Maternal Cannabis Use Alters Ventral Striatal Dopamine D2 Gene Regulation in the Offspring

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Background: Prenatal cannabis exposure has been linked to addiction vulnerability, but the neurobiology underlying this risk is unknown.

Methods: Striatal dopamine and opioid-related genes were studied in human fetal subjects exposed to cannabis (as well as cigarettes and alcohol). Cannabis-related gene disturbances observed in the human fetus were subsequently characterized with an animal model of prenatal Δ-9-tetrahydrocannabinol (THC) (.15 mg/kg) exposure.

Results: Prenatal cannabis exposure decreased dopamine receptor D2 (*DRD2*) messenger RNA expression in the human ventral striatum (nucleus accumbens [NAc]), a key brain reward region. No significant alterations were observed for the other genes in cannabis-exposed subjects. Maternal cigarette use was associated with reduced NAc prodynorphin messenger RNA expression, and alcohol exposure induced broad alterations primarily in the dorsal striatum of most genes. To explore the mechanisms underlying the cannabis-associated disturbances, we exposed pregnant rats to THC and examined the epigenetic regulation of the NAc *Drd2* gene in their offspring at postnatal day 2, comparable to the human fetal period studied, and in adulthood. Chromatin immunoprecipitation of the adult NAc revealed increased 2meH3K9 repressive mark and decreased 3meH3K4 and RNA polymerase II at the *Drd2* gene locus in the THC-exposed offspring. Decreased *Drd2* expression was accompanied by reduced dopamine D2 receptor (D_2R) binding sites and increased sensitivity to opiate reward in adulthood.

Conclusions: These data suggest that maternal cannabis use alters developmental regulation of mesolimbic D_2R in offspring through epigenetic mechanisms that regulate histone lysine methylation, and the ensuing reduction of D_2R might contribute to addiction vulnerability later in life.

Key Words: Addiction, D1 receptor, development, dynorphin, enkephalin, THC

The prenatal period is sensitive to environmental influences, due to dynamic neurobiological events that occur during this stage to ensure proper patterning of the nervous system. These processes are likely to be disrupted by maternal drug use and could have lifelong consequences for their children. In the United States, approximately 4% of pregnant women report using illegal drugs, with marijuana (*Cannabis sativa*) being the illicit drug most commonly abused during pregnancy (1). Cannabis is abused for its psychoactive properties and is prescribed as an antiemetic to treat nausea during pregnancy with the perception that no negative consequences will befall the developing fetus. However, a growing body of evidence suggests that maternal cannabis use can have long-lasting negative consequences on the cannabis-exposed individual, including increased risk for developing drug addiction and neuropsychiatric disorders (2–4). Animal studies have also demonstrated that developmental exposure to cannabis-mimetic compounds enhances sensitivity to drugs of abuse, including heroin and morphine in adulthood (5–8). Although these studies have been instrumental in establishing a link between prenatal cannabis exposure and addiction risk, little is known with regard to which neurobiological systems are vulnerable to early developmental cannabis exposure and how cannabis-induced alterations are maintained into adulthood to contribute to pathological behavior.

The striatal dopamine system has been implicated in the underlying pathogenesis of neuropsychiatric disorders (9). The dorsal striatum (caudate and putamen) is associated with motor control and habit formation, whereas the ventral striatum (nucleus accumbens [NAc]) is linked to goal-directed behavior and reward processing (10). Diverse populations of medium spiny neurons, which are enriched in cannabinoid receptors, constitute the striatal output pathways and are dissociated on the basis of their expression of dopamine receptors and neuropeptides. Dopamine receptor subtype 1 (D_1R) is preferentially localized to the striatonigral "direct" pathway that contains the opioid neuropeptide dynorphin, whereas dopamine receptor subtype 2 (D_2R) is abundant in striatopallidal "indirect" neurons that also express enkephalin (11). Reduced D_2R is a consistent feature observed in adult drug abusers (12) and has raised questions as to whether D_2R impairments could also predate adult drug use to increase addiction risk. On the basis of the significant number of women who smoke marijuana when pregnant and the preclinical studies emphasizing the long-term impact of prenatal cannabis exposure on addiction-related behavior, we hypothesized that in utero cannabis exposure could contribute to D_2R impairments characteristic of addiction vulnerability.

Here we studied whether maternal cannabis use alters regulation of dopamine receptor D2 (*DRD2*) and related striatal genes in the human fetal brain. Our findings demonstrate that maternal cannabis use decreases NAc *DRD2* gene expression in the fetus. With a Δ-9-tetrahydrocannabinol (THC) prenatal rat model, we show that disruption of NAc *Drd2* gene regulation persists into adulthood and is maintained by epigenetic alterations, which might contribute to increased opiate sensitivity in adulthood.

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Table 1. Demographic Data of the Human Fetal Samples in Cannabis-Exposed and Control Groups

	Control ($n = 25$)	Cannabis-Exposed ($n = 24$)	p
Mother's Age (yrs)	23.84 ± 1.2	22.25 ± .7	.2724
Mother's Education (yrs)	12.12 ± .4	11.87 ± .4	.6712
Mother's Race (White/Black/Hispanic)	2/21/2	0/19/5	—
Fetal Gender (male/female)	13/12	13/11	—
Fetal Age (gestation weeks)	20.36 ± .3	20.21 ± .3	.6961
Postmortem Interval (hours)	9.58 ± .7	7.97 ± .7	.1248
Maternal Cannabis Use (ADJ)	0	1.24 ± .2	<.0001
Maternal Cigarette Use (ADC)	2.16 ± .8	4.04 ± .8	.0983
Maternal Alcohol Use (ADV)	.30 ± .1	.30 ± .1	.9899

Maternal cannabis, cigarette, and alcohol use are represented as average daily joint (ADJ), average daily cigarette (ADC), and average daily volume (ADV), respectively. This metric was calculated by converting weekly use into daily use on the basis of the yearly pattern of use (e.g., seven joints/week equals an ADJ score of .90 [7 joints/week × 4 weeks/month]/[31 days/month]) (40). For alcohol use, 1 drink was equal to 12 oz of beer, 4 oz of wine, or 1.5 oz of liquor. Values are represented as mean ± SEM.

Methods and Materials

Human Fetal Brain Material

Fetal brain specimens (18–22 weeks gestation) were previously collected after saline-induced elective abortions under Institutional Review Board approval at SUNY Downstate Medical Center, Brooklyn, New York (13). Pregnant participants were interviewed to obtain information related to maternal demographic status, substance abuse history, and medical history. Fetal brain samples were examined by a pathologist and lightly fixed with 1% paraformaldehyde and frozen in isopentane. Maternal urine and fetal meconium were analyzed for cannabis, opiates, stimulants, and their respective metabolites to confirm information obtained by maternal self-report. Samples included in the cannabis-exposed group had positive maternal self-report and/or maternal urine that tested positive for THC and/or fetal meconium positive for THC. Maternal alcohol and cigarette use was evaluated on the basis of maternal report of average daily alcohol volume and number of cigarettes smoked daily (14). Subjects had no gross brain malformation. Characteristics of the entire fetal population have been published (13), and a subset from this collection (Table 1) was chosen on the basis of availability of tissue at the level of the NAc that had been cryosectioned in the coronal plane (20 μm-thick), slide-mounted, and stored at −30°C.

Drugs

The THC (10 mg/mL in ethyl alcohol 95%; National Institute on Drug Abuse) was evaporated under nitrogen gas and dissolved in saline with .3% Tween 80 to a concentration of 1.0 mg/mL. Morphine (Sigma-Aldrich, St. Louis, Missouri) was dissolved in saline.

Prenatal THC Rat Model

Adult male (226–250 g) and female (151–175 g) Long Evans rats (Charles River Laboratories) were housed under a 12-hour light/dark cycle. Females received surgical implantation with an IV jugular catheter and were treated postsurgically as previously described (5) with ampicillin (50 mg/kg in heparin 10 U, IV) and carprofen (5 mg/kg, SC) to prevent infection and manage pain, respectively. Animals were pair mated (2 females: 1 male) for 5 days to ensure that each female went through at least one estrous cycle (15). After mating, females were individually housed, and the day of separation was recorded as gestation day 1 (GD1). Females were treated with daily IV injections of either THC (.15 mg/kg) or vehicle (VEH) (.3 % Tween 80-sterile saline solution) from GD5 to postnatal day (PND)2. This treatment period corresponds to the neurodevelopmental period examined in our human fetal population (midgestation, approximately 20 weeks) (16). The dose of THC used in this paradigm is comparable to current estimates of low-dose cannabis cigarettes (approximately 16 mg of THC) (17). On PND2, litters were culled 8–10, and pups were fostered such that all pups were raised by VEH-exposed dams and no dams raised its own offspring. On PND21, pups were weaned and allowed to mature into adulthood. Only males were studied to reduce variability and to be consistent with previous work showing greater alterations in male fetuses with prenatal cannabis exposure (18,19). Two pups/litter were used for different experiments.

Preparation of Rat Tissue Sections for Gene Expression Studies

Adult (PND62) rats were anesthetized with CO_2 and decapitated, and neonates (PND2) were sacrificed with live decapitation. After decapitation, brains were frozen in isopentane and stored at −80°C. Coronal cryosections (20-μm thick) of the striatum were taken, and slide-mounted sections were stored at −30°C.

In Situ Hybridization Histochemistry

In situ hybridization histochemistry was used to study *DRD1*, *DRD2*, proenkephalin (PENK), and prodynorphin (PDYN) in the human fetus (18,19) and *Drd2* (NM_012547; sense probe: GAGAAGGCTTTGCAGACCAC, antisense probe: GGATGGATCAGGGAGAGTGA) in the rat. A detailed in situ hybridization histochemistry procedure has been published previously (20). Briefly, complementary DNA fragments were obtained from total RNA by reverse transcription polymerase chain reaction (PCR). The RNA probes were transcribed in the presence of [^{35}S]-uridine 5′-[α-thio]triphosphate (specific activity 1000–1500 Ci/mmol; New England Nuclear, Boston, Massachusetts). The labeled probe was applied to the brain sections at a concentration of 2×10^3 cpm/mm^2 of the coverslip area. Two adjacent sections/subject were studied at the level of the NAc (anatomical location based on Nissl stain and expression pattern of biochemical marks examined throughout the striatum for each subject). Slides were hybridized overnight at 55°C and apposed to Imaging Plates (Fujifilm, Tokyo, Japan) along with ^{14}C-standards (American Radiolabeled Chemicals, St. Louis, Missouri). Films were developed with FLA-7000 phosphoimaging analyzer (Fujifilm), and images were analyzed (MultiGauge software, Fujifilm). Relative messenger RNA (mRNA) expression levels were measured within the human NAc and putamen (dorsal striatum) on the basis of Human Atlas (21) and the rat NAc (+1.60 mm from Bregma) and caudate-putamen (+1.60 mm) in accordance with reference atlas (22). Values from duplicate brain sections for each subject were averaged and expressed as dpm/mg of tissue by reference to the co-exposed standard.

D2 [^{3}H]Raclopride Binding

Adult rat brain sections were incubated in 2 nmol/L [^{3}H]raclopride (80 Ci/mmol specific activity) in incubation buffer (50 mmol/L Tris, .1% ascorbic acid, 120 nmol/L NACl, 5 mmol/L KCl, 2 mmol/L CaCl2, 1 mmol/L MgCl2, pH 7.4) for 60 min. Nonspecific binding was determined on adjacent brain sections by adding 100 mmol/L unlabeled raclopride in the binding buffer. After incubation, the sections were rinsed in cold incubation buffer, rapid dipped in cold distilled water, and dried. The dried slides were made conductive by