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Prenatal cocaine exposure increases anxiety, impairs cognitive function and increases dendritic spine density in adult rats: influence of sex

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Abstract

Cocaine exposure during pregnancy can impact brain development and have long-term behavioral consequences. The present study examined the lasting consequences of prenatal cocaine (PN-COC) exposure on the performance of cognitive tasks and dendritic spine density in adult male and female rats. From gestational day 8 to 20, dams were treated daily with 30 mg/kg (*ip*) of cocaine HCl or saline. At 62 days of age, offspring were tested consecutively for anxiety, locomotion, visual memory and spatial memory. PN-COC exposure significantly increased anxiety in both sexes. Object recognition (OR) and placement (OP) tasks were used to assess cognitive function. Behavioral tests consisted of an exploration trial (T1) and a recognition trial (T2) that were separated by an inter-trial delay of varying lengths. Male PN-COC subjects displayed significantly less time investigating new objects or object location during T2 in both OR and OP tasks. By contrast, female PN-COC subjects exhibited impairments only in OR and only at the longest inter-trial delay interval. In addition, gestational cocaine increased dendritic spine density in the prefrontal cortex and nucleus accumbens in both genders, but only females had increased spine density in the CA1 region of the hippocampus. These data reveal that *in-utero* exposure to cocaine results in enduring alterations in anxiety, cognitive function and spine density in adulthood. Moreover, cognitive deficits were more profound in males than in females.

Keywords

sex differences; synaptic plasticity; prefrontal cortex; hippocampus; visual memory; spatial memory

Cocaine abuse during pregnancy is recognized as a major social problem (G. A. Richardson, 1998; L. T. Singer et al., 2002). Clinical reports reveal an adverse relationship between

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prenatal cocaine (PN-COC) and neurobehavioral function (R. L. Freedland et al., 1998; L. T. Singer et al., 2002; B. Cone-Wesson, 2005). Moreover, longitudinal studies have shown long-term consequences of PN-COC, including a significant decline in cognitive performance in complex behavioral tasks, resembling attention deficit hyperactivity disorder (L. C. Mayes et al., 1998; L. T. Singer et al., 2004; B. L. Thompson et al., 2009).

Rodents exposed to cocaine during gestation display deficits analogous to those found in humans such as increased stress reactivity, impaired spatial memory and social behavior (G. Battaglia et al., 1995; R. D. Wood and L. P. Spear, 1998; J. O. Campbell et al., 2000; B. A. Morrow et al., 2002; V. Bashkatova et al., 2005). PN-COC exposed animals are easily distracted by olfactory cues and have difficulty maintaining attentional focus in adulthood (M. H. Gendle et al., 2004). *In-utero* cocaine exposure increases vulnerability to stressors and other environmental manipulations (D. Dow-Edwards et al., 1999).

Animal models may be used to elucidate the neurobiological factors underlying the deleterious consequences of PN-COC exposure. Gestational exposure causes neurochemical and morphological changes in the prefrontal cortex (PFC) and other cortical areas that are involved in cognition (P. Levitt et al., 1997; J. A. Harvey et al., 2001; J. A. Harvey, 2004). In the rabbit, PN-COC causes a dysfunction in cortical dopamine receptor signal transduction, glutamate receptor trafficking and signaling as well as changes in γ -aminobutyric acid (GABA) levels and in expression of calcium binding proteins (L. B. Jones et al., 2000; X. Zhen et al., 2001; B. L. Thompson et al., 2009). Similar alterations have been reported in rodent models (J. E. Crandall et al., 2004; D. P. Buxhoeveden et al., 2006; J. M. Silvers et al., 2006; T. F. Tropea et al., 2008). PN-COC increases dendritic spine density and spine synapses in prepubertal and adolescent rodents and induces alterations in cortical neuronal development in juvenile rabbits (L. Jones et al., 1996; B. A. Morrow et al., 2007; M. Frankfurt et al., 2009).

Less is known about gender-specific effects of gestational cocaine exposure, even though the effects of cocaine exposure in adulthood are sex-dependent in both rodents and humans (S. M. Evans and R. W. Foltin, 2009). For example, adult female subjects self-administer psychostimulants at lower doses than do males, drug use escalates more rapidly to dependence, and females are at greater risk for relapse following withdrawal than males (J. B. Becker and M. Hu, 2008). Female rats are more sensitive to the effects of cocaine than males, and their behavioral responses persist for longer (V. Quinones-Jenab et al., 2001; E. D. Festa and V. Quinones-Jenab, 2004; E. D. Festa et al., 2004). The present experiments sought to determine whether *in-utero* cocaine results in lasting cognitive and morphological changes and if these are sex-specific.

Experimental Procedures

Animals and Prenatal Cocaine Administration

Fifteen timed-pregnant Sprague-Dawley rats (colony 208A, Harlan Inc, Frederick, MD) arrived on gestational day (GD) 2. Animals were singly housed in a temperature controlled colony room (± 21 - 22°C), under a 12:12 light dark cycle (lights on at 8:00am) with water and food (Harlan Teklab Rodent Chow) available *ad libitum*. From GD8 to GD20, five of the pregnant dams were treated daily (at approximately 10:00am) with an intraperitoneal (ip) injection of 30 mg/kg of cocaine HCl (Sigma-Aldrich, St. Louis, MO), while ten dams received daily saline injections. The cocaine was made fresh every three days. The volume of the injections ranged from 0.24 to 0.39 mls. Within 24 hrs of parturition the cocaine treated dams were sacrificed. All pups were cross-fostered to saline treated dams and litters were culled (4 males, 4 females per litter) in order to control for variability of maternal

behaviors due to cocaine treatment or litter size. Cocaine treatment did not affect the mother's or pup's body weight.

At postnatal day (P) 21 rats were weaned and pair housed. Two pups of each sex and litter were used for these experiments. Body weights of pups were assessed every five days, as well as the onset of puberty and the estrous cycle state was noted for all female offspring. No significant differences were found in any these parameters between the cocaine and saline exposed groups (data not shown). Beginning at P62 a series of behavioral tests were performed to assess the effects of PN-COC administration on cognition and anxiety and to determine possible sex differences. A total of 32 animals [saline exposed: male (n=9) and female (n=8), cocaine exposed: male (n=9) and female (n=6)] were consecutively tested on an elevated plus maze, open field, an object recognition task and object placement task (see below). After each subject was tested all mazes were cleaned with Coverage disinfectant spray (Steris; Mentor, OH) as well as with a 1% Nolvasan solution in water to minimize olfactory cues. All procedures used were approved by the IACUC at Hunter College of the City University of New York.

Anxiety - Elevated Plus Maze (EPM)

On P62, male and female subjects were tested on an EPM (adapted from S. Pellow et al., 1985) which consists of two open arms (50 cm×10 cm) and two closed arms (50 cm×10 cm×40 cm high) elevated 40 cm above the floor. The walls extend from a central neutral area (10×10 cm). Subjects were placed in the neutral area facing one of the open arms and given 5 min to explore the maze. The number and duration of entries into open and closed arms were recorded. A total of three paws inside of an arm were used as criteria for entry. Entries or exploration in the neutral were not counted.

Locomotion – Open Field (OF)

On P63, overall locomotor activity was assessed using an open field (OF) paradigm (R. E. Bowman et al., 2006). Rats were placed in a 15×15 cm box; the open field is 117×70×45 cm black, Plexiglas, open box, with the floor divided into a grid of 9 inch square grids (5×3). Each animal was placed in the center square of the box and allowed to explore the field. The number of outer sector visits (movements across squares and a measure of general locomotor activity), inner sector visits (an anxiety-related behavior), total activity (combined outer and inner crossings), wall climbs, rears and grooms were scored for 6 min.

Non-spatial, visual memory -- Object recognition (OR)

From P64 to 68, an object recognition (OR) task, a visual memory task known to be less hippocampal-dependent and requires prefrontal cortex activity (A. Ennaceur and J. P. Aggleton, 1997; A. Ennaceur et al., 1997; D. G. Mumby, 2001; B. D. Winters et al., 2008). Trials consisted of an exploration trial (T1) and a recognition trial (T2). The two trials were separated by an intertrial interval of varying lengths. In T1, two identical objects were placed at one end of the open field and length of time spent exploring the two objects was recorded for 3 min. For T2, or the recognition trial, a new object replaced one of the previous objects. In T2, the time spent exploring the old (familiar object) and the new (novel) object was recorded for 3 min. Exploration was defined by the subject sniffing, whisking, or looking at the object from no more than 2 cm away. The objects used for trials were different bottles, cans, and containers (such as soda cans and candlesticks). The position of the objects and the object replaced was counterbalanced across both sex and treatment. All animals had acclimation sessions with inter-trial delays of 1, 10, and 60 min, and were then tested for object recognition with a 2-h and 4-h delay. Two control males did not explore the objects during either T1 or T2 and were excluded from the data analysis.

Spatial Memory -- Object Placement (OP)

From PND 69 to 73, spatial memory was assessed using an object placement (OP) task, a spatial memory task known to be hippocampal dependent (A. Ennaceur et al., 1997; K. D. Beck and V. N. Luine, 2002; C. Li et al., 2004). This task is identical to the OR procedure, except that during T2, the recognition trial, one object was moved to a novel location within the field (instead of replacing one object with a new object). Time spent by each subject exploring objects at the old versus new location was recorded during T2. The new location was counterbalanced across sexes and treatments. All animals had acclimation sessions with inter-trial delays of 15, 40 and 60 mins, and were tested for object recognition with a 2-h and 4-h delay. Two control males did not explore the objects during either T1 or T2 and were excluded from the data analysis.

Golgi Preparation and Spine Density Analysis

48 hours after the last behavior test, all subjects were sacrificed by decapitation. Brains were cut into an anterior block (anterior to the optic chiasm) and a posterior block (between the optic chiasm and the brainstem) and placed in solutions provided in the FD Rapid GolgiStain Kit (FD NeuroTechnologies, Ellicott City, MD).

Golgi impregnation was performed using the kit from FD NeuroTechnologies, with minor modifications (V. Luine et al., 2006; M. Frankfurt et al., 2009). Blocks were placed directly into solutions A and B, without rinsing and remained there for two weeks in the dark at RT. Forty eight hours after placing blocks in Solution C (4°C), blocks were frozen on dry ice and stored at -70°C until sectioning. Cryostat sections (100µm) were cut at -25°C and mounted onto subbed slides. Slides were allowed to dry in the dark and the rest of the staining process was performed as previously described (M. Wallace et al., 2006).

Secondary basal dendrites and tertiary apical dendrites were analyzed from pyramidal cells from the CA1 region of the dorsal hippocampus and layer II/III of pyramidal cells of the PFC. In the core of the nucleus accumbens (NAC) dendrites from medium spiny neurons were analyzed. Regions were defined according to The Rat Brain Atlas in Stereotaxic Coordinates (G. Paxinos and C. Watson, 2007). Six cells per region were quantified for each subject. Cells chosen for analyses had to be well impregnated, clearly distinguishable from adjacent cells and have continuous, unbroken dendrites. The dendrite chosen was the most lateral dendrite for a given dendritic tree. Spines were counted under oil (100×), using a Leitz Diaplan microscope and the entire dendritic length visible measured using Image Pro Plus software (Media Cybernetics) with a Nikon DXM 1200F camera. Spine density was calculated by dividing the number of spines by the length of the dendrite and data expressed as number of spines/10 µm dendrite.

Statistical Analysis

Two-way ANOVAs were used to analyze behavioral performance and spine density (sex × treatment) for the EPM, OF and the average exploration time (T1); where appropriate post hoc comparisons by student's t-tests were used to determine differences between PN-COC and control subjects within each sex. For OP and OR repeated measures three-way ANOVAs were used to analyze cognitive function by sex, treatment and inter-trial delay times. Two-way ANOVAs, in each sex, determined sex by treatment interactions within each inter-trial delay time, followed by post hoc simple planned comparisons by a student's t-test. Statistical significance was considered when $p < 0.05$.

Results

Effects of PN-COC Exposure on Anxiety and Locomotor Activity

Using the EPM to assess the impact of prenatal cocaine exposure on anxiety levels, we show that PN-COC rats, both males and females, are significantly more anxious than control animals. Cocaine exposed animals spent less time in the open arms of the maze, as determined by ratio of time spent in the open arms (time spent in the open arms/time spent in open and closed arms; $F_{1,25} = 5.4$, $p = 0.03$; Fig 1A). Independent of treatment, females were significantly less anxious than males because they spent the least amount of time in the closed arms ($F_{1,25} = 22.4$, $p = 0.001$; Fig 1A). Treatment did not affect number of open arm visits, closed arm visits or the ratio of open arm visits. However, female subjects, independent of cocaine exposure, had a higher ratio of number of open arm visits (open arm visits/closed plus open arm visits) than males ($F_{1,25} = 24.1$, $p = 0.001$; data not shown). Thus, PN-COC exposure increased anxiety in both genders.

PN-COC animals visited the inner sectors in an open field (OF) task significantly less than control subjects ($F_{1,26} = 5.8$, $p = 0.02$; Fig 1B). In addition, PN-COC subjects displayed more wall climbs than control animals ($F_{1,26} = 4.2$, $p = 0.05$). Both measures suggest that gestational cocaine exposure results in increased anxiety, a pattern similar to EPM results. Compared to males, females displayed significantly more overall locomotor activity than male subjects (Table 1). Specifically, they exhibited significantly higher number of outer sector visits ($F_{1,26} = 4.9$, $p = 0.04$), inner sector visits ($F_{1,26} = 16.7$, $p = 0.004$), total visits ($F_{1,26} = 7.3$, $p = 0.01$) and a higher number of wall climbs ($F_{1,26} = 28.9$, $p < 0.0001$).

Effects of PN-COC Exposure on Exploration of Objects during the Exploration Trial (T1) of the Cognitive Tasks

PN-COC subjects spent significantly more time exploring objects in both the object recognition ($F_{1,20} = 4.4$; $p = 0.006$) and placement ($F_{1,24} = 6.7$, $p = 0.016$) tasks (Fig 2). Furthermore, females explored the objects significantly more than males in both the object recognition ($F_{1,20} = 4.4$; $p = 0.05$) and placement ($F_{1,24} = 4.3$; $p = 0.05$) tasks (Fig 2).

Effects of PN-COC Exposure on Visual Memory

On the object recognition (OR) task an overall main effect of cocaine ($F_{1,20} = 5.01$; $p = 0.04$) and a significant 3-way interaction ($F_{1,20} = 5.2$; $p = 0.03$) between sex, treatment, and inter-trial delay on the ratio of time spent with the new object during T2 was observed (Fig. 3A). Two-way ANOVAs (sex $\times$ treatment) were used to determine group differences in the OR task for two and four hour delay intervals. When subjects were tested after a two hour inter-trial delay, a sex by treatment interaction was observed ($F_{1,20} = 4.7$, $p = 0.04$) where only male PN-COC subjects had a significantly lower ratio of time spent with the new object (t -value = 2.4; $p = 0.03$) when compared to the other groups. No differences were observed between cocaine and saline treated females when tested following a two-hour intertribal delay. When the inter-trial delay was extended to four-hrs, a main effect of cocaine treatment was observed in both males and females. PN-COC subjects displayed significant impairments ($F_{1,20} = 6.1$, $p = 0.02$) when compared to saline-treated subjects. In addition, we assessed whether groups significantly discriminated between the old and new objects using a two-way ANOVA (group by object (old vs new)). Both control males and females significantly discriminated at the two-and-four hour inter-trial delay times ($p < 0.05$), while cocaine-treated males did not at both the two-and-four hour inter-trial delay times. Interestingly, cocaine-treated females did not discriminate between the old and new objects only in the four-hour inter-trial delay (data not shown). In summary, PN-COC exposure results in memory deficits in both male and female subjects; however, female rats displayed

deficiencies only when there was a higher cognitive demand as determined by the ratio of time spent investigating the new object.

Effects of PN-COC Exposure on Spatial Memory

Exposure to cocaine during gestation elicited a main effect ($F_{1,24} = 9.5$; $p = 0.001$) and a significant interaction between sex and treatment ($F_{1,24} = 9.02$; $P = 0.001$) on the object placement (OP) task (Fig. 3B). To determine group differences, two-way ANOVAs were performed for each individual inter-trial delay. A significant sex by treatment interaction demonstrated that only PN-COC males displayed deficits on the OP task at both two-hr ($F_{1,24} = 9.2$, $p = 0.006$; t -value = 4.2, $p = 0.001$) and four-hr ($F_{1,24} = 5.5$, $p = 0.03$; t -value = 3.3, $p = 0.006$) inter-trial delay as determined by the ratio of time spent investigating the object placed in the new location versus the previous location. Female cognitive function remained unaffected at both delay lengths; therefore, PN-COC impairs spatial memory only in males. In addition, we assessed whether groups significantly discriminated between the old and new location using a two-way ANOVA (group by object location (old vs new location)). Both control males and females significantly discriminated at the two-and-four hour inter-trial delay times ($p < 0.05$), while cocaine-treated males did not at both the two-and-four hour inter-trial delay times. As in the OR task, cocaine-treated females did not discriminate between the old and new objects only in the four-hour inter-trial delay (data not shown).

Effects of PN-COC Exposure on Dendritic Spine Density

PN-COC increased spine density in the PFC and NAC in both male and female subjects; however, only females exposed to COC had increased spine density in the CA1 region of the hippocampus (Fig. 4). Gestational cocaine exposure significantly increased PFC pyramidal cell basal dendrite spine density in both male and female subjects ($F_{1,25} = 5.2$, $p = 0.03$; Fig. 5). Increased spine density was also observed on NAC medium spiny neurons dendrites ($F_{1,28} = 14.6$, $p = 0.007$). ANOVAs also revealed a significant sex by treatment interaction on spine density in both apical ($F_{1,28} = 4.4$, $p = 0.05$) and basal dendrites ($F_{1,28} = 5.1$; $p = 0.03$) of CA1. PN-COC exposed females had significantly higher spine density in both apical (t -value = -2.4, $p = 0.03$) and basal dendrites (t -value = -4.1, $p = 0.002$) in pyramidal cells in CA1 when compared to control females and all male subjects. The spine density on dendrites in CA1 was not changed by PN-COC in male rats.

Discussion

The present experiments demonstrate that *in-utero* cocaine exposure elicits behavioral and structural changes that persist into adulthood. Exposure to PN-COC results in increased anxiety and impaired cognitive function. While anxiety was elevated in all cocaine exposed animals, the deficit in the memory was sex-specific. Males exposed to PN-COC exhibited impaired visual and spatial memory, while female rats showed less severe impairments in visual memory and no deficits in spatial memory. Interestingly, females exposed prenatally to cocaine showed increased spine density in basal and apical dendrites of pyramidal cells in CA1; these effects were not observed in males. Both males and females exhibited increases in spine density in apical dendrites of pyramidal cells in the PFC and in medium spiny neurons in the NAC. These studies demonstrate that gestational cocaine results in lasting gender-specific structural modifications in brain regions that are associated with cognitive function.

Anxiety and anxiety-related indexes in adulthood

Increased anxiety was noted in both the EPM and OF tasks in all rodents exposed to cocaine *in-utero*. In previous studies PN-COC resulted in either a decrease or no effect on anxiety

(D. H. Overstreet et al., 2000; S. K. Sobrian et al., 2003). The differences in these behavioral outcomes may be due to the age of the animals at the time of testing (P90 – P365). Previous studies employing different behavioral paradigms report that PN-COC results in enduring effects on emotional behavior and social anxiety in adulthood. In a forced swim test, a behavioral task useful in screening for anti-depressant drug activity, gestational cocaine exposure resulted in significant immobility when tested in adulthood. This effect was correlated to emotional changes seen in children exposed to cocaine prenatally (P. J. Bilitzke and M. W. Church, 1992; V. A. Molina et al., 1994; D. H. Overstreet et al., 2000). In another investigation, both male and female cocaine exposed rodents show context fear conditioning; however, sex differences were observed in context extinction (D. H. Brunzell et al., 2002). Furthermore, gestational cocaine exposure in rodents results in decreased contact time during social interactions, considered comparable to generalized anxiety disorder in humans (R. D. Wood et al., 1994; D. H. Overstreet et al., 2000). Collectively, these data suggest that *in-utero* cocaine exposure alters emotional responsiveness that may parallel behavioral consequences observed in children of cocaine abusing women (A. Molitor et al., 2003; E. Z. Tronick et al., 2005).

Although increased anxiety is observed in both males and female rats after gestational cocaine exposure, males were more anxious than females. This sex difference may be related to the fact that male rats are more anxious and display less locomotor behavior than females in both EPM and OF paradigms (A. L. Johnston and S. E. File, 1991; C. Fernandes et al., 1999; P. Palanza, 2001). *PN-COC* does not dramatically affect locomotor activity, consistent with our experimental results and others (D. H. Brunzell et al., 2002; C. Crozatier et al., 2003; A. Torres-Reveron and D. L. Dow-Edwards, 2006; C. J. Malanga et al., 2007). Thus, the impacts of gestational cocaine exposure on male and female anxiety are unrelated to motor function.

Sex-specific effects on cognitive function in adulthood

The direct comparison of two distinct cognitive tasks that gauge visual and spatial memory revealed sex-specific effects of gestational cocaine exposure. While, PN-COC exposed males displayed deficits in both visual and spatial memory tasks, females were deficient only in the visual memory paradigm. In keeping with the present findings, previous studies, employing a spontaneous OR task, reported that PN-COC affected non-spatial, short-term memory in males (B. A. Morrow et al., 2002). Furthermore, impaired visual memory was observed only in PN-COC females when there was high cognitive demand. Although an earlier study using the Morris water maze determined that PN-COC does not impair spatial memory (J. M. Johns et al., 1992), more recent findings reveal spatial memory impairments in PN-COC males tested during adolescence and adulthood (S. J. Choi et al., 1998; G. H. Trksak et al., 2007). In agreement with our findings, Choi *et al.* also showed that males exposed to PN-COC demonstrate impaired spatial memory in the OP task and in the Morris water maze, whereas females were unaffected. Taken together, these results suggest a greater vulnerability of males to the cognitive consequences of gestational cocaine exposure.

In both OR and OP tasks both PN-COC exposed male and female offspring explored objects during T1 significantly more than saline treated animals, suggesting that heightened anxiety was not responsible for the impaired memory. During the acclimation trials, which consisted of shorter inter-trial delays, cocaine exposed animals explored the new objects for longer than the old objects presented during T2, indicating that the experimental subjects can perform the objects tasks when there is less cognitive demand required from the subjects. Using appetitive conditioning and conditioned place preference it has been shown that PN-COC causes learning deficits in adolescent animals (L. P. Spear et al., 1989a; L. P. Spear et al., 1989b; C. J. Heyser et al., 1992). The present and previous findings indicate that PN-COC exposure affects learning, memory and emotional responsiveness.

Increases in spine density in neural structures associated with cognitive function

PN-COC exposure resulted in an increase in dendritic spine density that persisted into adulthood in PFC, CA1 and NAC. These results extend previous findings showing that PN-COC increased spine density in several cortical and subcortical brain regions in prepubertal animals, independent of sex (M. Frankfurt et al., 2009). In addition, twice as many spine synapses were found in the prelimbic cortex in both adolescent male and female rats exposed to PN-COC (B. A. Morrow et al., 2007). The increase in the number of synaptic inputs on dendrites during adolescence as well as the increase in spine density in adulthood following PN-COC exposure may contribute to the cognitive deficits noted in the current experiments.

Sex differences in spine density were found in CA1 after PN-COC. Female rats exposed to PN-COC had a greater spine density than any other group on both apical and basal dendrites. A correlation between spine density and ability to perform cognitive tasks has been suggested and the current results provides some support for this relationship (C. Li et al., 2004; M. Wallace et al., 2006). Memory deficits were still observed in cocaine exposed subjects; nonetheless, treatment effects were not as pronounced in females perhaps of the increase spine density in the CA1 region of the hippocampus. Another potential explanation for the observed sex differences in cognitive function is that dendritic spine dynamics, synapse formation and remodeling are differentially regulated in males and females. There is an increase in the number of stable spines, which form synapses, and a decrease in the total number of spines during development (V. A. Alvarez and B. L. Sabatini, 2007). In adult males, three-wks after the last cocaine dose of a chronic treatment regimen, an increase in spine density and a concomitant time-dependent change in cytoskeletal proteins and post-synaptic density proteins has been demonstrated (H. W. Shen et al., 2009). It is possible that dendritic spine dynamics, synapse formation and remodeling are mediated differently in male and females throughout development affecting the mechanism through which gestational cocaine regulates cognitive function in adulthood (A. Parducz et al., 2006; D. H. Bhatt et al., 2009).

Gestational cocaine exposure results in biochemical and physiological alterations, some of which are sex-dependent. Basal and electrically evoked dopamine release is reduced (S. J. Glatt et al., 2004) and decreased D2 receptor and increased D3 receptor are found only in PN-COC males (J. M. Silvers et al., 2006). PN-COC also induces sex-independent increases in density of alpha adrenergic receptors (F. J. Seidler and T. A. Slotkin, 1992), decreases glucose metabolism, increases dopamine D1 receptor density and sensitivity (D. L. Dow-Edwards et al., 1990), alters signal transduction in the D1 receptor system without apparent changes in D1R density (E. Friedman and H. Y. Wang, 1998) and dopamine D2R density (M. G. Henderson et al., 1991). Neurophysiological studies in the mPFC indicate that *in utero* cocaine exposure attenuates AMPAR-mediated long-term depression without affecting basal transmission at P21 suggesting cocaine alters signal transduction pathways of glutamatergic systems (K. Bakshi et al., 2009). PN-COC also facilitates activity-induced long-term potentiation at excitatory synapses from P16-P42 as a result of a change in surface expression of GABAR subunits (H. Lu et al., 2009). Thus, these neurochemical differences that play a role in neural plasticity may explain the long-term consequences on cognitive function after gestational cocaine exposure (reviewed in B. L. Thompson et al., 2009).

Conclusion

Cognitive and attentional deficits have been observed in infants, children and teens that were exposed to cocaine during gestation; however, the long term impact of *in-utero* cocaine in humans is yet to be determined (J. M. Struthers and R. L. Hansen, 1992; E. S. Bandstra et al., 2001; M. B. Pulsifer et al., 2004; J. Savage et al., 2005). By using an animal model of

PN-COC this study underscores the impact cocaine may have on the developing brain long after drug exposure. Furthermore, these results highlight the differential consequences of gestational drug exposure in male and female subjects affecting neural organization and cognitive function. Further studies on synaptic plasticity mechanisms are necessary to understand the repercussions of drug exposure on fetal development and subsequent adult behavior.

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A – Elevated Plus Maze

B – Open Field

Figure 1. Prenatal cocaine increased anxiety-related behaviors in an elevated plus maze and open field task

(A) Female rats were significantly less anxious than males as determined in the ratio of time spent in the open arms (open/closed + open time) and PN-COC subjects were significantly more anxious than controls. (B) In an open field, females were less anxious than males and PN-COC animals also showed increased anxiety by visiting the inner sectors of the field significantly less often than control subjects. Asterisks denote a significant difference ($p < 0.05$) between cocaine exposed subjects and control subjects or between male and female subjects.

Figure 2. Prenatal cocaine significantly increased the mean exploration time during the exploratory phase of both memory tasks

On average, PN-COC subjects spent more time exploring the objects in both the (A) object recognition and (B) placement tasks. In addition, females explored the objects significantly more than male subjects in both the (A) object recognition and (B) placement tasks.

Asterisks denote a significant difference ($p < 0.05$) between cocaine exposed subjects and control subjects or between male and female subjects.

A- Object Recognition

B- Object Placement

Figure 3. Prenatal cocaine exposure results in sex-specific memory deficits

(A) In the object recognition task, three-way repeated measures ANOVA revealed a significant three way interaction. M PN-COC display visual memory deficits at two- and four- hr intertrial delay times while F PN-COC only displayed memory deficits at a 4-hr intertrial delay. (B) In the object placement task, three-way repeated measures ANOVA revealed a significant interaction between sex and treatment. PN-COC exposure impairs spatial memory only in males at both two- and four-hour intertrial delay times, while females remain unaffected. Asterisks highlight male and female subjects, independent of treatment, which performed well on both visual and spatial memory tasks ($p < 0.05$).

Figure 4. Prenatal cocaine exposure increased the spine density in CA1, PFC and NAC of adult subjects

ANOVAs for CA1 region of the dorsal hippocampus revealed a significant sex by treatment interaction on spine density in both apical and basal dendrites of the CA1 region in the hippocampus. PN-COC females had significantly higher spine density in both apical and basal dendrites of the CA1 region of the hippocampus. PN-COC significantly increased spine density on basal dendrites in the PFC and in the dendrites of the NAC. Asterisks highlight treatment groups that showed a significant increase in spine density ($p < 0.05$).

Figure 5. Golgi impregnated neurons in the PFC

Photomicrograph illustrating the cocaine-induced increase in dendritic spines on layer III pyramidal cells. The dendrites of (B) PN-COC subjects have greater spine density than (A) control subject. Scale bar = 10µm.

Open field task

Generally, females were more active than males in most measures of locomotion, such as outer sector visits, inner sector visits, total visits and wall climbs. PN-COC subjects exhibited less inner sector visits, a measure of increased anxiety and a higher number of wall climbs, compared to control subjects.

Table 1

Behavior	Female Control	Female PN-COC	Male Control	Male PN-COC	Statistics (2-way ANOVA)
Number of Outer Visits (mean $\pm$ s.e.m.)	137.6 $\pm$ 4.4	137.7 $\pm$ 8.3	118.1 $\pm$ 6.9	125.4 $\pm$ 8.5	Sex: $p < 0.05$
Number of Inner Visits (mean $\pm$ s.e.m.)	9.5 $\pm$ 1.4	6.5 $\pm$ 0.7	4.9 $\pm$ 0.4	3.3 $\pm$ 1.0	Treatment: $p < 0.05$; sex: $p < 0.05$
Total Visits (outer + inner) (mean $\pm$ s.e.m.)	147.3 $\pm$ 5.0	145.8 $\pm$ 10.0	122.1 $\pm$ 7.0	128.6 $\pm$ 1.0	Sex: $p < 0.05$
Number of Wall Climbs (mean $\pm$ s.e.m.)	27.4 $\pm$ 1.8	32.5 $\pm$ 3.4	14.9 $\pm$ 1.3	20 $\pm$ 9.1	Treatment: $p, 0.05$; sex: $p < 0.05$
Number of Rears (mean $\pm$ s.e.m.)	2.13 $\pm$ 0.4	1.83 $\pm$ 0.5	1.88 $\pm$ 0.4	1.63 $\pm$ 0.4	n.s.
Number of Grooms (mean $\pm$ s.e.m.)	4.63 $\pm$ 0.9	5.0 $\pm$ 1.7	4.63 $\pm$ 1.4	2.63 $\pm$ 1.0	n.s.