

Neural Correlates of Aggression in Medication-Naive Children with ADHD: Multivariate Analysis of Morphometry and Tractography

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Aggression is widely observed in children with attention deficit/hyperactivity disorder (ADHD) and has been frequently linked to frustration or the unsatisfied anticipation of reward. Although animal studies and human functional neuroimaging implicate altered reward processing in aggressive behaviors, no previous studies have documented the relationship between fronto-accumbal circuitry—a critical cortical pathway to subcortical limbic regions—and aggression in medication-naive children with ADHD. To address this, we collected behavioral measures and parental reports of aggression and impulsivity, as well as structural and diffusion MRI, from 30 children with ADHD and 31 healthy controls (HC) (mean age, 10 ± 2.1 SD). Using grey matter morphometry and probabilistic tractography combined with multivariate statistical modeling (partial least squares regression and support vector regression), we identified anomalies within the fronto-accumbal circuit in childhood ADHD, which were associated with increased aggression. More specifically, children with ADHD showed reduced right accumbal volumes and frontal-accumbal white matter connectivity compared with HC. The magnitude of the accumbal volume reductions within the ADHD group was significantly correlated with increased aggression, an effect mediated by the relationship between the accumbal volume and impulsivity. Furthermore, aggression, but not impulsivity, was significantly explained by multivariate measures of fronto-accumbal white matter connectivity and cortical thickness within the orbitofrontal cortex. Our multi-modal imaging, combined with multivariate statistical modeling, indicates that the fronto-accumbal circuit is an important substrate of aggression in children with ADHD. These findings suggest that strategies aimed at probing the fronto-accumbal circuit may be beneficial for the treatment of aggressive behaviors in childhood ADHD.

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INTRODUCTION

Aggression is a common presenting concern in children with attention-deficit/hyperactivity disorder (ADHD) (Connor *et al*, 2002). Behavioral studies, as well as clinical observation, suggest a link between aggressive outbursts and frustration, or the unsatisfied anticipation of reward, in hyperactive children (Scime and Norvilitis, 2006). Aggression is therefore thought to be associated with the brain's reward system, yet this relationship has scarcely been examined in children with ADHD.

Fronto-accumbal circuitry encompassing the nucleus accumbens (NAcc) and the ventral prefrontal region (Alexander and

Crutcher, 1990; Posner *et al*, 2014) is associated with reward expectation (Knutson *et al*, 2003), reinforcement learning (Adcock *et al*, 2006), and frustration in response to anticipated, but unmet, rewards (Stern and Passingham, 1996; Blair, 2004). Functional neuroimaging implicates the fronto-accumbal circuitry in the pathophysiology of ADHD (reviewed in Marsh *et al*, 2009). For example, positron emission tomography (PET) reports that individuals with ADHD have reduced dopaminergic transmission in the NAcc (Volkow *et al*, 2009). Similarly, functional MRI (fMRI) studies report that ADHD is related to reduced activation of the NAcc in response to reward expectations (Scheres *et al*, 2007) and altered functional connectivity between the NAcc and the orbitofrontal cortex (OFC) (Posner *et al*, 2013). Nevertheless, it is unknown whether fronto-accumbal anomalies relate to aggression in ADHD and whether such associations are dissociable from impulsivity. For example, aggressive behaviors could simply represent a manifestation of fronto-accumbal circuit related impulsivity, or, conversely, fronto-accumbal anomalies may underlie aggression that is

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discrete from more general impulsive behaviors seen in children with ADHD (Sonuga-Barke, 2005). This distinction may help clarify whether aggression in childhood ADHD has neurobiological correlates distinct from those of impulsivity, at least based on the structural and connectivity neuroimaging techniques used in the current study.

The following methodological issues merit consideration when attempting to identify neural correlates of aggression. First, examining both the gray and white matter within a brain circuit is preferable over prioritizing one or the other. This is true when studying not only aggression but any psychiatric symptoms or conditions thought to arise from 'brain circuits' rather than discrete lesions (Insel *et al*, 2010). For example, in a previous multi-modal neuroimaging study, combination of gray and white matter measurements (e.g., structural and functional) provides a more comprehensive account of neural substrates of clinical anxiety (Cha *et al*, 2014). Second, to incorporate and account for multiple brain measures, statistically robust multivariate pattern analyses are preferable over traditional univariate statistical approaches. Without multivariate techniques, the combined effects or influences of the multiple brain measurements on the outcome of interest (in this case, aggression) may be missed. For example, multivariate techniques are critical when the behavior of interest may be associated with latent (unobserved) effects of inter-linked nodes of a circuit, rather than isolated or regional anomalies. Last, compared with univariate techniques, multivariate approaches could more readily increase the predictability and generalizability of findings to novel data because of the validation procedures available with multivariate approaches.

With these methodological concerns in mind, we examined morphometric and structural connectivity measures within the fronto-accumbal circuit in children with ADHD, and how these measures relate to aggression. We used structural and diffusion MRI to examine gray matter structure and white matter connectivity, respectively, in medication-naïve children with ADHD and healthy controls. First, we tested the hypothesis that childhood ADHD is associated with gray and white matter anomalies within the fronto-accumbal circuit. Then, we employed multivariate statistical models (partial least squares regression and a supervised machine learning-support vector regression) to test our second hypothesis that morphometric and structural connectivity measures of the fronto-accumbal circuit would correlate with aggression in ADHD.

PATIENTS AND METHODS

The Institutional Review Board of the New York State Psychiatric Institute (NYSPI) approved the study procedures. Child participants provided informed assent, and a legal guardian provided written informed consent.

Participants

Our sample comprised 30 children with ADHD and 31 healthy control (HC) participants between the ages of 6 and 13 (mean, 10.3 years; SD, 2.09). The ADHD participants fulfilled DSM-IV criteria for ADHD-combined type, ADHD-predominantly hyperactive-impulsive type, or ADHD-

predominantly inattentive type. HC participants were free of DSM-IV Axis I psychiatric disorders and were group matched to the ADHD patients by age, gender, and Tanner stage. All study participants were free of any previous exposure to psychotropic medication. A board-certified child psychiatrist confirmed diagnoses derived from the parent and child versions of the Kiddie-Schedule for Affective Disorders and Schizophrenia (K-SADS-PL 2009; <http://www.psychiatry.pitt.edu/node/8234>) (Kaufman *et al*, 1996), ADHD Rating Scale-IV (DuPaul, 1991), Conners' Parent and Teacher Rating Scales Revised (Conners *et al*, 1998), and the Child Behavior Checklist (Achenbach and Rescorla, 2001). ADHD participants were excluded if found to have a diagnosis of a pervasive development disorder, bipolar disorder, psychotic disorder, or substance use disorder. Other comorbid disorders such as mood or oppositional defiant disorders were not exclusionary, but were recorded and controlled for in subsequent analyses (Table 1). Additional exclusion criteria for both groups included neurological illness or significant head trauma (ie, loss of consciousness > 2 min); serious medical problems; and MRI contraindications (eg, braces).

Participants were administered the continuous performance task (CPT), which assesses sustained attention and impulsivity (Klee and Garfinkel, 1983) (A description of the CPT is provided in the Supplementary Material.). Participants were also administered the Wechsler Abbreviated Scale of Intelligence (Wechsler, 1999). Compared with the HC participants, the participants with ADHD had a significantly lower estimated mean IQ (Table 1), which is common in samples of children with ADHD. IQ was thus used as a confounding variable in the statistical analyses. Parents completed the ADHD Rating Scale-IV (DuPaul, 1991), Conners' Parent Rating Scales Revised (Conners *et al*, 1998), the Child Behavior Checklist (Achenbach and Rescorla, 2001), and the Hollingshead Index of Social Status (Hollingshead, 1975).

MRI Acquisition

We collected 61 anatomical MRI scans (30 ADHD and 31HC) and 38 diffusion MRI scans (19 ADHD and 19 HC). Only a subset of the participants had diffusion MR data collected because of limitations in the availability of funds to support MRI data collection and the availability of the MRI scanner. There were no significant differences in any demographic or clinical features (age, gender, IQ, race, and symptom severity; Table 1) between children with and without diffusion MRI scans. Images were acquired at the New York State Psychiatric Institute on a GE Signa 3.0 Tesla whole-body scanner. High-resolution T1-weighted images were acquired using a fast-spoiled gradient-recall sequence with 11° flip angle; 256 × 256 matrix; 25 cm field of view; and 1 mm isotropic acquisition. Diffusion MR images were acquired in two runs using following parameters: TR 10586 ms; TE 70 ms; slice thickness 2 mm (skip 0); voxel size 2 × 2 × 2 mm³; slices 70. The series included three initial images acquired without diffusion weighting and with diffusion weighting along 15 non-collinear directions ($b = 1,000 \text{ sm}^{-2}$).

Diffusion MRI Tractography

To investigate structural connectivity of the fronto-accumbal circuit, we performed probabilistic tractography on diffusion

Table 1 Demographic and Clinical Characteristics of the Sample

	ADHD (<i>n</i> = 30)			D. Healthy Controls (<i>n</i> = 31)	Test statistic (d.f.)	P-value ^a
	A. Total (<i>n</i> = 30)	B. Structural and diffusion (<i>n</i> = 19)*	C. Structural, but no diffusion (<i>n</i> = 11)			
Age in years	9.4 ± 2.0	10.1 ± 2.0	9.83 ± 2.1	10.5 ± 2.1	A vs D: <i>t</i> (60) = 1.8 A vs B: <i>t</i> (47) = 0.4 A vs C: <i>t</i> (39) = 0.6	0.075 0.658 0.542
Gender	24 m, 6 f	14 m, 5 f	10 m, 1 f	21 m, 10 f	A vs D: $\chi^2(1) = 0.9$ A vs B: $\chi^2(1) = 0.3$ A vs C: $\chi^2(1) = 0.7$	0.405 0.606 0.411
FSIQ	99.1 ± 15.9	103.4 ± 16.2	91.68 ± 12.6	109.1 ± 15.7	A vs D: <i>t</i> (59) = 2.3 A vs B: <i>t</i> (47) = 0.9 A vs C: <i>t</i> (39) = 1.4	0.022 0.362 0.169
Tanner stages average	2.2 ± 1.2	2.1 ± 1.5	2.7 ± 1	2.2 ± 0.9	A vs D: <i>t</i> (29) = 0.1 A vs B: <i>t</i> (28) = 0.3 A vs C: <i>t</i> (19) = 0.7	0.940 0.736 0.464
Race	20 Cau, 10 AA	12 Cau, 6 AA, 1 other	7 Cau, 4 AA	19 Cau, 5 AA, 1 A, 6 Other	A vs D: $\chi^2(3) = 3.8$ A vs B: $\chi^2(2) = 1.6$ A vs C: $\chi^2(1) = 0.3$	0.287 0.447 0.856
ADHD diagnoses	24 ADHD-C, 1 ADHD-HI, 5 ADHD-I	13 ADHD-C, 1 ADHD-HI, 5 ADHD-I	11 ADHD-C	NA	A vs B: $\chi^2(2) = 0.8$ A vs C: $\chi^2(1) = 0.2$	0.656 0.698
Comorbid diagnoses	9 ODD (0 CD), 3 MDD, 2 Enuresis, 1 SA, 1 GAD/MDD	5 ODD, 1 SA 1 GAD/MDD,	4 ODD	NA	A vs B: $\chi^2(1) = 0.1$ A vs C: $\chi^2(1) = 0.2$	0.781 0.698
CBCL-aggression	63.1 ± 1.7	61.5 ± 9.9	65.9 ± 7.9	NA	A vs B: <i>t</i> (44) = 0.5 A vs C: <i>t</i> (36) = 0.9	0.591 0.400
CBCL-inattention	69.1 ± 2.1	66.8 ± 9.1	74.7 ± 13.7	NA	A vs B: <i>t</i> (44) = 0.9 A vs C: <i>t</i> (36) = 1.1	0.383 0.261
CPT-commission rate	53.5 ± 1.2	54.7 ± 6.0	51.5 ± 5.8	NA	A vs B: <i>t</i> (41) = 0.6 A vs C: <i>t</i> (33) = 0.9	0.553 0.366
ADHD-rating scale-inattention	23.7 ± 0.9	22.9 ± 5.1	25.3 ± 4.3	NA	A vs B: <i>t</i> (47) = 0.6 A vs C: <i>t</i> (39) = 0.9	0.560 0.400
ADHD-rating scale-hyperactivity	22.1 ± 0.9	21.7 ± 5.8	22.6 ± 3.6	NA	A vs B: <i>t</i> (47) = 0.2 A vs C: <i>t</i> (39) = 0.3	0.835 0.735

Abbreviations: A, Asian; AA, African American; ADHD-C, attention deficit hyperactivity disorder-combined type; ADHD-HI, attention deficit hyperactivity disorder-hyperactive type; ADHD-I, attention deficit hyperactivity disorder-inattentive type; Cau, Caucasian; CBCL, child behavior checklist; CD, conduct disorder; CPT, continuous performance task; FSIQ, Full-scale IQ estimated by the Wechsler Abbreviated Scale of Intelligence (WASI); GAD, generalized anxiety disorder; ODD, oppositional defiant disorder; MDD, major depressive disorder; NA, not applicable; SA, separation anxiety. Confidence interval is a SD.

*Children with ADHD who participated in both structural and diffusion MRI sessions

^aTwo-tailed in case of *t*-tests.

MRI data. Diffusion MRI data were processed with the FSL 5.0 Brain's Diffusion Toolbox (FDT) (Smith *et al*, 2004). This pipeline includes skull stripping, eddy current correction, B-matrix rotation, multi-fiber probabilistic diffusion modeling, and affine registration of the T1-weighted, diffusion-weighted, and Freesurfer-segmented and parcellated images. White matter tracts were then estimated using 'probabilistic tracking with crossing fibers' (probtrackx2) in FSL 5.0 (Behrens *et al*, 2003). We used each individual's NAcc segmentation mask (conducted with Freesurfer) as a seed (each hemisphere separately) and the prefrontal masks from Freesurfer parcellations as regions of interest (ROIs; see ROI Definition below for more details regarding ROI subdivisions). We created an exclusion mask from each individual's segmentation masks of the cerebral spinal fluid (CSF) and all the ventricles. Five thousand streamline samples were generated for each tractography run from each ROI voxel to build a connectivity distribution. We calculated the number of streamline samples from the seed mask

successfully arriving at the target ROI mask proportional to the total number of samples. We first used individuals' cortical parcellation masks as the target ROIs, and then excluded ROIs whose probabilistic measures did not reach a threshold of 0.02% of the total estimated streamlines; this is a commonly used threshold in previous studies (Forstmann *et al*, 2011; Li *et al*, 2012; Chowdhury *et al*, 2013). Thus, the connectivity measures represent ROI-to-ROI probabilistic connectivity. Tractography analyses were performed on a Linux-based High-Performance Computing system at Columbia University's Advanced Research Computing Services.

MRI Morphometric Analysis

NAcc volume and ventral prefrontal thickness were measured with the FreeSurfer 5.3 image analysis suite (<http://surfer.nmr.mgh.harvard.edu/>). The technical details of these procedures are described in previous publications (Dale *et al*, 1999; Fischl *et al*, 1999; Fischl and Dale, 2000; Fischl *et al*,

2002; Desikan *et al*, 2006). In brief, image processing includes motion correction, removal of non-brain tissue, Talairach transformation, segmentation, intensity normalization, tessellation of the gray matter white matter boundary, topology correction, and surface deformation. Deformation procedures use both intensity and continuity information to produce representations of cortical thickness. The maps produced are not restricted to the voxel resolution and are thus capable of detecting submillimeter differences between groups. Cortical thickness measures have been validated against histological analysis (Rosas *et al*, 2002) and manual measurements (Kuperberg *et al*, 2003; Salat *et al*, 2004).

ROI Definition

To examine the white matter (eg, tractography) and the gray matter (eg, morphometry) of the fronto-accumbal system, we specified *a priori* target ventral prefrontal ROIs based on a recent human anatomical study (Rigoard *et al*, 2011). Anatomical masks were defined using the automated parcellation (Fischl *et al*, 2004) and the Desikan–Killiany Atlas (Desikan *et al*, 2006). Target ROI included the medial orbitofrontal (mOFC), lateral orbitofrontal (lOFC), pars orbitalis (Pars Orb), rostral anterior cingulate (rACC) cortex, frontal pole (FP), and medial prefrontal cortex (mPFC) (Supplementary Figure S1; see Supplementary Information for detailed anatomical delineation of ROI).

Measures of Aggressive Behaviors and Impulsivity

In our statistical models, we used the Child Behavior Checklist (CBCL) aggression subscale for aggression (Achenbach and Edelbrock, 1991) and two separate measures for impulsivity: (i) the continuous performance task (CPT) commission rate (Conners and Staff, 2000), and (ii) the hyperactive/impulsive symptoms derived from the ADHD Rating Scale (ADHD-HI) (DuPaul, 1991). We used both behavioral/neuropsychological assessment (ie, CPT) and symptom score (ie, ADHD-HI) of impulsivity to ensure that we were adequately phenotyping impulsivity. By assessing both aggression and impulsivity, we aimed to test the specificity of our MRI measures whether our MRI measures were specific in predicting aggression, but not impulsivity. We confirmed that the aggression and impulsivity measures are normally distributed ($P_s > 0.205$; Kolmogorov–Smirnov test) (Supplementary Figure S2).

Statistical analyses

To determine whether participants with ADHD have differential fronto-accumbal tract measures and cortical thickness/subcortical volume measures (eg, ventral prefrontal cortex and NAcc) relative to HCs, we used permutation testing with group as the response, the MRI measures as the predictors, and age, gender, IQ, and the total intra-cranial volume estimate (in case of gray matter metrics) as covariates. Effects of ADHD subtype or ODD were then examined using a separate model. In case of tract measures, we used the network-based statistics (NBS) to control the familywise error rate (Zalesky *et al*, 2010). Detailed procedures for NBS are described in Supplementary Information.

To test the hypothesis that fronto-accumbal connectivity is related to aggression in children with ADHD, we used two

different multivariate pattern regression analyses: partial least square (PLS) regression and support vector regression (SVR). Using two independent approaches allowed us to effectively confirm and corroborate the findings. First, PLS regression was used because it circumvents limitations of standard multiple regression such as overfitting or collinearity. PLS regression uses a latent variable approach to reduce the predictors to a smaller set of uncorrelated components and performs least squares regression on these components. It is therefore particularly useful when the predictors are collinear, or when the number of predictors relative to the number of observations is high and thus the model may fail to balance predictability and generalizability. We used the nonlinear iterative PLS algorithm (Wold *et al*, 1984) that reduces the number of predictors by extracting components which explain the maximum correlation between the predictors (ie, tract measures) and the response variable (ie, aggression). Least-squares regression coupled with leave-one-out cross-validation (LOOCV) was then performed to select the components that maximized predictability of the model. We used aggression as the response variable, and the MRI measures (a tract model with the 10 ROI-specific tractography measures in each hemisphere separately, and an OFC thickness model with the 5 ROI-specific thickness measures in each hemisphere separately) as the predictors. We also tested models with impulsivity (based on the CPT and ADHD-HI) as the response variable to examine whether association with the fronto-accumbal MRI measures was specific to aggression.

Second, we performed SVR with a strict cross-validation method because this approach allows inferences at the level of individual, and thus has high translational potential in medicine (Orri *et al*, 2012). In brief, aggression scores were fitted with a linear SVR machine with random feature elimination (Guyon *et al*, 2002) using either structural connectivity or morphometric measures as the predictive variables. To assess the predictive capacity of the variables, a two-tiered cross-validation scheme was implemented. First, the data were split into leave-one-out training and testing folds. For each training fold, an additional LOOCV was carried out to select model parameters, the soft margin of the SVM, and the number of features by applying RFE. Next, the SVR model was fitted to the entire training fold and was used to predict the aggression score of the test fold. This two-tiered LOOCV resulted in a vector of predictions, which could be compared with the actual scores via the correlation coefficient. The significance of the resulting correlation was assessed through bootstrapping where we permuted the aggression scores randomly 1000 times, and then applied the same CV procedure to each of the resulting data sets to yield an empirical estimate of the significance of the prediction. SVR was performed using Neuroclass (Fekete *et al*, 2013) and the Library for SVM (LIB-SVM) toolbox (Chang and Lin, 2011). PLS and SVR were performed within the ADHD group only. After demonstrating an association of the left NAcc-seeded tractography measures with aggression, as a confirmatory analysis, we examined a subset of the fronto-accumbal tractography measures that best predicted aggression using ordinary least squares multiple linear regression. Age and gender were used as covariates. IQ was excluded as a covariate as no correlations with aggression/impulsivity or connectivity measures were observed ($P_s > 0.14$, Spearman's correlation, two-tailed).

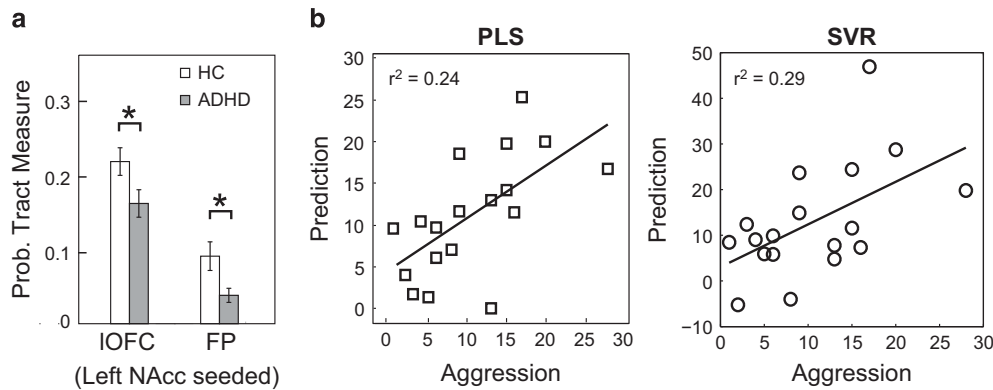


Figure 1 Decreased fronto-accumbal structural connectivity in childhood ADHD is associated with aggression. (a) Children with ADHD showed decreased probabilistic tractography measures between the left NAcc and the left IOFC and between the left NAcc and the left FP. (b) Fronto-accumbal probabilistic tract measures were associated with aggression in children with ADHD. In partial least squares (PLS) regression using the left fronto-accumbal tract measures, cross-validated prediction showed a linear pattern indicating that the model accounted for 24% of the variance in aggression (left). Similarly, in support vector regression (SVR), cross-validated prediction of aggression from the model based on the left fronto-accumbal tract measures accounted for 29% of the variance in aggression (right). * $P < 0.05$. FP, frontal pole; IOFC, lateral orbitofrontal cortex; NAcc, nucleus accumbens.

Previous research has suggested that decreased NAcc volume is associated with impulsivity in ADHD (Carmona et al, 2009). Based on this, we performed path modeling (mediation analysis) to test mediation effects of impulsivity on the relationship between NAcc volumes and aggression in ADHD. NAcc volume was normalized to each individual's intracranial volume. Significance of regression weight was determined using a two-tailed bootstrap bias-corrected percentile method. Path modeling was done using AMOS 22 (SPSS, Chicago, IL, USA).

RESULTS

White Matter, Fronto-Accumbal Structural Connectivity

Effects of ADHD. Initially, we estimated fronto-accumbal fiber tracts using DTI-probabilistic tractography. Using the NAcc as a seed region, our tract estimation across the entire sample yielded major tracts in the vicinity of the ventral and medial prefrontal regions (Supplementary Figure S3). Analysis of probabilistic connectivity measures in the prefrontal target regions revealed that children with ADHD, relative to HCs, exhibit a decrease in tract measures between the left NAcc and the left IOFC (effect size (partial η^2) = 0.14, $P < 0.01$, permutation test), and between the left NAcc and the left FP (effect size (partial η^2) = 0.13, $P < 0.032$) (Figure 1a). Neither the effects of ODD comorbidity ($P_s > 0.63$) nor ADHD subtype ($P_s > 0.50$) were observed in relation to these connectivity measures. The network-based statistics (NBS) confirmed that the reduction of the left NAcc-IOFC and the left NAcc-FP connectivity in ADHD was significant ($P_{FWE-corrected} < 0.034$; permutation testing).

Correlation with aggression. We next tested whether our estimated fronto-accumbal tract measures were associated with aggression or impulsivity in children with ADHD using multivariate partial least-squares (PLS) regression. A PLS model using aggression as the response variable and the left NAcc-seeded tract measure as the predictor accounted for 24% of the variance of aggression after cross validation

($F = 14.51$, $P < 0.001$; Figure 1b and Supplementary Figure S4). A model with the right NAcc-seeded tract did not predict aggression ($R^2 = 0$, cross validated). No PLS model significantly predicted impulsivity (either CPT-commissions or ADHD-HI) in relation to either the left or right NAcc-seeded tract measures ($R^2 = 0$, cross validated). Importantly, we were able to replicate these findings using support vector regression (SVR) as an alternative multivariate approach. SVR analysis revealed that the left fronto-accumbal tract measures accounted for 29% of the variance of aggression at a bootstrapping-corrected $P < 0.04$ (cross-validated). In this model, the left NAcc-mPFC (bilateral) tract explained 71% of the variance and the left NAcc-IOFC (bilateral) explained 16%. An SVR model using the right NAcc tract measures did not show significance.

Subsequent parametric multiple linear regression analyses showed a similar pattern of results. Stepwise estimation identified three tract measures as significant predictors of aggression: the left NAcc to left mPFC (semi-partial correlation = 0.70), the left NAcc to left rACC (semi-partial correlation = -0.50), and the left NAcc to right mOFC (semi-partial correlation = -0.39) (Supplementary Table S2 and Supplementary Figure S5). A model with the right NAcc tract measures selected no predictors.

Gray Matter, Fronto-Accumbal Morphometry

Effects of ADHD. We tested whether fronto-accumbal gray matter is altered in children with ADHD relative to HCs. We found that children with ADHD exhibit smaller right NAcc volumes compared with controls ($P = 0.019$; Kolmogorov-Smirnov test; Figure 2). This was significant after controlling for age, gender, IQ, and the total intracranial volume (effect size (partial η^2) = 0.09, $P = 0.033$, permutation test). Neither ODD comorbidity nor ADHD subtype had an effect on NAcc volumes ($P_s > 0.20$). No group differences in the left NAcc volumes were observed ($P > 0.41$). The ventral prefrontal regions that served as target ROIs for the NAcc-seeded tractography did not show group differences in cortical thickness ($P_s > 0.26$).

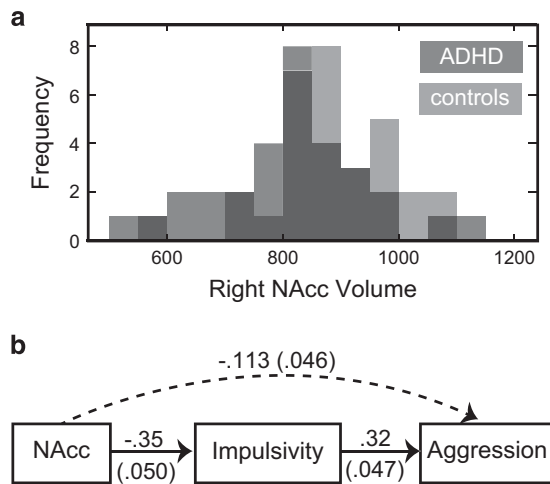


Figure 2 Reduced NAcc gray matter volume in ADHD is correlated with aggression. (a) Children with ADHD showed smaller right NAcc volumes than healthy controls ($P = 0.019$; Kolmogorov–Smirnov test). This effect was significant after controlling for age, IQ, and the total intracranial volume ($P = 0.033$, permutation test). (b) We conducted path modeling (mediation analysis) using the right NAcc volume (NAcc; normalized to the whole brain volume), impulsivity (indexed by CPT commissions), and aggression (indexed by parent ratings). The numbers represent standardized coefficients with Bias-corrected bootstrap significances (two-tailed) in brackets. The model shows acceptable model fit indices: $\chi^2 = 0.0$; GFI (goodness of fit index) = 1.0; RMR (root mean square residual) = 0.0, CFI (comparative fit index) = 1.0; RMSEA (root mean square error of approximation) = 0.206 (Hooper *et al*, 2008). NAcc; nucleus accumbens.

Correlation with Aggression. On the basis of the inverse correlation between NAcc volume and impulsivity in childhood ADHD (Carmona *et al*, 2009), we used path modeling (mediation analysis) to test the hypothesis that a smaller (right) NAcc volume leads to aggression by increasing impulsivity in ADHD. This analysis confirmed that right NAcc volumes predicts aggression in children with ADHD, an effect that is at least in part mediated through the impact of right NAcc volume on impulsivity (CPT commissions; Figure 2). Without the mediator, the NAcc volume has a non-significant effect on aggression ($P > 0.39$). A path model using impulsivity symptom scores (ADHD-HI) instead of CPT commissions showed a non-significant indirect effect of NAcc volume on aggression ($P > 0.35$).

In a PLS model, aggression was significantly explained by the ventral prefrontal cortical thickness in children with ADHD (right hemisphere: $F = 7.31$, $P = 0.001$, cross-validated $R^2 = 0.25$; left hemisphere: $F = 3.71$, $P = 0.018$, cross-validated $R^2 = 0.08$). In a subsequent multiple linear regression analysis, stepwise estimation identified cortical thickness in the left Pars Orb as a significant predictor of aggression (Figure 3). Association of the Pars Orb appeared to be specific to aggression because, in a linear regression model using the Pars Orb cortical thickness as the response, and aggression and impulsivity as the predictors, aggression predicted Pars Orb thickness (Robust Coefficient = -0.036 , $P = 0.004$), but not impulsivity (CPT commissions or ADHD-HI; $P_s > 0.2$). These results support the idea that variability in the cortical thickness of the ventral prefrontal gray matter is predictive of aggression in children with ADHD.

DISCUSSION

In the present study, we examined morphometry and structural connectivity of fronto-accumbal circuitry in medication-naïve children with ADHD. We demonstrate that (i) childhood ADHD is associated with reduced NAcc gray matter volumes and decreased fronto-accumbal structural connectivity and (ii) the patterns of gray and white matter structure (ie, morphometry and connectivity, respectively) in children with ADHD are predictive of the level of aggression in these children. This association may be particular to aggression, as it was not found to be attributable to impulsivity. With robust multivariate statistics, validation methods, and multi-modal neuroimaging, our results provide converging evidence supporting the role of fronto-accumbal circuitry in aggression in childhood ADHD.

Our finding of reduced NAcc volume in childhood ADHD is consistent with previous reports. For example, a recent study found reduced volumes of the ventral striatum (which encompasses the NAcc) in medication naïve as well as medicated children with ADHD. This volumetric abnormality correlated with levels of impulsivity (Carmona *et al*, 2009). Our study replicates these findings by demonstrating that ADHD-related reductions in NAcc volumes correlate with impulsivity as measured by a behavioral/neuropsychological assessment (CPT-commissions; Klee and Garfinkel, 1983); it further shows that the negative correlation between NAcc volume and impulsivity mediates the relationship between NAcc volume and aggression. This suggests that NAcc volumetric abnormalities may contribute to increased aggression in childhood ADHD indirectly, as this effect transmitted via the child's impulsivity. This path analysis thus specifies the role of the NAcc in aggression in childhood ADHD more precisely than has been previously reported.

Using probabilistic tractography, we found that fronto-accumbal structural connectivity is reduced in childhood ADHD. This is the first study to use DTI tractography to examine this circuit in ADHD, and our findings parallel previous studies that used resting-state fMRI to show reduced functional connectivity within this circuit in children with this disorder (Posner *et al*, 2013). Abnormalities in fronto-accumbal white matter connectivity are congruent with previous reports of prefrontal hypoplasia in childhood ADHD (Ridderinkhof *et al*, 2004; Shaw *et al*, 2007a). In addition, considering that delayed cortical (gray matter) maturation has been previously implicated in childhood ADHD (Shaw *et al*, 2007a), it is possible that our connectivity results reflect insufficient maturation of the white matter tracts (eg, deficits in myelination or fasciculation) in this circuit. However, the cross-sectional design of our study limits us from testing this directly, and thus future research using longitudinal investigations of white matter maturation may be useful to draw a more complete picture of white matter development in ADHD.

A few previous studies using DTI have reported increases in fractional anisotropy (FA) in inferior frontal regions in childhood ADHD (Silk *et al*, 2009; Li *et al*, 2010). Although these findings seemingly diverge from our tractography results, the increased frontal FA identified in these studies could relate to circuits other than the fronto-accumbal circuit in the present study: for example, fronto-amygdala

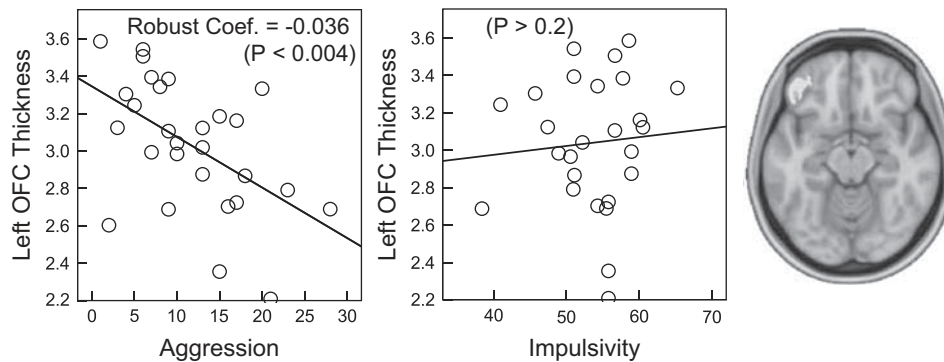


Figure 3 Ventral prefrontal cortical thickness is inversely correlated with aggression in childhood ADHD. Aggression significantly accounted for the variance in ventral prefrontal cortical thickness (left), whereas impulsivity did not (middle; $P_s > 0.2$). A group average ROI for the Pars Orb (yellow) is overlaid on the standard MNI 152 template (right). ROI, region of interest; Pars Orb, pars orbitalis. A full color version of this figure is available at the *Neuropsychopharmacology* journal online.

connections via the uncinate fasciculus. A limitation of voxel-wise analyses of FA (and related DTI measures) is that, unlike DTI-tractography, these approaches cannot identify the specific circuits exhibiting FA abnormalities.

The multivariate statistical models used in this study implicate the left fronto-accumbal circuit as a neural substrate of aggression in ADHD. Our results were significant in two independent multivariate statistical analyses (PLS regression and SVR) followed by strict cross-validation methods. Similar analyses based on ventral prefrontal cortical thickness demonstrated additional evidence for the role of the fronto-accumbal system in aggression in childhood ADHD. Taken together, our findings suggest that MRI measures of gray and white matter within fronto-accumbal circuits are predictive of aggression in childhood ADHD. Aggression—a multifaceted maladaptive behavior—may result from complex factors, as probed by patterns of MRI measures, rather than from a single MRI measure (eg, an increase/decrease in gray matter volume or voxel-wise diffusion measure of white matter). Such circuit-wide (ie, gray and white matter) MRI measures of aggression are analogous to the multiple MRI measures that account for OFC dysregulation in pathological anxiety (Cha *et al*, 2014). Our findings underscore the importance of coupling multi-modal MRI and multivariate statistical modeling to examine circuit-wide contributions to complex behaviors such as aggression.

Our morphometry and connectivity results do not provide coherent evidence for lateralized brain substrates of aggression. In animal literature, lateralization of aggression has been frequently reported, although the direction of asymmetry varies across species (eg, left-sided biases in horses (Austin and Rogers, 2012) and amphibians (Deckel, 1995; Robins *et al*, 1998) and right-sided in fish (Bisazza and de Santi, 2003)). In contrast, controversy exists in human literature: while earlier studies report valence-specific lateralization of the brain (eg, ‘positive emotions’ associated with left frontal activation and ‘negative emotions’ associated with right frontal activation (Davidson, 1992; Killgore and Yurgelun-Todd, 2007)), meta-analyses on neuroimaging data provide limited support for such claims (Wager *et al*, 2003). Of note, a recent transcranial magnetic stimulation study indicates that inter-hemispheric communication is important in determining aggression in healthy humans (Hofman

and Schutter, 2009). Although no firm conclusions can be made here, our multi-modal neuroimaging results provide novel, complementary information about the human neural correlates of pathological aggression and bilateral fronto-accumbal circuitry.

Limitations of the study merit consideration. First, our measure of aggression (CBCL-aggression subscale) did not differentiate reactive from proactive forms of aggression. Reactive aggression refers to sudden outbursts of destructive behaviors that follow the incitement of anger or frustration (Blair, 2004). Conversely, proactive aggression refers to dispassionate, unprovoked assaults and is often associated with sociopathy (Blair, 2004). Follow-up research using more refined assessments of aggression might test the hypothesis that the relationship between aggression and fronto-accumbal circuitry in ADHD is more specific to reactive *vs* proactive forms of aggression. Second, some of the MRI measures showed significant correlations with aggression within children with ADHD, but non-significant effects of ADHD compared with HC. Our multivariate models indicate that aggression is associated with patterns of connectivity and cortical thickness, not with discrete abnormalities within the fronto-accumbal circuit. Our findings should not be interpreted to mean that a single MRI measure of the fronto-accumbal circuitry is predictive of aggression. Rather, circuit-wide measures must be considered. Finally, given the relatively modest sample size of this study, despite the robust statistical procedures used here (ie, cross-validation and permutation testing), replication with a larger sample from multiple sites would help establish the reliability of the study findings. This is particularly important to fully exclude potentially confounding effects of ADHD subtype and comorbid disorders on fronto-accumbal circuitry. Though we did not detect effects related to ADHD subtype and/or comorbidity, our sample had limited statistical power to detect these effects.

CONCLUSIONS

Morphometry and structural connectivity MRI measures combined with robust statistical modeling revealed that fronto-accumbal circuitry has an important role in aggression in childhood ADHD. Furthermore, the fronto-accumbal

associations were specific to aggression and were not attributable to impulsivity, comorbid disorders, or ADHD subtype. These findings point to potential distinct and identifiable neurobiological correlates of aggression in ADHD. Future studies should examine whether pharmacological and/or behavioral treatments for aggression are mediated by their effects on fronto-accumbal circuitry. If so, fronto-accumbal circuitry would represent an important target for treatments to curtail aggression in childhood ADHD.

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