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.

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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 subcellular changes 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 ElectrosQuireporterator TB30 (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 anhydrous 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 Neuronlucida (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 ± 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 ± 6.2 neurons per animal) in saline group, 66 neurons from 4 offspring (16.5 ± 3.1 neurons per animal) in cocaine group and 122 neurons from 9 offspring (13.5 ± 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 habituation 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
### Table 1
Effects of prenatal cocaine or heroin exposure on maternal and offspring measures.

<table>
<thead>
<tr>
<th>Maternal measures</th>
<th>Offspring measures</th>
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<tbody>
<tr>
<td></td>
<td></td>
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<tr>
<td>Mother’s weights (g)</td>
<td>Gestation duration (days)</td>
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<tr>
<td>E0.5</td>
<td>Weights gain (E18.5–E8.5)</td>
</tr>
<tr>
<td>Saline</td>
<td>35.7 ± 1.0</td>
</tr>
<tr>
<td>Cocaine</td>
<td>33.5 ± 1.4</td>
</tr>
<tr>
<td>Heroin</td>
<td>35.3 ± 1.0</td>
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All results are presented as mean ± SEM. Embryonic day (E0) was the day of conception. Postnatal day (P0) was the day of birth. Gestational duration was determined from the day of conception to the day of birth. ***p < 0.001, as compared with saline group.

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![Figure 1](image.png)

**Fig. 1.** Effects of prenatal exposure to cocaine or heroin on dendrite morphogenesis of cortical neurons. Mouse embryos were electroporated in utero at E15.5 with the EYFP plasmids, and sections of P3 brain were visualized. (A) Confocal images of EYFP-positive neurons in layer II/III of the somatosensory cortex. Scale bar, 20 μm. (B) Reconstructions of transfected neurons using Neurolucida. Scale bar, 50 μm. (C) Quantification of total dendrite length and branch number of transfected neurons. 82 neurons from 4 prenatal saline-treated mice, 66 neurons from 4 prenatal cocaine-treated mice, 122 neurons from 9 prenatal heroin-treated mice were quantified. Data are mean ± SEM. One-way ANOVA. ***p < 0.001.
abortion rate. Maternal and offspring outcomes induced by gestational exposure to cocaine or heroin are summarized in Table 1. Prenatal exposure to heroin resulted in lower weight of offspring mice at P62–65 (p < 0.001), but there was no significant difference between those prenatally exposed to cocaine and saline. The other parameters, including maternal weight gain from E8.5–18.5, gestational period, and offspring weight at P0, showed no significant differences among the three groups.

3.2. The effect of prenatal exposure to cocaine or heroin on dendrite morphological development of cortical neurons in P3 offspring

To explore the effects of prenatal exposure to cocaine and heroin on dendritic development, in utero electroporation was used to visualize the detailed dendrite morphology in developing cortex. Saline, cocaine, or heroin was given to ICR mice from E8.5 to E17.5, and EVFP plasmids were electroporated into neuron progenitors at ventricular zone of embryonic E15.5 mice. The offspring were assigned to three groups according to the prenatal treatment. The morphogenesis of transfected neurons in somatosensory cortex was evaluated at P3. Morphological analysis of cortical neurons at layer II/III showed that prenatal cocaine exposure markedly promoted dendrite outgrowth, as longer and more branches were observed compared with those prenatally exposed to saline. However, prenatal heroin exposure resulted in simplified dendrite arborization, as indicated by the decreased dendrite number and length (Fig. 1A and B). The quantification analysis indicated that there are significant differences in total branch number (F(2,267) = 84.195, p < 0.001) and branch length (F(2,267) = 95.36, p < 0.001) among saline, cocaine and heroin groups (Fig. 1C). These data demonstrate that prenatal exposure to cocaine and heroin cause abnormality in dendrite morphogenesis, but the effects of cocaine and heroin are different.

3.3. The effect of prenatal exposure to cocaine or heroin on performance of adult offspring in object recognition and object location recognition tasks

To examine the effects of prenatal exposure to addictive drugs on the recognition memory, male adult offspring from mothers received saline, cocaine, or heroin treatment during pregnancy were subjected to novelty recognition tasks. In both object recognition (Fig. 2A) and object location recognition (Fig. 2C) tasks, there were no differences in the total exploration time of objects among three groups (data not shown), and no group of mice showed preference for any identical object in the training phase (Fig. 2B and D).

In the test phase of object recognition task, mouse offspring prenatally exposed to cocaine or heroin showed similar novel object preference to that observed in saline group (Fig. 2B), indicating that prenatal exposure to cocaine or heroin did not significantly affect object discrimination and short-term recognition memory for novel object. The object location recognition task was performed in these mice one week later. In the test phase, the offspring of saline group showed clear preference for the object placed to a novel location (Fig. 2D; F(5,37) = 5.928, p < 0.001). However, offspring prenatally exposed to cocaine exhibited no preference for object placed in novel location, as indicated by decreased preference index (Fig. 2D; F(5,37) = 5.928, p = 0.074). Mice in the heroin group showed a reduced tendency of exploration toward the object moved to a novel location, although they still showed preference for the object at a novel location (Fig. 2D; F(5,37) = 5.928, p = 0.03). These data suggest that prenatal exposure to addictive drugs may impair short-term spatial memory in adult offspring.

4. Discussion

It is well known that maternal cocaine or heroin abuse has long-term effects on health and development of children [19,27]. This underscores the importance of research on specific effects of prenatal cocaine or heroin exposure on neuronal development and behavior patterns of offspring. It has been demonstrated in several studies that prenatal cocaine abuse induces morphological alterations in certain brain regions [12,14,16,24,25], but the effect of heroin is largely unknown. In this study, our results show that
prenatal heroin exposure decreased dendrite length and number of branches of pyramidal neurons in developing cortex of offspring. These structural modifications induced by prenatal heroin exposure may be associated with development defects observed in clinic [27]. In contrast to heroin, we observed that prenatal cocaine exposure resulted in more complex dendrite arborization in somatosensory cortex. These results extend previous findings showing the prenatal cocaine exposure modulates dendrite growth in specific cortical and subcortical brain regions [12,13,16,24,25]. However, using Golgi–Cox method, it has been reported that prenatal cocaine exposure had no significant quantitative effect on dendritic tree in somatosensory cortex [16]. This inconsistency may be due to the distinct methods used. In utero electroporation has been proved to be a powerful technology of labeling and tracing specific type of neurons in certain regions of developing brain [2,17,21], by which very subtle alteration in morphology of individual neurons could be detected. Our data reveal distinct dendrite morphological patterns of cortical neurons in mice prenatally exposed to cocaine and heroin, and thus demonstrate the differential effects of the two drugs.

In previous studies, abnormal cognitive performances were detected after prenatal cocaine exposure using morris water maze, radial arm maze, or recognition tasks [10,20], but the results were inconsistent. Based on rodent’s innate preference for novelty, the novel object and novel object location recognition tests, which are without external stress, reward or punishment, provide more advantages over maze models [1]. Accumulating evidence shows that prenatal cocaine exposure induces alterations of DA release or DA receptors density or sensitivity [5,26], which might cause behavioral dysfunction, including changed interest in exploring the surrounding. Thus, we examined the performance of mouse offspring prenatally exposed to saline, heroin, or cocaine in open field and the total exploration time for objects in the training phase. Our results showed there was no difference in locomotor activity or exploration time for objects among the three groups (data not shown), in addition, there was no difference found in preference for novel object among the three groups. These data suggest that the decreased preference in novel location recognition task may be due to the spatial memory impairment.

As a derivative of morphine, heroin is rapidly metabolized to 6-MAM and morphine in vivo, which has high affinity for mu-opioid receptor. Studies showed that prenatal heroin treatment reduces birth weight in hamsters or human beings [4,9,31]. In the present study, a significant weight reduction was detected in adult mouse offspring prenatally exposed to heroin. This effect may be due to the interaction between heroin and the endogenous opioid system [18], or the neonatal opiate withdrawal symptoms [11], both of which cause the imbalance of energy intake and metabolism, leading to the lower weight gains in development.

5. Conclusion

The present study used in utero electroporation technology and novelty recognition models to evaluate the effects of prenatal cocaine and heroin on cortical dendrite morphogenesis and cognitive functions. Prenatal cocaine exposure increased the dendrite outgrowth of pyramidal neurons in the somatosensory cortex, while prenatal heroin exposure decreased dendrite length and branch number. The results of object location recognition task showed that mice prenatally exposed to cocaine exhibited no preference for the spatially displaced familiar object, and the mice prenatally exposed to heroin showed a tendency of reduced preference as well. Thus, we conclude that maternal administration of cocaine and heroin during pregnancy induces distinct morphological alterations in pyramidal neurons in the developing somatosensory cortex, and causes abnormal performance in object location recognition task in adult offspring.

Authors’ contributions

Ruhui Lu conducted in utero electroporation and behavioral experiments, analyzed data and wrote the manuscript. Hui Long conducted in utero electroporation, designed the experiment, analyzed data, and wrote the manuscript. Xing Liu analyzed data and wrote the manuscript. Lan Ma designed and supervised the experiments and wrote the manuscript.

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