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Amphetamine locomotor sensitization and conditioned place preference in adolescent male and female rats neonatally treated with quinpirole

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Abstract

Neonatal quinpirole treatment has been shown to produce an increase of dopamine D₂ - like receptor sensitivity that persists throughout the animal's lifetime. The objective was to analyze the effects of neonatal quinpirole treatment on effects of amphetamine in adolescent rats using locomotor sensitization and conditioned place preference (CPP) procedures. Sprague-Dawley rats were treated with quinpirole (1mg/kg) or saline from postnatal days (P)1-11 and raised to adolescence. For locomotor sensitization, animals were given amphetamine (1mg/kg) or saline every second day from P35-47 and placed into a locomotor arena. In females, neonatal quinpirole treatment enhanced amphetamine locomotor sensitization compared to quinpirole-free controls sensitized to amphetamine. Males demonstrated sensitization to amphetamine, but this was muted compared to females and unaffected by neonatal quinpirole. For CPP, animals were conditioned for eight consecutive days (P32-39) with amphetamine (1mg/kg) or saline and a drug-free preference test was performed at P40. Neonatal quinpirole enhanced time spent in the amphetamine-paired context compared to quinpirole-free controls conditioned with amphetamine, but only female controls conditioned with amphetamine spent more time in the drug-paired context compared to saline-treated controls. Increased D₂-like receptor sensitivity appears to have enhanced the behavioral effects of amphetamine, but these effects were more prevalent in adolescent females compared to males.

Keywords

Neonatal; quinpirole; Dopamine D₂-like receptors; locomotor sensitization; conditioned place preference; adolescence; rat

Introduction

Neonatal quinpirole treatment to rats has been shown to result in an increase of dopamine D₂-like receptor sensitivity that persists throughout the animal's lifetime, but does not result

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in any change in D₂ receptor number (Kostrzewa, 1995). We have shown in a series of studies that neonatal quinpirole treatment to rats results in a sensitized behavioral response to nicotine in adolescence (Sheppard et al 2009) as well as nicotine and amphetamine in adulthood (Perna et al 2007;Cope et al 2010). In addition, neonatal quinpirole treatment results in enhanced dopaminergic overflow in response to amphetamine in both the dorsal striatum (Nowak et al 2001) and nucleus accumbens core (Cope et al 2010). The effects of amphetamine on behavior or the dopaminergic system in adolescents has been lightly studied, but in general, findings have shown a muted response to amphetamine in adolescent males as compared to adolescent females (Bolanos, et al, 1998; Laviola et al, 2001; Mathews & McCormick, 2007; 2010;).

The focus of the current study was to analyze amphetamine locomotor sensitization and conditioned place preference in separate groups of adolescent male and female rats neonatally treated with quinpirole (dopamine D₂/D₃ agonist) or saline. The hypothesis was that increases in D₂-like receptor sensitivity produced by neonatal quinpirole treatment would ultimately result in increases in locomotor sensitization and CPP in response to amphetamine, but this effect would be more robust in females as compared to males, consistent with past work in adolescents as well as adults (for review, see Becker, et al 1999).

Methods

Subjects

A total of 15 untimed pregnant female Sprague-Dawley rats were obtained from Harlan Laboratories, Inc (Indianapolis, IN) and their offspring were used as subjects. One male and one female from each litter were assigned to each condition to control for within-litter variance (Holson & Pearce, 1992). All subjects were injected i.p. with either quinpirole HCl (1 mg/kg) or saline from postnatal days (P)1 to P 11, which results in an increase of dopamine D₂-like sensitivity persisting into adulthood (Kostrzewa 1995). There were a total of 6–7 males and females in each of four drug conditions for both locomotor sensitization and CPP. A total of 51 subjects were tested for locomotor sensitization, and 52 subjects were tested for CPP.

Locomotor Sensitization procedure

All animals were tested in a 40 cm-square arena-. After three 10-min habituation trials given from P31-33, animals began amphetamine sensitization on P35 and were administered d-amphetamine sulfate (1 mg/kg) or saline every second day between P35-P47. All trials were 10 min in length and behavioral tracking was performed by Any Maze software (Stoelting, Wood Dale, IL). For data analysis, separate factors included sex, neonatal drug treatment (quinpirole, saline) and adolescent drug treatment (amphetamine, saline). Only two days of behavior were analyzed (Days 1 and 7) although animals were identically treated and tested across all seven days of treatment. Horizontal activity counts served as the dependent measure and the Newman-Keuls test was used to analyze any significant interactions ($p = .05$).

Conditioned Place Preference (CPP) procedure

The CPP apparatus was constructed of plywood (76 cm long×46 cm wide×51 cm high) and consisted of two equally-sized chambers, one painted with textured black paint and the other with a glossy white paint; the bottom of the white chamber was covered with a plastic studded surface. A removable painted wooden divider separated these two chambers.

An initial pre-conditioning test was conducted at P31 with dividers removed. Animals demonstrated a significant bias towards the black context (see Figure 2, panels a and b). Therefore, a biased design was employed for conditioning. On each of eight consecutive days from P32-39 animals were given two trials/day following a similar procedure as Torrella et al (2006). Animals in the groups conditioned with amphetamine were always administered amphetamine in the white context, and saline in the black context. Saline was given in both contexts in all other groups. A drug-free preference test with dividers removed was administered on P40, with all animals injected i.p. with saline. All trials were 10 min in length. The paired context ratio, which served as the dependent measure, was calculated by the time spent in the drug-paired context divided by the total amount of time spent in both contexts on the preference test (Bardo et al 2003).

Results

Locomotor sensitization

There were no significant group differences in habituation (data not shown). An initial four-way ANOVA revealed several significant main effects and interactions, but most importantly a significant four-way interaction of sex×neonatal drug treatment×adolescent drug treatment×day of treatment $F(1,43) = 5.84, p < .025$. Post hoc analysis revealed that Female Group QA demonstrated significantly higher locomotor activity on day 7 compared to all other groups. Female Group SA also demonstrated significantly higher levels of activity at days 1 and 7 than male Group SA as well as controls (Group SS). Due to this and other significant interactions involving sex as a factor, males and females were analyzed separately.

Adolescent females are shown in Figure 1(a) with horizontal activity presented as a function of group and day of treatment. A $2 \times 2 \times 2$ three-way ANOVA revealed significant main effects of neonatal drug treatment $F(1,21) = 4.38, p < .05$, adolescent drug treatment $F(1,21) = 11.35, p < .005$, and day of treatment $F(1,21) = 20.98, p < .001$, a significant two-way interaction of adolescent drug treatment×day of treatment $F(1,21) = 12.73, p < .002$ and a significant three-way interaction of neonatal drug treatment×adolescent drug treatment×day of treatment $F(1,21) = 4.88, p < .05$. Most importantly, post hoc analysis of the significant three-way interaction revealed that female Group QA demonstrated significantly higher levels of activity than all other groups at day 7 of treatment, revealing that neonatal quinpirole enhanced amphetamine sensitization in adolescent females.

Adolescent males are shown in Figure 1(b) with horizontal activity presented as a function of group and day of treatment. A $2 \times 2 \times 2$ three-way ANOVA revealed a significant main effect of day of testing $F(1,21) = 13.81, p < .001$ and a two-way interaction of adolescent drug treatment×day of testing $F(1,21) = 21.44, p < .001$. Amphetamine produced a significant increase in locomotor activity at day 7 as compared to day 1 of treatment, but there were no other significant group differences.

Conditioned Place Preference

The paired context ratio is presented as a function of sex and neonatal drug condition in Figures 2(a: females) and 2(b: males) for the pre-conditioning test, and in Figures 2(c: females) and 2(d: males) for the post-conditioning test. Analysis of the pre-conditioning test revealed no significant main effects or interactions. For the post-conditioning test, A $2 \times 2 \times 2$ ANOVA revealed significant main effects of neonatal drug treatment $F(1,44) = 16.70, p < .001$ and adolescent drug treatment $F(1,44) = 22.41, p < .001$, and significant two-way interactions of sex×adolescent drug treatment $F(1,44) = 5.05, p < .05$, and neonatal drug treatment×adolescent drug treatment $F(1,44) = 4.62, p < .05$. Most importantly, analysis of

the neonatal drug treatment×adolescent drug treatment interaction revealed that animals conditioned with amphetamine and neonatally treated with quinpirole demonstrated a significantly higher paired context ratio than all other groups. Analysis of the sex×adolescent drug treatment interaction revealed that females conditioned with amphetamine demonstrated significantly higher paired context ratios compared to saline controls, but males conditioned with amphetamine did not spend significantly more time in the drug-paired context compared to saline controls.

Discussion

The results of the present study demonstrate that neonatal quinpirole treatment appears to enhance locomotor sensitization to amphetamine in adolescent females as well as time spent in an amphetamine-paired context using the CPP paradigm in both adolescent males and females. These results are consistent with past findings from our laboratory which showed that neonatal quinpirole treatment results in a sensitized locomotor and dopaminergic response in the striatum and nucleus accumbens core (Nowak et al 2001; Cope et al 2010), brain areas that mediate both locomotor sensitization and the associative effects of drugs (Volkow et al 2007). This result is also consistent with findings showing that neonatal quinpirole enhanced sensitization to the psychostimulant nicotine in adolescence (Sheppard et al 2009).

There have been relatively few published studies analyzing the adolescent behavioral response to amphetamine. Mathews & McCormick (2007) reported a lack of amphetamine sensitization in adolescent rats tested between the ages of P46 and 52, but only males were used as subjects. Laviola and colleagues (2001) showed that adolescents demonstrated a propensity to increase stereotypic behaviors, such as an increased head bobbing response, whereas adults demonstrated increased cage exploratory behavior when tested between the ages of P33-43. A challenge to a 2 mg/kg amphetamine dose after a five day abstinence period revealed that adolescent rats previously treated with the 2 mg/kg dose demonstrated a prominent increase of amphetamine-stimulated dopamine release compared to controls and rats administered the 10 mg/kg dose, whereas no such change was found in adult subjects. This result demonstrates an increased sensitivity to the effects of amphetamine on the dopamine system in adolescence, although this was not manifested in locomotor sensitization as analyzed through activity counts.

In agreement with past work, adolescent males demonstrated a muted behavioral response to amphetamine on both the locomotor sensitization and CPP behavioral tests (Bolanos et al 1998; Mathews & McCormick 2007; 2010), demonstrating a surprising sex difference in the response to amphetamine in adolescence, the basis of which is unknown. This finding may be related to sex differences in development of the dopamine system during adolescence. Andersen and Teicher (2000) have shown that males have significantly lower levels of striatal D₂ receptors compared to females at P25 and significantly higher levels of D₂ receptors by P40, although equal to females in this brain region at P60. In addition, males demonstrate higher levels of accumbal D₁ receptors compared to females throughout early postweanling and adolescent periods, and a robust increase in D₁ receptors in the striatum at P40. Thus, there appears to be sexually dimorphic development of the dopamine receptor system in adolescence. We have recently shown that adolescent males did not demonstrate any change in behavior compared to controls in response to the D₂/D₃ antagonist eticlopride or the D₃ antagonist nafadotride at P33, which was in contrast to adolescent females tested at the same age (Sheppard, et al 2009). Treatment with either antagonist ultimately resulted in a hypoactive behavioral response later in treatment, suggesting that the male dopaminergic system, especially the D₂-like receptor family, may be immature at this age. Bolanos et al (1998) has hypothesized that adolescent male rats may demonstrate behavioral

subsensitivity to amphetamine due to the transient elevation of dopamine transmission after amphetamine treatment, resulting in post-synaptic supersensitivity of cholinergic receptors and behavioral subsensitivity when challenged with psychostimulants. However, this hypothesis has yet to be tested.

In conclusion, results from the present study revealed that neonatal quinpirole enhanced amphetamine locomotor sensitization and CPP in adolescence, but there were significant sex differences in this effect. Interestingly, the muted response to amphetamine in adolescent males is also consistent with past work showing a muted locomotor behavioral response to the psychostimulant cocaine (Frantz et al 2007) as well as nicotine (Schochet et al 2004). Sex and age differences in the response to psychostimulants needs to be further explored to discover the mechanism of this effect.

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Figure 1.

All groups are indicated on the figures by neonatal drug treatment (quinpirole = Q, saline = S) followed by adolescent drug treatment (amphetamine = A, saline = S). (a) Horizontal activity for adolescent females are presented as a function of day and group. **Female Group QA demonstrates significantly higher levels of activity on day 7 as compared to all other groups. *Female Groups QS and SA demonstrated significantly higher levels of activity as compared to controls at days 1 and 7 (b) *Male groups QA and SA demonstrated significantly higher levels of horizontal activity as compared to Group SS at day 7. Both ** and * indicate effects at $p < .05$.

Figure 2.

All groups are indicated on the figures by neonatal drug treatment (quinpirole = Q, saline = S) followed by adolescent drug treatment (amphetamine = A, saline = S). For the pre-conditioning test there were no significant differences in paired context ratio in either females (a) or males (b) due to sex or neonatal drug treatment (c) The post-conditioning paired context ratio is presented as a function of group for adolescent females. **Group QA demonstrated a significantly higher paired context ratio compared to all other adolescent female groups, *Group SA also demonstrated a significant increased paired context ratio compared to Group SS. (d) The post-conditioning paired context ratio is presented as a function of group for adolescent males. **Group QA demonstrated a significantly higher paired context ratio than all other groups. Group SA did not significant differ from Group SS in adolescent males. Similar effects were seen in Group QA in females (c) and males (d). Both ** and * indicate effects at $p < .05$.