Research report

Cocaine exposure prior to pregnancy alters the psychomotor response to cocaine and transcriptional regulation of the dopamine D1 receptor in adult male offspring

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HIGHLIGHTS

- Cocaine prior to pregnancy enhanced psychomotor sensitivity to cocaine in adult male offspring.
- This enhanced sensitivity to cocaine in the offspring was associated with increased gene expression of DRD1 in mPFC.
- Cocaine prior to pregnancy had no effect on maternal behavior during lactation.
- Cocaine prior to pregnancy had no effect on CORT, GR or CRF gene expression in offspring.

ARTICLE INFO

ABSTRACT

There is evidence that maternal experience prior to pregnancy can play an important role in behavioral, physiological, and genetic programming of offspring. Likewise, exposure to cocaine in utero can result in marked changes in central nervous system function of offspring. In this study, we examined whether exposure of rat dams to cocaine *prior to* pregnancy subsequently alters indices of behavior, physiology, and gene expression in offspring. Multiple outcome measures were examined in adult male offspring: (1) behavioral expression of cocaine-induced psychomotor activation; (2) levels of corticosterone in response to immobilization stress; and (3) expression of multiple genes, including dopamine receptor D1 (DRD1) and D2 (DRD2), glucocorticoid receptor (GR), and corticotropin-releasing factor (CRF), in functionally relevant brain regions. Adult Sprague-Dawley females were exposed to cocaine (15–30 mg/kg, i.p.) or saline for 10 days, and were then mated to drug naïve males of the same strain. Separate groups of adult male offspring were tested for their acute psychomotor response to cocaine (0, 15, 30 mg/kg, i.p.), corticosterone responsivity to 20 min of immobilization stress, and expression of multiple genes using quantitative PCR. Offspring of dams exposed to cocaine prior to conception exhibited increased psychomotor sensitivity to cocaine, and upregulated gene expression of DRD1 in the medial prefrontal cortex (mPFC). Neither stress-induced corticosterone levels nor gene expression of GR or CRF genes were altered. These data suggest that cocaine exposure before pregnancy can serve to enhance psychomotor sensitivity to cocaine in offspring, possibly via alterations in dopamine function that include upregulation of the DRD1.

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1. Introduction

Prenatal exposure to cocaine can lead to detrimental effects in the physiology and central nervous system function of offspring. In humans, the most common outcomes of cocaine use during pregnancy include premature birth, lower than average birth weight, respiratory distress, and increased risk of seizures in offspring [1]. Prenatal exposure to cocaine has also been shown to affect the developing nervous system, by delaying structural brain maturation of dopamine-rich cortical and subcortical brain structures, such as the prefrontal cortex (PFC) and basal ganglia, respectively [2]. For example, in a recent study carried out in adolescents exposed to cocaine during pregnancy, significant impairments in structural brain maturation of the PFC were observed, possibly owing to cocaine-induced elevations in synaptic levels of serotonin and dopamine as a result of interference in monoamine reuptake [3].

Rodents exposed to cocaine prenatally display deficits analogous to those found in humans. For example, cocaine exposure during gestation leads to dose-dependent increases in maternal blood pressure and decreases in uterine blood flow, impairing oxygen transfer to the fetus; such impairment may in turn contribute to observed increases in fetal levels of cocaine and catecholamines [4]. Likewise, prenatal cocaine exposure leads to abnormalities in fetal brain development, particularly within the dopamine-rich neurons of the primary reward pathway projecting from the ventral tegmental area (VTA) to the nucleus accumbens (NAc) and PFC. In addition, prenatal cocaine exposure has been shown to delay the migration of GABA neurons during the embryonic period, leading to altered laminar positioning of neurons in the medial PFC [5] and marked reduction of GABAergic function in the medial PFC [6]. Prenatal cocaine exposure has also been linked to altered cocaine-primed dopamine release in the NAc in adulthood [7]. Taken together, these data indicate that prenatal exposure to cocaine in both humans and animals leads to physiological dysregulation and neural alteration in reward-related circuitry of offspring.

Importantly, cocaine crosses the placenta and is metabolized slowly in the fetus, which can lead to direct and prolonged exposure to significant levels of cocaine in the developing fetus [1]. Notably, in an in vitro model, fetal neuronal cells exposed to cocaine showed alterations in the expression of many genes involved in brain development [8]. Because gestational exposure to cocaine potentially involves direct effects of cocaine on both the mother and offspring, the maternally mediated effects of cocaine exposure are unclear.

The purpose of the present study was to determine whether exposure of adult rat dams to cocaine prior to conception changes the behavioral and transcriptional phenotypes of adult offspring. By studying the effects of cocaine exposure before pregnancy, we were able to disentangle the potential intergenerational effects of cocaine exposure in dams from the direct effects of cocaine to the developing fetus. To this end, we assessed three major outcome measures in the adult male offspring of dams given cocaine prior to conception, all of which can be influenced by a history of prior cocaine experience [9–20]: (1) cocaine-induced psychomotor activation; (2) stress-induced activation of the hypothalamic pituitary adrenal (HPA) axis, as reflected in plasma levels of corticosterone; and (3) expression of DRD1 and DRD2 genes within discrete regions of the brain reward circuitry (i.e., VTA, NAc, and mPFC), as well as expression of the stress-related glucocorticoid receptor (GR) and corticotropin releasing factor (CRF) genes in limbic regions (i.e., hypothalamus [HYP], hippocampus, [HC], and amygdala [AMG]).

2. Materials and methods

2.1. Animals

Twenty female Sprague-Dawley rats were purchased from Charles River Canada (St.-Constant, QC). Rats were pair-housed in cages in a humidity-controlled vivarium on a 12 h light–dark cycle. Standard rat chow and water were freely available. All rats were allowed at least 1 week of acclimatization to the vivarium before the start of the cocaine pre-exposure regimen, which consisted of once daily injections of cocaine for 10 days (see below). After the cocaine pre-exposure regimen, rats were left undisturbed for 5 days, after which time they were housed with sexually experienced male Sprague-Dawley rats. One male was housed with each pair of females for 7 days. After this mating period, males were returned to their home cages and females were singly housed.

Of the original 20 female subjects, 18 became pregnant: 9 cocaine pre-exposed and 9 saline pre-exposed. The offspring of six dams from each pre-exposure condition were randomly selected for the experiments. On Postnatal Day 1 (PND1), all litters were weighed and culled to six males and six females. After weaning on PND21, offspring were pair-housed with a littermate of the same sex.

All procedures were performed in accordance with the guidelines of the Canadian Council on Animal Care and were approved by the University of Toronto Animal Care Committee.

2.2. Procedures

2.2.1. Procedural manipulations of dams
2.2.1.1. *Cocaine pre-exposure.* The cocaine pre-exposure regimen was started when the rat dams were approximately 65 days of age. One day prior to the start of cocaine (or saline) injections, all rats were given a habituation session, to acclimatize them to the experimental apparatus and treatment procedures. During this session, rats were placed in locomotor activity chambers (26 cm × 48 cm × 21 cm) for a period of 30 min. Then they were given a saline (1 kg/ml i.p.) injection, after which they were replaced in the activity chambers for an additional 60 min. Locomotor activity both before and after the injection was monitored and recorded by a video tracking system that measured distance traveled during each min of the session (Ethovision, Noldus Information Technology, Inc., Leesburg, VA). In order to equate baseline levels of activity in the cocaine and saline pre-exposure conditions, animals were assigned to the conditions based on activity during the habituation session.

Over a subsequent and consecutive 10-day period, rats were given once daily injections of cocaine or saline. On Days 1 and 10, cocaine (15 mg/kg, i.p.) or saline injections were given in the locomotor activity chambers, under the same conditions described for the habituation session. Thus, rats were placed in the chambers for 30 min, injected with cocaine or saline, and placed back in the chambers for an additional 60-min recording period. On Days 2–9, cocaine (30 mg/kg, i.p.) or saline injections were given in the home cages. This is a cocaine dosing regimen that we and others have found previously to produce robust behavioral sensitization to cocaine [20,21].

2.2.1.2. *Test for cocaine sensitization.* Approximately 8 weeks after the termination of the cocaine exposure phase, and within 1 week of weaning (which occurred on PND21), all dams were tested for their locomotor response to a challenge injection of 15 mg/kg of cocaine (i.p.). For this test, rats were placed in the locomotor chambers for

30 min, injected with cocaine, and placed back in the locomotor chambers for an additional 60 min period.

2.2.1.3. Assessment of maternal behavior.

The maternal behavior of nine dams that had been exposed to cocaine before pregnancy, and nine dams that had been exposed to saline, was monitored on each of 5 days between PND1 and PND9 (i.e., PNDs1, 2, 5, 7, and 9). The date when the pups were born was regarded as PND0. The maternal monitoring procedures were a modified version of methods we have described previously [22]. Briefly, the behavior of each dam was video-recorded during four consecutive 1-h observation periods that occurred either in the morning (i.e., 0900–1000 h, 1000–1100 h, 1100–1200 h, and 1200–1300 h) or afternoon (1300–1400 h, 1400–1500 h, 1500–1600 h, and 1600–1700 h). The morning and afternoon observation periods alternated between observation days, and were counterbalanced between conditions. For each observation period, the individual video recordings for each dam were assessed in 5 min intervals (i.e., 12 observations/period × 4 periods per day = 48 observations/dam/day), using a computer-controlled behavioral coding and analysis system (Observer 4.1, Noldus Information Technology, Inc., Leesburg, VA). The frequency of maternal behaviors of licking and grooming of pups and/or nursing pups in an arched-back posture (LG ABN) was scored. Percentage scores for each behavior where generated by dividing the frequency score for that behavior by the total number of observations made on a given observation day. The coding of maternal behavior was carried out by five trained raters. Raters were blind to the subject's maternal history of cocaine exposure, and were determined to have an inter-rater reliability of more than 85%, using the reliability analysis tool in Observer 4.1 (Noldus Information Technology, VA, USA). The five raters were each assigned to one observation day per dam, in order to avoid observer bias for a particular dam.

2.2.2. Procedural manipulations of male offspring

An equal number of male offspring (1–2 per litter) from mothers with a history of cocaine and saline exposure, were used for the different tests described below. Each group of rats was selected from six out of nine litters per maternal condition (saline or cocaine).

2.2.2.1. Tests for cocaine-induced locomotor activity.

The acute psychomotor response to 0, 10 and 30 mg/kg (i.p.) of cocaine was measured in 12 male offspring of cocaine-exposed dams, and 12 male offspring of saline-exposed dams, starting on approximately PND75. One day before the start of testing, rats were habituated to the locomotor chambers. During this habituation session, rats were placed individually in the locomotor activity chambers for 30 min, after which they were given an i.p. injection of saline and placed back in the chambers for an additional 60 min. On each of three subsequent and consecutive days, rats were given i.p. injections of 0 mg/kg (i.e., saline), 10 mg/kg, or 30 mg/kg of cocaine between the 30 min pre-injection and 60-min post-injection sessions. All rats were tested under all three dose conditions, in a counterbalanced order. Two male offspring of cocaine pre-exposed dams were considered as outliers, and were excluded from the analyses; their activity scores in response to a saline injection were greater than two standard deviations from the group median. These rats also exhibited a reduced locomotor response to cocaine (10 mg/kg and 30 mg/kg, respectively) compared to saline.

2.2.2.2. Immobilization stress-induced corticosterone responsivity.

A separate subset of 24 male offspring (12 of cocaine- and 12 of saline-exposed dams) was used to assess for corticosterone responsivity to 20 min of immobilization stress; these assessments were made on approximately PND90. Rats were habituated to the procedure room and handled for several minutes per day for five consecutive

days, prior to the test day. On the day of testing, rats were habituated to the procedure room for 2 h, and then hand-restrained with a loosely fitting towel. Blood was immediately withdrawn into a tube from a small nick in the tail, and placed on ice. Rats were then placed into Plexiglas restrainers for 20 min (6.4 cm diameter × 21.6 cm length; Plas-Labs). After 20 min, a second sample of blood was withdrawn while rats were still in the restrainer. Rats were then returned to their home cage without their conspecific partner, and left undistured for 70 min. At the end of 70 min, rats were hand-restrained again, and a third sample of blood was withdrawn, after which they were returned to their home cage; at this time, they were also returned to their cage mate.

Blood was kept on ice for at least 30 min before being centrifuged at 4 °C, 4000 rpm, for 20 min. Serum was then extracted and stored at −80 °C. Levels of serum corticosterone were determined using commercially available radioimmunoassay kits with ^{125}I-labeled anti-corticosterone antibody (MP Biomedicals Inc., CA, USA: sensitivity 7.7 ng/ml and the intra-assay coefficient of variation was 5.4%). The data from one male offspring of a saline exposed dam was excluded from the analysis, due to premature interruption of the stress regimen and resulting failure to collect all samples.

2.2.2.3. Gene expression analyses in male offspring.

The brains of a separate subset of 12 male offspring (6 of cocaine- and 6 of saline-exposed dams), at approximately PND130, were collected and processed for expression of the following genes: DRD1, DRD2, GR and CRF. For brain collection, rats were deeply anesthetized with CO_2 and decapitated. Brains were rapidly removed and flash frozen in isopentane chilled with dry ice. Brains were stored at −80 °C prior to slice preparation and processing. Specific brain regions were dissected using a cryostat and the following coordinates relative to bregma [23]: mPFC (+4.2–2.2), NAc (+2.2–1.2), VTA (−5.2 to −6.4), AMG (−1.6 to −3.4), HYP (−1.6 to −3.6) and vHC (−4.16 to −6.3). Total RNA was isolated from the tissues using Qiazol followed by the RNeasy Plus kit, using manufacturer protocols (Qiagen Canada, Toronto, ON, Canada). The quality and quantity of RNA were measured using a NanoDrop spectrophotometer (Nanodrop ND-2000C, Thermo Scientific, MA, USA). Equal amounts of RNA per sample were converted to cDNA using a high-capacity cDNA reverse transcription kit (Life Technologies, CA, USA). Quantitative PCR was then performed using a StepOne Plus real time thermocycler and Fast SYBR Green Master Mix (Life Technologies, CA, USA). A standard curve was generated from 10 serial dilutions of a mixture of cDNA from all subjects, and gene expression was quantified relative to a geometric mean of three housekeeping genes (GAPDH, Actin B, and UBC), in order to avoid bias among any housekeeping gene. The samples from each rat were analyzed in triplicate, and a mean value for the group was generated from the average of the triplicates. Thus, for each of the two maternal conditions, a single data point from each of six rats was generated, for a total of 12 data points contributing to the analysis of each brain region. The relative transcript abundance was calculated by dividing the data from the gene of interest by the geometric mean of three housekeeping genes. Each housekeeping gene was examined in each brain region analyzed. The primers used in this study were designed using sequence information from GenBank at the National Center for Biotechnology Information (NCBI; www.ncbi.nlm.nih.gov), and a freely available online primer designing tool (Primer 3; http://primer3.sourceforge.net). The efficacy of each primer set was verified according to the manufacturer's protocol for a StepOne Plus real time thermocycler (Life Technologies, CA, USA) by ensuring efficiency levels greater than 90%. UBC: F: 5′-CACCAAGAAGGTCAAACAGGAA-3′; R: 5′-AAGACACCTCCCCATCAAACC-3′, GAPDH: F: 5′-ACATCAAAT GGGGTGATGCT-3′ and R: 5′-GTGGTTCACACCCATCACAA-3′, Actin B: F: 5′-TTTGAGACCTTCAACACCCC-3′ and R: 5′-ATAGCTCTTCTCC

AGGGAGG-3′, DRD1: F: 5′-TCCACTCTCCTGGGCAATAC-3′ and R: 5′-CAGGACAGCCACCAAGAGAT-3′, DRD2: F: 5′-TCCCAGCAGAA GGAGAAGAA-3′ and R: 5′-GTGGGATGTTGCAATCACAG-3′, GR: F: 5′-CTCGAAAGGCTCCACAAGCAATGT-3′ and R: 5′-GCAATGCTT TCTTCCAGAAGCCGA-3′, CRF: F: 5′-TTCCTGTTGCTGTGAGCTTG-3′ and R: 5′-TCACCTTCCACCTTCTGAGG-3′.

2.2.3. Data analyses

The data from the cocaine pre-exposure phase were analyzed using a two-way repeated measures ANOVA with pre-exposure (cocaine or saline) as the between subjects factor and pre-exposure day (1 and 10) as the within subjects factor. Student's t-tests were used for comparisons between Days 1 and 10.

The data from the test for cocaine sensitization were analyzed using Student's t-test for the factor of pre-exposure condition (cocaine or saline) in the first 30 min of testing. Although all test sessions were 60 min in duration, careful scrutiny of the behavioral data indicated that between-group differences were revealed only in the first 30 min of each session, after which time activity levels returned to baseline in both conditions; the restoration of baseline activity was, however, accompanied by a high level of variability that interfered in the expression of a significant time by condition interaction, overall. This pattern of results is very consistent with our previous work using similar conditioning protocols [24].

The data from the maternal coding were analyzed using a repeated measures ANOVA with pre-exposure (cocaine or saline) as the between subject factor and postnatal day (PND1, 3, 5, 7, and 9) as the within subjects factor. The data examining the offspring's acute response to cocaine were analyzed using a repeated measures ANOVA with maternal history (cocaine or saline) as a between-subjects factors and cocaine dose (0, 10, and 30 mg/kg) as a within subjects factor in the first 10 min of testing. In this case, careful scrutiny of behavioral data indicated that between-group differences were revealed only in the first 10 min of each session, after which activity levels rapidly returned to baseline levels under – as was the case in the test for sensitization in the dams – considerable variability that interfered in the expression of a significant time by condition interaction.

The data from stress-induced CORT were analyzed using a repeated measures ANOVA with maternal history (cocaine or saline) as the between subject factor and time after stress onset (0, 20, and 70 min) as the within subjects factor. The data from gene expression assays were analyzed using Student's t-tests for the factor of maternal history (cocaine or saline).

All analyses were performed using IBM SPSS Statistics version 21 for Mac OS. Data are presented as mean values ± SEM throughout. In all cases, statistical significance was set at $p \leq 0.05$. In cases where main effects and interactions were statistically significant, post hoc tests with a Bonferroni correction for multiple comparisons were used to clarify between-group differences.

3. Results

3.1. Effect of repeated cocaine exposure prior to conception on locomotor activity and maternal behavior in dams

3.1.1. Locomotor activity during cocaine pre-exposure

Fig. 1 shows the mean (±SEM) distance traveled (cm) by dams on Days 1 and 10 of the pre-exposure phase. Cocaine relative to saline pre-exposed dams exhibited a higher level of locomotor activity on both Day 1 and Day 10, and cocaine pre-exposed dams exhibited a higher level of activity on Day 10 relative to Day 1. Overall, repeated measures ANOVA revealed a significant main effect of drug pre-exposure [$F(1,16) = 31.79$, $p < .0001$], and a significant interaction of drug pre-exposure × day [$F(1,16) = 4.55$, $p < .05$] that
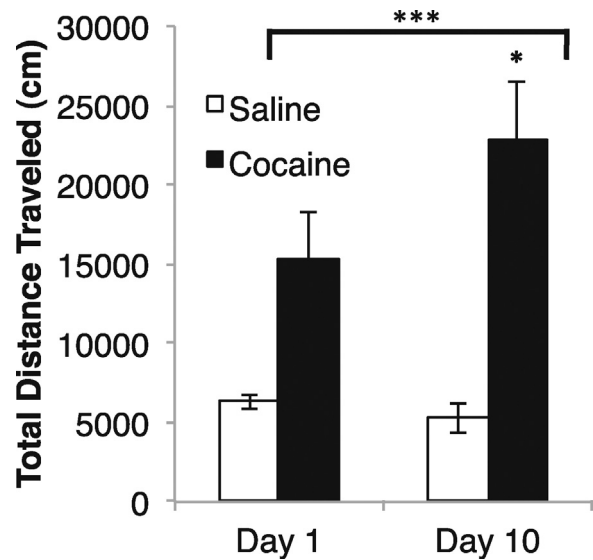

Fig. 1. Locomotor activity during cocaine pre-exposure in dams. Mean ± SEM distance traveled (cm) by dams in 30 min, during Days 1 and 10 of the cocaine pre-exposure phase. *Cocaine different than saline, $p < .001$; ***Day 1 different than D10 cocaine, $p < .05$; $n = 9$ per group.

can be attributed to an increase in activity between Day 1 to Day 10 in cocaine pre-exposed dams ($p < .05$).

3.1.2. Locomotor activity during test for cocaine sensitization

Approximately 8 weeks after the last pre-exposure, and within a week of weaning (i.e., PND21), all dams were given a test for locomotor sensitization in response to a cocaine challenge. Fig. 2 shows that, in the first 30 min of testing (see data analysis section for rationale for looking at first 30 min), dams pre-exposed to cocaine exhibited a significantly higher level of locomotor activity in response to an acute cocaine challenge relative to dams
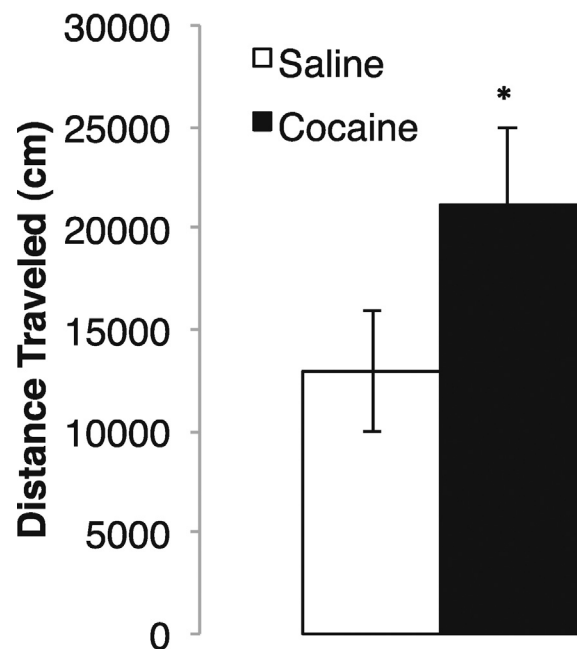

Fig. 2. Locomotor activity during test for cocaine sensitization in dams. Mean ± SEM distance traveled (cm) by dams in the first 30 min following a challenge injection of cocaine (15 mg/kg, i.p.). This test took place approximately 8 weeks after the last day of the pre-exposure phase, and within 1 week of weaning (which occurred on PND21). *Cocaine different than saline, $p < .05$; $n = 9$ per group.
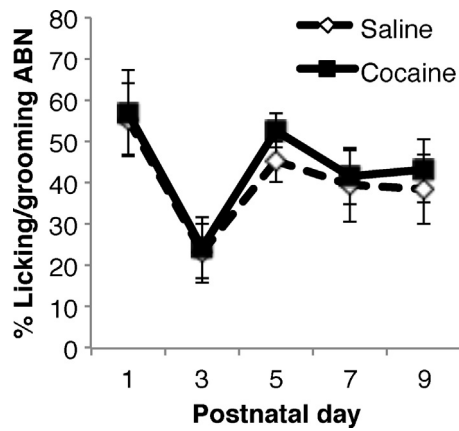
Fig. 3. Maternal behavior in dams. Mean percentage (%) ± SEM of the frequency of licking, grooming and/or arched-back nursing (ABN) on PND 1, 3, 5, 7, or 9; n = 9 per group.

pre-exposed to saline ($p < .05$). Thus, the effects of repeated cocaine exposure persisted over a significant period of time, that encompassed the conception, birth, and weaning of a generation of offspring.

3.1.3. Maternal behavior

Fig. 3 shows the mean (±SEM) percentage of maternal behavior across each 4-h observation period, over five observation days (see Section 2.2.1.3 for details on the calculation of these values). Dam licking and grooming of pup and/or nursing pup in an arched-back posture was measured, based on previous evidence that these behaviors can alter function of the HPA axis and response to stress in offspring [25]. Although there was a main effect of day [$F(4,68) = 3.47$, $p < .05$], there were no effects of maternal history (cocaine, saline).

3.2. Effect of repeated cocaine exposure prior to pregnancy on behavioral, physiological, and genetic responses in male offspring

3.2.1. Acute locomotor response to cocaine in male offspring

Fig. 4 shows the mean (±SEM) distance traveled (cm) by male offspring during the first 10 min of each locomotor test (see data
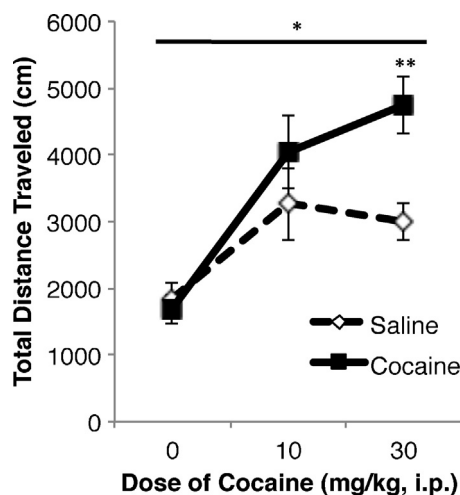
Fig. 4. Acute locomotor response to cocaine in male offspring. Mean ± SEM distance traveled (cm) by offspring in the first 10 min following an acute injection of cocaine (0, 10 and 30 mg/kg, i.p.). *Cocaine different than saline, $p < .05$; **30 mg/kg different than saline, $p < .01$; n = 12 for saline and n = 10 for cocaine.
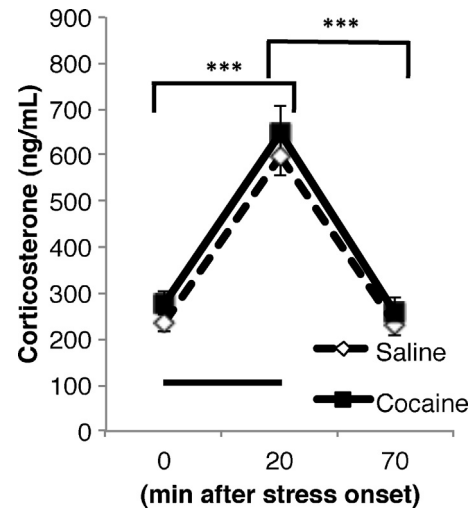
Fig. 5. Immobilization stress-induced CORT responsivity in male offspring. Mean ± SEM corticosterone (ng/mL) in offspring before (Time 0), 20 and 70 min after the onset of immobilization stress. Black line shows the duration of stress. ***Time 20 min different from Times 0 and 70, $p < .001$; n = 11 for saline and n = 12 for cocaine.

analysis section for rationale for looking at first 10 min). There were significant effects of cocaine dose (0, 10, 30 mg/kg, i.p.) [$F(2,40) = 22.82$, $p < .0001$] and maternal history (cocaine, saline) [$F(1,20) = 4.33$, $p = .05$], and a significant interaction of maternal history by dose [$F(2,40) = 3.81$, $p < .05$]. Post hoc tests revealed a significant difference in locomotor activity, with the high dose of cocaine (30 mg/kg, i.p.), between male offspring of cocaine and saline pre-exposed dams ($p < .01$).

3.2.2. Immobilization stress-induced CORT responsivity in male offspring

Fig. 5 shows the mean (±SEM) levels of plasma corticosterone (ng/ml) in the male offspring just prior to the onset of immobilization stress (0 min), just after the termination of the stress (20 min), and again 50 min later (70 min). The ANOVA revealed only a significant main effect of time [$F(2,42) = 62.62$, $p < .0001$]. As expected, there was a significant increase in plasma corticosterone levels between 0 and 20 min ($p < .0001$), and a significant decrease between 20- and 70-min ($p < .0001$). There was, however, no effect of maternal history.

3.2.3. Expression of dopamine receptor and stress-related genes in male offspring

Table 1 shows the mean (±SEM) relative abundance of transcript for each of the genes that were analyzed in several relevant brain regions, including mPFC, NAc, VTA, AMG, HYP and HC. Of the genes and regions assessed, a significant effect of maternal history was found only for the DRD1 transcript in the mPFC. Male offspring of dams that had been pre-exposed to cocaine, as compared to saline, before pregnancy showed a higher relative abundance of the DRD1 transcript in mPFC ($p < .01$).

4. Discussion

The present study yielded four major findings. First, maternal exposure to cocaine prior to pregnancy resulted in an enhanced sensitivity to the psychomotor activating effects of cocaine in the adult male offspring. Second, this enhanced sensitivity to cocaine in the male offspring was associated with a selective upregulation of the DRD1 gene in the mPFC. Third, maternal exposure to cocaine prior to pregnancy had no effect on maternal behavior during lactation, and prior to weaning; thus the positive findings

Table 1
Expression of dopamine receptor and stress-related genes in male offspring.

Gene	Region	Maternal drug treatment	
		Saline	Cocaine
DRD1	mPFC	0.87 ± 0.03	1.03 ± 0.03[a]
	NAC	0.92 ± 0.09	1.04 ± 0.75
	VTA	1.64 ± 0.41	1.39 ± 0.34
DRD2	mPFC	1.07 ± 0.05	1.00 ± 0.05
	NAC	1.07 ± 0.07	1.08 ± 0.10
	VTA	1.24 ± 0.24	1.34 ± 0.12
GR	AMY	1.38 ± 0.10	1.57 ± 0.18
	HYP	0.95 ± 0.05	1.03 ± 0.06
	HC	0.88 ± 0.03	0.92 ± 0.04
CRF	AMY	1.39 ± 0.18	1.49 ± 0.05
	HYP	0.97 ± 0.10	0.89 ± 0.10
	HC	0.88 ± 0.09	1.07 ± 0.11

Mean ± SEM relative abundance of transcripts for dopamine receptor D1 (DRD1) and dopamine receptor D2 (DRD2) in medial prefrontal cortex (mPFC), nucleus accumbens (NAC) and ventral tegmental area (VTA), and for glucocorticoid receptor (GR) and corticotropin releasing factor (CRF) in hypothalamus (HYP), amygdala (AMG), and hippocampus (HC). Gene expression was assessed in male offspring of dams that had been pre-exposed to cocaine or saline before pregnancy.

[a] DRD1 in mPFC greater in cocaine than in saline condition, $p < .01$; $n = 6$ per group.

cannot be attributed to differential rearing of offspring. Finally, maternal exposure to cocaine prior to pregnancy had no effect on stress-induced plasma corticosterone levels in male offspring, or on the expression of GR or CRF genes in key limbic regions of these offspring. Thus, under the conditions assessed in the present study, intergenerational transmission of the effects of maternal cocaine exposure occurred independent of direct exposure of offspring to the drug in utero; moreover, the effects were selective for a specific alteration in the gene expression of cortical dopamine neurons (i.e., upregulation of the DRD1 in mPFC), and a specific behavior that is known to rely on the integrity of the mesocorticolimbic dopamine system (i.e., acute cocaine-induced psychomotor activation).

Our effect of maternal cocaine exposure prior to pregnancy on the acute psychomotor response to cocaine in male offspring is consistent with several recent studies demonstrating similar effects of maternal exposure to cannabinoids or morphine on dopamine-related behaviors in a subsequent generation. For example, female rats exposed to cannabinioids during adolescence, and subsequently mated during adulthood, produced male offspring that expressed enhanced sensitivity to morphine-induced conditioned place preference, relative to controls [26]. Likewise, female rats exposed to morphine during adolescence, and subsequently mated during adulthood, produced offspring that expressed enhanced morphine-induced locomotor sensitization, relative to controls [27]. Of relevance to interpreting the present findings, both drug-induced conditioning of place preferences and drug-induced locomotor sensitization are behaviors that, like acute drug-induced psychomotor activation, are mediated by the meso-corticolimbic system [11].

To the best of our knowledge, transgenerational transmission of cocaine effects on behavior has only been examined in the offspring of sires (as opposed to dams) that had been exposed to cocaine prior to breeding and, moreover, only in sires that had self-administered cocaine (as opposed to been given non-contingent injections of cocaine). More specifically, male rats that had self-administered cocaine for 60 days, and were subsequently mated to drug naïve dams, produced male offspring that in fact acquired cocaine self-administration *more slowly* and consumed *less* cocaine relative to controls [28]. Whereas our finding of *enhanced* sensitivity to the psychomotor activating effects of cocaine in male offspring would seem at odds with what might

be expected based on these self-administration results, our finding is, as discussed, consistent with the previous results based on maternal exposure to morphine and cannabinoids prior to pregnancy. The differences in outcome between our study and that of Vassoler and colleagues [28] may be attributed to several factors, including whether transmission of cocaine effects occurred via dams or sires, the behavioral outcome assessed (psychomotor activation versus drug self-administration), the method of parental cocaine exposure (non-contingent injections versus self-administration), and the stringency of cocaine exposure (7 non-contingent injections versus 60 days of self-administration). Indeed, it is possible that any one or more of these factors would produce differential changes in the circuits mediating the outcomes observed.

Corresponding to an increase in sensitivity to the psychomotor activating effects of cocaine that we observed in male offspring, we found increased mRNA expression of DRD1 in the mPFC of these same offspring. This finding is consistent with several related findings pointing to an important role for cortical DRD1 in long-lasting behavioral effects of cocaine. Most notably, activation of DRD1 receptors in the mPFC has been found to play a critical role in the reinstatement of cocaine seeking in rats (e.g., [9–13]). Likewise, DRD1 in mPFC has been found to play a role in the expression of cocaine sensitization, though in a manner reflecting an inhibitory rather than facilitatory role [14,15]. Thus, although the results suggest a possible role for mPFC DRD1 in the transgenerational effects of maternal cocaine exposure, the extent to which this effect is functionally related to the increased sensitivity to the psychomotor activating effects of cocaine is unknown at this time. However, the DRD1 and corresponding behavioral effect associated with a history of maternal cocaine exposure prior to pregnancy, and thus in the absence of direct exposure of offspring to cocaine during development, suggest a potential role for epigenetic mechanisms in producing changes that are transmissible to a subsequent generation.

Of possible additional relevance to our finding of upregulated DRD1 in mPFC of male offspring is the recent finding of an increase in BDNF mRNA expression in the mPFC of male offspring of sires that had self-administered cocaine; this increased BDNF expression was attributed to epigenetic reprogramming of histone acetylation in the male germline and, moreover, it corresponded to an increased behavioral sensitivity to cocaine [28]. This result is noteworthy, given that dopamine receptors are thought to play an essential role in regulating BDNF expression in cortical regions, and that DRD1 signaling in the mPFC may be especially important in this regard (e.g., [29–31]). In the current study, our rationale for looking at mRNA levels was based on this and additional evidence that behavioral sensitivity to cocaine can be modified via epigenetic mechanisms that alter mRNA levels (e.g., [28,32]). Thus, given that changes in transcript levels are closely associated with these epigenetic mechanisms, we focused on gene regulation at the mRNA level. Future studies, however, should also examine functional changes in protein associated with transcriptional differences in the DRD1 gene.

In the present study, we monitored maternal behavior prior to weaning, in order to determine whether any effects in the next generation could be attributed to the effect of differential rearing based on maternal history. Indeed, maternal behavior (e.g., frequency of licking and grooming) is known to alter stress reactivity in pups via programming of the hypothalamic pituitary adrenal (HPA) axis [25], and these changes are, in turn, related to certain behavioral outcomes, such as increased cocaine-induced psychomotor activity of offspring in adulthood [16]. The lack of change in maternal behavior, either in the frequency of licking or grooming, that we observed in the present study is in fact consistent with the result of a previous experiment, in which a similar lack

of effect of pre-pregnancy cocaine exposure on maternal behavior was observed [33]. Thus, under the conditions of the present study, we can be reasonably assured that the positive effects of maternal history that we observed reflected epigenetic changes brought about by maternal exposure to cocaine prior to pregnancy, and not changes induced by differential rearing of offspring.

A final objective of our study was to determine whether maternal exposure to cocaine prior to pregnancy would alter, in her offspring, either the functionality of the HPA axis or the expression of stress-related genes in key regions of the limbic system. Our rationale for examining these measures was based on considerable evidence that exposure to psychostimulants, including cocaine, alters HPA function and/or stress-related neural circuits. For example, pharmacological manipulations of circulating corticosterone levels are altered by cocaine self-administration in rats [17], and acute cocaine administration induces activation of the HPA axis in rats (e.g., [18,19]). Likewise, repeated exposure to cocaine, under conditions similar to those used in the present study, induces a long-term sensitized locomotor response to an intracranial injection of CRF, and this sensitized response corresponds to increased neuronal activity in the amygdala [20]. Also of possible relevance for the present study, it has been found, both in humans and rodents, that in utero exposure to cocaine alters the functionality of the HPA axis in offspring [34–37]. Although we failed to show any transgenerational alterations in the stress-related markers that we examined, it must be born in mind that we did not carry out an exhaustive study of possible markers and, moreover, that our regimen of maternal cocaine exposure was relatively mild. Thus, the present findings do not allow us to rule out the possibility that maternal exposure to cocaine prior to pregnancy can produce transgenerational effects on the development and functionality of stress systems in the offspring. On the other hand, the present study does allow us to conclude that a relatively mild regimen of maternal cocaine exposure is sufficient to produce transgenerational alterations in a marker of dopamine function (i.e., upregulation of DRD1) and a related and relevant behavior (i.e., cocaine-induced psychomotor activation). Thus, a more extensive characterization of possible transgenerational effects of cocaine exposure (that might include more stringent cocaine exposure regimens) is warranted in future studies.

Acknowledgements

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