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ORIGINAL CONTRIBUTION

Combined stimulant and antipsychotic treatment in adolescents with attention-deficit/hyperactivity disorder: a cross-sectional observational structural MRI study

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Abstract Meta-analyses suggest normalizing effects of methylphenidate on structural fronto-striatal abnormalities in patients with attention-deficit/hyperactivity disorder (ADHD). A subgroup of patients receives atypical antipsychotics concurrent with methylphenidate. Long-term safety and efficacy of combined treatment are unknown. The current study provides an initial investigation of structural brain correlates of combined methylphenidate and antipsychotic treatment in patients with ADHD. Structural magnetic resonance imaging was obtained in 31 patients who had received combined methylphenidate and antipsychotic treatment, 31 matched patients who had received methylphenidate but not antipsychotics, and 31 healthy controls (M age 16.7 years). We analyzed between-group effects in total cortical and subcortical volume, and in seven frontal cortical and eight subcortical-limbic volumes of interest, each involved in dopaminergic neurotransmission. Patients in the combined treatment group, but not those in the methylphenidate only group, showed a reduction in total cortical volume compared to healthy controls (Cohen's $d = 0.69$, $p < 0.004$), which was apparent in most frontal volumes of interest. Further, the combined treatment group, but not the methylphenidate group, showed volume reduction in bilateral ventral diencephalon (*Left* Cohen's $d = 0.48$, $p < 0.04$; *Right* Cohen's $d = 0.46$, $p < 0.05$) and the left thalamus (Cohen's $d = 0.47$, $p < 0.04$). These findings may indicate antipsychotic treatment counteracting the normalizing effects of methylphenidate on brain structure. However, it cannot be ruled out that pre-existing clinical differences between both patient groups may have resulted in anatomical differences at the time of scanning. The absence of an untreated ADHD group hinders unequivocal interpretation and implications of our findings.

Keywords ADHD · Neuroimaging · Treatment · Antipsychotics · Adolescents

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Attention-deficit/hyperactivity disorder (ADHD) is characterized by age-inappropriate hyperactivity, impulsivity, and/or inattention. Subtle though widespread differences in brain morphology have been found in patients with ADHD, the most replicated being reduced volumes of the basal ganglia including the caudate, putamen, and globuspallidus, and of frontal regions [1–3]. Treatment with methylphenidate is the medical intervention of first choice [4]. Neuroimaging studies investigating the effect of methylphenidate on brain structure and function in children with ADHD consistently suggest normalizing effect. At least partial normalization has been reported for volumes of the

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anterior cingulate cortex, thalamus, inferior frontal gyrus, right precentral gyrus, right parieto-occipital gyrus, and the cerebellar vermis [5].

In a subgroup of patients with ADHD, stimulant treatment is combined with atypical antipsychotics such as risperidone or pipamperone. Antipsychotics have been recommended by an expert group for the treatment of comorbid disruptive behavior and severe aggression in ADHD [6]. Besides disruptive behavior, co-morbid pervasive developmental disorder (PDD) has been found to be predictive of the prescription of antipsychotics [7]. In the Netherlands, atypical antipsychotics are prescribed to 8 % of stimulant-treated children with ADHD [7]. Increasing prescription rates of atypical antipsychotics to children and adolescents [8] have raised concerns [9].

Abnormalities in dopaminergic neurotransmission have been reported in patients with ADHD and include increased levels of striatal dopamine auto-receptors and reduced dopamine metabolism in the frontal cortex [10]. Both methylphenidate and atypical antipsychotics exert their effects by interacting with dopaminergic neurotransmission, albeit with opposite modes of action. Methylphenidate blocks dopamine reuptake and stimulates dopamine release from the presynaptic cell, resulting in increased synaptic levels of dopamine [11]. Most atypical antipsychotics, by contrast, are dopamine antagonists blocking the effects of dopamine in the synapse by occupying the postsynaptic dopamine D2 receptors. It has been suggested that combined treatment with methylphenidate and antipsychotics may compromise the effects of each of the individual agents [12]. Large-scale studies on long-term safety and efficacy of combined treatment have not yet been performed [13].

Little is known about the possible effects of atypical antipsychotic treatment on structural brain development in children. The few studies investigating the neural effects of antipsychotics in pediatric populations have been limited to childhood-onset schizophrenia and pediatric bipolar disorder. Frazier et al. [14] reported a trend of normalizing subcortical volumes with clozapine treatment in the children with childhood-onset schizophrenia, but others found no such effect [15]. Despite increasing prescription rates, no studies have yet investigated the effects of atypical antipsychotic treatment on brain development in children with ADHD.

The current cross-sectional, observational MRI study investigated brain correlates of combined methylphenidate and atypical antipsychotics treatment in patients with ADHD, in comparison to patients who had received methylphenidate only, and to healthy control subjects. In the absence of a medication-naïve ADHD group and of pre-treatment measurements, we were unable to directly investigate whether concurrent antipsychotic treatment would counteract any normalizing effects of methylphenidate treatment on brain structure. However, based on the opposing synaptic effects of the two substances, we expected to find volume reductions in frontal-striatal regions in patients who had received combined treatment compared to healthy control participants, and that such reductions would be smaller or absent in patients who had received methylphenidate treatment only.

Methods

Participants

This study was part of NeuroIMAGE [16], the follow-up of the Dutch part of the International Multicenter ADHD Genetics (IMAGE) study [17]. The NeuroIMAGE sample consists of 1,045 children from 330 ADHD and 154 control families, who met the following inclusion criteria: age between 5 and 30 years, of European Caucasian descent, an IQ $\geq$70, and no diagnosis of autistic disorder, general learning difficulties, brain disorders, or known genetic disorders. All subjects who successfully underwent diagnostic assessment and structural MRI scanning were considered for inclusion in the current study. First, all participants with ADHD who received combined treatment with (1) any methylphenidate preparation and (2) atypical antipsychotics, either in the past or currently and for a minimum duration of thirty 30 days, were included in the study sample (MPH + AAP group). Next, two one-to-one age-and gender-matched control samples were drawn: a methylphenidate group (MPH group) consisting of participants with ADHD with current or past methylphenidate treatment with a minimum duration of 30 days and no treatment with antipsychotics and a healthy control group (HC group) consisting of participants with no psychiatric diagnosis and no current or past treatment with psychoactive medication of any type. Informed consent was signed by all participants (and parents) and the study had been approved by the local ethics committees. Detailed demographic, clinical and treatment characteristics of the three participant groups are presented in the results section and in Table 1.

Diagnostic assessment

The Dutch translation of the Schedule for Affective Disorders and Schizophrenia for School-Age Children—Present and Lifetime Version (K-SADS [18]), was administered. In addition, all participants were administered the Conners parent rating scale—Revised (Long version, CPRS-R:L [19]) combined with either the Conners Teacher Rating Scale—Revised (Long version, CTRS-R:L [20]) for participants <18 years old, or the Conners adult ADHD rating

Table 1 Clinical and phenotypical characteristics of both ADHD groups

	$ADHD_{MPH}$ ($N = 31$)		$ADHD_{MPH+AAP}$ ($N = 31$)		F	p
	Mean	SD	Mean	SD		
Total number of ADHD Symptoms	13.03	3.13	14.74	2.91	4.971	0.030*
IQ	99.71	14.65	92.52	15.28	3.580	0.063*
CGAS	63.23	7.91	59.68	9.48	2.560	0.041*
MPH treatment duration (years)	5.11	3.12	5.71	3.47	0.505	0.480*
MPH age of initiation (years)	9.35	3.33	8.35	3.39	1.340	0.252*
MPH age of cessation (years)	15.91	1.80	14.58	1.66	0.298	0.591*
MPH dose	32.60	2.44	30.89	2.40	0.250	0.619*
CSBQ score	28.38	17.86	32.77	16.09	1.005	0.320*
	N	%	*N*	%	Chi^2	*p*
ADHD type					2.761	0.430
Combined type	13	41.9	19	61.3		
Hyperactive type	3	9.7	3	9.7		
Inattentive type	14	45.2	8	25.8		
Mild ADHD	1	3.2	1	3.2		
Current users (< 3 months prior to scan)	20	64.5	18	58.1	0.272	0.602
Co-morbid diagnosis (any)	12	38.7	15	48.4	0.590	0.304
Co-morbid diagnosis (ODD/CD)	10	32.3	14	45.2	1.088	0.297
Medication other than MPH or AAP	17	54.8	24	77.4	3.528	0.060

Groups, including the HC group, are matched one-to-one on gender and age (*M* age = 16.7 years; 84 % male; no differences between groups)

MPH methylphenidate, *CGAS* children's global assessment scale, *CSBQ* children's social behavior questionnaire, *ODD* oppositional defiant disorder, *CD* conduct disorder

* $p < 0.05$

scales—self-report (Long Version, CAARS-S:L [21]) for participants ≥18 years old. For participants using medication, ratings reflected their functioning while they were off medication.

Scores on the K-SADS interview and Conners questionnaires were restructured to match the DSM 5criteria for ADHD. Participants with ADHD had to fulfill the following criteria: (1) six or more symptoms of hyperactivity/impulsivity and/or inattentiveness (five for participants ≥18 old), (2) meet DSM 5 criteria for pervasiveness of symptoms and impact on daily functioning, (3) symptom onset before the age of 12 years, and (4) $T \geq 63$ on at least one of the ADHD scales on either one of the Conners questionnaires. Two participants who fulfilled all criteria but one for a full ADHD diagnosis were classified as mild ADHD cases. ADHD type (predominantly hyperactive/impulsive, predominantly inattentive, or combined type), impairment in daily functioning (Children's Global Assessment Scale, CGAS [22]) and comorbidity were assessed using the K-SADS interview. Mild PDD symptoms were assessed with the Children's Social Behavior Questionnaire (CSBQ [23]). Healthy control participants were required to have less than three ADHD symptoms (two for participants ≥18 years old) and $T < 63$ on each of the scales of all Conners questionnaires.

From pharmacy transcripts, the following parameters were obtained for each type of psychoactive medication used: treatment duration, age of treatment initiation and cessation, mean daily dose, and current vs. past user. If pharmacy transcripts were incomplete ($n = 19$, 31 %), information from parent-report questionnaires was used.

MRI acquisition and analysis

MRI data were acquired at 1.5 Tesla on a Siemens Sonata scanner at the VU Medical Centre in Amsterdam and on a Siemens Avanto scanner at the Donders Centre for Cognitive Neuroimagingin Nijmegen (Siemens, Germany). A standard identical 8-channel phased array coil model was used at both sites and all scan parameters were matched as closely as possible. AT1-weighted 3D MP-RAGE scan was acquired with parallel imaging by generalized auto-calibrating partially parallel acquisition (GRAPPA; 176 sagittal slices, voxel size 1 × 1 × 1 mm, FOV = 256 × 256 × 176 mm).

Cortical reconstruction and volumetric segmentation were performed with FreeSurfer software version 5.3 with default settings (http://surfer.nmr.mgh.harvard.edu/). FreeSurfer is an image processing pipeline including a volume-based route to subcortical segmentation [24] and a surface-based route to create a 3D reconstruction and parcellation of the cortical sheet [25]. From FreeSurfer parcellations [26] and segmentations, we calculated total cortical and subcortical volume, and selected eight bilateral subcortical and limbic volumes of interest (VOIs; bilateral ventral diencephalon, putamen, caudate nucleus, globuspallidus,

nucleus accumbens area, hippocampus, amygdala, and thalamus) and seven bilateral frontal cortical VOIs [inferior frontal gyrus (IFG); sum of pars orbitalis, pars triangularis, and pars opercularis], [orbitofrontal gyrus (OFG); sum of medial orbitofrontal gyrus and lateral orbitofrontal gyrus], [middle frontal gyrus (MFG); sum of caudal middle frontal gyrus and rostral middle frontal gyrus], superior frontal gyrus (SFG), [anterior cingulate gyrus (ACC); sum of caudal anterior cingulate cortex and rostral anterior cingulate cortex], precentral gyrus, and the frontal pole, all implicated in dopaminergic pathways.

Statistical analyses

Treatment group (HC, MPH, or MPH + AAP) was entered in two univariate linear mixed regression models predicting standardized total cortical and subcortical volume. Dummy variables modeled between-group contrasts (MPH + AAP vs. HC, MPH vs. HC, and MPH + AAP vs. MPH; the third contrast was tested in a second run of the model). Age, gender, and scanner location were entered as fixed covariates. To correct for family relatedness within the sample, a random family intercept was modeled. Restricted Maximum Likelihood (REML) was applied for model estimation.

We analyzed between-group effects in (1) the subcortical-limbic VOIs, (2) the right, and (3) left frontal cortical VOIs in three multivariate linear mixed regression models, containing the same covariates and dummy variables for between-group contrasts. The VOI analyses were initially run without total cortical or subcortical volume as a covariate. If there was a significant ($\alpha < 0.05$) effect of treatment group on total cortical or subcortical volume, totals were added to the model to investigate the local effects that could not be accounted for by global effects. For each significant between-group effect, effect size (Cohen's d [27]) was calculated as the difference between the estimated marginal means divided by their pooled standard deviations. False discovery rate (FDR) procedures (maximum acceptable FDR of 5 %) accounted for multiple hypothesis testing [28].

Structural brain differences between groups may be mediated by pre-existing clinical differences between groups (e.g. in symptom severity), but adding such measures to the model as covariates eliminates variance of interest [29]. Continuous variables of significant difference between both treatment groups were thus entered as covariates only in a secondary step to provide an exploratory analysis of possible confounders. The contribution of the covariates to between-group differences was assessed for each contrast by calculating the range of changes in effect size (Cohen's $d_{\text{model with covariate}}$ − Cohen's $d_{\text{initial model}}$) and p values, and the average of absolute changes in effect size and p value, within brain volumes affected by treatment group. For categorical factors that may have confounded the results, sensitivity analyses were performed by repeating the analyses in each subgroup (e.g. patients with and without a history of atomoxetine treatment).

Results

Clinical sample characteristics

The sample consisted of 93 participants from 87 families, between the ages of 10 and 24 years with no age differences between the three participant groups (HC $M = 16.7$, SD = 3.3, range 10.6–24.8; ADHD_{MPH}: $M = 16.6$, SD = 3.0, range 10.6–22.3; $\text{ADHD}_{\text{MPH+AAP}}$: $M = 16.7$, SD = 3.3, range 10.2–24.2). Eighty-four percent of participants were males and 50 % participated in Amsterdam, both variables being equally distributed over the three groups ($\text{Chi}^2_{\text{gender}} = 0.000$, $p = 1.00$; $\text{Chi}^2_{\text{location}} = 2.409$, $p < 0.300$). IQ in the ADHD sample was lower than in the healthy control sample ($M_{\text{HC}} = 103.26$, $M_{\text{ADHD}} = 96.11$, $t = 2.235$, $p = 0.028$), as was CGAS-score of daily functioning ($M_{\text{HC}} = 89.67$, $M_{\text{ADHD}} = 61.45$, $t = 17.254$ $p = 0.001$). Patients in the MPH + AAP group had more ADHD symptoms than the MPH group. We found no other clinical differences (i.e. IQ, ADHD type, presence of comorbid diagnoses, scores on anautism spectrum questionnaire) between both ADHD groups (Table 1). Comorbid diagnoses included ODD/CD ($n_{\text{MPH}} = 10$; $n_{\text{MPH+AAP}} = 14$; $\text{Chi}^2 = 1.088$, $p = 0.297$), anxiety disorders ($n_{\text{MPH}} = 2$; $n_{\text{MPH+AAP}} = 0$; $\text{Chi}^2 = 2.067$, $p = 0.151$), and tic disorders ($n_{\text{MPH}} = 1$; $n_{\text{MPH+AAP}} = 1$; $\text{Chi}^2 = 0.000$, $p = 1.000$), which were equally distributed across the two groups.

Within all patients who had been medicated, 81 % had been prescribed immediate-release methylphenidate preparations ($n = 50$), 90 % extended-release methylphenidate preparations ($n = 56$), and 15 % dexamphetamine preparations ($n = 9$). The majority of patients had a treatment history of more than one stimulant type ($n = 46$, 74 %). Sixty-one percent ($n = 38$) of patients received stimulant treatment within 3 months prior to scan (current users). Stimulant treatment duration ranged from 0.1 to 12.1 years with a mean (SD) of 5.4 (3.3) years. We found no between-group differences regarding stimulant treatment duration, age of stimulant treatment onset, age of treatment cessation, mean daily dose, and the proportion of current users (Table 1). Although not significant, more patients in the MPH + AAP group had a history of psychotropic medication treatment other than MPH + AAP. Medication other than MPH or AAP included clonidine ($n_{\text{MPH}} = 1$; $n_{\text{MPH+AAP}} = 5$), atomoxetine ($n_{\text{MPH}} = 3$; $n_{\text{MPH+AAP}} = 15$), melatonin ($n_{\text{MPH}} = 13$; $n_{\text{MPH+AAP}} = 19$), antidepressants ($n_{\text{MPH}} = 1$; $n_{\text{MPH+AAP}} = 5$), and anxiolytics

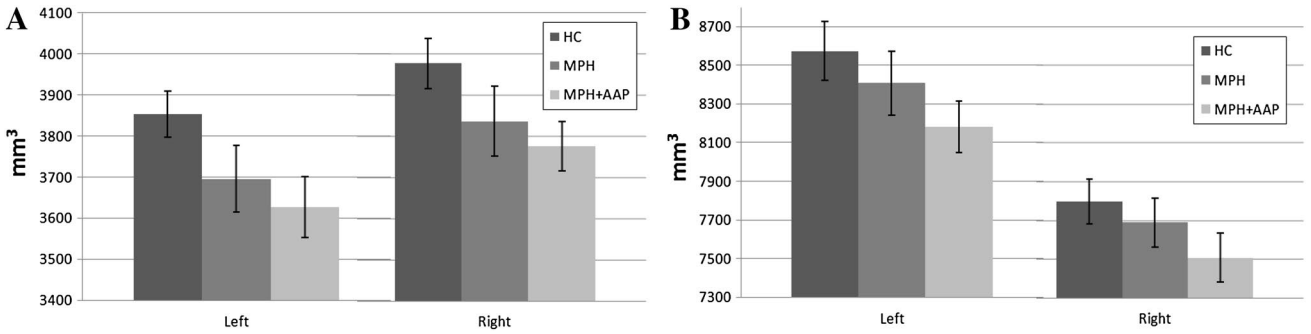

Fig. 1 Mean left and right ventral diencephalon volume (**a**) and left and right thalamus volume (**b**) of the three groups, with their standard errors. Uncorrected volumes are displayed in mm^3. The MPH + AAP group, but not the MPH group, showed significant volume decrease compared to the HC group

($n_{MPH} = 1$; $n_{MPH+AAP} = 1$). A history of atomoxetine use was significantly more prevalent in the combined treatment group compared to in the MPH only group ($Chi^2 = 11.3$, $p = 0.001$).

In the MPH + AAP group, most patients had been prescribed risperidone ($n = 24$), with a mean daily dose of 1.2 mg; other antipsychotics were pipamperone ($n = 8$), quetiapine ($n = 1$), olanzapine ($n = 1$), and aripiprazole ($n = 1$). Four participants had a history of two antipsychotic agents. Antipsychotic treatment duration ranged from 0.2 to 10.9 years (mean = 3.7, SD = 2.9), and age of antipsychotic treatment initiation ranged from 2.6 to 17.2 years (mean = 10.7, SD = 3.7). Fifty-eight percent of patients ($n = 18$) had ceased AAP treatment at least 3 months, and on average 3.8 years (SD = 2.7), prior to scan.

Subcortical-limbic volumes

There were no differences in total subcortical volume between the three groups. Local volume reductions approaching moderate effect sizes were found in the MPH + AAP group compared to the HC group (Fig. 1) in the left ($\beta = -0.5204$, $p_{uncorrected}$ <0.04, Cohen's $d = 0.48$) and right ($\beta = -0.4919$, $p_{uncorrected} = 0.05$, Cohen's $d = 0.46$)ventral diencephalon and the left thalamus($\beta = -0.5118$, $p_{uncorrected} = 0.04$, Cohen's $d = 0.47$). A similar effect size was found in the right thalamus, but this effect only approached significance ($\beta = -0.471$, $p_{uncorrected} = 0.058$, Cohen's $d = 0.44$). No between-group effects survived FDR-correction for multiple testing. There were no local volume differences between the MPH and HC group, or between the MPH and MPH + AAP group.

Cortical volumes

Total cortical volume was reduced in the MPH + AAP group compared to the HC group with moderate effect size ($p_{uncorrected} = 0.004$; Cohen's $d = 0.69$; $p_{FDR\text{-}corrected} < 0.075$), but not between the MPH and HC group or between the MPH and MPH + AAP group. Results from the cortical VOI analyses are summarized in Table 2 and Fig. 2a. The MPH + AAP group showed significant volume reductions compared to the HC group in bilateral precentral gyrus, IFG, OFG, SFG, MFG, and left ACC, with effect sizes ranging from small (Cohen's $d = 0.47$ in left MFG) to large (Cohen's $d = 0.80$ in left ACC and right precentral gyrus). Effects in the right precentral gyrus ($p_{FDR\text{-}corrected} = 0.028$) and left ACC ($p_{FDR\text{-}corrected} = 0.028$) survived correction for multiple testing. The MPH group showed volume reductions compared to HC in the right SFG, the right OFG, and the left ACC, with moderate effect sizes ranging from 0.47 to 0.77, of which left ACC volume reduction survived correction for multiple comparisons ($p_{FDR\text{-}corrected} = 0.028$). Comparing MPH to MPH + AAP yielded a significant difference of moderate effect size in the right precentral gyrus (Cohen's $d = 0.58$), which did not survive FDR-correction. Total cortical volume was added to the model to assess local rather than global effects. After adding total cortical volume, left ACC volume was reduced in both the MPH + AAP group ($p = 0.001$; Cohen's $d = 0.72$) and the MPH group ($p = 0.03$; Cohen's $d = 0.49$) compared to the HC group. Volume reduction in the MPH + AAP group survived FDR-correction ($p_{FDR\text{-}corrected} = 0.042$).

Exploratory analyses of clinical confounders

Since the MPH + AAP group displayed more ADHD symptoms, greater functional impairment, and more patients with a history of atomoxetine treatment than the MPH group, we explored the contribution of these factors to structural brain differences. Adding total symptom count to the model affected the effect sizes and p values in each contrast and in each brain region (Fig. 2b; Table 3). Average absolute change in effect sizes [Cohen's $d_{(model}$

Table 2 Between-group contrasts of total cortical volume and cortical volumes of interest

		MPH vs. HC		MPH + AAP vs. HC		MPH + AAP vs. MPH	
		β	$p^{*\dagger}$	β	$p^{*\dagger}$	β	p^{*}
Total cortical volume		−0.32	0.10*†	−0.60	0.01*†	0.28	0.16*
Middle frontal gyrus	R	−0.32	0.15*†	−0.55	0.02*†	0.23	0.31*
	L	−0.38	0.09*†	−0.46	0.04*†	0.08	0.71*
Orbitofrontal gyrus	R	−0.55	0.01*†	−0.54	0.02*†	−0.01	0.95*
	L	−0.22	0.32*†	−0.54	0.02*†	0.32	0.16*
Inferior frontal gyrus	R	−0.17	0.45*†	−0.58	0.01*†	0.42	0.06*
	L	−0.18	0.41*†	−0.59	0.01*†	0.40	0.07*
Precentral gyrus	R	−0.22	0.31*†	−0.79	0.01*†	0.57	0.01*
	L	−0.38	0.09*†	−0.64	0.01*†	0.27	0.23*
Superior frontal gyrus	R	−0.45	0.04*†	−0.53	0.02*†	0.08	0.73*
	L	−0.16	0.47*†	−0.55	0.02*†	0.39	0.08*
Anterior cingulate gyrus	R	0.04	0.85*†	−0.30	0.18*†	0.35	0.12*
	L	−0.75	0.01*†	−0.79	0.01*†	0.04	0.85*
Frontal pole	R	−0.29	0.19*†	−0.37	0.10*†	0.08	0.71*
	L	−0.12	0.59*†	−0.21	0.36*†	0.09	0.69*

β-estimates and corresponding p values for each between-group contrast for total cortical volume and cortical VOIs. Negative β-estimates indicate smaller volumes in the group first mentioned

R right, *L* left, *VOI* volume of interest

* $p_{\text{FDR-uncorrected}} < 0.05$,
† $p_{\text{FDR-corrected}} < 0.05$

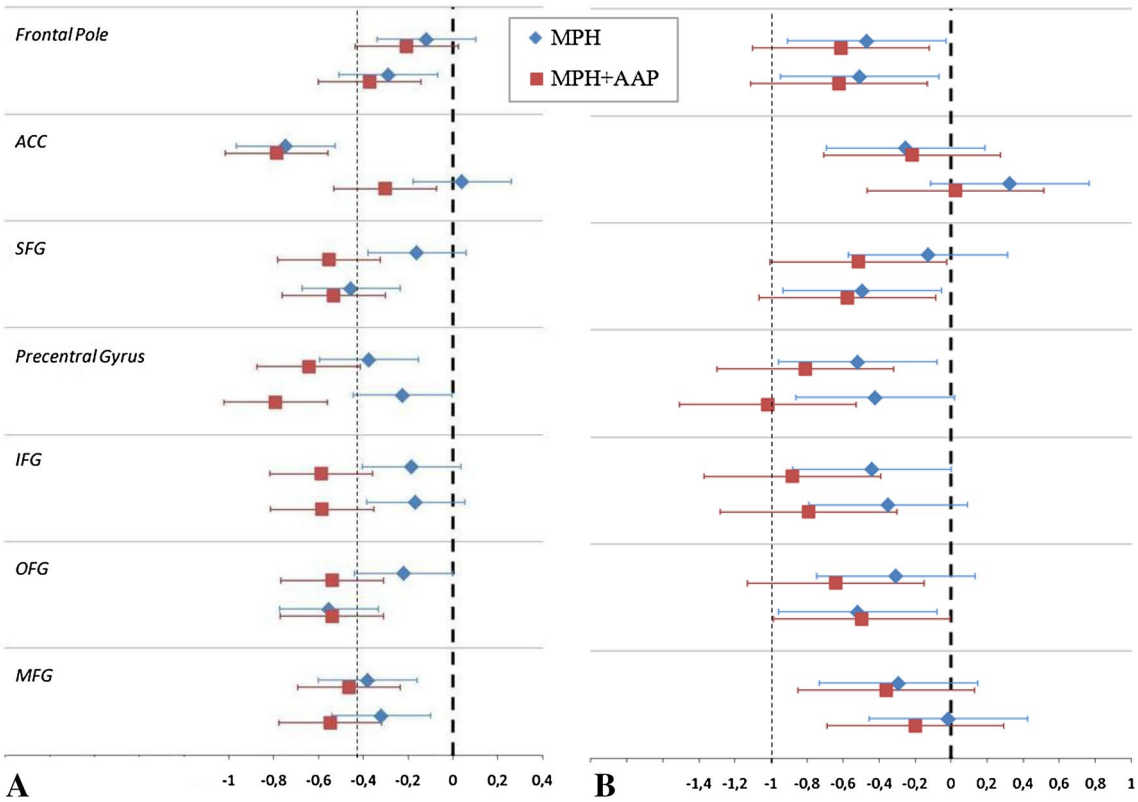

Fig. 2 Beta regression weights and their standard errors of the MPH group and the MPH + AAP group contrasted to the HC group (set to zero, *bold dashed line*), predicting cortical VOIs. Within each VOI, the *upper markers* represent the left hemisphere, and the lower markers the right hemisphere. The leftmost dashed line represents the α-level of 0.05 (FDR-uncorrected). **a** displays βs of the initial model, **b** displays βs of the model after adding total symptom count as a covariate

with symptom count)–Cohen's $d_{(\text{initial model})}$] across brain regions affected by treatment group was 0.23 for the MPH + AAP vs. HC contrast (range −0.66 to 0.21), 0.18 for the MPH vs. HC contrast (range −0.61 to 0.29) and 0.05 (range −0.02 to 0.10) for the MPH vs.MPH + AAP contrast. The absolute average change in p-values was 0.25 and 0.30 for

Table 3 Exploratory analyses of clinical between-group differences

		+ Total symptom count		+ Functional impairment	
		Range	Abs. average	Range	Abs. average
$ADHD_{MPH+AAP}$ vs. HC	$\Delta\ \beta$	−0.66–0.21	0.23	−0.56–0.09	0.20
	$\Delta\ p$	−0.03–0.77	0.25	0.03–0.60	0.21
$ADHD_{MPH}$ vs. HC	$\Delta\ \beta$	−0.61–0.29	0.18	−0.50–0.15	0.14
	$\Delta\ p$	−0.32–0.74	0.30	−0.15–0.44	0.24
$ADHD_{MPH+AAP}$ vs. $ADHD_{MPH}$	$\Delta\ \beta$	−0.02–0.10	0.05	−0.14–0.04	0.05
	$\Delta\ p$	−0.20–0.12	0.06	−0.09–0.05	0.03

Range of change ($\Delta_{min} - \Delta_{max}$) and average absolute change ($\mu|\Delta|$) in Cohen's d effect sizes and p-values across all 15 volumes of interest* where a significant (FDR-uncorrected) difference was found between any of the three groups, upon adding clinical, possibly confounding, factors to the regression model (model with clinical variable–initial model)

ADHD attention-deficit/hyperactivity disorder, *MPH* methylphenidate, *AAP* atypical antipsychotics, *HC* healthy control

* total cortical volume, left thalamic volume, bilateral diencephalic volume, bilateral middle frontal, inferior frontal, superior frontal, orbitofrontal, and precentral volume, and left anterior congulate cortex volume

MPH *vs.* MPH + AAP and MPH vs. HC, respectively, and 0.06 for the MPH vs. MPH + AAP contrast. Adding CGAS scores for daily functioning to the model had a very similar effect: average absolute change in effect size was 0.20 for MPH + AAP vs HC, 0.14 for MPH vs HC and 0.05 for MPH vs. MPH + AAP. Average absolute change in p-values was 0.21 for MPH + AAP vs HC, 0.24 for MPH vs HC and 0.03 for MPH *vs.* MPH + AAP. Thus, adding symptom count or functional impairment scores to the model influenced effect sizes and *p* values in contrasts involving HC subjects, but had a minimal impact on the contrast between the two treatment groups (Table 3).

Last, the combined treatment group contained more patients with a history of atomoxetine treatment. To evaluate the possible confounding effect of atomoxetine treatment history, all tests with significant results were repeated in atomoxetine-naïve patients only. Our findings remained unchanged: excluding atomoxetine users did not change the direction of effect in any VOI, and all but three (left thalamus in the MPH + AAP vs HC contrast, right superior frontal gyrus in the MPH vs HC contrast, and right precentral gyrus in the MPH vs MPH + AAP contrast) p-values remained significant. No other clinical differences between the two treatment groups were found.

Discussion

This study intended to provide an initial investigation of long-term structural brain correlates of combined methylphenidate and atypical antipsychotics treatment in adolescent patients with ADHD. Compared to unaffected peers, patients who had received combined treatment showed reduced total cortical volume, which was reflected in volume reductions across the frontal cortex. In addition, these patients showed reduced local volumes of the bilateral ventral diencephalon and the left thalamus. Patients treated with methylphenidate solely, by contrast, showed no reduction in total cortical or subcortical volume compared to unaffected peers, nor in any of the subcortical-limbic volumes of interest. Patients in the MPH + AAP group displayed more ADHD symptoms, functional impairment, and comprised a higher incidence of atomoxetine users compared to patients in the MPH only group. Adding these covariates to the model had minimal impact on differences in brain structure between the two ADHD groups.

Reduced total cortical volume and frontal cortical volumes are among the most replicated findings in ADHD and have repeatedly been shown to be normalized in patients using psycho-stimulants [3], [30], [31]. Within the frontal cortex, we found little evidence of spatial specificity. The reduction of total cortical volume in the MPH + AAP group is reflected in all frontal regions of interest except the frontal poles. All frontal volume reductions in the MPH + AAP group were driven by total cortical volume reduction, with the exception of left ACC volume reduction. However, left ACC volume reduction was also found in the MPH group. Cortical volume reduction associated with combined methylphenidate and antipsychotic treatment thus appears to be global rather than local. This is in line with previous studies suggesting that ADHD itself may be associated with global rather than local cortical changes [2]. It is less clear whether stimulants have local or global effects on brain structure, since the majority of previous studies adopted a region of interest approach. Future investigations of the effects of stimulants, antipsychotics, and combined treatment may benefit from a whole-brain approach.

In the subcortical-limbic regions, we found volume reduction in the bilateral ventral diencephalon and left thalamus in the combined treatment group, but not in

the methylphenidate only group. The ventral diencephalon includes the subthalamic nuclei and substantia nigra. The subthalamic nuclei are strongly connected within the basal ganglia and have been attributed an important role in response inhibition [32]. The substantia nigra is the largest dopaminergic nucleus in the human brain, strongly connected to the striatum, and is thought to play an important role in reward [33] and movement [34]. The ventrolateral portion of the thalamus relays and modulates neurotransmission in the frontostriatal circuits, connecting the cerebellum and basal ganglia to the cortical motor areas. Although these thalamic and subthalamic functions are highly relevant to the clinical presentation of ADHD, the role of thalamic and subthalamic nuclei in ADHD pathophysiology remains poorly understood. Thalamic volume reduction [35], altered left thalamic morphology and structural connectivity [36], and normalized thalamic morphology with methylphenidate treatment [35] have previously been reported in ADHD. We found no previous reports of thalamic or subthalamic changes with childhood antipsychotic treatment. Further investigation of thalamic and subthalamic structures in ADHD and their susceptibility to treatment is needed to interpret the thalamic and subthalamic changes we observed in the combined treatment group.

The current study was explorative and we warrant cautious interpretation of our findings. Participants in the MPH + AAP group showed more pronounced structural brain changes relative to healthy controls than stimulant-treated patients with ADHD, thereby resembling the non-medicated patient groups in previous studies [37]. Volume reductions in the combined treatment group but not in the methylphenidate only group could be indicative of counteractive effects of methylphenidate and atypical antipsychotics. That is, the opposing mechanism of action may compromise the synaptic effects of both individual agents, resulting in attenuation of structural normalization typically occurring with methylphenidate treatment. However, the current study did not allow direct investigation of this hypothesis and multiple interpretations are possible. Two shortcomings in study design prevent us from rejecting two plausible alternative interpretations.

First, our study was observational and cross-sectional. Our study is in many ways different from randomized controlled trials (RCTs), which are considered the gold standard when investigating treatment effects. First, in the current study no pre-treatment measurement was performed. The structural changes in the combined treatment group may be the cause rather than the result of medication intake (i.e. antipsychotic treatment may be assigned to patients at higher risk for cortical volume reduction). Pre-treatment measurements are needed to exclude this possibility. As a substitute to pre-treatment assessment, we explored the contribution of several clinical measures at time of scan. Our findings suggested that adding such variables to the model only minimally affected differences between the combined treatment group and the methylphenidate only group. However, the possibility of pre-treatment differences confounding treatment effects cannot be excluded, and the exploratory analyses we applied are not conclusive in this respect. Second, due to the absence of a matched stimulant-naïve patient sample, we were unable replicate previous findings of structural normalization with methylphenidate treatment in the current sample. Future studies would benefit from the inclusion of a stimulant naïve patient group, to enable the interpretation of the individual contributions of both stimulant and antipsychotic treatment on brain structure.

Besides these two essential caveats for correct interpretation of our findings, several other limitations should be kept in mind. The naturalistic study design resulted in a heterogeneous patient sample, and the age-range of participants was very wide. The combined treatment group included patients who were receiving treatment within a week prior to scanning as well as patients who had ceased treatment years before study participation, and patients who initiated treatment before the age of four as well as patients who initiated treatment after the age of 16. Due to limited power, we were unable to assess the effects of factors such as treatment duration, timing of treatment, co-medication and daily dose within the combined treatment group. Increased variance within our sample may have limited our ability to detect clinically meaningful effects. Apart from being heterogeneous, our sample was also relatively small, and subtle brain changes of small effect size may, therefore, have gone undetected. In line with this, most of our findings did not survive FDR correction for multiple testing. Last, as we were unable to obtain complete medication transcripts from all pharmacies, our data may have been subject to recall bias. Although a recent report suggested that recall bias may be limited in ADHD [38], it cannot be fully excluded, given our long-term retrospective study design.

At the same time, there are several advantages to our study design. Large-scale observational cohort studies, such as the current study, investigate patients that are representative of the heterogeneous clinical population, which enhances generalizability. Moreover, observational studies allow the investigation of complex treatment patterns in vulnerable patient groups, such as children with ADHD [39]. Results from observational studies are generally more consistent than results of RCTs and susceptibility bias does not typically result in an overestimation of treatment effects [40], [41]. Moreover, neuroimaging is a valid tool to investigate treatment safety and efficacy in observational study designs [42]. Therefore, while awaiting replication, the current study may have implications. Our findings, although

preliminary, raise concern and stress the need for clinical guidelines for the prescription of these agents [43]. Furthermore, they emphasize that future studies investigating the effects of methylphenidate on the developing brain should carefully document co-medication with dopaminergic agents, including atypical antipsychotics.

In conclusion, we found reduced total cortical volume that was reflected in local frontal volume reductions, as well as volume reductions in the bilateral ventral diencephalon and left thalamus, in patients with ADHD who had received combined methylphenidate and atypical antipsychotic treatment. These structural anomalies were smaller or absent in patients who were treated with methylphenidate solely. Longitudinal studies, including pre-treatment measurements and a stimulant-naïve patient group, are needed to allow more conclusive interpretation of potential mechanisms leading to these volume reductions.

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Conflict of interest Jan Buitelaar has been in the past 3 years a consultant to/member of advisory board of/and/or speaker for Janssen Cilag BV, Eli Lilly, Bristol-Myer Squibb, Schering Plough, UCB, Shire, Novartis and Servier. He is not an employee of any of these companies, and not a stock shareholder of any of these companies. He has no other financial or material support, including expert testimony, patents, royalties. Pieter Hoekstra has been a paid consultant to Shire and Eli Lilly. The other authors declare no conflicts of interest.

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