Anomalous Brain Development Is Evident in Preschoolers With Attention-Deficit/Hyperactivity Disorder

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Abstract

Objectives: Attention-deficit/hyperactivity disorder (ADHD) is a common neurological disorder with symptom onset early in childhood. Growing evidence suggests anomalous brain development across multiple brain regions is evident in school-aged children; however, few studies have examined whether such differences are notable in the preschool years when symptom onset typically occurs. **Methods:** High resolution anatomical (MPRAGE) images and cognitive and behavioral measures were analyzed in a total of 90 medication-naïve preschoolers, ages 4–5 years (52 with ADHD, 38 controls; 64.4% boys). **Results:** Results revealed reductions in bilateral frontal, parietal, and temporal lobe gray matter volumes in children with ADHD relative to typically developing children, with largest effect sizes noted for right frontal and left temporal lobe volumes. Examining frontal lobe sub-regions, the largest between group effect sizes were evident for left orbitofrontal cortex, left primary motor cortex (M1), and left supplementary motor complex (SMC). ADHD-related reductions in specific sub-regions (left prefrontal, left premotor, left frontal eye field, left M1, and right SMC) were significantly correlated with symptom severity, such that higher ratings of hyperactive/impulsive symptoms were associated with reduced cortical volumes. **Conclusions:** These findings represent the first comprehensive examination of cortical volume in preschool children with ADHD, providing evidence that anomalous brain structure in ADHD is evident very early in development. Furthermore, findings set the stage for developing our understanding of the way in which developmental trajectories of anomalous brain development are associated with the unfolding of symptoms in childhood ADHD. (*JINS*, 2018, *24*, 1–9)

Keywords: Attention, Magnetic resonance imaging, Behavior rating scale, Cognitive functioning, Children, Developmental disabilities

INTRODUCTION

Attention-deficit/hyperactivity disorder (ADHD) symptoms are common (Pastor, Reuben, Duran, Hawkins, 2015) and often evident early in development (Arnett, MacDonald, Pennington, 2013); by age 4 years, as many as 40% of children exhibit parent-reported problems with attention or hyperactivity-impulsivity corresponding to diagnostic criteria for ADHD (Smidts & Oosterlaan, 2007). In fact, ADHD is the most commonly diagnosed form of psychopathology during the preschool years (Armstrong & Nettleton, 2004), with onset during early childhood associated with significant long-term health and economic costs (Chorozoglou et al., 2015).

ADHD has been associated with widespread structural brain abnormalities in school-aged and adolescent youth, including smaller overall cerebral volumes (Friedman & Rapoport, 2015), reductions in total gray matter volumes (Batty et al., 2010), and delays in cortical maturation (Shaw et al., 2007). More specific findings have consistently centered on networks important for attentional control, including regional structural anomalies in prefrontal and premotor areas (Dirlikov et al., 2015; Shaw et al., 2006; Yang, Carrey, Bernier, & MacMaster, 2015), supplementary motor complex (Mahone, Ranta, et al., 2011), and basal ganglia (Nakao, Radua, Rubia, & Mataix-Cols, 2011; Qiu et al., 2009), with these differences evident into adulthood (Greven et al., 2015; Nakao et al., 2011). The rate of cortical maturation in prefrontal cortical regions, including middle, (right) inferior, and orbital frontal gyri (Shaw et al., 2011), as well as observed reductions in gray matter (e.g., cortical volumes

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and/or thickness) in right frontoparietal regions (Almeida Montes et al., 2013), premotor and primary motor cortex (Dirlikov et al., 2015), and supplementary motor complex (Mahone, Ranta, et al., 2011) have been associated with severity of ADHD symptoms in children and/or adolescents.

Most studies examining structural brain anomalies in children with ADHD have examined school-aged samples. Although early anomalous development in regions important for early attentional control may subsequently influence development of key structures in these networks (Limperopoulos, Chilingaryan, Guizard, Robertson, & Du Plessis, 2010), few studies have examined whether the brain anomalies seen in older children with ADHD are evident closer to symptom onset. The only studies to date in very young children with ADHD have found few differences in cortical thickness (Yang et al., 2015) or volumes (Mahone, Crocetti, et al., 2011); however, both samples were small and closer examination of frontal regions associated with ADHD-related dysfunction noted previously may yield differing results. Given dynamic changes in cortical volumes during the preschool period, particularly within frontal cortices (Brown & Jernigan, 2012), further investigation of regions important for development of attentional and response control is needed in well-powered samples of young children.

Given the early onset of ADHD symptoms and developmental nature of the observed brain anomalies, further examination of early brain differences in very young children with ADHD is critical for advancing understanding of the disorder. The present study represents a first step toward more comprehensively examining anatomical differences in brain development between preschoolers with ADHD and typically developing peers. We hypothesized that, consistent with findings in older children with ADHD, cortical, particularly frontal, development would be anomalous in young children with ADHD, relative to their typically developing peers. A preliminary investigation (Mahone, Crocetti et al., 2011) within a subsample ($N = 26$ preschoolers, 13 ADHD) of the present study previously failed to find significant group differences in cortical volumes, which they hypothesized was potentially reflective of lack of power given the small sample size.

Power calculations based upon data from the original pilot sample ($n = 23$) for lobar multivariate analyses of variance (MANOVAs) indicate power estimates of .108 (left hemisphere lobar volumes) and .169 (right hemisphere lobar volumes). Based upon Cohen's d calculated for each specific lobar group difference in the pilot sample, with assumption of $r = .90$ reliability for repeated MRI measurements, the sample sizes (per group) needed for power of .80 in the present study range from 13 (right frontal lobe) to 47 (left parietal lobe). The present study sought to more comprehensively investigate these differences in a much larger (and adequately powered) sample of preschoolers with ADHD ($N = 90$). In addition, the present study's methods took advantage of a sophisticated frontal lobe parcellation technique designed especially to examine frontal regions of interest in youth with ADHD (e.g., the Ranta atlas: Ranta et al., 2009, 2014).

The present study investigated differences in regionally specific cerebral volume among 90 medication-naïve preschool children (ages 4–5 years) with and without ADHD, exploring associations between particular regional brain volumes and symptom severity.

METHODS

Study Procedures

Participants were recruited from the community, daycare centers, and pediatricians' offices. Interested parents were provided with a study description, completed a telephone interview to determine participant eligibility, met in person with a research assistant to answer questions, then signed informed consent; children provided verbal assent. After enrollment, participants completed an assessment battery including measures of cognitive and language functioning, while parents completed behavior rating scales. Mock MRI scan training preceded scans, scheduled in conjunction with the assessment visit. All procedures were approved by the Johns Hopkins Hospital Institutional Review Board.

Participants

Participants were required to be between the ages of 4 years, 0 months and 5 years, 11 months at the study visit.

Inclusion and exclusion procedures

Participants were excluded for any of the following, established *via* review of medical/developmental history and/or by study assessment: (1) diagnosis of intellectual disability or autism spectrum disorder; (2) known visual impairment; (3) treatment for psychiatric disorder (other than ADHD) with medication; (4) history of DSM-IV diagnosis, other than oppositional defiant disorder or adjustment disorder; (5) neurological disorder (e.g., epilepsy, traumatic brain injury, Tourette syndrome); (6) documented hearing loss ≥ 25 dB; (7) history of physical, sexual, or emotional abuse; (8) medical contraindication to MRI; or (9) Full Scale IQ < 80. In addition, children were excluded if developmental language disorder (DLD) was determined either during initial phone screen, based on prior assessment (completed within 1 year of the current assessment), or during study visit. DLD exclusion is in deference to literature suggesting that language impairments may influence development of inhibitory control, response preparation, and working memory–core features of ADHD (Hagberg, Miniscalco, & Gillberg, 2010). Children who were unable to complete imaging after repeated mock scan training also were excluded.

Of those initially recruited ($N = 142$), 15 participants were determined to be ineligible for participation at telephone screening or based upon initial behavioral measures. Of those initially considered eligible, one participant elected to withdraw before study completion, leaving an initial enrollment

of 129 preschoolers. Of those who successfully completed both behavioral training ($n = 119$) and MR imaging ($n = 103$), five were subsequently determined to be ineligible based upon incidental imaging findings and software registration errors resulted in inability to use imaging data from eight children. The total remaining in the study with useable imaging and behavioral data included 90 preschoolers (38 typically developing, 52 with ADHD).

Diagnostic methods for group assignment were adapted from the NIH Preschoolers with Attention-deficit/Hyperactivity Disorder Treatment (PATS) Study (Kollins et al., 2006; Posner et al., 2007). Diagnosis of ADHD was made using modified DSM-IV-TR criteria, based on parent report on the Diagnostic Interview Schedule for Children-Young Child (YC-DISC; used for 4 year olds) (Lucas, Fisher, & Luby, 1998, 2008) or Diagnostic Interview for Children and Adolescents, Fourth Edition (DICA-IV; used for 5-year-olds) (Reich, Welner, & Herjanic, 1997), and the DSM-IV ADHD Scales (Scales L and M) of the Conners' Parent Rating Scales-Revised (CPRS) (Conners, 1997). The YC-DISC is a highly structured, computer-assisted diagnostic instrument that assesses common psychiatric disorders, as defined by DSM-IV, presenting in young children; the DICA-IV is the parallel version for older children and adolescents. For inclusion in the ADHD group, children had T-scores $\geq$ 65 on one or both of the CPRS DSM-IV ADHD Scales, presence of symptoms for at least 6 months as documented *via* structured interview, and evidence of cross-situational impairment (defined as parent report of problems at home and with peers, as not all children were enrolled in school).

Upon meeting eligibility/exclusion criteria, children were included in the control group only if they (1) did not have a sibling diagnosed with ADHD, (2) did not meet categorical diagnostic criteria for any disorder on the YC-DISC or DICA-IV, and (3) had T-scores < 65 on the CPRS DSM-IV ADHD scales.

Assessment Methods

Wechsler Preschool and Primary Scale of Intelligence-Third Edition (WPPSI-III) (Wechsler, 2002)

The WPPSI-III is an individually administered, norm-referenced test of early cognitive abilities used to assess IQ (FSIQ).

Clinical Evaluation of Language Functions-Preschool-2 (CELF-P2) (Wiig, Secord, & Semel, 2004)

The CELF-P2 is an individually administered, norm-referenced test used to identify language disorders in preschool children. Children scoring below -1.5 *SD* on either the Receptive Language or Expressive Language Index of the CELF-P2, or below -1.0 *SD* on both indices, were excluded.

Conners' Parent Rating Scales-Revised, Long Form (CPRS) (Conners, 1997)

Dimensional ratings of ADHD symptom severity were obtained using the DSM-IV oriented scales from the CPRS, including Scale L (DSM-IV Inattentive) and Scale M (DSM-IV Hyperactive/Impulsive).

MRI Methods

Preparation of preschoolers for scans

MRI scanning in children is inherently challenging, particularly for young children with behaviors associated with ADHD. Two problems must be overcome: first, getting children to enter the MRI environment willingly, and second, keeping their heads sufficiently still to acquire good data. Head motion is a particular challenge in young children with neurodevelopmental disorders. The present study used a brief (15–30 min) behavioral protocol involving practice with a mock MRI scanner, designed for young children and those with developmental disabilities (Slifer, Cataldo, Gerson, & Tucker, 1994); a full description of behavioral procedures used can be found in Mahone, Crocetti, et al. (2011). Scans took place in the afternoon of the second day of study visit, following completion of testing, a lunch break, and successful completion of mock scan procedures. Where a repeat session of mock scan practice was required, the second session and real scan took place in the afternoon of another day, scheduled at family convenience.

Imaging methods

All scanning was completed using a 3.0T Philips GyroscanNT scanner. Magnetization Prepared Rapid Gradient Recalled Echo (MPRAGE) images were used for volumetric assessment. Slice thickness = 1.0 mm; field of view = 26 cm; Matrix size: 256 × 256. A Sensitivity Encoding (SENSE) coil was used to address geometric distortion artifacts due to macroscopic magnetic susceptibility effects that can cause signal dropout at the air–tissue interface. Cortical reconstruction and volumetric segmentation was performed with the Freesurfer image analysis suite, documented and freely available for download online (http://surfer.nmr.mgh.harvard.edu). To ensure good data quality, a rigorous qualitative assessment of motion was used for each MPRAGE scan in three stages.

First, the MPRAGE was visually inspected for gross motion artifacts (e.g., ringing, blurred gray–white matter boundaries, frame shifts, pixelation) at the scanner and a new MPRAGE was attempted, following corrective feedback to the child, if excessive motion was detected. Additionally, a trained research team member sat with the child as needed to help minimize movement. Second, visual inspection for each MPRAGE was conducted by 2 raters and the quality rating on a scale of 1 ("good") to 5 ("poor") was based on their agreement. Images classified as good contained no visible signs of distortion, ringing, ghosting, shifts in subject position, signal-dropout, or blurring (i.e., partial-voluming due to compromised signal-to-noise). Images rated with scores of 2 through 5 present varying degrees of the motion artifact categories listed above; poor quality scans (ratings of 5) were

excluded from analyses. Third, if outliers were detected in the Freesurfer cortical statistics, parcellation maps were visually inspected for errors. Quality ratings were evenly distributed across diagnostic groups ($\chi^2_{(4)} = 2.634$, $p = .621$).

Total cerebral volume and regional measures of cortical volume were obtained using automated methods within Freesurfer. Freesurfer used a fully automated method to perform pre-processing steps including Talairach alignment, intensity normalization, and removal of skull and non-brain tissue with a hybrid watershed/surface deformation procedure, separation of the cerebellum and brainstem from the cerebrum, and splitting of the left and right hemispheres (Dale, Fischl, & Sereno, 1999; Segonne et al., 2004). A deformable surface algorithm was used to define inner (gray–white) and outer (gray-cerebrospinal fluid [CSF]) cortical surfaces (Dale et al., 1999). Automated topological correction, surface inflation and registration to a spherical atlas were also included in the processing stream (Dale et al., 1999; Fischl, Sereno, & Dale, 1999). Total cerebral volume (TCV) including total cerebral gray and white matter for each subject was estimated using the Freesurfer pial surface, excluding CSF.

Frontal lobe sub-regions were generated using an automated protocol developed in Freesurfer (Ranta et al., 2014), which applies a frontal lobe parcellation based on the highly reliable manual method described in Ranta et al. (2009) in which sulcal-gyral landmarks were used to manually delimit functionally relevant regions within the frontal lobe. Functional subdivisions of the frontal lobe include: primary motor cortex, anterior cingulate, premotor cortex regions (supplementary motor complex [SMC], frontal eye field, and lateral premotor cortex) and prefrontal cortex (PFC) regions (medial PFC, dorsolateral PFC, inferior PFC, lateral orbitofrontal cortex [OFC] and medial OFC). Regional volumes (e.g., premotor and prefrontal volumes) reflect summed raw volumes of parcellated sub-regions as specified above (i.e., left premotor cortex was calculated as the sum of the left SMC, left frontal eye field, and left lateral premotor cortex volumes; left PFC was calculated as the sum of the left medial PFC, left dorsolateral PFC, left inferior PFC, left lateral OFC, and left medial OFC volumes).

Regional and sub-regional volumes were normalized in all analyses to correct for within-group total cerebral volume differences, using the procedure recommended by Kramer et al.

(2007): multiplying the absolute ROI volume by the average total cerebral volume of the analysis group and dividing by the individual's total cerebral volume (Mahone, Ranta, et al., 2011; Ranta et al., 2009).

Data Analysis

Group differences (ADHD *vs.* TD) in TCV and adjusted lobar, regional, and sub-regional gray matter volumes were examined using serial multivariate analyses of variance (ANOVAs), and follow-up *post hoc* comparisons, with Bonferroni correction for multiple comparisons. Hierarchical analyses were conducted as follows: following initial examination of between-group differences in TCV, a MANOVA examined group differences in lobar volumes with two follow-up MANOVAs examining prefrontal and then premotor regions. Follow-up univariate ANOVAs examined specific sub-regions (left and right) within premotor and prefrontal regions for between group differences. Pearson correlations between regional volumes and symptom severity measures within the ADHD group examined brain-behavior relationships for regions and sub-regions previously found to show significant between group differences.

RESULTS

Sample Demographics

Mean participant age was 4.97 years ($SD = .58$; range = 4.05–5.99 years) (see Table 1). There were no differences between groups on sex ($\chi^2_{(1)} = .048$; $p = .827$), handedness ($\chi^2_{(2)} = .096$; $p = .953$; 85.6% right-handed), racial distribution ($\chi^2_{(4)} = 5.967$; $p = .202$), or socioeconomic status as estimated by the Hollingshead index ($p = .987$). Of note, the Hollingshead index, originally developed in the 1970s (Hollingshead, 1975) is based solely upon parent education and occupation, thus has limitations as a true measure of social risk. The sample included 58 boys (24 controls, 34 ADHD) and 32 girls (14 controls, 18 ADHD), matched on age, Full-Scale IQ, and overall language abilities. Participants were 83.3% Caucasian, 11.1% African-American, 3.3% Asian, and 1.1% Multiracial (1.1% unknown/not reported). None were prescribed stimulant medication at the

Table 1. Sample demographic and performance summary

	Control			ADHD				
	n	Mean	*SD*	*n*	Mean	*SD*	*p*	η^2_p
Age (years)	38	4.92	.58	52	5.01	.58	.454	.006
WPPSI-3 FSIQ	37	109.32	16.14	50	109.76	12.81	.889	.000
CELF-P2 Core Lang	38	105.81	11.82	49	107.67	11.92	.474	.006
CPRS Scale L	38	44.55	5.42	50	74.42	11.74	<.001	.711
CPRS Scale M	38	46.05	6.45	50	73.48	10.95	<.001	.687

Note: ADHD = Attention-deficit/hyperactivity disorder; η^2_p = effect size, partial eta-squared; WPPSI-3 = Wechsler Preschool and Primary Scale of Intelligence, 3rd Ed.; FSIQ = Full Scale IQ score; CELF-P2 = Clinical Evaluation of Language Fundamentals, Preschool, 2nd Ed.; Core Lang = Core Language score; CPRS = Conners' Parent Rating Scale-Revised, Scale L (DSM-IV Inattentive) T-score; Scale M (DSM-IV Hyperactive/Impulsive) T-score.

time of participation, although several began treatment shortly afterward.

Behavioral Training for Scans

The behavioral training protocol was effective for teaching 92.2% (119 of 129) of the study participants to participate in the MR imaging. Of those, 86.6% (103 of 119) were able to remain sufficiently motionless to successfully complete MR image acquisition without sedation. There was no difference between groups in participants' ability to successfully complete mock scan and MR image acquisition ($\chi^2_{(2)} = .420$; $p = .517$).

Total Cerebral Volume

There was a significant effect of group (ADHD < TD; $p = .011$, $\eta^2_p = .073$) and sex (girls < boys, $p = .011$; $\eta^2_p = .073$), but no sex-by-group interaction ($p = .859$) on TCV. Within the narrow age range of the sample, age was not correlated with TCV ($r = .184$; $p = .082$). Given the overall between group differences in TCV, regional and sub-regional volumes were normalized in all subsequent analyses.

Lobar Volumes

After normalization, lobar GM volumes were significantly reduced in children with ADHD, relative to controls, bilaterally in frontal, temporal, and parietal lobes, with largest effect sizes noted for right frontal ($\eta^2_p = .213$) and left temporal ($\eta^2_p = .216$) lobe volumes (see Table 2). There was no significant effect of sex, within groups, for any of the lobar volumes (e.g., frontal, temporal, parietal, or occipital; overall multivariate: TD $p = .858$; ADHD $p = .432$).

Frontal Lobe Sub-divisions

Premotor and prefrontal regions

Results of MANOVAs examining normalized frontal regions (e.g., prefrontal and premotor regions) revealed that the overall group effect was significant for both models, bilaterally (prefrontal region $p = .0004$; $\eta^2_p = .134$; premotor region $p < .0001$; $\eta^2_p = .202$; controls > ADHD; see Table 3). Age was not associated with regional volumes (prefrontal: $r = -.109$; $p = .309$; premotor: $r = .020$; $p = .855$).

Premotor and prefrontal sub-regions

Specific findings suggest multiple areas of significantly reduced GM volumes in children with ADHD within bilateral prefrontal and premotor regions (see Table 3). The largest effect sizes for group differences were observed in left LOFC, $\eta^2_p = .116$), left primary motor cortex (M1, $\eta^2_p = .129$), and left SMC ($\eta^2_p = .117$).

Brain–Behavior Associations

Examining brain–behavior correlations, a pattern of specific associations was observed between GM volumes and symptom severity within the ADHD group (see Table 4). Bilateral frontal and parietal lobe volumes, as well as right temporal lobe volume, were correlated with hyperactive/impulsive symptom severity (CPRS M scale), with specific associations evident within frontal sub-regions. Prefrontal (left: $r = -.332$; $p < .01$; right: $r = -.292$; $p < .05$) and premotor (left: $r = -.318$; $p < .05$; right: $r = -.254$; $p < .05$) volumes and specifically right DLPFC ($r = -.253$; $p < .05$), left FEF volume ($r = -.426$; $p < .01$), left primary motor volume ($r = -.279$; $p < .05$), and right SMC ($r = -.315$; $p < .05$) were significantly and negatively correlated with symptom ratings, such that higher ratings of hyperactivity (CPRS M scale) were associated with reduced cortical volumes (Figure 1). With regard to inattention ratings, only left parietal lobe volumes ($r = -.279$; $p < .05$) were associated with greater inattention severity (CPRS L scale).

DISCUSSION

These findings are among the first to reveal strong evidence for anomalous cortical development as early as the preschool

Table 2. Lobar cortical gray matter volumes (mm^3) (raw)

Region	Control		ADHD			
	Mean	*SD*	Mean	*SD*	*p*	η^2_p
Total cerebral volume	1004418.81	13588.21	959106.59	11615.89	.013	.068
Frontal lobe (left)	96640.13	9007.78	91965.12	8640.52	.0001	.161
Frontal lobe (right)	100563.13	10589.46	95235.87	9012.74	<.0001	.213
Temporal lobe (left)	73215.21	7336.15	69152.62	6558.72	<.0001	.216
Temporal lobe (right)	72067.08	6725.43	68822.08	6631.36	<.0001	.190
Parietal lobe (left)	85090.05	10074.98	81524.65	7795.27	.0008	.122
Parietal lobe (right)	86591.55	8397.67	82381.04	8072.81	.0001	.170
Occipital lobe (left)	20603.21	2512.52	20454.52	3254.29	.739	.001
Occipital lobe (right)	20494.58	2883.31	20559.96	2566.63	.853	.000

Note. ADHD = Attention-deficit/hyperactivity disorder; *p*: between group significance for normalized volumes; η^2_p = effect size, partial eta-squared for between group differences in normalized volumes.

Table 3. Sub-regional cortical gray matter volumes (mm^3) (raw)

| | Left | | | | | | Right | | | | | |
| | Control | | ADHD | | | | Control | | ADHD | | | |
Region	Mean	SD	Mean	SD	p*	η^2$_p$	Mean	SD	Mean	SD	p*	η^2$_p$
Prefrontal regions[a]	64920.58	6541.69	62568.31	6226.37	.0090	.075	68550.18	7045.15	65072.73	6621.68	.0001	.160
DLPFC	19296.84	2678.41	18743.79	2988.97	.203	.018	21589.11	2815.77	20045.23	3009.45	.001	.109
MPFC	15696.24	1731.07	15124.02	1787.93	.056	.041	16548.61	1690.60	15861.85	1963.46	.019	.061
ILPFC	12165.50	1987.78	11708.52	1515.31	.181	.020	12858.29	1915.00	12674.12	1814.04	.580	.003
MOFC	3999.53	949.50	4223.96	713.25	.119	.027	5778.66	927.86	5339.46	707.67	.003	.096
LOFC	13762.47	1778.73	12768.02	1607.71	.001	.116	11775.53	1808.88	11152.08	1173.14	.015	.065
Premotor regions[a]	16481.47	2261.65	15065.88	1872.85	.0002	.144	16128.53	2163.45	14764.50	2013.61	.0003	.139
FEF	2675.11	486.54	2336.29	548.03	.003	.093	2243.26	575.29	2041.96	590.02	.116	.028
LPM	7427.84	1449.44	6949.87	1084.99	.050	.043	8240.37	1333.33	7543.52	1368.83	.006	.081
SMC	6378.53	1006.95	5779.73	862.91	.001	.117	5644.89	883.02	5179.02	845.98	.005	.088
M1	13211.87	1726.35	12129.44	1761.93	.001	.129	14137.84	2550.26	13536.08	2057.20	.125	.026
ACC	7005.42	1127.20	6789.10	957.22	.296	.012	6721.42	1282.06	6555.12	1017.33	.534	.004

Note. ADHD = Attention-deficit/hyperactivity disorder; η^2$_p$ = partial eta-squared for differences in normalized volumes; DLPFC = dorsolateral prefrontal cortex; MPFC = medial prefrontal cortex; ILPFC = inferolateral prefrontal cortex; MOFC = medial orbital prefrontal cortex; LOFC = lateral orbital prefrontal cortex; FEF = frontal eye field; LPM = lateral premotor cortex; SMC = supplemental motor cortex; M1 = primary motor cortex; ACC = anterior cingulate cortex.
p*: between group significance for normalized volumes.
[a]Prefrontal and premotor region volumes calculated as sums of corresponding sub-regions.

period in a carefully characterized sample of children with ADHD. Results of comparisons between children with ADHD and typically developing peers showed widespread cortical volume reductions in young children with ADHD across frontal, parietal, and temporal cortices, with large regional and medium to large sub-regional effect sizes. Furthermore, the volumetric reductions showing greatest effect size were noted in left temporal and right frontal lobes and specific prefrontal and premotor regions important for response control and execution (e.g., SMC and M1), inhibitory control (lateral OFC), as well as planning and working memory (e.g., DLPFC). Reductions in regional and specific sub-regional volumes were significantly associated with reports of greater symptom severity.

Structural brain differences characteristic of older children with ADHD in total GM volumes (Batty et al., 2010) and specific prefrontal and premotor regions (Dirlikov et al., 2015; Yang et al., 2015), including SMC (Mahone, Ranta, et al., 2011), also appear evident in preschoolers with the disorder, with potentially larger effect sizes in preschoolers relative to those observed in these older samples. Furthermore, early anomalous brain development is associated with ADHD symptoms. Notably, however, volumetric differences in anterior cingulate cortex reported in older children with ADHD (Dirlikov et al., 2015; Villemonteix et al., 2015) were not observed in this sample. Additionally, although between-group differences in SMC were found bilaterally, only the right SMC and left FEF showed consistent associations with ADHD symptomatology; together, these findings suggest a dynamic unfolding of functional associations over time.

Developmental studies suggest a pattern of emerging functional asymmetries from the first year of life, with a right to left gradient of development of cerebral hemispheres and a further shift over time from right to left functional dominance.

Lateralization of function in children appears more variable than in adults, but the evidence suggests earlier emergence of right motor and premotor regions and later maturation of left

Table 4. Correlations between regional gray matter volume (raw) and symptom severity within ADHD group

	CPRS L	CPRS M
LH frontal	−.168	−.366**
RH frontal	−.137	−.304*
LH temporal	−.110	−.234
RH temporal	−.168	−.247*
LH parietal	−.279*	−.237*
RH parietal	−.206	−.239*
LH prefrontal	−.172	−.332**
RH prefrontal	−.113	−.292*
LH premotor	−.174	−.318*
RH premotor	−.161	−.254*
RH DLPFC	−.016	−.253*
RH MPFC	−.065	−.130
RH MOFC	−.063	−.123
LH LOFC	−.030	−.125
RH LOFC	.111	−.175
LH FEF	−.213	−.426**
RH LPM	−.086	−.054
LH SMC	−.038	−.159
RH SMC	−.160	−.315*
LH M1	−.050	−.279*

CPRS = Conners' Parent Rating Scale-Revised, Scale L (DSM-IV Inattentive) T-score, Scale M (DSM-IV Hyperactive/Impulsive) T-score; ADHD = Attention-deficit/hyperactivity disorder; LH = left hemisphere; RH = right hemisphere; DLPFC = dorsolateral prefrontal cortex; MPFC = medial prefrontal cortex; MOFC = medial orbital prefrontal cortex; LOFC = lateral orbital prefrontal cortex; FEF = frontal eye field; LPM = lateral premotor cortex; SMC = supplemental motor cortex; M1 = primary motor cortex; * p < .05, **p < .01, one-tailed;
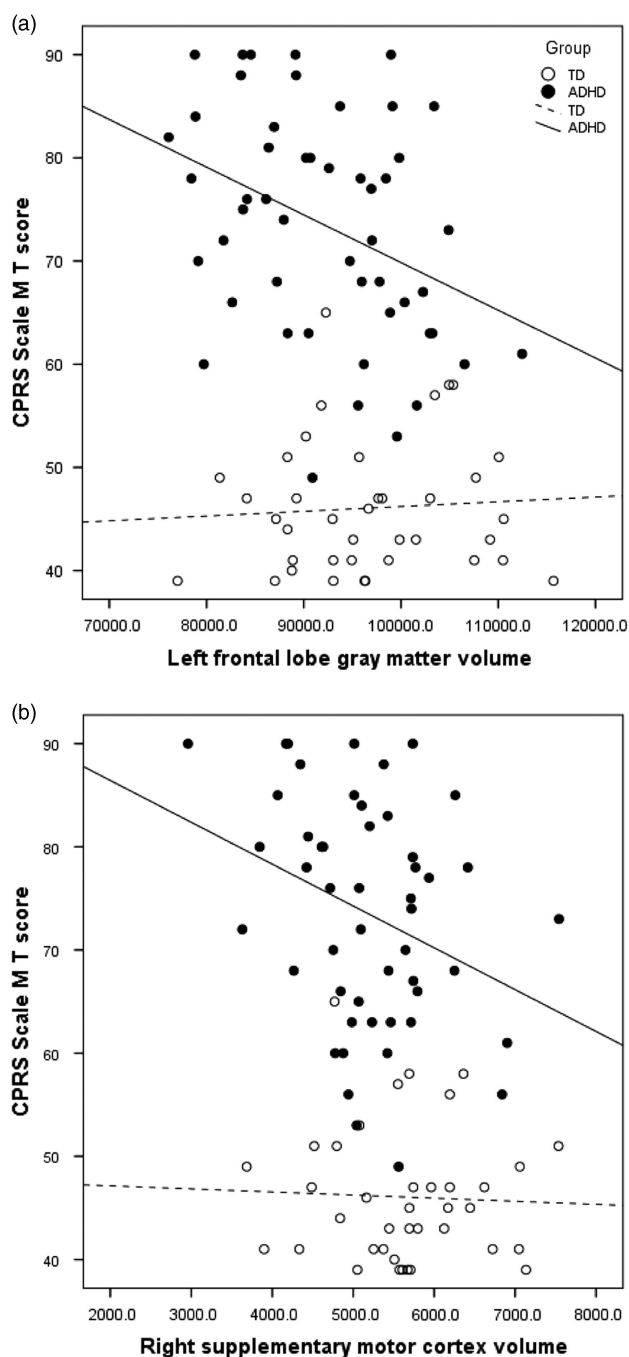
Fig. 1. Correlation between parent ratings of hyperactivity/impulsivity (CPRS Scale M) and a) left frontal gray matter volume (ADHD $R^2 = .134$) and b) right supplementary motor cortex volume (ADHD $R^2 = .099$).

frontal and premotor regions (Best, 1988). There is more specific evidence that orbital frontal regions develop earlier than DLPFC (e.g., Orzhekhovskaya, 1981), with right OFC integrity more strongly associated with social-behavioral functioning than left OFC (Bechara, 2004) and with global skills such as self-awareness (Happaney, Zelazo & Stuss, 2004), skills known to be affected in ADHD. It may also be that specificity of premotor function develops over time such that preschoolers have yet to fully "grow into the deficits" associated with

ADHD. Findings of largest between group effects in the left temporal lobe, SMC and M1, and lateral OFC may suggest the important role of developing language underlying working memory and inhibitory control in young children (e.g., internalization of self-speech, Barkley, 1997).

Furthermore, the variability in brain development at this point in development may help to explain the inconsistencies between the current findings and prior work in a smaller, and potentially underpowered, sample (Mahone, Crocetti, et al., 2011). Given the notable changes in PFC development occurring in all children during preschool (Brown & Jernigan, 2012), the present study provides a window into prefrontal structural differences between very young children with ADHD and their typical peers.

The identification of early biomarkers of ADHD is critical to developing more targeted interventions, both pharmacological and behavioral, to reduce the lifetime morbidity associated with the condition. Longitudinal studies of children with ADHD symptoms paint a concerning picture of functional outcomes and long-term risks associated with early onset of hyperactivity. Even when treated during preschool, children continue to have symptoms and functional impairment in elementary school (Riddle et al., 2013). Moreover, children identified as "hyperactive" at age 3 years show increased rates of functional impairment and psychopathology in adolescence relative to typical peers (Smith et al., 2016). Even as adults, those who were identified as "hyperactive" in preschool are reported to have 17 times higher annual healthcare costs (Chorozoglou et al., 2015).

Findings of the present study suggest that some neuroimaging biomarkers of ADHD may be age-dependent and set the stage for atypical behavioral development throughout childhood and adolescence. To this end, the continued longitudinal study of early biomarkers of ADHD may help researchers determine *how* early patterns of anomalous development (brain and behavior) affect later presentation of symptoms to identify what aspects of early brain development are associated with continued severity of symptoms, and which are associated with "recovery" from symptoms, incorporating knowledge gained from studying the patterns of "normalization" of structure and function in certain subgroups of children with ADHD (e.g., girls).

Strengths of the study include the careful group assignment/diagnostic strategy, modeled after the PATS studies (Kollins et al., 2006); matching of groups on age, sex, IQ, and language ability; and exclusion of common comorbidities to provide a clearer interpretation of findings specific to ADHD. At the same time, several limitations exist. Most notably, young children are quite variable in their day-to-day presentation with a large range of "normal" behavior at this age (Blackman, 1999; Byrne, Bawden, Beattie, & De Wolfe, 2000), a fact that is reflected in wide standard deviations and reduced reliability of performance measures (Baron & Anderson, 2012). As such, there is also a lack of stability in diagnostic classification over the preschool to early school-age time period (Chacko, Wakschlag, Hill, Danis, & Espy, 2009); given that data were collected at one point in time, we do not know how many of those within the ADHD group will

still meet diagnostic criteria at later assessments and thus prove "true positives" in terms of early diagnostic accuracy.

Given the sample age range and variability in school experience, teacher ratings were not available for some participants, limiting the ability to identify cross-setting impairment per DSM-5. Additionally, given the challenges of effectively scanning preschoolers without sedation, results may reflect only the degree of brain differences evident in children with only moderate symptom severity, for example, a degree of symptomatology that permitted them to lie still in the scanner long enough to yield useable imaging. It may be that greater between group differences would be evident if we were also able to include those children who were unable to successfully participate in MRI scanning. Furthermore, exclusion of common comorbidities, to the degree possible in children this age, may limit generalization to samples of children presenting with ADHD plus comorbidities. Exclusion of common comorbidities may also contribute to the high average IQ observed in the sample (Waber et al., 2007). Finally, the study did not include enough girls to permit well-powered between sex comparisons. Future work should examine the consistency of these early structural brain differences between sexes, to clarify the degree to which the pattern of sex differences evident in older children is present in preschoolers.

In summary, widespread reductions in cortical brain volumes are already evident in preschoolers with ADHD, with evidence for specific prefrontal and premotor regional anomalies. These data provide initial evidence that brain differences are evident at least as early as symptom onset, and add to earlier data suggesting subcortical anomalies as well (Mahone, Crocetti, et al., 2011). Furthermore, these findings provide groundwork for future work examining the developmental trajectory of cortical and subcortical development in preschoolers with ADHD, including analysis of structural and functional changes in white matter development, which will greatly expand our understanding of brain–behavior relationships in the disorder.

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