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Neonatal quinpirole treatment enhances locomotor activation and dopamine release in the nucleus accumbens core in response to amphetamine treatment in adulthood

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Abstract

Neonatal quinpirole treatment to rats produces long-term increases in D₂ receptor sensitivity that persists throughout the animal's lifetime, a phenomenon referred to as D₂ priming. Male and female Sprague-dawley rats were administered quinpirole (1mg/kg) or saline from postnatal days (P)1–11. At P60, all animals were given an injection of quinpirole (100 ug/kg), and results showed that rats neonatally treated with quinpirole demonstrated enhanced yawning in response to quinpirole, verifying D₂ receptor priming because yawning is a D₂ receptor mediated event. Beginning 1–3 days later, locomotor sensitization was tested through administration of d-amphetamine (1mg/kg) or saline every other day over 14 days, and horizontal activity and turning behavior were analyzed. Findings indicated that D₂-priming enhanced horizontal activity in response to amphetamine in females compared to males at days 1 and 4 of locomotor sensitization testing, and D₂-priming enhanced turning in response to amphetamine. Seven to 10 days after sensitization was complete, microdialysis of the NAcc core was performed using a cumulative dosing regimen of amphetamine (0.1 – 3.0 mg/kg). D₂-primed rats administered amphetamine demonstrated a 500% increase in accumbal DA overflow compared to control rats administered amphetamine. Additionally, amphetamine produced a significant increase in NE overflow compared to controls, but this was unaffected by D₂ priming. These results indicate that D₂ receptor priming as is produced by neonatal quinpirole treatment robustly enhances behavioral activation and accumbal DA overflow in response to amphetamine, which may underlie increases in psychostimulant use and abuse within the psychotic population where increased D₂ receptor sensitivity is a hallmark.

Keywords

dopamine; sensitization; quinpirole; amphetamine; microdialysis; D₂ receptor

Introduction

Past studies have shown that neonatal quinpirole (a dopamine D₂/D₃ agonist) treatment to rats result in a significant increase of dopamine D₂ receptor sensitivity that persists

throughout the animal's lifetime, which is a phenomenon that has also been referred to as 'D₂ priming' (Kostrzewa, et al., 1993; Thacker, et al., 2006). Interestingly, this change in receptor sensitivity is independent of a change in receptor number (Kostrzewa, 1995). Increases in D₂ receptor sensitivity are common in several behavioral disorders, including schizophrenia, bipolar disorder, obsessive-compulsive disorder, and D₂ receptor family genetic polymorphisms have been shown in attention deficit/hyperactivity disorder (ADHD) (Jimerson et al. 1987; Seeman et al. 2006; Kieling et al. 2006; Sery et al. 2006). Recently we have shown that neonatal quinpirole treatment results in a significant decrease of genetic expression of *Rgs9*, a regulator of G-protein signaling at the dopamine D₂ receptor, in the nucleus accumbens, striatum, and to a lesser extent, in the frontal cortex of adult rats (Maple, et al., 2007). This significant decrease in RGS9 expression is consistent with post-mortem findings in psychosis (Seeman, et al., 2007). Additionally, we have shown that treatment with the antipsychotic olanzapine alleviates cognitive impairment and significant decreases of neurotrophic factor protein produced by neonatal quinpirole treatment (Thacker, et al., 2006), also consistent with other preclinical findings (Fumagalli, et al., 2003; Angelucci, et al., 2004).

In psychotic disorders, a common comorbidity is a significant 2–5 increase in psychostimulant abuse compared to the normal population (LeDuc and Mittleman 1995; Lasser et al. 2000; Snyder et al. 2006). Psychostimulants, such as amphetamine and nicotine, are the most frequently abused drugs in these behavioral disorders. Despite these observations no definitive explanation for the high level of drug abuse in this population exists, and few investigations have been done into the neural correlates of psychostimulant abuse in these populations. Interestingly, imaging studies in schizophrenics have shown that treatment with amphetamine produces a significant increase in striatal dopamine release, which is most pronounced during episodes of illness exacerbation (Laruelle, et al., 1996; Abi Dargham, et al., 1998). This result suggests that schizophrenics may be vulnerable to abuse of psychostimulants due to an increased dopaminergic response to drugs of addiction, which presumably would produce increased behavioral activation and positive reinforcement in response to the drug. Consistent with this past work, a collaborating laboratory has shown that acute amphetamine to rats D₂-primed with quinpirole as neonates resulted in a four-fold increase in striatal dopamine release compared to controls administered amphetamine (Nowak, et al., 2001). However, this past study did not analyze sex differences or locomotor sensitization, and although the dorsal striatum plays a role in mediating locomotor activation and positive reinforcement, the nucleus accumbens has been shown to central to the reinforcement properties of addictive drugs as well as mediate behavioral activating aspects of psychostimulants (DiChiara, 1993).

The present study was designed to analyze amphetamine locomotor sensitization as well as DA and NE microdialysis in male and female rats D₂-primed as neonates with the D₂/D₃ agonist quinpirole. Locomotor sensitization has been prominently used as a behavioral assay to analyze underlying behavioral mechanisms of addiction (Robinson and Berridge, 1993; Pierce and Kalivas, 1995). An additional focus of this study was to analyze the effects of previous exposure to amphetamine on dopamine release in the nucleus accumbens core in animals D₂ receptor primed as neonates. The rationale for choosing the NAcc core is based on findings that have shown that the core, but not the NAcc shell, has been implicated in dopamine release in psychostimulant locomotor sensitization (Cadoni, et al., 2000; Ito, et al., 2000), and the NAcc core is preferentially innervated by nuclei that process motor information (Heimer, et al., 1991). Finally, direct injections of dopamine to this region produce a more robust locomotor response than the shell of the NAcc (Campbell, et al., 1997). Based on the focus of this study on locomotor behavioral sensitization and the role of the NAcc core in locomotor behavior, we chose to focus on the NAcc core as the brain area for microdialysis.

Male and female rats were used as subjects, as several past studies have reported a sex difference in both the behavioral and neurochemical response to amphetamine (Becker, et al., 1999; 2001). Additionally, both dopamine (DA) and norepinephrine (NE) levels were analyzed, because although increases in dopaminergic activity have been shown to be central to primary drug reinforcement and locomotor sensitization (for review, see Vetulani, 2001), amphetamine has also been shown to increase NE in the NAcc core which may be related to behavioral effects produced by amphetamine (McKittrick and Abercrombie, 2007). Further, several studies have shown alterations in the NE system in post-mortem analyses of the nucleus accumbens in psychosis, specifically in schizophrenia, (Farley, et al., 1978; Bridge, et al., 1985, for review see Yamamoto & Hornykiewicz, 2004) but it is unclear whether this change is an increase or decrease of noradrenergic functioning within the accumbens.

Materials and Methods

A total of 10 adult untimed pregnant female Sprague-dawley rats were ordered from Harlan, Inc (Indianapolis, IN) and the offspring were used as subjects. Rats were weaned from the female dam at P22, socially housed 2–3 per cage and allowed to age to adulthood (P60) without any further drug treatment, although animals were handled occasionally during this period. Only one male and one female from each litter were assigned to each drug condition to control for within litter variability (Holson & Pearce, 1992). Animals were kept in an Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) accredited climate-controlled animal colony with a 12-hour on/off light/dark cycle, and all testing was performed during the light cycle. All procedures in this experiment were approved by the University Committee on Animal Care at East Tennessee State University.

Neonatal injections and yawning behavior

Neonatal drug injections began on postnatal day (P)1, the day following birth, and administered once each day through P11. All neonatal injections were given ip at 1% of the animal's weight. At P60, D₂ receptor priming was behaviorally verified by testing for increased yawning behavior. Yawning has been shown to be a dopamine D₂-like receptor mediated event, with studies demonstrating that this behavior is mediated by either the D₂ (Cooper, et al., 1989), or D₃ subtype (Collins, et al., 2007). Prior to testing, each animal was administered an injection of 100 µg/kg quinpirole and then placed in an inverted cage without bedding as the presence of bedding can induce chewing that can supersede yawning. This procedure was done to behaviorally verify that the dopamine D₂ receptor has been primed by neonatal quinpirole treatment. This dose of quinpirole was chosen based on past work that has shown that this dose produces a maximal yawning response in adult rats (Nowak, et al., 2001). An observer blind to condition recorded the number of yawns for 1 hr in each subject.

Amphetamine sensitization

On the day following the yawning test, animals began amphetamine sensitization. Locomotor activity was recorded and measured using the Any Maze (Stoelting Co, Wood Dale, IL) tracking system. All animals were tested in a square arena, measuring 40 cm on a side, and the arena was painted flat black. On each day of habituation, all rats were i.p. administered 0.9% saline 15 min before being placed into the locomotor arena. Beginning at approximately P65, all animals were given three habituation trials, administered one each day for three consecutive days. All testing sessions were 10 min. The day following habituation, animals began amphetamine sensitization, and were administered either d-amphetamine sulfate (1 mg/kg) or saline every other day over a 14 day period for a total of seven exposures to amphetamine. Similar to habituation, amphetamine or saline was

administered 15 min before the animal was placed into the locomotor arena, and the animal's behavior was tracked for 10 min. The rationale for using an intermittent sensitization paradigm is based on work that has shown repeated intermittent exposure to psychostimulants produces a long-term enhancement in the drug's ability to increase locomotion and DA release compared to controls (Robinson & Camp, 1990; Saito, et al., 2005). Immediately after each day of behavioral testing during amphetamine sensitization and microdialysis, all females were vaginally swabbed to determine the stage of the estrous cycle, which has been shown to influence amphetamine locomotor sensitization and DA release in females (for review, see Becker, 1999). The number of animals behaviorally tested in each group is detailed below, with the first letter (Q or S for quinpirole or saline) referring to neonatal drug treatment and second letter (A or S; for amphetamine or saline) referring to drug treatment in adulthood. The number for females behaviorally tested for each group was as follows: Group QA = 10, Group QS = 10, Group SA = 9, Group SS = 9. The number of males behaviorally tested for each group was as follows: QA = 9, QS = 10, SA = 8, and SS = 10.

Behavioral dependent measures

There were two behavioral dependent measures analyzed in this study: horizontal activity to test behavioral activation, and turning behavior to test stereotypic behavior. For horizontal activity, the AnyMaze software program digitally superimposes a 5×5 cm grid across the floor of the apparatus. Each time the animal crosses a line on the grid, this is recorded as an activity count. The total line crosses for each trial provide a quantification of overall horizontal activity. For turning behavior, the definition of a clockwise or counterclockwise turn is a 360° rotation made by each animal, and this complete turn has to be made within a 5 second period. The rationale for choosing this measure is that an animal can turn within a radius that may not actually cross a line on the superimposed grid, so this dependent measure is another gauge of activity in the animal. Additionally, turning behavior is a type of stereotypic behavior, and increases in stereotypic behavior are often observed in cases of significant increases of dopamine activation after administration of psychostimulants (Christie and Crow, 1971; Glick, et al., 1983; Ali, et al., 1994; Koshikawa, 1994; Schwarting and Borta, 2004).

Surgical procedure

On the day after amphetamine sensitization was complete, the guide cannula was surgically implanted. For surgery, all rats were injected i.p. with a 1:1 ratio of ketamine (100mg/kg) and xylazine (10mg/kg) to produce preliminary surgical anesthesia and placed into a stereotaxic frame. Once the animal was placed into the ear bars and incisor plate, the rat's nose was placed into a Kopf stereotaxic mask (Kopf Instruments, Inc., Tujunga, CA) which was attached to an isoflurane/O₂ gas mixture delivered through an anesthesia machine (JD Medical VT-100, Phoenix, AZ). The dermis overlying the skull was shaved and incised. A small burr hole was drilled and a guide cannula (BioAnalytical, Lafayette, IN) was placed at the following coordinates aimed at the dorsal side of the NAcc core: AP = + 1.0 mm, ML = ± 1.2 mm, V = - 6.0 mm below the dura mater. The NAcc core is located at a depth of 6.0 to 8.0 mm ventral to the dura mater at these coordinates (Paxinos & Watson, 2006). The length of the active membrane for the dialysis probe was 2.0 mm, and the tip of the dialysis probe was lowered through the guide cannula to - 8.0 mm, which placed the probe on the ventral edge of the NAcc core, meaning that the active membrane of the probe extended the length of the NAcc core. This length of active membrane at this location has been shown to be effective for microdialysis of DA within the NAcc core (He, et al., 2004; Lecca, et al., 2004). The side of the brain analyzed in each subject was balanced across groups. Three stainless steel screws (Plastics One, Roanoke, VA) were mounted to the cranium and the

assembly fixed in place with dental cement. Animals were allowed at least six days to recover from implantation of the guide cannula before microdialysis was to commence.

Microdialysis procedure

Approximately 7–10 days after amphetamine sensitization was complete, and 6–9 days after the guide cannula was implanted, microdialysis was performed on each rat. For microdialysis, a pin style microdialysis probe (Bioanalytical, Lafayette, IN) was implanted through the guide cannula, with 2.0 mm of the active membrane exposed. All rats were placed into the identical arena used for amphetamine behavioral sensitization training. Once the animal was attached to the probe, artificial CSF was perfused continuously at 2.0 μ l/min through the dialysis probe for 1 h. Over the next two hours, six baseline samples were collected at 20 min intervals. After this interval, d-amphetamine sulfate was injected using a cumulative dosing procedure similar to that used by Schad, et al. (1996). Over the next four 20 min intervals, rats were given an injection of the following doses of d-amphetamine sulfate in this order: 0.1, 0.4, 1.0, and 1.5 mg/kg. This provided a dose-effect profile of amphetamine's effects in D₂-primed rats compared to saline controls, as doses of 0.1, 0.5 (0.1 + 0.4mg/kg), 1.5 (0.1 + 0.4 + 1.0 mg/kg) and 3.0 (0.1 + 0.4 + 1.0 + 1.5 mg/kg) were analyzed. Samples were collected every 20 min throughout drug treatment and 0.1 M perchloric acid (HClO₄) was added to each vial to counter degradation. Samples were stored at –80° C for later analysis.

Before experimental samples were analyzed, a series of standards for dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), and norepinephrine (NE) were prepared at various known concentrations to construct a standard curve from which concentrations of the target neurotransmitters or DA metabolites were calculated. Additionally, pure standards were injected onto the column to determine the retention time for each compound. The samples being tested were allowed to defrost to liquid phase and then placed into vials in an autosampler (ESA, Chelmsford, MA). The autosampler injected 40 μ l of each sample onto the detection column where the sample was separated into its respective constituents that cleared the column at its retention time. The area of each target peak was compared to the standard curve to calculate concentrations for each compound. The baseline concentrations of DA, DOPAC, HVA, and NE were all calculated in picograms (pg) per 10 μ l (presented in Table 1) and represent an average of the last three baseline samples taken (last hour of baseline testing). For microdialysis of all compounds after drug treatment, the percentage of baseline was calculated based on the concentration of the target compound at each time point.

Statistical analysis

A repeated measures four-way ANOVA was used as the primary statistic. There were a total of four factors in the experimental design: *sex* (male, female), *neonatal drug treatment* (quinpirole, Saline), *adulthood drug treatment* (amphetamine, saline), and *day of testing* (repeated measure: Days 1, 4, and 7 of drug treatment). It is important to note that animals were behaviorally tested all seven days of amphetamine treatment, but these three days for behavioral analysis were chosen for analysis because this provides a representation of initial, middle, and late assays of amphetamine sensitization. For statistical analysis of microdialysis, all of the same factors were analyzed, but the repeated measure increased to nine levels because this was the number of samples taken after the first drug injection (referred to as *time point*). An additional factor, stage of the estrous cycle (estrous, diestrus, proestrus, and metestrus), was added to the statistical analysis of both locomotor sensitization and microdialysis in females. After microdialysis was complete, the position of the probe was verified, and a subset of animals was eliminated from the study due to surgical problems or probe misplacement. Thus, the number of subjects per group tested for

microdialysis was a subset of the animals behaviorally tested. The number of animals tested in each group is detailed below, with the first letter (Q or S for quinpirole or saline) referring to neonatal drug treatment and second letter (A or S; for amphetamine or saline) referring to drug treatment in adulthood. The number of females in each group was: QA=6, QS=6, SA=6, and SS=5. For males, the number of animals tested in each group was exactly the same QA=6, QS=6, SA=6, and SS=5. For all post hoc analyses, the Newman-Keuls test was used ($p=.05$).

Results

Yawning behavior is presented as a function of sex and neonatal drug condition in Figure 1. A 2×2 ANOVA revealed significant main effects of sex $F(1,77) = 9.60$, $p<.003$, neonatal drug treatment $F(1,77) = 13.08$, $p<.001$ and a significant interaction of Sex $\times$ Neonatal Drug treatment $F(1,77) = 4.75$, $p<.032$. Post hoc analysis revealed that males neonatally treated with quinpirole demonstrated significantly more yawning in response to acute quinpirole compared to all other groups, and females neonatally treated with quinpirole demonstrated a significant increase in yawning as compared to female controls. This result verifies D_2 priming, and replicates past studies reporting that males demonstrate increased yawning as compared to females (Thacker, et al., 2006; Perna, et al., 2008).

For the initial statistical analysis of locomotor sensitization, A $2 \times 2 \times 2 \times 3$ three-way ANOVA revealed significant main effects of adulthood drug treatment $F(1,67) = 29.71$, $p<.001$ and day of testing $F(2,67) = 4.85$, $p<.009$, significant two-way interactions of Neonatal Drug Treatment $\times$ Adulthood Drug Treatment $F(1,67) = 7.34$, $p<.008$, Sex $\times$ Day of Testing $F(2,130)=4.88$, $p<.009$, Adulthood Drug Treatment $\times$ Day of Testing $F(2, 67) = 16.81$, $p<.001$, a significant three-way interaction of Neonatal Drug Treatment $\times$ Adulthood Drug Treatment $\times$ Day of Testing $F(2, 67) = 3.80$, $p<.025$, and a significant four-way interaction of sex $\times$ Neonatal Drug Treatment $\times$ Adulthood Drug Treatment $\times$ Day of Testing $F(2, 67) = 5.96$, $p<.003$. Based on the fact that males and females were analyzed separately below, post hoc analyses here will focus only on significant interactions involving sex as a factor to analyze sex differences. Post hoc analysis of the sex $\times$ Day of Testing interaction revealed that females were significantly more active than males on day 4, but were equivalent to males on days 1 and 7. Additionally, analysis of the significant four-way interaction revealed that female Group QA demonstrated significantly higher levels of activity compared to all other groups at day 4, and female Group SS demonstrated significantly higher levels of activity compared to male Groups QS and SS at day 7. To further analyze these significant interactions, we separately analyzed males and females below.

For females, an initial analysis including the stage of the estrous cycle revealed that estrous cycle stage did not produce any significant differences across days of testing in amphetamine-treated females and this factor was subsequently dropped from the analysis. Horizontal activity for females is presented as a function of drug treatment in Figure 2(a). A $2 \times 2 \times 3$ three-way ANOVA using neonatal drug treatment, adulthood drug treatment and day of testing as the three factors revealed a significant main effect of neonatal drug treatment $F(1,34) = 6.90$, $p<.013$, adulthood drug treatment $F(1,34) = 31.49$, $p<.004$, and significant two-way interactions of Neonatal Drug Treatment $\times$ Adulthood Drug Treatment $F(1,34)=6.75$, $p<.014$ and Adulthood Drug Treatment $\times$ Day of Testing $F(2,34) = 5.17$, $p<.029$. Post hoc analyses of these Neonatal Drug Treatment $\times$ Adulthood Drug Treatment interaction revealed that Group QA demonstrated significantly higher levels of horizontal activity compared to all other groups. Additionally, post hoc analysis of the Adulthood Drug Treatment $\times$ Day of Testing interaction demonstrated amphetamine produced a significant increase in horizontal activity at days 4 and 7 compared to females treated with saline. These

results show that D₂-priming as is produced by neonatal quinpirole treatment enhanced the behavioral response to amphetamine in females.

Horizontal activity for males is presented as a function of drug treatment in Figure 2(b). A $2 \times 2 \times 3$ three-way ANOVA revealed significant main effects of adulthood drug treatment $F(1,33) = 27.09$, $p < .01$, day of testing $F(2, 33) = 21.63$, $p < .001$, and significant two-way interactions of Neonatal Drug Treatment $\times$ Adulthood Drug Treatment $F(1,33) = 6.90$, $p < .013$, and Adulthood Drug Treatment $\times$ Day of Testing $F(2,33) = 4.91$, $p < .034$. Post hoc analysis of the significant Neonatal Drug Treatment $\times$ Adulthood Drug Treatment interaction revealed that identical to females, Group QA demonstrated significantly higher levels of horizontal activity as compared to all other groups. Analysis of the significant Adulthood Drug $\times$ Day of Testing revealed that amphetamine produced a significant increase in horizontal activity at days 4 and 7. Identical to females, it appears that neonatal quinpirole treatment resulted in an enhanced behavioral response to amphetamine.

For turning behavior, an initial analysis determined no significant differences in turning a specific direction across any of the groups, therefore, the number of clockwise and counterclockwise turns were summed. Turning behavior is presented as a function of drug treatment in for females in Figure 3(a) and males in Figure 3(b). However, males and females were analyzed together in this analysis based on the finding of only one statistically significant effect involving sex as a factor. A $2 \times 2 \times 3$ three-way ANOVA revealed a significant main effect of adulthood drug treatment $F(1, 67) = 29.82$, $p < .001$ and three significant two-way interactions of Neonatal Drug Treatment $\times$ Adulthood Drug Treatment $F(1,67) = 5.60$, $p < .02$, sex $\times$ Day of Testing $F(2, 67) = 3.91$, $p < .022$ and Adulthood Drug Treatment $\times$ Day of Testing $F(2, 67) = 4.70$, $p < .01$. Post hoc analysis of the three significant two-way interactions revealed that Group QA demonstrated significantly more turning behavior than all other groups. Additionally, analysis of the sex $\times$ Day of Testing interaction revealed that males demonstrated higher levels of turning as compared to females on day 7, and analysis of the Adulthood Drug Treatment $\times$ Day of Testing interaction revealed amphetamine significantly increased turning behavior at days 4 and 7 compared to saline controls. It appears that D₂-priming increased turning behavior, in response to amphetamine compared to non D₂-primed controls.

When brain tissue was removed, microdialysis probe placement was verified after the tissue was sectioned. All probes were found to be at locations between 1.0 and 1.2mm anterior to bregma, between 1.2 and 2.0 mm lateral from the midline and the 2 mm active membrane of the probe extended between 6 to 8 mm ventral to the dura mater (see Figure 4a). The other 6 probes were located at 1.2 mm anterior to bregma, between 1.2 and 1.8 mm and 6 to 8 mm ventral to the dura mater (see Figure 4b). It should be noted that of the 52 animals included for microdialysis, 46 of the probe placements were precisely at 1.0 mm anterior to bregma. All probes intersected the NAcc core (Paxinos and Watson, 2006). Any animals with probe placements outside of these measurements were not included in the study.

The mean percentage of baseline dopamine levels in the NAcc core are presented as a function of drug treatment condition in Table 1. In the initial analysis of dopamine (DA), homovanillic acid (HVA), DOPAC, and norepinephrine (NE), there were no statistically significant effects involving sex or stage of estrous cycle as a factor, therefore, these factors were dropped from the analysis. A 2×2 two-way ANOVA was performed on baseline levels for DA, HVA, or DOPAC, and there were no significant main effects or interactions. However, a 2×2 ANOVA on basal levels of NE revealed a significant main effect of neonatal drug treatment $F(1,45) = 5.62$, $p < .022$. It appears that neonatal quinpirole treatment produced a significant 57% decrease in NE levels in the NAcc core as compared to controls.

Dopamine levels are presented as a function of time point and drug treatment condition in Figure 5(a). A $2 \times 2 \times 9$ three-way ANOVA on DA levels calculated as a percentage of baseline after drug treatment revealed significant main effects of neonatal drug treatment $F(1,45) = 8.53$, $p < .005$, adulthood drug treatment $F(1,45) = 25.23$, $p < .001$, and time point $F(8,45) = 7.24$, $p < .001$, and significant two-way interactions of Neonatal Drug Treatment $\times$ Adulthood Drug Treatment $F(1,45) = 7.32$, $p < .009$, Neonatal Drug Treatment $\times$ Time Point $F(8, 45) = 2.26$, $p < .023$, and Adulthood Drug Treatment $\times$ Time Point $F(8, 45) = 6.06$, $p < .001$. Most importantly, post hoc analysis of the Neonatal Drug Treatment $\times$ Adulthood Drug Treatment interaction revealed that Group QA demonstrated significantly higher DA levels expressed as a percentage of baseline as compared to all other groups, and the increase was approximately 500% compared to Group SA at 60, 80, and 100 min after initial amphetamine treatment. This result shows that D_2 -priming as is produced by neonatal quinpirole treatment enhanced the DA response to amphetamine as compared to animals neonatally treated with saline. As expected, Group SA demonstrated significantly higher DA levels than Group SS, showing that amphetamine increased DA release compared to controls. Analyses of DOPAC (Figure 5b) and HVA (Figure 5c) did not reveal any significant main effects or interactions.

NE levels are presented as a function of time point and drug treatment condition in Figure 6. A $2 \times 2 \times 9$ three-way ANOVA treatment on NE levels in the NAcc core revealed a significant main effect of time point $F(8,43) = 13.66$, $p < .001$, a significant two-way interaction of Adulthood Drug Treatment $\times$ Time Point $F(8,43) = 5.39$, $p < .025$, and a significant three-way interaction of Neonatal Drug Treatment $\times$ Adulthood Drug Treatment $\times$ Time Point $F(8,43) = 6.43$, $p < .015$. Post hoc analysis of the two-way interaction of Adulthood Drug Treatment $\times$ Time Point demonstrated that adulthood amphetamine treatment produced a significant 200% increase in NE levels at 80, 100, and 120 min post drug treatment compared to animals given saline. This effect was clearly less than the effects on DA, but is consistent with past findings demonstrating that amphetamine produces a significant increase in NE in the NAcc core (McKittrick and Abercrombie, 2007), although the increase in NE reported here is less than that reported in this previous study. Post hoc analysis of the significant three-way interaction demonstrated a significant increase in NE levels in the control group, S-S, compared to all other groups but only at 20 min after amphetamine treatment had begun. This is a surprising effect, and may be due to an invariable NE baseline in this group. However, note that NE levels in this group were relatively stable as testing progressed.

Discussion

The results from the present study showed that rats neonatally treated with quinpirole demonstrated enhanced horizontal activity and turning behavior in response to d-amphetamine treatment in adulthood, and the effect on horizontal activity was more prominent in female as compared to male rats. Additionally, rats that received neonatal quinpirole treatment demonstrated a robustly enhanced dopaminergic response to amphetamine in the NAcc core compared to controls administered amphetamine, although there were no sex differences observed in this response. Therefore, it appears that increases in D_2 receptor sensitivity as a result of neonatal quinpirole treatment leads to an enhanced dopaminergic response to the psychostimulant amphetamine in the nucleus accumbens, a brain area known to mediate locomotor activation and primary drug reinforcement (Sellings and Clarke, 2003). This effect is consistent with clinical findings in schizophrenics, a population shown to demonstrate increased D_2 receptor occupancy and function (Abi-Dargham, 2004; Remington, et al., 2006), that have shown amphetamine treatment results in significantly larger increases in striatal DA concentrations of patients with schizophrenia compared to healthy controls (Laruelle, 1996; Abi-Dargham, 1998). These results together

suggest an increased effect of amphetamine on behavioral activation and possibly increased positive reinforcement in response to amphetamine in this population as compared to the normal population. This increased response of the brain's drug reinforcement system to amphetamine may ultimately lead to increased psychostimulant abuse in this population. Additionally, animals administered amphetamine demonstrated a significant increase in NE overflow in the NAcc core, consistent with past results (McKittrick and Abercrombie, 2007), although the increase in NE overflow was unaffected by increases in D₂ receptor sensitivity.

Results showed that D₂ primed animals exhibited a 500% increase in accumbal DA overflow compared to control animals when both were administered amphetamine. This increase in dopaminergic activity was presumably manifested in increased locomotor activation and turning behavior produced by amphetamine in D₂-primed animals. The behavioral effects of amphetamine have been hypothesized to be due to the drug's ability to increase DA concentrations in the brain, specifically in the NAcc (Pierce and Kalivas, 1997), but also the striatum (Paulson and Robinson, 1995). Past studies have shown that amphetamine may facilitate dopaminergic neurotransmission in a number of ways, including direct release of dopamine from presynaptic terminals, inhibition of dopamine uptake, and inhibition of monoamine oxidase-B (MAO-B) activity (Butcher, et al., 1988). Results have also shown that amphetamine produces subsensitivity of inhibitory A10 D₂ autoreceptors that is persistent for up to 3 to 4 days (Seutin, et al., 1991; Wolf, et al., 1993). Similar to the hypothesis of Nowak, et al., (2001), we hypothesize that the enhancement of dopamine overflow in D₂-primed rats produced by amphetamine was accompanied by subsensitivity of presynaptically located dopamine D₂ autoreceptors and by overt priming of postsynaptic DA D₂ receptors, resulting in an overall significant increased dopaminergic response.

Another mechanism that may be important in the increased dopaminergic response of D₂-primed rats could be related to the regulation of dopamine D₂ receptor-mediated cell signaling. A recent study published from our laboratory has shown that RGS9, a regulatory protein involved in D₂ receptor signaling, is significantly down-regulated by approximately 300% in the NAcc of rats neonatally treated with quinpirole (Maple, et al., 2007). RGS9 is a protein that has been shown to accelerate the termination of D₂ related events (Kovoor et al., 2005). Rahman, et al. (2003) has shown that viral-mediated overexpression of RGS9 in rat NAcc resulted in attenuation of locomotor responses to cocaine and apomorphine. Additionally, *Rgs9* expression is significantly decreased in the hippocampus of schizophrenics, although the NAcc has yet to be analyzed (Seeman, et al., 2007). A significant decrease of RGS9 function in the NAcc may be a mechanism through which the dopaminergic response is substantiated after amphetamine administration in D₂-primed animals.

Present findings also demonstrated a significant decrease of baseline NE levels in the NAcc core in D₂-primed rats compared to controls. This result is consistent with a past study that has shown quinpirole to inhibit electrically evoked accumbal NE release using a slice preparation in rats (Vanderschuren, et al., 1999). Obviously, this past work used an *in vitro* preparation and also utilized an acute dose of quinpirole, but it does appear that dopamine D₂ receptor stimulation decreases NE activity in the NAcc, and the present results were consistent with this past finding. Also consistent with past work, amphetamine produced a significant increase in NE overflow in the NAcc core (McKittrick and Abercrombie, 2007), but this increase was unaffected by D₂-priming as is produced by neonatal quinpirole treatment. Thus, there does not appear to be an interaction of increases in D₂ receptor sensitivity with NAcc NE overflow, whereas increases in D₂ receptor sensitivity affected the dopaminergic response in the NAcc to amphetamine. There is very little information on the interaction between accumbal NE and DA systems, and the few studies on this topic have

primarily investigated NE receptor control of the DA system, but not vice versa (Nurse, et al., 1984; Yavich, et al., 1997; Ihlainen & Tanila, 2004). Amphetamine-induced changes of accumbal NE may play a significant role in mediating both the physiological and behavioral responses to the drug. Indeed, recent studies in mice have demonstrated that the noradrenergic pathways that project to these areas are crucial regulators of opiate-mediated reward (Olson, et al. 2006).

There were several sex differences reported in the present study, but these effects were primarily on the horizontal activity measure and related to neonatal quinpirole treatment. Interestingly, D₂-primed females administered amphetamine demonstrated significantly higher levels of activity than all other groups at days 1 and 4. This result shows both a more robust response to amphetamine after acute treatment and more rapid sensitization to amphetamine in females as compared to males neonatally treated with quinpirole. This result is consistent with recent findings from our laboratory analyzing nicotine sensitization in adult rats neonatally treated with quinpirole, which reported D₂-primed females demonstrated stronger behavioral activation to nicotine as compared to males (Perna, et al., 2008). This result is also consistent with past studies analyzing the locomotor response to quinpirole, which have shown females demonstrated a stronger locomotor activating response to quinpirole than males (Szumlinski, et al., 2000; Schindler and Carmona, 2002). Interestingly, in controls administered amphetamine, there were no significant differences between males and females throughout testing. This result contradicts several past studies that have shown female rats are more sensitive to the locomotor activating effects of amphetamine (Becker, et al., 2001; Dafny & Yang, 2006; Milesi-Halle, et al., 2007). However, there are several methodological differences between the current study and these past studies, including route of administration and dosing differences. Additionally, stage of the estrous cycle in females is known to greatly influence the behavioral and dopaminergic response to amphetamine. In the current study, it was found that stage of the estrous cycle did not influence the behavioral or dopaminergic response to amphetamine, but the number of subjects is likely too low for sufficient statistical power to detect a statistical difference. Through observation, there were a low number of females in each of the four stages of estrous, although it should be noted that all four stages were represented throughout behavioral testing and on analysis of DA and NE overflow. In absolute terms, females demonstrated a slightly higher DA overflow in response to amphetamine as compared to males, although this increase did not approach statistical significance. Thus, a future study could analyze whether there is an effect of estrous cycle in D₂-primed females through increasing the number of females tested. This result suggests that behavioral activation to amphetamine in D₂-primed females is higher than that of males, which may have implications relative to sex differences in psychotic individuals that abuse psychostimulants.

In conclusion, there are several consistencies between the present study and other non-clinical and clinical literature analyzing the effects of amphetamine on DA levels in model of psychosis and in psychotic individuals. In pre-clinical work, past studies using the neonatal ventral hippocampal lesion (NVHL) model of schizophrenia in rats have also shown hyper-responsiveness to amphetamine both behaviorally and in enhanced DA release in the NAcc core (Lipska & Weinberger, 2000; Corda, et al., 2006). Clinically, both schizophrenics and schizotypal personality disorder have shown an enhanced DA response in the dorsal striatum measured using PET (Laruelle, et al., 1996; Abi-Dargham, et al., 1998). Moreover, these studies revealed a correlation between the exaggerated response of the striatal dopaminergic system to acute amphetamine and a transient exacerbation of positive symptoms in patients with schizophrenia, underscoring the clinical relevance of this alteration in DA-mediated transmission.

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Figure 1. Yawning behavior is presented as a function of sex and neonatal drug treatment. Quinpirole-treated males demonstrated significantly higher levels of yawning than all other groups (indicated by **), and quinpirole-treated females demonstrated higher levels of yawning as compared to saline-treated females (indicated by *).

Figure 2.

(a). Horizontal activity is presented as a function of neonatal and adulthood drug treatment for females. Group QA demonstrated significantly higher levels of horizontal activity compared to all other groups (indicated by **). Group SA demonstrated significantly higher levels of horizontal activity compared to female Group SS (indicated by *). (b). Horizontal activity is presented as a function of neonatal and adulthood drug treatment for males. Group QA demonstrated significantly higher levels of horizontal activity compared to all other male groups (indicated by **), and Group SA demonstrated significantly higher levels of horizontal activity compared male Group SS.

Figure 3.

The number of turns is presented as function of neonatal and adulthood drug treatment. Group QA demonstrated significantly higher levels of horizontal activity compared to all other groups (indicated by **). Group SA demonstrated significantly higher levels of horizontal activity compared to Group SS at days 4 and 7 (indicated by *).

Figure 4.

Rat brain stereotaxic atlas for (a) 1.0 mm anterior to bregma, and (b) 1.2 mm anterior to bregma. Note that AcbC in this figure represents the nucleus accumbens core. The two lines on either figure indicate the furthest lateral and medial a probe was placed at each location for the left and right hemisphere. Note that all probes intersected the nucleus accumbens core. A total of 46 of the 52 probes intersected the NAcc core at 1.0 mm anterior to bregma, and the other 6 probes intersected the NAcc core at 1.2 mm anterior to bregma. Figure was adapted from *The Rat Brain in Stereotaxic Coordinates*, 5th edition (Paxinos and Watson, 2006).

Figure 5.

(a). Dopamine (DA) is presented as a percentage of baseline as a function of minutes post adulthood drug treatment and group. The arrows along the x axis indicate the dose of d-amphetamine injected at these time points. Group QA demonstrated a significant increase in accumbal core DA overflow compared to all other groups (indicated by **). Group SA demonstrated a significant increase in accumbal DA overflow compared to controls (Group SS; indicated by *). (b) DOPAC is presented as a percentage of baseline as a function of minutes post drug treatment and group. Although it appears that Group QS demonstrated an absolute increase in DOPAC levels from time points 80 through 160, there were no significant group differences. (c) Homovanillic acid (HVA) is presented as a percentage of baseline as a function of minutes post adulthood drug treatment and group. Likewise, Group QA demonstrated an absolute increase towards the end of testing, but statistically, there were no significant group differences.

Figure 6.

Norepinephrine (NE) is presented as a percentage of baseline as a function of minutes post adulthood drug treatment and group. Groups QA and SA demonstrated a significant increase in accumbal core NE overflow at time points 80, 100, and 120 compared to Groups SS and QS (indicated by *). Group SS demonstrated a slight but significant increase in NE concentrations at time point 20 (indicated by **).

Table 1

Basal concentrations of dopamine, HVA, DOPAC, and NE in the NAcc core.

Neonatal Drug Treatment	Adulthood Drug Treatment	Dopamine (pg/ 10µl)	HVA (pg/10ul)	DOPAC (pg/10ul)	NE (pg/10ul)
Quinpirole	Saline	20.1 +/- .4.9	126.3 +/- 2.7.8	138.9 +/- 45.4	1.87 +/- .26
Quinpirole	Amphetamine	25.4 +/- .6.1	146.8 +/- 42.2	147.1 +/- 34.9	1.54 +/- .22
Saline	Saline	29.2 +/- .8.4	94.8 +/- 34.6	114.5 +/- 28.6	2.67 +/- .5*
Saline	Amphetamine	22.5 +/- .9.5	126.9 +/- 46.7	107.9 +/- 21.5	3.34 +/- 1.04*