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

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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 with the curve-shift method of brain stimulation-reward (BSR) threshold determination.

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

Key Words: Brain stimulation-reward, BSR, gestation, ICSS, in utero, intracranial self-stimulation, psychostimulant

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 (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) has 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. Although these clinical studies demonstrate associations and not causal mechanisms, they are sufficiently concerning to prompt further translational study.

Although many studies of gestational exposure to drugs of abuse have demonstrated changes in spontaneous exploratory behavior in animal models, preclinical investigations of alterations in the rewarding potency of drugs of abuse after 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 (ICSS) (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 after prenatal exposure to cocaine or other drugs of abuse are comparatively limited; however, increased intravenous self-administration of cocaine after gestational cocaine exposure (19, 20) and increased potentiation of rewarding electrical self-stimulation by cocaine after 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 with operant methods compared with classical methods. We explored this distinction further with a different operant behavioral method, 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).

Methods and Materials

Animal Care and Handling

All animal procedures were carried out according to the National Institutes of Health Guide to the Care and Use of

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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, Germantown, New York) were allowed access to food and water ad libitum and housed on a 12-hour (7:00 AM 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/cage) by gender on P21. For each prenatal treatment group, only one animal of each gender/litter was used for experiments to avoid oversampling bias or "litter effects" (28).

ICSS

Male and female mice at least P50 or weighing > 25g were anesthetized (ketamine/xylazine 120/18 mg/kg IP; Sigma, St. Louis, Missouri) and stereotaxically implanted with an insulated monopolar stainless steel electrode (0.1 mm diameter, Plastics One, Roanoke, Virginia) to the right median forebrain bundle in the lateral hypothalamus with coordinates derived from Paxinos and Franklin (1996): bregma −2.0 mm (anterior/posterior), sagital −0.8 mm (medial/lateral), and depth −4.5 mm (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 BSR in a 16 × 14 × 13 cm operant chamber with a wheel manipulandum and a house light (MedAssociates, St. Albans, Vermont). Each one-quarter-turn of the wheel earned a 500-msec train of unipolar cathodal square-wave current at a frequency of 158 Hz (pulse width = 100 μsec). Each one-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 −220 μA: 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 158 Hz to 19 Hz in discrete .05 $\log_{10}$ increments (i.e., $\log_{10}$[112Hz] = 2.05; $\log_{10}$[100Hz] = 2.00, etc.). At each frequency, five non-contingent priming stimuli were followed by 50 sec ad libitum access to BSR 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., 1 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). The 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 WAC) 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 3 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 IP. The BSR thresholds and maximal response rates were measured for four 15-min series after drug or vehicle injections and expressed as percent changes from baseline. After 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 euthanized with sodium pentobarbital (120 mg/kg) and perfused intracardially with 4% paraformaldehyde in 0.1 mol/L phosphate-buffered saline (PBS; pH = 7.4). Brains were cryoprotected in sucrose (30% in PBS) and frozen in crushed dry ice; 50 μm coronal sections were stained with cresyl violet for Nissl and photographed under low-power (5×) light microscopy for confirmation of electrode placements.

Results

The ICSS electrode tip positions are shown in Figure 1. No significant effects of gender [$F(1,44) = .05$, $p = .82$] or prenatal treatment [$F(1,44) = .11$, $p = .75$] were observed on baseline BSR thresholds (θ_0), determined as the total charge delivery in Coulombs (μA × μsec) at the stabilized baseline threshold before 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 [$F(5,95) = 213.79$, $p < .001$], time after injection [$F(3,95) = 62.08$, $p < .001$], and gender [$F(1,95) = 13.31$, $p < .001$] were observed. Although main effects of prenatal treatment were not evident, there was a significant interaction between cocaine dose and prenatal treatment [$F(5,95) = 3.04$, $p = .010$] indicating a difference in the cocaine dose-response relationship between SAL and COC40 mice and between dose and gender [$F(5,95) = 4.18$, $p < .001$], indicating a difference in the cocaine dose-response relationship between