Published in final edited form as:

Biol Psychiatry. 2008 January 15; 63(2): 214–221.

Prenatal Exposure to Cocaine Increases the Rewarding Potency of Cocaine and Selective Dopaminergic Agonists in Adult Mice

C.J. Malanga¹, Thorfinn T. Riday, William A. Carlezon Jr.², and Barry E. Kosofsky^{1,3}

¹Laboratory of Molecular and Developmental Neuroscience, Department of Neurology, Massachusetts General Hospital; Boston, MA

²Behavioral Genetics Laboratory, Mailman Research Center, McLean Hospital; Belmont, MA

Abstract

Background—Substance abuse during pregnancy results in persistent affective and behavioral deficits in drug-exposed children, and increased rates of substance abuse have been observed in young adults prenatally exposed to drugs of abuse. Animal models of prenatal cocaine exposure have yielded differing results depending on the behavioral method used to assess drug potency.

Methods—The effects of cocaine, the dopamine D1 agonists SKF-81297 and SKF-82958, and the D2 agonist quinpirole on intracranial self-stimulation were measured in adult Swiss-Webster mice exposed to cocaine *in utero* (40 mg/kg/day) and vehicle controls using the curve-shift method of brain stimulation-reward (BSR) threshold determination.

Results—The reward-potentiating effects of cocaine (0.3–30 mg/kg i.p.) and SKF-82958, but not SKF-81297, on BSR were increased in adult male but not female mice following prenatal cocaine exposure. Quinpirole exerted biphasic effects on BSR, both elevating (0.1–0.3 mg/kg i.p.) and lowering (1.0–10 mg/kg i.p.) reward thresholds. Both effects of quinpirole were also enhanced in adult male mice following prenatal cocaine exposure.

Conclusions—Prenatal cocaine exposure results in increased reward-potentiating potency of cocaine on BSR in adult mice in a sexually-dimorphic manner. This augmented rewarding effect of cocaine is also associated with increased sensitivity to both D1- and D2-selective agonists.

Keywords

In utero; gestation; psychostimulant; intracranial self-stimulation; ICSS; brain stimulation-reward; BSR

INTRODUCTION

Prenatal exposure to drugs of abuse, both legal and illicit, affects over 800,000 infants, or approximately 20% of all live-births, each year in the United States [1]. An estimated 45,000 infants each year are exposed to cocaine at least once during gestation, and the cost in special educational expenses alone for these children is estimated to exceed \$350 million annually

¹Corresponding Author: C.J. Malanga, M.D., Ph.D., Department of Neurology, University of North Carolina at Chapel Hill, 3114 Bioinformatics Bldg., CB 7025, Chapel Hill, NC 27599-7025, Tel: (919) 966-1683, Fax: (919) 843-4576, e-mail: malangacj@neurology.unc.edu.

³Current address: Barry E. Kosofsky, M.D., Ph.D., Department of Pediatrics, Weill Medical College of Cornell University, The New York Presbyterian Hospital, 525 East 68th St., New York, NY 10021, Tel: (212) 746-3278, Fax: (212) 746-8137, e-mail: bar2009@med.cornell.edu

FINANCIAL DISCLOSURES The authors have no conflict of interest, financial or otherwise, related directly or indirectly to the work presented in this manuscript.

[2]. Longitudinal clinical follow-up of cocaine-exposed infants has demonstrated that these children have persistent deficits in multiple developmental domains [3-5], as well as abnormalities of both attention and affect [6-8] suggesting persistent dysfunction of limbic forebrain systems implicated in control of motivation and reward. As addiction is increasingly conceptualized as a developmental disorder, one emerging question is whether drug exposure during vulnerable developmental periods, such as gestation and adolescence, can increase the liability for addictive behaviors later in life. Prenatal exposure to alcohol [9,10] or tobacco [11-13] have been shown to be associated with an increased risk of alcohol or nicotine abuse, respectively, in adolescents and young adults. Similar investigations of the rate of adolescent or adult drug abuse in cocaine-exposed children have not yet been published. While these clinical studies demonstrate associations and not causal mechanisms, they are sufficiently concerning to prompt further translational study.

While many studies of gestational exposure to drugs of abuse have demonstrated changes in spontaneous exploratory behavior in animal models, pre-clinical investigations of alterations in the rewarding potency of drugs of abuse following gestational exposure (reviewed in [14]) are much less common. Operant or instrumental behavioral methods such as drug self-administration [15,16] and intracranial self-stimulation [17,18] are useful in studying the reinforcing effects of drugs of abuse in animal models. Investigations employing operant behavioral measures of reward in adult animals following prenatal exposure to cocaine or other drugs of abuse are comparatively limited; however, increased intravenous self-administration of cocaine following gestational cocaine exposure [19,20] and increased potentiation of rewarding electrical self-stimulation by cocaine following neonatal cocaine exposure [21] have been demonstrated. Conversely, chronic non-contingent cocaine administration to adult animals exposed to cocaine *in utero* results in less locomotor sensitization [22-24] and impaired development of conditioned place-preference [25]. Therefore, an apparent dissociation exists between the effects of prenatal cocaine exposure on the subsequent potency of cocaine in adulthood using operant methods compared to classical methods. We explored this distinction further using a different operant behavioral method, intracranial self-stimulation (ICSS), in a series of experiments investigating the potency of cocaine and selective dopamine receptor agonists to potentiate brain stimulation-reward (BSR) in adult mice that were exposed to cocaine *in utero*. Preliminary results of these studies have been presented in abstract form [26].

MATERIALS AND METHODS

Animal care and handling

All animal procedures were carried out according to the NIH *Guide to the Care and Use of Laboratory Animals*, and were approved by the Subcommittee on Research Animal Care at Massachusetts General Hospital.

In utero cocaine exposure

Mice were exposed to cocaine *in utero* as previously described [27]. Adult timed-pregnant white Swiss-Webster dams (Taconic Labs) were allowed access to food and water *ad libitum* and housed on a 12-hour (7:00AM light–7:00 PM dark) cycle. Two pregnant dams of comparable weight were identified on embryonic day 6 (E6) as an experimental pair: one to receive cocaine (40 mg/kg/day, **COC40**) and one to receive saline (**SAL**). On E7 all pregnant dams were given free access to liquid chow (BioServ, #F1259SP), and consumption was recorded daily from E7 through term (E18-19). All dams were weighed and received twice-daily (7:00 AM and 7:00 PM) injections from E7 until parturition: COC40 dams received 20 mg/kg of cocaine HCl dissolved in sterile normal saline and SAL dams received an equivalent volume of sterile saline. All injections were subcutaneous and injection sites were rotated with

each administration. Our previous studies demonstrated that this cocaine regimen produces maternal serum cocaine concentrations that are similar to those measured in human cocaine addicts [27]. At birth (postnatal day zero, P0) each litter was fostered to an untreated surrogate black Swiss-Webster dam that had given birth within the preceding 24-72 hours. Pups were weaned and group-housed (4 per cage) by gender on P21. For each prenatal treatment group, only one animal of each gender per litter was used for experiments to avoid oversampling bias, or “litter effects” [28].

Intracranial Self-Stimulation (ICSS)

Male and female mice at least P50 or weighing >25g were anesthetized (ketamine/xylazine 120/18 mg/kg i.p.; Sigma) and stereotactically implanted with an insulated monopolar stainless steel electrode (0.1mm diameter, Plastics One) to the right median forebrain bundle in the lateral hypothalamus using coordinates derived from Paxinos and Franklin (1996): bregma -2.0mm (anterior/posterior), sagittal -0.8mm (medial/lateral) and depth -4.5mm (dorsal/ventral) [29]. A stainless steel screw (electrical ground) and the electrode assembly were secured to the skull with dental cement. After recovery animals were allowed access to food and water *ad libitum*.

One week after implantation mice were trained on a continuous (FR-1) schedule of reinforcement for brain stimulation-reward (BSR) in a 16 × 14 × 13 cm operant chamber with a wheel manipulandum and a house light (MedAssociates). Each quarter-turn of the wheel earned a 500 msec train of unipolar cathodal square-wave current at a frequency of 158Hz (pulse width = 100µsec). Each quarter-turn delivered one stimulus train, and activated the house light for 500 msec; subsequent responses during the 500 msec did not earn additional stimulation. Optimal stimulus intensity to sustain reliable responding (≥ 40 responses/min) was determined for animals individually during training, and varied between -90 and -220µA: current intensity was kept constant for each animal for all experiments.

Mice were then presented a series of training stimulus frequencies in descending order from 158Hz to 19Hz in discrete 0.05 log₁₀ increments (i.e., $\log_{10}\{112\text{Hz}\}=2.05$; $\log_{10}\{100\text{Hz}\}=2.00$, etc.). At each frequency, five non-contingent priming stimuli were followed by 50 sec *ad libitum* access to intracranial self-stimulation (ICSS) on an FR-1 schedule during which responses were measured. A 5 sec time-out period followed each trial frequency during which responses earned no additional stimulation. Mice were trained to complete four series of 15 trial frequencies (i.e., one hour daily). After training, the range of frequencies was adjusted for each mouse such that only the highest 4-6 frequencies would sustain responding. For each series of 15 stimulus frequencies, the rate of operant responding for BSR was plotted (i.e., the rate-frequency curve). BSR threshold (θ_0) was defined as the X-intercept of the least-squares regression line through frequencies that sustained responding at 20, 30, 40, 50 and 60% of the maximal response rate in each series, and was calculated automatically by custom-designed software (courtesy of W.A.C.) at the end of each experiment. This method of reward threshold determination is less sensitive to changes in response rate than other calculations, e.g., the frequency sustaining ½ -maximal response, or EF50 [30]. Drug testing began in each animal when its mean BSR threshold varied <10% over three consecutive days.

For each test, three series were acquired before and four after injection with saline vehicle or drug. The first series served as a warm-up and was discarded; θ_0 and maximum rate from the second and third rate-frequency curves were averaged and used as baselines for each animal daily. All drugs were dissolved in sterile normal saline and administered by intraperitoneal injection. BSR thresholds and maximal response rates were measured for four 15-minute series following drug or vehicle injections and expressed as percent changes from baseline. Following initial cocaine dose-response determinations, mice received all doses of the selective dopamine D1-like agonists SKF-81297 and SKF-82958 and the selective dopamine D2-like agonist

quinpirole in random order, and received only one dose of one drug on any day. Our prior work has shown that the BSR-potentiating effects of cocaine and SKF-82958 do not sensitize with repeated administration [31]. Animals were tested with drug and vehicle on alternating days to ensure stability of baseline BSR thresholds.

Histology

After ICSS experiments mice were deeply anesthetized with sodium pentobarbital (120 mg/kg) and perfused intracardially with 4% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS; pH=7.4). Brains were cryoprotected in sucrose (30% in PBS) and frozen in crushed dry ice; 50 μ m coronal sections were stained with cresyl violet for Nissl and photographed under low-power (5X) light microscopy for confirmation of electrode placements.

RESULTS

ICSS electrode tip positions are shown in Figure 1. No significant effects of gender ($F_{(1,44)}=0.05$, $P=0.82$) or prenatal treatment ($F_{(1,44)}=0.11$, $P=0.75$) were observed on baseline brain stimulation-reward (BSR) thresholds (θ_0), determined as the total charge delivery in Coulombs ($\mu\text{A} \times \mu\text{sec}$) at the stabilized baseline threshold prior to saline determinations (Table 1), indicating that prenatal cocaine exposure does not alter responding for BSR, and that ICSS does not differ between males and females.

The effects of cocaine on BSR thresholds (θ_0) are shown in Figures 2 and 3 and on maximal operant response rate in Figure 4. Significant main effects of cocaine dose ($F_{(5,95)}=213.79$, $P<0.001$), time after injection ($F_{(3,95)}=62.08$, $P<0.001$) and gender ($F_{(1,95)}=13.31$, $P<0.001$) were observed. While main effects of prenatal treatment were not evident, there was a significant interaction between cocaine dose and prenatal treatment ($F_{(5,95)}=3.04$, $P=0.010$), indicating a difference in the cocaine dose-response relationship between SAL and COC40 mice; and between dose and gender ($F_{(5,95)}=4.18$, $P<0.001$) indicating a difference in the cocaine dose-response relationship between male and female mice. Significant lowering of θ_0 (Tukey's $P<0.05$) compared to vehicle was observed for 60 minutes following 30 mg/kg cocaine in both SAL and COC40 males; however, following 10 mg/kg cocaine significant changes were observed in the first 15 minutes in SAL males but the entire 60 minute test in COC40 males (Figure 2). COC40 but not SAL males also showed a significant lowering of threshold following 3.0 mg/kg cocaine. Figure 3 demonstrates that in each 15 minute pass after cocaine injection the dose-response curve is shifted to the left in COC40 males relative to SAL males with no apparent change in maximum cocaine effect. No significant differences in the effects of cocaine were observed between prenatal groups in female mice. While main effects of cocaine dose ($F_{(5,95)}=7.87$, $P<0.001$) and time after injection ($F_{(3,95)}=42.58$, $P<0.001$) were observed on maximal response rates, Tukey's post-hoc analyses showed no significant differences from vehicle at any cocaine dose tested within any group (Figure 4).

To determine the pharmacological specificity of the enhanced reward-potentiating effect of cocaine in male COC40 mice, ICSS experiments were performed using the D1-like agonists SKF-81297 and SKF-82958, and the D2-like agonist quinpirole. The effects of SKF-81297 are shown in Figure 5. Significant main effects of dose ($F_{(5,95)}=9.64$, $P<0.001$) and time ($F_{(3,95)}=4.00$, $P<0.01$), but neither prenatal treatment nor gender, nor interactions of prenatal treatment and dose were observed on θ_0 . Neither 0.1 nor 0.3 mg/kg SKF-81297 produced significant changes during the 60 minute test session in any group (data not shown). At doses higher than 0.3 mg/kg the effects of SKF-81297 were highly variable. Simple pairwise comparisons showed significant ($P<0.05$) effects at multiple time points, particularly after 30 minutes post-injection; however, due to variability of responses, post-hoc Tukey's tests showed significant effects of SKF-81297 compared to vehicle only in COC40 males at 10 mg/kg and only between 30 and 45 minutes post-injection (Figure 5). No significant difference in

thresholds were observed in female mice at any time point following injection, and no significant effect of SKF-81297 on maximal response rate was observed in any prenatal treatment group (data not shown).

The effects of SKF-82958 are shown in Figure 6. Significant main effects of dose ($F_{(3,95)}=24.04$, $P<0.001$) and prenatal treatment ($F_{(3,95)}=5.42$, $P=0.02$) but neither gender nor time on θ_0 were observed; significant interactions among the four variables were also not observed. SKF-82958 was the only drug tested that did not show a significant effect of time after injection; data were therefore collapsed across the entire 60 minute post-injection test (Figure 6). In pilot studies from our laboratory, SKF-82958 doses above 1.0 mg/kg resulted in behavioral activation sufficient to damage to the electrode headstage and were therefore not subsequently administered; however, consistent with our previous findings [31], there was no significant effect of SKF-82958 dose on maximal operant response rate (data not shown).

The D2-like agonist quinpirole showed a markedly different response profile than either cocaine or the D1-like agonists on both θ_0 and maximal response rate, as shown in Figures 7 and 8. In males and females of both prenatal treatment groups, lower doses (0.1-0.3 mg/kg) and higher doses at early time points after injection (0-15 min.) decreased the value of BSR, shown in Figure 7 as increases (>100% of baseline) in θ_0 . At higher doses (1.0-10mg/kg), particularly at later time points (16-60 min.), lowering of θ_0 was observed. Significant main effects of dose ($F_{(5,95)}=57.92$, $P<0.001$) and time ($F_{(3,95)}=54.71$, $P<0.001$) and a significant interaction between dose and prenatal treatment ($F_{(5,95)}=5.75$, $P<0.001$) were observed on θ_0 , indicating differences in the quinpirole dose-response relationship between SAL and COC40 mice. COC40 males showed significantly greater potentiation of BSR by quinpirole at doses above 0.3 mg/kg than SAL males. In addition to its biphasic dose-response relationship on θ_0 , quinpirole was the only drug tested that resulted in clear dose-dependent changes in the maximal response rate (Figure 8). Significant main effects of dose ($F_{(5,95)}=121.67$, $P<0.001$), time ($F_{(3,95)}=3.33$, $P<0.02$) and prenatal treatment ($F_{(3,95)}=6.52$, $P<0.02$) on maximal response rates were observed, with COC40 males being the least sensitive of the four groups to the rate-suppressing effects of quinpirole at doses lower than 3.0 mg/kg.

DISCUSSION

Most preclinical investigations of consequences of prenatal exposure to drugs of abuse have employed passive behavioral methods, particularly open-field exploration or locomotor behavior. The relatively fewer studies examining appetitive behavior using either instrumental or classical conditioning methods have shown subtle but reproducible and consistent effects of gestational exposure to drugs of abuse (reviewed in [14]). Most investigations of operant behavior following such exposures have demonstrated increased potency of drug reinforcers [19-21,32,33], while most investigations of classical conditioning have demonstrated decreased association of behavior with specific drug-paired environments [25,34,35]. We sought to clarify this distinction in a well-characterized mouse model of prenatal cocaine exposure using intracranial self-stimulation (ICSS), a robust method of examining the function of neural circuitry mediating motivation and reward in the mammalian brain [17,18,36]. Unlike the other operant behavioral method primarily used to assess the rewarding potency of drugs of abuse, intravenous drug self-administration, ICSS bypasses the peripheral nervous system entirely, and as such the perception of brain stimulation-reward (BSR), and the processing of motor behaviors to obtain it, occur largely in the absence of sensory input [18]. It is therefore thought that ICSS involves a more selectively distributed set of cortical and subcortical brain circuits specific to motivated behavior than other behavioral methods.

We found that the potency of cocaine to lower BSR threshold was greater in male but not female mice exposed to cocaine *in utero*. One of the limitations of ICSS is that each data point,

defined as one response at a given drug dose, is collected over a time interval in which a range of stimulation frequencies is tested, in our case 15 minutes. Therefore, four data points corresponding to four 15-minute epochs are generated after each drug injection. The dose-response curve to cocaine is shifted to the left in COC 40 males compared to SAL males in each 15-minute epoch after acute cocaine administration, indicating an increase in the potency of cocaine to potentiate the value of BSR. That this effect is not due to changes in cocaine pharmacokinetics is supported by 1. an increase in potency at early time points (0-15 minutes) after administration at which impaired metabolism would be unlikely to produce significant changes in effect, as well as later time points; and 2. previous studies showing no effect of prenatal cocaine exposure on the distribution of cocaine and its primary metabolite, benzoylecgonine, in the adult brain [34] or of neonatal cocaine exposure on adult plasma cocaine levels [21]. We conclude from these data that prenatal cocaine exposure results in an increased rewarding potency of cocaine in adult animals measured by lowering of BSR threshold, and that this effect is sexually dimorphic, preferentially affecting cocaine-exposed male offspring. The gender effect we observe is consistent with prior observations that male offspring are typically more affected than females by prenatal cocaine exposure [37], suggesting that drug exposure may affect interactions between sex hormones and early brain development.

It is generally accepted that dopamine release from VTA projections to the limbic forebrain is necessary for the rewarding effects of ICSS [38-41]. Dopamine acts at two classes of receptors, D1-like (D1 and D5) and D2-like (D2, D3 and D4). We explored the pharmacological specificity of the increased potency of cocaine using the D1-like agonists SKF-81297 ($K_i=1.90$ nM at D1 vs. SCH-23390; >1000 nM at D2 vs. nemonapride) and SKF-82958 ($K_i=4.56$ nM at D1; 264 nM at D2 [42]) and the D2-like agonist quinpirole ($K_i=48$ nM at D2 vs. spiperone; >1000 nM at D1 vs. SCH-23390 [43]).

While most investigators agree that BSR threshold is elevated by systemic administration of the selective D1 antagonist SCH-23390, findings are inconsistent among the few studies that have examined the effect of systemically-administered D1 agonists on ICSS. Reports have shown decreased ICSS following SKF-38393 administration in a reward-summation procedure [44] and increased BSR threshold following SKF-81297 administration using a rate-independent method [45]; while others have shown decreased BSR threshold with SKF-38393 [46] and A-77636 [47] using the curve-shift method that we employ. We previously demonstrated in Swiss-Webster mice that systemic administration of SKF-82958 lowers BSR threshold in a dose-dependent manner [31]. The effect size we observe to both D1-like agonists is small, consistent with our previous data and with prior reports in mice. Given the wide variability in effect on ICSS we observe, it is not possible to statistically differentiate prenatally cocaine-exposed mice from controls on the basis of our SKF-81297 data. However, despite a small effect size, cocaine-exposed adult male mice demonstrate greater sensitivity to the threshold-lowering effect of SKF-82958 than controls, while females show no difference in the reward-potentiating effect of this D1-like agonist. We conclude that it is possible that increased sensitivity to D1 receptor-mediated effects may account in part for the increase in cocaine potency we observe in adult male mice following prenatal cocaine exposure.

Most investigators agree that agonists acting at dopamine D2-receptors potentiate the rewarding value of ICSS in rats [46-48]. However, one study [49] demonstrated similar increases in reward threshold using quinpirole at doses in the same range as those at which we also observe elevations in BSR threshold, although that study did not test doses higher than 0.066 mg/kg. Apomorphine, an agonist with similar affinity for D2 and D1 receptors [50,51], also increases BSR threshold at low doses (0.01-0.1 mg/kg) and decreases threshold at higher doses (0.3-1.0 mg/kg) [52]. We observe clear increases in BSR threshold following low doses of quinpirole (0.1-0.3 mg/kg) and decreases in threshold at higher doses (1.0-10 mg/kg). We

conclude from our biphasic dose-response data that it is likely that the D2-selective agonist quinpirole acts to suppress dopamine release from presynaptic terminals at low doses and to increase postsynaptic signaling at higher doses, consistent with the known pharmacology of D2-mediated actions in the nAc [53,54]. We observe enhanced potency of quinpirole to both increase threshold at low doses and decrease threshold at higher doses in cocaine-exposed male but not female mice, and conclude from these data that it is possible that increased D2 receptor sensitivity may also account in part for the increased potency of cocaine we observe in adult male mice following prenatal cocaine exposure.

The efficacies of SKF-82958 and SKF-81297 to stimulate adenylyl cyclase through the D1 receptor are similar [55,56]. However, it has also been shown that SKF-82958 possesses significantly more affinity for D2 receptors than other relatively D1-selective agonists [42]. While the locomotor-stimulating effects of systemically-administered SKF-82958 and SKF-81297 are similar [57-59] the potency of SKF-81297 is significantly less than that of SKF-82958 to produce conditioned place preference in rats [58,60]. Systemically-administered SKF-82958 but not SKF-81297 decreases mesencephalic dopaminergic neuronal firing in the rat, and this inhibition is blocked by D2 (haloperidol, eticlopride) but not D1 (SCH-23390) antagonists [61]. Taken together, these studies suggest that SKF-82958 may be a partial agonist at D2 receptors as well as a full agonist at D1 receptors. It is therefore possible that the increased potency of SKF-82958 to lower ICSS threshold that we observe may reflect adaptations at the D2 receptor or its signal transduction systems rather than changes in D1 signaling in males following gestational cocaine exposure. Investigation of the effects of D1 (e.g., SCH-23390) and D2 antagonists (e.g., eticlopride) on ICSS threshold, which are known to possess greater relative selectivity than available agonists, are currently addressing this question.

One of the most persistent criticisms of the curve-shift ICSS method is that the behavior measured is rate-dependent [17], and therefore changes in reward threshold cannot be reliably determined for drugs that may interfere with motoric performance of the operant response. In this study we demonstrate that administration of a drug that decreases the rate of operant response in a dose-dependent manner, the dopamine D2-selective agonist quinpirole, results in biphasic effects on BSR threshold in the same dose range in which we observe decreased maximum rates of operant response. While quinpirole is known to have different behavioral effects in rats and mice, this dissociation of pharmacological effects on BSR threshold and rate of responding indicates that the two measures are experimentally separable in mice, and supports the validity of rate-dependent ICSS methods.

In summary, convergent preclinical findings from several different lines of behavioral investigation of the rewarding properties of drugs of abuse suggests that early developmental exposure to cocaine increases the rewarding potency of cocaine in operant procedures, such as ICSS and drug self-administration, and decreases the rewarding potency of cocaine in procedures employing non-contingent administration and classical conditioning, such as locomotor sensitization and conditioned place-preference. Our current findings suggest that adaptations in dopamine D2 and possibly D1 receptor signaling may account in part for the changes we observe in the rewarding potency of cocaine in adult male mice following prenatal cocaine exposure. These changes in the function of brain reward circuitry persist into adulthood, and may be relevant to the vulnerability of adults to drug-seeking and drug-consuming behaviors following exposure to drugs of abuse occurring in prenatal life or during other critical developmental periods. We hope that these preclinical findings will facilitate the design of future clinical studies on later liability for drug-abusing behavior, and the development of early therapeutic intervention, in drug-exposed children.

Acknowledgements

The authors are grateful to Dr. Josephine Johns for her helpful comments in the preparation of this manuscript. This work was supported by grants from the National Institute on Drug Abuse DA015429 (CJM); DA012736 (WAC); DA000354 (BEK); DA008648 (BEK), and an award from the William Randolph Hearst Fund for Pre- and Perinatal Research at the Harvard Medical School (CJM).

References

1. National Institute on Drug Abuse. Research Findings. NIDA; Bethesda, MD: 1995. NIDA survey provides first national data on drug use during pregnancy.
2. Lester BM, LaGasse LL, Seifer R. Cocaine exposure and children: The meaning of subtle effects. *Science* 1998;282(5389):633–634. [PubMed: 9841414]
3. Singer LT, Arendt R, Minnes S, Farkas K, Salvator A, Kirchner HL, Kliegman R. Cognitive and motor outcomes of cocaine-exposed infants. *JAMA* 2002;287(15):1952–1960. [PubMed: 11960537]
4. Bandstra ES, Morrow CE, Vogel AL, Fifer RC, Ofir AY, Dausa AT, Xue L, Anthony JC. Longitudinal influence of prenatal cocaine exposure on child language functioning. *Neurotoxicol Teratol* 2002;24(3):297–308. [PubMed: 12009485]
5. Griffith DR, Azuma SC, Chasnoff IJ. Three-year outcome of children exposed prenatally to drugs. *J American Acad Child Psychiatry* 1994;33:20–27.
6. Leech SL, Richardson GA, Goldschmidt L, Day NL. Prenatal substance exposure: Effects on attention and impulsivity of 6-year-olds. *Neurotoxicol Teratol* 1999;21(2):109–118. [PubMed: 10192271]
7. Richardson GA, Conroy ML, Day NL. Prenatal cocaine exposure: Effects on the development of school-age children. *Neurotoxicol Teratol* 1997;18:627–634. [PubMed: 8947939]
8. Jacobson SW, Jacobson JL, Sokol RJ, Martier SS, Chiodo LM. New evidence for neurobehavioral effects of in utero cocaine exposure. *J Pediatrics* 1996;129:581–589.
9. Baer JS, Sampson PD, Barr HM, Connor PD, Streissguth AP. A 21-year longitudinal analysis of the effects of prenatal alcohol exposure on young adult drinking. *Arch Gen Psychiatry* 2003;60(4):377–385. [PubMed: 12695315]
10. Alati R, Al Mamun A, Williams GM, O'Callaghan M, Najman JM, Bor W. In utero alcohol exposure and prediction of alcohol disorders in early adulthood: A birth cohort study. *Arch Gen Psychiatry* 2006;63(9):1009–1016. [PubMed: 16953003]
11. Cornelius MD, Leech SL, Goldschmidt L, Day NL. Prenatal tobacco exposure: Is it a risk factor for early tobacco experimentation? *Nicotine Tob Res* 2000;2(1):45–52. [PubMed: 11072440]
12. Kandel DB, Wu P, Davies M. Maternal smoking during pregnancy and smoking by adolescent daughters. *Am J Public Health* 1994;84(9):1407–1413. [PubMed: 8092363]
13. Al Mamun A, O'Callaghan FV, Alati R, O'Callaghan M, Najman JM, Williams GM, Bor W. Does maternal smoking during pregnancy predict the smoking patterns of young adult offspring? A birth cohort study. *Tob Control* 2006;15(6):452–457. [PubMed: 17130374]
14. Malanga CJ, Kosofsky BE. Does drug abuse beget drug abuse? Behavioral analysis of addiction liability in animal models of prenatal drug exposure. *Brain Res Dev Brain Res* 2003;147(12):47–57.
15. Koob GF, Caine B, Markou A, Pulvirenti L, Weiss F. Role for the mesocortical dopamine system in the motivating effects of cocaine. *NIDA Res Monogr* 1994;145:1–18.
16. Carelli RM, Wightman RM. Functional microcircuitry in the accumbens underlying drug addiction: Insights from real-time signaling during behavior. *Curr Opin Neurobiol* 2004;14(6):763–768. [PubMed: 15582381]
17. Kornetsky C, Bain G. Brain-stimulation reward: A model for the study of the rewarding effects of abused drugs. *NIDA Res Monogr* 1992;124:73–93.
18. Wise RA. Brain reward circuitry: Insights from unsensed incentives. *Neuron* 2002;36(2):229–240. [PubMed: 12383779]
19. Rocha BA, Mead AN, Kosofsky BE. Increased vulnerability to self-administer cocaine in mice prenatally exposed to cocaine. *Psychopharmacology (Berl)* 2002;163(2):221–229. [PubMed: 12202969]
20. Keller RW Jr, LeFevre R, Raucci J, Carlson JN, Glick SD. Enhanced cocaine self-administration in adult rats prenatally exposed to cocaine. *Neurosci Lett* 1996;205(3):153–156. [PubMed: 8852581]

21. Lin D, Kellogg CK. Neonatal exposure to cocaine enhances the reward-potentiating properties of the drug in young adult animals. *Behav Neurosci* 1996;110(4):791–801. [PubMed: 8864269]
22. Byrnes JJ, Pritchard GA, Koff JM, Miller LG. Prenatal cocaine exposure: Decreased sensitization to cocaine and decreased striatal dopamine transporter binding in offspring. *Neuropharmacology* 1993;32(7):721–723. [PubMed: 8361584]
23. Crozatier C, Guerriero RM, Mathieu F, Giros B, Nosten-Bertrand M, Kosofsky BE. Altered cocaine-induced behavioral sensitization in adult mice exposed to cocaine in utero. *Brain Res Dev Brain Res* 2003;147(12):97–105.
24. Guerriero RM, Rajadhyaksha A, Crozatier C, Giros B, Nosten-Bertrand M, Kosofsky BE. Augmented constitutive creb expression in the nucleus accumbens and striatum may contribute to the altered behavioral response to cocaine of adult mice exposed to cocaine in utero. *Dev Neurosci* 2005;27(24):235–248. [PubMed: 16046859]
25. Heyser CJ, Miller JS, Spear NE, Spear LP. Prenatal exposure to cocaine disrupts cocaine-induced conditioned place preference in rats. *Neurotoxicol Teratol* 1992;14(1):57–64. [PubMed: 1593980]
26. Malanga C, Carlezon W, Kosofsky B. Prenatal cocaine exposure results in increased rewarding potency of cocaine in adult male but not female mice. *Soc for Neurosci Abstracts* 2003;28:857.813.
27. Wilkins AS, Genova LM, Posten W, Kosofsky BE. Transplacental cocaine exposure. 1: A rodent model. *Neurotoxicol Teratol* 1998;20(3):215–226. [PubMed: 9638679]
28. Holson RR, Pearce B. Principles and pitfalls in the analysis of prenatal treatment effects in multiparous species. *Neurotoxicol Teratol* 1992;14:221–228. [PubMed: 1635542]
29. Paxinos, GT.; Franklin, KB. *The Mouse Brain in Stereotaxic Coordinates*. 1. Academic Press; San Diego, CA: 1996.
30. Miliareisis E, Rompre PP, Laviolette P, Philippe L, Coulombe D. The curve-shift paradigm in self-stimulation. *Physiol Behav* 1986;37(1):85–91. [PubMed: 3016774]
31. Gilliss B, Malanga CJ, Pieper JO, Carlezon WA Jr. Cocaine and SKF-82958 potentiate brain stimulation reward in Swiss-Webster mice. *Psychopharmacology (Berl)* 2002;163(2):238–248. [PubMed: 12202971]
32. Ramsey NF, Niesink RJ, Van Ree JM. Prenatal exposure to morphine enhances cocaine and heroin self-administration in drug-naïve rats. *Drug Alcohol Depend* 1993;33(1):41–51. [PubMed: 8396529]
33. Hecht GS, Spear NE, Spear LP. Alterations in the reinforcing efficacy of cocaine in adult rats following prenatal exposure to cocaine. *Behav Neurosci* 1998;112(2):410–418. [PubMed: 9588487]
34. Heyser CJ, Rajachandran L, Spear NE, Spear LP. Responsiveness to cocaine challenge in adult rats following prenatal exposure to cocaine. *Psychopharmacology (Berl)* 1994;116(1):45–55. [PubMed: 7862930]
35. Estelles J, Rodriguez-Arias M, Maldonado C, Manzanedo C, Aguilar MA, Minarro J. Prenatal cocaine alters later responses to morphine in adult male mice. *Prog Neuropsychopharmacol Biol Psychiatry* 2006;30(6):1073–1082. [PubMed: 16737762]
36. Olds J, Milner P. Positive reinforcement produced by electrical stimulation of septal area and other regions of rat brain. *J Comp Physiol Psychol* 1954;47(6):419–427. [PubMed: 13233369]
37. Spear LP, Silveri MM, Casale M, Katovic NM, Campbell JO, Douglas LA. Cocaine and development: A retrospective perspective. *Neurotoxicol Teratol* 2002;24(3):321–327. [PubMed: 12009487]
38. Kornetsky C, Duvauchelle C. Dopamine, a common substrate for the rewarding effects of brain stimulation reward, cocaine, and morphine. *NIDA Res Monogr* 1994;145:19:39.
39. Wise RA. Forebrain substrates of reward and motivation. *J Comp Neurol* 2005;493(1):115–121. [PubMed: 16254990]
40. Phillips AG, Blaha CD, Fibiger HC. Neurochemical correlates of brain-stimulation reward measured by ex vivo and in vivo analyses. *Neurosci Biobehav Rev* 1989;13(23):99–104. [PubMed: 2530478]
41. Beninger RJ, Miller R. Dopamine d1-like receptors and reward-related incentive learning. *Neurosci Biobehav Rev* 1998;22(2):335–345. [PubMed: 9579323]
42. Neumeyer JL, Kula NS, Bergman J, Baldessarini RJ. Receptor affinities of dopamine D1 receptor-selective novel phenylbenzazepines. *Eur J Pharmacol* 2003;474(23):137–140. [PubMed: 12921854]
43. Mottola DM, Kilts JD, Lewis MM, Connery HS, Walker QD, Jones SR, Booth RG, Hyslop DK, Piercey M, Wightman RM, Lawler CP, Nichols DE, Mailman RB. Functional selectivity of dopamine

- receptor agonists. I. Selective activation of postsynaptic dopamine D2 receptors linked to adenylate cyclase. *J Pharmacol Exp Ther* 2002;301(3):1166–1178. [PubMed: 12023552]
44. Hunt GE, Atrens DM, Jackson DM. Reward summation and the effects of dopamine D1 and D2 agonists and antagonists on fixed-interval responding for brain stimulation. *Pharmacol Biochem Behav* 1994;48(4):853–862. [PubMed: 7972288]
 45. Baldo BA, Jain K, Veraldi L, Koob GF, Markou A. A dopamine D1 agonist elevates self-stimulation thresholds: Comparison to other dopamine-selective drugs. *Pharmacol Biochem Behav* 1999;62(4):659–672. [PubMed: 10208371]
 46. Nakajima S, O'Regan NB. The effects of dopaminergic agonists and antagonists on the frequency-response function for hypothalamic self-stimulation in the rat. *Pharmacol Biochem Behav* 1991;39(2):465–468. [PubMed: 1682950]
 47. Ranaldi R, Beninger RJ. The effects of systemic and intracerebral injections of D1 and D2 agonists on brain stimulation reward. *Brain Res* 1994;651(12):283–292. [PubMed: 7922577]
 48. Knapp CM, Kornetsky C. Bromocriptine, a D2 receptor agonist, lowers the threshold for rewarding brain stimulation. *Pharmacol Biochem Behav* 1994;49(4):901–904. [PubMed: 7886105]
 49. Hatcher JP, Hagan JJ. The effects of dopamine D3/D2 receptor agonists on intracranial self stimulation in the rat. *Psychopharmacology (Berl)* 1998;140(4):405–410. [PubMed: 9888614]
 50. Andersen PH, Gronvald FC, Jansen JA. A comparison between dopamine-stimulated adenylate cyclase and 3H-SCH 23390 binding in rat striatum. *Life Sci* 1985;37(21):1971–1983. [PubMed: 2415793]
 51. Billard W, Ruperto V, Crosby G, Iorio LC, Barnett A. Characterization of the binding of 3H-SCH 23390, a selective D-1 receptor antagonist ligand, in rat striatum. *Life Sci* 1984;35(18):1885–1893. [PubMed: 6387355]
 52. Fouriez G, Francis S. Apomorphine and electrical self-stimulation of rat brain. *Behav Brain Res* 1992;52(1):73–80. [PubMed: 1472289]
 53. Shen RY, Asdourian D, Chiodo LA. Microiontophoretic studies of the effects of D-1 and D-2 receptor agonists on type I caudate nucleus neurons: Lack of synergistic interaction. *Synapse* 1992;11(4):319–329. [PubMed: 1354398]
 54. White FJ, Wang RY. Electrophysiological evidence for the existence of both D-1 and D-2 dopamine receptors in the rat nucleus accumbens. *J Neurosci* 1986;6(1):274–280. [PubMed: 2868077]
 55. Izenwasser S, Katz JL. Differential efficacies of dopamine D1 receptor agonists for stimulating adenylyl cyclase in squirrel monkey and rat. *Eur J Pharmacol* 1993;246(1):39–44. [PubMed: 8102607]
 56. Undie AS, Weinstock J, Sarau HM, Friedman E. Evidence for a distinct D1-like dopamine receptor that couples to activation of phosphoinositide metabolism in brain. *J Neurochem* 1994;62(5):2045–2048. [PubMed: 7908949]
 57. Desai RI, Terry P, Katz JL. A comparison of the locomotor stimulant effects of D1-like receptor agonists in mice. *Pharmacol Biochem Behav* 2005;81(4):843–848. [PubMed: 16000217]
 58. Abrahams BS, Rutherford JD, Mallet PE, Beninger RJ. Place conditioning with the dopamine D1-like receptor agonist SKF 82958 but not SKF 81297 or SKF 77434. *Eur J Pharmacol* 1998;343(23):111–118. [PubMed: 9570457]
 59. Chausmer AL, Katz JL. Comparison of interactions of D1-like agonists, SKF 81297, SKF 82958 and A-77636, with cocaine: Locomotor activity and drug discrimination studies in rodents. *Psychopharmacology (Berl)* 2002;159(2):145–153. [PubMed: 11862342]
 60. Graham DL, Hoppenot R, Hendryx A, Self DW. Differential ability of D1 and D2 dopamine receptor agonists to induce and modulate expression and reinstatement of cocaine place preference in rats. *Psychopharmacology (Berl)*. 2006Online First 12 July 2006
 61. Ruskin DN, Rawji SS, Walters JR. Effects of full D1 dopamine receptor agonists on firing rates in the globus pallidus and substantia nigra pars compacta in vivo: Tests for D1 receptor selectivity and comparisons to the partial agonist SKF 38393. *J Pharmacol Exp Ther* 1998;286(1):272–281. [PubMed: 9655869]

Figure 1.

Intracranial self-stimulation electrode placements in the medial forebrain bundle of adult Swiss-Webster mice at the level of the lateral hypothalamus. Locations of electrode tips are plotted on templates from the standard mouse stereotaxic atlas [29] by manual inspection of Nissl-stained sections. Note that all electrodes were implanted on the right; tip placements in females are shown on the left for clarity. *Gray Circles* = SAL mice; *Black Circles* = COC40 mice. See Materials and Methods for details.

Figure 2.

Changes in intracranial self-stimulation (ICSS) thresholds (θ_0) following acute administration of cocaine or saline vehicle. Values at each time point are plotted as the mean percentage of the average of 30 minutes of pre-injection reward thresholds $\pm$ S.E.M. Displayed in this manner, values $>100\%$ indicate rightward shifts and values $<100\%$ indicate leftward shifts (i.e., potentiation) of the rate–frequency curve. $n = 7$ SAL males; 13 COC40 males; 12 SAL females; and 12 COC40 females. * = Tukey's $P < 0.05$ vs. saline; † = $P < 0.05$ vs. females. See Materials and Methods and Results for details.

Figure 3.

Dose-response curve for effects of cocaine on intracranial self-stimulation (ICSS) thresholds (θ_0). Each dose-response curve represents one 15-minute post-injection epoch. Values at each time point are plotted as the mean percentage of the average of 30 minutes of pre-injection thresholds $\pm$ S.E.M. $n = 7$ SAL males; 13 COC40 males; 12 SAL females; and 12 COC40 females. * = Tukey's $P < 0.05$ vs. saline; † = $P < 0.05$ vs. females. See Results for details.

Figure 4.

Changes in maximum rate of operant response following acute administration of cocaine or saline vehicle. Values at each time point are plotted as mean percentages of the average of 30 minutes of pre-injection maximum response rates $\pm$ S.E.M.; n values are identical to Figure 2. See Results for details.

Figure 5.

Changes in ICSS thresholds (θ_0) following acute administration of the D1-agonist SKF-81297 or saline vehicle. Values at each time point are plotted as a percentage of the average of 30 minutes of pre-injection reward thresholds $\pm$ S.E.M. Note the difference in y-axis scale between this figure and Figure 2. $n = 4$ SAL males; 7 COC40 males; 8 SAL females; and 8 COC40 females. * = Tukey's $P < 0.05$ vs. saline. See Results for details.

Figure 6.

Changes in ICSS thresholds (θ_0) over 60 minutes following acute administration of the D1-agonist SKF-82958 or saline vehicle. Values at each dose are plotted as a percentage of the average of 30 minutes of pre-injection reward thresholds $\pm$ S.E.M. $n = 7$ SAL males; 11 COC40 males; 11 SAL females; and 11 COC40 females. * = Tukey's $P < 0.05$ vs. saline. See Results for details.

Figure 7.

Changes in ICSS thresholds (θ_0) following acute administration of the D2-agonist quinpirole or saline vehicle. Each dose-response curve represents one 15-minute post-injection epoch. Values at each dose are plotted as a percentage of the average of 30 minutes of pre-injection reward thresholds $\pm$ S.E.M. $n = 7$ SAL males; 12 COC40 males; 11 SAL females; and 10 COC40 females. * = Tukey's $P < 0.05$ vs. saline; † = $P < 0.05$ vs. 0.1 mg/kg; ‡ = $P < 0.05$ vs. 0.3 mg/kg. See Results for details.

Figure 8.

Changes in maximum rate of operant response following acute administration of quinpirole or saline vehicle. Each dose-response curve represents one 15-minute post-injection epoch. Values at each dose are plotted as a percentage of the average of 30 minutes of pre-injection maximum response rates $\pm$ S.E.M.; n values are identical to Figure 9. * = Tukey's $P < 0.05$ vs. saline. See Results for details.

Table 1Mean ICSS Thresholds (θ_0) in Coulombs (C)

	MALE	FEMALE
SAL	$4.18 \pm 1.6 \times 10^{-7}$	$4.39 \pm 1.3 \times 10^{-7}$
COC40	$4.69 \pm 1.3 \times 10^{-7}$	$4.23 \pm 1.2 \times 10^{-7}$

All values expressed as means $\pm$ S.E.M.