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Neuroimaging of prenatal drug exposure

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A symposium report

This report summarizes the Symposium presented at the Annual Meeting of the Neurobehavioral Teratology Society, Vancouver, BC June 26, 2004.

The objective of this symposium was to bring together scientists who are studying the effects of prenatal substance abuse in humans and primates. A number of investigators studying cohorts of prenatally substance-exposed children have recently received funding from National Institute on Drug Abuse (NIDA) to examine these children using neuroimaging techniques. It was the purpose of this symposium to provide a platform for these individuals to present their work, assess the strengths and weaknesses of their neuroimaging and data processing techniques and discuss strategies that could facilitate the collection of valuable imaging data in exposed and control children.

In 2000, NIDA organized a meeting to evaluate the feasibility of utilizing imaging techniques to study children whose mothers used cocaine during pregnancy. There was extensive discussion about how to accommodate children in the imaging environment, especially children who may have difficulty in staying motionless in a scanner for the necessary time to complete a neuroimaging study. It was obvious that imaging of drug-exposed children was going to present many hurdles. However, neuroimaging had the potential to yield valuable insights into the subtle effects of prenatal cocaine exposure that might not otherwise be revealed.

In June, 2004, NIDA sponsored a symposium within the annual meeting of the Neurobehavioral Teratology Society in which some of the first magnetic resonance imaging data on cocaine-exposed children were presented (see Chang et al., [16]). Although the numbers of children studied were relatively small at that time (compared to the cohorts being followed), significant strides have been made since that 2000 meeting. The investigators who presented at this symposium have not only established large cohorts of drug exposed children, they have developed techniques to minimize motion artifacts, to make the children comfortable and cooperative in a scanning environment and to obtain reliable images. Through imaging brain structure, metabolites, and function, we can greatly enhance our understanding of how maternal substance abuse alters neurobehavior in the offspring as well as expand our understanding of how the normal brain functions during development.

The first paper by Helene Benveniste, M.D., Ph.D., describes the Brookhaven study utilizing magnetic resonance imaging (MRI) co-registered with positron emission tomography (PET) to study drug distribution and metabolism in fetal Macaque monkeys. Dr. Benveniste showed that these techniques can be used to study pharmacokinetics and pharmacodynamics of drugs of abuse such as cocaine as well as the resulting perturbations in primate brain development. The next paper by Marylou Behnke, M.D., and coworkers described the Gainesville cohort that has been longitudinally followed for 12 years and the techniques that their group has developed to allow them to successfully conduct neuroimaging studies on 11-year old children. Dr. Behnke's group has collected MRI and diffusion tensor imaging (DTI) data on 60 children. Emmalee Bandstra, M.D., then presented the characteristics of the Miami cohort and their neuroimaging

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study using anatomic MRI and 3-D whole-brain volumetric high-resolution proton magnetic resonance spectroscopy (MRS), which delineates metabolites reflecting neuronal viability, membrane status, and tissue energetics. These investigators also reported acclimatization strategies for making the children more comfortable with the scanning and data acquisition and processing techniques that reduce interference and improve signal quality. Lynn Singer, Ph.D., then presented the description of the Cleveland cohort and pilot data that they have collected showing alterations in the corpus callosum, occipital and parietal lobes and also described the possible relationships of these alterations to deficits in attentional, visual motor, cognitive and language skills that have been reported in their cohort of cocaine-exposed children. This approach holds great promise as a tool to begin early diagnosis which can be conducted as a screen and perhaps therapy for neuropsychological problems since MRI scanners are becoming readily available. Lastly, Yasmin Hurd, Ph.D., presented in situ hybridization histochemistry (ISHH) findings from a large study of 18–22 week gestation human fetal brains collected in Brooklyn, NY. Since marijuana was the drug most frequently used in the population from which this sample was obtained, she reported on cannabinoid-related changes in mRNA expression in the amygdala. While analyses of these tissues are ongoing, this study demonstrates that imaging the brain can reveal drug effects which are regionally selective and will certainly provide an incredible wealth of knowledge about human fetal brain development and the ways in which it is impacted by drugs of abuse.

Drug exposure in the womb: PET and MRI imaging of drug transfer in pregnant non-human primates

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Currently there is a significant gap in our knowledge of the developing human brain and the molecular, genetic and environmental factors that may contribute to the development of neurological and psychiatric disorders later in life. Epidemiological and experimental studies now increasingly indicate that diseases such as autism, schizophrenia, depression, addiction, obesity and certain forms of degeneration may have their origin early in life [48,87,92]. One way to further enhance our understanding of these complex interactions would be to longitudinally characterize the maturation of fetal brain receptors, the changes that might occur following various stressors (endogenous as well as exogenous) and most importantly, how such changes link to specific behaviors in the progeny later in life. However, most animal models developed for the study of fetal receptors and maternal–fetal drug exchanges are invasive or performed post-mortem [63,101]. For example, pregnant sheep or rhesus monkeys with chronically implanted maternal and fetal venous catheters have been used in pioneering studies on exchange of anesthetics, therapeutic drugs, drugs of abuse and physiological studies of the maternal–fetal circulation [21,63,76]. Similarly, the rodent models used to characterize maternal–fetal pharmacokinetics, pharmacodynamics and drug distribution patterns require post-mortem analysis of the fetus after maternal drug exposure [22,24].

Hartvig and coworkers were the first to implement the use of positron emission tomography (PET) as a potential tool to study maternal–fetal drug exchange non-invasively [40]. The initial hypothesis put forward by Lindberg et al. stated that non-invasive imaging like PET would potentially permit access to the fetal compartments without disturbing the normal physiology compared to the classical invasive models [8]. Another advantage was that the tracer technique would allow the study of drug exchange without the pharmacological effects of administered compounds [8]. In one of the first maternal–fetal studies, PET was used semi-invasively to visualize placental transfer of nutrients (^{11}C-methionine) [8] and labeled opioid compounds such as ^{11}C-morphine metabolites and ^{11}C-heroin in pregnant Rhesus monkeys [40]. In placenta, ^{11}C-morphine and ^{11}C-heroin rapidly reached high concentration within the first few minutes after administration. The transfer of ^{11}C-morphine-derived radioactivity to the fetus was also rapid, although there was a lag-time in relation to the placental uptake [40]. While a limited interpretation of pharmacokinetics could be made for ^{11}C heroin and other compounds with regards to the placenta, it was difficult to identify fetal organs in this study due to lack of spatial resolution in the PET images and thus limited fetal anatomical information was available.

We recently implemented and improved the PET imaging approach for visualization and quantitation of maternal–fetal drug exchange in the Macaques radiata species by combining it with magnetic resonance imaging (MRI) [7]. The multimodality imaging approach allowed a more accurate identification of fetal body organs because the anatomical MRI template of the pregnant monkey was used for co-registration to the PET images [7]. The anesthesia regimen was also designed to allow more stable and optimal physiological conditions for the mother and fetus while at the same time permitting a fast induction and emergence following the procedures. A combination of a short-acting hypnotic (propofol) and opioid (remifentanil) agents was used with careful attention to maternal hemodynamic parameters and the pregnant animals were intubated and mechanically ventilated during the imaging procedures. The mechanical ventilation was synchronized to the data acquisition during MRI scanning to avoid image motion artifacts. In 3rd trimester fetuses, motion artifacts were minimal except when the fetus was in breech position; in breech position, fetal head movements were more prominent on the MRI images. T2-weighted as well as proton density weighted

MRI images were acquired for each pregnant animal (3rd and 2nd trimester). Except for placement of two intravenous catheters and oro-tracheal intubation, the experiments were non-invasive and no fetal catheterization was performed. Following the imaging procedures, the animals were allowed to recover and later delivered normal offspring (only one of 18 pregnant Macaque radiata used in these imaging experiments over the past 2-years has delivered a stillborn; all others have delivered normal, full-term babies).

We first demonstrated the potential for non-invasive measurement of the transfer of 2-deoxy-2[^{18}F]fluoro-D-glucose (^{18}FDG) across the placenta and calculated ^{18}FDG standard uptake values (SUV) for maternal and fetal brain as well as other body organs using a whole body PET scanning approach [7]. The co-registration to the MRI image allowed the identification of the fetal forebrain, cerebellum, heart, lungs, liver, kidney (only 3rd trimester) and the placenta. The proton-density weighted MRI images of the 3rd trimester fetuses revealed vascular anatomical detail such as the umbilical vein, ductus venosus, hepatic circulation and inferior vena cava. However, during the MRI-to-PET imaging co-registration process (rigid warping using corresponding anatomical landmarks on the MRI and PET image), this detail was partially obliterated and could not be used in the analysis process (the spatial resolution of the MRI images was $0.78 \times 0.625 \times 2$ mm^3 while the PET image reconstructed resolution was $2.57 \times 2.57 \times 2.42$ mm^3).

The static whole body PET scan approach used in our first study did not permit the extraction of pharmacokinetic information because to obtain the time course of activity simultaneously in both maternal and fetal brain, both must be in the field-of-view (FOV) for the entire study. Since the axial FOV of our CTI HR+PET scanner is only 15.5 cm, this requires positioning the animal transverse to the scanner axis, such that it occupies nearly the entire transverse FOV of 56 cm. We therefore changed the positioning of the macaques from conventional cephalad-to-caudal axis transaxial to the center of the PET scanner to transverse positioning in order to be able to capture maternal and fetal brain time–activity curves in parallel. The dynamic PET studies using ^{18}FDG were used to

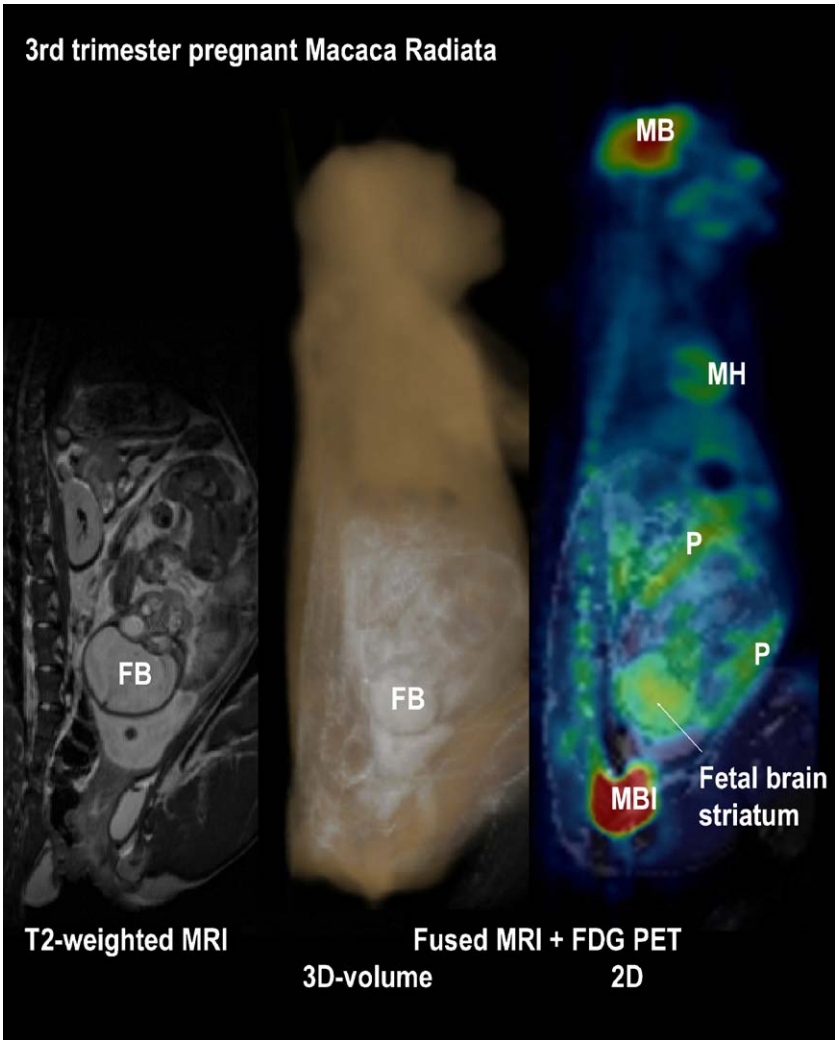

Fig. 1. MRI and PET images from the same 3rd trimester pregnant Macaque radiata. The figure shows the T2-weighted MRI acquired at a spatial resolution of $0.78 \times 0.625 \times 2$ mm^3 (left), the volume rendered fused MRI and corresponding FDG PET image (middle) and a 2D fused MRI and PET data set of this subject (right). FB = fetal brain; MB = maternal brain: MBI = maternal bladder: P = placenta.

obtain time–activity curves from maternal and fetal organs of interest following co-registration to their corresponding MRI images (Fig. 1). As expected, high ^{18}FDG uptake was observed at early time points in the maternal lungs, heart, kidneys and liver; in the uterine cavity, uptake was evident in the placenta 30-min following the injection of tracer. In the fetus, ^{18}FDG uptake was detected first in the fetal liver and later in the fetal brain and heart. We are in the process of characterizing the ^{18}FDG uptake in 2nd and 3rd trimester fetuses for subsequent kinetic modeling and calculation of rate constants which will allow an indirect assessment of fetal glucose metabolism (Logan et al., in preparation). Future studies are focused on characterizing maternal and fetal dopamine transporter (DAT) uptake sites using [^{11}C] cocaine and $\alpha_4\beta_2$ nicotinic receptor changes throughout 2nd and 3rd trimester.

Neuroimaging in Prenatally Cocaine-Exposed Children

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In general, the long-term effects of prenatal cocaine exposure identified in clinical samples have been subtle. Neuroimaging of children enrolled in prospective studies of prenatal cocaine exposure holds the promise of providing a more detailed look at the potential effects of cocaine on the developing brain in conjunction with access to a rich database of outcomes from birth to middle childhood.

The Parent Project [27,28]. The study was approved by our Institutional Review Board and a Certificate of Confidentiality was obtained from NIDA. Our sample was drawn from an understudied rural population of pregnant women who exhibited a wide range of cocaine use, primarily in the form of crack. A priori exclusion criteria included potentially confounding variables that occurred rarely in our population: major illnesses that had been diagnosed prior to pregnancy, the chronic use of prescription or over-the-counter drugs, any illicit drug use except for cocaine and marijuana, non-English speaking women, and those <18 years old. A priori matching criteria included more commonly occurring factors that were also thought to be potentially confounded with the effects of cocaine exposure: the location of the prenatal care which related to the perinatal risk of the mother, the level of socioeconomic status using an adaptation of the Hollingshead index, first born versus subsequent births and self-reported race categorized as Black and non-Black.

Drug use was identified using both self-reported history and drug testing. A detailed drug use history was obtained following each trimester of pregnancy and at each subsequent visit. Urine drug testing was performed at two unanticipated times: enrollment and delivery. The urine was screened for 8 drugs of abuse using an immunoassay with positive screens confirmed by GC/MS. Ultimately, 154 prenatal cocaine users were matched to 154 non-cocaine users for a total enrollment of 308 subjects. The majority of women were black, multiparous, and in the lowest Hollingshead category of socioeconomic status. A range of assessments have been performed at multiple timepoints since birth. About 90% of the surviving offspring have remained in the study to age 7. Data are currently being collected on the 10 and 12 year old children.

Psychometrically appropriate measures were chosen for the child evaluations which have been administered by licensed/certified evaluators, blinded to prenatal drug history. Transportation has been provided to the testing site or a mobile testing unit used at the child's school.

Primary caregivers have been interviewed during home visits at multiple timepoints. Approximately 1/2 of the exposed children are in foster care and do not reside with their biological parents. At each home visit, demographic information is updated, psychosocial surveys are administered to the caregiver, and the home environment is observed.

The Imaging Study. Several issues stimulated interest in exploring neuroimaging techniques in the cohort. First, there were theoretical issues surrounding the teratogenic effects of prenatal cocaine exposure. Studies in sheep and rodents have documented vasoconstrictive and hypoxic effects on the fetus after cocaine administration to the mother [79]. Evidence from other areas of research has indicated that chronic or intermittent hypoxia during pregnancy can result in neurobehavioral alterations [57]. The hippocampus is generally considered one of the most vulnerable areas of the brain to hypoxia. In addition, white matter development may be affected by hypoxia with an impact on interhemispheric communication (e.g., callosal integrity) and the efficiency of tract connections between the subcortex and the cortex [91]. Thus, chronic hypoxia can result in permanent effects, with information processing and problem solving being potentially vulnerable.

Cocaine also has a pharmacologic action on the monoaminergic systems and on pathways that innervate forebrain regions. Neurotransmitter receptors can be affected as well, with critical periods of development determining region specific effects [25,50,59,62,99]. Advanced MRI techniques appear to be able to more precisely investigate those areas of the brain hypothesized to be affected by prenatal cocaine exposure.

A second reason we became interested in neuroimaging was the repeated finding from the parent study that cocaine appears to affect outcome indirectly through head circumference measured at birth and adjusted for gestational age. After

controlling for other drug exposures, we found a negative correlation between the amounts of prenatal cocaine use reported and birth head circumference as well as newborn behavior. In addition, there was an interaction effect with tobacco such that cocaine users who also smoked tobacco had infants with smaller head circumferences and poorer scores on newborn behavioral testing [27,28]. Although we have reported direct effects of cocaine on development at birth using the Brazelton Neonatal Behavioral Assessment Scale and at 6 months using the Bayley Scales of Infant Development [6], we have not found any further direct effects between ages 3 and 7. However, important indirect effects of cocaine continue to manifest themselves at all of the timepoints between birth and age 7 such that more prenatal cocaine use is associated with smaller head circumference at birth, adjusted for gestational age, which in turn predicts poorer developmental/cognitive outcome.

Despite these findings, we were unable to demonstrate any drug group differences on 266 cranial ultrasounds performed at birth [5]. However MRI provides a more precise look at the brain itself, allowing volumetric measurements of various regions of interest as well as the application of newer techniques designed to look at white matter integrity.

Thus we embarked upon a multidisciplinary study of the outcome of children prenatally exposed to cocaine using 3 different MRI techniques: (1) structural analysis using conventional MR sequences; (2) volumetric analysis with volume and surface area measurements of areas of interest. The areas chosen for initial investigation were those that have been shown in other samples to be related to cognition and reading ability; (3) and finally, diffusion tensor imaging. DTI is a relatively new MRI technique that produces 3-D diffusion maps of the brain. It was chosen because of its sensitivity to microstructural changes in white matter integrity, an area of theoretical interest as mentioned earlier.

Imaging Methods. Sixty children from the parent project were chosen for inclusion in the imaging study, including 30 cocaine-exposed and 30 non-exposed who were about 11 years old at the time of the scanning. They were all right handed and the groups were balanced for gender and IQ. All of the girls were pre-menstrual.

Subjects were imaged using a Siemens 3T Allegra head scanner. Thirteen scans were not usable due to protocol violations, technical problems, motion artifact, children too big for the scanner and several children who did not wish to complete the imaging sequences. To date we have 51 structural analyses completed with preliminary data on 41 volumetric and 40 diffusion tensor analyses.

For the structural analyses, detailed, standardized reviews of the brains were made by the project neuroradiologist who was blind to drug exposure status. For the initial volumetric analyses, several areas of the brain were evaluated: the anterior cerebellum (shown in other samples to be related to dyslexia), the posterior–superior cerebellar vermis and the posterior–inferior cerebellar vermis (previously shown to be related to ADHD). In addition, auditory and language structures were measured which included Heschl's gyrus (primary auditory cortex), the pars triangularis (a left hemisphere substructure of Broca's area in the inferior frontal lobe associated with expressive language), and the planum.

For the diffusion tensor imaging, the scan parameters included 6-direction, 3 *b*-value diffusion tensor and SE-EPI pulse sequence. The processing parameters included standard rank-2 tensor model and mono-exponential Anisotropic diffusion coefficient (ADC) fitting with fractional anisotropy and average diffusion maps generated for analysis from regions of interest (ROI).

A methodological issue surfaced with DTI analyses. Initially, a core and hand drawn method was used but not found to be reliable. A third, revised core method, involves drawing four likely core regions inside the structure of interest and using the core with the highest mean for analysis. This method has proved to be highly reliable with intraclass correlations ranging from moderately reliable for 1 region of interest to highly reliable for 3 additional regions of interest. To date, only the genu and splenium of the corpus callosum have been reanalyzed using the reliable revised core method.

Imaging Results. Using structural analysis, 46 of 51 MRI scans were read as normal. Two children had abnormal craniofacial relationships indicating small brains (1 exposed; 1 non-exposed). Other abnormalities included abnormal signal intensity in the white matter of the right frontal lobe (1 exposed), periventricular leukomalacia (1 non-exposed), and mega cisterna magna vs. arachnoid cyst (1 exposed).

In preliminary analyses, only 1 significant finding has been identified in the volumetric measurements completed; to date, the cocaine-exposed group on average has smaller right anterior cerebella. This finding has been shown in other samples to be related to dyslexia [26,51]. No significant findings were identified in the DTI analyses for the splenium and genu of the corpus callosum using the more reliable revised core methodology.

Future work will include completing analyses of the diffusion data using the more reliable revised core method, investigating higher order tensor models, and evaluating other ROIs, including frontal areas thought to be sensitive to cocaine exposure. We then plan to correlate MRI findings with appropriate cognitive/behavioral outcomes. We have already found a correlation between the amount of cocaine exposure and some measures of brain asymmetry which may be important because asymmetry (rightward or no asymmetry) has been shown in other clinical samples to be related to reading and other cognitive abilities. Finally, if results of the above analyses are promising, we will attempt to extend the imaging to our entire subject population.

Neuroimaging with Anatomic Magnetic Resonance Imaging and Magnetic Resonance Spectroscopic Imaging in Prenatally Cocaine-exposed Children

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Introduction and Scientific Rationale

Few perinatal substances of abuse have garnered as much attention as cocaine and crack cocaine. In the Miami Prenatal Cocaine Study (PCS), a NIDA-funded longitudinal study of the effects of in utero cocaine exposure on the developing child, we have reported abnormalities in growth [3], infant neurobehavior [65], attentional processing [4], language [66], and an increased rate of learning disabilities [64]. Furthermore, we have reported that cocaine-associated neuropsychological deficits at age 7 years appear to be mediated by cocaine-associated deficits in birth head circumference [2]. State-of-the-art neuroimaging has the potential to elucidate the impact of in utero cocaine exposure upon brain structure, growth, chemistry, and function instead of relying solely on anthropometrics and neurodevelopmental assessments. In this presentation, we describe the rationale, study design, and pilot examinations from our NIDA-funded exploratory/technological development neuroimaging project in which anatomical MRI (aMRI) and whole brain magnetic resonance spectroscopic imaging (MRSI) are being performed in a subsample of the Miami PCS participants.

Echoencephalographic evidence of CNS abnormalities in response to prenatal cocaine exposure has been inconsistent, predominantly emanating from case reports, retrospective studies, and relatively small series. In a large prospective study, Behnke et al. reported no difference in the rate of abnormal cranial ultrasound results between cocaine-exposed and non-cocaine-exposed infants [5]. In another large prospective study, Frank et al. emphasized a greater risk for subependymal hemorrhages in the caudothalamic groove in heavily cocaine-exposed infants compared to non-exposed infants [31]. Unexplored or unascertainable dose and/or gestational timing effects may explain inconsistencies in prior studies.

Anatomic MRI is important in the assessment of normal and abnormal development in infants and children of all ages [72]. Brain structure is intimately related to neurochemistry and function and no study of the latter can reliably proceed without delineation of the anatomic correlation. Few investigators have reported cranial MRI findings in cocaine-exposed cases. Dominguez reported severe cerebral anomalies and cerebral infarcts diagnosed by computed tomography (CT) and/or MR in a small series of cocaine-exposed infants with ophthalmologic abnormalities and/or developmental delay ($n=10$) [23]. Link et al. found no MRI evidence of congenital abnormality, cerebral infarction, hemorrhage, or myelination abnormality among nine cocaine-exposed infants [56].

In vivo proton (^{1}H) MR spectroscopy (MRS), a unique non-invasive method for evaluation of tissue metabolites, may impart valuable information regarding the effects of in utero cocaine exposure since changes of metabolites may occur in the absence of structural abnormalities on MRI. In clinical investigations, even a relatively simple (i.e., long TE) ^{1}H MRS observation can provide considerable information on neuronal viability (via *N*-acetyl-aspartate), membrane status (via choline) and tissue energetics (via creatine and lactate). Short-TE measurements potentially enable additional metabolites to be observed, including myo-inositol, considered a glial marker. Smith et al. performed ^{1}H MRS on 14 prenatally cocaine-exposed and 12 unexposed preadolescent children [86]. They found that cocaine-exposed children had significantly higher creatine (Cr) (+13%), potentially signifying abnormal energy metabolism, in the frontal white matter despite normal brain structure and no significant differences in other metabolites or volumetric measurements. This finding parallels MRS results in abstinent previously heavy cocaine-using men, where elevated white matter creatine (CR) and myoinositol (MI) were observed in conjunction with normal *N*-acetyl aspartate (NAA), the latter suggesting no neuronal loss [16]. On the other hand, chronic cocaine use appears to induce a lower level of *N*-acetyl aspartate (NAA) in the left thalamus in adult chronic cocaine abusers [53].

Recent reviews [46,100] summarize MRS findings in normal brain development as well as various pediatric conditions. Localized MRS may be implemented using single volume measurements or by MR spectroscopic imaging (MRSI) techniques [77]. Although more difficult to implement, MRSI techniques obtain multiple spectra over a wide region, thereby enabling generation of clinically preferred images of metabolite distributions. Most ^{1}H MRSI studies in human brain have been carried out using more widely available 1.5 Tesla MR systems, typically with 1–2 cc voxel volumes acquired in 20–40 min. Due to increased sensitivity and spectral resolution at 3 Tesla, both data acquisition time and voxel volume can be decreased without compromising accurate metabolite quantification.

Methods

Sample. This neuroimaging study is being performed in 48 male and female 12-year-old children (24 cocaine-exposed; 24 non-cocaine-exposed) recruited from the Miami PCS, detailed elsewhere [2]. The follow-up study has 87% retention of the original 476 enrollees, currently ages 11–13 years. Prenatal drug exposure status was determined by maternal postpartum interview and toxicology assays of urine and meconium.

Exclusions were prematurity, maternal use of drugs other than cocaine, alcohol, tobacco, or marijuana, maternal HIV/AIDS, major congenital malformation or chromosomal defect, and overt disseminated congenital infection. For this MR study, children with metallic implants or tattoos, left-handed dominance, psychotropic medications, or IQ <70 were ineligible. Ten prior waves of age-appropriate child developmental assessments and caregiver interviews, performed blind to drug status, have been completed and both the 12-year-old assessment and this neuroimaging study are in progress. This study is performed with Institutional Review Board approval, parental consent and child assent, and a NIDA Certificate of Confidentiality.

Acclimatization Procedures and Incentives. Engagement procedures have been designed to motivate participation in the neuroimaging study, as well as to inform and acclimatize the child and the mother or primary caregiver to the MR process. During the consent process, all aspects are explained thoroughly by the Project Coordinator and a 5-min video is shown to explain the fascinating MR equipment, provide a demonstration of patient preparation for scanning, and play audio clips of actual MR sequences for desensitization to potentially intimidating aspects of the scan. In addition, there is an opportunity to tour the MR facilities. The Siemens 3.0 Tesla Magnetom Trio MR System is equipped with an 8-channel phased array head RF coil with integrated pre-amplifiers. An entertainment system allows the child to watch a pre-selected video through a screen mounted on the head-coil while the operator maintains audio contact with the child, introducing each new phase of the scan. Since the child must lie still for lengthy scanning sequences, scanning is performed in two sessions with an intermission for lunch or snack. The child is given a gift certificate for $25 per session and a keepsake certificate featuring a picture of his or her brain. Caregivers are reimbursed at $50/day and van transportation is provided.

Anatomic Magnetic Resonance Imaging. Technical development for this study began in September, 2003, after magnet installation and Siemens site licensure. Optimal acquisition sequence parameters have been determined for both anatomic (T1-weighted; MPRAGE) and dual-contrast (proton density and T2-weighted; double spin-echo) MRI sequences. All investigators are blind to the identity of the brains and characteristics of the participants. Conventional T1 and T2 images are interpreted diagnostically at the University of Miami by a neuroradiologist (B.B.) and surface area and volumetric measurements are performed by a neuroscientist (C.L.) on structural images electronically transferred to the University of Florida McKnight Brain Institute. Surface area measurements include corpus callosum (7 subparts), planum temporale, Heschl's gyri, pars triangularis in Broca's area (inferior frontal gyrus), pars opercularis in Broca's area (inferior frontal gyrus). Volume measurements include cerebral hemispheres (grey and white matter and cerebrospinal fluid), frontal lobe regions and insula, cerebellar vermis, hippocampus and amygdala, and basal ganglia. Volumes, surface areas, means, standard deviations, and average asymmetries are automatically accumulated in a data file for statistical analysis. Final scans have isotropic voxels measuring 1 mm in each dimension and each structure is measured twice by at least two different investigators. Assisted by our collaborators at the University of Florida (S.B., K.P.) and Johns Hopkins University (S.M.), we have also incorporated diffusion tensor imaging (DTI) data acquisition into the scanning protocol. Readers are referred to the accompanying symposium presentation by Behnke et al., with whom we are collaborating on the aMRI and DTI. Combined data from the two sites should yield improved statistical power for hypothesis testing.

3-D Whole Brain MRSI. Our University of Miami co-investigators in the MR Center have successfully implemented whole-brain volumetric high-resolution proton MRSI (HR-MRSI) sequences with the following typical acquisition parameters: field-of-view: 280×280×180 mm, slab thickness: 135 mm, acquired data matrix: 50×50, and 18 points, respectively, and a total data acquisition time: 25 min. The HR-MRSI acquisition method incorporates subcutaneous lipid signal nulling procedure to minimize signal contribution from the lipids which would otherwise contaminate brain metabolites of interest (i.e., *N*-acetyl aspartate, creatine and choline). The method also includes an approach whereby water reference signal is collected in an interleaved fashion to correct for magnetic field inhomogeneity of voxels across each slice in the slab. To account for partial volume signal losses due to CSF (no observable metabolite signal), anatomic MRIs are segmented into grey matter, white matter and CSF fractions using Statistical Parametric Mapping (http://www.fil.ion.ucl.ac.uk/spm/). For normalization and quantitation of observed metabolite signals in institutional absolute concentration units, a method is being developed which will use proton density weighted images and the segmented images. Despite the advances made thus far in developing whole-brain MRSI, obtaining reliable and good quality spectra from the inferior frontal lobes has remained difficult due to local magnetic field distortion caused by wide variation of magnetic field susceptibility in this anatomic region. After post-processing using our software [88], images showing distribution of metabolites across any slice can be formed (see Fig. 2). The quality of spectra obtained from a slice that includes superior frontal lobes is shown in Fig. 3. We have also included right and left frontal white matter single-voxel MRS samples (Fig. 4) to supplement the whole brain MRSI due to the importance of the region for correlation with neuropsychological testing.

Preliminary Study and Future Plans. Implementation of the protocol on the children was preceded by the above-mentioned technical development and scanning of 8 healthy adult volunteers to optimize sequences and refine the entire scanning protocol. Figs. 2 and 4 cited above are representative samples from the 3T MRS scanning session in a 19-year-old healthy male volunteer. Since the symposium presentation, we have successfully scanned the first two study children and the study is ongoing. Upon completion of the neuroimaging assessments on the 48 subjects in the preliminary study, we hope to expand the scanning protocol to include the entire follow-up cohort for correlation with specific neuropsychological outcomes and to conduct serial assessments throughout adolescence and young

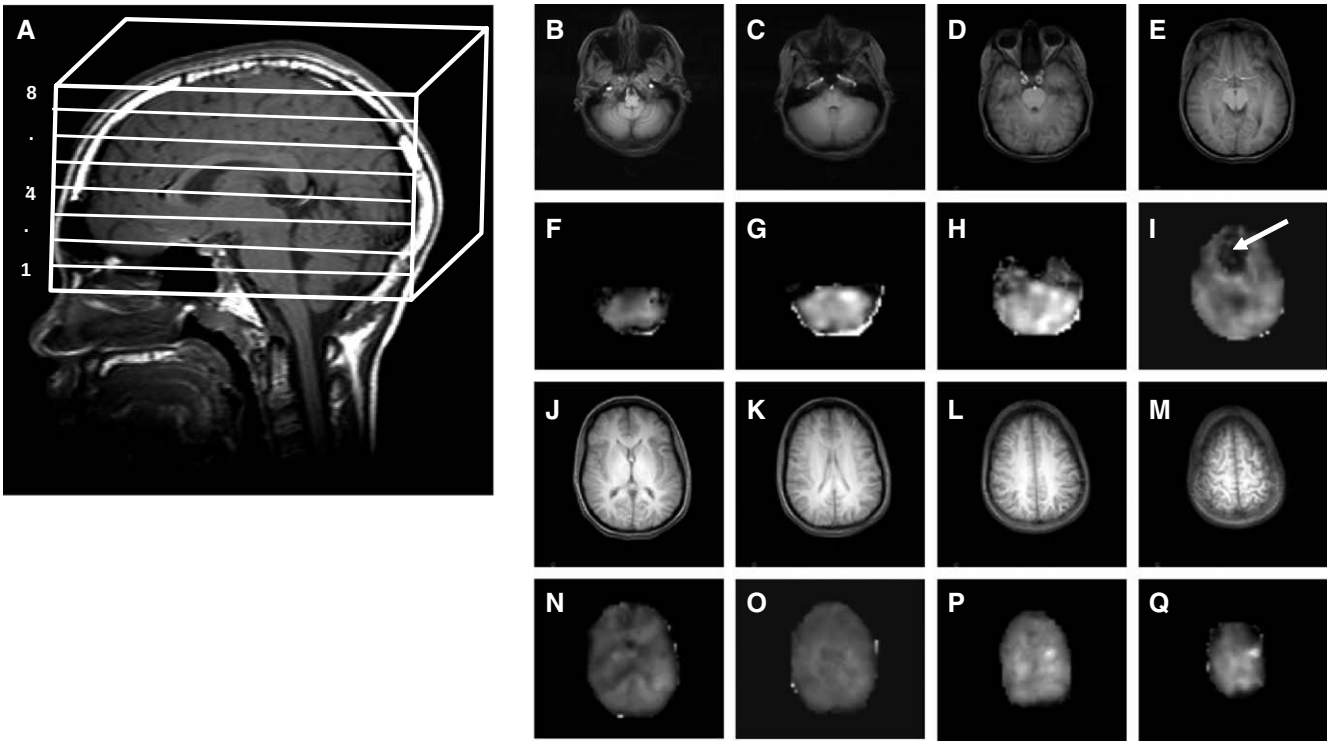

Fig. 2. Typical MRSI slab orientation used for our study (parallel to AC–PC plane) and approximate location of the slices (1–8) are shown on a sagittal MRI image (A). Images F–I and N–Q show the distribution of the metabolite, *N*-acetyl aspartate, across the 8 slices of 15 mm thickness, and their corresponding MRIs are shown in images B–E and J–M, respectively. Poor spectral quality, due to magnetic field susceptibility variation induced magnetic field changes, results in unreliable metabolite map in the frontal lobe as indicated by an arrow in I.

adulthood to delineate trajectories of brain growth and maturation within each group. Ultimately, these studies will be complemented with functional magnetic resonance imaging.

Neuroimaging of 7–8 year-old children exposed prenatally to cocaine

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Introduction

Recent advances in magnetic resonance imaging (MRI) and computational morphometry have opened up new opportunities for non-invasive quantitative assessments in brain structure of neurodevelopmental changes associated with teratogens that underlie cognitive and motor functioning in children [69]. The specific aims of the present study were to assess the feasibility of using MRI-based morphometry with early school aged cocaine-exposed children; to assess potential adverse effects of prenatal exposure on brain volume and morphology in 7–8 year old children; and to assess neurobehavioral correlates of brain volumes.

Subjects

Fifty cocaine-exposed and non-exposed children at ages 7–8 years were selected from a cohort participating in a longitudinal investigation of the effects of prenatal cocaine exposure [83,85]. During the course of the study, children were assessed at regular intervals (1 month, 6 months, 1, 2, 3, 4, and 6 years of age) on a variety of standardized, normative, or experimental neurodevelopmental tests. For inclusion in this study, children had to be >37 weeks gestation, have IQ scores >79, and must have lived with the same caregiver since birth. Children were also free of known genetic abnormalities, postnatal growth problems, and medical illnesses. Exposed children were matched to non-exposed children on age, race, gender, handedness, and the presence of heavy alcohol-exposure during pregnancy.

Of 50 children identified through the matching program, 38 were invited to participate. The remaining 12 could not be accommodated in the time frame of the study. One family refused and 37 children attempted the MRI studies. MRI was completed by 35 children (21 cocaine-exposed and 14 non-exposed). Informed written consent was obtained from all caregivers and written assent was given by all children.

MRI Assessment

Image Acquisition: MPRage T1-weighted structural MRIs were performed on a Siemens Symphony 1.5 T (Siemens Medical Systems, Erlangen, Germany). Three sequences were used: TR=1710 ms, TE=2.09 ms, Tl=1100 ms, flip angle=15. Axial proton density and T2-weighted images were also

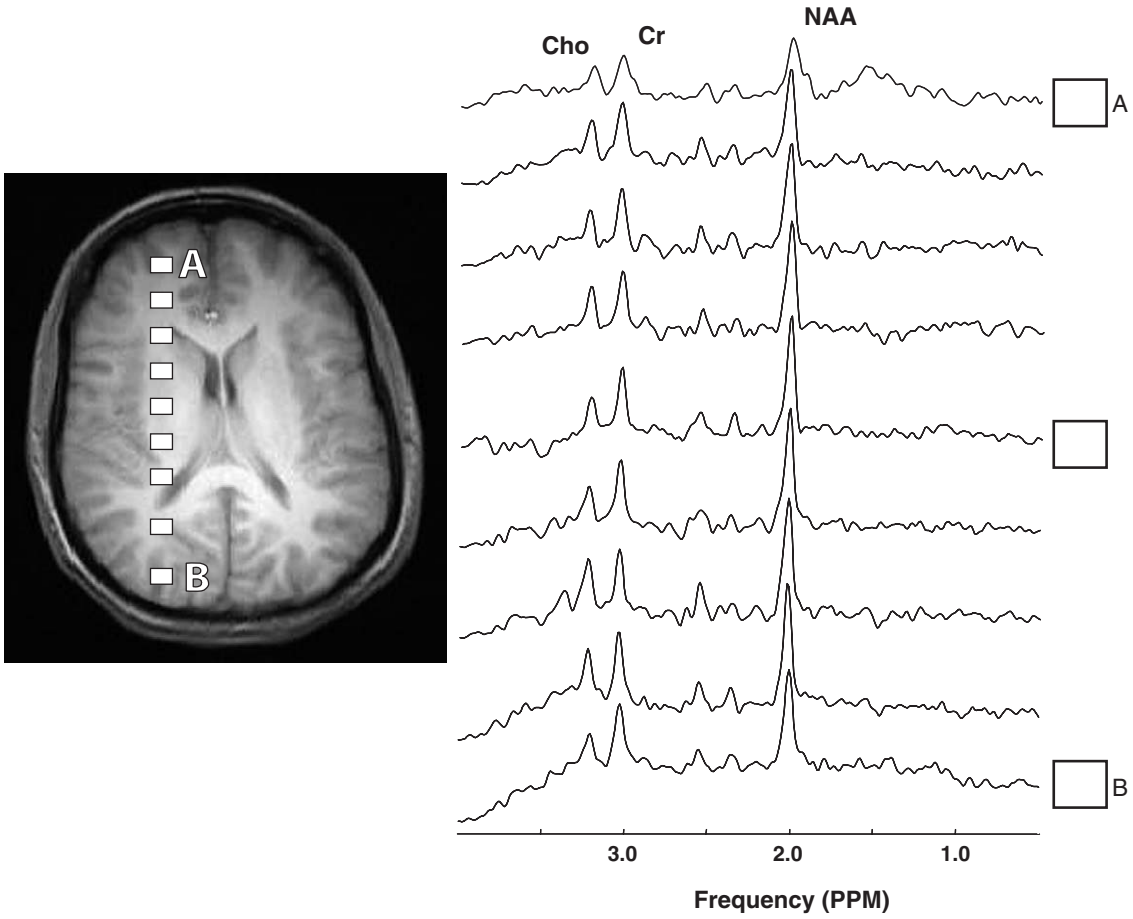

Fig. 3. 3 T 3-D whole brain magnetic resonance spectroscopic imaging (MRSI) performed in a healthy 19-year-old male. This figure shows typical quality of spectra obtained from multiple voxel locations, shown on the T1-weighted MRI, of a 15 mm thickness slice in a 3D whole brain MRSI data set. Spectra sampled from the frontal (A) to posterior (B) regions show peaks for the prominent cerebral metabolites, choline (Cho), creatine (Cr) and *N*-acetyl aspartate (NAA). Data were acquired using a 3D-EPSI sequence with a TE of 70 ms, TR of 1800 ms and an acquisition time of 26 min.

obtained and reviewed by a neuroradiologist to rule out clinically significant structural abnormalities. The entire imaging procedure took 1 1/2 h which included an introductory practice procedure. The child was in the MRI scanner for 15 to 20 min. No sedation was used.

Image preparation: DICOM images were converted to MINC format by Neuralyse, Inc., of Quebec, Canada [17,102]. Computational analysis of high-resolution structural magnetic resonance (MR) images was used to assess group- and outcome-related differences in brain structure, specifically, an Automatic Non-Linear Image Matching and Anatomical Labeling (ANIMAL) [17] of the cerebral lobes and the cerebellum to quantify regional volumes of grey and white matter. Voxel-based morphology (VBM) [102], which allows for the evaluation of possible group and outcome related differences in grey and white matter density were completed, but only limited results for illustration purposes are presented.

Data Analysis

MRI absolute volumes were compared by cocaine-exposure status using *t*-tests with and without relevant covariates, followed by regression analyses with the continuous independent variable (average cocaine-units per week during pregnancy). Volumetric data were also correlated with the cognitive outcomes collected at different time points during the longitudinal study. A generalized linear model (GLIM) was used to calculate *t*-statistics for regressions of voxel-based tissue density against the cocaine usage variable defined as units/week.

Results

Demographics and exposure to cocaine, alcohol, cigarettes and marijuana

For analyses of the automatic segmentation (ANIMAL) results, 21 cocaine-exposed children were compared to 14 non-exposed children. Both groups were seen at a mean age of 8.2±.5 years ($t=.18$, $p>.20$), and did not differ in gender, birthweight, birth head circumference, birth length, neonatal risk score, race, handedness, low birthweight, or incidence of intrauterine growth retardation. Cocaine-exposed children were exposed to more cigarettes per day prenatally (10.9±7 vs. 3.9±11, $t=-4.4$, $p<.0001$) as well as to more alcoholic drinks per week (6.8±11 vs. 1.0±3, $t=-2.3$, $p<.03$), but they did not differ in their exposure to the average number of marijuana joints per week during pregnancy (1.0±2 vs. 2.1±8, $t=-0.73$, $p<.47$). On average, cocaine-exposed children were exposed to 16.4±23 units (rocks) of cocaine per week prenatally.

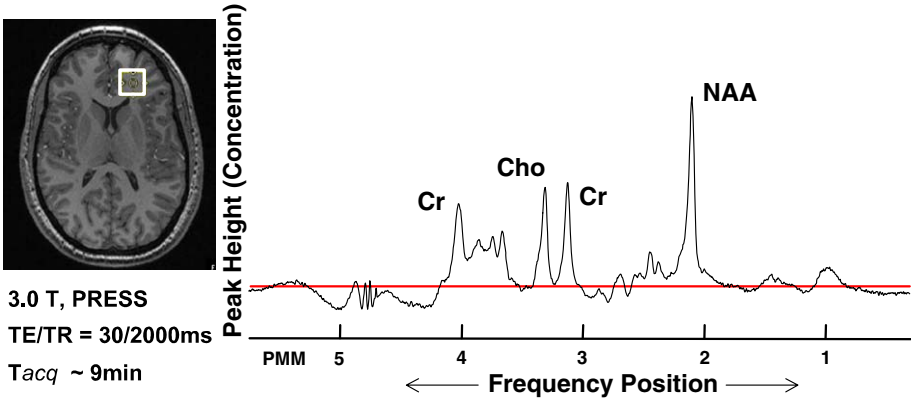

Fig. 4. 3 T left frontal lobe single-voxel magnetic resonance spectroscopy (MRS) performed in a healthy 19-year-old male. This figure depicts creatine (Cr), choline (Cho), and *N*-acetyl aspartate (NAA) peaks. Data were acquired using a single voxel point resolved spectroscopy (PRESS) sequence with a TE of 30 ms, TR of 2000 ms and an acquisition time of approximately 9 min.

Regional brain volumes

Cocaine-exposed children had smaller total area of the corpus callosum (M±SD; 571±93 mm^2 vs. 644±71 mm^2, $t=2.51$, $p<.05$), and smaller relative volume of grey matter of the left occipital lobe (51,062±3748 mm^3 vs. 55,770±7568 mm^3, $t=2.16$, $p<.05$) and the right parietal lobe (81,386±5921 mm^3 vs. 85,403±8070 mm^3, $t=1.60$, $p<.10$). The average amount of cocaine ingested by the mother during pregnancy also predicted the total area of the corpus callosum and remained significant after control for severity of alcohol, cigarette, and marijuana exposure (β (SE)=−32.1 (9.5), $p<.002$). VBM analyses revealed an overall grey matter increase with severity of cocaine exposure adjacent corpus callosum p ($t>3.2$, $df=26$) (see Fig. 5). The amount of cocaine exposure did not predict the above group differences in the occipital and parietal grey-matter.

We also correlated results from neurobehavioral assessments with the grey-matter volumes of the left occipital and the right parietal lobes. Grey-matter volume of the right parietal lobe was positively related to performance on the Developmental Neuropsychological Assessment (NEPSY) [49] at 6 years in visual attention ($r=.36$, $p<.04$), visual-motor precision ($r=.44$, $p<.01$), and sensorimotor core abilities ($r=.33$, $p<.06$), as well as Comprehensive Assessment of Spoken Language (CASL) [15] syntax construction ($r=.36$, $p<.04$). There were marginally significant trends for it to relate to 4-year WPPSI-R [97] Picture Completion performance ($r=.32$, $p<.07$), and to neonatal visual attention ($r=.35$, $p<.10$) [80]. Grey-matter volume of the left occipital lobe was positively related to NEPSY [49] visual motor performance ($r=.32$, $p<.07$) at 6 years, to neonatal visual attention [80] ($r=.52$, $p<.01$), and to visual recognition memory at 12 months ($r=.39$, $p<.05$).

Discussion

These pilot data on a subsample of children followed longitudinally from birth with fetal cocaine exposure demonstrate common trends for differences in the corpus callosum, and the occipital and parietal lobes between the cocaine-exposed and the non-exposed children.

Corpus-callosum abnormalities, ranging from complete callosal agenesis to a hypoplastic corpus callosum, have been found rather frequently in offspring exposed in utero to various teratogens, including alcohol [13], cocaine [23,68], anti-epileptic drugs [55], and corticosteroids [45].

Early postnatal exposure to cocaine in rats was related to a reduction in the size of the corpus callosum in males [68]. Several clinical reports indicate that the morphology of the callosum is related to psychological and behavioral functions such as attention deficit hyperactivity disorder [36] and performance on intelligence tests [89], both of which have been negatively affected in our cocaine-exposed cohort [54,82,84].

In our cohort, a number of functional outcomes have been negatively affected by cocaine exposure which correspond to the brain areas differentiated by MRI here. In particular, visual–spatial skills were negatively related to the amount of prenatal cocaine exposure as measured by performance on an object assembly task on the WPPSI-R at 4 years [85]. In addition, neonatal neurobehavioral abnormalities, including poorer visual attention and visual recognition memory [80], jitteriness and motor asymmetries [81], and receptive language deficits were seen in the first year of life [82]. Subsequently, we have detected generalized cognitive deficits at 2 years [83], and specific deficits in cognitive skills of arithmetic, visual spatial abilities, general knowledge [85], and language abilities at 4 years [52], and attention at 6 years [54].

Conclusions

Prenatal cocaine exposure was associated with common trends for differences in the corpus callosum, occipital and parietal lobes in six-year-old children that could not be accounted for by other drug exposures. These anatomical differences are consistent with previous findings in this cohort of cocaine-exposed children, which found deficits in attentional, visual–motor, cognitive, and language skills in comparison to non-exposed children, suggesting long-term alterations in brain structure associated with prenatal cocaine exposure. Continued follow-up and a larger sampling of the cohort will be important in assessing the permanence of these differences, their

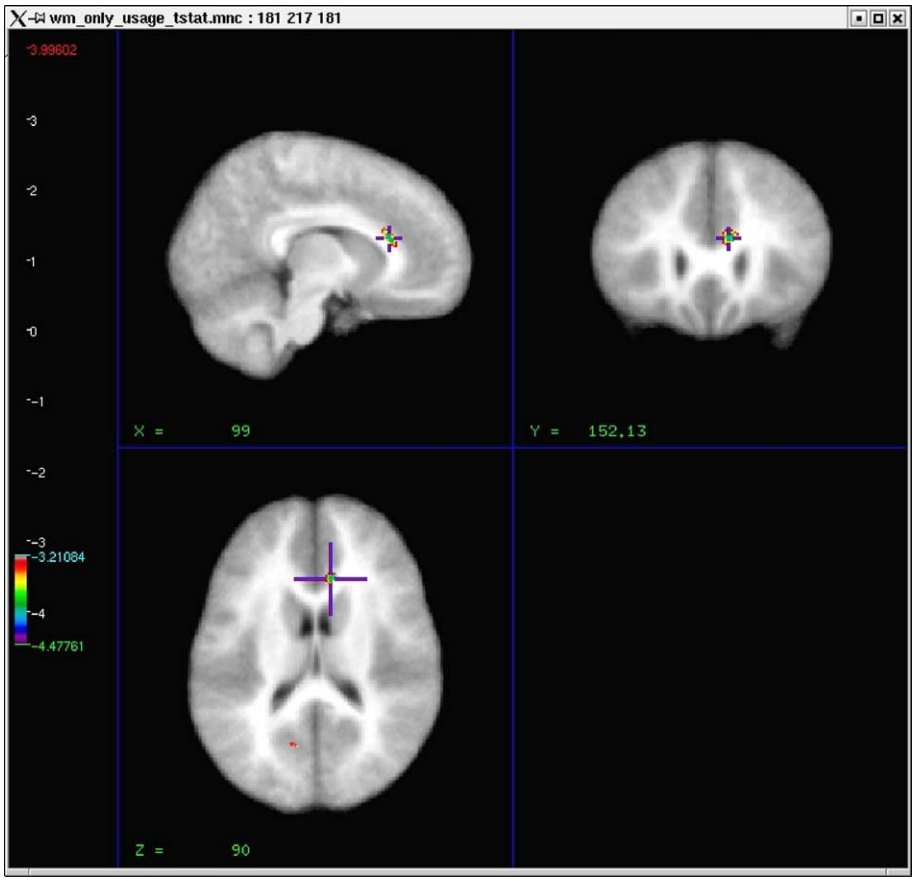

Fig. 5. Grey matter increase adjacent to the corpus callosum as a function of severity of cocaine usage.

relationship to functional outcomes, and the potential for environmental interventions to affect their developmental consequences.

Effects of Marijuana Exposure on the Human Fetal Brain: Molecular Imaging Studies

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Approximately 4% of women in the USA report using illicit drugs during pregnancy with marijuana (*Cannabis sativa*) being the most common (75%) drug used among pregnant women [78]. Δ^9-tetrahydrocannabinol (THC; the major psychoactive component of cannabis) readily crosses the placenta so maternal marijuana use can potentially affect the health and development of the fetus and offspring. Epidemiological human studies have in fact documented long-term disturbances in behavior (impulsivity, inattention, and social disturbances) and cognitive function (e.g., poor abstract/visual reasoning and planning as well as impairment of verbal skills and memory) in offspring of women who used marijuana during pregnancy [29,32,38,71]. Animal studies have also provided supportive evidence of a negative long-term impact of perinatal cannabis exposure. For example, adult animals exposed during gestation or lactation to cannabis exhibit persistent alterations in learning, social interactions, sexual behavior, motor activity, passive avoidance, behavioral response to novelty, and drug-seeking behavior (see, e.g., Navarro et al., 1995 [67]).

An important consideration regarding prenatal cannabis exposure is its potential impact on fetal growth that has significant implications for the long-term health of the offspring. There is accumulating evidence, though not unequivocal, that marijuana-exposed infants at birth have reduced weight [30,35,41,103] and/or head circumference [30] as well as decreased gestation length [19,33,35,41]. Most of these studies have examined newborns which reflect the cumulative impact of cannabis exposure throughout the entire prenatal developmental period. We recently investigated the potential effects of cannabis exposure on fetal growth during early stages of pregnancy. Developmental outcome variables (fetal weight, foot length, body length, and head circumference) were evaluated in women electing voluntary saline-induced abortions at a mid-gestational stage of pregnancy (weeks 17–22). Toxicological assays (maternal urine and fetal meconium) were used in conjunction with maternal report (detailed drug use and medical histories) to assign groups. Of 138 subjects who fulfilled the study inclusion criteria (e.g., no gross developmental genetic

disorder, valid maternal substance use report, expulsion time less than 24 h), approximately 32% were exposed in utero to cannabis. The cannabis group was defined based on having positive maternal report for cannabis use and/or mother's urine tested positive for THC and/or fetal meconium toxicology tested positive for THC. Similar to other studies [20,98], a high percentage of the cannabis users were found to smoke cigarettes (39%) and drink alcohol (39%) during pregnancy so statistical analyses were adjusted for maternal alcohol and cigarette use. Our findings revealed that cannabis-exposed fetuses had reduced weight (14 g) and foot length (0.1 cm) over the age range studied (weeks 17–22) [47]. These results substantiate the reports of a negative impact of early prenatal marijuana exposure on fetal growth even when adjusting for maternal use of other substances well known to impair fetal development.

THC mediates its physiological actions by binding to at least two receptor subtypes – CB_1 and CB_2 – which are inhibitory G-protein-coupled receptors (see e.g., [44]). Recent evidence suggest a potential third CB receptor, however, no "CB_3" gene has been discovered to date. The CB_1 is mainly found in the central nervous system and the CB_2 is located mainly in the immune system (see e.g., [44]). The CB_1 receptor (mRNA and receptor binding sites) is widely expressed in the adult brain in structures important for movement (e.g., basal ganglia, cerebellum), cognition and attention (e.g., cerebral cortex), as well as emotion and memory (e.g., amygdala, hippocampus) [42,43,58,61]. In contrast to the adult brain, only few CB_1 mRNA expression studies have been performed on the fetal brain, and these have mainly examined the rodent [9,10,14,75]. Such studies reveal that the CB_1 gene is already expressed at gestation day 14 in the rat [9]; comparable to early week 6 in humans. We have recently characterized the distribution of the CB_1 mRNA in the human fetus from 18 to 22 gestation weeks [95]. The most striking feature of the expression pattern in the human fetal brain was the predominance of the CB_1 mRNA in limbic structures, the amygdaloid complex (primarily the basal nuclear group) and hippocampus (in particular the CA2,3 regions) (Fig. 6). Low to moderate expression was also present in cell populations within the medial/ventral striatum with very low to undetectable expression in other brain regions. CB_1 receptor binding sites are also present during early human life [9,11,37] and they appear to represent functional receptors as evidenced by specific CB_1 agonist (WIN-55,212-2)-activated GTPγS binding at gestational day 16 in the rat [10]. The CB_1 receptors are also functional in the mid-gestational human fetal brain as verified by agonist-stimulated activation of GTPγS binding (Fig. 7); (Mato et al. [60]). Taken together, the anatomical imaging of the prenatal human CB_1 system carried out thus far clearly suggests that early exposure to cannabis could alter specific neural systems important for behaviors linked to emotional regulation and striatal function.

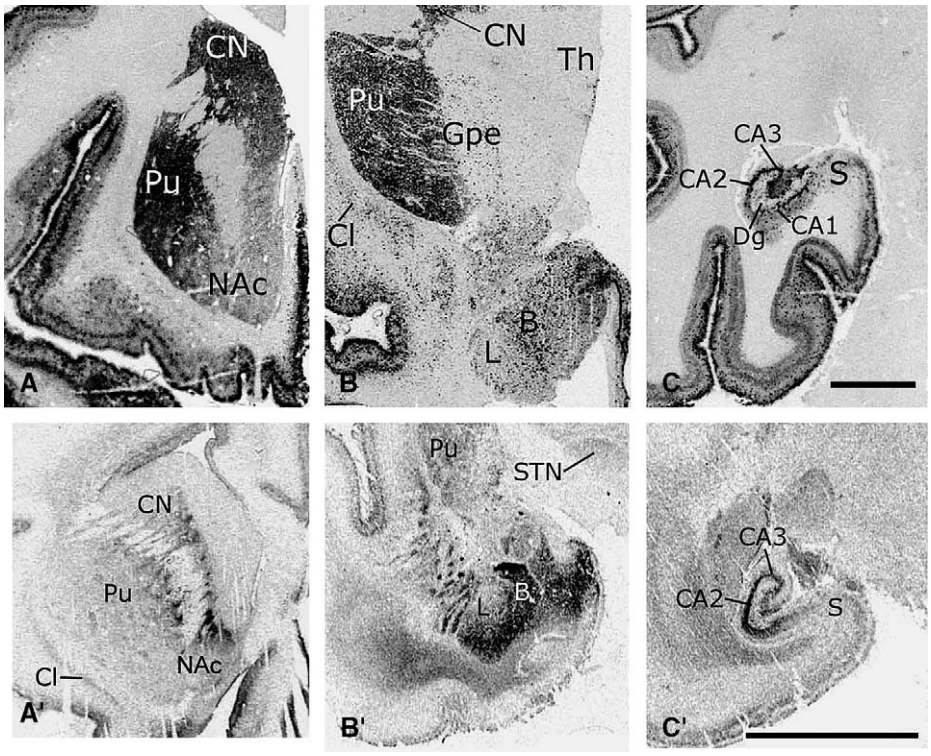

Fig. 6. Autoradiographic images showing the distribution of CB_1 mRNA expression in coronal sections in human adult (A, B, C) and mid-gestation fetal (A′, B′, C′) brains at the level of the nucleus accumbens (A, A′), amygdaloid complex (B, B′) and hippocampus (C, C′). Note the higher overall expression of the CB_1 mRNA to various structures in the adult versus fetal brain and the predominant expression of CB_1 mRNA to the fetal amygdaloid complex and hippocampus. B, basal amygdala nucleus; CA2/CA3, cornu ammoni 2/3; CL, claustrum; CN, caudate nucleus; Dg, dentate gyrus; GL, germinal layer; Gpe, globus pallidus external; Gpi, globus pallidus internal; L, lateral amygdala nucleus; Pu, putamen; S, subiculum. Scale bar = 1 cm. Modified from Wang et al. [94].

The majority of the data available regarding the effects of perinatal marijuana exposure arise from experimental animal studies. Of the existing animal data, there is evidence of a time- and brain region-dependent alteration for the effects of cannabis on neurotransmitter systems and there are significant sexual dimorphic differences in these effects. Perinatal cannabis exposure in animal models has been most associated with altered development of the dopamine and opioid neuropeptide systems. There are some inconsistent findings, but an overall greater number of studies report decreased tyrosine hydroxylase activity, content, immunoreactivity, and mRNA expression in subjects exposed perinatally to cannabinoids [12,73,74,90]. Such decreases have been predominantly evident in males. A number of studies have also provided evidence that dopamine receptors in the striatum and mesolimbic areas are also impaired as a consequence of THC exposure during perinatal development [34,73,74,93]. Impairment of the endogenous opioid neuropeptide system, primarily on enkephalin-related neural populations, is also evident in adulthood in association with early cannabis exposure. For example, perinatal THC leads to reduced proenkephalin mRNA levels in adult offspring [18].

Very limited information, however, currently exists as to the neurobiological consequences of in utero cannabis exposure in humans due to the technical challenges and complexity of studying the fetal human brain. As such, we recently modified the in situ hybridization histochemistry technique to image mRNA expression of neurobiological systems linked to cannabinoids in the human fetal brain. CB_1 as well as dopamine- and opioid neuropeptide-related genes were studied in a subgroup of our population of cannabis ($n=21$) and control ($n=21$) subjects. An important observation from these studies was that not all neural systems were affected by prenatal cannabis exposure. For example, no significant differences were detected between cannabis users and controls for the CB_1 mRNA in the caudal striatum (putamen), hippocampus, and amygdala or for the expression of dopamine D_1 and D_2 receptor mRNAs measured in the hippocampus and putamen. It is important to note, however, that maternal alcohol use did have a significant contribution to the D_1 and D_2 mRNA expression in the putamen. These findings are consistent with animal studies documenting significant prenatal alcohol effects on the striatal dopamine system [70]. A major finding of our investigation was that maternal marijuana use was significantly associated with reduced D_2 mRNA levels in the basolateral amygdala even when adjusting for alcohol exposure as a covariate [94]. Moreover, these alterations were predominantly evident in males. Another interesting observation was that the D_2 mRNA levels in the fetal amygdala were significantly correlated to the amount of marijuana use reported by the mothers. A large body of data has identified the amygdala as being critical for development of emotional behavior and negative mood states [96]. Depression symptoms have also been reported by children with prenatal marijuana exposure [39] and other amygdaloid-related abnormalities have been identified in marijuana-exposed children at school age (e.g., social behavior disturbances such as delinquency [38]. The abnormal D_2 mRNA expression evident in the human fetal amygdala may underlie some of the emotional and cognitive problems that were exhibited in the children with in utero exposure to cannabis since the amygdala is a key component of the mesocorticolimbic brain circuitry that processes information related to reward, motivation, emotion, and cognition [see Ref. [1]].

Consistent with previous animal studies, disturbances of the enkephalin opioid system are also evident in the human fetuses exposed in utero to cannabis. We recently observed reduced proenkephalin mRNA expression levels in the putamen (both in the patch, limbic-related, and matrix, sensorimotor-related compartments) in cannabis-exposed fetuses and the mRNA expression levels were directly correlated to maternal marijuana use. The data suggest that striatal enkephalin neuronal pathways that are involved in both limbic and motor functions are affected by early cannabis exposure. Our preliminary animal studies have in fact documented impaired heroin self-administration in adult rats exposed during development to THC. These findings support the hypothesis that early THC does have long-term influence on the development of the endogenous opioid neuropeptide system. We, however, observed no significant alterations for the dynorphin/kappa opioid system in either the putamen or amygdala in association with maternal cannabis use though there was a significant influence of alcohol exposure. These findings are again consistent with the well documented effects of prenatal alcohol on several neurotransmitter systems.

In summary, our studies show the potential to image mRNA expression of the human fetal brain which should help to

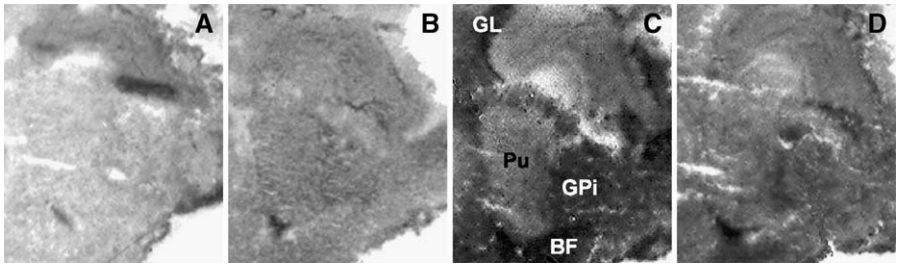

Fig. 7. CB_1 agonist (WIN 55, 212-2)-activated GTPγS binding in the human mid-gestational fetal brain. Non-specific binding (A); basal binding without agonist (B); agonist (10 μM WIN) activation (C); agonist and CB_1 antagonist (10 μM WIN+3 μM SR141716A; D). Note the activation of CB_1 agonist binding in, e.g., the globus pallidus (output structure of the striatum) which is blocked by CB_1 antagonist reflecting the functional coupling of the CB_1 receptors at this stage of fetal development. Abbreviation list in Fig. 6 legend.

provide insights about the early development of the human brain. Overall, the data to date strongly suggest that there are region- and gene-specific neural alterations associated with prenatal cannabis exposure in the human fetus.

Summary and Conclusions

The presentations in this symposium are representative of the first steps in what is likely to be a long path toward understanding the effects of exposure to drugs of the abuse on the human brain. But even the very preliminary findings presented here, gained in the study of a very small number of children, suggest that neuroimaging is likely to reveal effects on the brain and possible brain/behavioral correlations that are not likely to be appreciated through any other methodologies.

Dr. Benveniste's demonstration that PET and structural MRI can be combined in non-human primates points to the possibility that this model system can be used to detect, quantify, and localize drugs during fetal development as well as obtain metabolic data, and the effects of drugs on brain function, during the course of development. The paradigm that Dr. Benveniste and her colleagues are developing has enormous potential for longitudinal studies of in utero drug exposure and, moreover, by elegantly combining PET and MRI, the technique maximizes the strengths and minimizes the limitations of each of the modalities.

A number of NIDA-supported investigators have very comprehensively studied and documented the effects of prenatal drug exposure in cohorts of children that have been followed since before birth. Advances made in neuroimaging technologies afford the promise of linking empirical neurobiological findings with neurocognitive and behavioral characteristics that might be attributable to exposure to drugs of abuse. Dr. Marylou Behnke and her colleagues have followed a cohort of children in the Gainesville, Florida area since birth. These children, who are reaching adolescence, are now being studied with anatomical MRI, including DTI, a neuroimaging modality that has the potential to reveal abnormalities in the development of the connections among brain areas. In fact, the anatomical findings in this group thus far suggest alterations in brain areas related to neurocognitive functions that have now been shown to be affected by prenatal exposure to cocaine. Dr. Emmalee Bandstra is studying a cohort of cocaine-exposed children in the Miami area, also using structural MRI (including DTI) but along with MRS. Although these studies have really only just begun, the application of MRS to this cohort of children will add invaluable information on how prenatal exposure to cocaine might have long-lasting effects not only on the anatomical development of the brain but also on its metabolism and cellular integrity. Finally, Dr. Lynn Singer presented very intriguing anatomical MRI findings obtained in the study of a cohort of 7 to 8 year old cocaine-exposed children in the Cleveland area. Dr. Singer and her colleagues have demonstrated alterations in the area of the corpus callosum and the total grey matter volumes in the left occipital and right parietal lobes of the exposed children. The alterations in corpus callosum size were positively correlated with the amount of cocaine that the mother had taken but, more importantly perhaps, the alterations in brain structure were correlated with a number of neurocognitive measures that are known to involve those brain areas.

Finally, Dr. Yasmin Hurd presented preliminary data on the effects of marijuana exposure on human brain development in the context of the ontogeny of specific receptors associated with cannabinoid systems. The findings thus far indicate that exposure to marijuana alters dopamine function in the amygdala and related mesolimbic system, the brain circuits which mediate reward. There is more complexity in the effects than might have been expected, in that exposure to marijuana in utero differed in different regions of the brain and even as a function of the gender of the fetus.

The findings presented in this symposium, while very preliminary, strongly emphasize the promise that the evolution of neuroimaging methodologies holds for understanding the ways in which drug exposure affects the development of the brain. With the methodologies now available, we can begin to explore the detailed anatomy of the brain during development, the maturation of connections between brain areas, the metabolic and functional state of the brain and even the ontogeny of specific neurotransmitter systems. Moreover, the resolution and sensitivity of neuroimaging methods is continually improving, as are our capabilities in analyzing the data that they provide. Analyses along any single dimension, whether anatomical, chemical, functional, behavioral, or cognitive can only provide a limited perspective on human development and the ways in which it can be altered. Combinations and comparisons of data along many dimensions, such as the discovery of brain/behavior correlations obtained through neuroimaging studies like those described here, will undoubtedly provide otherwise unobtainable insights into the effects of drugs on human brain development.

Acknowledgements

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References

[1] R. Adolphs, Neural systems for recognizing emotion, Curr. Opin. Neurobiol. 12 (2002) 169–177.

[2] E.S. Bandstra, C.E. Morrow, J.C. Anthony, S.S. Churchill, D.D. Chitwood, B.M. Steele, A.Y. Ofir, L. Xue, Intrauterine growth of full-term infants: impact of prenatal cocaine exposure, Pediatrics 108 (2001) 1309–1319.

[3] E.S. Bandstra, C.E. Morrow, J.C. Anthony, V.H. Accornero, P.A. Fried, Longitudinal investigation of task persistence and sustained attention in children with prenatal cocaine exposure, Neurotoxicol. Teratol. 23 (2001) 545–559.

[4] E.S. Bandstra, C.E. Morrow, V.H. Accornero, A.L. Johnson, A.L. Vogel, L. Xue, J.C. Anthony, The Miami Prenatal Cocaine Study: neuropsychological function at age 7 years, "Cocaine Kids Go To School" at the Society for Research in Child Development Biennial Meeting, Tampa, FL, April, 2003.

[5] M. Behnke, F.D. Eyler, M. Conlon, K. Wobie, N.S. Woods, W. Cumming, Incidence and description of structural brain abnormalities in cocaine-exposed newborns, J. Pediatr. 132 (1998) 291–294.
[6] M. Behnke, F.D. Eyler, C.W. Garvan, K. Wobie, W. Hou, Cocaine exposure and developmental outcome from birth to 6 months, Neurotoxicol. Teratol. 24 (2002) 283–295.
[7] H. Benveniste, J.S. Fowler, W.D. Rooney, D.H. Moller, W.W. Backus, D. A. Warner, P. Carter, P. King, B. Scharf, D.A. Alexoff, Y. Ma, P. Vaska, D. Schlyer, N. Volkow, Maternal–fetal in vivo imaging: a combined PET and MRI study, J. Nucl. Med. 44 (2003) 1522–1530.
[8] L. Berglund, J. Andersson, A. Lilja, B.S. Lindberg, M. Gebre-Medhin, G. Antoni, P. Bjurling, B. Langstrom, H. Lundqvist, Amino acid transport across the placenta measured by positron emission tomography and analyzed by compartment modelling, J. Perinat. Med. 18 (1990) 89–100.
[9] F. Berrendero, L. Garcia-Gil, M.L. Hernandez, J. Romero, M. Cebeira, R. de Miguel, J.A. Ramos, J.J. Fernandez-Ruiz, Localization of mRNA expression and activation of signal transduction mechanisms for cannabinoid receptor in rat brain during fetal development, Development 125 (1998) 3179–3188.
[10] F. Berrendero, N. Sepe, J.A. Ramos, V. Di Marzo, J.J. Fernandez-Ruiz, Analysis of cannabinoid receptor binding and mRNA expression and endogenous cannabinoid contents in the developing rat brain during late gestation and early postnatal period, Synapse 33 (1999) 181–191.
[11] A. Biegon, I.A. Kerman, Autoradiographic study of pre- and postnatal distribution of cannabinoid receptors in human brain, Neuroimage 4 (2001) 1463–1468.
[12] A. Bonnin, R. de Miguel, J.G. Castro, J.A. Ramos, J.J. Fernandez-Ruiz, Effects of perinatal exposure to delta 9-tetrahydrocannabinol on the fetal and early postnatal development of tyrosine hydroxylase-containing neurons in rat brain, J. Mol. Neurosci. 7 (1996) 291–308.
[13] F.L. Bookstein, P.D. Sampson, A.P. Streissguth, P.D. Connor, Geometric morphometrics of corpus callosum and subcortical structures in the fetal-alcohol-affected brain, Teratology 64 (2001) 4–32.
[14] N.E. Buckley, S. Hansson, G. Harta, E. Mezey, Expression of the CB1 and CB2 receptor messenger RNAs during embryonic development in the rat, Neuroscience 82 (1998) 1131–1149.
[15] E. Carrow-Woolfolk, Comprehensive Assessment of Spoken Language (CASL), American Guidance Service, Circle Pines, MN, 1999.
[16] L. Chang, C.M. Mehringer, T. Ernst, R. Melchor, H. Myers, D. Forney, P. Satz, Neurochemical alterations in asymptomatic abstinent cocaine users: a proton magnetic resonance spectroscopy study, Biol. Psychiatry 42 (1997) 1105–1114.
[17] D.L. Collins, A.P. Zijdenbos, W.F.C. Baare, and A.C. Evans, ANIMAL+ INSECT: improved cortical structure segmentation. Proceedings of the 16th International Conference on Information Processing in Medical Imaging (IPMI), vol 1613 of LNCS, pp. 210–223.
[18] J. Corchero, L. Garcia-Gil, J. Manzanares, J.J. Fernandez-Ruiz, J.A. Fuentes, J.A. Ramos, Perinatal delta9-tetrahydrocannabinol exposure reduces proenkephalin gene expression in the caudate-putamen of adult female rats, Life Sci. 63 (1998) 843–850.
[19] M.D. Cornelius, P.M. Taylor, D. Geva, N.L. Day, Prenatal tobacco and marijuana use among adolescents: effects on offspring gestational age, growth, and morphology, Pediatrics 95 (1995) 738–743.
[20] N.L. Day, G.A. Richardson, Prenatal marijuana use: epidemiology, methodologic issues, and infant outcome, Clin. Perinatol. 18 (1991) 77–91.
[21] C.L. DeVane, J.W. Simpkins, R.L. Miller, S.B. Braun, Tissue distribution of cocaine in the pregnant rat, Life Sci. 45 (1989) 1271–1276.
[22] C.L. DeVane, D.J. Burchfield, R.M. Abrams, R.L. Miller, S.B. Braun, Disposition of cocaine in pregnant sheep: I. Pharmacokinetics, Dev. Pharmacol. Ther. 16 (1991) 123–129.
[23] R. Dominguez, A. Aguirre Vila-Coro, J.M. Slopis, T.P. Bohan, Brain and ocular abnormalities in infants with in utero exposure to cocaine and other street drugs, Am. J. Dis. Child. 145 (1991) 688–695.
[24] D. Dow-Edwards, Fetal and maternal cocaine levels peak rapidly following intragastric administration in the rat, J. Subst. Abuse 2 (1990) 427–437.
[25] D.L. Dow-Edwards, Developmental toxicity of cocaine: mechanisms of action, in: M. Lewis, M. Bendersky (Eds.), Mothers, Babies, and Cocaine: The Role of Toxins in Development, Lawrence Erlbaum Associates, Inc., Hillsdale, NJ, 1995, pp. 5–17.
[26] M.A. Eckert, C.M. Leonard, T.L. Richards, E.H. Aylward, J. Thompson, V.W. Berninger, Anatomical correlates of dyslexia: frontal and cerebellar findings, Brain 126 (2003) 482–494.
[27] F.D. Eyler, M. Behnke, M. Conlon, N.S. Woods, K. Wobie, Birth outcome from a prospective, matched study of prenatal crack/cocaine use: I. Interactive and dose effects on health and growth, Pediatrics 101 (1998) 229–237.
[28] F.D. Eyler, M. Behnke, M. Conlon, N.S. Woods, K. Wobie, Birth outcome from a prospective, matched study of prenatal crack/cocaine use: II. Interactive and dose effects on neurobehavioral assessment, Pediatrics 101 (1998) 237–241.
[29] V.B. Faden, B.I. Graubard, Maternal substance use during pregnancy and developmental outcome at age three, J. Subst. Abuse 12 (2000) 329–340.
[30] D.M. Fergusson, L.J. Horwood, K. Northstone, Maternal use of cannabis and pregnancy outcome, BJOG 109 (2002) 21–27.
[31] D.A. Frank, K.M. McCarten, C.D. Robson, M. Mirochnick, H. Cabral, H. Park, B. Zuckerman, Level of in utero cocaine exposure and neonatal ultrasound findings, Pediatrics 104 (1999) 1101–1105.
[32] P.A. Fried, A.M. Smith, A literature review of the consequences of prenatal marijuana exposure. An emerging theme of a deficiency in aspects of executive function, Neurotoxicol. Teratol. 23 (2001) 1–11.
[33] P.A. Fried, B. Watkinson, A. Willan, Marijuana use during pregnancy and decreased length of gestation, Am. J. Obstet. Gynecol. 150 (1984) 23–27.
[34] L. Garcia, R. de Miguel, J.A. Ramos, J.J. Fernandez-Ruiz, Perinatal delta 9-tetrahydrocannabinol exposure in rats modifies the responsiveness of midbrain dopaminergic neurons in adulthood to a variety of challenges with dopaminergic drugs, Drug Alcohol Depend. 42 (1996) 155–166.
[35] G.T. Gibson, P.A. Baghurst, D.P. Colley, Maternal alcohol, tobacco and cannabis consumption and the outcome of pregnancy, Aust. N. Z. J. Obstet. Gynaecol. 23 (1983) 15–19.
[36] J.N. Giedd, F.X. Castellanos, B.J. Casey, et al., Quantitative morphology of the corpus callosum in attention deficit hyperactivity disorder, Am. J. Psychiatry 151 (1994) 665–669.
[37] M. Glass, M. Dragnunow, R.L. Faull, Cannabinoid receptors in the human brain: a detailed anatomical quantitative autoradiographic study in the fetal, neonatal and adult human brain, Neuroscience 77 (1997) 299–318.
[38] L. Goldschmidt, N.L. Day, G.A. Richardson, Effects of prenatal marijuana exposure on child behavior problems at age 10, Neurotoxicol.Teratol. 22 (2000) 325–336.
[39] K.A. Gray, G.A. Richardson, N.L. Day, Prenatal marijuana use and child depression at age ten, Neurotoxicol. Teratol. 19 (1997) 245.
[40] P. Hartvig, B.S. Lindberg, A. Lilja, H. Lundqvist, B. Langstrom, A. Rane, Positron emission tomography in studies on fetomaternal disposition of opioids, Dev. Pharmacol. Ther. 12 (1989) 74–80.
[41] E.E. Hatch, M.B. Bracken, Effect of marijuana use in pregnancy on fetal growth, Am. J. Epidemiol. 124 (1986) 986–993.
[42] M. Herkenham, A. Lynn, M. Little, M. Johnson, L. Melvin, B. De Costa, K. Rice, Cannabinoid receptor localization in brain, Proc. Natl. Acad. Sci. U. S. A. 87 (1990) 1932.
[43] M. Herkenham, A.B. Lynn, M.R. Johnson, L.S. Melvin, B.R. de Costa, K.C. Rice, Characterization and localization of cannabinoid receptors in rat brain: a quantitative in vitro autoradiographic study, J. Neurosci. 11 (1991) 563–583.
[44] A.C. Howlett, F. Barth, T.I. Bonner, G. Cabral, P. Casellas, W.A. Devane, C.C. Felder, M. Herkenham, K. Mackie, B.R. Martin, R. Mechoulam, R.G. Pertwee, International Union of Pharmacology. XXVII. Classification of cannabinoid receptors, Pharmacol. Rev. 54 (2002) 161–202.
[45] W.L. Huang, C.G. Harper, S.F. Evans, J.P. Newnham, S.A. Dunlop, Repeated prenatal corticosteroid administration delays myelination of

the corpus callosum in fetal sheep, Int. J. Dev. Neurosci. 19 (2001) 415–425.
[46] J.V. Hunter, Z.J. Wang, MR spectroscopy in pediatric neuroradiology, Magn. Reson. Imaging Clin. N. Am. 9 (2001) 165–189 (ix).
[47] Y.L. Hurd, X. Wang, V. Anderson, O. Beck, H. Minkoff, D. Dow-Edwards, Marijuana impairs growth in mid-gestation fetuses, Neurotoxicol. Teratol. (2005) 221–229.
[48] D.B. Kandel, K. Chen, Extent of smoking and nicotine dependence in the United States: 1991–1993, Nicotine Tob. Res. 2 (2000) 263–274.
[49] M. Korkman, U. Kirk, S. Kemp, NEPSY: A Developmental Neuropsychological Assessment Manual, The Psychological Corporation, San Antonio, TX, 1998.
[50] J.M. Lauder, Discussion: neuroteratology of cocaine-relationship to developing monoamine systems, in: M.M. Kilbey, J. Ashgar (Eds.), Methodological Issues in Controlled Studies on Effects of Prenatal Exposure to Drug Abuse, NIDA Res. Monogr. vol. 114, 1991, pp. 233–247.
[51] C.M. Leonard, M.A. Eckert, L.J. Lombardino, R. Oakland, J. Kranzler, C. M. Mohr, W.M. King, A. Freeman, Anatomical risk factors for phonological dyslexia, Cereb. Cortex 11 (2001) 148–157.
[52] B.A. Lewis, L.T. Singer, S. Minnes, R. Arendt, P. Weishampel, E.J. Short, N. Klein, M.O. Min, Four-year language outcomes of children exposed to cocaine in utero, Neurotoxicol. Teratol. 26 (2004) 617–627.
[53] S.J. Li, Y. Wang, J. Pankiewicz, E.A. Stein, Neurochemical adaptation to cocaine abuse: reduction of N-acetyl aspartate in thalamus of human cocaine abusers, Biol. Psychiatry 45 (1999) 1481–1487.
[54] T.J. Linares, L.T. Singer, H.L. Kirchner, M.O. Min, S. Minnes, Mental health outcomes in cocaine exposed children at age six, J. Pediatr. Psychol. 1 (2006) 85–97.
[55] D. Lindhout, J.G. Omtzigt, M.C. Cornel, Spectrum of neural-tube defects in 34 infants prenatally exposed to antiepileptic drugs, Neurology 42 (Suppl. 5) (1992) 111–118.
[56] E.A. Link, D.E. Weese-Mayer, S.E. Bryd, Magnetic resonance imaging in infants exposed to cocaine prenatally: a preliminary report, Clin. Pediatr. 30 (1991) 506–508.
[57] C.F. Mactutus, L.D. Fechter, Perinatal hypoxia: implications for mammalian development, in: E.P. Riley, C.V. Vorhees (Eds.), Handbook of Behavioral Teratology, Plenum, New York, 1986, pp. 427–470.
[58] P. Mailleux, M. Parmentier, J.J. Vanderhaeghen, Distribution of cannabinoid receptor messenger RNA in the human brain: an in situ hybridization histochemistry with oligonucleotides, Neurosci. Lett. 143 (1992) 200–204.
[59] C.J. Malanga, B.E. Kosofsky, Mechanisms of action of drugs of abuse on the developing fetal brain, Clin. Perinatol. 26 (1999) 17–37.
[60] S. Mato, E. Del Olmo, A. Pazos, Ontogenetic development of cannabinoid receptor expression and signal transduction functionality in the human brain, Eur. J. Neurosci. 17 (2003) 1747–1754.
[61] L.A. Matsuda, T.I. Bonner, S.J. Lolait, Localization of cannabinoid receptor mRNA in rat brain, J. Comp. Neurol. 327 (1993) 535–550.
[62] L.C. Mayes, Developing brain and in utero cocaine exposure: effects on neural ontogeny, Dev. Psychopathol. 11 (1999) 685–714.
[63] H.O. Morishima, M.A. Heymann, A.M. Rudolph, C.T. Barrett, L.S. James, Transfer of lidocaine across the sheep placenta to the fetus, Am. J. Obstet. Gynecol. 122 (1975) 581–588.
[64] C.E. Morrow, E.S. Bandstra, J.C. Anthony, A.Y. Ofir, L. Xue, M. Reyes, Influence of prenatal cocaine exposure on full-term infant neurobehavioral functioning, Neurotoxicol. Teratol. 23 (2001) 533–544.
[65] C.E. Morrow, E.S. Bandstra, J.C. Anthony, A.Y. Ofir, L.H. Xue, M.B. Reyes, Influence of prenatal cocaine exposure on early language development: longitudinal findings from four months to three years of age, J. Dev. Behav. Pediatr. 24 (2003) 39–50.
[66] C.E. Morrow, J. Culbertson, V.H. Accornero, L. Xue, J.C. Anthony, E.S. Bandstra, Estimated risk of developing a learning disability by age 7 among prenatally cocaine-exposed children, Presented at the International Neuropsychological Society Annual Scientific Meeting, Baltimore, Maryland, February, 2004, 2004.
[67] M. Navarro, P. Rubio, F.R. de Fonseca, Behavioral consequences of maternal exposure to natural cannabinoids in rats, Psychopharmacology 122 (1995) 1–14.
[68] K. Ojima, H. Abiru, H. Matsumoto, Y. Fukui, Effects of postnatal exposure to cocaine on the development of the rat corpus callosum, Reprod. Toxicol. 10 (1996) 221–225.
[69] T. Paus, N. Otaky, Z. Caramanos, D. MacDonald, A. Zijdenbos, D. D'Avirro, D. Gutmans, C. Holmes, F. Tomaiuolo, A.C. Evans, In vivo morphometry of the intrasulcal gray matter in the human cingulate, paracingulate, and superior-rostral sulci: hemispheric asymmetries, gender differences, and probability maps, J. Comp. Neurol. 376 (1996) 664–673.
[70] S. Randall, J.H. Hannigan, In utero alcohol and postnatal methylphenidate: locomotion and dopamine receptors, Neurotoxicol. Teratol. 21 (1999) 587–593.
[71] G.A. Richardson, C. Ryan, J. Willford, N.L. Day, L. Goldschmidt, Prenatal alcohol and marijuana exposure. Effects on neuropsychological outcomes at 10 years, Neurotoxicol. Teratol. 24 (2002) 309–320.
[72] M.J. Rivkin, Developmental neuroimaging of children using magnetic resonance techniques, Ment. Retard. Dev. Disabil. Res. Rev. 6 (2000) 68–80.
[73] F. Rodriguez de Fonseca, M. Cebeira, M.L. Hernandez, J.A. Ramos, J.J. Fernandez-Ruiz, Changes in brain dopaminergic indices induced by perinatal exposure to cannabinoids in rats, Dev. Brain Res. 51 (1990) 237–240.
[74] F. Rodriguez de Fonseca, M. Cebeira, J.J. Fernandez-Ruiz, M. Navarro, J. A. Ramos, Effects of pre- and perinatal exposure to hashish extracts on the ontogeny of brain dopaminergic neurons, Neuroscience 43 (1991) 713–723.
[75] J. Romero, E. Garcia-Palomero, F. Berrendero, L. Garcia-Gil, M.L. Hernandez, J.A. Ramos, J.J. Fernandez-Ruiz, Atypical location of cannabinoid receptors in white matter areas during rat brain development, Synapse 26 (1997) 317–323.
[76] A.M. Rudolph, M.A. Heymann, The circulation of the fetus in utero, Circ. Res. 21 (1967) 163–184.
[77] N. Salibi, M. Brown, Clinical MR Spectroscopy: First Principles, John-Wiley, New York, 1998.
[78] SAMHSA, Substance Abuse and Mental Health Service Administration, Results from the 2001 National Household Survey on Drug Abuse NHSDA Series H-17, DHHS Publication No. SMA 02-3758, Rockville, MD, 2002.
[79] M.D. Schreiber, Fetal cerebral vascular effects of cocaine exposure, in: P. V. Thadani (Ed.), NIDA Res. Monogr., vol. 158, 1995, pp. 67–87.
[80] L. Singer, R. Arendt, J. Fagan, S. Minnes, A. Salvator, T. Bolek, M. Becker, Neonatal visual information processing in cocaine-exposed and non-exposed infants, Inf. Behav. Dev. 22 (1999) 1–15.
[81] L.T. Singer, R. Arendt, S. Minnes, K. Farkas, A. Salvator, Neurobehavioral outcomes of cocaine-exposed infants, Neurotoxicol. Teratol. 22 (2000) 1–14.
[82] L.T. Singer, R. Arendt, S. Minnes, A. Salvator, A.C. Siegel, A. Lewis, Developing language outcomes of cocaine exposed infants, Pediatrics 107 (2001) 1–8.
[83] L.T. Singer, R. Arendt, S. Minnes, K. Farkas, A. Salvator, H.L. Kirchner, R. Kliegman, Cognitive and motor outcomes of cocaine-exposed infants, JAMA 287 (2002) 1952–1960.
[84] L.T. Singer, A. Salvator, R. Arendt, S. Minnes, K. Farkas, R. Kliegman, Effects of cocaine/polydrug exposure and maternal psychological distress on infant birth outcomes, Neurotoxicol. Teratol. 24 (2002) 127–135.
[85] L.T. Singer, S. Minnes, R.E. Arendt, K. Farkas, E. Short, B. Lewis, N. Klein, S. Russ, M.O. Min, H.L Kirchner, Cognitive outcomes of preschool children with prenatal cocaine exposure, JAMA 291 (2004) 2448–2456.
[86] L.M. Smith, L. Chang, M.L. Yonekura, K. Gilbride, J. Kuo, R.E. Poland, I. Walot, T. Ernst, Brain proton magnetic resonance spectroscopy and imaging in children exposed to cocaine in utero, Pediatrics 107 (2001) 227–231.
[87] D.A. Snowdon, S.J. Kemper, J.A. Mortimer, L.H. Greiner, D.R. Wekstein, W.R. Markesbery, Linguistic ability in early life and cognitive

function and Alzheimer's disease in late life. Findings from the Nun Study, JAMA 275 (1996) 528–532.
[88] B.J. Soher, K. Young, V. Govindaraju, A.A. Maudsley, Automated spectral analysis III: Application to in vivo proton MR spectroscopy and spectroscopic imaging, Magn. Reson. Med. 40 (1998) 822–831.
[89] E. Strauss, J. Wada, M. Hunter, Callosal morphology and performance on intelligence test, J. Clin. Exp. Neuropsychol. 16 (1994) 79–83.
[90] I. Suarez, G. Bodega, J.A. Ramos, J.J. Fernandez-Ruiz, B. Fernandez, Neuronal and astroglial response to pre- and perinatal exposure to delta-9-tetra-hydrocannabinol in the rat substantia nigra, Dev. Neurosci. 22 (2000) 253–263.
[91] J.J. Volpe, Neurology of the Newborn, W.B. Saunders, Co., Philadelphia, PA, 1987.
[92] F.A. Wagner, J.C. Anthony, From first drug use to drug dependence; developmental periods of risk for dependence upon marijuana, cocaine, and alcohol, Neuropsychopharmacology 26 (2002) 479–488.
[93] D.E. Walters, L.A. Carr, Perinatal exposure to cannabinoids alters neurochemical development in rat brain, Pharmacol. Biochem. Behav. 29 (1988) 213–216.
[94] X. Wang, D. Dow-Edwards, E. Keller, Y.L. Hurd, Preferential limbic expression of the cannabinoid receptor mRNA in the human fetal brain, Neuroscience 118 (2003) 681–694.
[95] X. Wang, D. Dow-Edwards, V. Andersen, H. Minkoff, Y.L. Hurd, In utero marijuana exposure associated with abnormal amygdala D2 gene expression in the human fetus, Biol. Psychiatry 56 (2004) 819–825.
[96] D. Wechsler, Wechsler Preschool and Primary Scale of Intelligence-Revised (WPPSI-R) Manual, Psychological Corporation, New York, NY, 1989.
[97] P.J. Whalen, L.M. Shin, L.H. Somerville, A.A. McLean, H. Kim, Functional neuroimaging studies of the amygdala in depression, Semin. Clin. Neuropsychiatry 7 (2002) 234–242.
[98] F.R. Witter, J.R. Niebyl, Marijuana use in pregnancy and pregnancy outcome, Am. J. Perinatol. 7 (1990) 36–38.
[99] J.R. Woods Jr., Adverse consequences of prenatal illicit drug exposure, Curr. Opin. Obstet. Gynecol. 8 (1996) 403–411.
[100] D. Yurgelun-Todd, P. Renshaw, MRS in childhood psychiatric disorders, in: M. Ernst, J. Rumsey (Eds.), Functional Neuroimaging in Child Psychiatry, Cambridge University Press, Cambridge, UK, 2000, pp. 59–76.
[101] M. Zhou, Z.M. Song, M.S. Lidow, Pharmacokinetics of cocaine in maternal and fetal rhesus monkeys at mid-gestation, J. Pharmacol. Exp. Ther. 297 (2001) 556–562.
[102] A.P. Zijdenbos, R. Forghani, A.C. Evans, Automatic "pipeline" analysis of 3-D MRI data for clinical trials: application to multiple sclerosis, IEEE Trans. Med. Imag. 21 (2002) 1280–1291.
[103] B. Zuckerman, D.A. Frank, R. Hingson, H. Amaro, S.M. Levenson, H. Kayne, S. Parker, R. Vinci, K. Aboagye, L.E. Fried, et al., Effects of maternal marijuana and cocaine use on fetal growth, N. Engl. J. Med. 320 (1989) 762–768.