



## Smaller subcortical volumes and cognitive deficits in children with prenatal methamphetamine exposure

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### Abstract

The purpose of this pilot study was to examine possible neurotoxic effects of prenatal methamphetamine (Meth) exposure on the developing brain and on cognition. Meth-exposed children ( $n=13$ ) and unexposed control subjects ( $n=15$ ) were evaluated with MRI. Global brain volumes and regional brain structures were quantified. Ten Meth-exposed and nine unexposed children also completed neurocognitive assessments. Meth-exposed children scored lower on measures of visual motor integration, attention, verbal memory and long-term spatial memory. There were no differences among the groups in motor skills, short delay spatial memory or measures of non-verbal intelligence. Despite comparable whole brain volumes in each group, the Meth-exposed children had smaller putamen bilaterally ( $-17.7\%$ ), smaller globus pallidus (left:  $-27\%$ , right:  $30\%$ ), smaller hippocampus volumes (left:  $-19\%$ , right:  $-20\%$ ) and a trend for a smaller caudate bilaterally ( $-13\%$ ). The reduction in these brain structures correlated with poorer performance on sustained attention and delayed verbal memory. No group differences in volumes were noted in the thalamus, midbrain or the cerebellum. In summary, compared with the control group, children exposed to Meth prenatally exhibit smaller subcortical volumes and associated neurocognitive deficits. These preliminary findings suggest prenatal Meth exposure may be neurotoxic to the developing brain.

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## 1. Introduction

Methamphetamine (Meth) is a major drug of abuse in the United States and in Asia. It is a potent sympathomimetic that causes a massive release of dopamine in the brain, inducing experiences of euphoria, increased alertness and confidence in users. Women using Meth during pregnancy have an increased rate of premature delivery and placental abruption (Eriksson et al., 1978). Cases of cardiovascular collapse, seizures and death have also been reported with maternal Meth use (Elliott and Rees, 1990). In addition, prenatal Meth exposure has been associated with intrauterine growth restriction, cardiac defects and clefting (Plessinger, 1998), as well as abnormal brain metabolism (Smith et al., 2001).

Meth-using adults exhibit significant neurocognitive dysfunction, including deficits in memory, attention and manipulation of information (Simon et al., 2000). The effects of prenatal Meth exposure on the developing fetal central nervous system are unknown. Hansen et al. (1993) found that a group of Meth and cocaine exposed newborns demonstrated significantly reduced visual recognition memory, a measure correlated with subsequent IQ, compared with unexposed controls. The scant developmental data available in Meth-exposed children suggest that they have disorders of executive function manifested by aggressive behavior and hyperactivity (Billing et al., 1994).

Limited information is available regarding brain structural alterations in Meth-exposed children. Cranial ultrasound studies of children exposed to prenatal Meth and cocaine demonstrated an increased incidence of intraventricular hemorrhage and white matter densities (Dixon and Bejar, 1989). To our knowledge, no studies of brain anatomy utilizing MRI or computerized tomography have been conducted in children exposed primarily to Meth in utero. Extending our prior observation of abnormal metabolism in children exposed to Meth in utero (Smith et al., 2001), the present study examined brain morphometry on MRI of children exposed to Meth in utero compared with brains of unexposed children. For this preliminary study, we evaluated global intracerebral brain volumes, subcortical brain structures and cerebellum, especially since prior neuroimaging studies of adult methamphetamine abusers showed potential neurotoxic effects in the subcortical brain structures, namely

decreased dopamine transporters, brain metabolism and perfusion (Ernst et al., 2000; Volkow et al., 2001a,b,c,d; Chang et al., 2002). In addition, a battery of neurocognitive tests were administered assessing measures of global cognitive functioning, with additional emphasis on tests of attention and motor function. Based on the known effects of methamphetamine on the dopaminergic terminals, we hypothesized that children with prenatal methamphetamine exposure would show smaller striatal structures (putamen, caudate, globus pallidus) and cerebellum, and that these changes would be related to their cognitive deficits, especially on tasks that involve attention and motor integration.

## 2. Methods

Study participants included 13 children with a history of Meth exposure in utero ( $6.9 \pm 3.5$  years, range 3–16 years, 9 females and 4 males) and a healthy control group of 15 children without a history of drug exposure ( $7.8 \pm 3.2$  years, range 3–15 years, 9 females, 6 males). Some of the subjects from each group participated in a brain proton magnetic resonance spectroscopy study previously reported (Smith et al., 2001). Subjects from the two groups were matched only by age and gender proportion, but both groups were recruited from the same population of predominately lower and middle socioeconomic status. Children were included in the exposed group only if the mother was Meth-dependent by DSM-IV criteria for at least two thirds of the pregnancy. Diagnosis of Meth dependence was determined by detailed interviews and follow-up visits with the mothers and positive urine tests for Meth during the prenatal period when available. This information was available since the majority of children were recruited from the greater Los Angeles area from a state-funded drug treatment program at the Harbor-UCLA Medical Center. Drug-using women were referred to this drug treatment program from local area hospitals and community clinics. After complete description of the study, written informed consent, approved by the Institutional Review Board at Harbor-UCLA Medical Center, was obtained from the parent or the legal guardian for each of the children, and children older than 8 years also signed an assent form.

Exclusion criteria for the Meth-exposed children included: (1) infantile gestation age less than 37 weeks; (2) diagnosis of developmental delay, impaired growth, seizure disorders or attention deficit hyperactivity disorder; (3) presence of implanted metallic objects; (4) significant maternal illness during gestation (e.g., sickle cell disease, mental retardation), dependence on other illicit drugs (except Meth) or alcohol. Exclusion criteria for the unexposed children included 1–4 and any exposure to illicit drugs during gestation. The primary language was English for all children participating in the study.

### 2.1. MRI

The MRI studies were performed on a clinical 1.5 T General Electric scanner (Sigma 5.8, Milwaukee, WI). The MRI began with a sagittal T<sub>1</sub>-weighted localizer (Echo Time/Relaxation Time=11/500 ms, 4-mm slice thickness, 1-mm gap, 24-cm field of view), followed by a coronal fast double spin echo scan (Echo Time<sub>1</sub>/Echo Time<sub>2</sub>/Relaxation Time=17/102/4000 ms, 5-mm slice thickness, no gap, 24-cm field of view). Next, an axial fast inversion recovery scan was performed (Echo Time/Inversion Time/Relaxation Time=32/120/4000 ms, 3.5-mm slice thickness, no gap, 24-cm field of view). The sagittal and coronal images were used to screen for possible gross brain abnormalities (e.g., silent infarcts or vascular malformations). The inversion recovery sequence yielded contiguous brain slices and excellent contrast between the signal intensities from white matter, gray matter and cerebrospinal fluid (CSF); therefore, these images were used for morphometric analyses. Chloral hydrate (50 mg/kg) was required for sedation during the MR studies in only one Meth-exposed child and none of the unexposed children.

### 2.2. MRI morphometry

MR images were transferred to a DEC-ALPHA (Digital Equipment Corporation; Maynard, MA) workstation. Using an MRI segmentation program developed at our laboratory, the inversion recovery scans were processed automatically to segment the brain from the surrounding brain structures (skull, muscle, skin, etc.) (Itti et al., 1997). From the ex-

tracted brain, the global intracerebral brain volume and global CSF volume were determined automatically, and the whole brain tissue volume was calculated by subtracting the global CSF volume (including intradural and intraventricular CSF) from the global intracerebral brain volume. Gray and white matter were not segmented; only brain parenchyma was separated from CSF. Right and left brain regions of interest (ROIs), including the midbrain, thalamus, globus pallidus, caudate, putamen, cerebellar hemispheres and hippocampus, were manually outlined on each slice where these structures were well visualized and enlarged for the measurements (Fig. 1). The ROIs also were expressed as percent (%) of total brain tissue volume (excluding global CSF). To minimize multiple comparisons, we focused our measurements only on these brain regions; the regions were also selected based on prior imaging studies of adult methamphetamine abusers that showed potential neurotoxic effects in the subcortical brain structures, namely decreased dopamine transporters, brain metabolism and perfusion (Ernst et al., 2000; Volkow et al., 2001a,b,c,d; Chang et al., 2002), as well as in regions that have high densities of dopamine transporters (putamen) or dopamine receptors (putamen, cerebellum). To minimize potential bias, the ROIs were delineated by a trained investigator blinded to the drug exposure status of the subjects, and were checked by another investigator, also blinded to the subject status. For each brain structure, the area of each slice was multiplied by the slice thickness to determine the volume within each slice, and all slices containing the same structure were then summed to yield the volume of each structure. The intra-subject volumes, segmented by the same observer 2 weeks apart, showed high reproducibility ( $r=0.98$ ), and the inter-subject reproducibility was also high ( $r=0.96$ ) as reported previously (Chang et al., 2000).

### 2.3. Neuropsychological assessments

Ten of the Meth-exposed children and nine unexposed control children also completed a battery of neuropsychological tests, designed by the pediatric neuropsychologist (CL), within 1 month of the MRI. The testing time was approximately 2 h, and drug

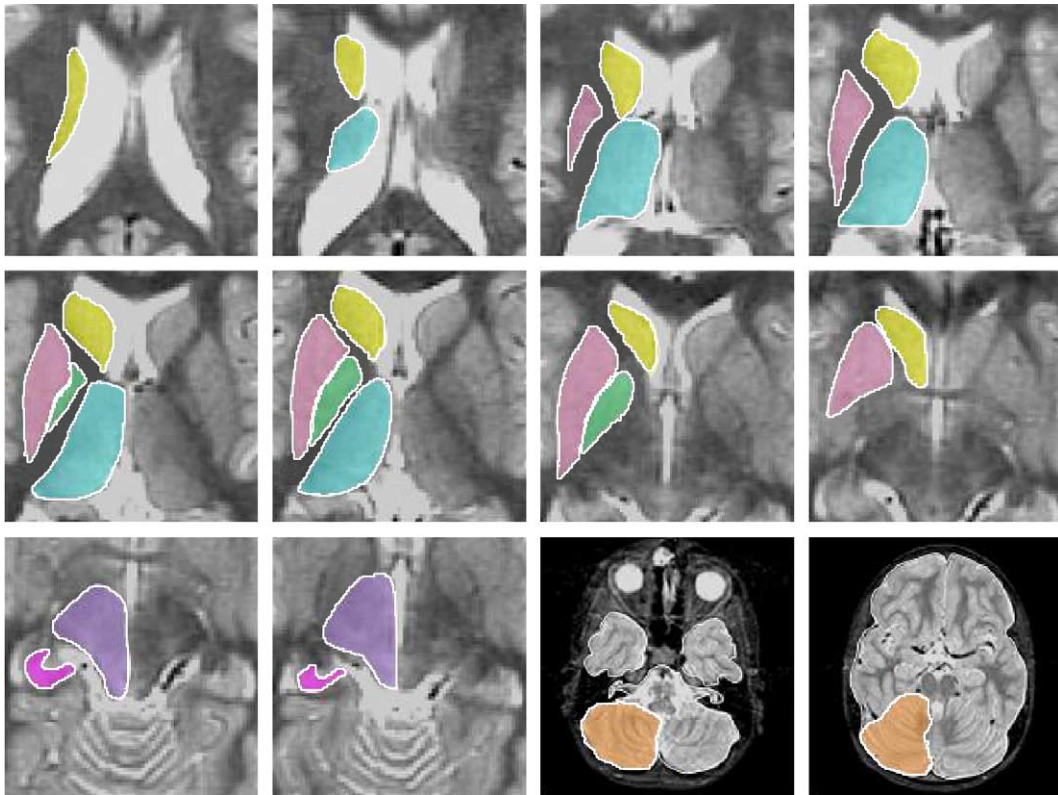


Fig. 1. Axial MR images (inversion recovery sequence, TE/TI/TR=32/120/4000 ms, 3.5-mm slice thickness, no gap, 24-cm field of view) showing representative slices for the regions of interest (ROIs) measured: caudate (yellow), putamen (pink), globus pallidus (green), thalamus (blue), hippocampus (magenta), mid-brain (purple), cerebellum (peach); only the right side is illustrated with semi-transparent colors filling the ROIs.

exposure status of the subjects was blinded. The test battery included the following measures:

1. *Visual motor integration* [Beery Test of Visual Motor Integration (Beery and Buktenica, 1989)]: Subjects were instructed to copy 24 geometric figures from a model presented in a booklet: figures were arranged in order of increasing difficulty.
2. *Motor function* [Purdue Pegboard (Purdue Research Foundation, Lafayette, IN)]: This test assessed fine motor skills in the dominant and non-dominant hand by having the subject insert as many pins as possible into successive holes during a 30-s period.
3. *Sustained attention* [Test of Variable Attention (TOVA) (Greenberg, 1987)]: Assessments include (1) failure to respond (omission errors), interpreted as a measure of attention; (2) Response to a non-target stimulus (commission errors), interpreted as a measure of impulsivity; (3) response time variability (the standard deviation of the mean correct response times), interpreted as a measure of consistency of attention.
4. *Visual attention/visuomotor tasks* [The Developmental Neuropsychological Assessment (NEPSY) Visual Attention subtest (Korkman, 1988) for children under age 6 and the Halsted-Reitan Trail Making tests (Trails A and B) (Reitan and Wolfson, 1985) for children 6 years or older]: Both tests involve visual scanning, attention and psychomotor speed. The Trail Making test also has a sequencing and set shifting component (Sattler, 1990; Spreen and Strauss, 1998).
5. *Verbal memory* [The Wechsler Preschool and Primary Scale of Intelligence-Revised (WPPSI-R)

Sentence Repetition (Wechsler, 1989) for children under 6 years of age; California Verbal Learning Test for Children (CVLT-C) (Delis et al., 1994) for children 6 and older]: The WPPSI-R Sentence Repetition subtest was utilized to assess verbal memory. The task was to repeat sentences of increasing difficulty given orally by the examiner. The CVLT-C consists of a 15-item (List A) word list, which is semantically related to three categories. The list was presented five times, with each presentation followed by a free recall trial for the list. A second word list (List B) was then presented as a distracter trial. Following the second list recall trial, a short delay free and category cued recall of List A was conducted. Free and cued recall of List A was measured again after a long delay (20 min) (Spreen and Strauss, 1998).

6. *Spatial memory* [Children's Memory Scale-Dot Location subtest (Cohen, 1997)]: This subtest assessed the ability to learn the spatial location of an array of dots over three learning trials. Following the initial presentation and recall of the learning trials, a single distracter array was presented. Recall of the first dot array followed the presentation and recall of the distracter array. In the delayed portion, recall of the original dot array occurred.
7. *Naming/word retrieval* [Expressive One Word Picture Vocabulary Test-Revised (EOWPVT-R)]: The EOWPVT-R, is a naming task consisting of 143 items that assessed expressive language ability (Gardner, 1983).
8. *Comprehension vocabulary* [Peabody Picture Vocabulary Test-Third Edition (PPVT-III) (Dunn and Dunn, 1981)]: The PPVT-III consists of 175 test items designed to measure auditory receptive language. The subject was asked to identify a stimulus word from a selection of four simple black-and-white illustrations.
9. *Verbal fluency* [Controlled Oral Word Association Test-FAS (Benton et al., 1983)]: This test consisted of timed tasks evaluating animal and food naming in children under age 6 years. For children age 6 and older, animal naming, food naming and words beginning with the letters F, A and S within a time limit were used. These tasks evaluated spontaneous production of words

beginning with a specific letter or in a specific category.

10. *Intelligence* [Vocabulary and Block Design-Wechsler Intelligence Scale for Children-Third Edition (WISC-III) and Wechsler Preschool and Primary Scale of Intelligence-Revised (WPPSI-R) for children older and younger than 6 years, respectively]: The WISC-III and WPPSI-R consist of both verbal and performance (visual nonverbal) subtests (Wechsler, 1989). A short form of the WISC-III and WPPSI-R (Vocabulary and Block Design) was used to estimate IQ from normative data (Sattler, 1990).
11. *Mood* [Children's Depression Inventory (CDI) (Kovacs, 1992) for children age 7 and older]: The CDI is a commonly employed self-report measure of depression in clinical and research settings. It contains 27 items covering the affective, cognitive and somatic aspects of depression.

#### 2.4. Statistical analysis

Neuropsychological testing scores among groups were compared using Student's *t* tests (two-tailed). The neuropsychological test scores were transformed into percentile (%) using age-appropriate normative data, such that higher % indicates better performance. A modified Bonferroni procedure, in which individual *P* values are ranked and compared with an ascending list of significance levels to determine statistical significance of individual tests, was performed to correct for multiple comparisons (Simes, 1986). All values are reported as mean  $\pm$  S.D. Morphometric data were analyzed using repeated-measures analysis of variance (ANOVA), with Meth status as a between-subjects variable and brain hemisphere as a within-subject variable. An additional repeated-measures ANOVA was performed, in which the four subcortical gray matter regions (globus pallidus, putamen, caudate and thalamus) were treated as a second within-subject variable. All volumetric data were expressed in percent to the whole brain volume, to eliminate the influence of overall brain size among subjects. The relationship between each regional brain volume and performance of neurodevelopmental testing was explored with analysis of covariance (ANCOVA), using regional volume (both hemispheres combined) as a covariate, Meth status as a categorical variable,

and performance as the dependent variable. These ANCOVAs were performed only for volumetric and neurocognitive variables that showed significant group differences. When the ANCOVA showed no significant interaction between Meth status and regional brain volume on cognitive tests, data from both groups were combined for a simple linear regression analysis between regional brain volume and neurodevelopmental test scores.

### 3. Results

Of the 13 drug-exposed children, six were also exposed to nicotine ( $22 \pm 7$  cigarettes/day). None of the children had been medicated with methylphenidate. Only two women reported light alcohol use during pregnancy and all reported the equivalent of less than 0.5 oz. of absolute alcohol/day. Two women reported “trying” cocaine during the pregnancy on a limited number of occasions (<5 times). None of the women in the group reported any other illicit drug exposure during their pregnancy. No prenatal alcohol exposure was reported in the control group. Only 1 of the 15 control children was exposed to nicotine during pregnancy. None of the children studied were pre-

viously diagnosed with or exhibited physical characteristics of fetal alcohol syndrome. Furthermore, none of the children had reported drug use or a psychological disorder.

#### 3.1. Structural MRI and volumetrics

All Meth-exposed and comparison subjects had normal-appearing MRI scans without any brain lesions on visual inspection by a Board-certified neuroradiologist (IW). Regional brain volumes are shown in Table 1, and regions showing significant group differences are shown in Fig. 2. On the repeated-measures ANOVA, the Meth-exposed children showed smaller volumes (relative to the whole brain) across all four subcortical gray matter volumes and independent of hemisphere (main effect of methamphetamine status:  $F=6.0$ ,  $df=1,78$ ,  $P=0.022$ ). On region-specific ANOVAs, significant Meth effects on the relative volume were found in the putamen ( $F=7.5$ ,  $df=1,26$ ,  $P=0.011$ ), and in the globus pallidus ( $F=6.0$ ,  $df=1,26$ ,  $P=0.021$ ). In addition, Meth-exposed children had smaller hippocampal volumes bilaterally ( $F=6.7$ ,  $df=1,26$ ,  $P=0.016$ ). No interaction between Meth status and hemisphere was found in any brain region.

Table 1  
Brain region volumes in milliliters (% whole brain, mean  $\pm$  S.D.) in children exposed to Meth prenatally and in healthy unexposed controls

Brain region (% whole brain)	Controls ( $n=15$ )		Meth-exposed ( $n=13$ )		Meth vs. controls
	Right	Left	Right	Left	$P$ Values* (right/left)
Putamen	6.36 $\pm$ 0.78 (0.48 $\pm$ 0.09)	6.43 $\pm$ 0.72 (0.48 $\pm$ 0.08)	5.23 $\pm$ 0.78 (0.41 $\pm$ 0.06)	5.29 $\pm$ 0.88 (0.42 $\pm$ 0.07)	0.0007*/0.0009* (0.01/0.01)
Globus pallidus	2.27 $\pm$ 0.71 (0.17 $\pm$ 0.06)	2.16 $\pm$ 0.76 (0.16 $\pm$ 0.06)	1.58 $\pm$ 0.56 (0.12 $\pm$ 0.04)	1.58 $\pm$ 0.58 (0.12 $\pm$ 0.04)	0.009*/0.037 (0.01/0.04)
Caudate	4.86 $\pm$ 0.97 (0.38 $\pm$ 0.08)	5.00 $\pm$ 0.87 (0.38 $\pm$ 0.07)	4.21 $\pm$ 0.65 (0.33 $\pm$ 0.06)	4.37 $\pm$ 0.86 (0.35 $\pm$ 0.07)	0.05/0.06 (0.1/0.1)
Thalamus	7.79 $\pm$ 1.5 (0.61 $\pm$ 0.13)	7.69 $\pm$ 1.3 (0.59 $\pm$ 0.1)	7.05 $\pm$ 0.47 (0.56 $\pm$ 0.07)	6.97 $\pm$ 0.56 (0.55 $\pm$ 0.07)	0.10/0.07 (n.s./n.s.)
Midbrain	3.65 $\pm$ 0.38 (0.29 $\pm$ 0.05)	3.71 $\pm$ 0.30 (0.29 $\pm$ 0.05)	3.78 $\pm$ 0.62 (0.30 $\pm$ 0.05)	3.65 $\pm$ 0.55 (0.29 $\pm$ 0.05)	n.s./n.s. (n.s./n.s.)
Hippocampus	0.82 $\pm$ 0.18 (0.064 $\pm$ 0.013)	0.78 $\pm$ 0.23 (0.060 $\pm$ 0.013)	0.66 $\pm$ 0.13 (0.051 $\pm$ 0.01)	0.62 $\pm$ 0.14 (0.047 $\pm$ 0.010)	0.02/0.04 (0.03/0.02)
Cerebellum	70.4 $\pm$ 9.1 (5.5 $\pm$ 1.0)	69.6 $\pm$ 9.8 (5.4 $\pm$ 1.0)	64.7 $\pm$ 6.5 (5.1 $\pm$ 0.6)	66.0 $\pm$ 6.3 (5.2 $\pm$ 0.5)	0.07/n.s. (n.s./n.s.)
Whole brain <sup>a</sup> (ml)	1314 $\pm$ 168		1273 $\pm$ 138		n.s.
Global CSF (ml)	93.2 $\pm$ 33		93.2 $\pm$ 67		n.s.

<sup>a</sup> Whole brain volume = global intracerebral brain volume – global CSF volume.

\*  $P$  Values are from post hoc ANOVA; asterisks indicate significance after Bonferroni correction (Simes, 1986).

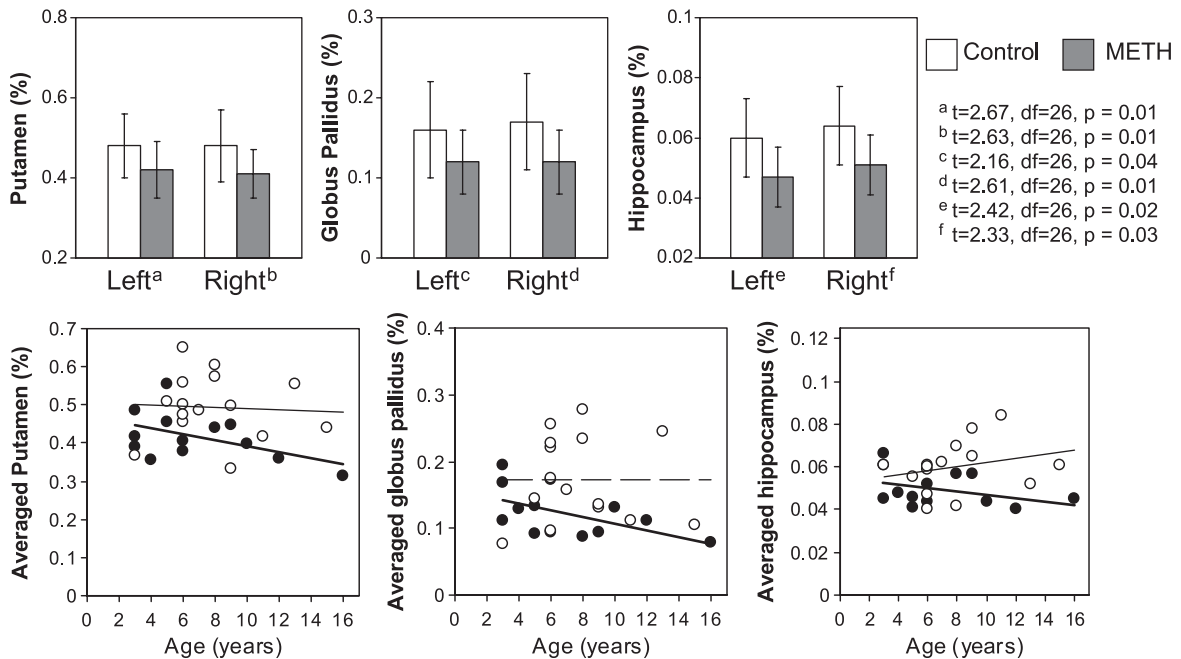


Fig. 2. Top row: Bar graphs showing relative brain volumes (% relative to whole brain, mean  $\pm$  S.D.) for the left and right putamen, globus pallidus and hippocampus of unexposed (control, white bars) and methamphetamine-exposed (Meth, black bars) children. Bottom row: Scatterplots showing age-related brain volumes in comparison (open circles) and Meth (black circles) children; left and right sides are averaged for each region.

### 3.2. Neurocognitive performance

The results from the neurocognitive tests are shown in Table 2. Scores for all tests were converted to standard scores with a mean of 100 and a standard deviation of 15. For all cognitive tests, a higher score indicates a better performance.

#### 3.2.1. Mood

The CDI was obtained on all of the subjects to determine mood as well as possible effects on the cognitive performance. We found no differences in depression scores in the Meth-exposed and control children.

#### 3.2.2. Visual motor integration and fine motor dexterity

The Meth-exposed children performed worse on the VMI than those in the unexposed group ( $-18\%$ ,  $t=3.6$ ,  $df=17$ ,  $P=0.002$ ). There were no differences in motor function as assessed by the Purdue Pegboard for either group.

#### 3.2.3. Attention and psychomotor speed

The Meth-exposed children performed significantly worse than the unexposed children on the sustained attention test (TOVA). Scores for errors of omission ( $-24\%$ ,  $t=6.2$ ,  $df=13$ ,  $P<0.0001$ ), commission ( $-21\%$ ,  $t=3.4$ ,  $df=13$ ,  $P=0.005$ ) and response time variability ( $-25\%$ ,  $t=3.1$ ,  $df=13$ ,  $P=0.009$ ) were all lower in the Meth-exposed children. There were no differences in visual attention and psychomotor speed on the Trails A and B or the NEPSY visual attention tests between the Meth-exposed and unexposed children.

#### 3.2.4. Memory

The Meth-exposed group had significantly poorer long delay verbal memory ( $-26\%$ ,  $t=3.7$ ,  $df=14$ ,  $P=0.002$ ) and a trend for poorer short delay verbal memory ( $-11\%$ ,  $t=2.1$ ,  $df=13$ ,  $P=0.06$ ) on the CVLT-C. Long delay spatial memory scores in the exposed group were lower than in the unexposed group ( $-9\%$ ,  $t=3.0$ ,  $df=14$ ,  $P=0.01$ ), but both groups performed within normal limits. There were no differences between the groups in short delay spatial memory.

Table 2

Results from the neurocognitive tests of children exposed to prenatal methamphetamine and healthy unexposed controls (% relative to normative data, mean±S.D.)

	Control	Meth	<i>P</i> Value/ <i>t</i>
Children's Depression Inventory	93.1±11.7	101.7±17.4	n.s.
<i>Motor</i>			
Visual motor integration	102.8±10.7	83.9±12.0	0.002*/ <i>t</i> <sub>17</sub> =3.6
Purdue Pegboard			
Dominant hand	79.7±11.7	86.5±8.8	n.s.
Non-dominant hand	81.8±12.3	92.4±20.4	n.s.
<i>Attention and psychomotor speed</i>			
TOVA: errors of omission	102.1±5.7	77.2±10.1	<0.0001*/ <i>t</i> <sub>13</sub> =6.2
TOVA: errors of commission	102.7±12.5	81.5±10.9	0.005*/ <i>t</i> <sub>13</sub> =3.4
TOVA: response time variability	96.9±15.5	72.5±14.1	0.009*/ <i>t</i> <sub>13</sub> =3.1
Visual: Trail A	91.5±14.5	91.0±23.6	n.s.
Visual: Trail B	103.9±14.2	103.2±7.6	n.s.
<i>Memory</i>			
Verbal: short delay (CVLT)	106.4±12.5	95.2±6.1	(0.06)/ <i>t</i> <sub>13</sub> =2.1
Verbal: long delay (CVLT)	111.5±17.5	82.7±9.5	0.002*/ <i>t</i> <sub>14</sub> =3.7
Spatial: short delay (CMS)	113.1±10.7	106.0±9.6	n.s.
Spatial: long delay (CMS)	116.5±4.1	105.8±10.2	0.01*/ <i>t</i> <sub>14</sub> =3.0
<i>Vocabulary</i>			
Naming word retrieval (EOWPVT-R)	120.7±21.4	105.4±11.8	(0.08)/ <i>t</i> <sub>14</sub> =1.8
Comprehension vocabulary (PPVT-III)	106.7±14.1	98.8±13.5	n.s.
Verbal fluency (FAS)	103.2±25.0	98.3±15.9	n.s.
Verbal fluency (category)	95.3±13.2	92.5±9.3	n.s.
<i>Intelligence</i>			
Block design (WISC OR WPPSI)	98.0±13.6	91.3±14.8	n.s.
Vocabulary (WISC OR WPPSI)	106.5±20.7	91.3±7.9	(0.07)/ <i>t</i> <sub>16</sub> =2.0

\* *P*<0.05 after a modified Bonferroni correction (Simes, 1986).

### 3.2.5. Naming word retrieval and comprehension vocabulary

Naming word retrieval showed a trend to be lower in the Meth-exposed children relative to the unexposed children, although both groups scored within the normal range. There were no differences in comprehensive vocabulary performance or phonetic/semantic fluency between the groups.

### 3.2.6. Intelligence

Both groups performed within the normal range for block design and vocabulary. However, the Meth group had a trend for lower scores on the vocabulary test than the control group (−14%, *t*=2.0, *df*=16, *P*=0.07).

### 3.3. Relationship between brain volume and neurocognitive performance

Meth-exposed children and the comparison subjects showed an interaction between brain volume and Meth status on performance only in the normalized putamen in relation to one of the sustained attention tasks (TOVA, omission, ANCOVA, *F*=6.6, *df*=1,14, *P*=0.03). Since no other interactions between group status and brain volumes were observed in relation to performance, the data from the two groups were combined to explore the relationships between brain volumes and cognitive tests that were abnormal in the Meth group. Poorer performance on several of the individual TOVA measures was related to relative volumes in the hippocampus (TOVA omission: *r*=0.68, *F*=11.2, *df*=1,14, *P*=0.005\*; commission: *r*=0.80, *F*=23.5, *df*=1,14, *P*=0.0003\*), in the putamen (omission: *r*=0.62, *F*=8.3, *df*=1,14, *P*=0.01\*; commission: *r*=0.55, *F*=5.7, *df*=1,14, *P*=0.03; variability: *r*=0.57, *F*=6.3, *df*=1,14, *P*=0.03) and in the globus pallidus (omission: *r*=0.53, *F*=5.2, *df*=1,14, *P*=0.04). Furthermore, poorer delayed verbal memory (CVLT) was associated with smaller relative putamen (*r*=0.75, *F*=16.6, *df*=1,14, *P*=0.0013\*) and smaller relative globus pallidus (*r*=0.61, *F*=7.6, *df*=1,14, *P*=0.016). Poorer visual motor integration was also associated with smaller globus pallidus (*r*=0.47, *F*=4.7, *df*=1,17, *P*=0.045). Significant correlations after correction for multiple comparisons (Simes, 1986) are shown in Fig. 3, and these *P* values are indicated with an asterisk.

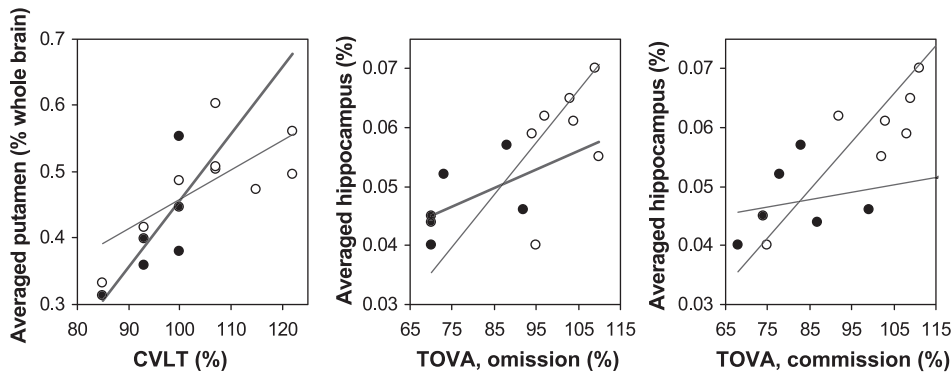


Fig. 3. Since there were no significant interactions between group status and brain morphometry on performance, the two groups were combined to evaluate these relationships. Poorer delayed verbal memory (CVLT) was associated with smaller relative putamen (averaged between right and left,  $r=0.75$ ,  $F=16.6$ ,  $df=1,14$ ,  $P=0.0013^*$ ) while poorer performance on TOVA (omission:  $r=0.68$ ,  $F=11.2$ ,  $df=1,14$ ,  $P=0.005^*$ ; commission:  $r=0.80$ ,  $F=23.5$ ,  $df=1,14$ ,  $P=0.0003$ ) was associated with smaller hippocampus. Meth (black circles) and unexposed children (open circles).

#### 4. Discussion

This study provides evidence of neurocognitive deficits and smaller subcortical brain volumes in children exposed to Meth in utero. MRI-based morphometry showed smaller putamen, globus pallidus and hippocampus in Meth-exposed children compared with control children. These findings were noted in the presence of normal appearing MRIs, while previous cranial ultrasound studies evaluated neonates with gross structural abnormalities (e.g., intraventricular hemorrhage) (Dixon and Bejar, 1989). In addition, neurocognitive deficits were found in attention and memory domains, and visuomotor integration for the Meth-exposed group, compared with both the control group and published normative data. Therefore, this preliminary study suggests children exposed to Meth in utero have neurocognitive deficits and structural alterations despite normal appearing MRIs on routine clinical evaluations.

MR-based volumetric assessments were used in numerous studies of children including a cohort of cocaine- and amphetamine-dependent young adults (Bartzokis et al., 2000), but none evaluated brain structural changes in prenatal Meth-exposed children. The smaller globus pallidus in the Meth-exposed children is similar to that observed in children with attention-deficit disorder (Aylward et al., 1996; Castellanos et al., 1996). Since both smaller putamen

and globus pallidus were associated with poorer attentional task performance (on TOVA), these findings suggest that these basal ganglia structures are involved in attention. A recent study of children with putamenal strokes found they had increased risk for ADHD traits (Max et al., 2002). Lastly, MR spectroscopy studies also demonstrated metabolite alterations suggestive of neuronal dysfunction in the striatum of chronic Meth-dependent adults, even during abstinence (Ernst et al., 2000; Sekine et al., 2002).

It is unclear if the changes in brain volumes observed in the Meth-exposed children will persist into adulthood. PET studies in abstinent adult Meth users have demonstrated decreased dopamine transporters suggesting damage to the dopaminergic nerve terminals (Volkow et al., 2001c,d). However, significant recovery of these transporters was observed both in humans and in monkeys after protracted abstinence (Melega et al., 1997; Volkow et al., 2001d). Nevertheless, children who were exposed in utero may be more vulnerable to the effects of Meth since their brains were still developing.

The neurocognitive effects of prenatal Meth exposure remain unclear. One prospective longitudinal study followed Meth-exposed children to 15 years of age, and found that these children exhibit aggressive behavior (Billing et al., 1988) and delays in language, mathematics and physical fitness acti-

vities (Cernerud et al., 1996). The limitations of this earlier study, however, included concomitant prenatal exposure to alcohol and cigarettes, as well as the lack of a comparison group. Compared with non-drug users, adults actively using Meth (mean duration of use=135 months) had lower performance on word and picture recall and decreased attention (Simon et al., 2000), and abstinent Meth users also showed slower psychomotor speed on computerized tasks (Chang et al., 2002). Consistent with these findings in adults, we found that Meth-exposed children had abnormalities in verbal memory and attention/vigilance impulsivity. Although our subjects did not have clinical diagnoses of ADHD, their performance on the attention tests, particularly in the area of vigilance, suggests that these processing difficulties have not yet been identified in the school setting, but very likely are interfering with efficient learning. Furthermore, the correlations of performance on sustained attention (TOVA) and delayed verbal memory (CVLT) with the subcortical structures (putamen and hippocampus) support the hypothesis that smaller striatal brain volume may contribute to the poorer learning in Meth-exposed children. The significant correlations between the attention and delayed memory problem and the smaller putamen and hippocampus would be consistent with deficits in the dopaminergic system, which are likely due to the prenatal Meth exposure.

Our findings of a smaller subcortical brain structures are consistent with clinical and preclinical observations in Meth-induced neurotoxicity. Rhesus monkeys demonstrate reduced brain monoamines up to 4 years after the last drug exposure (Woolverton et al., 1989). Several studies in rodents also showed that repeated exposure to Meth is toxic to dopaminergic and serotonergic neurons (Fuller and Hemrick-Luecke, 1982; Pu and Vorhees, 1993). PET studies demonstrated decreased dopamine transporters (Volkow et al., 2001a,b,c,d) and D2 receptors (Volkow et al., 2001a,b,c,d), while MR studies reported decreased neuronal markers (Ernst et al., 2000) and perfusion (Chang et al., 2002), all in the striatum of abstinent Meth-dependent adults. Furthermore, since chronic blockade of dopamine D2 receptors may lead to increased striatal trophic activity during development (Bjorklund and Stromberg, 1998), as well as increased striatal volume in patients after neuroleptic treatments

(Chakos et al., 1994), the decreased basal ganglia volume in the Meth-exposed children would suggest deficient dopamine function.

In addition to the direct neurotoxic effects of the drug, additional mechanism(s) of injury to the fetus may occur secondary to maternal Meth use. In the ovine model, Meth results in decreased uterine blood flow and increased uterine vascular resistance leading to fetal hypoxia (Stek et al., 1995) and restriction of nutritional substrate to the developing fetus. In addition, Meth is an anorectic agent, which might lead to poor maternal–fetal nutrition. Collectively, these maternal/placental effects of Meth could alter antenatal and postnatal neurodevelopment.

There are several limitations to the current study; therefore, these preliminary results should be interpreted with caution. First, despite the significant brain volume changes, our sample size is small. Second, our brain slices were relatively thick (3.5 mm), and although many of these structures appeared on multiple slices, the smaller structures such as the hippocampus would have larger potential errors. Therefore, this preliminary finding of smaller hippocampus in the Meth-exposed children should be considered cautiously; a larger validation study using thinner slices oriented along the hippocampus is needed. Third, although we recruited both the Meth-exposed children and the control subjects from similar lower and middle socioeconomic status groups, we did not match for parental education level, which might influence the subjects' cognitive performance. Fourth, we also did not take into account possible differences in living situations or actual school performance of these children. However, the children did not present with significant performance deficits in the public schools they attended, which might not have the resources to monitor their development carefully. In addition, some of the children were exposed to cigarettes and low doses of alcohol. Both could have contributed to the neurocognitive deficits, as shown in a meta-analysis of the effects of prenatal cocaine exposure, which found alcohol, poor environment and tobacco as likely contributors to the adverse outcomes found in cocaine-exposed children (Frank et al., 2001). However, given the profound neurotoxic effects of Meth in adult users and animal models, exposure to Meth in utero is a likely contributor to our findings.

This preliminary study demonstrates neurocognitive deficits and smaller subcortical volumes in children exposed to Meth in utero. MR-based volumetric assessments are more sensitive than visual inspection of structural MRI for evaluating brain changes. We found smaller putamen, globus pallidus and hippocampus volumes, despite comparable whole brain volumes, with associated memory and attention deficits in Meth-exposed children. These findings suggest possible neurotoxic effects of Meth to the developing striatum (globus pallidus and putamen). Due to the small sample size of this study, however, future studies correlating neuropsychological tests with structural imaging in larger longitudinal cohorts are needed to validate these initial observations.

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