Neurotoxicology and Teratology 47 (2015) 80–88

Contents lists available at ScienceDirect

Neurotoxicology and Teratology

journal homepage: www.elsevier.com/locate/neutera

Effects of prenatal cocaine exposure on early postnatal rodent brain structure and diffusion properties

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ARTICLE INFO

Article history:
Received 19 June 2014
Received in revised form 24 October 2014
Accepted 16 November 2014
Available online 22 November 2014

Keywords:
Prenatal cocaine
Diffusion tensor imaging
Magnetic resonance histology
Development
Rats

ABSTRACT

Prenatal cocaine exposure has been associated with numerous behavioral phenotypes in clinical populations, including impulsivity, reduced attention, alterations in social behaviors, and delayed language and sensory-motor development. Detecting associated changes in brain structure in these populations has proven difficult, and results have been inconclusive and inconsistent. Due to their more controlled designs, animal models may shed light on the neuroanatomical changes caused by prenatal cocaine; however, to maximize clinical relevance, data must be carefully collected using translational methods. The goal of this study was two-fold: (1) to determine if prenatal cocaine alters developmental neuroanatomy using methods that are available to human researchers, specifically structural MRI and diffusion tensor imaging, and (2) to determine the feasibility of rodent in vivo neuroimaging for usage in longitudinal studies of developmental disorders. Cocaine-exposed (prenatal days 1–20, 30 mg/kg/day) rat pups were sedated and imaged live using diffusion tensor imaging and postmortem (fixed) using magnetic resonance histology on postnatal day 14. Volume and diffusion properties in whole brain as well as specific regions of interest were then assessed from the resulting images. Whole brain analyses revealed that cocaine-exposed animals showed no change in whole brain volume. Additionally, we found alterations in fractional anisotropy across regions associated with reward processing and emotional regulation, especially in the thalamus and globus pallidus, as well as sex-dependent effects of cocaine in the right cortex. Reductions in fractional anisotropy were paired with reductions only in axial diffusivity, which preliminarily suggests that the changes observed here may be due to axonal damage, as opposed to reductions in myelination of the affected regions/pathways. Our data indicate that prenatal cocaine may target a number of developing brain structures but does not result in overt changes to brain volumes. These results highlight not only the brain alterations that result from prenatal cocaine but also the advancements in live imaging that allow longitudinal study designs in other models.

1. Introduction

Despite concerted public health efforts, epidemiological rates of prenatal exposure to illicit drugs have remained relatively consistent over the last 5 years (Substance Abuse and Mental Health Services Administration, 2012). While the exact percentage of infants exposed specifically to cocaine is currently unclear (Lambert and Bauer, 2012), prenatal cocaine exposure may reduce motor performance in the early postpartum period (Held et al., 1999) and lower scores on measures of neurobehavioral functioning, attention, and speech and language development (Bandstra et al., 2010; Frank et al., 2001). Clinical investigations of the impact of prenatal cocaine exposure on brain development have shown a reduction in birth weight and head circumference compared to controls (Gouin et al., 2011; Nordstrom-Klee et al., 2002) and alterations in cortical volumes (Grewen et al., 2014). However, replications of such findings are relatively rare, with most studies showing a myriad of results, perhaps due to methodological inconsistencies, small sample sizes, and restricted end points. Importantly, clinical research on prenatal cocaine is often confounded by variation in maternal drug history (i.e., poly-drug abuse, dose, route, and frequency), poor maternal nutrition, and socio-economic factors, all of which likely contribute to variability in results and reduced effect sizes.

Animal models allow for tighter controls as well as more invasive research methods. Unfortunately, like clinical research, results from preclinical studies have proven inconsistent, falling prey to methodological inconsistencies, small sample sizes, and restricted end points.

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http://dx.doi.org/10.1016/j.ntt.2014.11.007
0892-0362/

However, some trends have begun to emerge showing alterations in the development of both the neocortex (He and Lidow, 2004; Jones et al., 1996; Kosofsky et al., 1994; Lidow and Song, 2001a,b; Ren et al., 2004) and hippocampus (Baraban et al., 1999), as well as disruptions in central myelination (Wiggins and Ruiz, 1990) following in utero cocaine exposure.

Much of the preclinical work has relied upon microscopy and traditional slice histology techniques to allow for more detailed analyses, but these approaches limit the translational value of findings since similar methods cannot be employed in living human subjects. Thus, the primary purpose of this study was to explore the impact of full-term prenatal cocaine exposure on a 14-day-old rat neuroanatomy using neuroimaging methods available to clinical researchers. The time point chosen for imaging, postnatal day (PND) 14, was selected due to its approximate similarity to a 6-month-old human infant in many regions of interest (Gerig et al., 2011; Watson et al., 2006), allowing for comparison with the earlier clinical work described above, which examined early childhood. Given the relatively consistent behavioral deficits reported for both human and non-human subjects exposed to cocaine, our study has specifically examined brain regions associated with reward processing, emotional regulation, and motor development that may show alterations in volume and diffusion parameters. However, the nature of this study is purely exploratory.

A secondary goal of this study was to develop methods for live anesthetized developmental neuroimaging in rodents. Here, diffusion tensor imaging (DTI) was employed on live animals, demonstrating that viable data can be obtained from live rodent subjects even at such young ages, with an image resolution and quality that allows for the quantification of within-region organization. An additional set of high-resolution MR histology structural images (3D volumetric measurements) were also produced from postmortem (fixed) tissue, allowing for higher resolution images resistant to changes in diffusion parameters, and thus a more precise quantification of volume. Such methods are more typically used in animal work, providing a reference to previously validated methods.

2. Methods

2.1. Subjects and treatment

Individually housed Sprague–Dawley nulliparous female rats (200 g, Charles River, Raleigh, NC) were kept on a 12:12 reverse light cycle (8:00 AM dark) for 1 week and then mated until conception was noted by the presence of a vaginal plug and sperm in a vaginal smear (gestation day (GD) 0). Following conception, females were randomly assigned to chronic cocaine or untreated groups as they became pregnant. Chronic cocaine-treated dams received twice-daily subcutaneous injections of 15 mg/kg of cocaine hydrochloride (total 30 mg/kg dose calculated as free base, 2 ml total volume, Sigma, St. Louis, MO) dissolved in normal saline at approximately 9:00 AM and 4:00 PM throughout gestation (GD 1–20) and not thereafter. Untreated dams received no injections (neither drug nor vehicle) or food restriction during gestation or during the postpartum period but were weighed daily to control for the effects of handling. Weight gain was measured daily for all animals throughout gestation. Water and chow was available ad libitum for all rat dams. Seven days following conception (GD 7), females were moved to a colony room and individually housed on a regular 12:12 light:dark cycle with lights on at 7:00 AM. This procedure results in the majority of dams delivering in the normal daylight hours (Mayer and Rosenblatt, 1998). PPD 1 was defined as the calendar day during which delivery was completed. Following delivery, litters were culled to 10 pups (5 males, 5 females), and pups were returned to their own biological mothers. On PND14, one male and female sibling pair was selected from each litter for imaging. Littermates underwent the same imaging modality (DTI or MR Histology), and each dam provided pups for only one imaging modality (10 dams provided pups for DTI imaging, while a separate set of 14 dams provided pups for MR Histology). Subjects selected for DTI were transported to the imaging facility for imaging, while subjects selected for MR histology were rapidly perfused via cardiac puncture perfusion with 4% paraformaldehyde in PBS containing 1:100 Prohance (Bracco Diagnostics Inc., Princeton, NJ). Following perfusion, the intact head was placed in PBS with 1:200 Prohance and stored at 4 °C for at least 12 h before imaging. Specific imaging protocols are detailed below.

This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Institutional Animal Care and Use Committee at the University of North Carolina. All efforts were made to minimize suffering throughout the experiment.

2.2. Image acquisition

2.2.1. In vivo diffusion tensor imaging

DTI images were collected from 19 cocaine-exposed (10 males, 9 females) and 23 untreated (13 males, 10 females) animals in the final dataset. A representative subject image from each group can be found in Fig. 1A and B, respectively. Animal respiration and surface body temperature were continuously monitored using a MR compatible small-animal monitoring system SAII 1025 L (SAII Instruments, Inc, Stony Brook, NY 11790, USA). In this study, body surface temperatures were obtained from abdomen region, and maintained at 33 °C (±1 °C) using circulating water heating systems. Respiratory rates were maintained at approximately 30 expirations per min.

For this study, a 3D DTI RARE sequence (Cai et al., 2011) with twin navigator echoes was implemented on a Bruker horizontal bore 9.4 T scanner (BioSpec 9.4/30 USR, Bruker Biospin, Billerica, MA, USA). A rat head phase array surface coil (Bruker, Billerica, MA, USA) was used for acquiring the images. The acquisition parameters were as follows: TR = 700 ms; the first RARE echo was assigned to the k-space center, effective TE = 23.662 ms; RARE echo spacing = 11.9 ms. Six non-collinear diffusion encoding directions with $b = 1000$ s/mm^2 images and one baseline reference $b = 0$ image were acquired for the in vivo DTI scans. The total scan time was 3 h 11 min per subject.

Other acquisition parameters were diffusion gradient duration $\delta =$ 6.5 ms, diffusion gradient separation $n\Delta = 12.72$ ms, field of view (FOV) = 27 mm × 19.2 mm × 11 mm, matrix size = 180 × 128 × 55, the resolution = 0.15 mm × 0.15 mm × 0.2 mm, readout direction: H–F, phase encoding direction: L–R, slab encoding direction: A–P. The final data were interpolated to the final matrix size 360 × 256 × 128 to achieve a nominal spatial resolution around 0.075 mm × 0.075 mm × 0.1 mm.

2.2.2. Fixed brain MR histology

MR histology was acquired for 10 cocaine-exposed and 10 untreated subject heads (5 males and 5 females each) on PND14 at the Duke University Center for in Vivo Microscopy (an NIBIB Biomedical Technology Resource), using a 9.4 T superconducting magnet equipped with 200 G/cm resonance research gradient coils (BFG-73/45-100) and controlled with a General Electric Signa console (GE Medical Systems, Milwaukee, WI, USA). Representative subject images for both cocaine and untreated animals can be found in Fig. 1 C and D, respectively. Prior to imaging, subject heads were placed in custom-made, MRI-compatible tubes and immersed in Fomblin liquid fluorocarbon for susceptibility matching and to prevent tissue dehydration. All imaging experiments were performed with the intact brain in the neurocranium to preserve tissue integrity and spatial relationships. RF excitation and reception were accomplished using 21 mm Birdcage coil (m2m Imaging Corporation, Cleveland, OH), with 3D RF refocused spin echo sequence (TR = 50 ms and TE = 6.2 ms) as in (Johnson et al., 2007). The data were fully sampled in Fourier space with an asymmetric acquisition matrix of 768 (frequency) × 384 (phase) × 384 (phase), zero filled to