Maternal Separation and Handling Affects Cocaine Self-Administration in Both the Treated Pups as Adults and the Dams.

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Abbreviations: MS, maternal separation; NH, non-handled; H, handling; AFR, animal facility reared; PN, postnatal; ANOVA, analysis of variance; CES, carboxylesterase; DAT, dopamine transporter; ip, intraperitoneal

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ABSTRACT

Repeated maternal separation of pups from dams is often used as an early life stressor that causes profound neurochemical and behavioral changes in the pups that persist into adulthood. The effects of maternal separation on both the dams and the treated pups as adults on cocaine self-administration were examined using four separation conditions: 15 or 180 minute separation (MS15, MS180), brief handling without separation (MS0) and a non-handled group (NH). The separations and handling occurred daily on post natal days 2-15. The acquisition of cocaine self-administration (0.0625-1.0 mg/kg/infusion) was evaluated in the treated pups as adults. The MS180 group acquired cocaine self-administration at the lowest dose tested (0.0625 mg/kg/infusion) while the MS15s did not respond for cocaine at rates greater than that seen with saline administration. The NH group received the greatest number of infusions and intake at the highest doses. Following self-administration, no differences were observed between groups in activity of two liver carboxylesterases involved in the inactivation of cocaine, ES10 and ES4. Maternal separation affected cocaine self-administration in the dams as well. Although there was an overall significant affect of treatment on cocaine self-administration, the length of separation (15 or 180 minutes) did not affect cocaine self-administration on the dams. The MS0 dams averaged a greater number of infusions per session than NH group during the first week of acquisition. These data suggests that in addition to the profound changes that occur in pups as result of maternal separation, the dams are also susceptible to alterations in behaviors.
INTRODUCTION

Traumatic or stressful events experienced in childhood can have profound behavioral effects in adults. It is therefore not surprising that early adverse events in humans are associated with increased vulnerability to drug abuse as adults. McFarlane et al (2005) found an association of adverse early life experiences with nicotine dependence. Felitti et al (1998) found a strong relationship between household dysfunction during childhood and drug abuse, and others have similar results (Dube et al., 2003). Because of the relatively common and serious nature of this problem, developing an animal model has been important.

Animal models of early life stress, such as maternal separation and neonatal isolation, have been developed in an attempt to elucidate the neurochemical and behavioral alterations resulting from exposure to the early life stressor. In animal models, perinatal stress affects behavioral response to various drugs of abuse. Neonatal isolation and prolonged maternal separation alter the self-administration of cocaine in rats (Matthews et al., 1999; Flagel et al., 2003; Kosten et al., 2004; Zhang et al., 2005). Early life stress also alters conditioned place preference for amphetamine (Campbell and Spear, 1999), cocaine-induced locomotor activity (Brake et al., 2004), behavioral sensitization to cocaine (Li et al., 2003), ethanol preference and consumption (Ploj et al., 2003; Jaworski et al., 2005) and responses to morphine (Kalinichev et al., 2003). In addition, several neurochemical alterations in the dopamine mesolimbic system and the serotonergic system have been reported that may underlie some of the observed behavioral changes (Hall et al., 1999; Meaney et al., 2002; Brake et al., 2004; Vincentic et al., in press). Most of the above studies utilize 2 or 3 groups of experimental animals.
that sometimes include separated, handled and non handled animals with varying definitions of those groups. Also in experiments using neonatal isolation, the pups are separated individually from the dam while in maternal separation the litter as a whole is separated from the dam.

In our experimental approach, we utilize 4 groups of litters with variations in the time of separation and variations in handling, which provides additional data for interpretation (Jaworski et al 2005). The various litters of pups were: picked up and put back down with no separation into different cages (MS0), separated from their mother for 15 min/day (MS15), separated from their mother for 180 min/day (MS180), or were handled only once for a bedding change (NH) which is required by the Emory IACUC.

In addition to studying the effects of maternal separation on cocaine self-administration in the treated pups as adults, we also looked at potential pharmacokinetic differences. The rapid hydrolysis of cocaine to two major inactive metabolites, benzoylecgonine and ecgonine methyl ester, is an important step in the inactivation of cocaine (Bosron et al., 1997). Serum cholinesterase catalyzes the hydrolysis of cocaine to ecgonine methyl ester, while human carboxysylesterase-1 (hCE-1) hydrolyzes the methyl ester group of cocaine to form benzoylecogine (Bosron et al., 1997). In this study we measure two major class 1 isoenzymes, ES10 and ES4, that play a crucial role in rat liver carboxylesterase activity (Sanghani et al., 2002).

While the effects of maternal separation on the pups as adults have been studied, there is little known about the effects of maternal separation on the dams. Human mothers who had been separated from their children were more likely to have a
substance abuse disorder (Zlotnick et al., 2003). Therefore, there is a definite need to understand the effects of separation on the vulnerability to drug abuse in mothers.

Our hypothesis is that separation of pups from dams (MS) and variations in handling during the perinatal period will produce changes in cocaine self-administration behavior in pups as adults, and in the dams as well. Understanding the mechanisms of these changes could lead to new strategies for developing treatments of addictive disorders resulting from MS/H in the perinatal period.
METHODS

Animals: Thirty-three timed-pregnant Long Evans rats (Charles River Laboratories, Wilmington, MA) were received at the animal facility on gestation days 12-13. The dams were singly housed in static cages (27 cm x 48 cm x 20 cm) in a temperature- and humidity-controlled animal care facility and maintained on a 12-h light/dark schedule (lights on at 0700) with food and water available ad libitum. The pups were group housed by sex in static cages following weaning until 3 months of age at which time they were individually housed. Water was available ad libitum throughout the experiment. Food was available ad libitum from weaning until venous catheter implantation at 3 months of age. The treated adults were food-restricted following surgery through the conclusion of the food training sessions at which time free access to food was restored. All procedures were approved by the Emory animal care and use committee and in accordance with the “Principles of Laboratory Animal Care (NIH publication No. 85-23).

Separation and handling procedures: As the pups were born, the litter was randomly assigned to one of four groups (Jaworski et al 2005). The experimental groups are as follows: (1) Non-handled (NH)– pups and dams were handled only on PN11 for a cage change and not separated (2) MS0- the dam was picked up and moved to the opposite end of the cage followed by the individual handling of each pup by picking the pup up and moving it to the opposite end of the cage with no separation into different cages (3) MS15 – the pups in this group were exposed to 15 minutes of maternal separation from PN2-15, (4) MS180 - the pups were exposed to 180 minutes of maternal separation from PN2-15. The MS0, MS15 and MS180 groups provide time course data on the effects of maternal separation and handling. The MS0 and NH groups provide data to control for
handling during the separation procedure. Another group that is often used in maternal separation as a control group is the animal facility reared (AFR) group. In this group the pups are subjected only to routine handling by the animal facility staff. The data obtained from an AFR group is often difficult to compare between laboratories due to the variations in care at each institution’s animal facility and was therefore not used in the current study. It has been suggested that separation and handling during the second, not the first week, is critical in lasting behavioral effects (Flagel et al., 2003). To ensure that the separation occurred during a critical point in the pups’ life, the pups were separated daily during the first two weeks of life. Eight litters were assigned to each treatment group with the extra litter assigned to the MS15 group. One litter assigned to the MS180 group died during separation on PN2, the dam was sacrificed.

The manipulation of the pups in the MS15 and MS180 was initiated at 10:00 a.m. by removal of the dams from the maternity cage and placing them in individual cage until the end of the manipulation. Following removal of the dams, the litters were removed from the nest, weighed and placed in a cage assigned to that litter for the remainder of the experiment. The cage contained 3cm of bedding material (Bed O’ Cobs; The Andersons, Maumee, OH) and was placed on a heating pad set to maintain 37˚C. At the conclusion of the separation period, the pups were returned to their maternity cage and the dam was returned. The bedding material in the maternity cages was changed on PN11 for all the groups as required by Emory University’s IACUC. The pups were weaned on PN21 and group housed by sex and by litter. A one-way ANOVA determined that the average litter size (NH: 11.88 ± 0.67, MS0: 11.88 ± 0.55, MS15: 12.22 ± 0.32 and MS180: 11.43 ± 0.72), or the sex ratio (NH: 1.39 ± 0.36, MS0: 1.05 ± 0.16, MS15: 1.28 ± 0.19 and
MS180: 1.43 ± 0.72), did not significantly vary by group [litter size: F (3, 32) = 0.3296, p = 0.80; sex ratio: F (3, 32) = 0.6845, p = 57]. The average pup weight showed a trend for greater average weight in the MS180 group (46.94 ± 2.33) compared to the other groups (NH: 41.30 ± 0.99, MS0: 42.93 ± 1.13 and MS15: 41.40 ± 1.40) but did not reach significance [F (3, 32) = 2.870, p = 0.054].

Venous Catheterization and drug delivery: The dams were implanted with indwelling jugular catheters 5-8 days after weaning of the pups. The treated pups were implanted with a jugular catheter around 3 months of age. Under anesthesia induced by a combination of ketamine (70 mg/kg, i.p.) and medetomidine (0.5 mg/kg, i.p.), the catheter (0.3048 mm i.d. x 0.635 mm o.d., silicone tubing) was inserted into the right posterior facial vein and pushed down into the jugular until it terminated outside the right atrium. The catheter was anchored to the surrounding tissue and continued subcutaneously to the back where it exited at the base of the skull. The catheter was connected to a 22-gauge guide cannula (Plastics One Inc., Roanoke, VA) which was mounted to the top of the skull using stainless steel screws and cranioplastic cement (Plastics One Inc., Roanoke, VA) for attachment to a leash. Following the surgery the rats were given atipamezole (0.2 mg/kg, s.c.) to reverse the sedative effects of medetomidine.

The leash was attached to the guide cannula assembly and to a fluid swivel suspended above the operant chamber. The swivel and leash assembly were counterbalanced to allow relatively unrestricted movement in the operant chamber. Tubing ran from the swivel to a 20-ml syringe in a motor driven pump (Razel, St. Albans, VT) located outside the sound-attenuating chamber. Prior to the start of the behavioral
session, blood was obtained from the catheter to ensure patency. If blood was not obtained the rat was injected via the catheter with methohexital sodium (1.5mg, i.v.) at the conclusion of the experiment. An immediate light anesthesia indicated the catheter was patent. Following the session the rats were disconnected from the leash assembly and flushed with streptokinase (0.67 mg/ml) and ticarcillin/clavulanic acid mixture (67 mg/ml) in a volume of 0.1 ml.

**Apparatuses:** Module test cages (12"Wx10"Dx12"H) contained within sound-attenuating enclosures (Coulbourn Instruments, Allentown, PA) were used for the self-administration experiments. Each chamber was equipped with two response levers located 6 cm above the floor and 13 cm apart. A stimulus light was located 8 cm above each of the response levers. The house light was mounted 19 cm above the chamber floor on the wall opposite the response levers. Sucrose pellets (45 mg, Research Diets, New Brunswick, NJ) were dispensed into a trough located between the two response levers. The chambers were connected to a PC-compatible computer via Lab Linc V interface (Coulbourn Instruments, Allentown, PA). The experiments were programmed and the data collected using Graphic State 3 software (Coulbourn Instruments, Allentown, PA).

Locomotor activity was measured in a 40 x 40 x 30 cm plexiglass cage (Omnitech Electronics, Columbus, OH) equipped with 16 photobeams front to back and 16 photobeams side to side spaced 2.4 cm apart. The activity cages were located in stainless steel sound-attenuating chambers equipped with an exhaust fan and a 10-W light. The experimental data were collected and analyzed on a PC-compatible computer using Digipro software (Omnitech Electronics, Columbus, OH).
Acquisition of Cocaine Self-Administration - Dams: The dams were given between 5 to 7 days to recover from surgery before the start of the two-week self-administration sessions. The sessions were conducted between 0800 and 1800, seven days a week for 2 hours a day. At the start of the session the lever light above the active drug lever was illuminated signaling that drug was available for self-administration. Responding on the active lever (FR1) resulted in the darkening of the lever light and the illumination of the house light for the duration of the infusion (0.2 ml over 6 s). There was a 20 s time-out following the infusions during which all lights were darkened and responses on either lever were recorded but had no programmed consequences. At the end of the time-out the lever light was once again illuminated and drug was available for self-administration. Responses on the inactive lever were recorded but had no programmed consequences at any time during the self-administration session.

Food Training – Treated Pups at Adulthood: The treated pups at adulthood were given 7-12 days to recover from surgery before the start of food training. During the recovery time the MS adults were slowly food restricted to 85% of their free-feeding weight. All behavioral test sessions were conducted between 0800 and 2000, Monday through Friday. The rats were divided into 4 groups with an equal number of rats from each treatment condition (i.e. NH, MS0, MS15 and MS180). Each group of rats was run at the same time each day. The food sessions lasted for one hour or until 100 food pellets were earned. At the start of the session, the lever light above the active food lever was illuminated. Responses on the lever (FR1) resulted in the presentation of a food pellet and darkening of the lever light. Following a 10 s time-out, during which lever responses were recorded but had no consequences, the lever light above the active was once again illuminated.
illuminated. All responses on the inactive lever were recorded. The food sessions were conducted daily for 2 weeks at which time self-administration sessions began.

**Cocaine Self-Administration - Treated Pups at Adulthood:** In the self-administration sessions, the lever formerly associated with food reinforced responding (the food active lever) was assigned as the inactive lever and the lever previously assigned as the inactive lever during food training became the active drug lever. The lever light above the active drug lever was illuminated at the start of the session indicating that drug was available for self-administration. The conditions during self-administration for the treated pups at adulthood were identical to those used for the dams. Briefly, responses on the active lever (FR1) resulted in the illumination of the house light for the duration of infusion (0.2 ml over 6 s) followed by a 20 second time-out. The rats were allowed to self-administer saline during the first week to establish a base-line of responding. During the following weeks cocaine was made available for self-administration starting at 0.0625 through 1.0 mg/kg/infusion. Each week a single dose was available for self-administration with the dose doubling each successive week.

**Food Test Session- Treated Pups at Adulthood:** One week following the conclusion of self-administration the MS, handled and non-handled rats were re-tested on the food program used during food training. Briefly, the rats were able to respond on a FR1 schedule of food reinforcement until 100 food pellets were earned or 1 hour had elapsed. In this experiment the lever assigned as the active lever during cocaine self-administration was reassigned as the inactive lever.

**Cocaine-Induced Locomotor Activity – Treated Pups at Adulthood:** Two weeks following the conclusions of self-administration, cocaine-induced locomotor activity was
measured. On the first day of testing the rats were given 2 hours to habituate to the activity chamber during which time the total distance was measured. On each of the next three days the rats received either saline, 5 or 15 mg/kg cocaine (i.p.) following a 30 minute habituation period in the activity chamber. Each rat received both doses of cocaine and saline, each on separate occasions in a counter balanced order. The total distance traveled was measured for 2 hours following the injection after which the animals were returned to their home cages for 22 hours.

**Carboxylesterase Assays:** One week following self-administration, the treated pups as adults were decapitated and the livers removed, placed on dry ice and stored at -80°C. Frozen rat liver was powdered in liquid N$_2$. Approximately 100 mg weight was homogenized in 50 mM Na phosphate buffer at pH 7.4 with 1 mM EDTA, 1 mM Benzamidine and 0.05% Triton X-100. The suspension was sonicated briefly and centrifuged. Six individual liver specimens each from the four treatment groups were analyzed. Carboxylesterase activity in rat liver supernatants was assayed by UV spectroscopy with 4-methylumbelliferyl acetate as substrate and total protein was assayed by the Bio-Rad (Hercules, CA) Bradford protein assay with bovine serum albumin as standard.

Forty micrograms of total protein was analyzed for the expression of individual carboxylesterase isoenzyme by non-denaturing polyacrylamide gel electrophoresis. Gels were stained for activity with 4-methylumbelliferyl acetate as substrate with fluorescence detection followed by total protein staining with coomassie blue dye (Sanghani et al., 2002). The activity staining pattern resembled that seen by Sanghani et al., (2002) in rat liver extracts. The fluorescence band densities of the two major rat liver
carboxylesterases, ES10 and ES4, and the control carboxylesterase loaded on each gel was determined. The ratio of ES10 and ES4 to that of the control was determined and compared across all groups.

**Drugs:** Ketamine (Fort Dodge Animal Health, Fort Dodge, Iowa) and medetomidine (Pfizer Animal Health, Exton, PA) were given i.p. prior to surgery. Following surgery, atipamezole (Pfizer Animal Health, Exton, PA) was used to reverse the sedative effects of medetomidine. Cocaine was a gift from the National Insitute on Drug Abuse (NIDA; National Institute of Health, Bethesda, MD) and was dissolved in bacteriostatic, heparinized 0.9% saline. The catheters were maintained by daily infusions of a ticarcillin/clavulanic acid (GlaxoSmithKline, Research Triangle Park, NC) and streptokinase (American Diagnostica Inc., Stamford, CT) cocktail. Catheter patency was checked by infusion with methohexital sodium (Monarch Pharmaceuticals, Bristol, TN)

**Statistical Analysis:** All statistical analyses were performed using either SPSS 13.0 for Windows or GraphPad Prism v 4.0 for Windows. Comparisons were made by either a one-way or two-way with repeated measures analysis of variance (ANOVA) as stated in the results section. Post hoc comparisons were made using Tukey’s Multiple Comparison Test. Statistical significance is stated as a p value was less than 0.05.
RESULTS

Maternal Separation and Handling: Pregnant dams were placed into one of four groups according to separation and handling as described in Methods. Following weaning on PN21, the litter size, average pup weight and the body of the weight of the dams were measured (Table 1). One-way ANOVAs were used to analyze the average litter size, dam body weights and pup weights. The average litter size did not significantly vary by group [litter size: \( F(3, 32) = 0.3296, p = 0.80 \)]. Although there were no significant differences in dam body weight between the groups at weaning or during self-administration, the average pup weight showed a trend for greater average weight in the MS180 group (46.94 ± 2.33) compared to the other groups (NH: 41.30 ± 0.99, MS0: 42.93 ± 1.13 and MS15: 41.40 ± 1.40) but did not reach significance [\( F(3, 32) = 2.870, p = 0.054 \)]. The trend of MS180 group weighing more than the other groups continued into adulthood (see below). Thus the treatments had only a very minor effect on body weight and no effect on litter size at weaning.

Maternally Separated and Non-handled Adult Self-Administration:

When the pups reached adulthood, they were prepared for the self-administration paradigm as described in Methods.

Food Training: The rats were trained to respond for food on a continuous schedule of reinforcement during daily session. All four groups acquired food maintained responding in the two weeks of training; however there was a trend for a significant effect of treatment (data not shown, \( p = 0.064 \)). The average number of days to earn \( \geq 90 \) food pellets in a session (Table 1) did not differ between groups (One-Way ANOVA [\( F(3, 32) = 2.612, p > 0.07 \)]. Although there was a trend for the impaired acquisition of food-
reinforced responding in the MS15 group; neither measure reached significance and all rats acquired food-reinforced responding in the 2 week span. In addition, the MS15 group had comparable levels of food-reinforced responding measured at the completion of the self-administration experiment (see below).

There were no significant differences in body weight on the start of food training (One-Way Anova, \(F (3, 33) = 2.708, p = 0.0628\)), however significant differences in weight were seen at the start of self-administration (One-Way Anova, \(F (3,33) = 3.627, \ p < 0.05\)) with the MS180 group weighing more than the NH group (\(p < 0.05\)) (Table 1). The differences seen in body weight were no longer evident by the end of self-administration (One-Way Anova, \(F (3, 32) = 2.112, p = 0.1204\)). Again, there were no marked differences among the groups.

**Cocaine Self-Administration:** The self-administration data is presented as the mean number of infusions per session for the 5 sessions conducted for each dose. Figure 1 shows the typical inverted “U” cocaine dose-response curves for the adults from the four groups. A two-way repeated measures ANOVA revealed a significant effect of DOSE \(F (5,29) = 7.851, \ p < 0.001\). The effect of DOSE alone did not account for the differences in curves as indicated by a GROUP x DOSE interaction \(F (15, 29) = 2.602, \ p = 0.002\).

Although the MS180 group appeared to receive more infusions at the 0.0625 mg/kg/infusion dose than the other groups this was only a trend and failed to reach significance (\(p = 0.078\)). Separation of the curves was most evident at the two highest doses tested with the NH group taking significantly more infusions than the MS15 group.
at the 0.5 (p < 0.05) and the 1.0 (p < 0.01) mg/kg/infusion doses. Thus the animals did show some differences in their sensitivity to cocaine.

Figure 2 illustrates the acquisition of cocaine self-administration for each of the four groups (redrawn from Figure 1). Here we define acquisition as the first dose at which the number of infusions per session is significantly greater than saline. Interestingly, neither the MS0 nor the MS15 groups acquired at any of the doses tested. The MS0 group showed a slight inverted “U” shaped dose-response curve; however, the MS15s maintained a relatively low level of responding throughout the experiment. The NH group first acquired at the 0.125 mg/kg/infusion dose (p < 0.001 vs. saline) and continued to respond at higher than saline levels for the remaining doses [0.25 (p < 0.001), 0.5 (p < 0.001) and 1.0 mg/kg/infusion (p < 0.05)]. The dose-response curve for the MS180 group appeared to be shifted to the left compared to the NH group. The MS180 group acquired at the lowest dose tested, 0.0625 mg/kg/infusion (p < 0.01 vs. saline) and continued to self-administer cocaine up to the 0.125 mg/kg/infusion dose (p < 0.05). The average infusions per session for the other three doses did not significantly differ from saline for the MS180 group.

The average intake (mg) per session (Figure 3) varied by DOSE [F (4, 29) = 135.7, p < 0.001] and by GROUP [F (3, 29) = 4.123, p < 0.05]. There was also a significant DOSE * GROUP interaction [F (12, 29) = 3.562, p < 0.001]. At the lowest dose tested there was a trend for the MS180 group to have a higher average daily intake than the MS15 group (p = 0.068). Differences between the MS180 and MS15 groups were evident at the 0.125 mg/kg/infusion dose, with the MS180 group having significantly higher intake (p < 0.05). Similar to what was seen with the average
infusions per session, the NH group had significantly higher levels of cocaine intake at the 0.5 and 1.0 mg/kg/infusion doses compared to the MS15 group (p < 0.05 and p < 0.01, respectively). These findings again indicate that the animals differed in their responses to cocaine.

Despite the low level of responding by the MS15 group, the rats were able to discriminate between the active and inactive levers although there was a significant effect of dose [LEVER F (1, 14) = 3.095, p < 0.05; DOSE F (5,14) = 3.578, p < 0.01] evident in Figure 4. The MS180 was the only other group able to discriminate between levers [LEVER F (1, 14) = 7.213, p < 0.05; DOSE F (5,14) = 3.025, p < 0.05]. Both the NH and MS0 groups showed an increase in inactive lever responding as the dose of cocaine self-administered increased. The increased intake in cocaine may have led to non-specific locomotor effects (DOSE effect reported above) which could explain the increase in inactive lever responding.

Post Self-Administration Food Testing: To ensure that the MS15 group was capable of responding for a reinforcer, the rats were retested on the same food program used during training (Figure 5). There were no significant differences between groups in the number of food pellets earned [One-Way ANOVA, F(3,31) = 0.95, p = 0.43], active lever presses [F (3,31) = 1.382, p = 0.27] or inactive lever presses [F (3,31) = 0.478, p = 0.70]. Although the MS15 adults did not respond at high rates for cocaine infusions, they had rates of food reinforced responding that were similar to those of the other groups.
Cocaine-Induced Locomotor Activity: In order to examine another cocaine-induced activity, locomotor activity was measured after the injection of cocaine in various doses. Figure 6 shows the total distance traveled during habituation and after saline, 5 or 15 mg/kg cocaine. There were no differences between groups during habituation, saline or 5 mg/kg cocaine. At the 15 mg/kg dose, however, the MS180 group showed significantly less locomotor activity compared to the MS0 group (p < 0.05). The MS15 group did not differ in its response to cocaine at either dose compared to the other groups. Thus there were no differences in locomotor activity that paralleled the differences in self-administration.

The finding that the MS180 had significantly less locomotor activity at the highest dose tested is interesting. There were no significant differences in locomotor activity between groups following 5 mg/kg cocaine, saline or during habituation. The results are somewhat difficult to interpret due to the unique histories of cocaine self-administration. Future experiments examining cocaine-induced locomotor activity in cocaine naïve animals are warranted.

Carboxylesterase Assay: The ratio of the density of activity bands for the two major carboxylesterase isoenzymes, ES10 and ES4, as well as the specific activity are presented in Figure 7. One-way ANOVAs showed no significant differences in ES10 \( F(3, 22) = 1.413; p = 0.2700 \), ES4 \( F(3,22) = 0.4473; p = 0.7221 \) or specific activity \( F(3,22) = 1.690, p = 0.2029 \). These data suggest that the differences in cocaine self-administration are due to pharmacodynamic differences rather than any pharmacokinetic differences.
**Dam Self-Administration:** The length of separation from the pups did not appear to affect the acquisition of cocaine self-administration in the dams. Table 2 shows the average number of infusions per session for the MS15 and MS180 groups, there were no statistical differences between the two [Two-Way Repeated Measures (1, 12) = 0.118, p = 0.74; SESSION F (13,12) = 4.453, p < 0.001; INTERACTION F (13, 12) = 0.5479, p = 0.89]. Therefore, the data from the MS15 and MS180 groups were combined into a MS group for statistical analysis of the average number of infusions per session during the 14 days of testing is shown in Figure 8. The rate of acquisition was analyzed using a two-way ANOVA with repeated measures looking at the effects of SESSION and GROUP on the average number of infusions per session. The average number of infusions per session increased as sessions progressed [SESSION, F (13, 25) = 10.28, p < 0.001]. Maternal separation, handling or non-handling had a significant affect on cocaine self-administration [GROUP, F (2, 25) = 3.807, p = 0.036]. Differences between groups are also seen when comparing the groups during the first week of acquisition [One-Way ANOVA; F (2, 27) = 4.025, p < 0.05] (Figure 8). The MS0 group had a greater number of average infusions compared to the NH group during the first week (p < 0.05). By the second week of acquisition there were no significant differences between groups [One-Way ANOVA; F (2, 27) = 2.941, p = 0.07]. All three groups averaged greater infusions per session during the second week compared to the first (NH p < 0.01; MS0 p <0.05 and MS p < 0.01). It appears that the length of maternal separation (the MS group) does not impact the acquisition of cocaine self-administration in the dams, however, the group of dams who were handled and were present while their pups were removed (MS0) showed greater levels of self-administration during both weeks compared to the non-handled
(NH) dams. The differences between in the acquisition of cocaine self-administration in the dams do not mirror the differences seen in the maternally separated, handled and non-handled adults. These data indicate that maternal separation has unique effects on both the dams and the pups.
DISCUSSION

Animal models of early life stressors such as maternal separation have been used in an attempt to elucidate the neurochemical and behavioral effects of the stressor in later life. Previous work has shown an effect of maternal separation on cocaine-induced locomotor activity (Brake et al., 2004), cocaine self-administration (Matthews et al., 1999), responses to primary and conditioned incentives (Matthews and Robbins, 2003), cocaine sensitization in rats (Li et al., 2003), and pharmacologically altered lateral hypothalamic intracranial electric self-stimulation thresholds (Matthews and Robbins, 2003). Another animal model of early life stress, neonatal isolation, has been demonstrated to enhance the acquisition of cocaine self-administration in rats (Kosten et al., 2004) and to impair amphetamine-induced conditioned place preference (Campbell and Spear, 1999).

Our data significantly extends this body of literature by the use of additional separation groups allowing for time course data to be gathered (MS0, MS15 and MS180), the inclusion of a non-handled (NH) group, a complete dose-response curve for cocaine self-administration, and the interesting new findings concerning the dams. In the present experiments, maternal separation had a significant impact on cocaine self-administration of the treated pups as adults. The MS180 group appeared to have a leftward shift in the dose response curve, with acquisition occurring at the 0.0625 mg/kg/infusion dose. Although the MS0 group did not fully acquire by the definition used, an inverted “U” dose-response curve was evident. The NH group acquired at the 0.125 mg/kg/infusion dose and continued a high level of responding through the highest dose tested. Interestingly, the MS15 had a relatively flat dose-response curve with responding at any
dose no greater than what was seen with saline. The lack of responding for cocaine did not seem to be related to an inability to complete the operant task, as the MS15 group had similar rates of responding to the other groups during both the initial food training and the food test session at the end of the experiment. The MS15 group was also able to discriminate between the active and inactive lever by preferentially responding on the active lever. The lack of cocaine-reinforced responding by the MS15 group suggests that the reinforcing effects of cocaine may possibly be blunted in this group.

Carboxylesterases are non-specific ester hydrolases with broad substrate specificity. Human liver has two major carboxylesterase isoenzymes, CES1 and CES2. CES1 is a class I isoenzyme and is responsible for hydrolysis of methyl ester of cocaine resulting in formation of benzoylecgonine (Brzezinski et al., 1994). The human class II enzyme (CES2) is responsible for hydrolysis of the benzoyl ester of cocaine (Pindel et al., 1997) and formation of ecgonine methylester. In comparison, the rat liver has at least four class 1 isoforms and three class 2 isoforms (Sanghani et al., 2002). A cocaine pharmacokinetic study in rats shows that benzoylecgonine is a major metabolite (Mets et al., 1999). Two class I enzymes, ES10 and ES4, account for >90% of total rat liver esterase activity and are likely to be responsible for formation of benzoylecgonine. There were no differences in the levels of CES activity or isoenzyme pattern of CESs among the four treatment groups following cocaine self-administration testing. Therefore, the differences between the various groups in cocaine-reinforced responding are unlikely to be caused by differences in pharmacokinetics.

Potential mechanisms of these changes have been examined. Others (Plotsky and Meaney, 1993; Pryce and Feldon, 2003) have shown a correlation with stress
responses and the different paradigms and proposed that the relative stress among the groups is the major factor in their changes. In this case, the neurochemical responses to glucocorticoids are proposed to somehow cause the changes (Huot et al., 2002; Vazquez et al., 2002; Ploj et al., 2003). MS15 rats had a lower HPA response to acute and chronic stress in adulthood compared to the MS180s or NHs (Meaney et al., 1991; Plotsky and Meaney, 1993; Meaney, 2001). Stress and the subsequent activation of the HPA axis facilitate the acquisition of cocaine self-administration (Tidey and Miczek, 1997; Mantsch et al., 1998); manipulations of the HPA axis resulting in a reduction of function through pharmacological or surgical means can attenuate the acquisition of cocaine self-administration in rats (Goeders and Guerin, 1996). In the current experiment, we show that the MS15 group did not acquire cocaine self-administration and this could possibly be attributed to or contributed by a reduction of HPA axis reactivity.

Some studies have examined the cellular and molecular mechanisms that might underlie the changes in the effects of psychostimulants. Decreases in DAT, D3 and D1 receptors in maternally separated rats compared to controls have been demonstrated (Meaney et al., 2002; Brake et al., 2004). Enhancement of stress and psychostimulant-induced dopamine release in the nucleus accumbens of rats that underwent prolonged maternal separation (Hall et al., 1999) or neonatal isolation (Kosten et al., 2005) has also been shown. The differences seen in the acquisition of cocaine self-administration may be a result of the altered dopaminergic system as dopamine is important in psychostimulant self-administration. The MS15 group may have a blunted accumbal dopamine response to cocaine, thereby reducing the rewarding properties of cocaine;
whereas the MS180 group may have enhanced dopamine release facilitating the acquisition of cocaine self-administration (Hooks et al., 1991; Rouge-Pont et al., 1993).

In the current study we demonstrate that maternal separation affects the acquisition of cocaine self-administration in the dams. The length of separation appeared not to affect the dams’ behavior. However, the dams whose pups were but not separated (MS0) averaged more infusions per session during the first week than the NH group, but this effect was not seen in the second week. This may suggest that the effect of maternal separation on the dams will be lost over time. Future experiments will explore the duration of the effects of maternal separation on cocaine self-administration in the dams.

While it has been shown repeatedly that maternal separation causes neurochemical and behavioral alterations in the pups that persist into adulthood, less is known about how the separation procedure affects the dams. Interestingly, while the dams in the MS15 and MS180 groups did not differ in the acquisition of cocaine self-administration, these conditions had an impact on the behavior of the pups. The differences seen in the acquisition of cocaine self-administration in the dams may be attributable to the stress experienced by the separation procedure. The key stressful event would be the removal of the dams from their home cage. Both the MS15 and MS180 dams experience the same procedure by first being removed and placed in a separate cage and later reunited with the pups. The NH dams never experience the separation or the handling. However, the MS0 dams were present while the pups were being handled and the intrusion of the experimenter’s hand is a potentially powerful stressor.

Some evidence suggests that the reactions of the dams to separation and handling are important and actually produce the changes in the pups (Denenberg, 1999;
Kalinichev et al., 2000; Champagne et al., 2004). Huot et al (2004), utilizing fostering of litters suggested that effects of MS may result largely from alterations in the quality of maternal care rather than from direct effects of the separation per se on the pups. Kalinichev et al (2000) reported anxiety-like behavior in dams in the MS paradigm. Weaver et al (2004) found that pups of mothers exhibiting high rates of licking and grooming and arched-back nursing had differences in DNA methylation in a glucocorticoid gene promoter in the hippocampus. These differences occurred in the first week of life, were reversed by cross fostering, and persisted into adulthood.

MS produced interesting changes in self-administration behavior in both the dams and the pups as adults. In the male adults, MS facilitated the acquisition of cocaine self-administration in the MS180 group while the MS15 group did not self-administer cocaine at rates that differed from saline administration. The length of separation did not have a significant effect on cocaine self-administration in the dams however; enhanced acquisition was seen in the MS0 group. Further study is warranted to investigate the mechanisms by which MS produces changes in vulnerability to drug abuse in both the dams and offspring.

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REFERENCES


Matthews K and Robbins TW (2003) Early experience as a determinant of adult
behavioural responses to reward: the effects of repeated maternal separation in the

separation alters intravenous cocaine self-administration in adult rats.
Psychopharmacology (Berl) 141:123-134.

McFarlane A, Clark CR, Bryant RA, Williams LM, Niaura R, Paul RH, Hitsman BL,
psychophysiological, personality and behavioral measures in 740 non-clinical

differences in stress reactivity across generations. Annu Rev Neurosci 24:1161-
1192.

development of mesolimbic dopamine systems: a neurobiological mechanism for

Meaney MJ, Mitchell JB, Aitken DH, Bhatnagar S, Bodnoff SR, Iny LJ and Sarrieau A
(1991) The effects of neonatal handling on the development of the adrenocortical
response to stress: implications for neuropathology and cognitive deficits in later

Mets B, Diaz J, Soo E and Jamdar S (1999) Cocaine, norcocaine, ecgonine methylester


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LEGENDS FOR FIGURES

**Figure 1:** Dose-response curve for cocaine self-administration in maternally separated (MS15 and MS180), handled (MS0) and non-handled (NH) adults. The data points represent the group mean ± SEM of the average number of infusions per session at each of the doses tested.

* p < 0.05 vs MS15
** p < 0.01 vs MS15

**Figure 2:** Acquisition of cocaine self-administration presented by group. The data are redrawn from figure one and presented as the group means ± SEM of the average number of infusions per session at each of the doses tested.

* p < 0.05 vs saline
** p < 0.01 vs saline
*** p < 0.001 vs saline

**Figure 3:** The effects of experimental treatment on the average cocaine intake (mg) per session of MS0, MS15, MS180 and NH adults at each of the doses tested. The data points represent the group means ± SEM.

* p < 0.05 vs MS15 at the same dose
** p < 0.01 vs MS15 at the same dose
Figure 4: Lever discrimination during cocaine self-administration for each group. The data points represent the average number of active and inactive lever presses per session (means ± SEM) at each of the doses tested.

Figure 5: Lever responses and food pellets earned during the food reinforced test session conducted at the end of cocaine self-administration testing. The data are presented as the mean ± SEM.

Figure 6: Cocaine-induced locomotor activity in MS0, MS15, MS180 and NH adult rats. The bars represent the mean total distance traveled ± SEM.

* p < 0.05 vs NH

Figure 7: Lack of differences in the ratio of the density of activity bands for the two major carboxylesterase isoenzymes, ES10 and ES4, as well as the specific activity (U/mg) in the treated pups as adults following self-administration. The data are presented as the mean ± SEM.

Figure 8: Acquisition of cocaine self-administration by the dams. Data represent the mean number of infusions per session ± SEM. There was a significant overall effect of both SESSION (p < 0.001) and GROUP (p < 0.05).
Figure 9: Average number of cocaine infusions per session by the dams during the first and second weeks of self-administration. Data are presented as the mean ± SEM.

* $p < 0.05$ vs NH during week 1
† $p < 0.05$ vs week 1 value of the same group
‡‡ $p < 0.01$ vs week 1 value of the same group
Table 1: Body weights at weaning and self-administration for the dams and treated pups.

<table>
<thead>
<tr>
<th></th>
<th>NH</th>
<th>MS0</th>
<th>MS15</th>
<th>MS180</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Weaning</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body Weights (g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dam Wt.</td>
<td>313.9 ± 5.2</td>
<td>314.5 ± 8.8</td>
<td>312.4 ± 7.5</td>
<td>320.6 ± 10.3</td>
</tr>
<tr>
<td>Avg. Pup Wt.</td>
<td>41.3 ± 1.0</td>
<td>42.9 ± 1.1</td>
<td>41.4 ± 1.4</td>
<td>46.9 ± 2.3</td>
</tr>
<tr>
<td>Litter Size</td>
<td>11.9 ± 0.7</td>
<td>11.9 ± 0.5</td>
<td>12.2 ± 0.3</td>
<td>11.4 ± 0.7</td>
</tr>
<tr>
<td><strong>Dam Self-Administration</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body Weights (g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First Day</td>
<td>285.0 ± 6.5</td>
<td>291.4 ± 8.8</td>
<td>290.0 ± 7.6</td>
<td>298.6 ± 7.4</td>
</tr>
<tr>
<td>Last Day</td>
<td>291.1 ± 5.2</td>
<td>303.1 ± 9.6</td>
<td>302.3 ± 4.5</td>
<td>309.9 ± 7.5</td>
</tr>
<tr>
<td><strong>MS, Handled and Non-Handled Adults Self-Administration</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body Weights (g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Food Training</td>
<td>410.6 ± 7.5</td>
<td>428.7 ± 7.5</td>
<td>429.5 ± 11.8</td>
<td>444.0 ± 5.4</td>
</tr>
<tr>
<td>First Day</td>
<td>403.8 ± 5.4</td>
<td>421.3 ± 7.7</td>
<td>412.8 ± 6.8</td>
<td>434.3 ± 6.5 *</td>
</tr>
<tr>
<td>Last Day</td>
<td>510.5 ± 10.4</td>
<td>533.2 ± 9.9</td>
<td>530.5 ± 16.1</td>
<td>555.8 ± 13.4</td>
</tr>
<tr>
<td>Days until Food</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Criteria are Met</td>
<td>6.0 ± 0.8</td>
<td>6.7 ± 0.6</td>
<td>8.3 ± 0.8</td>
<td>5.6 ± 0.7</td>
</tr>
</tbody>
</table>
Maternal separation and handling did not affect the body weights of the dams and pups or the average litter size measured at weaning. The average number of days until acquisition of food-reinforced responding in treated adults was not different between groups. The body weights of the dams are given on the first (session 1) and the last (session 14) days of self-administration. The body weights of the treated adults are given on the first day of food training, the first day of saline self-administration (First Day) and the last day of cocaine self-administration at the highest dose tested (Last Day). The data are presented as the mean ± SEM.
Table 2: The average infusions per session for the MS15 and MS180 dams.

<table>
<thead>
<tr>
<th>Session</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>MS15</td>
<td>16.9</td>
<td>25.6</td>
<td>38.0</td>
<td>46.1</td>
<td>47.3</td>
<td>73.0</td>
<td>63.1</td>
</tr>
<tr>
<td></td>
<td>(8.1)</td>
<td>(12.3)</td>
<td>(12.7)</td>
<td>(13.3)</td>
<td>(16.8)</td>
<td>(27.7)</td>
<td>(24.9)</td>
</tr>
<tr>
<td>MS180</td>
<td>29.9</td>
<td>34.6</td>
<td>29.0</td>
<td>51.1</td>
<td>59.6</td>
<td>60.0</td>
<td>60.6</td>
</tr>
<tr>
<td></td>
<td>(12.4)</td>
<td>(18.1)</td>
<td>(12.9)</td>
<td>(14.3)</td>
<td>(11.5)</td>
<td>(3.9)</td>
<td>(9.6)</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>9</td>
<td>10</td>
<td>11</td>
<td>12</td>
<td>13</td>
<td>14</td>
</tr>
<tr>
<td>MS15</td>
<td>57.9</td>
<td>57.0</td>
<td>63.6</td>
<td>61.1</td>
<td>63.9</td>
<td>56.9</td>
<td>56.1</td>
</tr>
<tr>
<td></td>
<td>(24.3)</td>
<td>(16.1)</td>
<td>(15.1)</td>
<td>(12.5)</td>
<td>(19.6)</td>
<td>(21.8)</td>
<td>(18.3)</td>
</tr>
<tr>
<td>MS180</td>
<td>67.7</td>
<td>57.0</td>
<td>65.4</td>
<td>74.1</td>
<td>80.9</td>
<td>86.6</td>
<td>59.9</td>
</tr>
<tr>
<td></td>
<td>(10.1)</td>
<td>(9.6)</td>
<td>(20.9)</td>
<td>(19.3)</td>
<td>(23.6)</td>
<td>(21.2)</td>
<td>(15.2)</td>
</tr>
</tbody>
</table>

The duration of separation from the pups did not significantly affect cocaine self-administration. There were no significant differences between MS15 and MS180 groups in the mean number of infusions per session (± SEM) for the 14 days of testing.
Figure 2

[Graph showing the number of infusions per session for different cocaine doses in NH, MS0, MS15, and MS180 groups.]
Figure 4

NH

MS0

MS15

MS180

Lever Presses/Session vs. Cocaine (mg/kg/hr)

Levels of Active and Inactive lever pressing are shown for different doses of cocaine for NH, MS0, MS15, and MS180 groups.

SAL 0.0625 0.125 0.25 0.5 1.0

Cocaine (mg/kg/hr)