

Time and psychostimulants: opposing long-term structural effects in the adult ADHD brain.

A longitudinal MR study.

Short title: Effects of psychostimulants on the adult ADHD brain.

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Abstract

Long-term effects of psychostimulants such as methylphenidate on ADHD patients have been proved to be difficult to capture in cross-sectional studies comparing medicated and non-medicated samples and in longitudinal studies with children, with age-related maturational processes possibly confounding independent effects of medication. However, chronic psychostimulant administration at therapeutic doses has been proven to yield profound neuroadaptive changes in rodent models. Here, we present for the first time the effect of psychostimulant treatment on brain volumes in a sample of medication-naïve adult ADHD patients. We investigated grey matter volume changes in a sample of 41 medication-naïve adult ADHD patients before and after three years of psychostimulant treatment (N = 25) or no treatment (N = 16) compared to healthy adults (N = 25). We found a significant group x time interaction effect on left putamen grey matter volumes, with a decrease in left putamen volumes in the non-medicated group compared to both the medicated group and controls, and no differences between the medicated group and controls. Our results suggest a [normalizing](#) effect of psychostimulant treatment on the left putamen volume loss detected in non-medicated ADHD patients.

1. Introduction

Psychostimulant medication is the treatment of choice for patients diagnosed with Attention Deficit and Hyperactivity Disorder (ADHD), a typical childhood disorder characterized by inattention, impulsivity and hyperactivity (American Psychiatric Association, 2000) that prevails in around 4.4% of the adults worldwide (Kessler et al., 2006). Although functional and structural effects of psychostimulant treatment ADHD have been explored by means of cross-sectional between-group designs both in adults and children (see Rubia et al., 2014 for a review), no studies to date have directly measured the long-term effects of sustained psychostimulant treatment on adult ADHD brain structure within longitudinal study designs. Therefore, the question of whether psychostimulant medication effectively “normalizes” brain structure alterations in adult ADHD remains open.

At a cellular level, methylphenidate (MPH) has been shown to increase dopamine levels in the striatum by blocking dopamine transporters (Volkow et al., 1998), thus increasing extracellular dopamine (Volkow et al., 2001). In turn, these cellular processes have been associated with long-term ADHD symptom improvement (Rosa-Neto et al., 2005). Indeed, magnetic resonance studies seem to support striatal sensitivity to MPH and, more generally, psychostimulants. For instance, our previous neuroimaging study (Hoekzema et al., 2014) revealed decreased [nucleus accumbens \(Nacc\)](#) grey matter volumes in a sample of adult ADHD patients treated with psychostimulants compared to a medication-naïve ADHD sample. Moreover, a second measure after 1-2 years of MPH treatment in 10 of the patients revealed a recovery of [Nacc](#) volume abnormalities compared to controls (Hoekzema et al., 2014). Although these results seem to support the notion that psychostimulants [normalize](#) ADHD structural alterations, comparisons against non-treated ADHD patients were missing. Thus, we cannot rule out that the striatal volume recovery may have been a temporal effect.

Furthermore, other studies have identified reduced basal ganglia grey matter volumes (see Ellison-Wright et al., 2008 for a meta-analysis), and particularly [putamen and caudate](#) volumes (Soliva et al., 2010; Tremols et al., 2008; Wellington et al., 2006), in ADHD patients compared to

controls, differences that have been suggested to normalize in ADHD patients treated with psychostimulants (Frodl and Skokauskas, 2012; Nakao et al., 2011). Moreover, acute doses of MPH have been proved to raise basal ganglia activity and functional connectivity to normal levels in ADHD patients (Rubia et al., 2011, 2009b). However, these conclusions have been drawn from cross-sectional between-group comparisons including stimulant-treated vs. medication-naïve ADHD patients, with no longitudinal studies targeting within-subject structural changes in the adult ADHD brain. In addition, other studies did not find significant basal ganglia volume differences associated with psychostimulant medication (Castellanos et al., 2002; Hoogman et al., 2017; Norman et al., 2016; Shaw et al., 2014).

Given these partly conflicting results and the lack of longitudinal studies on adult ADHD treatment, the aim of the present study was to test whether psychostimulant medication affects brain structure within-subjects in a sample of adult ADHD patients. For this purpose, we conducted a longitudinal magnetic resonance study, comparing structural brain images from a group of adult ADHD patients before and after 3 years of psychostimulant treatment with a group of non-pharmacologically treated ADHD patients and a group of healthy controls. If brain structural changes were to be attributed to psychostimulant treatment, we would expect significant interaction effects between session and group, thus eliminating temporal confounds that could be related to ADHD but not psychostimulant treatment. Since the basal ganglia conform a specific target for psychostimulant pharmacological action, we expected grey matter volume in the basal ganglia and, particularly, the Nacc, the putamen and the caudate, to be **normalized** only in the ADHD sample treated with psychostimulants.

2. Experimental procedures

Participants

This research was designed as a prospective cohort study including forty-one medication naïve adults with combined ADHD (27 men) who were asked to complete a structural MRI acquisition just after being diagnosed with ADHD (medication-naïve) and again after 3 years of either pharma-

cological treatment with psychostimulants (*ADHD med*, 25 participants, 16 men, 2 medicated with lysdexamphetamine, 1 with both lysdexamphetamine and MPH, and the rest with MPH) or no treatment with psychostimulants (*ADHD non-med*, 16 participants, 11 men). The two ADHD groups were compared with a sample of 25 healthy subjects (12 men) that underwent the two MRI acquisition protocols in parallel, adding up to a total of 66 participants completing a pre-post exploration. The three final groups (controls, *ADHD med*, *ADHD non-med*) were matched for age, gender and IQ (see demographics in Table 1). The ADHD patients were carefully selected by a specialized team of psychiatrists and psychologists from *the outpatient Adult ADHD Program of Hospital Universitari Vall d'Hebron* in Barcelona (Spain). All of them met the DSM-IV criteria (American Psychiatric Association, 2000) for ADHD combined subtype and were right-handed. ADHD patients in the non-medicated group were those who voluntarily decided not to take medication after receiving the diagnosis. These were included on psychoeducational treatment of the Adult ADHD Program (Estrada et al., 2013) and held regular visits with their psychiatrist during the duration of the study. [The non-medicated group](#) did not undergo any pharmacological treatment [and neither of the ADHD groups underwent](#) cognitive-behavioral therapy.

The ADHD Rating Scale (DuPaul et al., 1998) was administered twice in the three groups, once during the baseline measurements and once after 3 years. Baseline ADHD scores were significantly different between groups ($F(2) = 71.29, p < .001$), with pairwise comparisons showing higher scores in each of the two ADHD samples against controls ($p < .001$, see Table 1). In average, the second MRI session took place 2.85 years after the baseline measure.

Exclusion criteria included comorbidity with other psychiatric diseases or personality disorders, assessed by the Structured Clinical Interview for Axis I (SCID-I) (First et al., 1997) and Axis II (First et al., 1994). Participants with substance abuse disorder, including those who consumed tobacco and cannabis within the last 6 months, were also excluded. Participants with an estimated IQ lower than 80 as assessed by means of the Wechsler Adult Intelligence Scale (WAIS-III, Wechsler, 1997) were not included. The study was approved by the *Hospital Universitari Vall d'Hebron Ethics Committee* and informed consent was obtained from all participants before taking part in the study.

Behavioral analysis

The pre-post ADHD rating scale scores were analyzed on SPSS (SPSS Inc., PASW Statistics for Windows, Version 18.0) by means of a 3x2 ANOVA analysis including group as 3-level independent factor, session as 2-level dependent factor and age, sex, and between-session time difference in years as covariates.

MRI image acquisition and analysis

High-resolution anatomical MRI images were acquired in a Philips Achieva 3T scanner by means of a T1-weighted FSPGR sequence (TR: 8.2 ms, TE: 3.7ms, FA: 88, matrix size: 256 x 256 x 180, voxel size: 0.94 x 0.94 1.00 mm, gap: 0mm). An unexpected technical problem during the first MRI acquisition lead to the replacement of the radio frequency head coil in 27 of the participants. However, the replacement head coil was equally frequent between-groups and the same head coil was employed in the first and second MRI acquisition for each participant, thus eliminating possible confounds associated with the head coil in within-subject pre-post measurements.

Longitudinal structural MRI data were analyzed with the software package SPM12 (Wellcome Department of Cognitive Neurology, UCL, London, United Kingdom) using the Computational Anatomy Toolbox (CAT12) for longitudinal voxel-based morphometry (VBM) data (Christian Gaser & Robert Dahnke, Jena University Hospital, Departments of Psychiatry and Neurology). Image quality was assessed using sample homogeneity for VMB data. After a preliminary intra-subject realignment, the mean subject image was employed as the reference image for a consecutive realignment and bias-correction, thus correcting for motion-related artifacts and signal inhomogeneities. Segmentation of the mean image into GM, WM and CSF followed, yielding the parameter estimates used for the MNI normalization. Finally, images were spatially smoothed with an 8mm full-width-at-half-maximum Gaussian kernel.

Preprocessed GM images were then introduced into a flexible factorial model including subject as an independent factor with as many levels as subjects (66), group as a 3-level independent factor (*ADHD-med/controls/ADHD non-med*) and session as a 2-level dependent factor (baseline/after 3 years). In addition to the resulting 66 subject regressors, 3 group regressors, 2 session regressors, and 6 group x session interaction regressors, an intracranial total volume regressor, a regressor for age, for sex and for head coil type (replacement or not) was added. The main effects of group and session, together with the group x session interaction were explored.

In addition, a region-of-interest (ROI) morphometric analysis was conducted for the basal ganglia, including the putamen, the caudate and the nucleus accumbens by making use of the CAT12 tissue volume estimations based on the *Neuromorphometrics* atlas. The ROI volumes were submitted to a 3x2 ANOVA analysis including group as 3-level independent factor, session as 2-level dependent factor and total intracranial volume, age, sex, head coil type and between-session time difference in years as covariates. [Moreover, in order to examine the predictive power of the behavioral scale over MRI changes in ADHD, we conducted a regression analysis with ADHD rating scale scores and psychostimulant treatment as predictors of within-subject pre-post ROI volume differences controlling for age and sex.](#)

3. Results

Behavioral results

Data from the ADHD rating scales in the second data acquisition (after 3 years) were lost for 10 participants from the *ADHD med* group. This subsample included only men and was comparable in age but received lower baseline ADHD rating scale scores ($t(23) = 4.80, p < .001$) compared to the rest of the *ADHD med* group that completed the scale in the second session. The ADHD rating scale 3x2 ANOVA resulted in a significant group x session interaction (Wilk's Lambda = 8.06, $F(51,2) = 6.15, p = .004$). Post-hoc pairwise comparisons corrected for multiple comparisons by means of the Bonferroni correction revealed no difference in improvement in the ADHD rating

scale between the *ADHD med* and *ADHD non-med* group (mean = -5.56, sd = 10.94). This result should nevertheless be taken with caution, provided the lack of 10 ratings from the second session in the medicated group (see Figure 1).

Neuroimaging results

Whole-brain results

Table 2a presents the effect of the group x session interaction in the 3x2 ANOVA including the three groups along the two sessions. The interaction effect was significant in three different clusters ($T = 3.20$, $p < .05$ FWE_c, single-voxel $p < .001$), including the right middle frontal and inferior frontal gyrus pars triangularis (Figure 2 in green), the left putamen (Figure 2 in red) and the right cerebellum (Figure 2 in blue) at a whole-brain level. A detailed description of the three clusters together with the main effects of group and session is presented in Table S1a.

Post-hoc pairwise comparison analysis by means of three 2x2 ANOVAs yielded significant interaction effects only in the comparison between the *ADHD med* and *ADHD non-med* group, revealing a significant group x session effect on left putamen volume ($T = 3.20$, $p < .05$ FWE_c, single voxel $p < .001$,) at a whole-brain level (see Figure 3 and Table 2b). A summary of the main effects associated with group and time in the three 2x2 ANOVA comparisons is presented in Table S1b-2d.

Parameter estimates within each of the resulting clusters found in the whole-brain 3x2 ANOVA were extracted for each group by means of a one-sample t-test (see Table 3). Parameter estimate differences over time were evaluated for each cluster and between-groups by means of a *post-hoc* one-way ANOVA. This analysis revealed, on one hand, volume increases in the right middle frontal cluster in the *ADHD med* both compared to the *ADHD non-med* group (mean difference = 0.07, std error = .019, $p = .002$) and controls (mean difference = 0.05, std error = .016, $p = .015$), and in the right cerebellum cluster in *ADHD med* patients compared to the *ADHD non-med* group (mean difference = .094, std error = .028, $p = .004$) after Bonferroni correction. On the other hand, left puta-

men volumes increased in *ADHD med* patients compared to the *ADHD non-med* group (mean difference = .091, std error = .027, $p = .004$), with controls exhibiting intermediate volumes that were not significantly different from the other two groups (see graphs on the right side of Figure 2). However, it is important to note that between-subjects left putamen volume differences over time could be both due to volume increases within the *ADHD med* group as well as volume decreases within the *ADHD non-med* group.

Groups did not differ in total intracranial volume (*ADHD med*: mean = 1592.92, sd = 160.26; *ADHD non-med*: 1497.07, sd = 142.19; Controls: mean = 1493.25, sd = 167.98), in total grey matter volume (*ADHD med*: mean = 697.85, sd = 77.04; *ADHD non-med*: 651.90, sd = 57.27; Controls: mean = 664.40, sd = 65.60), nor in total white matter volume (*ADHD med*: mean = 545.54, sd = 69.48; *ADHD non-med*: 506.00, sd = 49.68; Controls: mean = 514.34, sd = 68.83) and none of these measures did significantly change over time.

A stepwise linear multiple regression analysis on medicated and non-medicated ADHD patients with medication, age, sex and ADHD rating scale scores as independent factors and left putamen volume change as the dependent variable, showed a significant effect of medication (standardized beta = -3.31) and baseline ADHD rating scale scores (standardized beta = -3.06) ($F(40, 2) = 14.13$, $p < .001$, with an $R^2 = .653$). Indeed, when evaluating medicated and non-medicated ADHD patients separately, a predictive effect of the ADHD rating scale scores on left putamen volume change could only be observed in medicated patients, so that lower baseline ADHD rating scale scores predicted higher left putamen increase after 3 years (see Figure 5)."

All the structural images obtained during the 3-year period received good quality scores (see Table S2).

ROI analysis results

The group x session interaction effect on the left putamen was corroborated by the ROI 3x2 ANOVA conducted on the extracted basal ganglia volumes using *neuromorphometrics* anatomical ROIs and controlling for total brain volume, head coil type, age and sex (Wilk's Lambda = .800, $F(490, 2) = 6.14$, $p = .004$). A post-hoc one-way ANOVA with group as the between-groups factor and left putamen volume change as the dependent variable confirmed the effect of group on left putamen volume change ($F(2) = 5.60$, $p = .006$) and revealed higher left putamen volume **increases** in *ADHD med* patients compared to the *ADHD non-med* group (mean difference = $-.336$, $sd = .104$, $p = .006$), and no differences between *ADHD med* and controls after Bonferroni correction (see Figure 4).

4. Discussion

This is the first longitudinal neuroimaging study specifically evaluating the effect of psychostimulant treatment on brain volumes in a sample of medication-naïve adult ADHD patients before and after three years of either psychostimulant treatment (*ADHD med*) or no treatment (*ADHD non-med*) compared to a group of healthy adults. The three-group comparison yielded significant differences in right middle frontal gyrus, left putamen and right cerebellum volume change associated with group. The post-hoc analysis showed significant differences in left putamen volume change between the *ADHD med* and the *ADHD non-med* group at a whole brain level. Whereas left putamen ROI volume **significantly increased in the *ADHD med* group** compared to the *ADHD non-med* after three years, **there were** no differences between *ADHD med* patients and controls. **In turn, medication predicted higher left putamen volume increases in lower baseline ADHD rating scale scorers, suggesting a stronger effect of medication on brain structure in ADHD patients with lower symptom severity. Medication was not associated with symptom severity improvement, although we did not count on the full sample for this particular analysis.**

Altogether, two different **but simultaneously operating processes could account for the differences observed in our data.** On one side, a reduction of grey matter volumes in the left putamen associated with ADHD and, on the other side, an increase of left putamen volumes associated with

psychostimulant treatment in ADHD, especially in patients with lower symptom scores. These results are in line with cross-sectional group comparisons highlighting smaller putamen volumes and putamen inward surface deformations associated with ADHD diagnosis, and significant basal ganglia outward surface deformations associated with psychostimulant medication in young ADHD patients (Sobel et al., 2010). In addition, our results are partly supported by a meta-analysis of cross-sectional studies conducted by Nakao et al. (2011) pointing to an effect of age and psychostimulant medication on the normalization of, respectively, right putamen and right caudate volumes in both adults and children with ADHD. Moreover, in more recent meta-analyses, reduced basal ganglia volumes in children with ADHD compared to controls that were, nevertheless, neither detected in adults (Hoogman et al., 2017) nor related to psychostimulant treatment (Norman et al., 2016). The fact that volume changes in our sample affected the left putamen unilaterally is in line with Wellington et al. (2006), who found reversed asymmetry in the putamen in ADHD patients (smaller left vs. right putamen volumes) in contrast to controls (larger left vs. right putamen volumes). Moreover, asymmetry differences in ADHD seem not to be exclusive to this structure. For instance, the right but not the left caudate has also shown to be reduced in ADHD compared to controls (Soliva et al., 2010; Tremols et al., 2008).

In turn, in a cross-sectional study, Greven et al. (2015) report smaller basal ganglia volumes (caudate and putamen) in ADHD patients compared to controls during childhood and early adolescence, but larger basal ganglia volumes during early adulthood. Moreover, whereas volumes in controls decrease with age, no interaction with age was found in ADHD patients. The authors attribute these differences to delays in developmental trajectories in ADHD, with basal ganglia volumes normalizing with age in ADHD patients independently of medication. However, all participants were taking medication. In the present longitudinal study, we provide evidence that non-medicated ADHD patients show a decline in putamen volumes over time and, conversely, medicated ADHD patients experience volume increases. Thus, the increased volumes in early adulthood in ADHD found by Greven et al. (2015) could be at least partly explained by exposure to medication.

Along the same lines, longitudinal studies on children diagnosed with ADHD do not seem to provide conclusive findings about the effect of psychostimulant treatment, pointing to either a normalization of reduced caudate volumes associated with age but not psychostimulants (Castellanos et al., 2002) or putamen and caudate surface reductions persisting into adolescence regardless of pharmacological treatment (Shaw et al., 2014). However, longitudinal neuroimaging studies using rodents revealed a hypertrophic effect of psychostimulant treatment on left striatal volumes (Biezonski et al., 2016), which could partly explain the normalizing effect of psychostimulants observed in our sample. Therefore, although psychostimulant effects could not be detected to date in any of the longitudinal studies conducted with children, our results point for the first time to a normalizing effect of psychostimulant treatment on left putamen volume in adults with ADHD.

This finding suggests differential responses to psychostimulant administration in mature versus developing brains in ADHD patients, in line with the hypothesis that adult ADHD could have different biological causes and trajectories compared to ADHD in children (Lugo-Candelas et al., 2017). For instance, it could be that whereas a proportion of children diagnosed with ADHD could potentially attain normal striatal volumes in early adulthood independently of medication, others - presumably part of the 65% who still meet ADHD criteria in adulthood (Faraone et al., 2000) - might be able to do so only with a pharmaceutical intervention. If this was the case, longitudinal studies on children with ADHD would not clearly show an effect of psychostimulants on brain structure (Castellanos et al., 2002), since a part of the non-medicated sample would undergo a belated but naturally occurring normalization of putamen volumes with age.

Increased putamen function in medicated ADHD patients has been emphasized in several functional MRI studies (see Rubia et al., 2014 for a review). For instance, the putamen was recruited to a greater extent in methylphenidate (MPH) vs. placebo treated adolescents with ADHD both in a divided attention task (Shafritz, 2004) and in a time discrimination task (Rubia et al., 2009a). At a cellular level, such short-term changes in putamen function could be due to the acute post-synaptic DA increase in the striatum after MPH intake (Schiffer et al., 2006). However, prolonged improvement of ADHD symptoms with chronic MPH treatment is likely to rely rather on long-term structural

changes such as the ones observed in the present study, which could possibly be driven by MPH-induced neuroadaptive changes in striatal gene expression through the activation of specific transcription factors (Adriani et al., 2006; Chase et al., 2005; Yano and Steiner, 2007).

It is worth mentioning that our findings could at least partly be confounded by changes in cerebral blood flow (CBF) associated with medication intake, as observed in a longitudinal study run by Franklin et al. (2013). In that study, participants exhibited an implausible decrease in anterior cingulate VBM signaling in T1-weighted images after acute baclofen administration, likely confounded by CBF changes. Indeed, SPECT studies show CBF increases in frontal gyri, caudate and thalamus in children with ADHD 90min after MPH administration (Kim et al., 2001) and CBF increases in middle and superior frontal gyri together with CBF decreases in temporal and occipital regions after 4-5 weeks of MPH treatment (Lee et al., 2005). However, the VBM effects found in the present study are substantially stronger than the CBF effects found in association with MPH, which can only be detected using very permissive thresholds such as $P < 0.001$ (Kim et al., 2001) and even $P < .01$ (Lee et al. 2005) uncorrected for whole-brain comparisons. Moreover, the brain areas affected by CBF changes do not overlap with the ones presented here.

The presented results are bound by two main limitations. On one side, the number of participants in the *ADHD non-med* group ($N = 16$) was substantially lower than in the other two groups ($N = 25$ respectively). This was due to the fact that not all participants in the *ADHD non-med* group were kept psychostimulant free within the three-year period, thus having to eliminate them from the second measure. However, post-hoc ROI volume analyses with reduced but equal sample sizes for each group (16 randomly chosen participants per group) did not compromise group x session interaction effects on left putamen volume, thus discarding confounds associated with sample size differences. On the other side, this study was not conceived as a clinical trial and participants were not randomly assigned to different medication groups. Given that the decision to take psychostimulants or not was voluntary, the effects of individual factors bound to that decision cannot be ruled out. Therefore, and despite the aforementioned limitations, we provide evidence that

psychostimulants have a significant effect in the long-term preservation of left putamen volumes in adult ADHD.

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Table 1. Demographic and clinical data of the two ADHD groups and the control sample. Between-group comparisons conducted by means of a one-way ANOVA and post-hoc pairwise comparisons using Bonferroni multiple comparisons correction.

Characteristic	MPH (N=12)	non-MPH (N=19)	controls (N = 19)		MPH vs. non- MPH	MPH vs. controls	non-MPH vs. con- trols
	Mean (SD)	Mean (SD)	Mean (SD)	F(p- value)	p-value	p-value	p-value
Age (range 19 to 53)	35.92 (11.23)	37.44 (9.83)	34.64 (8.75)	.384(n.s)	n.s.	n.s	n.s.
ADHD Rating Scale	29.28 (11.04)	35.06 (7.38)	6.04 (5.98)	71.292 (p<.001)	.116	<.001	<.001

Table 2. Effect of the interaction between group and time with a threshold of $p < 0.05$ FWE cluster-level corrected (single voxel $p < 0.001$) in the 3x2 ANOVA between the three groups (a) and the post-hoc 2x2 ANOVA pairwise comparison between *ADHD med* and *ADHD non-med* patients (b).

	Peak MNI co-ordinates			N of voxels	highest Z-score	FWE-corrected p-value
	x	y	z			
a) ADHD med, controls and ADHD non-med						
Group x session interaction						
R middle frontal/inferior frontal triangularis	50	35	3 5	618	4.77	.017
R cerebellum crus 1&2	51	-45	- 3 5	643	4.23	.014
L putamen/pallidum	-32	-5	8	588	3.74	.021
b) ADHD med and ADHD non-med						
Group x session interaction						
L putamen	-29	5	2	595	4.11	.023

Table 3. Parameter estimates for each of the three clusters obtained in the 3x2 whole-brain ANOVA.

		L putamen			R middle frontal gyrus			R cerebellum		
		Con- trols	<i>ADH</i> <i>D</i> <i>med</i>	<i>ADHD</i> <i>non-med</i>	Con- trols	<i>ADHD</i> <i>med</i>	<i>ADHD</i> <i>non-med</i>	Con- trols	<i>ADHD</i> <i>med</i>	<i>ADHD</i> <i>non-med</i>
baseli- ne	me- an	4,27	4,18	4,47	1,89	1,82	2,00	2,07	1,95	2,13
	sd	0,63	0,63	0,61	0,29	0,38	0,42	0,55	0,48	0,57
after 3 years	me- an	4,31	4,58	3,96	1,89	2,29	1,81	1,79	2,31	1,55
	sd	0,73	0,74	0,60	0,31	0,66	0,24	0,35	0,98	0,25

Table S1. Main effect of group and time, and effect of the interaction between group and time with a threshold of $p < 0.05$ FWE cluster-level corrected (single voxel $p < 0.001$) in the 3x2 ANOVA between the three groups (a) and the three post-hoc 2x2 ANOVA pairwise comparisons (b, c, d). Given the large amount of results associated with the main effect of time, which are not the focus of the present paper, only results surviving voxel-level $p < 0.05$ FWE correction and a minimum extent of $k = 100^*$ or $k = 500^{**}$ are presented.

	Peak MNI coordinates			N of voxels	highest Z-score	FWE-corrected p-value
	x	y	z			
a) ADHD med, controls and ADHD non-med						
Negative main effect of group						
L superior frontal medial	-3	48	21	728	5.00	.008
Vermis 1, 2 & 10	-2	-33	-20	1464	4.90	<.001
L Caudate	0	2	6	510	4.29	.037
Positive main effect of time**						
L temporal superior/insula	-38	-30	6	988	6.51	<.001
R precuneus	21	-53	15	743	6.12	<.001
Negative main effect of time**						
L superior frontal medial/superior frontal	-6	68	15	3951	6.95	<.001
L caudate	-14	23	5	915	6.80	<.001
R superior temporal	41	-33	14	2015	6.76	<.001
L calcarine/cuneus	5	-99	5	623	6.58	<.001
L calcarine/superior occipital	-15	-53	12	601	5.96	<.001
Group x session interaction						
R middle frontal/inferior frontal triangularis	50	35	35	618	4.77	.017
R cerebellum crus 1&2	51	-45	-35	643	4.23	.014
L putamen/pallidum	-32	-5	8	588	3.74	.021
b) Controls and ADHD med						
Positive main effect of group						
R middle frontal/inferior frontal triangularis	44	48	0	528	4.48	.036
Negative main effect of group						
vermis/bilat cerebellum lobes 4&5	0	-39	-9	5075	5.78	<.001
L superior frontal medial/ant cingulate	0	47	3	741	4.26	.008

Positive effect of time**

L superior temporal/insula	-38	-30	6	711	5.79	<.001
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Negative effect of time**

L rectus/caudate	-15	23	-11	537	6.39	<.001
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L superior/middle frontal	-17	71	9	1963	6.30	<.001
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R superior temporal	42	-32	12	1426	5.95	<.001
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c) Controls and ADHD non-med**Positive main effect of group**

L cerebellum lobe 6	-33	-41	-36	855	4.92	.004
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Positive main effect of time*

close to L amygdala	-35	0	-26	151	5.92	<.001
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Negative main effect of time*

L superior frontal medial/superior frontal	-5	68	17	182	5.44	<.001
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d) ADHD med and ADHD non-med**Positive main effect of time****

L superior temporal	-36	-29	3	513	5.67	<.001
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Negative main effect of time**

L superior frontal medial/superior frontal	-6	69	8	836	5.84	<.001
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R superior temporal	39	-35	17	673	5.61	<.001
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Group x session interaction

L Putamen	-29	5	2	595	4.11	.023
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Table S2. CAT12 image quality results (weighted average of resolution, noise and inhomogeneity indices ranging from unacceptable/F/<50% to excellent/A+/100%) of the anatomical acquisitions for each participant identified with “ID” both in the baseline session (S = 1) and after 3 years (S = 2).

ID	S	Data quality												
1001	1	86.58% (B)	1022	1	85.82% (B)	1043	1	86.73% (B+)	2024	1	85.64% (B)	2057	1	85.43% (B)
1001	2	86.48% (B)	1022	2	86.57% (B)	1043	2	86.74% (B+)	2024	2	86.45% (B)	2057	2	85.69% (B)
1002	1	86.35% (B)	1023	1	86.05% (B)	1046	1	85.66% (B)	2033	1	80.83% (B-)	2058	1	85.66% (B)
1002	2	86.44% (B)	1023	2	86.35% (B)	1046	2	85.28% (B)	2033	2	82.41% (B-)	2058	2	82.67% (B-)
1003	1	86.26% (B)	1025	1	85.60% (B)	2003	1	86.46% (B)	2036	1	83.32% (B-)	2059	1	85.81% (B)
1003	2	86.18% (B)	1025	2	85.26% (B)	2003	2	86.14% (B)	2036	2	86.25% (B)	2059	2	85.29% (B)
1004	1	86.14% (B)	1026	1	76.04% (C)	2004	1	83.19% (B-)	2037	1	85.60% (B)	2060	1	86.53% (B)
1004	2	86.64% (B)	1026	2	86.19% (B)	2004	2	85.87% (B)	2037	2	85.86% (B)	2060	2	86.54% (B)
1005	1	85.19% (B)	1027	1	86.28% (B)	2005	1	85.90% (B)	2040	1	81.90% (B-)	2061	1	86.55% (B)
1005	2	84.85% (B)	1027	2	85.46% (B)	2005	2	86.25% (B)	2040	2	86.99% (B+)	2061	2	86.32% (B)
1006	1	85.66% (B)	1028	1	85.88% (B)	2006	1	85.12% (B)	2042	1	86.23% (B)	2062	1	86.55% (B)
1006	2	85.78% (B)	1028	2	86.30% (B)	2006	2	85.74% (B)	2042	2	85.72% (B)	2062	2	86.49% (B)
1007	1	86.10% (B)	1030	1	86.46% (B)	2007	1	83.61% (B)	2045	1	85.18% (B)	2063	1	86.49% (B)
1007	2	85.88% (B)	1030	2	86.39% (B)	2007	2	86.03% (B)	2045	2	85.39% (B)	2063	2	86.45% (B)
1009	1	85.97% (B)	1032	1	79.24% (C+)	2008	1	85.78% (B)	2046	1	85.38% (B)	2064	1	86.53% (B)
1009	2	84.74% (B)	1032	2	85.76% (B)	2008	2	86.55% (B)	2046	2	84.58% (B)	2064	2	86.53% (B)
1011	1	84.50% (B)	1033	1	86.62% (B)	2011	1	86.15% (B)	2047	1	85.47% (B)	2065	1	86.59% (B)
1011	2	86.54% (B)	1033	2	86.91% (B+)	2011	2	86.26% (B)	2047	2	84.59% (B)	2065	2	86.60% (B)
1012	1	86.64% (B)	1034	1	85.86% (B)	2012	1	84.85% (B)	2048	1	85.55% (B)	2066	1	86.55% (B)
1012	2	86.62% (B)	1034	2	86.27% (B)	2012	2	84.15% (B)	2048	2	85.76% (B)	2066	2	86.59% (B)
1013	1	86.23% (B)	1037	1	85.71% (B)	2013	1	86.04% (B)	2050	1	85.15% (B)	2067	1	86.45% (B)
1013	2	85.76% (B)	1037	2	86.43% (B)	2013	2	84.19% (B)	2050	2	86.14% (B)	2067	2	85.51% (B)
1014	1	86.17% (B)	1038	1	86.24% (B)	2014	1	85.07% (B)	2051	1	76.15% (C)	2068	1	86.58% (B)
1014	2	85.76% (B)	1038	2	86.21% (B)	2014	2	86.60% (B)	2051	2	86.20% (B)	2068	2	86.59% (B)
1017	1	86.37% (B)	1039	1	86.56% (B)	2015	1	83.12% (B-)	2052	1	85.24% (B)	2069	1	85.57% (B)
1017	2	83.86% (B)	1039	2	86.53% (B)	2015	2	86.63% (B)	2052	2	85.13% (B)	2069	2	85.89% (B)
1018	1	85.66% (B)	1041	1	85.69% (B)	2017	1	86.16% (B)	2054	1	85.63% (B)			

1018	2	86.13% (B)	1041	2	85.79% (B)	2017	2	84.56% (B)	2054	2	86.12% (B)
1019	1	86.29% (B)	1042	1	85.82% (B)	2021	1	85.18% (B)	2056	1	83.32% (B-)
1019	2	86.07% (B)	1042	2	86.00% (B)	2021	2	86.44% (B)	2056	2	86.03% (B)

Figure 1. The 3x2 ANOVA with ADHD rating scale as a dependent variable resulted in a significant effect of the group x session interaction (Wilk’s Lambda = 8.06, $F(51,2) = 6.15$, $p = .004$), with post-hoc pairwise comparisons pointing at a significant improvement in ADHD symptom scores in the *ADHD med* ($p = .009$) compared to controls after 3 years, and no difference in improvement between the *ADHD non-med* and the control nor the *ADHD med* group after Bonferroni correction.

Figure 2. Three different clusters exhibited between-group differences in grey matter volume change between sessions in the 3x2 ANOVA ($T = 3.20$, $p < .05$ FWEc, single-voxel $p < .001$), including the right middle frontal gyrus (in green), the left putamen (in red) and the right cerebellum (in blue). Graphs on the right side of the image represent VBM volumes expressed in mm^3 in the baseline acquisition and after 3 years for each cluster detected in the 3x2 ANOVA. Y-axis ranges are slightly shifted for left putamen volumes so that between-group differences can be better appreciated. Significant results are marked with an asterisk.

Figure 3. Post-hoc pairwise between-group comparisons resulted in a significant group x session interaction effect between the *ADHD med* and the *ADHD non-med* group in the left putamen ($T = 3.20$, $p < .05$ FWEc, single-voxel $p < .001$), with a significant decrease in ROI left putamen volume in the *ADHD non-med* group compared to the *ADHD med* group ($p < .002$). The graph in the bottom right corner represent left putamen volumes expressed in mm^3 in the baseline acquisition and after 3 years obtained with CAT12 by means of *neuromorphometrics* anatomical masks. Significant results are marked with an asterisk.

Figure 4. The ROI 3x2 ANOVA confirmed the whole-brain level results, with a significant group x session interaction in the left putamen (Wilk’s Lambda = .800, $F(2, 49) = 6.14$, $p = .004$). Post-hoc pairwise comparisons revealed greater between-sessions volume change in the *ADHD non-med* group compared to the *ADHD med* group ($p = .004$) after Bonferroni correction.

Figure 5. Baseline ADHD rating scale scores significantly predicted left putamen ROI volume change in the ADHD med but not the ADHD non-med group with an $R^2 = .33$, so that medicated ADHD patients with lower baseline ADHD rating scale scores underwent significantly higher left putamen ROI volume increases after three years. Positive values in the y-axis refer to left putamen volume increases, whereas negative values represent volume decreases.

Abstract

Long-term effects of psychostimulants such as methylphenidate on ADHD patients have been proved to be difficult to capture in cross-sectional studies comparing medicated and non-medicated samples and in longitudinal studies with children, with age-related maturational processes possibly confounding independent effects of medication. However, chronic psychostimulant administration at therapeutic doses has been proven to yield profound neuroadaptive changes in rodent models. Here, we present for the first time the effect of psychostimulant treatment on brain volumes in a sample of medication-naïve adult ADHD patients. We investigated grey matter volume changes in a sample of 41 medication-naïve adult ADHD patients before and after three years of psychostimulant treatment (N = 25) or no treatment (N = 16) compared to healthy adults (N = 25). We found a significant group x time interaction effect on left putamen grey matter volumes, with a decrease in left putamen volumes in the non-medicated group compared to both the medicated group and controls, and no differences between the medicated group and controls. Our results suggest a compensatory normalizing effect of psychostimulant treatment on the left putamen volume loss detected in non-medicated ADHD patients.

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1. Introduction

Psychostimulant medication is the treatment of choice for patients diagnosed with Attention Deficit and Hyperactivity Disorder (ADHD), a typical childhood disorder characterized by inattention, impulsivity and hyperactivity (American Psychiatric Association, 2000) that prevails in around 4.4% of the adults worldwide (Kessler et al., 2006)(Kessler et al., 2006). Although functional and structural effects of psychostimulant treatment ADHD have been explored by means of cross-sectional between-group designs both in adults and children (see Rubia et al., 2014 for a review), no studies to date have directly measured the long-term effects of sustained psychostimulant treatment on adult ADHD brain structure within longitudinal study designs. Therefore, the question of whether psychostimulant medication effectively “normalizes” brain structure alterations in adult ADHD remains open.

~~At a cellular level, methylphenidate (MPH) has been shown to increase dopamine levels in the striatum by blocking dopamine transporters (Volkow et al., 1998), thus increasing extracellular dopamine (Volkow et al., 2001). In turn, these cellular processes have been associated with long-term ADHD symptomatology improvement (Rosa Neto et al., 2005). Indeed, magnetic resonance studies seem to support striatal sensitivity to MPH and, more generally, psychostimulants. For instance, our previous neuroimaging study (Hoekzema et al., 2014) revealed decreased ventral striatum (Vstr) grey matter volumes in a sample of adult ADHD patients treated with psychostimulants compared to a medication-naïve ADHD sample. Moreover, a second measure after 1-2 years of MPH treatment in 10 of the patients revealed a recovery of Vstr volume abnormalities compared to controls (Hoekzema et al., 2014). Although these results seem to support the notion that psychostimulants compensate ADHD structural alterations, comparisons against non-treated ADHD patients were missing. Thus, we cannot rule out that the striatal volume recovery may have been a temporal effect.~~

~~Furthermore, other studies have identified reduced basal ganglia grey matter volumes (see Ellison-Wright, Ellison-Wright, & Bullmore, 2008 for a meta-analysis), and particularly dorsal striatal volumes (Soliva et al., 2010; Trembls et al., 2008; Wellington et al., 2006), in ADHD patients compared to controls, differences that have been suggested to normalize in ADHD patients treated~~

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with psychostimulants (Frodl and Skokauskas, 2012). Moreover, acute doses of MPH have been proved to raise basal ganglia activity and functional connectivity to normal levels in ADHD patients (Rubia et al., 2011, 2009b). However, these conclusions have been drawn from cross-sectional between-group comparisons including stimulant-treated vs. medication-naïve ADHD patients, with no longitudinal studies targeting within-subject structural changes in the adult ADHD brain. In addition, other studies did not find significant basal ganglia volume differences associated with psychostimulant medication (Castellanos et al., 2002; Hoogman et al., 2017; Shaw et al., 2014).

Given these partly conflicting results and the lack of longitudinal studies on adult ADHD treatment, the aim of the present study was to test whether psychostimulant medication affects brain structure within subjects in a sample of adult ADHD patients. For this purpose, we conducted a longitudinal magnetic resonance study, comparing structural brain images from a group of adult ADHD patients before and after 3 years of psychostimulant treatment with a group of non-pharmacologically treated ADHD patients and a group of healthy controls. If brain structural changes were to be attributed to psychostimulant treatment, we would expect significant interaction effects between session and group, thus eliminating temporal confounds that could be related to ADHD but not psychostimulant treatment. Since the basal ganglia conform a specific target for psychostimulant pharmacological action, we expected grey matter volume in the basal ganglia and, particularly, the dorsal and ventral striatum, to be compensated only in the ADHD sample treated with psychostimulants.

At a cellular level, methylphenidate (MPH) has been shown to increase dopamine levels in the striatum by blocking dopamine transporters (Volkow et al., 1998), thus increasing extracellular dopamine (Volkow et al., 2001). In turn, these cellular processes have been associated with long-term ADHD symptom improvement (Rosa-Neto et al., 2005). Indeed, magnetic resonance studies seem to support striatal sensitivity to MPH and, more generally, psychostimulants. For instance, our previous neuroimaging study (Hoekzema et al., 2014) revealed decreased nucleus accumbens (Nacc) grey matter volumes in a sample of adult ADHD patients treated with psychostimulants compared to a medication-naïve ADHD sample. Moreover, a second measure after 1-2 years of MPH treatment in 10 of the patients revealed a recovery of Nacc volume abnormalities compared

to controls (Hoekzema et al., 2014). Although these results seem to support the notion that psychostimulants normalize ADHD structural alterations, comparisons against non-treated ADHD patients were missing. Thus, we cannot rule out that the striatal volume recovery may have been a temporal effect.

Furthermore, other studies have identified reduced basal ganglia grey matter volumes (see Ellison-Wright et al., 2008 for a meta-analysis), and particularly putamen and caudate volumes (Soliva et al., 2010; Tremols et al., 2008; Wellington et al., 2006), in ADHD patients compared to controls, differences that have been suggested to normalize in ADHD patients treated with psychostimulants (Frodl and Skokauskas, 2012; Nakao et al., 2011). Moreover, acute doses of MPH have been proved to raise basal ganglia activity and functional connectivity to normal levels in ADHD patients (Rubia et al., 2011, 2009b). However, these conclusions have been drawn from cross-sectional between-group comparisons including stimulant-treated vs. medication-naïve ADHD patients, with no longitudinal studies targeting within-subject structural changes in the adult ADHD brain. In addition, other studies did not find significant basal ganglia volume differences associated with psychostimulant medication (Castellanos et al., 2002; Hoogman et al., 2017; Norman et al., 2016; Shaw et al., 2014).

Given these partly conflicting results and the lack of longitudinal studies on adult ADHD treatment, the aim of the present study was to test whether psychostimulant medication affects brain structure within-subjects in a sample of adult ADHD patients. For this purpose, we conducted a longitudinal magnetic resonance study, comparing structural brain images from a group of adult ADHD patients before and after 3 years of psychostimulant treatment with a group of non-pharmacologically treated ADHD patients and a group of healthy controls. If brain structural changes were to be attributed to psychostimulant treatment, we would expect significant interaction effects between session and group, thus eliminating temporal confounds that could be related to ADHD but not psychostimulant treatment. Since the basal ganglia conform a specific target for psychostimulant pharmacological action, we expected grey matter volume in the basal ganglia and, particularly, the Nacc, the putamen and the caudate, to be normalized only in the ADHD sample treated with psychostimulants.

2. Experimental procedures

Participants

This research was designed as a prospective cohort study including forty-one medication naïve adults with combined ADHD (27 men) who were asked to complete a structural MRI acquisition just after being diagnosed with ADHD (medication-naïve) and again after 3 years of either pharmacological treatment with psychostimulants (*ADHD med*, 25 participants, 16 men, 2 medicated with lysdexamphetamine, 1 with both lysdexamphetamine and MPH, and the rest with MPH) or no treatment with psychostimulants (*ADHD non-med*, 16 participants, 11 men). The two ADHD groups were compared with a sample of 25 healthy subjects (12 men) that underwent the two MRI acquisition protocols in parallel, adding up to a total of 66 participants completing a pre-post exploration. The three final groups (controls, *ADHD med*, *ADHD non-med*) were matched for age, gender and IQ (see demographics in Table 1). The ADHD patients were carefully selected by a specialized team of psychiatrists and psychologists from the *outpatient Adult ADHD Program of Hospital Universitari Vall d'Hebron* in Barcelona (Spain). All of them met the DSM-IV criteria (American Psychiatric Association, 2000) for ADHD combined subtype and were right-handed. ADHD patients in the non-medicated group were those who voluntarily decided not to take medication after receiving the diagnosis. These were included on psychoeducational treatment of the Adult ADHD Program as treatment for ADHD (Estrada et al., 2013) (Estrada et al., 2013) and held regular visits with their psychiatrist during the duration of the study. The non-medicated group did not undergo any pharmacological treatment and neither of the ADHD groups underwent cognitive-behavioral therapy.

The ADHD Rating Scale (DuPaul, et al., 1998) (DuPaul et al., 1998) was administered twice in the three groups, once during the baseline measurements and once after 3 years. Baseline ADHD scores were significantly different between groups ($F(2) = 71.29, p < .001$), with pairwise comparisons showing higher scores in each of the two ADHD samples against controls ($p < .001$), and in

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~~non-medicated against medicated ADHD patients ($p < .039$, see Table 1).~~ In average, the second MRI session took place 2.85 years after the baseline measure.

Exclusion criteria included comorbidity with other psychiatric diseases or personality disorders, assessed by the Structured Clinical Interview for Axis I (SCID-I) (First et al., 1997) and Axis II (First et al., 1994). ~~Participants with substance abuse disorder, including those who consumed tobacco and cannabis within the last 6 months, were also excluded. Participants with an estimated IQ lower than 80 as assessed by means of the Wechsler Adult Intelligence Scale (WAIS-III,(First et al., 1997) and Axis II (First et al., 1994). Participants with substance abuse disorder, including those who consumed tobacco and cannabis within the last 6 months, were also excluded. Participants with an estimated IQ lower than 80 as assessed by means of the Wechsler Adult Intelligence Scale (WAIS-III. (Wechsler, 1997) were not included.~~ The study was approved by the *Hospital Universitari Vall d'Hebron Ethics Committee* and informed consent was obtained from all participants before taking part in the study.

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Behavioral analysis

The pre-post ADHD rating scale scores were analyzed on SPSS (~~SPSS Inc., PASW Statistics for Windows, Version 18.0~~)(SPSS Inc., PASW Statistics for Windows, Version 18.0) by means of a 3x2 ANOVA analysis including group as 3-level independent factor, session as 2-level dependent factor and age, sex, and between-session time difference in years as covariates.

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MRI image acquisition and analysis

High-resolution anatomical MRI images were acquired in a Philips Achieva 3T scanner by means of a T1-weighted FSPGR sequence (TR: 8.2 ms, TE: 3.7ms, FA: 88, matrix size: 256 x 256 x 180, voxel size: 0.94 x 0.94 1.00 mm, gap: 0mm). An unexpected technical problem during the first MRI acquisition lead to the replacement of the radio frequency head coil in 27 of the participants. However, the replacement head coil was equally frequent between-groups and the same head coil was

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employed in the first and second MRI acquisition for each participant, thus eliminating possible confounds associated with the head coil in within-subject pre-post measurements.

Longitudinal structural MRI data were analyzed with the software package SPM12 (Wellcome Department of Cognitive Neurology, UCL, London, United Kingdom) using the Computational Anatomy Toolbox (CAT12) for longitudinal voxel-based morphometry (VBM) data (Christian Gaser & Robert Dahnke, Jena University Hospital, Departments of Psychiatry and Neurology). Image quality was assessed using sample homogeneity for VMB data. After a preliminary intra-subject realignment, the mean subject image was employed as the reference image for a consecutive realignment and bias-correction, thus correcting for motion-related artifacts and signal inhomogeneities. Segmentation of the mean image into GM, WM and CSF followed, yielding the parameter estimates used for the MNI normalization. Finally, images were spatially smoothed with an 8mm full-width-at-half-maximum Gaussian kernel.

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Preprocessed GM images were then introduced into a flexible factorial model including subject as an independent factor with as many levels as subjects (66), group as a 3-level independent factor (*ADHD-med/controls/ADHD non-med*) and session as a 2-level dependent factor (baseline/after 3 years). In addition to the resulting 66 subject regressors, 3 group regressors, 2 session regressors, and 6 group x session interaction regressors, an intracranial total volume regressor, a regressor for age, for sex and for head coil type (replacement or not) was added. The main effects of group and session, together with the group x session interaction were explored.

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In addition, a region-of-interest (ROI) morphometric analysis was conducted for the basal ganglia, including the putamen, the caudate and the nucleus accumbens by making use of the CAT12 tissue volume estimations based on the *Neuromorphometrics* atlas. The ROI volumes were submitted to a 3x2 ANOVA analysis including group as 3-level independent factor, session as 2-level dependent factor and total intracranial volume, age, sex, head coil type and between-session time difference in years as covariates. Moreover, in order to examine the predictive power of the behavioral scale over MRI changes in ADHD, we conducted a regression analysis with ADHD rating

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scale scores and psychostimulant treatment as predictors of within-subject pre-post ROI volume differences controlling for age and sex.

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3. Results

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Behavioral results

Data from the ADHD rating scales in the second data acquisition (after 3 years) were lost for 10 participants from the *ADHD med* group. This subsample included only men and was comparable in age but received lower baseline ADHD rating scale scores ($t(23) = 4.80, p < .001$) compared to the rest of the *ADHD med* group that completed the scale in the second session. The ADHD rating scale 3x2 ANOVA resulted in a significant group x session interaction (Wilk's Lambda = 8.06, $F(51,2) = 6.15, p = .004$). Post-hoc pairwise comparisons corrected for multiple comparisons by means of the Bonferroni correction revealed no difference in improvement in the ADHD rating scale between the *ADHD med* and *ADHD non-med* group (mean = -5.56, sd = 10.94). This result should nevertheless be taken with caution, provided the lack of 10 ratings from the second session in the medicated group (see Figure 1).

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Neuroimaging results

Whole-brain results

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Table 2a presents the effect of the group x session interaction in the 3x2 ANOVA including the three groups along the two sessions. The interaction effect was significant in three different clusters ($T = 3.20, p < .05$ FWEc, single-voxel $p < .001$), including the right middle frontal and inferior frontal gyrus pars triangularis (Figure 2 in green), the left putamen (Figure 2 in red) and the right cerebellum (Figure 2 in blue) at a whole-brain level. A detailed description of the three clusters together with the main effects of group and session is presented in Table S1a.

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Post-hoc pairwise comparison analysis by means of three 2x2 ANOVAs yielded significant interaction effects only in the comparison between the *ADHD med* and *ADHD non-med* group, revealing a significant group x session effect on left putamen volume ($T = 3.20$, $p < .05$ FWEc, single voxel $p < .001$.) at a whole-brain level (see Figure 3 and Table 2b). A summary of the main effects associated with group and time in the three 2x2 ANOVA comparisons is presented in Table S1b-2d.

Parameter estimates within each of the resulting clusters found in the whole-brain 3x2 ANOVA were extracted for each group by means of a one-sample t-test (see Table 3). Parameter estimate differences over time were evaluated for each cluster and between-groups by means of a *post-hoc* one-way ANOVA. This analysis revealed, on one hand, volume increases in the right middle frontal cluster in the *ADHD med* both compared to the *ADHD non-med* group (mean difference = 0.07, std error = .019, $p = .002$) and controls (mean difference = 0.05, std error = .016, $p = .015$), and in the right cerebellum cluster in *ADHD med* patients both compared to the *ADHD non-med* group (mean difference = .094, std error = .028, $p = .004$) and controls (mean difference = -.064, std error = .025, $p = .039$) after Bonferroni correction. On the other hand, the left putamen was smaller volumes increased in *ADHD non-med* patients compared to the *ADHD non-med* group (mean difference = .091, std error = .027, $p = .004$), with controls exhibiting intermediate volumes that were not significantly different from the other two groups (see graphs on the right side of Figure 2). However, it is important to note that between-subjects left putamen volume differences over time could be both due to volume increases within the *ADHD med* group as well as volume decreases within the *ADHD non-med* group.

Groups did not differ in total intracranial volume (*ADHD med*: mean = 1592.92, sd = 160.26; *ADHD non-med*: 1497.07, sd = 142.19; Controls: mean = 1493.25, sd = 167.98), in total grey matter volume (*ADHD med*: mean = 697.85, sd = 77.04; *ADHD non-med*: 651.90, sd = 57.27; Controls: mean = 664.40, sd = 65.60), nor in total white matter volume (*ADHD med*: mean = 545.54, sd = 69.48; *ADHD non-med*: 506.00, sd = 49.68; Controls: mean = 514.34, sd = 68.83) and none of these measures did significantly change over time.

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A stepwise linear multiple regression analysis on medicated and non-medicated ADHD patients with medication, age, sex and ADHD rating scale scores as independent factors and left putamen volume change as the dependent variable, showed a significant effect of medication (standardized beta = -3.31) and baseline ADHD rating scale scores (standardized beta = -3.06) ($F(40, 2) = 14.13$, $p < .001$, with an $R^2 = .653$). Indeed, when evaluating medicated and non-medicated ADHD patients separately, a predictive effect of the ADHD rating scale scores on left putamen volume change could only be observed in medicated patients, so that lower baseline ADHD rating scale scores predicted higher left putamen increase after 3 years (see Figure 5)."

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All the structural images obtained during the 3-year period received good quality scores (see Table S2).

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ROI analysis results

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The group x session interaction effect on the left putamen was corroborated by the ROI 3x2 ANOVA conducted on the extracted basal ganglia volumes using *neuromorphometrics* anatomical ROIs and controlling for total brain volume, head coil type, age and sex (Wilk's Lambda = .800, $F(490, 2) = 6.14$, $p = .004$). A post-hoc one-way ANOVA with group as the between-groups factor and left putamen volume change as the dependent variable confirmed the effect of group on left putamen volume change ($F(2) = 5.60$, $p = .006$) and revealed higher left putamen volume ~~reduction~~ ~~increases~~ in ADHD ~~non-med~~ patients compared to the ADHD ~~non-med~~ group (mean difference = -.336, $sd = .104$, $p = .006$) ~~and compared to controls (mean difference = -.272, $sd = .104$, $p = .033$)~~, and no differences between ADHD med and controls after Bonferroni correction (see Figure 4).

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4. Discussion

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This is the first longitudinal neuroimaging study specifically evaluating the effect of psychostimulant treatment on brain volumes in a sample of medication-naïve adult ADHD patients before and after

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three years of either psychostimulant treatment (*ADHD med*) or no treatment (*ADHD non-med*) compared to a group of healthy adults. The three-group comparison yielded significant differences in right middle frontal gyrus, left putamen and right cerebellum volume change associated with group. The post-hoc analysis showed significant differences in left putamen volume change between the *ADHD med* and the *ADHD non-med* group at a whole brain level. Whereas left putamen ROI volume ~~was significantly reduced~~ increased in the *ADHD non-med* group compared to both the *ADHD non-med* and the control group after three years, there were no differences were found between *ADHD med* patients and controls. In turn, medication predicted higher left putamen volume increases in lower baseline ADHD rating scale scorers, suggesting a stronger effect of medication on brain structure in ADHD patients with lower symptom severity. Medication was not associated with symptom severity improvement, although we did not count on the full sample for this particular analysis.

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~~Thereby, our results suggest the presence of two different processes. On one side, a reduction of grey matter volumes in the left putamen associated with ADHD and, on the other side, a compensatory increase of left putamen volumes associated with psychostimulant treatment in ADHD. These results are in line with cross-sectional group comparisons highlighting smaller putamen volumes and putamen inward surface deformations associated with ADHD diagnosis, and significant basal ganglia outward surface deformations associated with psychostimulant medication in young ADHD patients (Sobel et al., 2010). In addition, our results are partly supported by a meta-analysis of cross-sectional studies conducted by Nakao, Radua, Rubia, & Mataix-Cols (2011) pointing to an effect of age and psychostimulant medication on the normalization of, respectively, right putamen and right caudate volumes in both adults and children with ADHD. Moreover, in a mega-analysis including 1713 ADHD patients, Hoogman and colleagues (2017) found reduced basal ganglia volumes in children with ADHD compared to controls that were, nevertheless, neither detected in adults nor related to psychostimulant treatment.~~

~~Along the same lines, longitudinal studies on children diagnosed with ADHD do not seem to provide conclusive findings about the effect of psychostimulant treatment, pointing to either a normali-~~

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zation of reduced caudate volumes associated with age but not psychostimulants (Castellanos et al., 2002) or dorsal striatal surface reductions persisting into adolescence regardless of pharmacological treatment (Shaw et al., 2014). However, longitudinal neuroimaging studies using rodents revealed a hypertrophic effect of psychostimulant treatment on left striatal volumes (Biezonski et al., 2016), which could partly explain the compensatory effect of psychostimulants observed in our sample. Therefore, although psychostimulant effects could not be detected to date in any of the longitudinal studies conducted with children, our results point for the first time to a compensatory effect of psychostimulant treatment on left putamen volume in adults with ADHD. This finding suggests differential responses to psychostimulant administration in mature versus developing brains in ADHD patients, in line with the hypothesis that adult ADHD could have different biological causes and trajectories compared to ADHD in children (Lugo-Candelas et al., 2017).

Increased putamen function in medicated ADHD patients has been emphasized in several functional MRI studies. For instance, the putamen was recruited to a greater extent in methylphenidate (MPH) vs. placebo treated adolescents with ADHD both in a divided attention task (Shafritz, 2004) and in a time discrimination task (Rubia et al., 2009a). At a cellular level, such short-term changes in putamen function could be due to the acute post-synaptic DA increase in the dorsal striatum after MPH intake (Schiffer et al., 2006). However, prolonged improvement of ADHD symptoms with chronic MPH treatment is likely to rely rather on long-term structural changes such as the ones observed in the present study, which could possibly be driven by MPH-induced neuroadaptive changes in striatal gene expression through the activation of specific transcription factors (Adriani et al., 2006; Chase et al., 2005; Yano and Steiner, 2007).

Finally, volumetric differences in the right middle frontal gyrus and the right cerebellum associated with group yielded by the three-group comparison could not be retrieved in the post-hoc pairwise comparisons analysis. However, parameter estimates were significantly higher in the *ADHD med* compared to the *ADHD non-med* group and controls, pointing at an effect of MPH (but no effect of the disorder) on these structures. Along these lines, the dorsal prefrontal cortex has been described to be structurally (Hoekzema et al., 2011) and functionally (Hart et al., 2012; Smith et al.,

2008) affected in ADHD and susceptible to MPH (Rubia et al., 2011, 2009a). In addition, smaller GM volumes in ADHD versus controls (Castellanos et al., 2002; Shaw et al., 2014) and susceptibility to MPH (Rubia et al., 2011) has also been reported for the cerebellum. Therefore, it is possible that larger samples may allow detecting volume changes associated with ADHD and with psychostimulant treatment in these areas.

Altogether, two different but simultaneously operating processes could account for the differences observed in our data. On one side, a reduction of grey matter volumes in the left putamen associated with ADHD and, on the other side, an increase of left putamen volumes associated with psychostimulant treatment in ADHD, especially in patients with lower symptom scores. These results are in line with cross-sectional group comparisons highlighting smaller putamen volumes and putamen inward surface deformations associated with ADHD diagnosis, and significant basal ganglia outward surface deformations associated with psychostimulant medication in young ADHD patients (Sobel et al., 2010). In addition, our results are partly supported by a meta-analysis of cross-sectional studies conducted by Nakao et al. (2011) pointing to an effect of age and psychostimulant medication on the normalization of, respectively, right putamen and right caudate volumes in both adults and children with ADHD. Moreover, in more recent meta-analyses, reduced basal ganglia volumes in children with ADHD compared to controls that were, nevertheless, neither detected in adults (Hoogman et al., 2017) nor related to psychostimulant treatment (Norman et al., 2016). The fact that volume changes in our sample affected the left putamen unilaterally is in line with Wellington et al. (2006), who found reversed asymmetry in the putamen in ADHD patients (smaller left vs. right putamen volumes) in contrast to controls (larger left vs. right putamen volumes). Moreover, asymmetry differences in ADHD seem not to be exclusive to this structure. For instance, the right but not the left caudate has also shown to be reduced in ADHD compared to controls (Soliva et al., 2010; Tremols et al., 2008).

In turn, in a cross-sectional study, Greven et al. (2015) report smaller basal ganglia volumes (caudate and putamen) in ADHD patients compared to controls during childhood and early adolescence, but larger basal ganglia volumes during early adulthood. Moreover, whereas volumes in controls decrease with age, no interaction with age was found in ADHD patients. The authors at-

tribute these differences to delays in developmental trajectories in ADHD, with basal ganglia volumes normalizing with age in ADHD patients independently of medication. However, all participants were taking medication. In the present longitudinal study, we provide evidence that non-medicated ADHD patients show a decline in putamen volumes over time and, conversely, medicated ADHD patients experience volume increases. Thus, the increased volumes in early adulthood in ADHD found by Greven et al. (2015) could be at least partly explained by exposure to medication.

Along the same lines, longitudinal studies on children diagnosed with ADHD do not seem to provide conclusive findings about the effect of psychostimulant treatment, pointing to either a normalization of reduced caudate volumes associated with age but not psychostimulants (Castellanos et al., 2002) or putamen and caudate surface reductions persisting into adolescence regardless of pharmacological treatment (Shaw et al., 2014). However, longitudinal neuroimaging studies using rodents revealed a hypertrophic effect of psychostimulant treatment on left striatal volumes (Biezonski et al., 2016), which could partly explain the normalizing effect of psychostimulants observed in our sample. Therefore, although psychostimulant effects could not be detected to date in any of the longitudinal studies conducted with children, our results point for the first time to a normalizing effect of psychostimulant treatment on left putamen volume in adults with ADHD.

This finding suggests differential responses to psychostimulant administration in mature versus developing brains in ADHD patients, in line with the hypothesis that adult ADHD could have different biological causes and trajectories compared to ADHD in children (Lugo-Candelas et al., 2017). For instance, it could be that whereas a proportion of children diagnosed with ADHD could potentially attain normal striatal volumes in early adulthood independently of medication, others - presumably part of the 65% who still meet ADHD criteria in adulthood (Faraone et al., 2000) - might be able to do so only with a pharmaceutical intervention. If this was the case, longitudinal studies on children with ADHD would not clearly show an effect of psychostimulants on brain structure (Castellanos et al., 2002), since a part of the non-medicated sample would undergo a belated but naturally occurring normalization of putamen volumes with age.

Increased putamen function in medicated ADHD patients has been emphasized in several functional MRI studies (see Rubia et al., 2014 for a review). For instance, the putamen was recruited to a greater extent in methylphenidate (MPH) vs. placebo treated adolescents with ADHD both in a divided attention task (Shafritz, 2004) and in a time discrimination task (Rubia et al., 2009a). At a cellular level, such short-term changes in putamen function could be due to the acute post-synaptic DA increase in the striatum after MPH intake (Schiffer et al., 2006). However, prolonged improvement of ADHD symptoms with chronic MPH treatment is likely to rely rather on long-term structural changes such as the ones observed in the present study, which could possibly be driven by MPH-induced neuroadaptive changes in striatal gene expression through the activation of specific transcription factors (Adriani et al., 2006; Chase et al., 2005; Yano and Steiner, 2007).

It is worth mentioning that our findings could at least partly be confounded by changes in cerebral blood flow (CBF) associated with medication intake, as observed in a longitudinal study run by Franklin et al. (2013). In that study, participants exhibited an implausible decrease in anterior cingulate VBM signaling in T1-weighted images after acute baclofen administration, likely confounded by CBF changes. Indeed, SPECT studies show CBF increases in frontal gyri, caudate and thalamus in children with ADHD 90min after MPH administration (Kim et al., 2001) and CBF increases in middle and superior frontal gyri together with CBF decreases in temporal and occipital regions after 4-5 weeks of MPH treatment (Lee et al., 2005). However, the VBM effects found in the present study are substantially stronger than the CBF effects found in association with MPH, which can only be detected using very permissive thresholds such as $P < 0.001$ (Kim et al., 2001) and even $P < .01$ (Lee et al. 2005) uncorrected for whole-brain comparisons. Moreover, the brain areas affected by CBF changes do not overlap with the ones presented here.

The presented results are bound by two main limitations. On one side, the number of participants in the *ADHD non-med* group ($N = 16$) was substantially lower than in the other two groups ($N = 25$ respectively). This was due to the fact that not all participants in the *ADHD non-med* group were kept psychostimulant free within the three-year period, thus having to eliminate them from the se-

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cond measure. However, post-hoc ROI volume analyses with reduced but equal sample sizes for each group (16 randomly chosen participants per group) did not compromise group x session interaction effects on left putamen volume, thus discarding confounds associated with sample size differences. On the other side, this study was not conceived as a clinical trial and participants were not randomly assigned to different medication groups. Given that the decision to take psychostimulants or not was voluntary, the effects of individual factors bound to that decision cannot be ruled out. Therefore, and despite the aforementioned limitations, we provide evidence that psychostimulants have a significant effect in the long-term preservation of left putamen volumes in adult ADHD.

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Figure 1

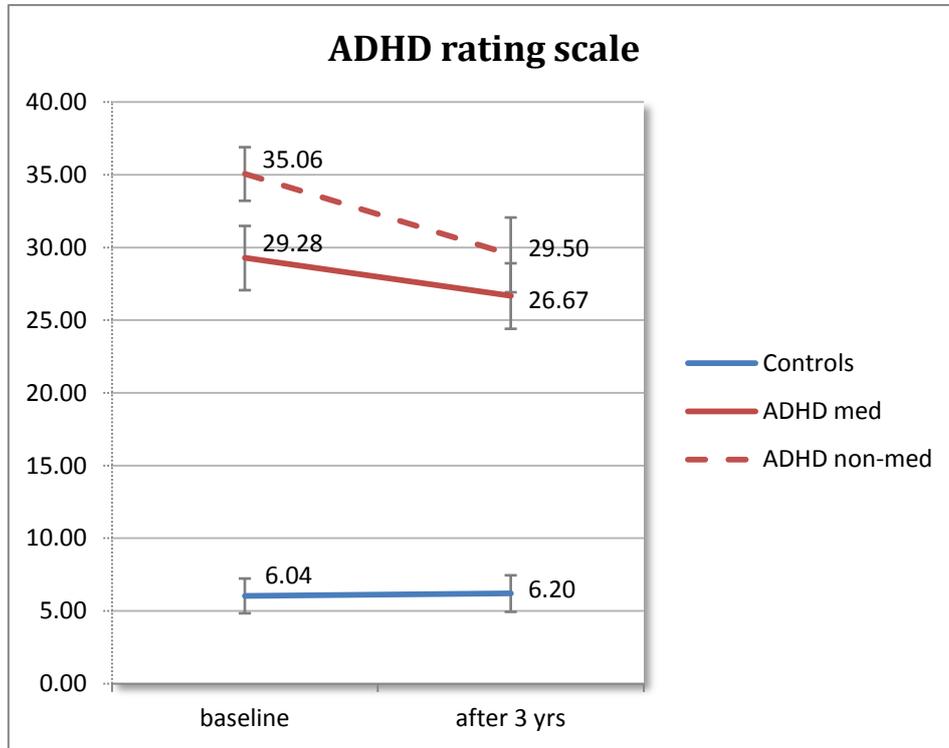


Figure 2

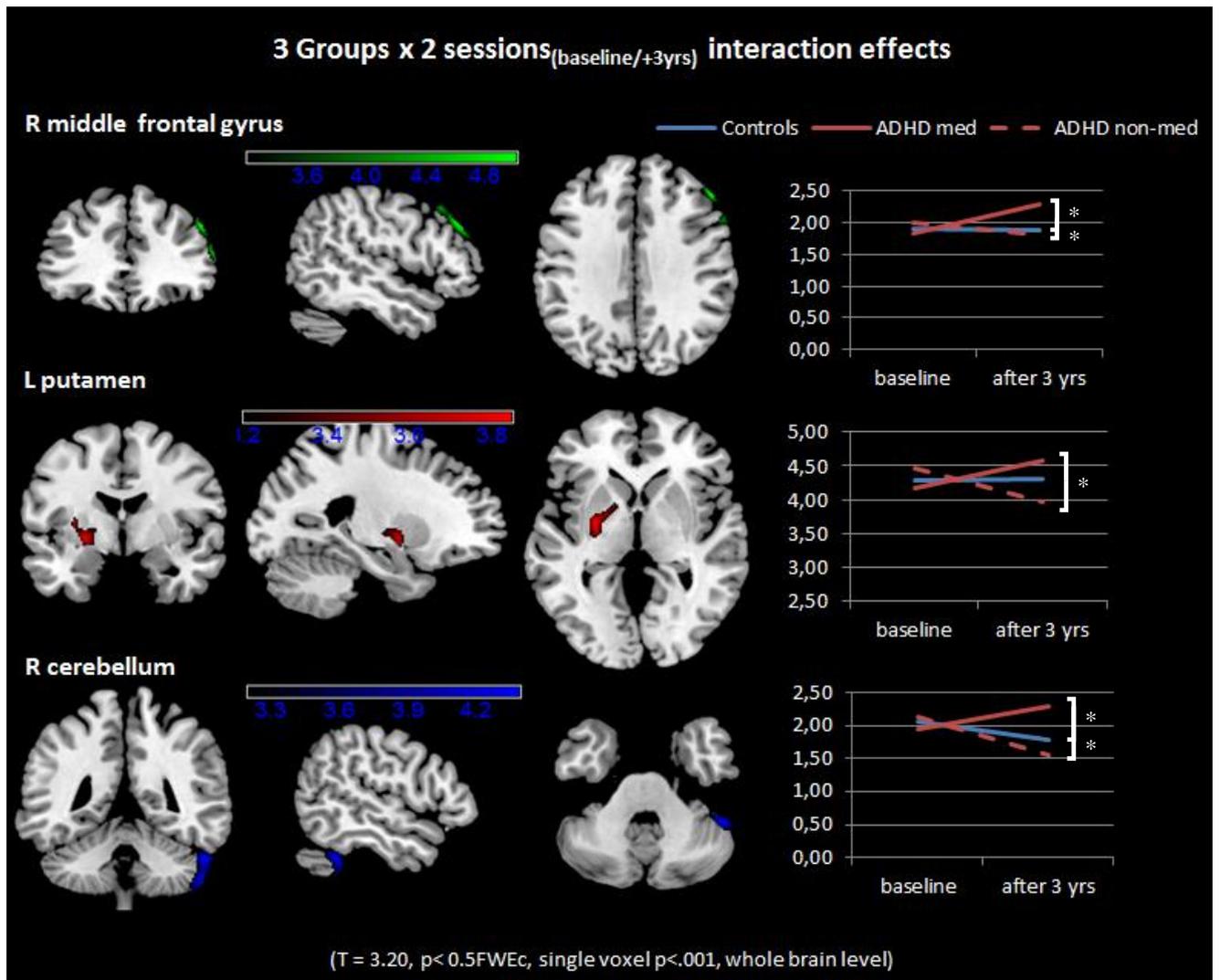


Figure 3

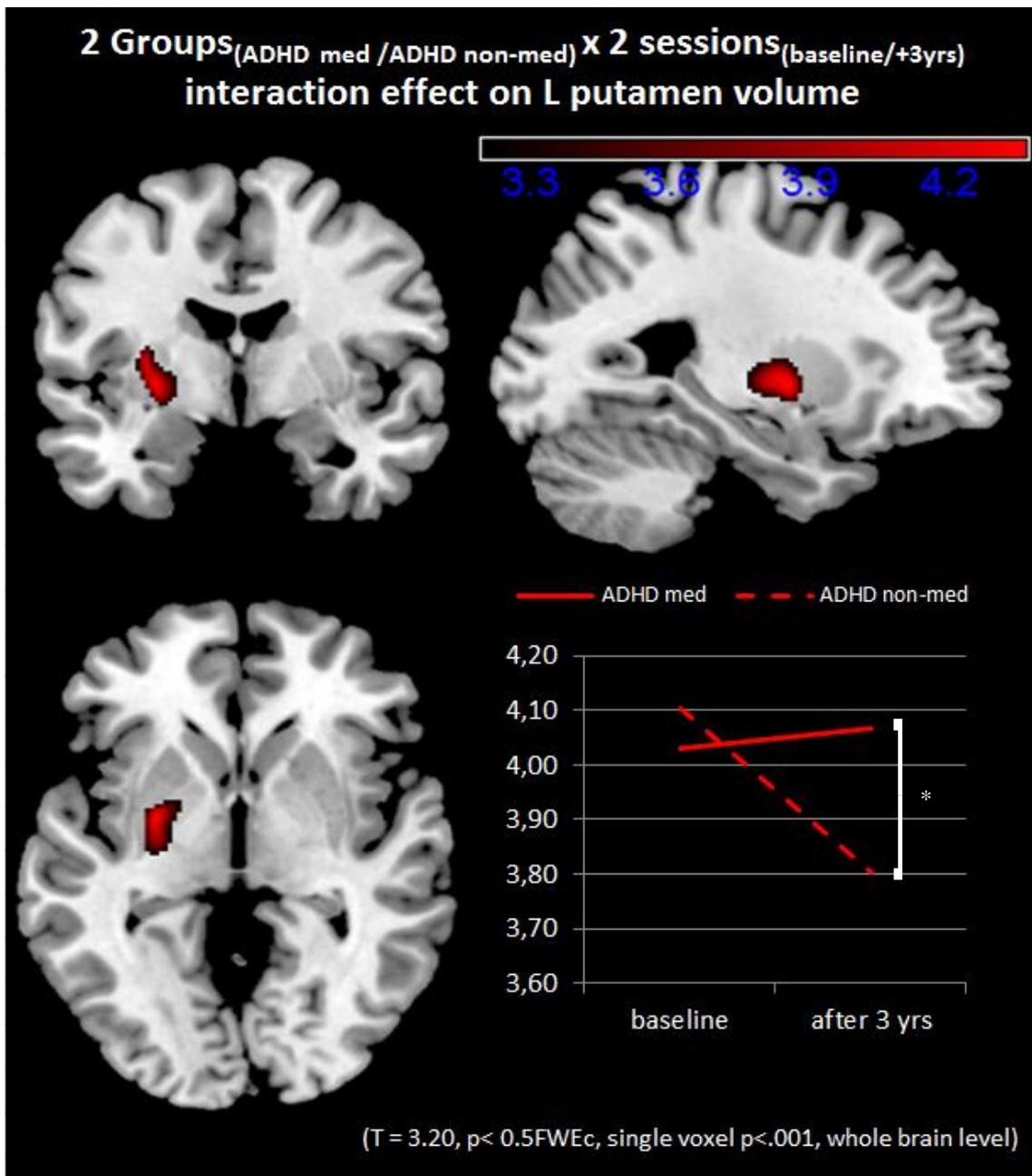


Figure 4

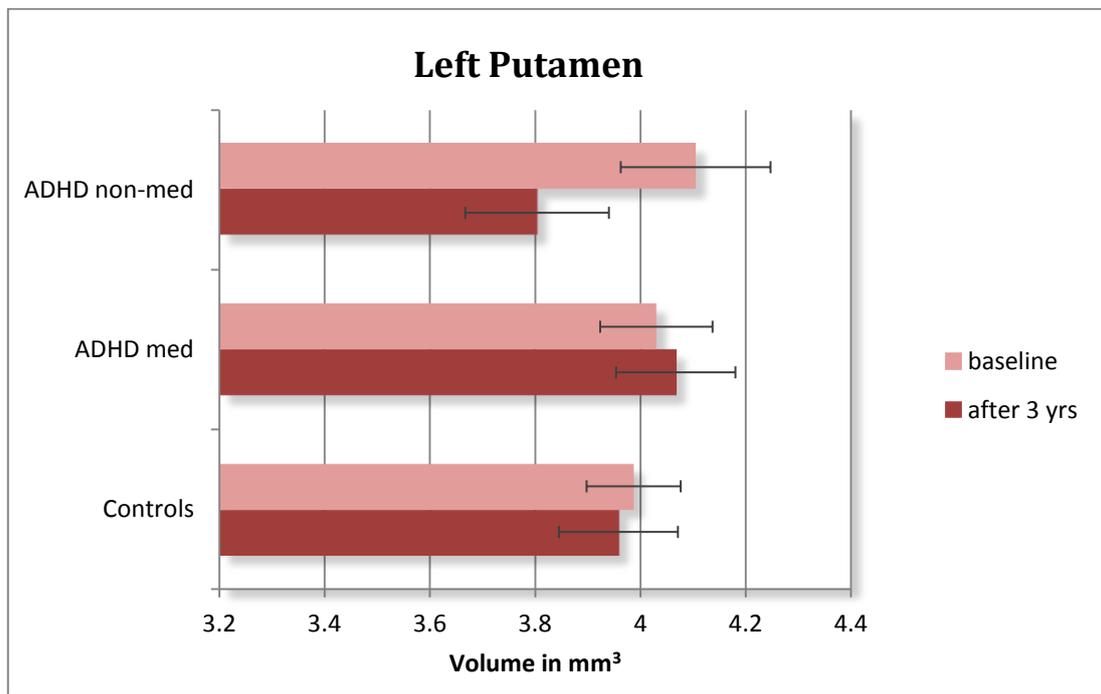
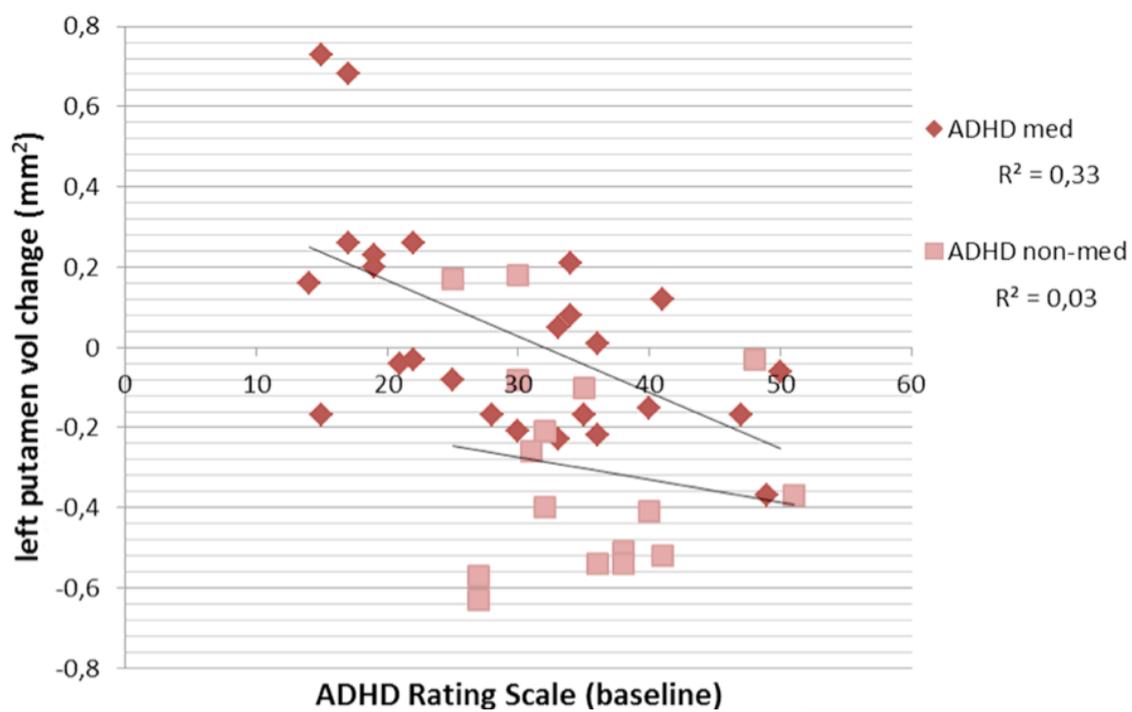


Figure 5



Role of the funding source

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Contributors

Oscar Vilarroya and Antoni Ramos-Quiroga designed the study and wrote the protocol. Vanesa Richarte and Montse Corrales selected the psychiatric sample. Marisol Picado selected the control sample. Clara Pretus and Marisol Picado carried out the data acquisition. Clara Pretus undertook the statistical analysis and wrote the first draft of the manuscript. Susanna Carmona contributed to the statistical analysis. All authors contributed to and have approved the final manuscript.

Conflict of Interest

Dr. Ramos-Quiroga has served on the speakers' bureau and acted as consultant for Eli Lilly and Co., Janssen-Cilag, Novartis, Lundbeck, Shire, Ferrer, and Laboratorios Rubió. He has received travel awards from Eli Lilly and Co., Janssen-Cilag, and Shire for participating in psychiatric meetings. The ADHD Program has received unrestricted educational and research support from Eli Lilly and Co., Janssen-Cilag, Shire, Rovi, and Laboratorios Rubió in the past two years.

Dra. Richarte has served on the speakers for Eli Lilly, Janssen-Cilag and Shire. She has received travel awards from Eli Lilly and Co., Janssen-Cilag, and Shire for participating in psychiatric meetings. The ADHD Program has received unrestricted educational and research support from Eli Lilly and Co., Janssen-Cilag, Shire, Rovi, and Laboratorios Rubió in the past two years.

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