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# Effects of prenatal cocaine and heroin exposure on neuronal dendrite morphogenesis and spatial recognition memory in mice

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## HIGHLIGHTS

- Prenatal cocaine exposure increased cortical dendrite morphogenesis.
- Prenatal heroin exposure decreased cortical dendrite morphogenesis.
- Abnormal recognition was observed after prenatal cocaine or heroin exposure.

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## ABSTRACT

Cocaine and heroin are psychoactive substances frequently used by woman abusers of childbearing age. In this study, we used in utero electroporation labeling technique and novelty recognition models to evaluate the effects of prenatal exposure of mice to cocaine or heroin on the morphological development of cortical neurons and postnatal cognitive functions. Our results showed that prenatal cocaine exposure increased dendrite outgrowth, and prenatal heroin exposure decreased dendrite length and branch number in pyramidal neurons in the somatosensory cortex. Furthermore, although no effects of prenatal cocaine or heroin exposure on novel object recognition were observed, offspring prenatally exposed to cocaine exhibited no exploration preference for objects placed in novel locations, and mice prenatally exposed to heroin showed a reduced tendency of exploration for objects in novel locations. These data demonstrate that maternal cocaine or heroin administration during pregnancy causes morphological alterations in pyramidal neurons in the somatosensory cortex and suggest that prenatal administration of addictive substances may impair short-term spatial memory in adult offspring.



## 1. Introduction

Cocaine and heroin are popular psychoactive substances used by woman abusers of childbearing age. Deficiencies in the central nervous system and long-term dysfunction in intellectual ability, cognition, and social interactions have been reported in infants born to cocaine- or heroin-addicted mothers [19,27,28]. Although cocaine and heroin exert effects via distinct targets in the brain, the active metabolites of both drugs can penetrate the fetal blood–brain barrier and interfere with early neuronal cell development, and, thereby influence postnatal behavior [15,19]. Because women abusers often use multiple drugs, the deficiencies in nervous system development and behavior in offspring frequently reflect the combined impacts of complex exposures. Research on the specific effects of prenatal exposure to a particular drug in animal models can provide important insights into the complex neurodevelopmental alterations and the disrupted behaviors.

Given that drug-induced effects persist long after prenatal exposure [14,27,28], it is likely that maternal drug abuse induces morphological alterations in the fetal brain, which subsequently contributes to postnatal behavioral dysfunction. Previous studies have reported that prenatal exposure to cocaine causes morphological defects in dendrites in specific brain regions and behavioral impairments in the adult mice. In several species, prenatal cocaine exposure has been shown to increase dendritic length in cortical neurons of the medial prefrontal cortex [16,24,25], the anterior cingulate cortex [12,13], entorhinal, and piriform cortices [25]. In contrast, other studies have reported the inhibitory effects of prenatal cocaine exposure on neurite outgrowth in the locus coeruleus [3] and striatum [24]. Together, these studies suggest that cocaine abuse during pregnancy can differentially modify dendrite morphology of neurons in the specific brain regions. Behavioral abnormalities caused by prenatal cocaine exposure have been also reported in various animal models, including dysfunction in attention, emotional reactivity, and recognition tasks [6,22,23,29].

*Abbreviations:* E, embryonic; P, postnatal; ANOVA, analysis of variance; EYFP, enhanced yellow fluorescent protein.

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Although abnormalities in cholinergic innervation-related biochemistry and behaviors have been demonstrated in several studies [8,30,32], the neurobiological alterations and behavioral outcomes induced by prenatal heroin exposure are largely unknown.

In the present study, we used in utero electroporation technique, which allows transfection of plasmid encoding enhanced yellow fluorescent protein (EYFP) and visualization of subtle change in morphogenesis of an individual neuron in developing cortex [21], to examine dendrite arborization patterns of cortical neurons prenatally exposed to cocaine or heroin. We further investigated the potential impacts of cocaine or heroin on novelty recognition memory in prenatally exposed adult mice.

## 2. Materials and methods

### 2.1. Animals and drug administration

ICR mice were provided by SLAC Laboratory Animal Co. Ltd. The day on which a vaginal plug was observed was designated as embryonic day 0 (E0) and maternal body weights were measured at E0.5, E8.5 and E18.5. The day of birth was designated as postnatal day 0 (P0), and offspring body weights were measured at P0 and P62–P65. At E0.5, dams were randomly assigned to three groups according to the prenatal treatment: control, cocaine and heroin group. An injection of 20 mg/kg of cocaine hydrochloride (Qinghai Pharmaceutical Firm, China), 10 mg/kg of heroin (National Laboratory of Narcotics, Beijing, China), or equivalent volume of saline were administered subcutaneously twice daily from E8.5 to E17.5. All animal treatments were strictly in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

### 2.2. In utero electroporation and brain section preparation

Plasmids were transfected using in utero electroporation as reported previously [2,17,21]. In brief, ICR mice at E15.5 of gestation were anesthetized with 10% chloral hydrate (4 ml/kg of body weight). 1–2 μl EYFP plasmid mixed with 2 mg/ml Fast Green (Sigma) were injected by trans-uterus pressure microinjection into the lateral ventricle of embryos. Then, electric pulses generated by the ElectroSquireportator T830 (BTX) were applied to the cerebral wall at 5 repeats of 30 V for 50 ms with an interval of 950 ms. Then, the uterine horns were repositioned in the abdominal cavity to allow the embryos to grow and be delivered naturally. The survival rate after in utero electroporation surgery was about 70%. The offspring at P3 randomly selected without sexual discrimination were put on ice to anesthetize and decapitated. Brains were removed and immediately fixed in 4% formaldehyde solution followed by anhydration with 20% sucrose solution. Brain sections were sliced at 50 μm thickness with a cryostat microtome (Leica).

### 2.3. Image acquisition and morphological analysis

Fixed brain slices were viewed and imaged using a confocal microscope (LSM 510; Carl Zeiss) with a 40× oil objective (Carl Zeiss). For morphological analysis, the z-series stacks of confocal images (5–10 optical sections were collected at 0.5 μm intervals) were traced and analyzed using Neurolucida (MicroBrightField, Inc.) as described in our previous reports [2,17]. Dendritic protrusions ≥10 μm were identified as branches. At least 4 mouse offspring from 3 to 4 dams were included in each group, and 10–28 ($15.9 \pm 4.6$) pyramidal neurons in the somatosensory regions (ranging from bregma 1.35 mm to 0.00 mm) were traced per animal. Total 82 neurons from 4 offspring ($20.5 \pm 6.2$ neurons per animal) in saline group, 66 neurons from 4 offspring ($16.5 \pm 3.1$ neurons per animal) in cocaine group and 122 neurons from 9 offspring ($13.5 \pm 2.7$ neurons per animal) in heroin group were traced.

### 2.4. Object recognition task

At P120, 1–2 male offspring from each litter (total 10 litters) were selected randomly for object recognition and object location recognition tests [2,7]. The mice which climbed on top of the object or did not explore either of objects at the training phase or test phase were excluded.

In object recognition experiment, mice were divided into saline ($n = 20$), cocaine ($n = 12$), and heroin ($n = 13$) groups. The exploration arena was an open-topped box (60 cm × 60 cm × 40 cm; made of gray-painted wood with a floor covered with sawdust) placed in a dimly illuminated room. Object A was a glass cube, and object B was a plastic cylinder. They were cleaned thoroughly between sessions to eliminate olfactory cues. The procedure consisted of a training phase and a preference test phase with one-hour interval. Mice were initially allowed to explore the box for 20 min per day for two days for habitation before training. In the training phase, mice were allowed to explore two of the same copies of object A (A1 and A2) placed in diagonal corners of the arena (10 cm from each adjacent wall). In the test phase, A1 was substituted by another copy A3, and A2 was substituted by object B1 (novel object) placed in the original position. The time for exploring each individual object (nose pointing toward the object at a distance ≤ 1 cm) in the training phase (10 min) and the test phase (5 min) was recorded. Novel object preference index was calculated as $T_{A2}/(T_{A1} + T_{A2}) \times 100$ (%) in training phase or $T_{B1}/(T_{A3} + T_{B1}) \times 100$ (%) in the test phase.

### 2.5. Object location recognition task

In the object location recognition experiment, mice were divided into saline ($n = 15$), cocaine ($n = 13$), and heroin ($n = 10$) groups. The exploration arena was an open-topped box (60 cm × 60 cm × 40 cm; made of wood) with different spatial cues attached on three walls to indicate different directions. The procedure and intervals were similar to those for the object recognition task. In the preference test, object C1 and C2 were substituted by another copy C3 and C4. C3 was placed in the same position as C1 and C4 was placed in the adjacent corner as a novel location. The exploration time was 10 min in training phase and 5 min in test phase. The time for exploring each object in the training phase and test phase was recorded. Novel location preference index was calculated as $T_{C2}/(T_{C1} + T_{C2}) \times 100$ (%) in training phase or $T_{C4}/(T_{C3} + T_{C4}) \times 100$ (%) in test phase.

### 2.6. Statistical analysis

Values were presented as mean ± standard error. Comparisons of multiple groups were performed by one-way analysis of variance (ANOVA) followed by Fisher LSD test. Student's *t*-test was used to measure significance of differences between two groups. Differences with $p < 0.05$ were considered statistically significant.

## 3. Results

### 3.1. The effect of prenatal exposure to heroin on weight of adult offspring

Dams were randomly assigned to one of three pregnant treatment groups: control, cocaine and heroin group ($n = 10$ dams per group). Subcutaneous injections of saline, cocaine (20 mg/kg per dose), or heroin (10 mg/kg per dose) twice daily from E8.5 to E17.5 did not alter the maternal or offspring mortality and dam