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Behavioral effects of dopamine receptor inactivation during the adolescent period: age-dependent changes in dorsal striatal D2^{High} receptors

Sanders A. McDougall, Joseph M. Valentine, Ashley E. Gonzalez, Danielle E. Humphrey, Crystal B. Widarma, and Cynthia A. Crawford

Department of Psychology, 5500 University Parkway, California State University, San Bernardino, CA 92407, USA

Abstract

Rationale—Dopamine (DA) receptor inactivation produces opposing behavioral effects across ontogeny. For example, inactivating DA receptors in the dorsal striatum attenuates DA agonist-induced behaviors of adult rats, while potentiating the locomotor activity of preweanling rats.

Objective—The purpose of this study was to determine if DA receptor inactivation potentiates the DA agonist-induced locomotor activity of adolescent rats, and whether alterations in D2^{High} receptors are responsible for this effect.

Methods—In the behavioral experiment, the irreversible receptor antagonist N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) or its vehicle (100% dimethylsulphoxide, DMSO) were bilaterally infused into the dorsal striatum on postnatal day (PD) 39. On PD 40, adolescent rats were given intrastriatal infusions of the DA agonist R(–)-propylnorapomorphine (NPA) or vehicle and locomotor activity was measured for 40 min. In the receptor binding experiment, rats received IP injections of EEDQ or DMSO (1:1 (v/v) in distilled water) on PD 17, PD 39, or PD 84. One day later, striatal samples were taken and subsequently assayed for D2 specific binding and D2^{High} receptors using [³H]-domperidone.

Results—Unlike what is observed during the preweanling period, EEDQ attenuated the NPA-induced locomotor activity of adolescent rats. EEDQ reduced D2 receptor levels in the dorsal striatum of all age groups, while increasing the proportion of D2^{High} receptors. Regardless of pretreatment condition (i.e., DMSO or EEDQ), preweanling rats had a greater percentage of D2^{High} receptors than adolescent or adult rats.

Conclusions—DA receptor inactivation affects the behaviors of preweanling and older rats differently. The DA supersensitivity exhibited by EEDQ-treated preweanling rats may result from an excess of D2^{High} receptors.

Keywords

Dorsal striatum; N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ); Locomotor activity; Ontogeny; D2^{High} receptors

Introduction

Dopamine (DA) agonist- and antagonist-induced behaviors, as well as the neural mechanisms that mediate these behaviors, often exhibit maturational changes across early ontogeny (Moody and Spear 1992; Lepekina and Tsitsurina 2007; for a review, see Spear 1979). For example, dorsal striatal DA content, VMAT₂, and plasma membrane DA transporters increase linearly from birth into adulthood (Coyle and Campochiaro 1976; Giorgi et al. 1987; Broaddus and Bennett 1990; Tarazi et al. 1998; Truong et al. 2005; Kuperstein et al. 2008). DA receptors exhibit a different pattern of postnatal development, because D1 and D2 binding sites increase in number from birth through the preweanling period (a developmental epoch analogous to childhood; Murrin and Zeng 1986, 1990; Kuperstein et al. 2008), are overproduced during adolescence (Giorgi et al. 1987; Teicher et al. 1995; Andersen et al. 1997), and then gradually decline by up to 35–50% until adult-like levels are reached (for reviews, see Andersen and Teicher 2000; Tarazi and Baldessarini 2000; Andersen 2003). Interestingly, Andersen et al. (1997) suggest that perturbations in the overproduction of DA receptors, and their subsequent pruning, may underlie various psychopathologies in humans.

The transient overabundance of D1 and D2 receptors during the adolescent period may also be responsible for ontogenetic changes in the acute behavioral effects of DA agonists and antagonists. These ontogenetic behavioral differences are not simply the result of a monotonic increase or decrease in drug effectiveness, because indirect DA agonists (e.g., cocaine and amphetamine) cause a hypoactive response in adolescent rats and mice when compared to younger and older age groups (Bolanos et al. 1998; Adriani and Laviola 2000; Collins and Izenwasser 2002; Vasilev et al. 2003; Frantz et al. 2006; for reviews, see Spear and Brake 1983; Laviola et al. 1999; Spear 2000). Curiously, other researchers report a nearly opposite pattern of effects, with acute and repeated cocaine treatment causing hyperresponsiveness, rather than hyporesponsiveness, in male rats during the adolescent period (Caster et al. 2005, 2007; Parylak et al. 2008; see also Walker et al. 2010). There are no ready explanations for these discordant findings, but these results do illustrate that adolescence is a period characterized by a unique pattern of drug responsivity.

As is the case with DA agonist drugs, reversible and irreversible DA antagonists differentially affect behavior across ontogeny. In terms of reversible antagonists, haloperidol and perphenazine produce exaggerated cataleptogenic effects in young rats when compared to older age groups (Campbell and Baldessarini 1981; Brus et al. 1983; Campbell et al. 1988). Irreversible receptor antagonists, on the other hand, induce qualitatively different behavioral effects in young and adult rats. Specifically, systemic and intrastriatal injections of N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) attenuate the DA agonist-induced behaviors of adult rats (Arnt et al. 1988; Bordi et al. 1989; Meller et al. 1989; Giorgi and Biggio 1990a,b; McDougall et al. 1992; Der-Ghazarian et al. 2012), whereas EEDQ potentiates R-propylnorapomorphine- (NPA) and quinpirole-induced locomotor activity in preweanling rats (McDougall et al. 1992, 1993; Der-Ghazarian et al. 2012, 2013). These behavioral differences are apparent even though EEDQ causes a substantial reduction in the D1 and D2 receptor levels of both age groups (Leff et al. 1984; Crawford et al. 1992, 1994; Kula et al. 1992; Der-Ghazarian et al. 2012, 2013). Whether EEDQ attenuates or potentiates the locomotor activity of adolescent rats had not been assessed.

Why DA receptor inactivation augments the agonist-induced locomotor activity of preweanling rats remains uncertain. Our working hypothesis is that a greater percentage of DA receptors survive EEDQ-induced alkylation in young rats, relative to adults, and a disproportionate number of these surviving receptors exist in a high affinity state. Stimulation of these high affinity receptors is hypothesized to produce the potentiated

locomotor response exhibited by young rats. Evidence that EEDQ causes DA supersensitivity is sparse, but includes the following findings: (a) intrastriatal infusions of EEDQ increase the basal locomotor activity of preweanling rats (Der-Ghazarian 2012, 2013); (b) very low doses of EEDQ (0.8 mg/kg) cause D1 receptor supersensitivity in the prefrontal cortex of adult rats (Trovero et al. 1992); and (c) EEDQ decreases DA levels in the dorsal striatum (Crawford et al. 1992, 1994), thus producing a state often associated with DA supersensitivity (Arnt and Hyttel 1984; Farley et al. 2006; Carvalho et al. 2009). Unfortunately, there is no direct evidence showing that EEDQ alters the percentage of high affinity receptors or that such an effect might vary according to the age of the animal being tested.

The purpose of the present study was twofold: First, to determine whether DA receptor inactivation potentiates or attenuates the DA agonist-induced locomotor activity of adolescent rats. To examine this research question, adolescent rats were tested using the identical procedures and drug doses employed with preweanling and adult rats (Der-Ghazarian et al. 2012, 2013). It was predicted that EEDQ would potentiate the NPA-induced locomotion of adolescent rats because the overabundance of dorsal striatal DA receptors, which is characteristic of the adolescent period, was expected to mitigate the effects of EEDQ. Seeman et al. (2005) reported that manipulations resulting in DA supersensitivity are associated with increased levels of the D2^{High} receptor. Therefore, a second goal of this study was to determine whether those age groups exhibiting EEDQ-induced behavioral potentiation have a greater proportion of dorsal striatal D2^{High} receptors than adult rats. D2 receptors were the focus of this study because previous experiments using selective D1 and D2 agonists, as well as receptor protection experiments, have shown that EEDQ's paradoxical behavioral effects are due to actions at the D2 receptor (McDougall et al. 1993; Der-Ghazarian et al. 2012, 2013). In the current study, tissue extractions for the receptor binding assays and behavioral testing occurred 24 h after EEDQ treatment (see also Arnt et al. 1988; Gnanalingham et al. 1994; Der-Ghazarian et al. 2012, 2013). By this time point, EEDQ levels in serum and brain are minimal (Leff et al. 1984), thus rats are tested in an antagonist-free state and acute motoric incapacity is not evident.

Materials and methods

Subjects

Subjects were 225 preweanling, adolescent, and adult rats. Male and female adult rats ($N = 24$) were purchased from Charles River (Hollister, CA); whereas, male and female adolescent ($N = 177$) and preweanling ($N = 24$) rats were born and bred at California State University, San Bernardino (CSUSB). Litters were culled to 10 pups on postnatal day (PD) 3 and weaned on PD 23. Preweanling rats were kept with the dam and littermates, whereas adolescent and adult rats were group housed with conspecifics. All rats were housed on racks in large polycarbonate maternity cages ($56 \times 34 \times 22$ cm) with wire lids. Food and water were freely available. The colony room was maintained at $22\text{--}23^\circ\text{C}$ and kept under a 12 L:12 D cycle. Subjects were cared for according to the "Guide for the Care and Use of Laboratory Animals" (National Research Council, 2010) under a research protocol approved by the Institutional Animal Care and Use Committee of CSUSB.

Apparatus

Behavioral testing was done in commercially available (Coulbourn Instruments, Allentown, PA) activity monitoring chambers, consisting of acrylic walls, a plastic floor, and an open top ($41 \times 41 \times 41$ cm). Each chamber included an X–Y photobeam array, with 16 photocells and detectors, that was used to determine distance traveled (locomotor activity).

Drugs

For the behavioral experiment, NPA hydrochloride was dissolved in distilled water vehicle containing 0.1% metabisulfite (an antioxidant); whereas, EEDQ was dissolved in 100% dimethyl sulfoxide (DMSO). EEDQ was microinjected at a volume of 0.75 μ l per side, while NPA was infused at a volume of 0.5 μ l per side. A relatively greater volume of EEDQ was administered in order to ensure that NPA was not stimulating DA receptors located outside the area of EEDQ-induced alkylation (Der-Ghazarian et al. 2012, 2013). For the receptor binding experiment, EEDQ was dissolved in a 50% DMSO solution (1:1 (v/v) in distilled water) and injected intraperitoneally (IP) at a volume of 5 ml/kg (preweanling rats) or 1 ml/kg (adolescent and adult rats). (–)-Sulpiride was dissolved in a minimal amount of glacial acetic acid and diluted with distilled water. Nonlabeled ligands were purchased from Sigma-Aldrich (St. Louis, MO), whereas [3 H]-domperidone (25 Ci/mmol) was purchased from American Radiolabeled Chemicals (St. Louis, MO).

Surgery

On PD 38, anesthesia was induced by isoflurane (2.5–5%) mixed with oxygen. A topical lidocaine solution (1%) was applied to the scalp and ibuprofen (2 mg/kg IP) was administered. Rats were placed in a standard Kopf stereotaxic apparatus and the scalp was incised to reveal the skull. For the behavioral experiments, two craniotomies were performed and stainless steel guide cannulae (22 gauge; Plastics One, Roanoke, VA) were implanted in the dorsal striatum (A/P +0.20, M/L $\pm$ 2.8, D/V –4.2 mm from bregma; Paxinos and Watson 1998). Guide cannulae were implanted 1 mm above the target location and were fixed in place using cyanoacrylate gel followed by dental cement. Stainless steel stylets (Plastics One) were used to seal guide cannulae until time of testing. Rats were allowed to recover in a temperature-controlled chamber (30°C) until fully responsive.

Behavioral procedures

On PD 39 (24 h after surgery), adolescent rats ($N = 144$) were randomly divided into three conditions, after which the stainless steel stylets were removed and DMSO or EEDQ (50 or 100 μ g) was bilaterally microinjected into the dorsal striatum at a volume of 0.75 μ l per side. Similar doses of EEDQ have been microinjected into various basal ganglia and limbic structures of preweanling and adult rats (Cory-Slechta et al. 2002; Hemsley and Crocker 2001; Der-Ghazarian et al. 2012, 2013). Drugs were delivered at a constant rate over a 60 s period and the infusion cannulae were subsequently left in place for 2 min.

On PD 40, rats ($n = 8$ males and 8 females per group) were habituated to the testing chambers for 40 min. NPA (10 or 20 μ g) or vehicle was bilaterally infused into the dorsal striatum at a volume of 0.5 μ l per side. After drug infusions, rats were returned to the testing chambers for 40 min. Distance traveled (a measure of horizontal locomotor activity) was assessed continuously across the 80-min session. These procedures are identical to those used to test the effects of EEDQ and NPA in preweanling and adult rats (Der-Ghazarian et al. 2012, 2013).

D2 competition assays using DA and [3 H]-domperidone

On PD 17 (preweanling), PD 39 (adolescent), or PD 84 (adult), rats were given an IP injection of vehicle or EEDQ (2.5 or 7.5 mg/kg). After 24 h, rats ($n = 4$ males and 4 females per group) were killed by rapid decapitation and dorsal striatal sections were dissected bilaterally on an ice-cold dissection plate and stored at –80°C. Tissue was prepared and assays were conducted as described in Seeman (2008). Briefly, striatal tissue was added to buffer (6 mg tissue per 1 ml buffer) consisting of 50 mM Tris-HCl (pH 7.4), 1 mM EDTA, 1.5 mM CaCl_2 , 5 mM KCl, 120 mM NaCl, and 4 mM MgCl_2 . Tissue was then homogenized

using a motorized Teflon-glass homogenizer. Procedures for the three age groups were identical, with the exception that homogenates from PD 18 rats consisted of two pooled tissue samples.

For the competition assays, duplicate incubation tubes contained 0.15 ml of striatal homogenate, 1.2 nM [^3H]-domperidone, and various concentrations of DA. Nonspecific binding was determined in the presence of 10 μM (–)-sulpiride. Total volume for each tube was 0.5 ml. The tubes were then incubated at room temperature for 2 h. Incubation was terminated by vacuum filtration over glass fiber filters (Whatman GF/C, presoaked in assay buffer). Radioactivity was measured by liquid scintillation spectrometry.

Histology

After behavioral testing, adolescent rats were given an overdose of sodium pentobarbital and brains were fixed in 4% paraformaldehyde. Brains were cryoprotected in a 20% sucrose solution, sectioned coronally (70 μm) using a cryostat, and then stained with thionin. Histological assessment of cannula placements was done by observers blind to drug treatment conditions. Overall, 94.7% of rats had proper guide cannula placements in the dorsal striatum. Data from animals with inappropriate cannula placements were not included in the statistical analyses and replacement rats were added as needed. A photomicrograph and schematic showing guide cannula placements can be seen in Fig. 1.

Additional adolescent rats ($N = 9$) received unilateral microinjections (0.75 μl per side) of 50 or 100 μg EEDQ (DMSO was administered in the opposite hemisphere) and D1 receptor autoradiography was used to determine dispersion. D1 autoradiography was used because the low levels of background radiation afforded a more precise assessment of dispersion. Briefly, 20 μm slices from the dorsal striatum were assayed for D1 binding sites with [^3H]-SCH23390 and apposed to x-ray film for 10 weeks (Crawford et al. 2011; Der-Ghazarian et al. 2013). EEDQ spread in a generally concentric pattern that encompassed the medial and most of the lateral portion of the dorsal caudate-putamen (Fig. 2). To determine dispersion of NPA, additional preweanling and adult rats received bilateral microinjections (0.5 μl per side) of crystal violet and brains were removed 5 or 30 min later. Examination of coronal sections indicated that dye was restricted within the dorsal striatum.

Data analysis

Litter effects were minimized by assigning no more than one subject from each litter to a particular group (for a discussion of litter effects, see Zorrilla 1997). For the behavioral experiments, separate repeated measures (5-min time blocks) analyses of variance (ANOVAs) were used for statistical analysis of time blocks 1–8 (habituation) and 9–16 (testing). When required, significant higher order interactions (e.g., Pretreatment $\times$ Agonist $\times$ Sex $\times$ Time block) were further analyzed using lower order ANOVAs. When the assumption of sphericity was violated, as determined by Mauchly's test of sphericity, the Huynh-Feldt epsilon statistic was used to adjust degrees of freedom (Huynh and Feldt 1976). Corrected degrees of freedom were rounded to the nearest whole number and are indicated by a superscripted "a". For the homogenate ligand binding assays, separate $3 \times 2 \times 3$ (age $\times$ sex $\times$ pretreatment) ANOVAs were used to analyze specific binding (fmol/mg wet weight tissue) and the fraction of D2 receptors in the high affinity state (i.e., D2^{High} receptors). The percentage of D2^{High} receptors for each [^3H]-domperidone/DA competition assay was determined using nonlinear regression for a two-site model (GraphPad Software, San Diego, CA). Post hoc analysis of both the behavioral and receptor binding data was done using Tukey tests ($P < 0.05$).

Results

Behavioral experiment: effects of EEDQ on the NPA-induced locomotor activity of adolescent rats

During both the habituation and test phases, adolescent female rats had greater distance traveled scores than male rats [Sex main effects, $F_{1,138} = 14.45$, $P < 0.001$; $F_{1,138} = 8.90$, $P < 0.01$; $F_{1,126} = 8.68$, $P < 0.01$, respectively]. For this reason, the drug-induced behavioral effects of male and female rats were assessed in separate statistical analyses.

Female rats—During time blocks 1–4 and 5–8 of the habituation phase, adolescent female rats given EEDQ (50 or 100 μg) exhibited less locomotor activity than female rats microinjected with DMSO (left panels, Fig. 3) [Pretreatment main effects, $F_{2,69} = 36.00$, $P < 0.001$; $F_{2,69} = 12.16$, $P < 0.001$, respectively]. Differences between the DMSO and EEDQ groups were apparent on each time block of the habituation phase [Pretreatment $\times$ Time Block interaction, $F_{6,207} = 3.25$, $P < 0.01$].

During the testing phase (i.e., time blocks 9–16), both doses of NPA (10 and 20 μg) increased the distance traveled scores of adolescent female rats (Fig. 3) [Agonist main effect, $F_{2,63} = 20.93$, $P < 0.001$], with statistically significant differences between the NPA and vehicle (i.e., 0 μg NPA) groups occurring on time blocks 10–16 [^aAgonist $\times$ Time Block interaction, $F_{8,269} = 11.04$, $P < 0.001$]. The effects of the agonist variable were moderated by pretreatment condition [Pretreatment $\times$ Agonist interaction, $F_{4,63} = 4.08$, $P < 0.01$; ^aPretreatment $\times$ Agonist $\times$ Time Block interaction, $F_{17,269} = 4.02$, $P < 0.001$]. Among female rats given a vehicle injection on the test day (upper graph, Fig. 3), both doses of EEDQ reduced distance traveled scores on time blocks 9–12 [Tukey tests, $P < 0.05$]. Among NPA-treated rats, only the highest dose of EEDQ (100 μg) significantly decreased distance traveled scores. Specifically, pretreatment with 100 μg EEDQ attenuated the distance traveled scores of female rats given 10 μg NPA (middle graph) or 20 μg NPA (lower graph) on time blocks 10–16 [Tukey tests, $P < 0.05$].

Male rats—During time blocks 1–4 of the habituation phase, EEDQ (50 or 100 μg) decreased the distance traveled scores of adolescent male rats (left panels, Fig. 4) [Pretreatment main effect, $F_{2,69} = 13.92$, $P < 0.001$]. EEDQ pretreatment did not alter locomotion during the final 20 min of the habituation phase.

Although male rats exhibited less locomotor activity than female rats (Fig. 4), both doses of NPA (10 and 20 μg) enhanced the distance traveled scores of adolescent male rats during the testing phase [Agonist main effect, $F_{2,63} = 23.91$, $P < 0.001$]. Relative to vehicle controls (i.e., the 0 μg NPA groups), 10 and 20 μg NPA increased distance traveled scores on time blocks 10–16 [^aAgonist $\times$ Time Block interaction, $F_{12,23} = 6.37$, $P < 0.001$]; however, the locomotor activating effects of NPA varied according to pretreatment condition [^aPretreatment $\times$ Agonist $\times$ Time Block interaction, $F_{23,364} = 2.37$, $P < 0.001$]. In terms of the control rats (upper graph, Fig. 4), EEDQ (50 and 100 μg) reduced the distance traveled scores of vehicle-treated rats on time block 9 [Tukey tests, $P < 0.05$], after which all of the vehicle groups exhibited minimal locomotion. Although the 50 μg dose of EEDQ was without significant effect (relative to the DMSO controls), a pretreatment infusion of 100 μg EEDQ attenuated the locomotor activity of NPA-treated adolescent male rats. Specifically, 100 μg EEDQ reduced the distance traveled scores of rats given 10 μg NPA on time blocks 9–12 as well as time blocks 15 and 16 (middle graph, Fig. 4) [Tukey tests, $P < 0.05$]; whereas, the same dose of EEDQ (100 μg) reduced the locomotor activating effects of 20 μg NPA on time blocks 14–16 (lower graph, Fig. 4) [Tukey tests, $P < 0.05$].

D2 competition assays: age-dependent effects of EEDQ on D2^{High} receptors

Specific binding—Among control rats (i.e., rats not receiving EEDQ), D2 specific binding in the dorsal striatum varied according to age (Table 1). Preweanling rats (PD 18) had significantly less dorsal striatal D2 specific binding sites than adult rats (PD 85), while adolescent rats (PD 40) had greater D2 specific binding than the other two age groups [Age main effect, $F_{2,21} = 21.81$, $P < 0.001$]. When expressed as the percent of same-age DMSO controls, EEDQ (2.5 and 7.5 mg/kg) significantly decreased D2 specific binding in the dorsal striatum regardless of age (Table 2) [Pretreatment main effect, $F_{2,63} = 127.96$, $P < 0.001$]. A separate statistical analysis assessing only rats given EEDQ showed that D2 receptor inactivation was significantly more pronounced in adolescent rats than preweanling rats [Age main effect, $F_{1,42} = 4.09$, $P < 0.05$]. D2 specific binding in the dorsal striatum of male and female rats did not differ.

Percent D2^{High} receptors—The effects of DMSO and EEDQ (2.5 or 7.5 mg/kg) on the percent of D2^{High} receptors in the various age groups are shown in Figure 5. As can be seen, pretreating rats with either dose of EEDQ significantly increased the percentage of D2^{High} receptors (Table 3) [Pretreatment main effect, $F_{2,63} = 7.50$, $P < 0.001$]. D2^{High} receptors also varied according to age, because preweanling rats had a significantly greater percentage of these high affinity receptors than adolescent or adult rats [Age main effect, $F_{2,63} = 65.17$, $P < 0.001$]. The Age $\times$ Pretreatment interaction did not approach statistical significance, indicating that the proportion of dorsal striatal D2^{High} receptors was consistently elevated in preweanling rats, relative to the other age groups, regardless of pretreatment condition (i.e., DMSO or EEDQ). D2^{High} receptors did not vary according to sex.

Discussion

DA receptor inactivation differentially affects the expression of behavior across ontogeny. When administered systemically, EEDQ blocks the DA-mediated behaviors of adult rats (Arnt et al. 1988; Bordini et al. 1989; Meller et al. 1989; McDougall et al. 1992), while leaving the NPA- and quinpirole-induced locomotor activity of preweanling rats unaffected (McDougall et al. 1992, 1993; Mestlin and McDougall 1993). This paradoxical behavioral effect is even more pronounced when drugs are microinjected into the dorsal striatum, because EEDQ-treated preweanling rats exhibit a potentiated locomotor response after NPA or quinpirole infusions (Der-Ghazarian et al. 2012, 2013). Using the same basic methodology, we examined the effects of inactivating DA receptors in the dorsal striatum of male and female adolescent rats. Adolescence is a developmental stage characterized by both an altered responsiveness to dopaminergic drugs (Spear and Brake 1983; Laviola et al. 1999; Spear 2000) and a transient overabundance of DA receptors in the dorsal striatum (Andersen and Teicher 2000; Tarazi and Baldessarini 2000; Andersen 2003). Both sets of findings led us to hypothesize that EEDQ would potentiate the NPA-induced locomotor activity of adolescent rats. This hypothesis was not supported by the data, because 100 μ g EEDQ (the same dose of EEDQ administered to preweanling and adult rats) attenuated the locomotor activating effects of dorsal striatal NPA (10 or 20 μ g) infusions. EEDQ's ability to reduce NPA-induced locomotion was apparent in both male and female adolescent rats, and is essentially the same pattern of results reported for adult rats. Therefore, it appears that adolescent and adult rats respond in a similar manner after DA receptor inactivation; it is only during the earlier preweanling period that EEDQ causes a paradoxical increase in basal and DA agonist-induced locomotor activity.

The neural basis for EEDQ's age-dependent behavioral effects has been difficult to determine, however, we speculated that the potentiated behavioral response characteristic of EEDQ-treated preweanling rats might result from the combined influence of two factors.

First, there is conflicting evidence that a greater percentage of D2 receptors survive EEDQ-induced alkylation in young rats relative to adults. Second, and more importantly, we proposed that in younger animals a disproportionate number of these surviving or newly repopulated D2 receptors exist in a high affinity state. Stimulation of these D2^{High} receptors is hypothesized to produce the potentiated locomotor response exhibited by young rats. In terms of the first factor, Crawford et al. (1992) reported that systemically administered EEDQ (7.5 mg/kg) inactivated more dorsal striatal D2 receptors in adult rats (i.e., a 80% decline) than preweanling rats (i.e., a 61% decline). Intrastriatal infusions of 100 µg EEDQ produced similar declines in the D2 receptor levels of preweanling rats (Der-Ghazarian et al. 2013). In the present study, 7.5 mg/kg EEDQ caused a somewhat less robust reduction in D2 receptors than previously reported, but the difference between preweanling rats (i.e., a 53% decline) and adolescent rats (i.e., a 67% decline) was statistically significant (adult rats were intermediate between the two other age groups). Consistent with these findings, Leff et al. (1984) reported that adult rats showed a greater reduction of striatal D2 receptors than young rats (PD 22–30) when measured 28–30 h after EEDQ treatment, although their time-course analysis indicated that some of this effect was due to a more rapid repopulation of D2 receptors in the younger age group. It is tempting to speculate that a significant percentage of these newly repopulated receptors might be in a high affinity state. In partial contrast, Kula et al. (1992) showed that the D2 binding sites of young (PD 28) and adult rats exhibited a similar decline when measured one day after EEDQ administration, but the rate of receptor recovery was again greater in young rats. When these studies are considered together, it appears that EEDQ causes somewhat less D2 receptor inactivation in preweanling rats than older animals, and the rate of D2 receptor repopulation is greater in younger age groups.

In terms of the second factor, it was hypothesized that EEDQ would increase the percentage of dorsal striatal D2^{High} receptors in all age groups, but the greatest proportional increase in high affinity receptors would be evident in preweanling rats. The predicted pattern of results was obtained, suggesting that a relative excess of D2^{High} receptors may produce a state of DA supersensitivity in which NPA causes an exaggerated locomotor response. This explanation is consistent with the work of Seeman et al. (2005) who showed that manipulations producing DA supersensitivity invariably increase the proportion of D2^{High} receptors. Even so, an abundance of high affinity D2 receptors does not necessarily mean that rats will exhibit a supersensitive response (Seeman et al., 2005). In the present study, for example, 100 µg EEDQ increased the proportion of D2^{High} receptors in adolescent and adult rats, but behavioral supersensitivity was not apparent. It is possible that the number of D2^{High} receptors was insufficient to induce DA supersensitivity in the older age groups, and/or that too many low and high affinity D2 receptors were inactivated to allow for a normal or potentiated behavioral response to DA agonist treatment.

The mechanism by which EEDQ produces a relative excess of D2^{High} receptors is uncertain, but it is possible that EEDQ increases the proportion of D2^{High} receptors by preferentially inactivating low affinity receptors. Endogenous DA may mediate this effect, because DA selectively binds to high affinity sites and protects receptors from EEDQ-induced inactivation (Lahti and Tamminga 1999). It is also possible that newly synthesized receptors are predominately of the D2^{High} variety. Whether EEDQ is capable of shifting low affinity D2 sites to a high affinity conformational state is uncertain, but such alterations have been proposed (see Levant 1995). Interestingly, Seeman et al. (2005) have suggested that the shift from D2_{Low} to D2^{High} states occurs as a generalized response to brain impairment. Although these authors were referring to events like repeated amphetamine treatment, hippocampal lesions, ethanol withdrawal, and birth by Cesarean-section, it is possible that EEDQ-induced receptor alkylation falls under the general rubric of brain impairment.

A disadvantage of using EEDQ to study the effects of DA receptor inactivation is that this compound lacks specificity. For example, EEDQ alkylates various receptor types, including muscarinic M₁ and M₂, α_2 -adrenergic, GABA_A, 5-HT_{1A} and 5-HT_{2A} receptors (Norman et al. 1989; Miller et al. 1991; Sallés et al. 1993; Ribas et al. 1998; Kettle et al. 1999; Vinod et al. 2001). Even so, protection experiments, in which D1 and D2 receptors are selectively spared from EEDQ-induced inactivation by SCH23390 and sulpiride pretreatment, clearly show that EEDQ's ability to enhance the DA agonist-induced locomotor activity of preweanling rats is due to the inactivation of DA receptors (McDougall et al. 1992, 1993; Der-Ghazarian et al. 2012, 2013). Microinjection studies using selective D1 (SKF82958) and D2 (quinpirole) agonists go a step further and indicate that the D2 receptor is responsible for EEDQ's paradoxical locomotor activating effects in preweanling rats (Der-Ghazarian et al. 2013). In terms of adult rats, protection experiments have shown that DA receptor inactivation is primarily responsible for blocking the behavioral effects of DA agonist drugs (Cameron and Crocker 1988; Meller et al. 1989; Giorgi and Biggio 1990a). To date, no studies using adolescent rats have confirmed that EEDQ's ability to attenuate NPA-induced locomotor activity is due to the inactivation of DA receptors and not some other receptor type.

Spear (1979, 2000) has long contended that drug sensitivity varies across ontogeny. In terms of direct DA receptor agonists and antagonists, behavioral responsiveness is greater during the late preweanling period than in adulthood (Campbell and Baldessarini 1981; Brus et al. 1983; Frantz and Van Hartesveldt 1999). This ontogenetic effect cannot be explained in terms of overall receptor numbers, since D2 binding sites have not reached adult-like levels by the end of the preweanling period (Broaddus and Bennett 1990; Rao et al. 1991). Instead, it is possible that age-dependent differences in high affinity D2 receptors might account for ontogenetic changes in drug sensitivity. More specifically, nonEEDQ-treated preweanling rats had proportionally more D2^{High} receptors in the dorsal striatum than adolescent or adult rats. Maturational changes in D2^{High} receptors may be sufficient to explain the age-dependent differences in psychopharmacological responsiveness exhibited by preweanling and adult rats.

Adolescence, on the other hand, is a developmental period characterized by a unique pattern of responding to dopaminergic drugs (Spear and Brake 1983; Laviola et al. 1999) and an overabundance of D1 and D2 binding sites (Tarazi and Baldessarini 2000; Andersen 2003). We replicated the latter result by showing that the D2 specific binding of nonEEDQ-treated adolescent rats was 52% and 27% greater than in preweanling and adult rats, respectively. In contrast, adolescent and adult rats had a nearly identical proportion of dorsal striatal D2^{High} receptors, while neither D2 specific binding nor the proportion of D2^{High} receptors varied according to sex. The lack of sex differences is not in agreement with Andersen et al. (1997), who reported that the transient overabundance of dorsal striatal D2 receptors was substantially more pronounced in adolescent male rats than female rats (for a review, see Andersen and Teicher 2000). The absence of significant sex effects may have been a consequence of assay sensitivity, insufficient statistical power (there were only 4 males and 4 females per group), or not controlling for the hormonal cycling of female rats (i.e., the number of striatal D2 binding sites varies depending on circulating estrogen levels; Bazzett and Becker 1994). On the other hand, sex differences were prominent in the behavioral experiment, as adolescent female rats exhibited more basal and NPA-induced locomotion than adolescent male rats. These results are consistent with studies showing that DA agonists stimulate more locomotor activity in female adolescent and adult rats, as compared to same-aged male rats (Schindler and Carmona 2002; Festa et al. 2004; Milesi-Hallé et al. 2005; Brown et al. 2012).

In summary, selectively inactivating D2 receptors in the dorsal striatum blocks the NPA-induced behaviors of adolescent and adult rats, while paradoxically increasing the DA agonist-induced locomotor activity of preweanling rats. Although EEDQ produced a similar reduction in the D2 specific binding of all three age groups, the proportion of dorsal striatal D2^{High} receptors was significantly elevated in preweanling rats relative to adolescent and adult rats. Whether age-dependent alterations in D2^{High} receptors are fully, or partially, responsible for the paradoxical locomotor activity of EEDQ-treated preweanling rats remains to be determined.

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Fig. 1. Schematic representations (a), as well as a typical photomicrograph (b), of cannula placements in the dorsal striatum of adolescent rats from the behavioral experiment. In all cases, numbers on the right indicate distance (mm) from Bregma using coordinates from the rat brain atlas of Paxinos and Watson (1998).

Fig. 2. Representative autoradiograms showing the dispersion of 75 μ l EEDQ (left hemisphere) or DMSO (right hemisphere) in the dorsal striatum on PD 39. (a) 50 μ g EEDQ; (b) 100 μ g EEDQ.

Fig. 3.

Mean distance traveled ($\pm$ SEM) of adolescent female rats ($n = 8$ per group) during the 80-min behavioral testing session. Rats had been given bilateral infusions of EEDQ (50 or 100 μ g) or DMSO into the dorsal CPu 24 h earlier. At the conclusion of time block 8 (indicated by the dashed line), EEDQ- and DMSO-treated rats received bilateral infusions of NPA (0, 10 or 20 μ g).

^aSignificant difference between the DMSO and 100 μ g EEDQ groups on the same time block.

^bSignificant difference between the DMSO and 50 μ g EEDQ groups on the same time block.

^cSignificant difference between the 50 μ g EEDQ and 100 μ g EEDQ groups on the same time block.

^dSignificantly different from rats given 0 μ g NPA (i.e., vehicle).

Fig. 4.

Mean distance traveled ($\pm$ SEM) of adolescent male rats ($n = 8$ per group) during the 80-min behavioral testing session. Rats had been given bilateral infusions of EEDQ (50 or 100 μ g) or DMSO into the dorsal CPu 24 h earlier. At the conclusion of time block 8 (indicated by the dashed line), EEDQ- and DMSO-treated rats received bilateral infusions of NPA (0, 10 or 20 μ g).

^aSignificant difference between the DMSO and 100 μ g EEDQ groups on the same time block.

^bSignificant difference between the DMSO and 50 μ g EEDQ groups on the same time block.

^cSignificant difference between the 50 μ g EEDQ and 100 μ g EEDQ groups on the same time block.

^dSignificantly different from rats given 0 μ g NPA (i.e., vehicle).

Fig. 5. Percent D_2^{High} receptors in the dorsal striatum of preweanling, adolescent, and adult rats ($n = 8$ per group) using DA/ $[^3H]$ -domperidone competition assays. Nonlinear regression for a two-site model was used to determine percentages. Rats were pretreated with vehicle (○), 2.5 mg/kg EEDQ (▲), or 7.5 mg/kg EEDQ (▼) 24 h earlier.

Table 1

Mean D2 specific binding in the dorsal striatum of DMSO-treated preweanling, adolescent, and adult rats ($n = 8$ per group).

Age group	Specific Binding
Preweanling	4.16 (± 0.15) ^a
Adolescent	8.42 (± 0.68) ^{ab}
Adult	6.18 (± 0.38)

Specific binding (mean $\pm$ SEM) is expressed as fmol/mg wet weight tissue.

^aSignificantly different from adult rats ($P < 0.05$).

^bSignificantly different from preweanling rats ($P < 0.05$).

Table 2

Mean D2 specific binding in the dorsal striatum of EEDQ-treated preweanling, adolescent, and adult rats expressed as the percent of DMSO controls ($n = 8$ per group).

Age	EEDQ		
	0 mg/kg	2.5 mg/kg	7.5 mg/kg
Preweanling	100% (+3.6)	56.35% (+4.0)	46.80% (+4.9)
Adolescent	100% (+8.0)	47.17% (+4.8) ^b	33.34% (+2.3) ^b
Adult	100% (+4.7)	54.38% (+3.1)	40.18% (+4.3)
$\bar{X}_{(P, A, A)}$	100% (+3.4)	52.63% (+2.4) ^a	40.11% (+2.5) ^a

^a Significantly different from the 0 mg/kg EEDQ group (i.e., DMSO group) of the same age ($P < 0.05$).

^b Significantly different from preweanling rats ($P < 0.05$).

Table 3

Percent D2^{High} receptors in the dorsal striatum of EEDQ-treated preweanling, adolescent, and adult rats ($n = 8$ per group).

Age	EEDQ		
	0 mg/kg	2.5 mg/kg	7.5 mg/kg
Preweanling	29.01 (+2.5) ^b	51.51 (+3.7) ^b	51.31 (+3.0) ^b
Adolescent	21.62 (+1.1)	42.75 (+1.6)	45.75 (+4.0)
Adult	20.82 (+1.4)	42.10 (+3.0)	44.80 (+3.2)
$\bar{X}_{(P, A, A)}$	23.82 (+1.2)	45.45 (+1.8) ^a	47.29 (+2.0) ^a

^aSignificantly different from the 0 mg/kg EEDQ group (i.e., DMSO group, $P < 0.05$).

^bSignificantly different from adolescent and adult rats ($P < 0.05$).