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Neurochemical and Behavioral Responses to Cocaine in Adult Male Rats with Neonatal Isolation Experience*

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Running title: Neonatal isolation enhances NAc DA responses to cocaine

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Abbreviations: Neonatal isolation (ISO); Non-handled (NH); Post-natal (PN); Dopamine (DA); Nucleus accumbens (NAc); fixed ratio (FR); Analysis of Variance (ANOVA); Analysis of covariance (ANOCOVA)

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Abstract

Our research demonstrates that neonatal isolation (ISO; 1 hr/day isolation; Postnatal days 2-9) enhances extracellular, ventral striatal dopamine (DA) responses to psychostimulants in infant and juvenile rats. In adult rats, we find ISO facilitates acquisition and maintenance of cocaine self-administration. We now test whether ISO enhances cocaine-induced accumbens DA levels in adults using in vivo microdialysis. Behavioral responses to cocaine and DA antagonists were also examined. Adult male rats were derived from litters subjected to ISO or non-handled (NH) control conditions. In Experiment 1, microdialysis probes were aimed at accumbens core and separate groups administered vehicle or cocaine (5, 10 mg/kg; IP). Samples were analyzed for DA levels via HPLC. In Experiment 2, ISO and NH rats were administered one of these cocaine doses and locomotor activity assessed. Effects of cocaine (0.3-30 mg/kg), the D₁ antagonist, SCH 23390 (0.003-0.03 mg/kg), and the D₂ antagonist, eticlopride (0.01-0.1 mg/kg) on disruption of responding for food were examined in Experiment 3. Cocaine plasma levels were assessed in Experiment 4. ISO enhanced cocaine-induced increases in accumbens DA levels. Further, the D₂, but not D₁, antagonist disrupted behavior to a greater extent in ISO vs NH rats. Yet, ISO did not significantly alter behavioral responses to cocaine or cocaine plasma levels. These data show that the ability of ISO to enhance accumbens DA responses to cocaine endures into adulthood. Moreover, that ISO rats are more sensitive to a D₂ antagonist may reflect decreased levels of this receptor type as we showed previously in infant rats.
Introduction

Over 1.8 million people in the United States use cocaine (SAMHSA, 1999) but not all will develop addiction. The propensity to develop addiction likely involves genetic factors. However, familial studies suggest that more than two-thirds of the overall variance is due to environmental factors (Pickens and Svikis 1988; Rounsaville et al. 1991; Tsuang et al. 1998; vandenBree et al. 1998). One such environmental factor may be stress exposure. Indeed, stress experienced early in life appears to be an important vulnerability factor for developing or relapsing to addiction (Gordon, 2002). The ability of stress to alter brain regions linked to psychoactive effects may contribute to this enhanced predisposition to addiction.

Cocaine acts as an indirect dopamine (DA) agonist. It increases synaptic DA levels in the nucleus accumbens (NAc) (Pettit and Justice, 1989) via its actions at the DA transporter inhibiting uptake into the presynaptic terminals (Harris and Baldessarini, 1973). Many of the behavioral effects of cocaine, including its ability to act as a reinforcer, are linked to this neurochemical effect (Ritz et al., 1987; Bergman et al., 1989). Like cocaine, stress increases DA neurotransmission (Deutch et al., 1985; Thierry et al., 1986; Kalivas and Stewart, 1991; Sorg and Kalivas, 1991). These and other effects common to cocaine and stress exposures may contribute to the ability of stress to enhance the behavioral effects of psychostimulant drugs (Antelman et al., 1980; Robinson, 1988; Kalivas and Stewart, 1991).

We have demonstrated that the chronic early life stress of neonatal isolation (ISO) has immediate and enduring behavioral and neurochemical effects in rats. ISO facilitates the acquisition (Kosten et al., 2000; Kosten et al., 2004a) and maintenance (Zhang et al., 2005) of cocaine self-administration and increases acute amphetamine-induced activity (Kehoe et al., 1998b) in adult male rats. Infant and juvenile rats with ISO experience show enhanced ventral
striatal DA levels after psychostimulant administration (Kehoe et al., 1996b; Kehoe et al., 1998a; Kosten et al., 2003) compared to control non-handled rats. Yet, ISO does not affect baseline DA levels in ventral striatum of pups or juveniles (Kehoe et al., 1996b; Kehoe et al., 1998a; McCormick et al., 2002; Kosten et al., 2003) and does not alter baseline locomotor activity levels at any age tested (Kehoe et al., 1996b; Kehoe et al., 1998b; Kosten et al., 2000; Kosten et al., 2004a; Kosten et al., 2005).

The main purpose of this study is to determine whether the ability of ISO to enhance cocaine-induced increases in extracellular DA levels in ventral striatum endures into adulthood. Thus, the study extends from our previous work in infants with ISO experience (Kosten et al., 2003). In addition to examining the acute neurochemical response to cocaine, we compare the acute behavioral effects of cocaine in ISO and NH rats using locomotor and schedule-controlled responding procedures. Our previous behavioral studies showing enhanced effects of cocaine in adult ISO rats utilized self-administration procedures (Kosten et al., 2000; Kosten et al., 2004a; Zhang et al., 2005). Data obtained from the present study will illuminate on whether our previous behavioral findings reflect, in part, an ISO-induced enhancement in the activational effects of cocaine. Further, we begin to investigate whether altered responses to cocaine may reflect changes in D₁- or D₂-like effects by examining the effects of DA antagonists on schedule-controlled responding. Previously, we showed that a single period of isolation in a novel environment decreased D₂ receptor binding in ventral striatum of 10-day old rat pups (Kehoe et al., 1996a). Lower D₂ levels predicts greater disruptions in responding by a D₂ antagonist as we showed previously in inbred rat strains that differ in levels of this receptor sub-type. (Haile and Kosten, 2001). Finally, although we have no a priori reason to suspect that ISO alters cocaine metabolism, we nonetheless compared plasma cocaine levels in ISO vs NH rats.
Methods

General Methods

Subjects and setting

Adult (70-120 days old) male rats were derived from litters born to Sprague-Dawley female rats mated with Sprague-Dawley rats (Charles River Laboratory; Wilmington, MA). Female rats from these litters were employed in other studies. Litters were either subjected to neonatal isolation (ISO), described below, or were non-handled (NH). Only one rat per litter was assigned to a study or dose group. Procedures were approved by the Institutional Animal Care and Use Committee and followed the “Principles of Laboratory Animal Care” (NIH publication No. 85-23, revised 1996)

After birth, rats were housed with littermates and dam in polypropylene cages in a temperature- and humidity-controlled colony room maintained on a 12:12 light/dark cycle (lights on at 0700) until weaning on post-natal day 25 (PN25). They were then housed in same sex pairs of the same treatment condition in hanging, stainless steel cages. Food and water were available ad libitum except for rats in Experiments 3. These rats were maintained on a food-restricted diet so that their body weights were at 85% of free-feeding rats.

Neonatal isolation procedure

The procedure used for neonatal isolation was as we described previously (Kosten et al., 2000; Kosten et al., 2003; Kosten et al., 2004a; Kosten et al., 2005). Litters born before 1700 hr were considered born on PN0. The following day (PN1), both ISO and NH litters were culled to 6 male and 6 female pups or as close to this ratio as possible. On PN2, all pups in the ISO litters began the 8-day isolation procedure that continued through PN9. On these days, each pup was
placed into an individual opaque plastic container (9-cm diameter and 8-cm deep) that contained no bedding for 1 hr (between 0900 and 1200). Containers were placed 20-30 cm apart within a chamber located in a heated (30° C), humidity-controlled room with white noise to mask other pups’ calls. On these days, NH pups remained in the cage with the dam and litter and were not disturbed even for cage cleanings.

Drugs

Cocaine, Ecgonine methyl ester benzoate hydrochloride, was provided by the National Institute on Drug Abuse (Research Triangle Institute, Research Triangle Park, NC). Eticlopride, S(-)-3-Chloro-5-ethyl-N-[(1-ethyl-2-pyrrolidinyl)methyl]-6-hydroxy-2-methoxybenzamide hydrochloride, and SCH 23390, R(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydrox-1H-3-benzazepine hydrochloride, were obtained from Research Biochemicals, Inc (RBI; Natick, MA). Solutions were dissolved in sterile isotonic saline and were administered IP in a volume of 1 ml/kg.

Experiment 1: Effects of cocaine on extracellular levels of DA in NAc.

Subjects

A total of 31 adult, male rats with probes verified to be in the NAc are reported on in this study. Data from four other rats were excluded due to placements outside of the NAc core. The sample included 17 NH rats of which 5 each were assigned to the vehicle and 10 mg/kg cocaine dose groups and 7 were assigned to the 5 mg/kg cocaine dose group. The 14 ISO rats included 5 each assigned to the vehicle and 5 mg/kg cocaine dose groups and 4 assigned to the 10 mg/kg cocaine dose group.

In vivo microdialysis procedure
The procedure for in vivo microdialysis was performed using a CMA 120 system for freely moving animals (CMA, Acton, MA). Rats were surgically implanted with a dialysis guide cannula aimed at the NAc core (AP = +2.2; ML = +1.8; DV = -7.0 mm) under nembutal (50 mg/kg; Abbott Laboratory, Chicago IL) anesthesia. A modified, pre-used probe (0.5 mm diameter, CMA/11) was inserted with the tip of the probe extending 1 mm beyond the guide cannula tubing. Penetration of the tissue with a pre-used probe, prior to testing, significantly reduces acute injury-induced neurochemical efflux (Devine et al., 1993). After a recovery period of at least 3-days, an unused probe was inserted into the cannula and a solution of artificial cerebrospinal fluid (150 mM Na; 3.0 mM K; 1.4 Ca; 0.8 Mg; 1.0 P; 155 Cl; pH = 7.4; Harvard Apparatus, Holliston, MA, USA ) was perfused through the probe constantly at a rate of 1.7 µl/min. Following a 1-hr “wash-out” period, dialysate samples were collected continuously into 250-µl polyethylene vials that contained 10 µl of 0.1% perchloric acid. Samples were collected every 15-min for a 2.5-hr baseline period. Following this baseline period, rats were injected (IP) with cocaine according to their group assignment and returned to the microdialysis chamber. Dialysate samples were collected every 15-min for another 2.5-hr post-cocaine administration period. The first sample obtained after injection was not used in the analysis to minimize effects of handling and injection. Rats were habituated to the chamber for 1-hr per day for 3 days prior to the test. Tests were conducted between 0800 and 1600 hr in a test room separate from the main laboratory under dim illumination.

Analysis of dialysate samples

Dialysate samples were analyzed for dopamine (DA) using high performance liquid chromatography (HPLC) with electrochemical detection, as described previously (McCormick et al., 2002; Kosten et al., 2003; Kosten et al., 2004b). Samples were run using a Gilson auto-
injection unit, an MD-150 column (ESA), electrochemical detector (Coulochem II-ESA; triple detector electrode system) with the electrochemical signal acquired via an ESA 501 chromatography software package. The first guard detector was set at +350 mV, the second detector at -175 mV and the third at +175 mV. The mobile phase (75 mM NaH₂PO₄·H₂O, 1.4mM OSA, 10 μM EDTA, 10% acetonitrile, pH 3.1 with H₃PO₄) was delivered via an ESA 580 HPLC pump at a flow rate of 0.6 ml/min. Levels of detected neurochemicals were expressed as pg/20 µl of dialysis.

**Histology of brains for probe placement**

Rats were decapitated rapidly under anesthesia (Nembutal) and brains were removed. A section of the brain (approximately 2-mm thick) around the cannula was placed in buffered 10% formalin solution. Brain tissue samples were frozen and sliced (40µ thickness) and then stained with cresyl violet. The samples were viewed under a light microscope to verify the placement of the probe with the aid of a brain atlas for rats (Paxinos and Watson, 1998).

**Data analysis**

Extracellular DA levels obtained after cocaine administration were analyzed using a 2 X 3 X 9 analysis of co-variance (ANOCOVA) representing the between group factors of Treatment condition and Cocaine dose with repeated measures on Time. The last baseline value obtained prior to cocaine administration was used as the co-variante. Significant treatment condition effects were followed up by post-hoc comparisons using Fischer’s exact tests.

**Experiment 2: Effects of cocaine on locomotor activity.**

**Subjects and groups**

A total of 92 adult male rats were employed in this study. This sample included 32 NH rats and 26 ISO rats. These rats were assigned to one of three cocaine dose groups in a non-
systematic manner. These groups were administered 0 (isotonic saline vehicle), 5, or 10 mg/kg cocaine (IP). The number of subjects per group ranged from 8 to 11 with a median of 9.5 rats per group.

**Apparatus**

The locomotor apparatus was a square, opaque chamber (120-cm X 120-cm X 45-cm) made of Plexiglas and equipped with a video-tracking system (Coulbourn Instruments; Allentown, PA). The apparatus was located in a separate, test room that was sound-attenuated and dimly lit. Locomotor activity was automatically tabulated.

**Procedure**

On the test day, the rat was injected with its assigned cocaine dose and placed immediately into the center of the apparatus. The dependent measure used was distance traveled (cm), a reflection of ambulatory locomotor activity. Data were collected in 5-min time bins for a 40-min session.

**Data analysis**

Distance traveled (cm) by 5-min time blocks was analyzed using a 2 X 3 X 8 ANOVA representing the between group factors of Treatment condition (ISO vs. NH) and Cocaine dose with repeated measures on Time. Significance was set at P<0.05.

**Experiment 3: Effects of cocaine and DA antagonists on schedule-controlled responding for food.**

**Subjects and groups**

A total of 18 adult, male rats were employed in this study. This sample included 8 ISO and 10 NH rats. Cocaine tests were conducted with 8 NH rats and all ISO rats.

**Apparatus**
Standard operant chambers (Coulbourn Instruments) housed in ventilated sound-attenuating cubicles (Coulbourn Instruments) equipped with fans to mask outside noise were utilized in this study. There were two response levers located on either side of a food trough on a wall in each chamber. The house light was located at the top of this wall and there were three cue lights positioned directly above each lever. Downward pressure (about 25 g) on either lever could result in a programmed consequence. Data on lever presses and rate of responding (# presses/sec) were recorded using a software program (Graphic State Notation; Coulbourn Instruments) and hardware system (Coulbourn Instruments) interfaced to a PC computer.

Training

Initially, rats were trained to press either lever to obtain a food pellet (45 mg; Bio-Serv, Inc. Frenchtown, NJ) under a fixed-ratio 1 (FR1) schedule of reinforcement. A total of 50 reinforcers could be obtained during daily sessions. The initiation of the sessions was signaled by the illumination of the house light and both sets of cue lights and terminated after 50 reinforcers were earned or 30-min elapsed whichever occurred first. The FR requirement was gradually increased until a FR15 schedule was achieved. Test sessions began once the rat showed consistent response rates (<20% variance over 3 consecutive days).

Testing

Test sessions were performed twice weekly (Tuesday and Friday) between 0800 and 1000. Training sessions continued on Monday, Wednesday, and Thursday as described above. The effects of pretreatment (15-min) with vehicle and with cocaine (0.3-30 mg/kg IP) on operant response rates (# lever presses/sec) were assessed. The effects of pretreatment with the D₁ DA antagonist, SCH 23390 (0.003-0.03 mg/kg; IP), and the D₂ DA antagonist, eticlopride (0.01-0.1
mg/kg; IP), were also assessed. Drugs and doses were tested in a non-systematic manner across rats. Each dose was tested twice for each rat and the means of these data points were used.

Data analysis

The dependent measure was response rate (lever presses/sec) during the test session. Data from the cocaine tests and the two DA antagonist drug tests were analyzed in three separate ANOCOVAs. The cocaine data were analyzed using a 2 X 5 ANOCOVA with the between group factor of Treatment condition (ISO vs. NH) with repeated measures of Dose using the response rates under the vehicle pretreatment condition as a co-variate. The SCH 23390 data and the eticlopride data were analyzed in two separate 2 X 3 ANOCOVAs with between group factor of Treatment condition and repeated measures on Cocaine dose. Rates obtained under the vehicle condition were used as a co-variate. Post-hoc comparisons were conducted using Fisher’s Exact test if the main effect of Treatment condition was significant. Significance was set at P<0.05.

Experiment 4: Effects of neonatal isolation on plasma cocaine levels

Subjects

A total of 23 rats were employed in this study. Of these, 13 were NH rats and included 7 rats tested 15-min post cocaine administration and 6 rats tested at 30-min post cocaine administration. Ten ISO rats were tested at either 15- or 30-min post cocaine administration (N=5 ea).

Procedure

Cocaine (10 mg/kg; IP) was administered to separate sets of NH and ISO rats that had trunk blood collected either 15- or 30-min later. These time points were chosen based on concentration vs. time profiles observed in our previous work (Guitart et al., 1992). Blood was
collected in vacutainer tubes containing sodium fluoride (5 mg) and potassium oxalate (4 mg) and samples were centrifuged at 1200 g for 20-min. Cocaine concentrations were determined by reversed phase HPLC with UV detection at 235-nm (Jatlow and Nadim, 1990).

Data analysis

To examine neonatal isolation effects on plasma levels of cocaine (ng/ml), data were analyzed using a 2 X 2 ANOVA representing the between group factors of Treatment condition and Time. Significance was set at P<0.05.

Results

Experiment 1: Effects of cocaine on extracellular levels of DA in NAc.

Baseline characteristics

Prior to probe implants, there was no group difference in body weight (P>0.10). The mean (+ S.E.M.) body weight of NH rats was 434.9 ± 8.1 g and the mean body weight of ISO rats was 445.7 ± 5.0 g. Levels of dopamine (DA) obtained from the last 15-min time period prior to cocaine administration are presented in Fig. 1. These data are shown for NH (Fig. 1A) and ISO (Fig. 1B) rats assigned to each cocaine dose administration group. The baseline DA levels do not differ between Treatment groups or across cocaine dose groups (P’s > 0.10).

Cocaine administration effects on dopamine levels

The extracellular levels of DA in NAc obtained over the nine 15-min time points sampled after cocaine administration are presented by Cocaine dose group in Fig. 1. Data obtained from NH groups are shown in Fig. 1A and data obtained from ISO groups are shown in Fig. 1B. ISO groups show greater DA levels after cocaine administration compared to NH groups. This is supported by the significant Treatment group effect, F(1,24)=6.53; P<0.02. Post-hoc comparisons show that the ISO group administered 10 mg/kg cocaine exhibits higher
extracellular DA levels than both saline groups and the 5 mg/kg cocaine NH group (P’s<0.05) and a trend towards greater levels than the 10 mg/kg cocaine NH group (P<0.10). All other main effects and interaction terms were not significant (P’s>0.10).

**Experiment 2: Effects of cocaine on locomotor activity.**

Cocaine increases locomotor activity as assessed by distance traveled. This statement is supported by the significant effect of Dose, F(2,52)=5.77; P<0.01, and shown in Fig. 2. Activity levels are high initially and decrease over time. This statement is supported by the significant Time effect, F(7,364)=23.99; P<0.0001. The main effect of Treatment condition and all its interactions are not significant (P’s>0.10).

**Experiment 3: Acute effects of cocaine on schedule-controlled responding for food.**

**Baseline characteristics**

Prior to food restriction, there was no Treatment condition effect on body weight (P>0.10). The mean (± S.E.M.) body weight of NH rats was 432.3 ± 7.8 g and the mean body weight of ISO rats was 456.2 ± 7.8 g. Baseline response rates (# lever presses/sec under vehicle pretreatment condition) are lower in ISO rats compared to NH rats, t(14)=2.20; P<0.05. The mean (± S.E.M.) response rates are 0.79 (± 0.06) for the ISO group and 1.07 (± 0.11) for the NH group. The baseline rate is used as a co-variate for the subsequent analyses with cocaine and the DA antagonist administrations.

**Cocaine effects**

Cocaine leads to a dose-related decrease in operant response rates in NH and ISO rats as seen in Fig. 3. This statement is supported by the significant Dose effect, F(4,56)= 48.25; P<0.0001. There is no significant effect of Treatment condition on change in response rates with cocaine administration (P> 0.10) but the Treatment condition X Dose interaction shows a trend towards
significance, $F(4,56)=2.30; P<0.07$. This likely reflects that ISO rats exhibit a greater decrease in responding at the highest cocaine dose (30 mg/kg) compared to NH rats, $F(1,14)=4.51; P=0.05$ as seen in Fig. 3. All other dose comparisons are not significant ($P's>0.10$).

**DA antagonist effects**

The effects of the D$_1$ antagonist, SCH 23390, and D$_2$ antagonist, eticlopride on response rates are shown in Fig. 4. As seen in Fig 4A, pretreatment with SCH 23390 leads to a dose-related decrease in responding as supported by the significant Dose effect, $F(2,26)=11.83; P<0.0001$. There is no significant main effect of Treatment condition or its interaction with Dose on response rates obtained under SCH 23390 treatments ($P's>0.10$). Pretreatment with eticlopride also decreases responding as seen in Fig. 4B and supported by the significant Dose effect, $F(2,26)=5.69; P<0.01$. Eticlopride has a greater effect on response rates in ISO rats compared to NH rats as seen in Fig. 4B and supported by the significant Treatment condition effect, $F(1,12)=5.70; P<0.05$. The Treatment condition X dose interaction term fails to reach significance ($P<0.12$).

**Experiment 4: Effects of neonatal isolation on plasma cocaine levels**

Plasma cocaine levels obtained at 15- and 30-min post cocaine administration are shown in Table 1 for NH and ISO rats. As seen in Table 1, there is no significant Treatment effect on plasma cocaine levels ($P's>0.10$). There is a trend for a significant decrease in levels over time, $F(1,21)=3.51; P<0.08$.

**Discussion**

The results of the present study demonstrate that cocaine increases dopamine (DA) levels in nucleus accumbens (NAc) to a greater extent in adult male rats with neonatal isolation (ISO) experience compared to non-handled (NH) control male rats. This finding confirms and expands
upon our previous work in infant rats (Kosten et al., 2003) and extends our research findings with amphetamine in infant and juvenile rats (Kehoe et al., 1996b; Kehoe et al., 1998a). We now show that the ability of the early life stress of ISO to increase ventral striatal DA responses to psychostimulants endures into adulthood. Thus, across ages and psychostimulant drugs, ISO enhances extracellular DA levels in ventral striatum in response to pharmacological challenges. Yet, ISO does not significantly alter cocaine-induced locomotor activity although there is a trend for cocaine to cause a greater disruption in ongoing response rates in ISO vs NH control rats.

There is no ISO effect on cocaine plasma levels after administration of the cocaine dose that is close to the ED50 in the schedule-controlled responding study and is the higher dose used in the locomotor and microdialysis studies. ISO does, however, increase sensitivity to a D2, but not a D1, antagonist compared to NH rats.

Extracellular DA levels in NAc are higher in ISO vs. NH rats with cocaine administration. And, similar to our previous studies (Kehoe et al., 1996b; Kehoe et al., 1998a; McCormick et al., 2002; Kosten et al., 2003), we find no differences between ISO and NH rats in basal DA levels. The highest cocaine dose used in the present study (10 mg/kg) is associated with greater DA levels in ISO rats compared to both groups administered vehicle and to the NH group administered the low cocaine dose (5 mg/kg). This group also shows a trend towards significantly greater DA levels than the NH group administered the same dose (10 mg/kg). Only low and moderate doses were assessed in this study because we predicted that ISO would enhance the ability of cocaine to increase NAc DA levels based on our previous work. Indeed, our study with infant rats that shows that ISO enhances extracellular DA levels with cocaine administration, used a similar dose range (Kosten et al., 2003). In the prior study, there is also little effect of the 5 mg/kg cocaine dose in NH rats. Many studies that examine the effects of
cocaine on extracellular DA levels in adult rats use cocaine doses that are well above threshold for behavioral activation (Pettit and Justice, 1989; Sorg and Kalivas, 1991; Reith et al., 1997; Andrews and Lucki, 2001). Thus, the lack of a significant main effect of cocaine dose in the present study is probably due to the use of more moderate cocaine doses.

The ability of the early life stress of ISO to enhance extracellular NAc DA levels in response to cocaine is similar to the effects of chronic stress exposure during adulthood (Sorg and Kalivas, 1991). In the Sorg and Kalivas study, chronic foot shock stress enhanced cocaine-induced increases in NAc DA levels and also increased locomotor activity to cocaine administration. The latter effect contrasts to the lack of ISO effect on cocaine-induced behaviors observed in the present study but may reflect different cocaine doses, differences in stressor type, length of time assessments were made after termination of stress, or developmental differences. Indeed, another early life stress exposure, prolonged maternal separation, enhances extracellular DA levels in NAc in response to amphetamine in adult rats (Hall et al., 1999). Interestingly, this group finds that the same manipulation decreases amphetamine-induced locomotor activity (Matthews et al., 1996). Across studies, it does not appear that stimulant-induced increases in NAc DA levels and stimulant-induced locomotor activity are correlated as suggested previously (Darracq et al., 1998).

The results showing that ISO fails to alter the acute locomotor effects of cocaine contrast with our previous studies that demonstrate that ISO enhances amphetamine-induced locomotor activity (Kehoe et al., 1996b; Kehoe et al., 1998b). This may reflect differences between cocaine and amphetamine or different neonatal isolation procedures. The former studies employed a mixed-litter design in which some pups were isolated and other pups remained with the dam. In the present study, a between-litter design was used in which all pups in a litter were isolated (or
One probable difference between these two procedures is the effect on the dam of the ISO litters. In the mixed-litter design, the dam has pups available whereas in the between-litter design she has none. The latter situation may constitute a greater stressor to the dam. Interestingly, in maternal separation, the dam is separated from the litter which remains in a huddle and this manipulation is associated with decreased locomotor responses to amphetamine (Zimmerberg and Shartrand, 1992; Matthews et al., 1996) but not in all cases (Weiss et al., 2001).

Rates of responding for food are lower in ISO rats compared to NH rats. And, there is a trend for cocaine to cause a greater disruption in responding in ISO vs. NH rats. Yet, we find ISO rats respond at higher rates than NH rats for cocaine under both fixed and progressive ratio schedules. Further, there are no group differences in responding for food under progressive ratio schedules (Zhang et al., 2005). Thus, the effect of ISO on response rates depends upon both the reinforcer type and the schedule of reinforcement.

Pretreatment with the D2 antagonist, eticlopride, causes a greater disruption of scheduled responding for food in ISO rats compared to NH rats. Yet, there are no group differences with the effect of pretreatment with the D1 antagonist. Similarly, Matthews and colleagues (Matthews et al., 1996) find that prolonged maternal separation increases sensitivity to a D2 but not a D1 antagonist in adult rats. This enhanced sensitivity to D2 antagonists suggests that ISO decreases post-synaptic levels of this receptor sub-type. Consistent with this notion, we previously showed greater effects of eticlopride in Lewis inbred rats (Haile and Kosten, 2001) that have lower D2 levels (Flores et al., 1998) and decreased Gi_{al/2} (Haile et al., 2001), the G-protein linked to D2 receptors, in NAc compared to Fischer rats. ISO may also decrease levels of these proteins, levels of the DA transporter, or have presynaptic effects. Indeed, our prior study in 10-day pups
showed that a brief isolation decreases D_2 levels in ventral striatum as shown by both *in vivo* and *ex vivo* ligand binding techniques (Kehoe et al., 1996a). Interestingly, both Lewis rats and ISO rats showed enhanced acquisition of cocaine self-administration relative to Fischer and NH rats respectively (Kosten et al., 1997; Kosten et al., 2000). Lower D_2 levels are also seen in cocaine addicts (Volkow et al., 1997) suggesting a link between this receptor sub-type and propensity to addiction.

The results of the present study demonstrate that ISO has enduring neurochemical effects on DA responses to cocaine administration in NAc, a region that is linked to the reinforcing effects of this drug (Ritz et al., 1987; Bergman et al., 1989). These neurochemical changes may contribute to the ability of ISO to enhance acquisition (Kosten et al., 2000; Kosten et al., 2004a) and maintenance (Zhang et al., 2005) of cocaine self-administration in adult male rats. Because early life stress may increase vulnerability to addiction (Gordon, 2002), this suggests that drug addiction prevention and treatment strategies be tailored for male addicts based on the presence or absence of early life stress.
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References


Deutch AY, Tam SY and Roth RH (1985) Footshock and conditioned stress increase 3,4-dihydroxyphenylacetic acid (DOPAC) in the ventral tegmental area but not substantia nigra. *Brain Research* **616**:89-98.


Footnote

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Legends for Figures

1. Mean (± S.E.M.) DA levels (pg/20 µl) obtained after pretreatment with cocaine (0, 5, or 10 mg/kg) are shown for NH (A) and ISO (B) rats. Cocaine administration leads to greater increases in DA levels in ISO vs. NH rats (see text for statistics).

2. Mean (± S.E.M.) distance traveled (cm) are shown for NH (A) and ISO (B) rats by cocaine (0, 5, or 10 mg/kg) administration group. Cocaine administration leads to dose-related increases in activity. There is no significant effect of neonatal isolation.

3. Mean (± S.E.M.) response rates (lever presses/sec) are shown for NH (closed symbols) and ISO (open symbols) rats by cocaine dose. Increasing cocaine doses lead to decreased response rates. There is no significant effect of neonatal isolation.

4. Mean (± S.E.M.) response rates (lever presses/sec) are shown for NH (closed symbols) and ISO (open symbols) rats by SCH 23390 dose in A. There is no significant effect of neonatal isolation on the effects of SCH 23390 on response rates. Response rates obtained after pretreatment with eticlopride are shown in B. Eticlopride disrupts responding to a greater extent in ISO vs. NH rats (see text for statistics).
**Table 1:** Mean ± S.E.M. (n) cocaine plasma levels (ng/ml) obtained 15- and 30-min after cocaine administration (10 mg/kg; IP) in non-handled and isolate male rats.

<table>
<thead>
<tr>
<th>Minutes post-injection</th>
<th>Non-handled</th>
<th>Isolate</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>395 ± 53 (7)</td>
<td>441 ± 143 (5)</td>
</tr>
<tr>
<td>30</td>
<td>275 ± 49 (6)</td>
<td>248 ± 98 (5)</td>
</tr>
</tbody>
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Fig. 1
Fig. 2

Non-handled Groups

- NH-0
- NH-5
- NH-10

Neonatal Isolation Groups

- ISO-0
- ISO-5
- ISO-10

5-min Time Block

Distance traveled (cm)
Fig. 3
Fig. 4