

Cortical alterations associated with lower response to methylphenidate in adults with ADHD

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Neurodevelopmental conditions such as attention deficit hyperactivity disorder (ADHD) vary among individuals. For instance, variation exists in clinical response to methylphenidate (MPH) treatment, especially in adults, but the biological basis of this variability is poorly understood. In this longitudinal structural imaging study, we showed that pre-treatment neuroanatomical measures were associated with response to MPH at two months in 60 adults with ADHD. We compared anatomy with that of 23 controls and examined whether differences were enriched for genes linked to MPH dynamics and brain cells. Individuals with ADHD differed from controls in cortical volume and thickness, predominantly in temporo-parietal regions. Treatment non-responders differed from responders and controls in fronto-temporo-parieto-occipital regions and such differences were associated with reduced improvement on inattentive symptoms. These novel findings suggest that variation in neuroanatomy is associated with varying treatment responses. Group differences in cortical thickness were enriched for biologically plausible genes, including those supporting noradrenaline transport, a target of MPH.

Attention deficit hyperactivity disorder (ADHD) is a common neurodevelopmental condition characterized by inattentive and/or hyperactive-impulsive symptoms. These typically manifest in childhood but persist in up to 65% of adults¹. The prevalence of adult ADHD is approximately² 4%. Stimulants such as methylphenidate (MPH) are the first line of treatment. They modulate dopamine and noradrenaline transmission in striato-cortical regions^{3–5}. Specifically, MPH blocks both the dopamine transporter (DAT) and noradrenaline (norepinephrine) transporter (NET) through allosteric binding (that is, at a different site from that of the endogenous neurotransmitter), and thus inhibits both catecholamines reuptake and increases their availability in the synaptic

cleft^{3,6,7}. This in turn increases the endogenous stimulation of dopamine and noradrenaline receptors, which optimizes striato-cortical function (for example, top-down regulation of attention, response inhibition and motivation)^{8–10}. Methylphenidate is generally effective in improving ADHD symptoms, but randomized controlled trials have reported that more than one-third of adults do not respond¹¹. A recent meta-analysis also confirmed lower response rates in adults than in children¹². This has been associated with an increased risk of negative outcomes, including occupational failure, criminal behavior and substance misuse^{13,14}. Yet the biological mechanisms underlying variation in adult treatment response are poorly understood.

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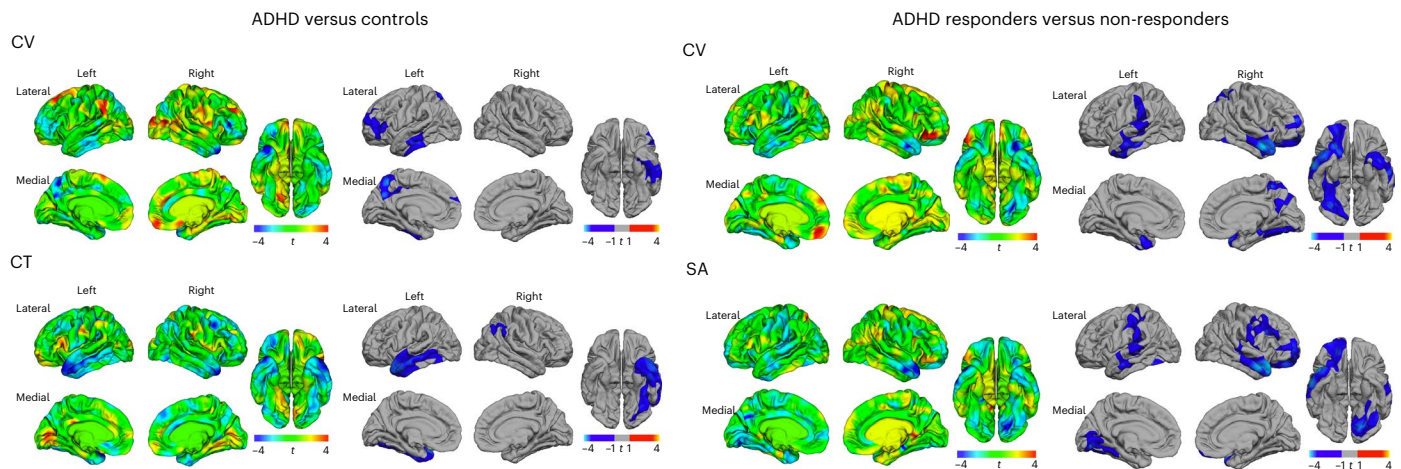


Fig. 1 | Vertex-wise comparisons between ADHD ($N = 60$) and controls ($N = 23$), and between ADHD responders ($N = 42$) and non-responders ($N = 18$). Vertex-wise comparisons between the whole ADHD group and neurotypical controls identified significant CV reductions in the ADHD group in three clusters, and lower CT in the ADHD group in two clusters. Compared

with ADHD responders, ADHD non-responders had significant reductions in CV across five clusters and significantly lower cortical SA in a further five clusters. For each contrast, uncorrected t -maps of effects are shown on the left, cluster maps after RFT correction are shown on the right. Cluster coordinates are reported in Table 1a,b.

Different neurobiological characteristics have been suggested to contribute to the varying treatment response in ADHD, including variants of genes encoding DAT (*SLC6A3*) and NET (*SLC6A2*)¹⁵; DAT epigenetic alterations¹⁶; theta oscillations^{17,18}; and resting-state frontostriatal functional connectivity¹⁹. However, the studies were primarily focused on children²⁰ and thus the findings may not apply to the adult population²¹. Here we test whether heterogeneity in brain anatomy may contribute to variation in adult treatment response. Most structural magnetic resonance imaging (MRI) studies have so far focused on ADHD versus control comparisons without investigating the treatment response, and mainly included children. For instance, meta-analyses have highlighted lower total and regional grey matter volumes in the basal ganglia and in fronto-parieto-occipital regions in young people with ADHD compared with neurotypical controls^{22–26}. They also suggested that increasing age is associated with less evident volumetric alterations^{24,25}. Recent large multicentre studies also confirmed diffuse but subtle morphometric alterations in cortico-subcortical regions in children with ADHD, but could not identify group differences in adults^{27,28}. The former study focused on the total intracranial volume and subcortical structures, and reported smaller total and subcortical volumes, including in the amygdala, accumbens, putamen, caudate and hippocampus. The latter study reported lower surface area measures in fronto-temporo-cingulate regions, and lower cortical thickness in the temporal pole and fusiform gyrus. Findings were not affected by current stimulant treatment^{27,28}. These observations are in line with the hypothesis that ADHD symptoms are related to a maturational delay of brain regions involved in cognitive and motor functions^{27,29}. Nevertheless, ADHD is a highly heterogeneous condition and past studies have suggested that anatomical variation exists (even in adults), and that more pronounced alterations may be associated with co-morbidities or symptom persistence^{30,31}. In summary, most neuroanatomical investigations have focused on group comparisons with controls, yielding inconsistent or no results, especially in adults. There is therefore an increasing need to parse neuroanatomical heterogeneity, perhaps especially regarding variation in treatment response.

There are limited structural imaging studies on individuals with ADHD that compare treatment responders and non-responders. A small study in children reported that the volume of the caudate and nucleus accumbens were reduced in non-responders compared with responders³². Similarly, a more recent retrospective MRI study including both children and adults observed smaller left putamen

and larger precuneus volumes in non-responders than responders³³. These studies represent valuable first steps and suggest that anatomical differences may underpin the variation in treatment response. We hypothesize that this may also apply to adults with ADHD because, although case-control brain anatomical differences decrease with age—perhaps especially in the basal ganglia^{24,25}—cortical alterations have even been reported in adults. For instance, a meta-analysis of whole brain voxel-based morphometry studies indicated lower volumes of the ventromedial prefrontal cortex in both children and adults with ADHD³⁴. Unfortunately, the study by Hoogman and colleagues²⁷ did not investigate case-control differences in regional cortical volumes, and thus these cannot be excluded in adults²⁷. In line with this observation, a past study from our group suggested that anatomical connectivity among fronto-parietal cortical regions—but not frontostriatal connectivity—was associated with treatment response in adults with ADHD³⁵. Finally, there is preliminary evidence that biological factors implicated in treatment response may at least partly differ from those underlying differences between ADHD and neurotypical controls^{20,36}. Unfortunately, anatomical studies comparing responders and non-responders so far: (1) include only children, or a mixed sample of children and adults; (2) did not include neurotypical controls for comparison; and (3) have mostly been based on volumetric measures. Cortical volume (CV) is a product of two distinct parameters, cortical thickness (CT) and surface area (SA), that, in turn, have distinct genetic and developmental origins^{37,38}. However, no study has yet investigated the relationship between regional differences in CV, SA and CT, and adult treatment outcome. Hence, it remains unclear what the brain anatomical associates of adult treatment response are, whether these involve surface-based cortical measures, and how these relate to potential underpinning cellular mechanisms.

To address these questions, this prospective study uses structural MRI to first compare CV, CT and SA in adult neurotypical controls and adults with ADHD, and then separately in ADHD responders and non-responders to two months of MPH treatment. It also tested whether differences between responders and non-responders were associated with improvement on inattentive and/or hyperactive-impulsive symptoms. Finally, we performed a virtual histology analysis to aid interpretation of the group morphometric differences. Specifically, following a previously published approach^{39,40}, we tested whether regional group differences were enriched for genes involved in MPH pharmacodynamics or expressed by different brain cell types.

Results

Sample

The sample included 60 adults with ADHD and 23 matched neurotypical controls. At follow-up, 42 individuals with ADHD were identified as responders and 18 as non-responders to MPH. The two subgroups did not significantly differ in clinical presentation, baseline severity and MPH dose at follow-up (refer to page 2 of the Supplementary Information). All of the participants were included in the following analyses.

Structural MRI analysis

Global brain measures. Non-responders had significantly lower total intracranial volume ($t = 2.51$, $P = 0.015$) and mean SA ($t = 3.146$, $P = 0.003$) than responders. We did not observe other significant group differences (Supplementary Table 1).

Vertex-wise comparison between ADHD and controls. When compared with the controls, individuals with ADHD (combined responders and non-responders) had significantly smaller CV in three clusters in the left hemisphere (random field theory (RFT) $P < 0.05$). These clusters included: (1) the posterior cingulate cortex, precuneus and superior parietal lobule; (2) the pars triangularis and middle/superior frontal gyri; (3) and the fusiform gyrus and inferior/middle temporal gyri (Fig. 1 and Table 1a). Furthermore, adults with ADHD had significantly lower CT in two clusters (RFT $P < 0.05$): (1) the left fusiform gyrus, temporal pole and gyri; (2) and the right supramarginal gyrus and parietal lobules (Fig. 1 and Table 1a). No significant differences in SA were observed.

Vertex-wise comparison between responders and non-responders. Non-responders showed much smaller CV across five clusters (RFT $P < 0.05$) than ADHD treatment responders. These clusters included: (1) the left temporo-parietal-insular regions; (2) the right precuneus and parietal lobules; (3) the right orbitofrontal cortex and middle frontal gyrus; (4) the right insula, temporal pole and middle/superior temporal gyri; and (5) the fusiform, lingual and parahippocampal gyri, and occipital cortex (Fig. 1 and Table 1b). Non-responders also had significantly lower cortical SA (RFT $P < 0.05$) in five clusters partially overlapping with those showing significant group differences in CV. These included the right fronto-parietal and temporal regions, and the left temporal, occipital and parietal regions (Fig. 1 and Table 1b). No significant differences in CT were observed between responders and non-responders.

Secondary statistical analysis

Subgroup comparisons. We first considered cortical regions that significantly differed between the whole ADHD group and controls. To determine the separate contribution of the ADHD subgroups to these significant differences, we used independent-sample t -tests to compare morphometric measures in the above five clusters between controls, and (separately) between responders and non-responders. We observed that both ADHD non-responders and responders showed significantly lower CV and CT than the controls in these five clusters. The two clinical groups only significantly differed in the volume of cluster 3 (that is, the left fusiform gyrus and inferior/middle temporal gyri), which was smaller in non-responders ($t = -2.337$, $P = 0.023$; Fig. 2 and Supplementary Table 2).

We then considered the cortical regions that significantly differed between responders and non-responders. Independent-sample t -tests revealed no differences in these ten clusters between ADHD responders and controls; however, significant differences in CV and SA were noted between non-responders and controls in all ten clusters (Fig. 3 and Supplementary Table 3).

Associations with symptom improvement. We used linear regression to determine whether the observed cortical differences between ADHD treatment responders and non-responders were associated with

individual improvement on either inattentive or hyperactive-impulsive symptoms after two months of treatment. After correcting for multiple comparisons ($P \leq 0.005$), out of the ten clusters in which significant differences between responders and non-responders were observed, one-fifth of the volumetric and three-fifths of the SA clusters showed significant positive associations with improvement on inattentive symptoms (Fig. 4 and Supplementary Table 4).

Genomic analyses (virtual histology)

Finally, we investigated the genomic associates of the observed morphometric differences between individuals with ADHD and neurotypical controls, and between ADHD responders and non-responders to MPH. Differences in CT between the whole ADHD group and controls were enriched for genes associated with noradrenaline transport (CT odds ratio (OR) = 13.012, $pFDR = 0.028$), and for genes expressed by astrocytes (CT OR = 2.93, $pFDR = 0.014$). We did not observe any significant enrichment of the cortical differences between responders and non-responders. See Supplementary Figs. 1 and 2, and Supplementary Tables 5 and 6 for the complete results.

Discussion

Compared with neurotypical controls, individuals with ADHD (responders and non-responders combined) had significantly smaller CV in the left fronto-temporo-parietal regions, and CT in the left temporal and right parietal regions. These group differences were driven by both ADHD treatment responders and non-responders, although the latter had more prominent temporo-occipital volumetric alterations. When directly compared, non-responders exhibited lower total and regional CV and SA than responders, especially in fronto-temporal regions; however, only the former significantly differed from the controls in these measures, and only the volumetric differences in the left temporo-occipital cortex partially overlapped with those observed in the ADHD versus controls comparison. Thus, non-responders had more pronounced volumetric differences and additional morphometric alterations not related to an ADHD diagnosis per se than responders.

The fact that the identified cortical areas are related to lower treatment response is in line with their role in supporting brain functions implicated in ADHD. For instance, the superior and inferior parietal cortices are part of the dorsal and ventral fronto-parietal networks, which support attentive functions^{41,42}. The right middle prefrontal cortex is also important to reorient attention to relevant stimuli⁴³, whereas the orbitofrontal cortex—together with anterior temporal regions—has been related to decision-making, emotion regulation and reward processing^{44–46}. The inferior and middle temporal gyri are involved in several cognitive processes, including memory, visual perception and multimodal integration⁴⁷. Furthermore, these brain regions have been implicated in functional MRI studies. For instance, functional MRI meta-analyses have identified consistently reduced activation in fronto-parietal areas during response inhibition, attention, and timing in adults and children with ADHD compared with controls^{48,49}. Finally, there is evidence that MPH modulates the activity of these brain areas. For example, past meta-analyses reported upregulation of fronto-parieto-temporo-occipital areas during cognitive tasks after MPH administration in youths with ADHD^{50,51}. In summary, the observed fronto-parieto-temporo-occipital morphometric differences between responders and non-responders are in line with the functional roles of the identified regions and the modulatory action of MPH.

This work adds to past findings from structural MRI studies. These have mostly focused on comparisons between ADHD and neurotypical individuals, and have reported reduced total and regional cortico-subcortical volumes, especially in children with ADHD²⁶. However, heterogeneity exists even in adults, and neuroanatomical alterations have been associated with ADHD persistence and co-morbidities^{30,31}. We observed that morphometric heterogeneity is also associated with

Table 1 | a,b. Clusters of significant differences between ADHD (N=60) and controls (N=23), and between ADHD responders (N=42) and non-responders (N=18)

Table 1a. Clusters of significant differences between ADHD and controls									
Measure	Cluster	Region labels	Talairach						
			Hemisphere	Vertices	x	y	z	t_{max}	$P_{cluster}$
Cortical volume									
	1	Posterior-cingulate cortex, precuneus, superior parietal cortex	L	4,751	-10	-57	50	-3.96	5.85×10^{-3}
	2	Pars triangularis, rostral middle frontal gyrus, superior frontal gyrus	L	4,248	-15	56	11	-2.78	2.62×10^{-2}
	3	Fusiform gyrus, inferior and middle temporal gyri	L	4,149	-51	-30	-16	-2.96	4.13×10^{-2}
Cortical thickness									
	1	Fusiform gyrus, temporal pole, inferior, middle and superior temporal gyri	L	13,269	-40	8	-25	-3.53	4.38×10^{-6}
	2	Supramarginal gyrus, inferior and superior parietal cortices	R	4,100	46	-49	42	-3.4	7.51×10^{-3}
Table 1b. Clusters of significant differences between ADHD responders and non-responders									
Measure	Cluster	Region labels	Talairach						
			Hemisphere	Vertices	x	y	z	t_{max}	$P_{cluster}$
Cortical volume									
	1	Banks superior temporal sulcus, inferior temporal gyrus, insula, middle temporal gyrus, post-central gyrus, superior temporal gyrus, supramarginal gyrus, temporal pole, transverse temporal cortex	L	13,629	-44	-26	32	-3.57	1.11×10^{-5}
	2	Precuneus, inferior and superior parietal cortices	R	5,683	9	-46	54	-3.44	4.16×10^{-3}
	3	Lateral orbital frontal cortex, rostral middle frontal gyrus	R	5,373	23	24	-13	-3.55	5.25×10^{-3}
	4	Insula, temporal pole, middle and superior temporal gyri	R	5,551	54	-5	-21	-5.07	1.02×10^{-2}
	5	Fusiform gyrus, lateral occipital cortex, lingual gyrus, parahippocampal gyrus	R	5,615	33	-69	-7	-3.4	1.44×10^{-2}
Surface area									
	1	Caudal middle frontal gyrus, insula, lateral orbital frontal cortex, pars triangularis, post-central gyrus, precentral gyrus, rostral middle frontal gyrus	R	13,817	29	50	-7	-3.17	2.87×10^{-5}
	2	Banks superior temporal sulcus, insula, middle temporal gyrus, superior temporal gyrus, supramarginal gyrus, transverse temporal cortex	L	8,171	-41	-28	14	-3.22	4.87×10^{-4}
	3	Banks superior temporal sulcus, middle and superior temporal gyri	R	7,082	45	13	-23	-4.57	9.28×10^{-4}
	4	Fusiform gyrus, lateral occipital cortex, lingual gyrus, pericalcarine cortex	L	6,260	-19	-75	-3	-3.74	2.00×10^{-2}
	5	Post-central gyrus, superior parietal cortex	L	4,199	-32	-35	49	-2.73	4.49×10^{-2}

For the comparisons between the controls and participants with ADHD, parameter estimates were obtained by regression of a generalised linear model at each vertex i , with age as a continuous co-variate (that is, $Y_i = \beta_0 + \beta_1 \text{Group} + \beta_2 \text{Age}$, where ϵ_i is the residual error). To examine the differences between ADHD treatment responders and non-responders in each morphometric measure, we also included their age, full-scale IQ, Barkley Adult ADHD Rating Scale-IV (BAARS-IV) baseline total score, MPH dose and weight as continuous co-variables, as well as handedness as a categorical variable (that is, $Y_i = \beta_0 + \beta_1 \text{Group} + \beta_2 \text{Age} + \beta_3 \text{IQ} + \beta_4 \text{BAARS-IV} + \beta_5 \text{Dose} + \beta_6 \text{Weight} + \beta_7 \text{Handedness} + \epsilon_i$, where ϵ_i is the residual error). Effects of interest were estimated from co-efficient β_i , normalized by the corresponding standard error. Corrections for multiple comparisons were performed using a RFT-based cluster analysis for non-isotropic images at a cluster-threshold of $P < 0.05$ (two-tailed).

variation in treatment response. Past structural MRI studies comparing ADHD responders and non-responders to treatment are limited. One such recent study in a mixed pediatric and adult sample reported reduced left putamen and increased bilateral precuneus volumes in non-responders compared with responders, and that these (and additional volumetric differences in fronto-parieto-occipital cortical regions) could differentiate between responders and non-responders using a machine learning approach³³. These cortical areas partially overlap with those identified in our study, albeit we did not observe regions of increased volume. Inconsistencies may be related to differences in both the sample and study characteristics. For instance, they

included both children and adults, but increasing age is associated with less evident volumetric alterations^{24,25,27,28}. Further, in this previous retrospective study, individuals with ADHD were classified as responders or non-responders on the basis of a global clinical improvement after a variable treatment duration, whereas, in our prospective interventional study, we classified individuals with ADHD as responders or non-responders on the basis of an ADHD symptom scale after a fixed period of treatment (two months). Our results add to these previous findings, as we observed that differences in cortical structure between responders and non-responders were associated with variation in improvement on inattentive symptoms.

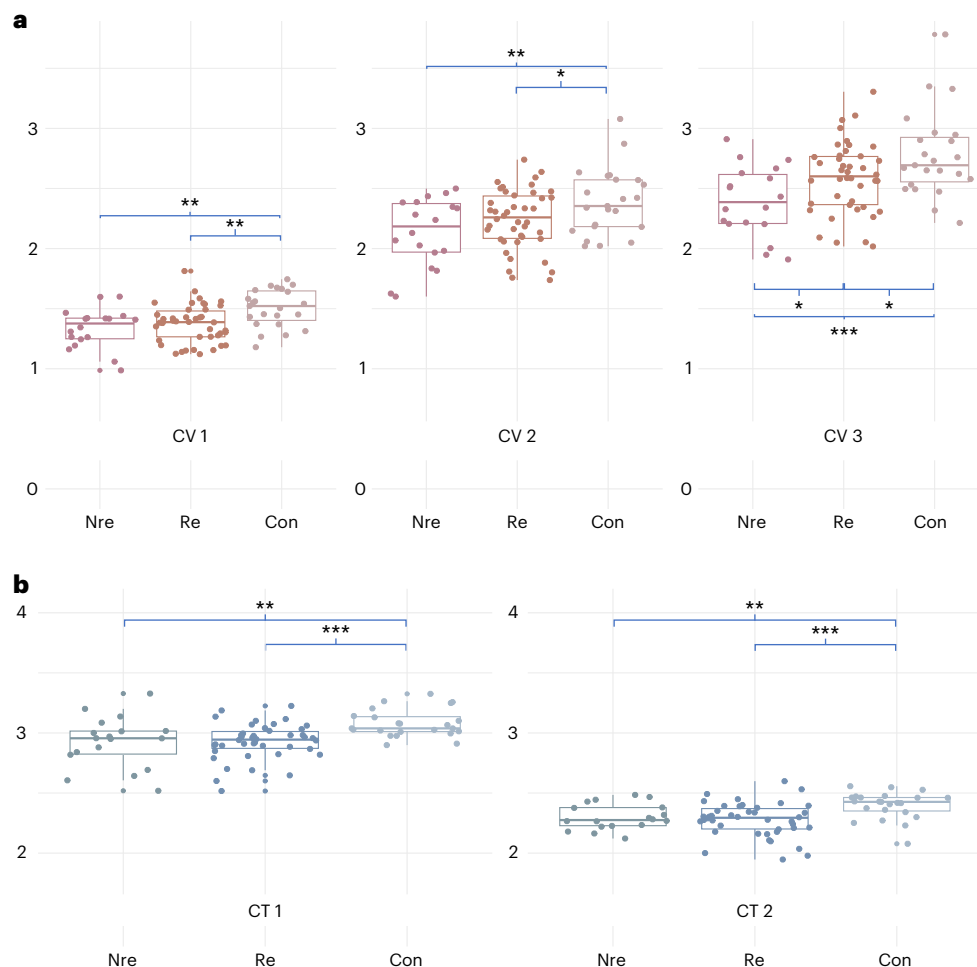


Fig. 2 | Subgroup comparisons for significantly different clusters between ADHD ($N = 60$) and controls ($N = 23$). a,b, Subgroup comparisons for CV (a) and CT (b). Both responders and non-responders significantly differed from the controls in all of the five clusters that were significantly different between the whole ADHD sample and controls. Furthermore, non-responders had lower CV in cluster 3 (that is, the left fusiform gyrus and inferior and middle temporal gyri)

than responders. Individual participants' data plotted as dots. Bounds of upper and lower whiskers represent maxima and minima, respectively; the center boxes represents the interquartile range, with the median represented by the middle horizontal lines. Significance: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Full statistical results are reported in Supplementary Table 2. Con, controls; Nre, non-responders; Re, responders.

Finally, to aid the interpretation of the observed morphometric group differences, we used a virtual histology approach to map our neuroanatomical results to putative biological processes involved in MPH pharmacodynamics and brain cell types. This exploratory analysis built on previous work showing that morphometric measures rely on different developmental processes^{37,38}, which, in turn, are regulated by a complex gene–environment interplay^{52–54}. We observed that ADHD versus controls differences in CT were enriched for genes involved in the transport of noradrenaline. These preliminary results corroborate previous evidence that links noradrenaline dysregulation to ADHD pathophysiology, and that substantiates the therapeutic use of MPH⁵⁵. Animal studies suggest that negative early life events may impact on brain development partly through disrupting noradrenaline signaling⁵⁶. For instance, exposure to nicotine during pregnancy, which is a known risk factor for ADHD⁵⁷, has been associated with increased levels of cortical noradrenaline in infant mice⁵⁸. Furthermore, animal studies suggested that therapeutic doses of stimulants mainly affect catecholamine levels at a cortical level, and especially those of NA^{4,59}. Taken together, our current work and previous findings suggest that variation in noradrenaline transport may contribute to altered brain development and heterogeneity in the treatment response.

We also observed an enrichment for genes expressed by astrocytes. These are glial cells that contribute to neurotransmitter homeostasis,

synaptic development and modulation, and the blood brain barrier⁶⁰. Astrocyte integrity is also required to support myelination during development⁶¹. Accordingly, alterations to astrocytes may hinder myelination and affect white matter tract development. In line with this, meta-analyses of diffusion imaging studies have identified several brain connectivity alterations in individuals with ADHD, especially in the splenium and body of the corpus callosum, which connect posterior cortical areas such as those identified in this structural analysis⁶². Thus, altered astrocyte development might potentially represent a shared mechanism underlying both grey and white matter pathology in ADHD. Furthermore, as they support optimal synaptic functioning, an alteration at this level may potentially contribute to lower treatment response to MPH. In summary, our virtual histology approach suggested that differences in CT between ADHD and controls were enriched for genes involved in noradrenaline transport and expressed by astrocytes. Further studies are needed to elucidate the biological pathways linking variation in genomic expression to neuroanatomy and this to treatment response. For instance, the single nucleotide polymorphism rs28386840 of the *SLC6A2* gene has been associated with increased noradrenaline transporter binding in the thalamus of individuals with ADHD compared with that of controls⁶³, and also with response to MPH in a meta-analysis¹⁵. Unfortunately, this variant was not tested in the only imaging genetic study investigating the

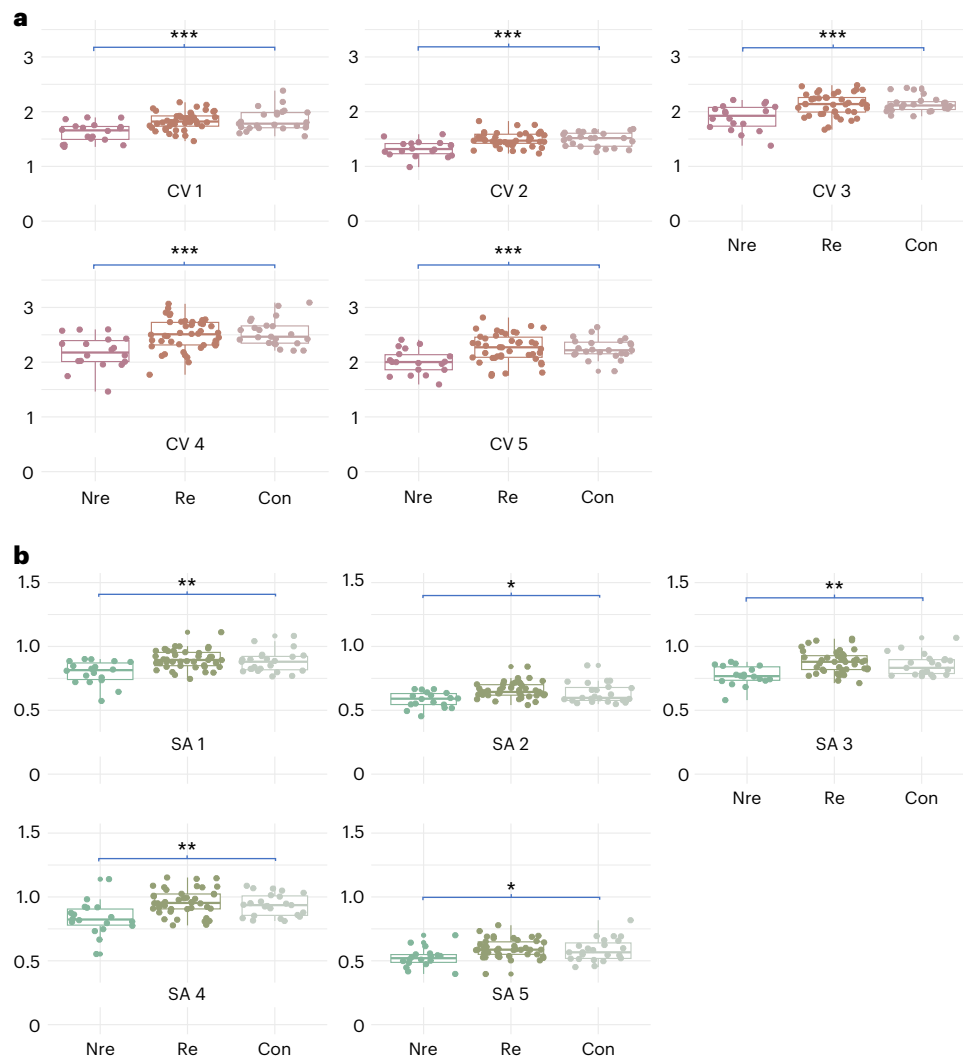


Fig. 3 | Subgroup comparisons for clusters significantly different between ADHD responders ($N = 42$) and non-responders ($N = 18$). Only non-responders significantly differed from the controls in the ten clusters significantly different between ADHD responders and non-responders.

Responders did not significantly differ from controls in these clusters. **a,b**, Display comparisons for CV (**a**) and SA (**b**). Individual participants' data plotted as dots. Significance: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Full statistical results are reported in Supplementary Table 3.

relationship between *SLC6A2* polymorphism and cerebral volume or thickness⁶⁴, but it warrants further investigation.

This study has several strengths. We specifically investigated the morphometric differences between responders and non-responders to MPH in a sample of adults with ADHD. The interventional study design allowed us to overcome limitations of naturalistic studies in ADHD, which mainly include patients on treatment and thus are enriched in responders. The study also benefitted from the homogeneity in treatment formulation and duration; the inclusion of controls for comparative analyses; and the investigation of both volumetric and surface-based cortical differences. However, limitations should be taken into consideration. We included a small percentage of individuals with ADHD who had previously been exposed to medication. Previous exposure to medication has been suggested to have a normalizing effect on brain structure²⁴, although other reports suggest that this may not be the case⁶⁵. Our findings suggest that the observed normalizing effect of long-term treatment on brain anatomy may be at least in part dependent on a selection bias, as responders to treatment have less severe/fewer pre-treatment brain alterations. We only recruited males because ADHD is more commonly diagnosed in males⁶⁶, and there is preliminary evidence of sex differences in brain anatomy and

biological response to stimulants^{67–69}. Further, it is not known whether sex differences in brain morphometry may relate to sex differences in treatment response. We therefore only included males to avoid potential sex-related confounding on our morphometric analyses. Nonetheless, we encourage future studies to extend our analyses to the female population with ADHD. Similarly, we only included participants without current co-morbid disorders because there is evidence that differences in neuroanatomy exist between individuals with/without co-morbidities⁷⁰. However, our results should be validated in clinical samples also including participants with co-morbidities. Furthermore, we focused our analyses on cortical morphometric characteristics. We made this choice because a previous structural connectivity study from our group identified cortico-cortical but not fronto-striatal anatomical characteristics related to treatment response in adult ADHD³⁵. This is also in line with prior meta-analytic studies showing that increasing age was associated with less evident structural abnormalities in the basal ganglia^{24,25}. Nevertheless, future studies would benefit from investigating subcortical regions in relation to adult treatment response. Moreover, although we recruited a relatively large sample of adults with ADHD and included controls for comparative analyses, findings need to be validated in larger samples to ensure generalizability. For

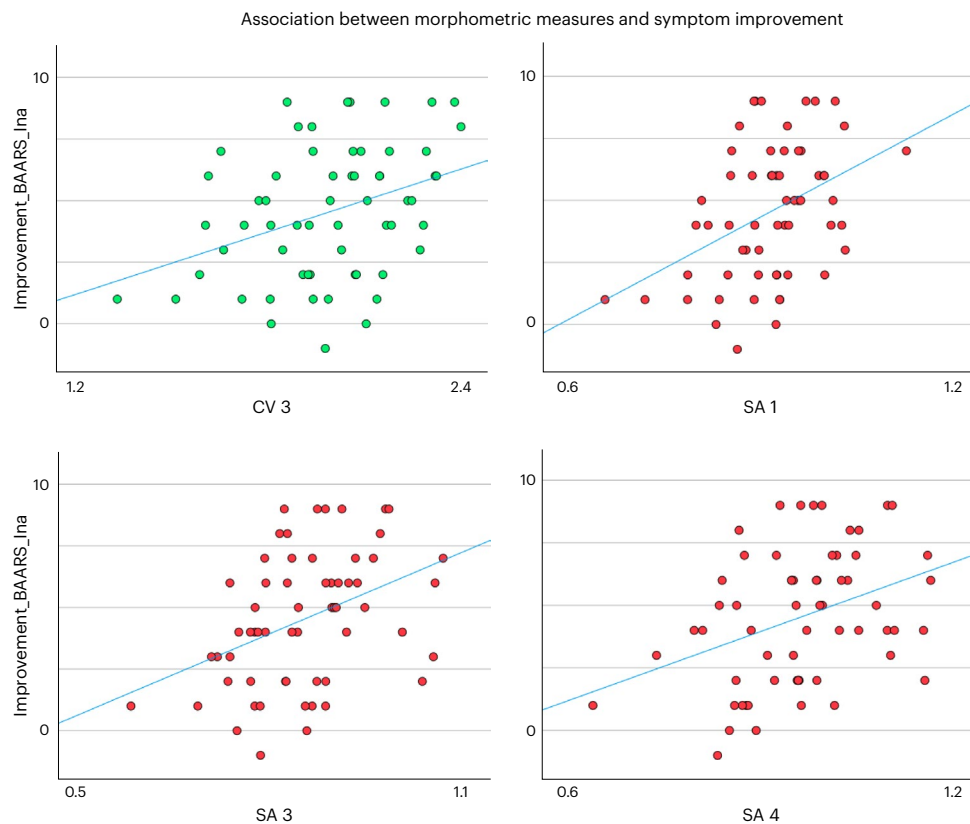


Fig. 4 | Association between morphometric measures and symptom improvement. After correcting for multiple comparisons, the morphometric differences between responders ($N = 42$) and non-responders ($N = 18$) in four

clusters were significantly associated with an improvement on inattentive symptoms at two months. Full statistical results are reported in Supplementary Table 4. BAARS-ina, Barkley-inattentive score.

instance, our prospective study identified a limited number of non-responders ($N = 18$). The observed proportion of non-responders is in line with previous randomized clinical trials¹¹ and thus representative of the actual ADHD clinical population. However, the small sample size together with the limited number of genes identified during the coding phase may have limited our power to explore genetic enrichment within ADHD subgroups. Furthermore, despite offering a unique opportunity to map neuroanatomy to neurobiology, virtual histology approaches remain limited in their spatial coverage and resolution (especially compared with structural MRI data). Consequently, future studies should validate our analysis once high-resolution gene expression datasets become available and in larger samples. We considered a two-month follow-up as this is the median duration of randomized controlled trials of extended-release MPH in adults with ADHD⁷¹. This timeframe is usually sufficient for dose optimization, nevertheless, studies with longer follow-ups are encouraged. They may also clarify how treatment-related changes may be potentially related to varying response over time. Finally, we established associations between brain anatomical characteristics and known genetic expression maps, however, future longitudinal imaging genetic studies are needed to clarify potential causal mechanisms.

Conclusion

We provide evidence that adults with ADHD who do not respond to MPH have significant differences in cortical morphometry compared with those who respond. Responders and non-responders may therefore represent different biological subgroups within the heterogeneous population with ADHD. Further, ADHD versus control group differences in cortical regions were enriched for genes expressed by astrocytes and involved in the transport of noradrenaline. Parsing neuroanatomic heterogeneity is critically important to better understand the mechanisms

underlying variation in clinical presentation and outcome³¹. These results need to be replicated and extended in independent samples and, preferably, confirmed by meta-analyses⁷². In the future, this knowledge may help advance the development of clinical interventions, for example, by identifying treatment resistant individuals in the context of clinical trials of new treatments.

Methods

Sample and research protocol

This study is part of a single-blind placebo-controlled cross-over study, followed by a longitudinal open-label phase (NCT 03709940), which was conducted at the South London and Maudsley NHS Foundation Trust (United Kingdom). Details on the sample and study protocol (NCT 03709940) have been described in a previous work³⁵. In this report we focused on the structural MRI data. We included 60 participants with a clinical diagnosis of ADHD according to the DSM-5 criteria⁷³, aged 18–45 years, with no current co-morbid disorders, and a full-scale IQ of above 70. The sample size was determined based on a power calculation (Supplementary Information). Attention deficit hyperactivity disorder is more commonly diagnosed in males⁶⁶, and there is preliminary evidence of sex differences in brain morphometry and biological response to stimulants^{67–69}, we therefore only included males to enhance sample homogeneity (see the 'Discussion' section for potential limitations). Recruitment focused on the inclusion of medication-naïve adults, but we also included a small minority of individuals who were previously medicated and had stopped treatment at least one year before the start of the study (see the 'Results' section). The severity of the symptoms was measured at the baseline and also at follow-up using BAARS-IV⁷⁴, which provided three scores (ADHD total score, ADHD inattention and ADHD hyperactivity-impulsivity). IQ was measured using the Wechsler Abbreviated Scale of Intelligence⁷⁵,

whereas handedness was measured using a modified version of the Edinburgh Handedness Inventory⁷⁶.

Participants with ADHD completed MRI scanning before starting routine treatment with the same long-acting formulation of MPH (Concerta XL, titrated up to 54 mg). During titration, side effects were monitored, and the dose was adjusted if needed. The dose was considered as a co-variate. Treatment response was measured at two months. According to previously published criteria, we used a categorical definition of treatment response based on a symptomatic improvement of at least 30%, as measured by the BAARS-IV total score at follow-up as compared with the baseline^{11,77}. We identified a subgroup with a high average and a subgroup with a low average treatment response, which we labelled as responders and non-responders, respectively. We also included 23 neurotypical adults matched for sex, age and IQ for comparative analyses. All participants gave written informed consent. We assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008. Participants received £50 after the scanning sessions as a compensation for their time. Ethical approval was obtained by Camden and Islington Research Ethics Committee (12/LO/0630).

Structural MRI data acquisition and analysis

MRI acquisition parameters. High-resolution T_1 -weighted structural images were acquired on a 3 T MR750 GE scanner (General Electric, Milwaukee) for each patient with the following parameters: 196 slices, 1.2 mm thickness with 1.2 mm gap, repetition time (TR) = 7.312 ms, time to echo (TE) = 3.016 ms and flip angle = 11°.

Cortical reconstruction using FreeSurfer. FreeSurfer v.5.3.0 software⁷⁸ was used to derive tessellated models of the cortical surface for each T_1 -weighted image. These well-validated and fully automated procedures have been extensively described elsewhere^{79–83}. Resulting surface models were visually inspected for reconstruction errors. Surface reconstructions with visible inaccuracies were further excluded from the statistical analysis and are not described in this study. Three separate vertex based morphometric cortical features were calculated from the individual surface reconstructions: (1) the cortical volume, which is composed of (2) CT⁸³ and (3) pial SA⁸⁴. Before further analysis, all features were smoothed using a 15 mm surface-based full-width at half-maximum Gaussian kernel.

Vertex-wise between-group comparison of cortical measures.

Vertex-wise statistical analyses of each cortical feature were conducted using the SurfStat toolbox⁸⁵ for MATLAB (R2014a, The Mathworks).

For the comparisons between the controls and participants with ADHD, parameter estimates were obtained by regression of a generalised linear model at each vertex i , with age as a continuous co-variate, that is,

$$Y_i = \beta_0 + \beta_1 \text{Group} + \beta_2 \text{Age}$$

where ε_i is the residual error.

To examine differences between ADHD treatment responders and non-responders in each morphometric measure, we also included age, full-scale IQ, BAARS-IV baseline total score, MPH dose and weight as continuous co-variables, and handedness as a categorical variable, that is,

$$Y_i = \beta_0 + \beta_1 \text{Group} + \beta_2 \text{Age} + \beta_3 \text{IQ} + \beta_4 \text{BAARS-IV} \\ + \beta_5 \text{Dose} + \beta_6 \text{Weight} + \beta_7 \text{Handedness} + \varepsilon_i,$$

where ε_i is the residual error. Effects of interest were estimated from coefficient β_1 -normalized by the corresponding standard error. Co-variables were selected as previously associated with treatment response³⁵.

Corrections for multiple comparisons were performed using a RFT-based cluster analysis for non-isotropic images at a cluster-threshold of $P < 0.05$ (two-tailed)⁸⁶. Imaging files are publicly available on OSF (<https://osf.io/mw4y3/>).

Secondary statistical analysis

Subgroup comparisons. To test whether significant vertex-wise differences between the whole ADHD group and controls were driven by either responders or non-responders (or both), we ran independent-sample t -tests (two-tailed) using SPSS (v28, IBM) and compared the morphometric measures in the identified clusters separately between controls and responders, and between controls and non-responders. For completeness, we also compared CT, CV and SA between clinical subgroups. Similarly, where we observed significant vertex-wise differences between responders and non-responders, we ran independent sample t -tests to compare morphometric measures in these identified clusters separately between responders and controls and non-responders and controls. We considered these secondary analyses significant at $P < 0.05$.

Associations with symptom improvement. We ran linear regressions to determine whether differences between responders and non-responders were associated with individual improvement on either inattentive or hyperactive-impulsive symptoms. Morphometric measures were the independent variables, whereas the BAARS-IV inattentive and hyperactive-impulsive scores at follow-up, as compared with the baseline, were the dependent variables. We applied Bonferroni correction for multiple comparisons (number of clusters = 10, $P \leq 0.005$).

Genomic analyses (virtual histology)

Finally, we conducted an exploratory analysis to map our neuroanatomical results to putative biological processes and cell-types involved in MPH treatment response and brain development. This analysis included two steps: decoding and enrichment.

Decoding. First, we identified those genes whose spatial expression was significantly similar to our neuroanatomical patterns of interest (neuroanatomical differences between the participants with ADHD and the controls, and between the responders and non-responders). Following a previously published approach^{39,40}, we leveraged the Allen Human Brain Atlas of gene expression⁸⁷, which is currently the most comprehensive publicly available atlas of gene-expression in the brain, and Neurovault (<https://neurovault.org>), a Python code embedded within Neurovault and Neurosynth (<https://neurosynth.org>).

Enrichment. Next we tested how the resulting genes were enriched for: (1) genes previously linked to the regulation of the metabolism and signaling of key neurotransmitters potentially involved in MPH pharmacodynamics; and (2) genes expressed in brain cell types during development.

Specifically, in line with the mechanism of action of MPH, we focused on genes involved in the synthesis and degradation of dopamine and noradrenaline, as well as their receptors and transporters⁵⁵. We also included genes expressed within serotonergic pathways, as there is some evidence that MPH may also bind the 5-HT1A receptor; however, its biological significance is unclear⁸⁸. Finally, we tested genes regulating nitric oxide signaling, which has been suggested to be affected by MPH treatment⁸⁹. The tested genes were based on gene-set annotations listed in the human gene ontology database (<http://www.gsea-msigdb.org/>)⁹⁰. The gene list overlap was computed using a specific R (v.4.3) code written by M.V. Lombardo (github.com/mvlombardo/utills/blob/master/genelistOverlap.R)³⁹.

Finally, due to the developmental nature of ADHD, we explored if the identified genes overlapped with those expressed by brain cell types during development, including progenitor cells, microglia, astrocytes

and neuronal cells (excitatory and inhibitory). The cell type-specific gene lists were based on a past work²¹ and are available at (<http://www.gsea-msigdb.org/>). We corrected each of these enrichment analyses for multiple comparisons across all contrasts and gene sets ($P_{\text{FDR}} < 0.05$).

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

Imaging files are publicly available via OSF at <https://doi.org/10.17605/OSF.IO/MW4Y3> (ref. 92).

Code availability

Magnetic resonance imaging data analysis was performed using the FreeSurfer v.5.3.0 software⁷⁸, which was used to derive tessellated models of the cortical surface for each T1-weighted image. Vertex-wise statistical analyses of each cortical feature were conducted using the SurfStat toolbox⁹² for MATLAB (R2014a, The Mathworks). Following a previously published approach^{39,40}, we leveraged the Allen Human Brain Atlas (AHBA) of gene expression⁸⁷, currently the most comprehensive publicly available atlas of gene-expression in the brain, and Neurovault (<https://neurovault.org>), a Python code embedded within Neurovault and Neurosynth (<https://neurosynth.org>), for decoding. For the enrichment analysis, the tested genes were based on gene-set annotations listed in the human gene ontology database (<http://www.gsea-msigdb.org/>)⁹⁰; the gene list overlap was computed using a specific R (v.4.3) code written by M.V. Lombardo (github.com/mvlombardo/utills/blob/master/genelistOverlap.R)^{39,40}.

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Author contributions

All of the authors made a substantial contribution to the study. V.P. was responsible for the recruitment of participants, data acquisition and analysis, and writing the paper. D.S.A. was responsible for the data analysis and writing. C.M.P. and M.A. were responsible for the genomic analysis and writing. E.D. and C.E. contributed to study design and supervision. D.G.M. contributed to study design, supervision and critical revision of the paper.

Competing interests

D.M. was funded by two IMI initiatives (EU AIMS and AIMS-2-TRIALS) under grant agreement nos.115300 and 777394. The other authors have no conflicts of interest to declare.

Additional information

Supplementary information The online version contains supplementary material available at <https://doi.org/10.1038/s44220-024-00228-y>.

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Reporting Summary

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Software and code

Policy information about [availability of computer code](#)

Data collection

Data analysis

Freesurfer:

FreeSurfer v5.3.0 software (<http://surfer.nmr.mgh.harvard.edu/>) was used to derive tessellated models of the cortical surface for each T1-weighted image. Vertex-wise statistical analyses of each cortical feature were conducted using the SurfStat toolbox (www.math.mcgill.ca/keith/surfstat/) for MATLAB (R2014a, The Mathworks, Massachusetts).

Statistics:

We used SPSS (v28, IBM) to run the statistical analyses.

Decoding:

Following a previously published approach (Pretzsch et al 2023, Pretzsch et al 2022), we leveraged the Allen Human Brain Atlas (AHBA) of gene expression (Hawrylycz et al 2012) and Neurovault (<https://neurovault.org>), a python code embedded within Neurovault and Neurosynth (<https://neurosynth.org>).

Enrichment:

The gene list overlap was computed using a specific R (v4.3) code written by MV Lombardo, as previously described (github.com/mvlombardo/utis/blob/master/genelistOverlap.R)(Pretzsch et al 2023).

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio [guidelines for submitting code & software](#) for further information.

Data

Policy information about [availability of data](#)

All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our [policy](#)

Methods:

Decoding:

We used the Allen Human Brain Atlas (AHBA) of gene expression (Hawrylycz et al 2012), currently the most comprehensive publicly available atlas of gene-expression in the brain (<http://www.brain-map.org>).

Enrichment:

The tested genes were based on gene-set annotations listed in the human gene ontology database (<http://www.gsea-msigdb.org/>) (The Gene Ontology 2019).

Imaging:

Imaging files are publicly available on OSF (<http://osf.io/mw4y3/>).

Human research participants

Policy information about [studies involving human research participants and Sex and Gender in Research](#).

Reporting on sex and gender

Methods: We only recruited males, because ADHD is more commonly diagnosed in males (Young et al., 2020) and there is preliminary evidence of sex differences in brain morphometry and biological response to stimulants (Carucci et al 2023, Duffy & Epperson 2022, Manza et al., 2022), thus we included only males to enhance sample homogeneity (see Discussion for potential limitations).

Population characteristics

Sample characteristics are detailed in suppl. material (page 2) as follows:

Sample characteristics

60 male adults with ADHD completed the study. They mostly identified as White British (71%), had a mean (\pm SD) age of 28 (± 7) years and a full-scale IQ of 109 (± 12); most of them was right-handed (78%) and medication-naïve (77%). Controls were matched for age, sex and IQ, and were predominantly right-handed (90%).

Concerta XL was titrated up to 54 mg in most ADHD individuals but, due to side effects, the dose was reduced to 36 mgs in 24% of cases and to 18 mgs in 7% of cases. Only two individuals increased the dose. We included dose among covariates in our analyses (see Methods).

Most participants (72%) were not assessed for ADHD before taking part into this study. None had a clinically diagnosed co-occurrent condition; however, one of them was diagnosed with Oppositional Defiant Disorder (ODD) and 8 with Dyslexia as children. Most of them were ADHD medication-naïve (77%); and none was on any psychotropic medication at the time of the study. Only 14 individuals were previously treated with ADHD medication. Among these, only three were classified as responders during this study.

Abstinence from illicit drugs was tested using urine drug screening at the time of the scan. Out of the 56 completed urine drug screenings, 46 were negative (82%). Treatment adherence was tested by carrying out MPH assays at follow-up. Out of the 55 completed MPH assays, 49 were positive (89%). All 60 ADHD participants were analyzed according to an intention to treat approach.

At follow-up, 42 participants were classified as responders and 18 as non-responders based on their total BAARS-IV score (reduction above or below 30% as compared to baseline). Average improvement of symptoms was 19.9 (± 7.85) in responders and 4.17 (± 4.17) in non-responders. The two groups did not significantly differ in ethnicity, age, total IQ, handedness, clinical presentation, baseline severity, and MPH dose at follow-up. Full details are reported in [3].

Recruitment

Details on recruitment and inclusion criteria are provided in Methods ('Sample and research protocol') and potential limitations in the Discussion.

Ethics oversight

Methods: Ethical approval by Camden and Islington Research Ethics Committee (12/LO/0630).

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

- Life sciences Behavioural & social sciences Ecological, evolutionary & environmental sciences

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	Methods: the sample size was determined based on a power calculation (supplementary material).
Data exclusions	No participant was excluded. See also note in suppl. material (page 2): all 60 participants were analyzed according to an intention to treat approach.
Replication	Conclusion: These results need to be replicated and extended in independent samples and, preferably, confirmed by meta-analyses (Fusar-Poli et al 2018).
Randomization	Methods: This study is part of a single-blind placebo-controlled cross-over study, followed by a longitudinal open-label phase (NCT 03709940). This was not a randomized controlled trial. We divided the ADHD sample in responders and non-responders as described in Methods: According to previously published criteria, we used a categorical definition of treatment response based on a symptomatic improvement of at least 30%, as measured by the BAARS-IV total score at follow-up as compared to baseline (Biederman et al 2006, Rosler et al 2009).
Blinding	Methods: This study is part of a single-blind placebo-controlled cross-over study, followed by a longitudinal open-label phase (NCT 03709940). This was not a randomized controlled trial.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

- n/a Involved in the study
- Antibodies
 - Eukaryotic cell lines
 - Palaeontology and archaeology
 - Animals and other organisms
 - Clinical data
 - Dual use research of concern

Methods

- n/a Involved in the study
- ChIP-seq
 - Flow cytometry
 - MRI-based neuroimaging

Magnetic resonance imaging

Experimental design

Design type	Methods: This study is part of a single-blind placebo-controlled cross-over study, followed by a longitudinal open-label phase (NCT 03709940).
Design specifications	Methods: In this report, we focused on the structural MRI data. We included 60 adults with ADHD [...]. They completed MRI scanning before starting routine treatment with the same long-acting formulation of MPH.
Behavioral performance measures	No fMRI task.

Acquisition

Imaging type(s)	T1 weighted
Field strength	3T
Sequence & imaging parameters	parameters: 196 slices, 1.2 mm thickness with 1.2 mm gap, TR= 7.312 ms, TE= 3.016 ms, and flip angle= 11°.
Area of acquisition	Whole brain
Diffusion MRI	<input type="checkbox"/> Used <input checked="" type="checkbox"/> Not used

Preprocessing

Preprocessing software	FreeSurfer v5.3.0 software (http://surfer.nmr.mgh.harvard.edu/) was used to derive tessellated models of the cortical
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Preprocessing software	surface for each T1-weighted image.
Normalization	Individual freesurfer data was normalized into standard fsaverage space using mris_preproc.
Normalization template	fsaverage (full resolution)
Noise and artifact removal	no additional beyond standard freesurfer recon-all pipeline
Volume censoring	not applied

Statistical modeling & inference

Model type and settings	general linear model ($Y_i = \beta_0 + \beta_1 \text{Group} + \beta_2 \text{Age}$) and ($Y_i = \beta_0 + \beta_1 \text{Group} + \beta_2 \text{Age} + \beta_3 \text{IQ} + \beta_4 \text{BAARS-IV} + \beta_5 \text{Dose} + \beta_6 \text{Weight} + \beta_7 \text{Handedness} + \epsilon_i$). Default settings for the surfstat package were used to estimate models.
Effect(s) tested	group (i.e., ADHD v control), treatment response (i.e., responders v non-responders)
Specify type of analysis:	<input checked="" type="checkbox"/> Whole brain <input type="checkbox"/> ROI-based <input type="checkbox"/> Both
Statistic type for inference (See Eklund et al. 2016)	'random field theory' (RFT)-based cluster analysis for non-isotropic images at a cluster-threshold of $p < 0.05$ (two-tailed) (Worsley et al 1999)
Correction	corrections for multiple comparisons were performed using a 'random field theory' (RFT)-based cluster analysis

Models & analysis

n/a	Involvement in the study
<input checked="" type="checkbox"/>	<input type="checkbox"/> Functional and/or effective connectivity
<input checked="" type="checkbox"/>	<input type="checkbox"/> Graph analysis
<input checked="" type="checkbox"/>	<input type="checkbox"/> Multivariate modeling or predictive analysis