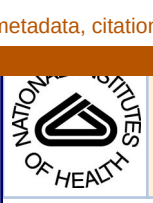

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Effects of prenatal cocaine exposure on early postnatal rodent brain structure and diffusion properties

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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) 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, 30mg/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 palladus, 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.

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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.

Keywords

Prenatal Cocaine; Diffusion Tensor Imaging; Magnetic Resonance Histology; Development; Rats

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 endpoints. Importantly, clinical research on prenatal cocaine is often confounded by variation in maternal drug history (ie. 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. 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, 2001b; 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 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 six 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 grams, Charles River, Raleigh, NC) were kept on a 12:12 reverse light cycle (8:00 AM dark) for one 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, 2ml 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 male, 5 female) and pups were returned to their own biological mothers. On PND14, one male and female sibling pair was selected from each litter for imaging. Litter-mates 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 Figure 1 A and B, respectively. Animal respiration and surface body temperature were continuously monitored using a MR compatible small-animal monitoring system SAII 1025L (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: 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 3h 11min per subject.

Other acquisition parameters were diffusion gradient duration δ = 6.5 ms, diffusion gradient separation nΔ=12.72ms, field-of-view (FOV) = 27mm × 19.2mm × 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 9.4T 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 Figure 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 21mm Birdcage coil (m2m Imaging Corporation, Cleveland, OH), with 3D RF refocused spin echo sequence (TR = 50ms and TE = 6.2ms) 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 1024 × 512 × 512, over a 40 × 20 ×

20 mm^3 field of view, and yielding an isotropic resolution of 39μm^3. Approximate scan time was 2.5h per specimen.

2.3. Image Processing

All data were processed using an in-house processing pipeline (Budin et al., 2013). Images were first rigidly registered to an external template image, in this case the Developmental Rat Atlas (Rumple et al., 2013) generated specifically for this study, which is based on high resolution postmortem images of subjects closely matched in all aspects to the subject population used here. Rigid registration, as opposed to affine or deformable registration methods, was used at this stage to keep the size of the brain constant for the computation of volume and shape statistics. Once subject images were rigidly registered, skull-stripping was completed using previously published automated methods (Oguz et al., 2011) followed by manual fine-tuning. All the skull-stripped images were histogram-matched and affinely registered to the first-case image, resulting in the first average image.

To minimize bias based in the ordering of the data, a second average was computed by affinely registering all the images to the first average obtained. An unbiased atlas was calculated using a greedy fluid-based registration algorithm performing diffeomorphic mapping (Joshi et al., 2004). In addition to the average image, this step generated deformation fields from each individual image in the population to that average image. Segmentation of the population average was obtained by registering the Developmental Rat Atlas to the population average via symmetric diffeomorphic image registration with cross-correlation (Avants et al., 2008). All DTI registrations were completed using the mean diffusivity (MD) images as the input, and employed the unbiased atlas building algorithm via greedy fluid flow registration (Joshi et al., 2004), implemented in the publicly available AtlasWerks package. MR histology registration used the symmetric diffeomorphic atlas building algorithm (Avants et al., 2008) implemented in the publicly available ANTS package.

The obtained transformation was then applied to the Developmental Rat Atlas (Rumple et al., 2013) in order to obtain the population average segmentation. Slices from the final average images are shown in Figure 2. The segmentation of the population average was then propagated into each individual subject in the population using the inverse deformation fields obtained during individual subject fluid registration. Each subject's final segmentation was visually checked by our anatomical expert for quality control. Region-based statistics were calculated for each subject and included volumes, means and standard deviations of the intensity in the segmented regions or over the whole mask. Regions of interest (ROIs) included Hippocampus, Cerebellum, Olfactory Bulb, External Capsule and Corpus Callosum, Hypothalamus, Brainstem, Thalamus, Cingulum, Ventricles, Striatum, Internal Capsule, Anterior Commissure, Globus Pallidus, Fimbria, Fornix, Midbrain, Colliculus, Left Cortex, Right Cortex, and Ventral Forebrain. Additionally, relevant regions were combined to form two composite ROIs, associated with Reward Processing and Emotional Regulation. ROIs associated with Reward Processing included Ventral Midbrain, Striatum, Globus Pallidus, and Thalamus. ROIs associated with Emotional Regulation included

Hypothalamus, Hippocampus, and Amygdala. Aside from ROI analysis, whole brain volume was also computed from both the DTI and MR histology datasets.

2.4. Statistical Analysis

Gestational measures were analyzed using t-tests, and pup measures were analyzed using 2-factor analysis of variance (ANOVA), which included [treatment] and [sex]. Statistical analyses of imaging data were conducted using up to five linear mixed models in which the pup weight and litter identification were included as covariates. All analyses initially included [treatment], [sex] and [treatment x sex] interactions, but [sex] and the [treatment x sex] interaction were eliminated if not significant, and sex was included as a covariate in our analyses of [treatment] effects. This was done to reduce the number of comparisons. The first two mixed models examined ROI volumes and fractional anisotropy (FA). If FA was found to be statistically different in any given region, this difference was further explored by subsequent mixed model analyses of MD, radial diffusivity (RD), and axial diffusivity (AD). Alpha levels were set at 0.05 and all p values were corrected for multiple comparisons using the false discovery rate (FDR) method. Means and standard errors are presented in figures, with significance denoted only for comparisons that survived the correction for multiple comparisons.

3. Results

3.1. Gestational Data

There was no difference in dam weights prior to breeding, and there was no difference in gestational duration or number of pups per litter. Gestational weight gain did not statistically differ between treatment groups in the MR histology study; however, cocaine-treated dams in the DTI study gained less weight over the gestational period ($t=-3.74$, $p<0.001$; Figure 3A), and thus weighed less than untreated dams on postpartum day 1 following delivery ($t=-2.86$, $p=0.008$). Culled litter weight at birth did not differ between groups or study. Additionally, individual pup body weight did not differ between groups or sexes on postnatal day 14 prior to imaging or perfusion. While not statistically significant, average body weights from pups used in the MR histology study were slightly higher than those used in the DTI study (Figure 3B).

3.2. Whole Brain

Whole brain volume was measured using both MR histology and DTI in separate subjects and is presented in Figure 1. Average whole brain volume was slightly higher in subjects that underwent MR histology than those who underwent DTI. This difference is reflective of slightly higher subject weights in the MR histology group. However, within imaging modality, there were no treatment or sex differences in whole brain volumes or the volume of whole brain grey or white matter (Figure 3C).

3.3. ROI Analysis

In the DTI images, no ROIs were found to have significant differences in volume due to sex, treatment, or their interaction. Despite this, cocaine exposure was associated with reductions in FA (see Figure 4A) in the Hypothalamus ($p\leq0.03$), Thalamus ($p\leq0.002$), Striatum

(p≤0.01), Internal Capsule (p≤0.05), Anterior Commissure (p≤0.004), Globus Pallidus (p≤0.001), Midbrain (p≤0.04), and Ventral Forebrain (p≤0.03). Furthermore, FA increases were seen in the Cingulum following prenatal cocaine-exposure (p≤0.02). Many of the reductions seen in FA were paired with reductions in AD (Figure 4B), but no differences in MD or RD. Specifically, reductions in AD were seen in the Thalamus (p≤0.01), Striatum (p≤0.01), Anterior Commissure (p≤0.05), Globus Pallidus (p≤0.02), and Midbrain (p≤0.04). However, after correction for multiple comparisons, only the reductions in FA of the Thalamus (corrected p=0.04) and Globus Pallidus (corrected p=0.03) remained, and no effects in AD remained. In addition to these treatment effects, there was a significant interaction between sex and treatment in the FA of the Right Cortex [$F(1,28)=4.17$, $p=0.04$], with post-hocs showing that cocaine-exposed females had lower FA values than untreated females (p≤0.01; Figure 5).

In the MR Histology images, no ROIs were found to have differences in volume due to cocaine exposure. However, in these images, females were found to have larger volumes than males in the Hippocampus (p≤0.03) and External Capsule/Corpus Callosum (p≤0.04), but these effects did not survive correction for multiple comparisons. There were no significant treatment by sex interactions in the MR Histology images.

3.4. Reward Processing and Emotional Regulation Circuits

Aside from differences in specific ROIs, specific circuits were also explored by combining across member ROIs. Specifically, Ventral Midbrain, Striatum, Globus Pallidus, and Thalamus were combined within animal to create a reward processing circuit, and Hypothalamus, Hippocampus, and Amygdala were combined within animal to create an emotional regulation circuit. These circuits were only explored in the DTI images. Like the specific ROIs, the volume of these circuits did not differ due to cocaine-exposure nor sex; however, cocaine-exposure was associated with reduced FA in both of these circuits (reward: p≤0.01; emotion: p≤0.03; Figure 6A). Additionally, the deficit in FA seen in these circuits was paired with a deficit in AD (reward: p≤0.01; emotion: p≤0.04; Figure 6B). After the correction for multiple comparisons, only the treatment effects described for the reward circuit remained (FA: corrected p=0.03; AD: corrected p=0.04).

4. Discussion

Here, we examined both the impact of prenatal cocaine exposure on early neonatal brain development using structural MRI and diffusion tensor imaging in both live and postmortem subjects, and demonstrated the feasibility of doing in vivo studies in developmental rodents, highlighting the potential for longitudinal studies. With respect to prenatal cocaine's effects, we found no volumetric reductions in regions of interest following prenatal cocaine exposure, but changes in diffusion parameters of live subjects in a number of brain regions. The regions affected are associated with a wide variety of behaviors, but particularly reward processing, suggesting that prenatal cocaine may alter such function. In their review of the neuroanatomical effects of cocaine exposure (Derauf et al., 2009), Derauf and colleagues postulated that a similar pattern of effects would exist. Here we provide evidence supporting this theory, which are in line with previous reports of behavioral deficits following cocaine, including social reinforcement (Johns and Noonan, 1995; Johns et al., 1998; Overstreet et

al., 2000), drug seeking (Malanga et al., 2007), reward sensitivity (Estelles et al., 2006; Malanga et al., 2008), and impulsivity (Liu et al., 2013). The specific neuroanatomical changes in these regions that result in these behavioral alterations bears further investigation. Thus, further histological investigations will be necessary to confirm and expand upon the results seen here; however, our data provide at least preliminary evidence that observable changes exist in these regions.

Volumes were measured in both the postmortem fixed brain MR histology and in the live anesthetized DTI images. These two imaging modalities provided us with a number of contrasts, resolutions, and image qualities, allowing us to measure volume in a number of manners. It is encouraging that relative volume measurements were consistent across imaging modalities; however, the lack of volumetric differences between treatment groups is surprising. Recent structural analyses of human infants (Grewen et al, 2014) found relatively profuse differences in cortical volumes following specifically prenatal cocaine exposure, especially in frontal and prefrontal cortex, but no effects in the volume of subcortical regions. Here we report an opposite pattern of effects, with minimal cortical differences and robust subcortical differences (in diffusion, not volume). These differences may be due to a number of factors, including both methodological and biological. Our methods were not able to parse subregions of the cortex, and perhaps the treatment of the cortex as a single ROI masked local effects in some subregions. Additionally, the focus of human work tends to be more cortical, and rarely are subcortical regions examined in such detail as we examine them here. Thus, the human studies may not have been able to accurately resolve the affected subcortical regions. Aside from methodological considerations, the different pattern of effects observed in these two studies may reflect differences in species-specific development. Despite our best effort to match the time point we selected for imaging with the time point used in the Grewen study and others, it is highly likely that developmental differences contribute to the different profile of effects. Further studies of regional connectivity may shed light on how to better match developmental age, and whether or not volumetric deficits exist in similar pathways.

It is important to note that the automated segmentation used here indirectly enforces some regularization between imaging modalities, which should therefore be resistant to image intensity differences. However, such resistance would depend on the amount and location of any intensity abnormalities, as well as presence and extent of any imaging artifacts. Manual segmentation of the images might allow experts to successfully account for boundaries where the automated methods failed, thus providing a more accurate segmentation. However, manual segmentation would also introduce its own set of confounds and bias. Since manual segmentation is entirely reliant on the contrast of the boundaries between regions, and not their spatial relationship with other regions, manual segmentation may be even less resistant to boundary “blurring” than automated methods. Regardless of the cause, poor boundary clarity would likely result in an increase in the variance of the affected region. Thus, the lack of volumetric differences found in our images does not negate differences seen in other studies or definitively demonstrate that no differences exist. Ideally, DTI images and MR Histology would have been collected form the same subjects, allowing a direct comparison between imaging modalities to be made. However, such a study design would have meant euthanizing our subjects immediately following imaging,

which would have prohibited our investigation of pup viability following live DTI imaging. Despite this necessary design limitation, the consistency in our findings across imaging modalities would suggest that volumetric differences either do not exist or that their detection requires much higher resolution than was used by our studies. Others have reported on the comparability of various imaging methods, and found relatively high correspondence of results (Bedea et at., 2012)

While our data show no changes in the ROI volume, we do show changes in diffusion parameters, the majority of which are reductions in FA and AD. Reductions in FA can be caused by alterations in AD, RD, or both. Increases in RD are typically associated with demyelination (Song et al., 2005), while decreases in AD may be related to axonal damage (Budde et al., 2009). Here, we show decreases in AD in many regions and no significant changes in RD. Thus, the combined reductions in both FA and AD reported here suggest that alterations in myelination are unlikely, and that instead, the changes seen in FA may result from some level of axonal damage. This speculative theory clearly bears further investigation using traditional histological techniques, since at this point MRI cannot definitively address this research topic. Thus, the findings reported here are preliminary in nature, and require additional follow-up studies.

Aside from the effects of prenatal cocaine exposure, we were also able to examine sex differences at this important early time point. In general, there were no sex differences in regional volume or organization, and only one instance of a treatment x sex interaction (entire Right Cortex). This is in contrast with a number of studies completed in humans at similar developmental ages, which showed larger callosum volumes in males (De Bellis et al., 2001; Sowell et al., 2002). This likely reflects a key difference between the species in the development of these regions and represents an important step towards an understanding of male and female differences in brain anatomy, which has recently gained considerable attention from NIH and others (Clayton and Collins, 2014).

Our approach is not without its limitations, and is thus very preliminary in nature. For example, many effects reported here are in large areas of the brain that lacked the contrast necessary to separate specific subregions (for example the Thalamus). Thus, we merged across regions in these instances, even though diffusion may not have been uniform. The differences found in these combined regions are likely driven by significant changes in only a few subregions, as opposed to the entire larger region. This is especially important when considering regions containing both white matter and grey matter subregions, such as the hypothalamus. Furthermore, while our decision to use only 6 directions in our DTI study was pivotal to our ability to reduce scan time to as little as possible (thus promoting pup viability), it also prohibited our ability to perform tractography studies and parse highly complex regions containing multiple crossing fibers. Future studies should attempt longer scans with more directions, to improve both our understanding of development and how it is impacted by cocaine.

In addition to the image acquisition and analysis shortcomings described above, one other limitation of our study was that all subjects were housed with their biological mothers. Prior work from our lab has shown this to be a less than optimal rearing environment (Johns et al.,

2005; Nelson et al., 1998), and thus it is possible that the results presented here reflect poorer maternal care by cocaine-treated dams, as well as the direct effects of prenatal cocaine exposure on the pups. Usage of cross-fostering models may allow follow-up studies to circumvent these potential effects. Lastly, our usage of an untreated group may not be an effective control for the non-drug effects experienced by the cocaine group, such as malnutrition and fetal stress. Future studies should explore how fetal stress alone can impact brain development.

Aside from our findings regarding prenatal cocaine's effects, here we were also able to demonstrate the practical feasibility of in vivo developmental DTI studies. This is an important advancement in DTI methods, which will allow us to perform longitudinal investigations of not only cocaine's effects, but also of other manipulations, including animal models of psychiatric disease. Once our imaging was completed, pups were returned to their biological mothers with no apparent ill effects. While we did not observe any overt differences in maternal care after returning pups to the litter, without specific testing for maternal behavior over an extended period, our data cannot speak to the presence of subtle differences in care. Despite this, and considering the dire need for tools to assess neurobiological development longitudinally, the 3 hour imaging window used here provides a good compromise between the need to collect such data and the need to maintain pup viability for longitudinal study. An extension of this imaging time may allow for higher resolution images or more directions, but may sacrifice pup viability. At this age (PND 14), pups are generally less dependent on dams for care than at earlier ages, so caution should be used when imaging at earlier developmental ages.

The results presented here represent an important advancement in both our understanding of cocaine's effects, but also demonstrate methods to collect viable neuroanatomical data from very young living subjects. Non-survival imaging methods currently dominate the field; however, the advancements in live imaging methods shown here and elsewhere (Cai et al., 2011; Harsan et al., 2013; Wu et al., 2013) allow for examination of development across the lifespan of a single subject. This incredibly powerful approach will improve our understanding of psychiatric diseases, many of which are developmental in nature (Bale et al., 2010). For example, the comprehensive database of regional effects presented here suggests new targets to explore, as well as potential mechanisms (ie. axonal damage/reduced axon density in the case of cocaine). Future studies of cocaine exposure and other developmental disorders should continue to leverage these live imaging techniques to improve the translational value of findings.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Highlights

- Translational studies of the neuroanatomical effects of prenatal cocaine in rats are lacking
- We investigated cocaine's effects using MR Histology and Diffusion Tensor imaging
- Prenatal cocaine did not alter the volume of any region examined
- Prenatal cocaine changed diffusion parameters in reward and emotion circuits
- Results highlight cocaine's effects and the feasibility of live developmental DTI

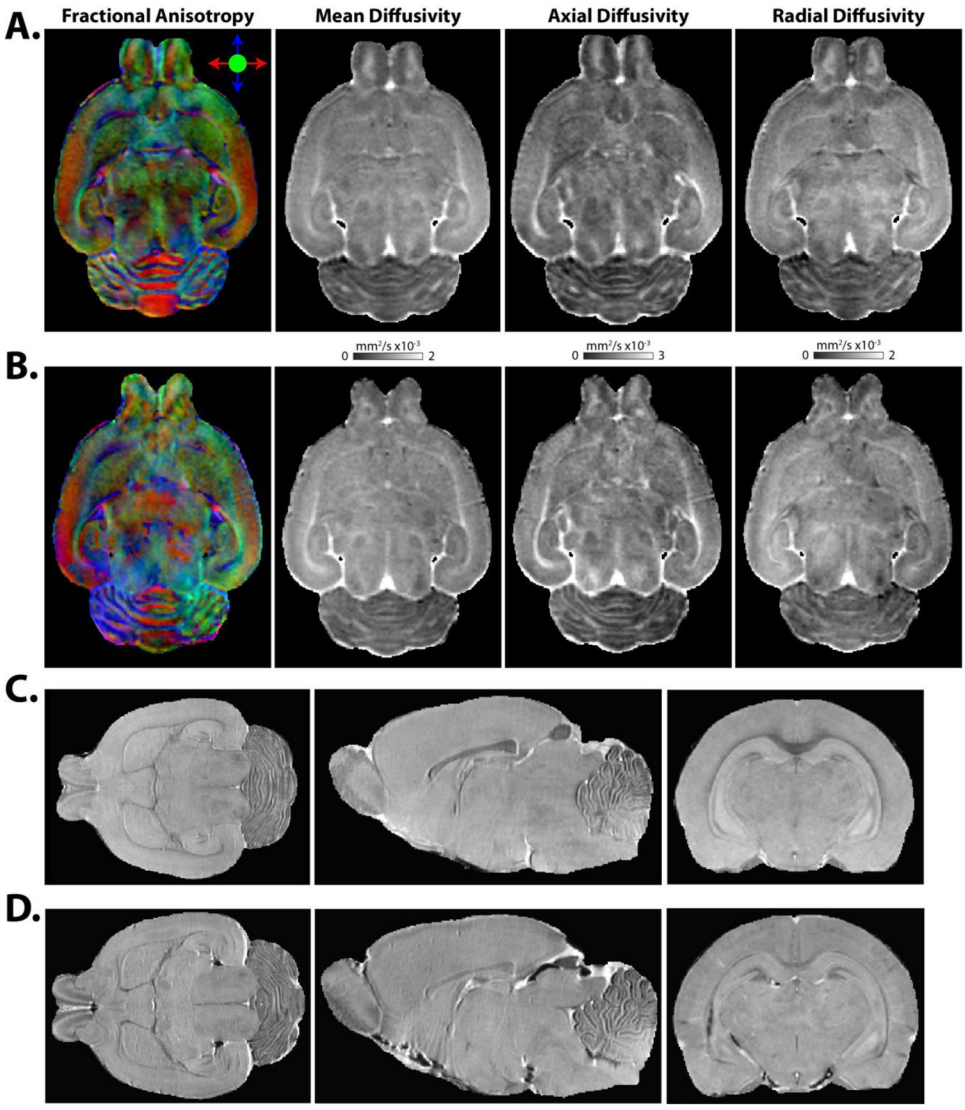

Figure 1.

Representative horizontal slices depicting Color-coded Fractional Anisotropy (FA), Mean Diffusivity (MD), Axial Diffusivity (AD), and Radial Diffusivity (RD) from a single male prenatal cocaine-exposed pup (A) and a single male untreated pup (B). Also included are representative MR Histology images in axial, sagittal, and coronal slices from a single female prenatal cocaine-exposed pup (C) and a single female untreated pup (D). As indicated in the top left panel (A), pixel color in FA parametric maps are representative of both the strength and the direction of diffusion. Red intensities indicate medial-lateral flow, while blue indicates dorsal-ventral, and green indicated anterior-posterior.

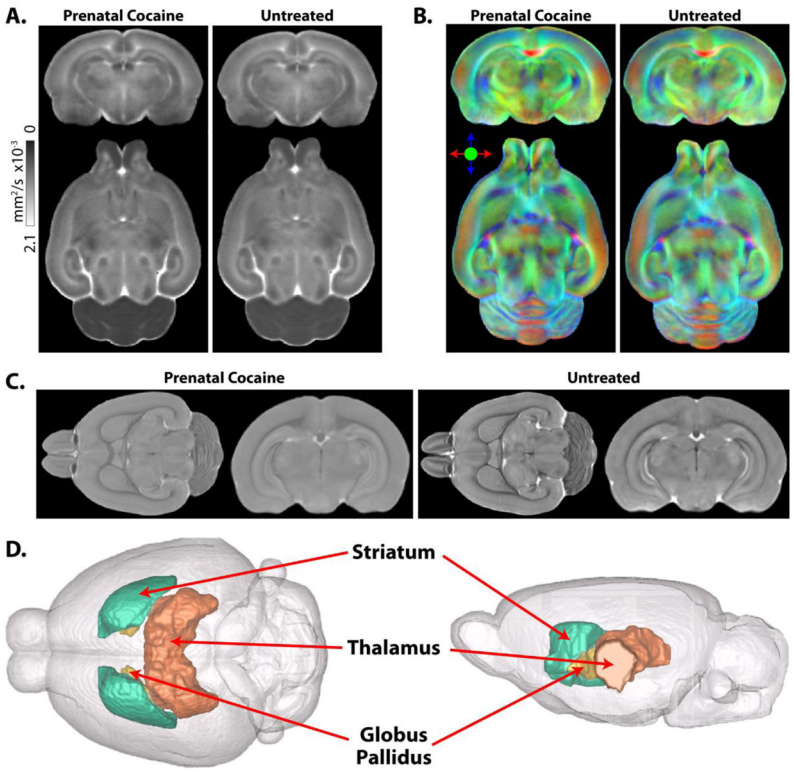

Figure 2.

Representative morphology for both prenatal cocaine-exposed and untreated pups, shown via average parametric maps of each population. Resulting average Mean Diffusivity maps are presented in (A), while Fractional Anisotropy maps are presented in (B). Parametric maps presented in (A) and (B) are scanned in our in-vivo animal cohort. Average MR Histology images can be found in (C), computed in our ex-vivo animal cohort. As indicated in the left panel of (B), pixel color in FA maps are representative of both the strength and the direction of diffusion. Red intensities indicate medial-lateral flow, while blue indicates, dorsal-ventral and green indicated anterior-posterior. Three-dimensional morphology of thalamus, globus pallidus, and striatum are represented in the population average of our in-vivo animal cohort (D). Among the regions of interest examined, these three showed significant differences between prenatal cocaine-exposed and untreated animals.

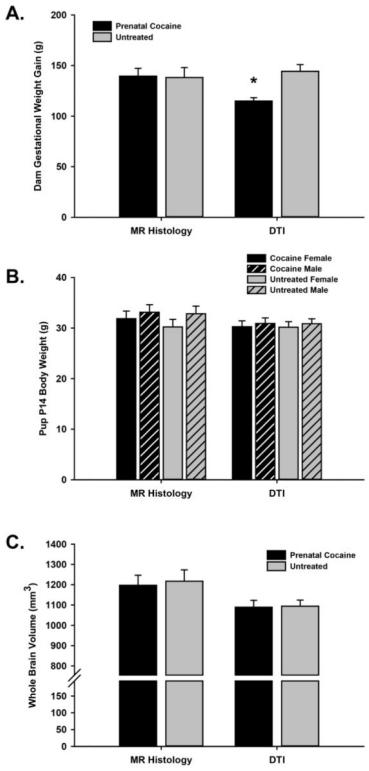

Figure 3.

Effect of cocaine on dam weight gain, pup weight, and pup brain volume. Cocaine-treated dams in the DTI study gained less weight over the gestational period, but there was no difference in dam weight gain in the MR Histology Study (A). There were no significant differences pup weight at the time of image collection (B). Pup whole brain volume also did not differ (C). Asterisk denotes $p \leq 0.05$.

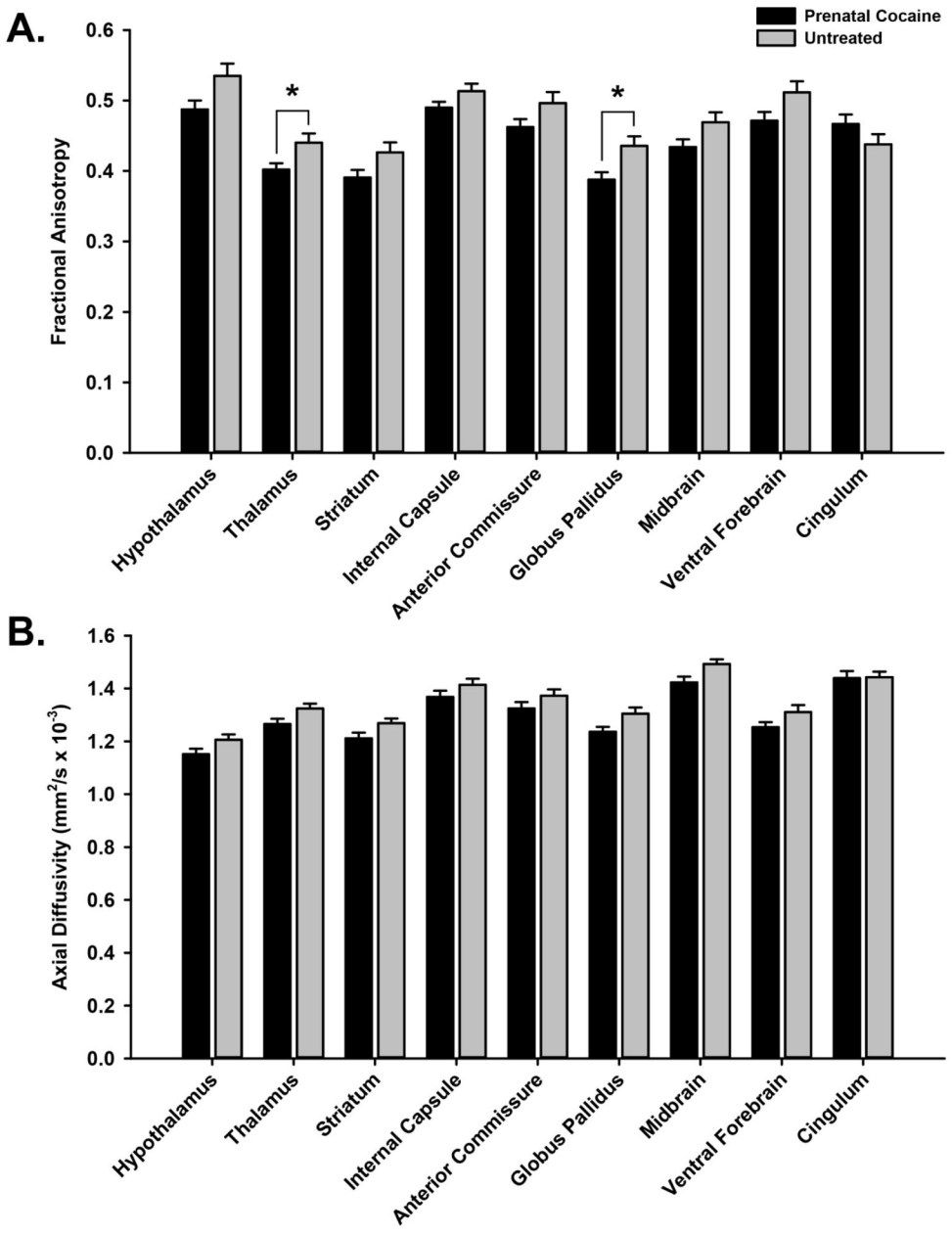

Figure 4.

Effect of prenatal cocaine on diffusion parameters within regions of interest. While a number of other regions were examined, those presented are those which were significantly different before correcting for multiple comparisons. Among these regions, Fractional Anisotropy is reduced following prenatal cocaine, but after correcting for multiple comparisons, significant reductions were only seen in the Thalamus and Globus Pallidus (A). These reductions were paired with reductions in Axial Diffusivity; however, after correcting for multiple comparisons, no differences remained statistically significant (B). Asterisks denote $p \leq 0.05$.

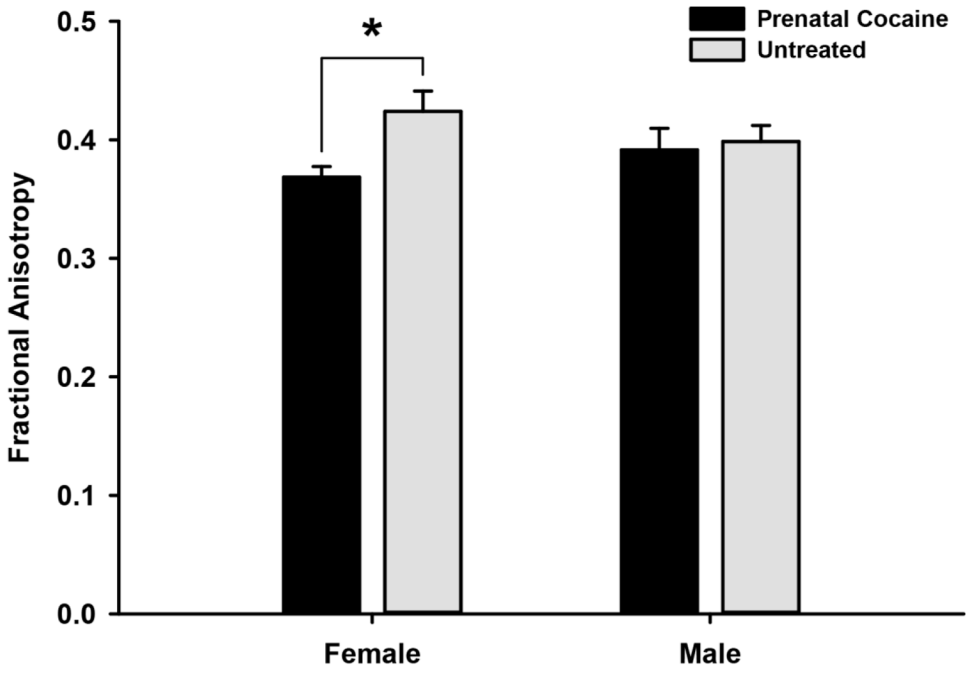

Figure 5.

Fractional Anisotropy is reduced in the Right cortex of cocaine-exposed females, but is unchanged in cocaine-exposed males. Asterisk denotes p≤0.05.

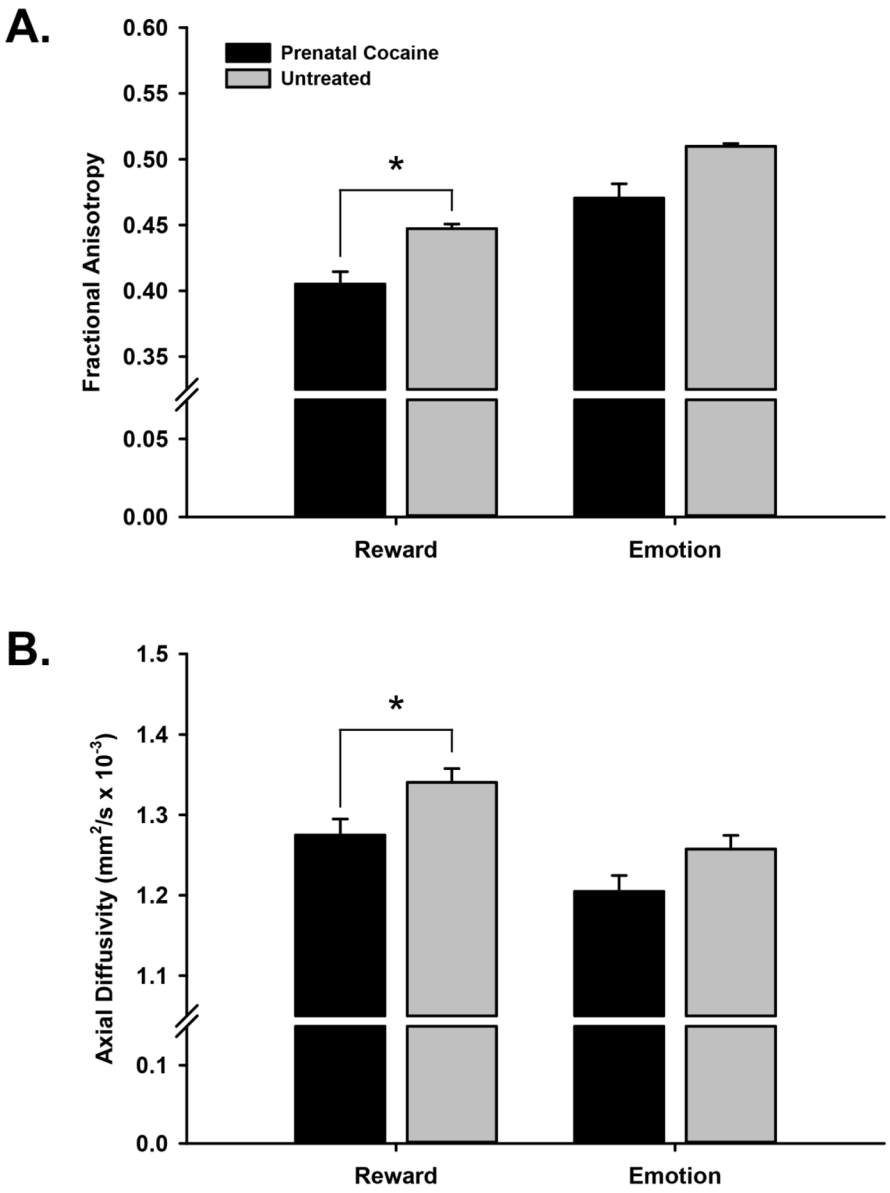

Figure 6.

Effect of prenatal cocaine on diffusion parameters within Reward and Emotion-regulation circuits. Across regions associated with reward processing, cocaine-exposure is associated with reductions in both fractional anisotropy (A) and axial diffusivity (B). Asterisks denote $p \leq 0.05$.