0.5, 1, or 5 mg/kg) followed, 15 min later, by an injection of 30 mg/kg cocaine. Rats in the saline-saline group (i.e., the acute control group) were given two injections of saline. After the second injection, rats were placed in activity chambers where distance traveled was measured for 30 min. Experiment 1b was designed to further assess the effects of a moderate dose of raclopride on the induction of behavioral sensitization. Rats ($N=40$) were administered 0 or 0.5 mg/kg raclopride on the pretreatment day followed, 15 min later, by an injection of saline or 30 mg/kg cocaine. The procedures for Experiment 1c were similar to those just described, with the exception that rats ($N=24$) were given two injections of saline or a cocktail of SCH23390+raclopride (0.5 mg/kg each) on the pretreatment day (PD 20). These injections were followed, 15 min later, by an injection of 30 mg/kg cocaine. Rats were then placed in the activity chambers for 30 min.

For these experiments, the test day occurred 24 h later (i.e., on PD 21). On the test day, all rats ($n=4$ males and 4 females per group) were injected with 20 mg/kg cocaine and placed in activity chambers for 120 min. This general methodology (i.e., administering antagonists 15 min prior to DA agonist treatment and testing rats 24 hr later) is similar to past studies [18,19,35]. One-trial sensitization studies typically use higher doses of cocaine than multi-trial studies. For example, parametric studies manipulating the pretreatment and test day doses of cocaine have shown that 30 and 20 mg/kg cocaine (pretreatment day and test day, respectively) produces strong one-trial locomotor sensitization in preweanling rats [23,48], whereas 40 and 10 mg/kg cocaine, respectively, causes robust one-trial locomotor sensitization in adult rats [18,19].

2.5. Experiment 2: Effects of EEDQ on the induction of cocaine-induced behavioral sensitization

On the preinjection day (PD 18), rats ($N=48$) were injected with EEDQ (0, 7.5, or 15 mg/kg) and immediately returned to their home cage. On the pretreatment day, which occurred 24 h later (i.e., on PD 19), the groups were further subdivided with half of the rats

being injected with saline and the other half with 30 mg/kg cocaine. Immediately afterwards, rats were placed in activity chambers for 30 min and distance traveled was recorded. On the test day (PD 21), all rats ($n = 4$ males and 4 females per group) were injected with 20 mg/kg cocaine and placed in activity chambers for 120 min.

2.6. Experiment 3: The use of receptor protection to further examine the effects of EEDQ on the induction of cocaine-induced behavioral sensitization

On the preinjection day (PD 18), rats ($N = 80$) received a single injection of saline (nonprotected group), 1 mg/kg SCH23390 (D1 protected group), 10 mg/kg raclopride (D2 protected group), or a cocktail of SCH23390+raclopride. Only these high doses of SCH23390 and raclopride are sufficient to protect D1 and/or D2 receptors from EEDQ-induced alkylation [45,46,49,50]. After 30 min, rats were injected with 7.5 mg/kg EEDQ. For comparison purposes, separate groups of nonprotected rats were injected with vehicle rather than EEDQ.

On the pretreatment day, which occurred 24 h later (i.e., on PD 19), the groups were further subdivided with half of the rats being injected with saline and the other half with 30 mg/kg cocaine. Immediately afterwards, rats were placed in activity chambers and distance traveled was recorded for 30 min. On the test day (PD 21), all rats ($n = 4$ males and 4 females per group) were injected with 20 mg/kg cocaine and placed in activity chambers for 120 min. In summary, there were 10 groups in this experiment receiving different drug sequences across four days (Table 1).

2.7. Experiments 4a and 4b: Effects of D1, D2, or D1/D2 receptor antagonism on the expression of cocaine-induced behavioral sensitization

On the pretreatment day (PD 20), half of the rats were injected with saline and the other half were given a single injection of 30 mg/kg cocaine. Immediately afterwards, rats were placed in activity chambers for 30 min.

On the test day, which occurred 24 h later (i.e., on PD 21), an equal number of saline- and cocaine-pretreated rats were injected with 0, 0.1, or 0.5 mg/kg raclopride (Experiment 4a). After 15 min, all rats ($N = 48$) were given a test day injection of 20 mg/kg cocaine and placed in activity chambers for 120 min. In Experiment 4b, saline- and cocaine-pretreated rats were injected with SCH23390 (0, 0.1, or 0.5 mg/kg) or cocktails of SCH23390+raclopride (0.1 or 0.5 mg/kg each). After 15 min, all rats ($N = 80$) were injected with 20 mg/kg cocaine and distance traveled was measured for 120 min.

2.8. Experiments 5: Effects of EEDQ on the expression of cocaine-induced behavioral sensitization

On the pretreatment day (PD 19), rats ($N = 48$) were injected with saline or 30 mg/kg cocaine and placed in activity chambers for 30 min. On the preinjection day, which occurred 24 h later (i.e., on PD 20), an equal number of saline- and cocaine-pretreated rats were injected with 0, 7.5, or 15 mg/kg EEDQ and returned to their home cage. On the test day (PD 21), all rats ($n = 4$ males and 4 females per group) were injected with 20 mg/kg cocaine and placed in activity chambers for 120 min. No companion receptor protection experiment

was reported, because the high doses of SCH23390 and raclopride needed to protect D1 and D2 receptors from EEDQ-induced inactivation altered behavior on the test day (data not shown).

2.9. Experiment 6: Dopamine D1 and D2 receptor densities in the dorsal striatum after EEDQ and/or receptor protection

On the preinjection day, an equal number of male and female rats ($N = 30$ per assay) received a single injection of saline (nonprotected group), 1 mg/kg SCH23390 (D1 protected group), 10 mg/kg raclopride (D2 protected group), or a cocktail of SCH23390+raclopride. After 30 min, rats were injected with vehicle or 7.5 mg/kg EEDQ. Age at preinjection (PD 18 or PD 20) was counterbalanced according to group.

After 24 h, rats were rapidly decapitated and dorsal striatal sections were dissected bilaterally and stored at -80°C . On the day of assay, tissue was homogenized in 100 volumes of 50 mM Tris-HCl buffer (pH 7.4) for approximately 20 s using a Brinkmann Polytron. Homogenates were then centrifuged at $20,000 \times g$ for 20 min. The pellet was resuspended in 100 volumes of the same buffer and centrifuged again at $20,000 \times g$ for 20 min. The final pellet was suspended in approximately 30 volumes of buffer (pH 7.4). Protein concentrations for the final pellet were determined using the Bio-Rad Protein Assay.

For both the D1 and D2 receptor binding assays, tissue suspensions (35–70 $\mu\text{g}/\text{protein}$) were added to duplicate tubes containing 50 mM Tris, 2 mM NaCl_2 , 5 mM KCl, 1 mM MgSO_4 , and 2 mM CaCl_2 (pH 7.4) at a final volume of 1 ml. For the D1 assay, tubes included [^3H]-SCH23390 in concentrations ranging from 0.01 to 10 nM. Nonspecific binding was determined in the presence of 10 μM (+)-butaclamol. To prevent binding of [^3H]-SCH23390 to serotonin receptors 100 nM mianserin was added to all tubes. For the D2 assay, tubes included [^3H]-raclopride in concentrations ranging from 0.15 to 20 nM. Nonspecific binding was determined in the presence of 100 μM (–)-sulpiride. Incubation time for the D1 assay was 30 min at 37°C , while incubation time for the D2 assay was 45 min at 32°C . Incubation was terminated by vacuum filtration over glass fiber filters (Whatman GF/B, pretreated with 0.1% polyethylenimine). Filters were washed twice with ice-cold Tris-HCl buffer and radioactivity was measured by liquid scintillation spectrometry.

2.10. Data Analysis

Distance traveled data were analyzed using one- or two-way analyses of variance (ANOVA) depending on experiment, while separate one-way ANOVAs were used to analyze D1 and D2 binding site (B_{max}) and affinity (K_d) data from the homogenate ligand binding assays. B_{max} and K_d values were determined using nonlinear regression with Prism (GraphPad Software, San Diego, CA). Post hoc analysis of behavioral and receptor binding data was made using Tukey tests ($p < 0.05$).

Litter effects were minimized by assigning no more than one subject from each litter to a particular group [51]. When this procedure was not possible, a single litter mean was calculated from multiple littermates assigned to the same group [51,52]. Litter effects were also controlled through statistical procedures, as litter was used as the unit of analysis for statistical purposes [51]. With this statistical model each litter, rather than each rat, is treated

as an independent observation (i.e., a within-subject analysis using one value/condition/litter). This statistical approach does not allow sex to be included as a factor in the analyses. Because an equal number of males and females were assigned to each group, between-subject ANOVAs were used to statistically analyze the sex variable.

3. Results

3.1. Experiment 1a: Effects of D2 receptor antagonism on the induction of cocaine-induced behavioral sensitization

3.1.1. Pretreatment day—On the pretreatment day, rats injected with cocaine alone (i.e., the 0 mg/kg Raclopride–Cocaine group) or 0.1 mg/kg raclopride and cocaine showed more locomotor activity than the saline controls (Figure 1, upper graph) [Drug main effect, $F(5, 35) = 7.99$, $p < 0.001$; and Tukey tests, $p < 0.05$]. Rats injected with cocaine and either of the two highest doses of raclopride (1 and 5 mg/kg) exhibited less locomotion than rats given cocaine alone [Tukey tests, $p < 0.05$].

3.1.2. Test day—On the test day, behavioral sensitization was evident since rats treated with cocaine alone (i.e., 0 mg/kg raclopride and cocaine) exhibited more locomotor activity than the acute control group (Figure 1, lower graph) [Drug main effect, $F(5, 35) = 3.83$, $p < 0.01$; and Tukey tests, $p < 0.05$]. Although no dose of raclopride (0.1–5 mg/kg) significantly reduced the locomotor activity of cocaine-pretreated rats, the omnibus ANOVA suggested that only rats treated with 0.1 mg/kg raclopride and cocaine exhibited more locomotor activity than the acute controls. A more targeted ANOVA, which included only the high-dose raclopride groups, showed that locomotor activity was also significantly elevated, relative to the acute control group, in rats receiving 1 or 5 mg/kg raclopride plus cocaine [Drug main effect, $F(2, 14) = 5.29$, $p < 0.05$; and Tukey tests, $p < 0.05$]. The effects of 0.5 mg/kg raclopride were more ambiguous, since this group neither differed from the acute control group nor the cocaine alone group. To clarify this finding, Experiment 1b was conducted to determine whether 0.5 mg/kg raclopride blocks the induction of behavioral sensitization or leaves the sensitized responding of preweanling rats unaffected. On neither the pretreatment day nor the test day did distance traveled scores vary according to sex.

3.2. Experiment 1b: Effects of 0.5 mg/kg raclopride on the induction of cocaine-induced behavioral sensitization

3.2.1. Pretreatment day—On the pretreatment day, rats injected with cocaine (i.e., the 0 mg/kg Raclopride–Cocaine group and the 0.5 mg/kg Raclopride–Cocaine group) exhibited more locomotor activity than the saline controls (Figure 2, upper graph) [Pretreatment $\times$ Drug interaction, $F(1, 7) = 5.89$, $p < 0.05$; and Tukey tests, $p < 0.05$]. Raclopride (0.5 mg/kg) significantly reduced the cocaine-induced locomotor activity of preweanling rats [Tukey tests, $p < 0.05$].

3.2.2. Test day—On the test day (Figure 2, lower graph), locomotor sensitization occurred since cocaine-pretreated rats showed more locomotor activity than the acute controls [Drug main effect, $F(1, 7) = 26.01$, $p < 0.001$]. Administering 0.5 mg/kg raclopride on the pretreatment day did not reduce the locomotor activity of the acute control or sensitization

groups. Thus, administering 0.5 mg/kg raclopride on the pretreatment day neither blocked the induction of cocaine sensitization, nor carried over and affected basal locomotor activity on the test day. Sensitized responding did not differ according to sex.

3.3. Experiment 1c: Effects of D1/D2 receptor antagonism on the induction of cocaine-induced behavioral sensitization

3.3.1. Pretreatment day—On the pretreatment day (Figure 3, upper graph), rats given only cocaine (i.e., the 0 mg/kg SCH23390+Raclopride-Cocaine group) showed more locomotor activity than the saline controls or rats pretreated with a cocktail of 0.5 mg/kg SCH23390+raclopride and cocaine [Drug main effect, $F(2, 14) = 25.65$, $p < 0.001$; and Tukey tests, $p < 0.05$].

3.3.2. Test day—On the test day (Figure 3, lower graph), rats pretreated with cocaine evidenced more locomotor activity than the acute control group [Drug main effect, $F(2, 35) = 22.62$, $p < 0.001$; and Tukey tests, $p < 0.05$]. A pretreatment injection of SCH23390+raclopride did not diminish the strength of the sensitized response. No sex differences were apparent.

3.4. Experiment 2: Effects of EEDQ on the induction of cocaine-induced behavioral sensitization

3.4.1. Pretreatment day—When compared to the saline groups, cocaine-treated rats showed elevated locomotor activity on the pretreatment day (Figure 4, upper graph) [Drug main effect, $F(1, 7) = 126.08$, $p < 0.001$; and Tukey tests, $p < 0.05$]. EEDQ did not significantly affect locomotion, although the higher dose of EEDQ (15 mg/kg) appeared to induce a nonsignificant decline in the locomotor activity of cocaine-treated rats.

3.4.2. Test day—On the test day (Figure 4, lower graph), locomotor sensitization was apparent since the cocaine alone group (i.e., the 0 mg/kg EEDQ-Sensitization group; the black bar) exhibited more locomotor activity than the acute control group given 0 mg/kg EEDQ (the white bar) [Preinjection $\times$ Drug interaction, $F(2, 14) = 13.60$, $p < 0.001$; and Tukey tests, $p < 0.05$]. Both doses of EEDQ (7.5 and 15 mg/kg) fully attenuated the sensitized locomotor response [Tukey tests, $P < 0.05$]. A between-subject ANOVA indicated that the sex variable did not interact with the preinjection or drug variables to affect behavior.

3.5. Experiment 3: The use of receptor protection to further examine the effects of EEDQ on the induction of cocaine-induced behavioral sensitization

3.5.1. Pretreatment day—Cocaine significantly increased the locomotor activity of young rats on the pretreatment day (Figure 5, upper graph) [Drug main effect, $F(1, 7) = 35.42$, $p < 0.001$]. The locomotor activity exhibited by saline- and cocaine-treated rats was not altered by EEDQ or any of the protection treatments (i.e., SCH23390, raclopride, or SCH/RAC).

3.5.2. Test day—On the test day (Figure 5, lower graph), a sensitized locomotor response was evident, since the cocaine alone group (i.e., the Nonprotected + Vehicle-Sensitization

group; the black bar) exhibited greater locomotor activity than the acute control group in the Nonprotected + Vehicle condition (the white bar) [Preinjection $\times$ Drug interaction, $F(4, 28) = 6.68$, $p < 0.001$; and Tukey tests, $p < 0.05$]. EEDQ significantly reduced the test day locomotor activity of cocaine-pretreated rats (Figure 5, lower graph, right panel), but did not affect the locomotion of saline-pretreated rats (left panel) [Preinjection $\times$ Drug interaction; and Tukey tests, $p < 0.05$]. Importantly, D1 or D2 protection (i.e., SCH23390, raclopride, or SCH+RAC treatment) did not impact the ability of EEDQ to attenuate cocaine's actions. Also, the sex variable did not differentially affect locomotor activity on the pretreatment day or sensitized responding on the test day.

3.6. Experiment 4a: Effects of D2 receptor antagonism on the expression of cocaine-induced behavioral sensitization

On the pretreatment day, cocaine ($M = 8,835$ cm, $SEM = 520$) stimulated more locomotor activity than saline ($M = 2,270$ cm, $SEM = 192$) [Drug effect, $t(7) = 10.72$, $p < 0.001$]. On the test day, cocaine-pretreated rats exhibited significantly more locomotor activity than saline-pretreated rats (Figure 6) [Drug main effect, $F(1, 7) = 195.97$, $p < 0.001$]. The greater dose of raclopride (0.5 mg/kg) significantly decreased the locomotion of preweanling rats [Treatment main effect, $F(2, 14) = 11.10$, $p < 0.001$], with planned comparisons showing that 0.5 mg/kg raclopride significantly reduced the locomotor activity of rats in both the acute control (saline-pretreated) and sensitization (cocaine-pretreated) groups [Tukey tests, $p < 0.05$]. The expression of behavioral sensitization did not differ according to sex.

3.7. Experiment 4b: Effects of D1 or D1/D2 receptor antagonism on the expression of cocaine-induced behavioral sensitization

On the pretreatment day, cocaine-treated rats ($M = 8,394$ cm, $SEM = 350$) had greater locomotor activity scores than saline-treated rats ($M = 3,114$ cm, $SEM = 332$) [Drug effect, $t(7) = 12.29$, $p < 0.001$]. A sensitized locomotor response was apparent on the test day (Figure 7), since the cocaine alone group (i.e., the 0 mg/kg SCH23390-Sensitization group; the black bar) exhibited more locomotor activity than the acute control group receiving 0 mg/kg SCH23390 (the white bar) [Treatment $\times$ Drug interaction, $F(4, 28) = 13.51$, $p < 0.001$; and Tukey tests, $p < 0.05$]. Among the acute control groups, the higher dose of SCH23390 (0.5 mg/kg) and the combined doses of SCH23390+raclopride (0.1 and 0.5 mg/kg) significantly reduced test day locomotor activity. Among the sensitization groups, both doses of SCH23390 (0.1 and 0.5 mg/kg) decreased locomotor activity on the test day; whereas, combined treatment with SCH23390+raclopride (0.1 and 0.5 mg/kg) caused a potentiated decline in locomotion [Tukey tests, $p < 0.05$]. Once again, no sex differences were apparent.

3.8. Experiment 5: Effects of EEDQ on the expression of cocaine-induced behavioral sensitization

On the pretreatment day, rats injected with cocaine ($M = 8,055$ cm, $SEM = 343$) exhibited more locomotor activity than rats given saline ($M = 2,118$ cm, $SEM = 218$) [Drug effect, $t(7) = 10.81$, $p < 0.001$]. On the test day (Figure 8), behavioral sensitization was evident since rats treated with cocaine alone (i.e., the 0 mg/kg EEDQ-Sensitization group; the black bar) were significantly more active than the acute control group injected with 0 mg/kg EEDQ

(the white bar) [Preinjection $\times$ Drug interaction, $F(2, 14) = 9.16$, $p < 0.01$; and Tukey tests, $p < 0.05$]. EEDQ, regardless of dose, blocked expression of the sensitized response, as the cocaine groups preinjected with EEDQ both exhibited less locomotor activity than the cocaine alone group and did not differ from the acute control groups [Preinjection $\times$ Drug interaction; and Tukey tests, $p < 0.05$]. Neither EEDQ nor cocaine differentially affected the locomotor activity of male and female rats.

3.9. Experiment 6: Dopamine D1 and D2 receptor densities in the dorsal striatum after EEDQ and/or receptor protection

Administering EEDQ alone (i.e., the Saline–EEDQ group) significantly decreased the dorsal striatal D1 and D2 binding sites ($B_{\max}$) of preweanling rats (Table 2) [Treatment effects, $F(4, 20) = 31.74$, $p < 0.001$; $F(4, 20) = 10.97$, $p < 0.001$, respectively]. EEDQ caused a 67% depletion of D1 binding sites, and a 57% depletion of D2 sites. SCH23390 (1 mg/kg) and raclopride (10 mg/kg), given either alone or in combination, fully protected D1 and D2 receptors, respectively, from EEDQ-induced receptor inactivation. K_d values (i.e., receptor affinity) were not affected by EEDQ or pretreatment injections of SCH23390 or raclopride (data not shown). Neither $B_{\max}$ nor K_d was differentially affected by sex or age at drug injection (PD 18 or PD 20).

4. Discussion

4.1. Behavioral effects of reversible DA receptor antagonists

4.1.1. Induction of behavioral sensitization—The purpose of the present study was to determine the role of D1 and D2 receptors for the induction and expression of short-term cocaine-induced behavioral sensitization during the preweanling period. The preweanling period was of interest because it is approximately analogous to late childhood in humans [8], and is a time when early-onset drug use often begins [1–3]. In terms of the induction process, pharmacological blockade of D2 receptors did not prevent the development of cocaine sensitization. Specifically, neither low, moderate, or high doses of raclopride (0.1–5 mg/kg) were able to significantly attenuate the cocaine-induced sensitized responding of preweanling rats. At higher doses (> 0.5 mg/kg), raclopride has nonspecific actions, but the present results serve to emphasize that nearly maximal D2 receptor blockade did not eliminate or even significantly reduce the strength of the sensitized response in preweanling rats. Combined antagonism of D1 and D2 receptors with 0.5 mg/kg raclopride and SCH23390 was also unable to prevent the induction of cocaine sensitization. Therefore, when the results of the present experiments are considered together with past studies, it appears that selective blockade of D1 receptors alone [35], D2 receptors alone (present study), or both D1 and D2 receptors (present study) does not disrupt the induction of one-trial cocaine-induced behavioral sensitization during the late preweanling period.

Surprisingly, adult rat and mice actually exhibit a more inconsistent pattern of antagonist-induced effects than younger animals, as D1 and D2 receptor antagonists block the induction of one-trial [18,19], but not multi-trial [29–32], cocaine sensitization during adulthood. Various authors have explained this inconsistency by suggesting that DA receptors mediate the associative components of behavioral sensitization in adult rats and mice. Because the

one-trial behavioral sensitization of older rats is context-dependent [18,20,21,53], only this form of sensitization should be sensitive to D1 and D2 receptor antagonism [19,32]. According to the same logic, D1 and D2 receptor antagonists should not disrupt the induction process during the preweanling period since the one-trial behavioral sensitization of preweanling rats is context-independent [23–26].

4.1.2. Expression of behavioral sensitization—In terms of expression, analysis of the present data indicates that D2 receptor stimulation is not necessary for the expression of behavioral sensitization during the preweanling period. Specifically, treating rats with 0.1 mg/kg raclopride before a challenge injection of cocaine did not alter expression of the sensitized response. Administering a higher dose of raclopride (0.5 mg/kg) decreased the test day locomotor activity of both saline- and cocaine-pretreated rats, suggesting that D2 receptor antagonism caused a disruption of motoric ability independent of the sensitization process. In a like manner, the motoric and sensitization inhibiting effects of D1 receptor antagonism were confounded when a higher dose of SCH23390 (0.5 mg/kg) was administered on the test day. Importantly, 0.1 mg/kg SCH23390 prevented expression of the sensitized response, without significantly altering the locomotor activity of rats treated acutely with cocaine (i.e., the acute control groups). Therefore, data gained using the lower dose of SCH23390 provides unambiguous evidence that D1 receptor stimulation is necessary for the expression of a sensitized response in preweanling rats. In many respects these results are similar to what is found during adulthood, since D2 receptor antagonists do not disrupt the expression of one- or multi-trial cocaine sensitization in adult rats [18,19,32,34]. D1 receptor stimulation, on the other hand, is necessary for the expression of multi-trial cocaine sensitization [32], but not one-trial sensitization [19]. The former study used a higher dose of SCH23390 (0.1 vs. 0.02 mg/kg) than the latter, leaving open the possibility that 0.02 mg/kg SCH23390 was insufficient to prevent the expression of one-trial cocaine sensitization in adult rats.

Co-administering 0.1 mg/kg SCH23390 and raclopride on the test day caused a further reduction in the locomotor activity of cocaine-pretreated rats, as well as a modest decline in the motoric performance of saline-pretreated rats (the same doses did not affect basal locomotion when given alone). This pattern of results suggests that combined D1/D2 receptor blockade caused a reduction in motoric capacity, although it remains possible that SCH23390's ability to inhibit expression is potentiated under conditions of reduced D2-mediated neurotransmission. In an additional experiment (data not shown), administering a lower dose cocktail of D1 and D2 receptor antagonists (0.05 mg/kg SCH23390 and 0.1 mg/kg raclopride) did not prevent the expression of cocaine-induced behavioral sensitization on PD 21.

4.1.3. Importance of drug-induced locomotor activity on the pretreatment day—In terms of the pretreatment day, raclopride (0.5–5 mg/kg) or combined treatment with SCH23390 plus raclopride (0.5 mg/kg each) caused a significant reduction in the cocaine-induced locomotor activity of preweanling rats. In Experiments 4a and 4b, a similar decline in locomotor activity was observed when SCH23390 (0.5 mg/kg) or raclopride (0.5 mg/kg) was administered prior to an acute injection of 20 mg/kg cocaine [54–56]. Importantly, the

expression of behavioral sensitization on the test day is not dependent on whether psychostimulant-induced locomotor activity was present or absent on the pretreatment day. For example, an antagonist may leave locomotion unaffected on the pretreatment day, yet still attenuate sensitized responding on the test day [32,57]. Conversely, a full sensitized response may be evident on the test day despite psychostimulant-induced locomotor activity being reduced or prevented on the pretreatment day [29–32,57]. In perhaps the most extreme example, preweanling and adult rats anesthetized during the pretreatment phase (i.e., no locomotor activity was possible) exhibit behavioral sensitization on the test day [23,58]. In the present study, both raclopride (0.5–5 mg/kg) and SCH23390+raclopride (0.5 mg/kg) significantly reduced the acute locomotor activating effects of cocaine on the pretreatment day, yet did not prevent the eventual occurrence of behavioral sensitization on the test day.

4.2. Behavioral and neural effects of EEDQ

Results from the EEDQ experiments are generally consistent with findings from the reversible antagonist experiments just discussed. For example, the expression of cocaine-induced behavioral sensitization was prevented by both EEDQ and SCH23390. Moreover, the finding that raclopride did not prevent the induction of locomotor sensitization is generally consistent with Experiment 3, which showed that EEDQ pretreatment blocked cocaine sensitization through a nondopaminergic mechanism. Although protection experiments can be used to determine receptor specificity, EEDQ is relatively nonselective and inactivates various receptor types, including 5-HT_{1A}, 5-HT_{2A}, and α_2 -adrenergic receptors among others [40–42]. For this reason, it is possible that EEDQ disrupted behavioral sensitization by inactivating one or more of these nondopaminergic receptor types. Consistent with this line of reasoning, antagonists of various serotonin receptors (e.g., 5-HT_{1A}, 5-HT_{2C}, and 5-HT₃) prevent the induction of cocaine-induced sensitization in adult rats and mice [59–61]. In fact, Auclair et al. [62] proposed that the induction of psychostimulant-induced behavioral sensitization is entirely mediated by 5-HT_{2A} and α_{1B} -adrenergic receptors, which they suggest are components of semi-redundant pathways that do not require DA receptor stimulation.

When the pretreatment day and test day are close together in time, it is important to consider whether DA antagonist treatment is exclusively affecting the neural mechanisms responsible for induction or expression, or whether the drugs are affecting both processes. In terms of reversible DA antagonists, Kuribara [63–65] has shown that these drugs are capable of affecting the induction process when administered 3 h, but not 6 h or 24 h, *after* psychostimulant pretreatment. In the present study, test day injections of SCH23390 and raclopride were administered 24 h after cocaine pretreatment, so any antagonist-induced effects involved the process of expression rather than induction. The same evidence suggests that administering EEDQ 24 h after cocaine pretreatment would be exclusively affecting expression. The same cannot be said when EEDQ is administered 24 h prior to cocaine pretreatment. Although DA receptors repopulate quickly, especially in younger animals, injecting EEDQ on PD 18 (one day prior to cocaine pretreatment) would still result in modest levels of DA receptor depletion on PD 21 (i.e., the sensitization test day). For example, Leff et al. [66] reported that D2 receptors had recovered to 80% of control values after 96 h, while Crawford et al. [49] found that the D1 receptors of 16-day-old rats had

recovered to age-matched control levels 96 h after EEDQ treatment. For these reasons, we believe that administering EEDQ 24 h prior to cocaine pretreatment primarily affected induction processes, although it remains possible that mechanisms underlying expression were also affected. If so, the protection experiment indicates that these EEDQ-induced effects were not DA receptor-mediated.

A notable feature of these results is that EEDQ did not depress the locomotor activity of young rats on the pretreatment day [67–69], even though EEDQ reduces the basal and DA agonist-induced locomotion of adult rats [45,67,70]. This ontogenetic difference is not due to EEDQ being ineffective in preweanling rats, since 7.5 mg/kg EEDQ produced a 67% and 57% reduction, respectively, in dorsal striatal D1 and D2 receptors when measured 24 h post-injection (Table 2). Consistent with past studies, we found that administering high doses of SCH23390 (1 mg/kg) and raclopride (10 mg/kg) prior to EEDQ treatment effectively protected D1 and D2 receptors from EEDQ-induced receptor alkylation. The effectiveness of this receptor protection protocol allows EEDQ to be used with some level of receptor specificity. For example, the lack of a sensitized response in the SCH23390 and raclopride protection groups indicates that D1 and D2 receptor stimulation is not necessary for the expression of cocaine-induced behavioral sensitization in preweanling rats.

4.3. Absence of sex-dependent differences

Acute and repeated treatment with psychostimulant drugs typically causes sex-dependent differences in adult animals. In adult rats, for example, females are often hyperresponsive to an acute injection of cocaine [71,72], and show a more pronounced sensitized response than do male rats [73,74]. Prepubescent rats, on the other hand, do not typically exhibit sex differences in locomotion or sensitized responding after DA agonist treatment [75–80]. Consistent with these past studies, we found that cocaine did not differentially affect the locomotor activity of male and female preweanling rats on the pretreatment day, or cause sex-dependent differences in sensitized responding on the test day.

4.4. Conclusions

In conclusion, we used converging evidence from two experimental paradigms to show that D1 and D2 receptor stimulation is not necessary for the initial induction of one-trial behavioral sensitization in preweanling rats. EEDQ (7.5 and 15 mg/kg) did disrupt the sensitization process, however, suggesting that an undetermined receptor-type sensitive to EEDQ modulates the induction of cocaine sensitization. In contrast, expression of behavioral sensitization does have a dopaminergic component, as a test day injection of SCH23390 prevented the expression of a sensitized response in preweanling rats. Overall, the pattern of antagonist-induced effects just described is similar to what is observed in adult rats. In some ways these findings are surprising, since the characteristics of cocaine sensitization differ substantially between young and adult rats (e.g., strength and longevity of the sensitized response, importance of contextual associations, etc.). The present results suggest that these age-dependent differences in the manifestation of cocaine sensitization are not caused by maturational changes in DA receptor systems. Instead, it is possible that ontogenetic changes in receptor systems (e.g., 5-HT₂, α_{1B} -adrenergic, etc.) that are more

fundamentally involved in the induction process are responsible for age-dependent differences in behavioral sensitization.

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Figure 1.

Mean distance traveled ($\pm$ SEM) on the pretreatment and test day. On the pretreatment day (PD 20), rats were injected with saline alone (0 mg/kg raclopride–Saline) or raclopride and cocaine. Behavioral assessment lasted 30 min. On the test day (PD 21), all rats were challenged with 20 mg/kg cocaine before a 120 min testing session.

* Significantly different from the 0 mg/kg Raclopride–Saline group (acute control group; open bars)

† Significantly different from the 0 mg/kg Raclopride–Cocaine group (black bars).

Figure 2.

Mean distance traveled ($\pm$ SEM) on the pretreatment and test day. On the pretreatment day (PD 20), rats were injected with raclopride (0 or 0.5 mg/kg) followed by saline or cocaine. Behavioral assessment lasted 30 min. On the test day (PD21), all rats were challenged with 20 mg/kg cocaine before a 120 min testing session.

* Significantly different from the 0 mg/kg Raclopride–Saline group (acute control group; open bars)

† Significantly different from the 0 mg/kg Raclopride–Cocaine group (cocaine alone group; black bars).

Figure 3.

Mean distance traveled ($\pm$ SEM) on the pretreatment and test day. On the pretreatment day (PD 20), rats were injected with saline alone (0 mg/kg SCH23390+Raclopride–Saline) or a cocktail of SCH23390+Raclopride and cocaine. Behavioral assessment lasted 30 min. On the test day (PD21), all rats were challenged with 20 mg/kg cocaine before a 120 min testing session.

* Significantly different from the 0 mg/kg SCH23390+Raclopride–Saline group (acute control group; open bars)

† Significantly different from the 0 mg/kg SCH23390+Raclopride–Cocaine group (cocaine alone group; filled circles and black bars).

Figure 4.

Mean distance traveled ($\pm$ SEM) on the pretreatment and test day. On the preinjection day (PD 18), rats were injected with EEDQ (0, 7.5, or 15 mg/kg). On the pretreatment day (PD 19), rats were injected with saline or 30 mg/kg cocaine, with behavioral assessment lasting 30 min. On the test day (PD 21), all rats were challenged with 20 mg/kg cocaine before a 120 min testing session.

* Significantly different from the 0 mg/kg EEDQ–Saline group (open bars)

† Significantly different from the 0 mg/kg EEDQ–Cocaine group (black bars).

Figure 5.

Mean distance traveled ($\pm$ SEM) on the pretreatment and test day. On the preinjection day (PD 18), rats were injected with saline (nonprotected, NP), SCH23390 (D1 protected), raclopride (D2 protected), or SCH+RAC (D1/D2 protected), followed by an injection of vehicle or 7.5 mg/kg EEDQ. On the pretreatment day (PD 19), rats were injected with saline or 30 mg/kg cocaine, with behavioral assessment lasting 30 min. On the test day (PD 21), all rats were challenged with 20 mg/kg cocaine before a 120 min testing session.

* Significantly different from the Nonprotected+Vehicle–Saline group (acute control group; open bars)

† Significantly different from the Nonprotected+Vehicle–Cocaine group (cocaine alone group; black bars).

Figure 6.

Mean distance traveled ($\pm$ SEM) on the test day. On the pretreatment day (PD 20), rats were injected with saline or cocaine, with behavioral assessment lasting 30 min. On the test day (PD 21), rats were treated with raclopride (0, 0.1, or 0.5 mg/kg) 15 min before a challenge injection of 20 mg/kg cocaine. The testing session lasted 120 min.

* Significantly different from the acute control groups.

† Significantly different from the 0 mg/kg Raclopride–Cocaine group (cocaine alone group; black bars).

‡ Significantly different from the 0 mg/kg Raclopride–Saline group (acute control group; open bars)

Figure 7.

Mean distance traveled ($\pm$ SEM) on the test day. On the pretreatment day (PD 20), rats were injected with saline or cocaine, with behavioral assessment lasting 30 min. On the test day (PD 21), rats were treated with SCH23390 (0, 0.1, or 0.5 mg/kg) or a cocktail of SCH23390+Raclopride (S+R, 0.1 or 0.5 mg/kg) 15 min before a challenge injection of 20 mg/kg cocaine. The testing session lasted 120 min.

* Significantly different from the 0 mg/kg SCH23390–Saline group (acute control group; open bars)

† Significantly different from the 0 mg/kg SCH23390–Cocaine group (cocaine alone group; black bars).

‡ Significantly different from the 0.1 mg/kg SCH23390–Cocaine group.

Figure 8.

Mean distance traveled ($\pm$ SEM) on the test day. On the pretreatment day (PD 19), rats were injected with saline or 30 mg/kg cocaine, with behavioral assessment lasting 30 min (data not shown). On the preinjection day (PD 20), rats were injected with EEDQ (0, 7.5, or 15 mg/kg). On the test day (PD 21), all rats were challenged with 20 mg/kg cocaine. The testing session lasted 120 min.

* Significantly different from the 0 mg/kg EEDQ–Saline group (open bars)

† Significantly different from the 0 mg/kg EEDQ–Cocaine group (black bars).

Table 1

Design of Experiment 3

Group	Treatment (Age)			
	Protection (PD 18)	Preinjection (PD 18)	Pretreatment Day (PD 19)	Test Day (PD 21)
Acute Control group	Saline	Vehicle	Saline	Cocaine
Nonprotected-EEDQ Acute Control	Saline	EEDQ	Saline	Cocaine
D1 Protected-EEDQ Acute Control	SCH23390	EEDQ	Saline	Cocaine
D2 Protected-EEDQ Acute Control	Raclopride	EEDQ	Saline	Cocaine
D1/D2 Protected-EEDQ Acute Control	SCH/RAC	EEDQ	Saline	Cocaine
Sensitization control group	Saline	Vehicle	Cocaine	Cocaine
Nonprotected-EEDQ Sensitization	Saline	EEDQ	Cocaine	Cocaine
D1 Protected-EEDQ Sensitization	SCH23390	EEDQ	Cocaine	Cocaine
D2 Protected-EEDQ Sensitization	Raclopride	EEDQ	Cocaine	Cocaine
D1/D2-EEDQ Protected Sensitization	SCH/RAC	EEDQ	Cocaine	Cocaine

Table 2Effects of EEDQ and various receptor protection treatments on dorsal striatal $B_{\max}$ values (fmol/mg protein).

Condition	D1 Receptors		D2 Receptors	
	$B_{\max}$	%Sal–Vehicle	$B_{\max}$	%Sal–
Saline–Vehicle	1676.7 ($\pm$ 160.4)	100%	692.71 ($\pm$ 68.7)	100%
SCH23390–Vehicle	1983.3 ($\pm$ 217.7)	118%	Not included	
Raclopride–Vehicle	Not included		774.44 ($\pm$ 96.7)	112%
Saline–EEDQ	546.2* ($\pm$ 113.5)	33%	297.50* ($\pm$ 38.5)	43%
SCH23390–EEDQ	1793.0 ($\pm$ 89.7)	107%	Not included	
Raclopride–EEDQ	Not included		765.57 ($\pm$ 36.4)	111%
SCH/RAC–EEDQ	1852.7 ($\pm$ 197.0)	110%	689.11 ($\pm$ 64.1)	99%

Note: The dorsal striatum was dissected and frozen 24 h after EEDQ (7.5 mg/kg) or vehicle treatment.

* Significantly different from the Saline–Vehicle group.